

LETTERS

Coupling of surface temperatures and atmospheric CO₂ concentrations during the Palaeozoic era

Rosemarie E. Came¹, John M. Eiler¹, Ján Veizer², Karem Azmy³, Uwe Brand⁴ & Christopher R. Weidman⁵

Atmospheric carbon dioxide concentrations seem to have been several times modern levels during much of the Palaeozoic era (543–248 million years ago), but decreased during the Carboniferous period to concentrations similar to that of today^{1–3}. Given that carbon dioxide is a greenhouse gas, it has been proposed that surface temperatures were significantly higher during the earlier portions of the Palaeozoic era¹. A reconstruction of tropical sea surface temperatures based on the $\delta^{18}\text{O}$ of carbonate fossils indicates, however, that the magnitude of temperature variability throughout this period was small⁴, suggesting that global climate may be independent of variations in atmospheric carbon dioxide concentration. Here we present estimates of sea surface temperatures that were obtained from fossil brachiopod and mollusc shells using the ‘carbonate clumped isotope’ method⁵—an approach that, unlike the $\delta^{18}\text{O}$ method, does not require independent estimates of the isotopic composition of the Palaeozoic ocean. Our results indicate that tropical sea surface temperatures were significantly higher than today during the Early Silurian period (443–423 Myr ago), when carbon dioxide concentrations are thought to have been relatively high, and were broadly similar to today during the Late Carboniferous period (314–300 Myr ago), when carbon dioxide concentrations are thought to have been similar to the present-day value. Our results are consistent with the proposal that increased atmospheric carbon dioxide concentrations drive or amplify increased global temperatures^{1,6}.

The link between atmospheric CO₂ concentrations and Earth surface temperatures is central to our understanding of environmental change at many times in Earth history⁷. Among the most puzzling times for our understanding of the climatic consequences of CO₂ is the Palaeozoic era (the period between 543 and 248 Myr ago) that saw the emergence and diversification of the major classes of large-bodied animal and plant life forms.

Modelled atmospheric CO₂ concentrations between the mid-Cambrian and latest Silurian (530–417 Myr ago; that is, the early Palaeozoic) were 12–17 times higher than the modern atmosphere, and were followed by far lower levels (comparable to the modern atmosphere) during the Carboniferous period (354–290 Myr ago; that is, the late Palaeozoic)^{1,2}. These model estimates are supported by independent geochemical proxies of atmospheric CO₂ (refs 8–10). In addition, the large decrease in atmospheric CO₂ that these models propose for the beginning of the Carboniferous period is a plausible cause of the extensive Carboniferous glaciation^{11–13}.

However, northern Africa experienced extensive glaciation during the Late Ordovician and Early Silurian^{12,13}, when atmospheric CO₂ is inferred to have been 12–17 times modern values^{1,2,10}. This suggests that either model reconstructions of atmospheric CO₂ levels are prone to large errors or that climate can vary dramatically, independent of variations in atmospheric CO₂. Furthermore, Veizer *et al.*⁴

have reconstructed tropical shallow-marine temperatures during the Palaeozoic by applying the oxygen isotope carbonate-water thermometer to well-preserved carbonate fossils from sediments deposited in shallow water at low latitude (less than about 30° of latitude). Their results suggest that tropical shallow-marine temperatures were similar to each other and within ~5 °C of modern conditions during times of high inferred atmospheric CO₂, such as the Silurian, and during times of lower inferred atmospheric CO₂, such as the Carboniferous. The Phanerozoic temperature trend implied by Veizer *et al.*⁴ has four ‘icehouse/greenhouse’ modes and resembles the sedimentological and palaeontological climate reconstruction of Scotese (see ref. 14 and www.scotese.com/climate.htm). Veizer *et al.*⁴ suggest on this basis that global climate is not well coupled with atmospheric CO₂ concentrations over the timescale of the Phanerozoic eon⁴.

This debate regarding climatic conditions during the Palaeozoic suffers from two uncertainties. First, geologic evidence for the spatial and temporal distribution of sediments and fossils provides qualitative constraints on climate, but cannot be easily translated into a measure of global temperature and therefore does not clearly and directly test models of global climate. Second, oxygen isotope constraints on surface temperature are vulnerable to artefacts from diagenetic or burial-metamorphic overprints^{4,15} and require assumptions or independent constraints on the oxygen isotopic compositions of the waters in which carbonate fossils grew. These issues have led to decades-long uncertainty as to whether the systematic temporal variations in oxygen isotope compositions of Phanerozoic marine carbonate fossils reflects climate change, variation in the $\delta^{18}\text{O}$ of sea water, or post-depositional alteration^{15,16}.

We address these issues by applying the carbonate clumped-isotope thermometer to aragonite and low-Mg calcite fossils of Palaeozoic age. This thermometer examines ordering, or ‘clumping’, of ¹³C and ¹⁸O into bonds with each other in the carbonate mineral lattice. This isotope effect is temperature dependent, and can be examined by analysis of ¹³C¹⁸O¹⁶O in CO₂ released from carbonates by phosphoric acid digestion. Importantly, it provides a temperature constraint that depends only on the isotopic composition of carbonate and is independent of the isotopic composition of the water in which the carbonate grew⁵ (see Methods). Furthermore, our approach permits us to estimate the $\delta^{18}\text{O}$ of sea water on the basis of known growth temperatures and $\delta^{18}\text{O}$ values of carbonate fossils.

