



Implications of high-precision measurements of ^{13}C – ^{18}O bond ordering in CO_2 for thermometry in modern bivalved mollusc shells

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Abstract

We report a temperature calibration for Δ_{47} from bivalve carbonate that lies within error of theoretical predictions (Schuble et al., 2006; Guo et al., 2009). The temperature sensitivity of this calibration is lower than several different earlier calibrations determined using either inorganic calcite, corals, foraminiferans and coccoliths, or brachiopods and bivalved molluscs, but it agrees with more recent Δ_{47} -temperature relationships determined from measurements of clumped-isotopes in mollusc and brachiopod shells.

We demonstrate that mollusc shell temperature calibrations originating from different laboratories that have not been corrected for instrument backgrounds may differ by as much as $\sim 0.07\%$ in Δ_{47} over the ~ 0 to 30°C temperature range even where $d\Delta_{47}/dT$ agree. Because recent calibrations for Δ_{47} vs. T from several different laboratories agree for bivalved mollusc shells, yet differ from an early calibration for bivalved molluscs, we suggest it is unlikely that temperature- Δ_{47} variability is attributable to phylum-specific vital effects, and instead conclude that differences in calibration slope between phyla and/or inorganic calcite are more easily explained by variability in measurements made in different laboratories. Discrepancies in both calibration slopes and/or intercepts indicate that Δ_{47} values measured in natural materials may be more significantly influenced by instrument-specific effects, as well as effects from sample preparation and handling and purification of CO_2 than current techniques are able to correct for, and therefore, temperatures obtained by comparing measurements of Δ_{47} to independently determined calcite calibrations may err by a far greater amount than acknowledged in previous studies.

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1. INTRODUCTION

The considerable investment in time and resources devoted to the application of clumped-isotope thermometry to naturally-occurring carbonates is testament to the potential of this proxy as a wholly independent intra-mineral paleothermometer. However, following the first published calibration of the clumped-isotope thermometer (Ghosh et al., 2006), and subsequent early application of clumped-

isotopes to natural materials (Ghosh et al., 2006; Came et al., 2007; Eagle et al., 2010; Tripathi et al., 2010; Thiagarajan et al., 2011), inconsistencies in the Δ_{47} vs. T relationship in natural materials have been reported (Dennis and Schrag, 2010; Dennis et al., 2013; Henkes et al., 2013; Eagle et al., 2013). At issue is whether these inconsistencies represent real differences between materials in the sensitivity of their ^{12}C – ^{18}O bond ordering to temperature or instead whether the inconsistencies are spurious analytical artifacts.

Potential inaccuracies arise because of two effects: (1) the dependency of Δ_{47} on $\delta^{47}\text{CO}_2$, (referred to as a compositional “non-linearity”) where measured Δ_{47} exhibits a positive correlation with $\delta^{47}\text{CO}_2$ relative to the reference

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gas ($\delta^{47}\text{CO}_2 \approx \delta^{13}\text{C} + 2 \delta^{18}\text{O}$, where $\delta^{13}\text{C}$ or $\delta^{18}\text{O} = [(R_{\text{smp}} - R_{\text{std}})/R_{\text{std}}] \times 10^3$ and where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{18}\text{O}/{}^{16}\text{O}$, smp = sample, std = reference standard); (2) inter-laboratory discrepancies in the differences in Δ_{47} measured between CO_2 samples representing two different temperatures (referred to by some workers as “scale compression”). For example, the difference in Δ_{47} between CO_2 samples of identical isotopic composition equilibrated at 1000 and 25 °C may be determined to be 0.90‰ in one laboratory and 0.76‰ in another (Table 1 in Dennis et al., 2011). What is more, these differences can evolve with time. Initial efforts to resolve these discrepancies have employed a strategy of characterizing these effects by analyzing temperature-equilibrated CO_2 for each instrument as a function of time (Huntington et al., 2009; Dennis et al., 2011). This approach may incur additional sources of error from imperfect equilibration and analysis of the reference CO_2 samples.

Although originally attributed to scrambling of analyte ions in the source of the mass spectrometers (Affek et al., 2008; Huntington et al., 2009), recent work suggests that the $\Delta_{47}/\delta^{47}\text{CO}_2$ non-linearity results from an apparent reduction in positive ion beam voltage that is caused by the inclusion of negative background voltage in the total measured by the Faraday cups during sample analysis (He et al., 2012; Yeung et al., 2012; Bernasconi et al., 2013; Petrizzo and Young, 2014). Experiments demonstrate that this negative voltage is due to secondary electrons sputtered from metallic surfaces inside the mass spectrometer. As expected, the effect is more pronounced for Faraday cups with higher amplification (e.g., resistance $\sim 1 \times 10^{12} \Omega$). The method commonly used to overcome $\Delta_{47}/\delta^{47}\text{CO}_2$ non-linearity is to perform a heated gas line (HGL) correction where CO_2 samples of different isotopic composition are re-equilibrated at 1000 °C to impart a stochastic distribution of isotopologues on the samples (i.e., $\Delta_{47} \sim 0$). Deviations in Δ_{47} from zero among these samples are attributable to compositional non-linearity. Plots of Δ_{47} vs. $\delta^{47}\text{CO}_2$ for high- T equilibrated gases can be used to correct for non-linearity. However, this compositional dependence is more easily eliminated by subtracting backgrounds that have been measured immediately before and immediately after each sample is measured (He et al., 2012; Yeung et al., 2012; Petrizzo and Young, 2014). Yoshida et al., (2013) have shown that secondary electrons may not be a problem on some kinds of mass spectrometers, as they observed no compositional dependence for Δ_{47} using a Thermo Delta XP whereas virtually all work to date has been performed on the larger geometry Thermo MAT 253.