We examined two suites of relatively well preserved carbonate shells of shallow-water marine organisms that lived at palaeolatitudes within 20° of the Equator: (1) early Silurian brachiopods consisting of low-Mg calcite, collected from the Telychian-age Jupiter Formation on Anticosti Island, Canada^{17,18}; and (2) Carboniferous (Middle Pennsylvanian) aragonitic molluscs, collected from the

¹Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California 91125, USA. ²Ottawa-Carleton Geoscience Centre, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada. ³Department of Earth Sciences, Memorial University of Newfoundland, St John's, Newfoundland A1B 3X5, Canada. ⁴Department of Earth Sciences, Brock University, St Catharines, Ontario L2S 3A1, Canada. ⁵Waquoit Bay National Estuarine Research Reserve, Waquoit, Massachusetts 02536, USA.

Boggy Formation, in southern Oklahoma, USA¹⁹. Both suites include equal numbers of samples that appear, on independent evidence (visual, microscopic¹⁸, X-ray diffraction and/or trace-element analysis^{20,21}), to be well preserved, and samples that appear to be moderately altered by post-depositional processes. These two sub-sets of each suite were selected so that we could systematically examine the effects of alteration on the isotopic record (see Supplementary Information for further details).

Pennsylvanian samples exhibit a positive correlation between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values (Table 1 and Fig. 1). The high- $\delta^{18}\text{O}$, high- $\delta^{13}\text{C}$ end of this trend is associated with elevated Fe abundances, Mn abundances and/or proportions of secondary calcite, suggesting that post-depositional alteration caused increases in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in this suite. The direction of this trend is contrary to common expectations that alteration leads to decreases in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (refs 15, 16, 21), but the mineralogical, textural and trace element attributes of the studied samples argue for such an interpretation. Silurian samples exhibit little variation in $\delta^{18}\text{O}$ but significant variability in $\delta^{13}\text{C}$ (Table 1 and Fig. 1). On the basis of visual evidence for recrystallization¹⁸, lower $\delta^{13}\text{C}$ values are associated with increasing post-depositional alteration (this result, though supported by relatively straightforward observations, is also contrary to common inferences regarding the isotopic effects of diagenesis and burial metamorphism).

The apparent temperatures of carbonate growth based on clumped isotope thermometry and the calculated $\delta^{18}\text{O}$ values of water in equilibrium with our samples at those apparent temperatures are presented in Table 1 and Fig. 1. Pennsylvanian samples exhibit a positive correlation between temperature and $\delta^{18}\text{O}$ of water, and a

clear association of altered samples with higher temperatures and higher water $\delta^{18}\text{O}$. The data reinforce our interpretation of the correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ for these samples, and indicate that the low-Fe, low-Mn, aragonite-rich sub-set of this suite (samples B81-21, B81-18 and B81-06x) most closely preserve their depositional isotopic compositions. Silurian samples yield a bimodal distribution of apparent temperatures and values of water $\delta^{18}\text{O}$: all but one of the nominally unaltered samples (based on visual inspection; that is, samples A1380b-30, A1391b-31, A1391b-06 and A1356a-37) group tightly around a mean temperature near $\sim 35^\circ\text{C}$. Visibly altered samples exhibit a positive correlation between temperature and $\delta^{18}\text{O}$ of water, and an association of altered samples with higher temperatures and higher water $\delta^{18}\text{O}$. These data also reinforce our interpretation that alteration is associated with low carbonate $\delta^{13}\text{C}$ in this sample suite.

We suggest that the average apparent temperatures and water $\delta^{18}\text{O}$ values recorded by the least altered subset of each sample suite best represent the original fossil growth conditions. These data yield nominal growth conditions of $24.9 \pm 1.7^\circ\text{C}$ and seawater $\delta^{18}\text{O}$ of $-1.6 \pm 0.5\text{‰}$ (both ± 1 s.e.) for the Middle Pennsylvanian, and $34.9 \pm 0.4^\circ\text{C}$ and seawater $\delta^{18}\text{O}$ of $-1.2 \pm 0.1\text{‰}$ for the Early Silurian. It is possible that even these averages are influenced by subtle post-depositional alteration and thus over-estimate depositional temperatures and seawater $\delta^{18}\text{O}$ values. It is not clear how one could ever strictly disprove this possibility. Nevertheless, all samples that contribute to these averages pass conventional criteria for a high level of preservation, each group is homogeneous within analytical precision, the Silurian data are consistent with previous measurements of the $\delta^{18}\text{O}$ values of well-preserved conodont fossils²², and both of our suites yield seawater $\delta^{18}\text{O}$ similar to that of the modern ocean, after adding back the water currently stored as polar ice. This is in accord with models of the global water isotopic budget that suggest nearly constant $\delta^{18}\text{O}$ of sea water throughout the Phanerozoic eon^{23,24} (but see ref. 25 for an alternative model that permits variations in the $\delta^{18}\text{O}$ of sea water). Finally, we also note that average growth temperatures and water $\delta^{18}\text{O}$ values for nominally unaltered samples from each suite may be influenced by ecological variability, and thus may be offset from true averages for their respective palaeolatitudes and ages. This possibility could be investigated further through more detailed studies covering a wider range of locations and depositional conditions for a given time period.

Our results provide a basis for discriminating between previous competing hypotheses regarding the character of Palaeozoic climate change and the $\delta^{18}\text{O}$ of the Phanerozoic ocean. First, we find that when atmospheric CO_2 is inferred to have been highly elevated compared to modern levels—that is, during the Early Silurian—shallow-marine temperatures were markedly elevated, and when atmospheric CO_2 was nearly as low as modern values—during the Middle Pennsylvanian—shallow-marine temperatures were similar to modern values^{1,2}. This result is consistent with the proposition that variations in atmospheric CO_2 concentration from the Silurian to the Pennsylvanian drove large variations in Earth surface temperatures¹ (Fig. 2; but note this raises a new question as to how such warm temperatures could be consistent with geological evidence for high-latitude glaciation during the earliest Silurian^{12,13}). More generally, our results are consistent with the hypothesis that elevated CO_2 concentrations are capable of producing Earth surface temperatures substantially ($5\text{--}11^\circ\text{C}$) higher than modern values. Second, our results support previous arguments that the $\delta^{18}\text{O}$ of sea water has varied within a narrow range throughout the Phanerozoic eon^{23,24} and argue against suggestions that it was several per mil lower during the Palaeozoic^{15,17}. Although there are many differences between Palaeozoic and modern climates, the suggestion our results give of a link between increased CO_2 and a large temperature increase provides a point of reference for models of projected climate change associated with currently rising concentrations of atmospheric greenhouse gases.

Table 1 | Stable isotope and temperature data

Sample ID	Species	$\delta^{18}\text{O}_\text{C}$ (‰, PDB)	$\delta^{13}\text{C}_\text{C}$ (‰, PDB)	Δ_{47} (‰)	T (°C)	$\delta^{18}\text{O}$ of water (‰, SMOW)
Unaltered Middle Pennsylvanian samples						
B81-21	<i>Domatoceras</i> sp.	-3.15	0.49	0.66	21.6	-2.77
B81-21	<i>Domatoceras</i> sp.	-3.26	0.55	0.65	24.0	-2.37
B81-21	<i>Domatoceras</i> sp.	-2.81	0.52	0.67	19.0	-2.97
B81-18	<i>Metacoceras cornutum</i>	-2.38	1.92	0.64	26.8	-0.92
B81-18	<i>Metacoceras cornutum</i>	-2.33	1.97	0.64	26.1	-0.99
B81-06x	<i>Orthoceras unicamera</i>	-2.99	2.00	0.65	25.1	-1.86
B81-06x	<i>Orthoceras unicamera</i>	-2.74	2.02	0.62	30.2	-0.58
B81-06x	<i>Orthoceras unicamera</i>	-2.41	2.02	0.64	27.0	-0.89
B81-06x	<i>Orthoceras unicamera</i>	-2.67	2.03	0.65	24.6	-1.65
Middle Pennsylvanian samples suspected of alteration						
B0-01	Cephalopod fragment	-1.43	3.25	0.64	26.8	0.04
B0-01	Cephalopod fragment	-1.43	3.22	0.63	27.9	0.25
B0-04	Cephalopod fragment	-1.13	3.50	0.65	23.4	-0.37
B0-04	Cephalopod fragment	-1.07	3.52	0.65	24.2	-0.14
Altered Middle Pennsylvanian samples						
B81-27	Cephalopod fragment	-0.25	5.60	0.63	29.3	1.72
B81-27	Cephalopod fragment	-0.28	5.61	0.63	29.2	1.69
B81-09	<i>Domatoceras</i> sp.	-0.76	3.74	0.62	30.5	1.46
B81-09	<i>Domatoceras</i> sp.	-0.78	3.66	0.63	29.7	1.27
B81-10	<i>Domatoceras</i> sp.	-0.28	3.52	0.61	34.5	2.74
B81-10	<i>Domatoceras</i> sp.	-0.73	3.51	0.62	32.0	1.79
B81-40	<i>Pseudorthoceras knoxense</i>	0.28	6.36	0.60	37.0	3.79
Unaltered Silurian samples						
A1380b-30	<i>Pentamerus</i> sp.	-5.34	1.94	0.61	33.7	-1.50
A1391b-31	<i>Pentamerus</i> sp.	-5.00	2.08	0.60	34.8	-0.95
A1391b-31	<i>Pentamerus</i> sp.	-4.94	2.03	0.60	35.3	-0.79
A1391b-06	<i>Pentamerus</i> sp.	-5.11	1.78	0.60	35.7	-0.88
A1391b-06	<i>Pentamerus</i> sp.	-5.12	1.71	0.61	34.2	-1.19
A1356a-37	<i>Pentamerus</i> sp.	-5.63	1.82	0.60	35.8	-1.39
Silurian samples suspected of alteration						
A1356a-07	<i>Pentamerus</i> sp.	-5.07	1.73	0.57	42.9	0.48
A1356a-07	<i>Pentamerus</i> sp.	-5.10	1.71	0.57	43.9	0.62
Altered Silurian samples						
A-551 alt	<i>Stricklandia planirostrata</i>	-5.22	-1.13	0.50	63.8	3.89
A-958	<i>Ehlersella davidsoni</i>	-5.12	0.18	0.51	62.4	3.75
A-958-2 alt	<i>Ehlersella davidsoni</i>	-5.20	-0.03	0.51	61.3	3.49
A-958-3	<i>Ehlersella davidsoni</i>	-4.88	0.86	0.54	53.0	2.43
A-958-4 alt	<i>Ehlersella davidsoni</i>	-4.88	0.21	0.52	56.7	3.07

$\delta^{18}\text{O}_\text{C}$ and $\delta^{13}\text{C}_\text{C}$ are the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of carbonate. The Δ_{47} is defined as the difference in ‰ 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 of CO_2 (ref. 27) (see Methods) Here T is the Δ_{47} -derived temperature in degrees Celsius.

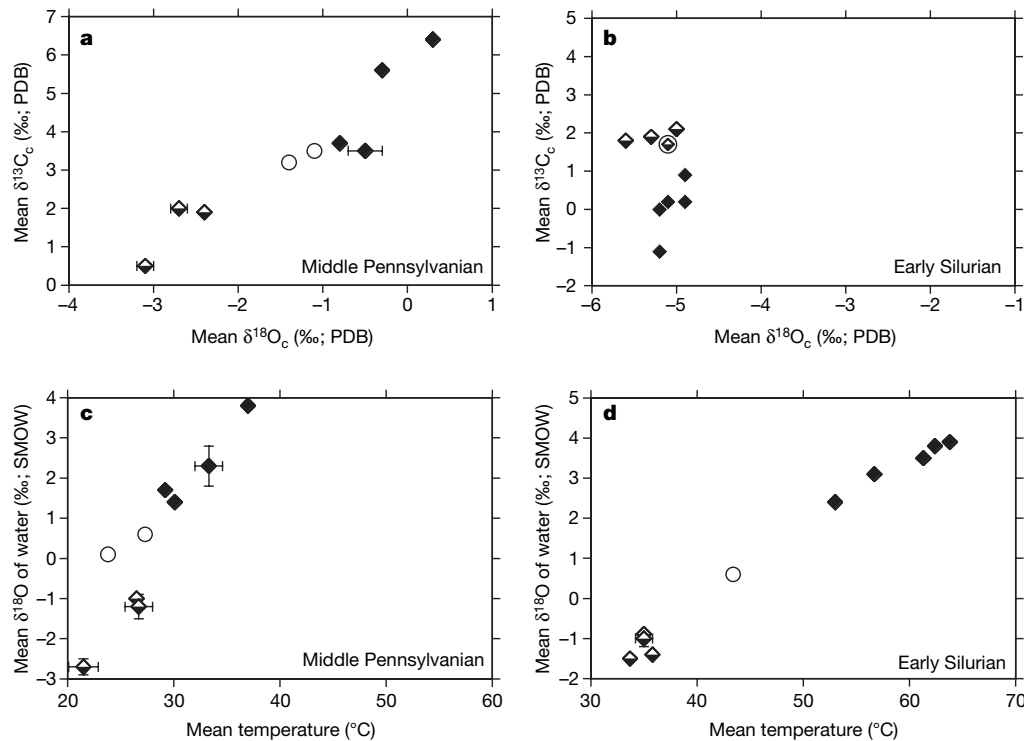


Figure 1 | Isotopic compositions and inferred crystallization temperatures of Pennsylvanian and Silurian fossils. **a**, Mean $\delta^{13}\text{C}_c$ (‰; PDB) versus mean $\delta^{18}\text{O}_c$ (‰; PDB) for Pennsylvanian aragonitic molluscs; **b**, same for Silurian calcitic brachiopods. **c**, Mean Δ_{47} -derived temperatures versus the calculated $\delta^{18}\text{O}$ (SMOW) of sea water and/or diagenetic waters for

Pennsylvanian aragonitic molluscs; **d**, same for Silurian calcitic brachiopods. In all panels, well-preserved samples are represented by half-filled diamonds; diagenetically altered samples by filled diamonds; and samples suspected of alteration by open circles. Error bars represent ± 1 s.e. on replicate analyses.

Note that the discrepancy between our results and the more subtle temperature variations in the reconstruction of Veizer *et al.* (Fig. 2) primarily reflects the fact that this previous study assumed large ice volume variations in the $\delta^{18}\text{O}$ of the ocean, whereas our data suggest such changes were minimal. Thus, our results suggest both positive and negative things about previous attempts at palaeothermometry based on the $\delta^{18}\text{O}$ of Palaeozoic carbonate fossils. On the

one hand, we confirm that carefully selected fossils of this age are characterized by isotopic compositions that reflect their conditions of deposition (that is, the previously proposed criteria for identifying well-preserved samples are mostly predictive for samples that have experienced minimal re-crystallization and/or isotopic exchange). On the other hand, these earlier studies suggested that the variable and, on average, low values of $\delta^{18}\text{O}$ of well-preserved Palaeozoic fossils should be interpreted as evidence for secular variation in the $\delta^{18}\text{O}$ of sea water in an ocean that varied little in average temperature, whereas our data suggest the converse (Figs 2 and 3); that is, the results of this study support the data but contradict the interpretation of Veizer *et al.*

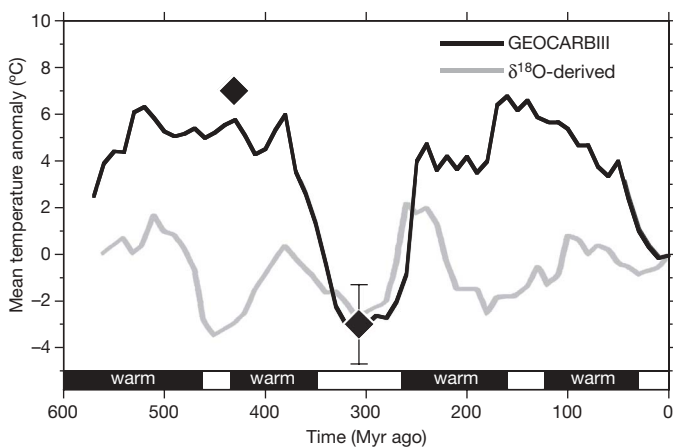


Figure 2 | Estimates of tropical temperature anomalies relative to today. The grey⁴ curve represents temperature estimates based on $\delta^{18}\text{O}$ of well-preserved carbonate fossils from palaeotropical seas; the black² curve represents GEOCARBIII model estimates of mean global temperatures based on reconstructions of atmospheric CO_2 levels; black bars (www.scotese.com/climate.htm) at the bottom represent time intervals during which global temperatures were as much as 10°C warmer than today based on the climate reconstruction of Scotese; filled diamonds represent our estimates of carbonate fossil growth temperatures. Error bars represent ± 1 s.e. on the Δ_{47} temperatures of well-preserved samples.

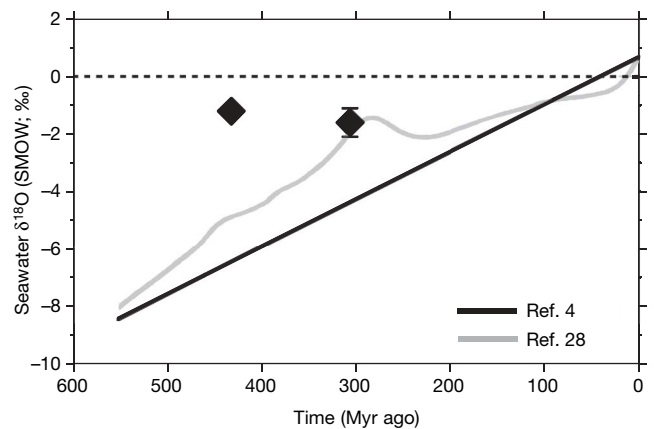


Figure 3 | Estimates of the oxygen isotopic composition of Phanerozoic sea water. The black⁴ and grey²⁸ curves represent previous model estimates; the dashed line represents modern seawater $\delta^{18}\text{O}$ (SMOW); filled diamonds represent new estimates of Pennsylvanian and Silurian seawater $\delta^{18}\text{O}$ calculated from carbonate $\delta^{18}\text{O}$ and Δ_{47} temperatures. Error bars represent ± 1 s.e. on the seawater $\delta^{18}\text{O}$ of well-preserved samples.

Our re-interpretation of the $\delta^{18}\text{O}$ values of Silurian and Pennsylvanian carbonate fossils also may apply to other parts of the Palaeozoic. However, there remain several marked discrepancies between climate reconstructions using the GEOCARB model versus those implied by the Scotese geological record and the Veizer *et al.* oxygen isotope record (which generally agree with each other, at least in timing of climate variations), and it is difficult to imagine that all time periods will be resolved in the same way as those examined in this study. For example, many carbonate fossils from the Cambrian and early Ordovician are so negative in $\delta^{18}\text{O}_{\text{PDB}}$ (in the range -8% to -10%) that they cannot plausibly represent precipitation from an ocean with seawater $\delta^{18}\text{O}_{\text{SMOW}} \approx 0\%$, because in that case they would imply growth temperatures ($54\text{--}67\text{ }^\circ\text{C}$) far in excess of the maximum temperature at which shallow-marine organisms can survive ($37\text{ }^\circ\text{C}$)²⁶. Application of carbonate clumped isotope thermometry to these extreme samples could reveal whether their low $\delta^{18}\text{O}$ values reflect consistently high levels of post-depositional alteration or low $\delta^{18}\text{O}$ values of sea water.

METHODS SUMMARY

CO_2 was extracted from all samples by phosphoric acid digestion using the laboratory methods described in ref. 5. Product CO_2 was analysed using a Finnigan MAT 253 gas source mass spectrometer configured to collect masses 44–49, inclusive, and standardized by comparison with CO_2 gases of known isotopic composition that had been heated for two hours at $1,000\text{ }^\circ\text{C}$ to achieve a stochastic isotopic distribution²⁷ (see Methods). Several heated gas standards, spanning a range of bulk stable isotope compositions, were analysed to minimize the potential errors associated with mass spectrometric nonlinearities, which are observable when the compositions of samples and standards differ by more than 20–30‰ in any given isotope ratio²⁷ (see Methods). Masses 48 and 49 were monitored to assure adequate sample purification. Each measurement consisted of 6–9 acquisitions, with typical standard deviations (acquisition-to-acquisition) of 0.02‰ to 0.05‰ in A_{47} (see Table 1 footnote). Values of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were acquired as part of each analysis. Measured values of A_{47} were used to estimate carbonate growth temperature (T , in kelvin) using the relationship⁵:

$$A_{47} = 0.0592(10^6 T^{-2}) - 0.02.$$

Analyses of modern molluscs and brachiopods establish that this relationship holds for these forms of biogenic carbonate (see Methods). Paired temperature and carbonate $\delta^{18}\text{O}$ data were used to calculate the $\delta^{18}\text{O}$ value of formation and/or diagenetic waters using previously published calibrations of the temperature dependence of carbonate-water fractionations (see Methods).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 15 April; accepted 3 July 2007.

- Berner, R. A. GEOCARBII: A revised model of atmospheric CO_2 over Phanerozoic time. *Am. J. Sci.* **294**, 56–91 (1994).
- Berner, R. A. & Kothavala, Z. GEOCARBIII: A revised model of atmospheric CO_2 over Phanerozoic time. *Am. J. Sci.* **301**, 182–204 (2001).
- François, L. M. & Walker, J. C. G. Modelling the Phanerozoic carbon cycle and climate: Constraints from the $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic signature of seawater. *Am. J. Sci.* **292**, 81–135 (1992).
- Veizer, J., Godderis, Y. & François, L. M. Evidence for decoupling of atmospheric CO_2 and global climate during the Phanerozoic eon. *Nature* **408**, 698–701 (2000).

- Ghosh, P. *et al.* ^{13}C – ^{18}O bonds in carbonate minerals: A new kind of paleothermometer. *Geochim. Cosmochim. Acta* **70**, 1439–1456 (2006).
- Royer, D. L., Berner, R. A. & Park, J. Climate sensitivity constrained by CO_2 concentrations over the past 420 million years. *Nature* **446**, 530–532 (2007).
- Ruddiman, W. F. *Earth's Climate: Past and Future* (Freeman, New York, 2001).
- Montañez, I. P. *et al.* CO_2 -forced climate and vegetation instability during Late Paleozoic deglaciation. *Science* **315**, 87–91 (2007).
- Mora, C. I., Driese, S. G. & Colarusso, L. A. Middle to late Paleozoic atmospheric CO_2 levels from soil carbonate and organic matter. *Science* **271**, 1105–1107 (1996).
- Yapp, C. J. & Poths, H. Ancient atmospheric CO_2 pressures inferred from natural goethites. *Nature* **355**, 342–344 (1992).
- Crowley, T. J. & North, G. R. *Paleoclimatology* (Oxford Univ. Press, Oxford, 1991).
- Caputo, M. V. & Crowell, J. C. Migration of glacial centers across Gondwana during Paleozoic Era. *Geol. Soc. Am. Bull.* **96**, 1020–1036 (1985).
- Frakes, L. A. & Francis, J. E. A guide to Phanerozoic cold polar climates from high-latitude ice-rafting in the Cretaceous. *Nature* **333**, 547–549 (1988).
- Boucot, A. J., Xu, C. & Scotese, C. R. Phanerozoic climate zones and paleogeography with a consideration of atmospheric CO_2 levels. *Paleont. J.* **38**, 115–122 (2004).
- Veizer, J. *et al.* $^{87}\text{Sr}/^{86}\text{Sr}$, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ evolution of Phanerozoic seawater. *Chem. Geol.* **161**, 59–88 (1999).
- Land, L. S. Comment on “Oxygen and carbon isotopic composition of Ordovician brachiopods: Implications for coeval seawater” by H. Qing and J. Veizer. *Geochim. Cosmochim. Acta* **59**, 2843–2844 (1995).
- Azmy, K., Veizer, J., Bassett, M. G. & Copper, P. Oxygen and carbon isotopic composition of Silurian brachiopods: Implications for coeval seawater and glaciations. *Geol. Soc. Am. Bull.* **110**, 1499–1512 (1998).
- Azmy, K., Veizer, J., Jin, J., Copper, P. & Brand, U. Paleobathymetry of a Silurian shelf based on brachiopod assemblages: An oxygen isotope test. *Can. J. Earth Sci.* **43**, 281–293 (2006).
- Squires, R. L. *Burial Environment, Diagenesis, Mineralogy and Mg & Sr Contents of Skeletal Carbonates in the Buckhorn Asphalt of Middle Pennsylvanian Age, Arbuckle Mountains, Oklahoma*. PhD thesis, California Inst. Technol. (1973).
- Brand, U. The oxygen and carbon isotopic composition of Carboniferous fossil components: Sea-water effects. *Sedimentology* **29**, 139–147 (1982).
- Brand, U. Aragonite-calcite transformation based on Pennsylvanian molluscs. *Geol. Soc. Am. Bull.* **101**, 377–390 (1989).
- Wenzel, B., Lécuyer, C. & Joachimski, M. M. Comparing oxygen isotope records of Silurian calcite and phosphate— $\delta^{18}\text{O}$ compositions of brachiopods and conodonts. *Geochim. Cosmochim. Acta* **64**, 1859–1872 (2000).
- Gregory, R. T. & Taylor, H. P. An oxygen isotope profile in a section of Cretaceous oceanic crust, Samail ophiolite, Oman – Evidence for $\delta^{18}\text{O}$ buffering of the oceans by deep (less than 5 km) seawater-hydrothermal circulation at mid-ocean ridges. *J. Geophys. Res.* **86**, 2737–2755 (1981).
- Muehlenbachs, K. The oxygen isotopic composition of the oceans, sediments and the seafloor. *Chem. Geol.* **145**, 263–273 (1998).
- Kasting, J. F. *et al.* Paleoclimates, ocean depth, and the oxygen isotopic composition of seawater. *Earth Planet. Sci. Lett.* **252**, 82–93 (2006).
- Brock, T. D. Life at high temperatures. *Science* **230**, 132–138 (1985).
- Eiler, J. M. & Schauble, E. ^{18}O – ^{13}C – ^{16}O in Earth's atmosphere. *Geochim. Cosmochim. Acta* **68**, 4767–4777 (2004).
- Wallmann, K. Impact of atmospheric CO_2 and galactic cosmic radiation on Phanerozoic climate change and the marine $\delta^{18}\text{O}$ record. *Geochem. Geophys. Geosys.* **5**, Q06004 10.1029/2003GC000683 (2004).

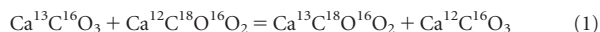
Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank H. Affek, W. Guo and P. Ghosh for laboratory advice, and A. Wanamaker for assistance with samples.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to R.E.C. (rcame@gps.caltech.edu).

METHODS

Clumped isotope geochemistry. The carbonate ‘clumped isotope’ palaeothermometer involves the temperature-dependent ‘clumping’ of ^{13}C and ^{18}O (that is, the formation of bonds between these two rare isotopes) within the carbonate mineral lattice. The abundance of ^{13}C – ^{18}O bonds in carbonate minerals depends on a thermodynamically controlled stable isotopic exchange equilibrium among various carbonate isotopologues, for example:



Importantly, the temperature-dependent equilibrium constant for this reaction can be calculated on the basis of the isotopic composition of carbonate alone and does not require knowledge of the isotopic composition of the water in which the carbonate formed⁵. For this reason, carbonate clumped isotope thermometry can be applied rigorously to times and settings where the oxygen isotope composition of water is unknown.

The equilibrium constant for reaction (1) is reconstructed by isotopic analysis of CO_2 produced by reaction of carbonate with anhydrous phosphoric acid. These analyses involve simultaneous collection of ion beams corresponding to masses 44, 45, 46 (as for conventional measurements of the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of carbonates) and 47. The mass-47 ion beam includes contributions from three isotopologues: $^{18}\text{O}^{13}\text{C}^{16}\text{O}$, $^{17}\text{O}^{12}\text{C}^{18}\text{O}$ and $^{17}\text{O}^{13}\text{C}^{17}\text{O}$. This population is overwhelmingly dominated by $^{18}\text{O}^{13}\text{C}^{16}\text{O}$, and so largely reflects the abundance of ^{13}C – ^{18}O bonds in reactant carbonate. We define R^{47} as the ratio of the mass-47 isotopologues of CO_2 to the light isotopologue of CO_2 ($^{16}\text{O}^{12}\text{C}^{16}\text{O}$)²⁷:

$$R^{47} = (\text{mass } 47)/(\text{mass } 44) \quad (2)$$

We report variations in R^{47} by comparison with the ‘stochastic distribution’; that is, the relative abundance of isotopologues expected for a random distribution of all rare isotopes among all possible isotopologues. For a given set of $\delta^{18}\text{O}$, $\delta^{17}\text{O}$ and $\delta^{13}\text{C}$ values, R^{47} for the stochastic distribution is defined by²⁷:

$$R^{47}_{\text{stochastic}} = (2[18][16][13] + [17]^2[13] + 2[18][17][12]) / ([16]^2[12]) \quad (3)$$

where [12] and [13] are the concentrations of ^{12}C and ^{13}C within the pool of all carbon atoms in the analysed CO_2 , and [16], [17] and [18] are the concentrations of ^{16}O , ^{17}O and ^{18}O within the pool of all oxygen atoms in the analysed CO_2 .

Finally, we use Δ_{47} to report how measured values of R^{47} differ from the stochastic distribution. Δ_{47} is defined as the difference in ‰ between the measured R^{47} value of the sample and the R^{47} value expected for that sample if its stable carbon and oxygen isotopes were randomly distributed among all isotopologues^{27,29}:

$$\Delta_{47} = (R^{47}_{\text{measured}}/R^{47}_{\text{stochastic}} - 1) \times 1,000 \quad (4)$$

Laboratory methods. CO_2 was extracted from all samples using the laboratory methods described in ref. 5, which are an extension of well-established methods of phosphoric-acid digestion^{30,31}. Product CO_2 was analysed using a Finnigan MAT 253 gas source mass spectrometer configured to collect masses 44–49 and standardized by comparison with CO_2 gases of known isotopic composition that had been heated for two hours at 1,000 °C to achieve a stochastic isotopic distribution²⁷. Several heated gas standards, spanning a range of bulk stable isotope compositions, were analysed to minimize the potential errors associated with mass spectrometric nonlinearities, which are observable when the compositions of samples and standards differ by more than 20–30‰ in any given isotope ratio²⁷. Masses 48 and 49 were monitored to assure adequate sample purification. Each measurement consisted of 6–9 acquisitions, with typical standard deviations (acquisition-to-acquisition) of 0.02‰ to 0.05‰ in Δ_{47} . As part of the analyses, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were simultaneously acquired. The Δ_{47} values were converted to carbonate growth temperature using the relationship⁵:

$$\Delta_{47} = 0.0592(10^6 T^{-2}) - 0.02 \quad (5)$$

Paired temperature and carbonate $\delta^{18}\text{O}$ data were used to calculate the $\delta^{18}\text{O}$ value of water from which the carbonates grew using the equation³²:

$$10^3 \ln \alpha_{\text{calcite-water}} = 18.03(10^3 T^{-1}) - 32.42 + 0.25 \quad (6)$$

for calcitic brachiopods, and³³:

$$10^3 \ln \alpha_{\text{aragonite-water}} = 18.45(10^3 T^{-1}) - 32.54 \quad (7)$$

for aragonitic molluscs, where α is the fractionation factor, T is temperature (in K) and $\delta^{18}\text{O}$ values are versus SMOW.

Appropriateness of the inorganic Δ_{47} -temperature calibration for brachiopods and molluscs. Ghosh *et al.*⁵ determined the relationship between Δ_{47} and temperature (equation (5)) by analysing the CO_2 extracted from synthetic calcites grown in the laboratory at known, controlled temperatures. In addition, they analysed natural surface-dwelling corals (*Porites*) and deep-sea corals (*Desmophyllum dianthus*), which grew at known, approximately constant temperatures⁵. Their results indicate that the vital effects that influence the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of surface-dwelling and deep-sea corals do not influence the Δ_{47} values of the CO_2 extracted from that carbonate⁵. This result is consistent with models for vital effects³⁴, which describe the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ offsets as reservoir effects, rather than kinetic fractionations. Recent calibration work³⁵ reveals that fish otolith carbonate, which also suffers from vital effects in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, does not exhibit a significant offset from the Ghosh *et al.*⁵ calibration of the relationship between growth temperature and the Δ_{47} of CO_2 .

As part of the current study, we analysed naturally occurring brachiopods and molluscs (the two phyla from which we obtained Palaeozoic temperatures based on carbonate clumped isotope thermometry) that grew at known temperatures in the modern ocean (see Supplementary Table 1 and Supplementary Fig. 1). Our modern calibration materials agree very well (mean deviation of $\pm 0.009\text{‰}$ in Δ_{47} of CO_2) with the Ghosh *et al.*⁵ temperature relationship for synthetic calcites.

Previous work on brachiopods and molluscs has shown that these organisms generally precipitate carbonate shells in isotopic equilibrium with the waters in which they form^{36,37}, without any apparent vital effects. Given this previous evidence for equilibrium carbonate growth in molluscs and brachiopods, and our new calibration results (see Supplementary Information), we suggest that vital effects do not influence the temperature estimates obtained for fossil molluscs and brachiopods based on carbonate clumped isotope thermometry.

29. Wang, Z., Schauble, E. A. & Eiler, J. M. Equilibrium thermodynamics of multiply substituted isotopologues of molecular gases. *Geochim. Cosmochim. Acta* **68**, 4779–4797 (2004).
30. McCrea, J. M. On the isotopic chemistry of carbonates and a paleotemperature scale. *J. Chem. Phys.* **18**, 849–857 (1950).
31. Swart, P. K., Burns, S. J. & Leder, J. J. Fractionation of the stable isotopes of oxygen and carbon in carbon dioxide during the reaction of calcite with phosphoric acid as a function of temperature and technique. *Chem. Geol.* **86**, 89–96 (1991).
32. Kim, S.-T. & O’Neil, J. R. Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochim. Cosmochim. Acta* **61**, 3461–3475 (1997).
33. Böhm, F. E. *et al.* Oxygen isotope fractionation in marine aragonite of coralline sponges. *Geochim. Cosmochim. Acta* **64**, 1695–1703 (2000).
34. Adkins, J. F., Boyle, E. A., Curry, W. B. & Lutringer, A. Stable isotopes in deep sea corals and a new mechanism for ‘vital effect’. *Geochim. Cosmochim. Acta* **67**, 1129–1143 (2003).
35. Ghosh, P., Eiler, J. M., Campana, S. E. & Feeney, R. F. Calibration of the carbonate ‘clumped isotope’ paleothermometer for otoliths. *Geochim. Cosmochim. Acta* **71**, 2736–2744 (2007).
36. Brand, U., Logan, A., Hiller, N. & Richardson, J. Geochemistry of modern brachiopods: Applications and implications for oceanography and paleoceanography. *Chem. Geol.* **198**, 305–334 (2003).
37. Wanamaker, A. D. *et al.* An aquaculture-based method for calibrated bivalve isotope paleothermometry. *Geochim. Geophys. Geosyst.* **7**, Q09011, doi:10.1029/2005GC001189 (2006).