Inter-laboratory discrepancies in scale compression can in principle be nullified by correcting measured Δ_{47} values to values expected from theory. This calibration is referred to variously as the “absolute reference frame” or carbon dioxide equilibrium scale (CDES) of Dennis et al. (2011). The calibration curve relating measured Δ_{47} to theoretical Δ_{47} invokes the theoretically calculated relationship between Δ_{47} and temperature obtained by Wang et al. (2004). Similar to the HGL, the calibration line necessary for moving data to the CDES scale must be determined in each laboratory during the period of study.

Despite these efforts, questions remain. For example, are there clear differences in Δ_{47} vs. temperature relationships

between inorganic carbonate and organically deposited carbonate, and if so, why? Also, laboratories analyzing shells of the same species that grew at the same temperature report very different Δ_{47} values. A first step toward a better understanding of Δ_{47}/T in natural carbonates is to achieve inter-laboratory agreement for various species.

Here we report measurements of Δ_{47} in bivalved mollusc shells making use of background subtraction. We compare these results to the uncorrected measurements of the same aliquots of CO_2 , derived from the mollusc shells as well as to Δ_{47} measured in bivalved mollusc shells in other studies (Eagle et al., 2013; Henkes et al., 2013). It is shown that intra- and inter-laboratory problems in clumped-isotope thermometry are at least partly attributable to lack of attention to backgrounds that are variable in time on the scale of weeks (Rosenheim et al., 2013).

2. MATERIALS AND METHODS

2.1. Modern shell samples

The calibration lines shown in Fig. 1 come from the carbonate shells of six modern bivalve species, which together represent a wide range of marine environments and water temperatures. Shells of deep-water species were alive when dredged; those from near-shore environments were disarticulated valves (deceased) collected from intertidal settings.

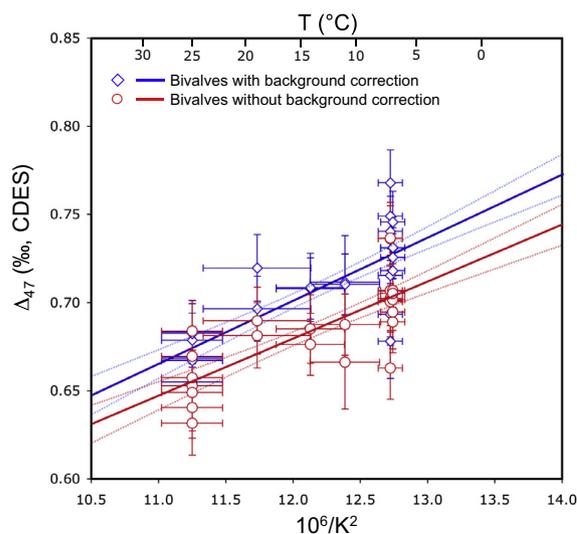


Fig. 1. All measurements of Δ_{47} in CO_2 liberated from bivalve shells grown at known temperature. Measurements corrected for backgrounds (blue diamonds) appear to be $\sim 0.025\text{‰}$ enriched in Δ_{47} against uncorrected measurements (red circles) of the same CO_2 samples. Each data point represents a separate phosphoric acid digestion at 25 °C, analyzed using 5–10 off-peak background/on-peak sample/off-peak background blocks (exact details in Petrizzo and Young, 2014). Error bars represent standard error of the mean ($=1\sigma/\sqrt{n}$). Error envelopes are 95% confidence level. Standard error in the mean for each measurement is generally unaffected when backgrounds are included, although Δ_{47} for one sample in this study moved relative to its calibration line by as much as 0.03‰. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Isotopic composition ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^{47}\text{CO}_2$, Δ_{47}) and known growth temperatures of marine bivalves.

Species (ID – run #)	Growth Range temp. ($\pm^\circ\text{C}$)		$\delta^{18}\text{O}$ (PDB)	$\delta^{13}\text{C}$ (PDB)	$\delta^{47}\text{CO}_2$ (‰, ref)	$\delta^{47}\text{CO}_{2\text{BG}}$ (‰, ref) ^a		
<i>Propeamussium dalli</i>								
CIS004 – 1	7.0	± 1.0	2.75 (± 0.006)	2.10 (± 0.003)	24.95 (± 0.016)	24.73 (± 0.016)		
CIS004 – 2	7.0	± 1.0	2.68 (± 0.009)	2.03 (± 0.002)	24.80 (± 0.016)	24.59 (± 0.016)		
CIS004 – 3	7.0	± 1.0	2.74 (± 0.009)	2.01 (± 0.003)	24.83 (± 0.016)	24.62 (± 0.016)		
CIS004 – 4	7.0	± 1.0	2.87 (± 0.008)	2.00 (± 0.003)	24.97 (± 0.015)	24.74 (± 0.015)		
CIS004 – 5	7.0	± 1.0	2.79 (± 0.007)	1.98 (± 0.004)	24.95 (± 0.019)	24.63 (± 0.019)		
Mean			2.77	2.02	24.88	24.66		
1 standard error			0.031	0.021	0.034	0.030		
<i>Euciroa pacifica</i>								
CIS006 – 1	7.2	± 1.0	3.00 (± 0.008)	0.07 (± 0.003)	23.18 (± 0.018)	22.98 (± 0.018)		
CIS006 – 2	7.2	± 1.0	2.81 (± 0.006)	–0.34 (± 0.003)	22.60 (± 0.017)	22.41 (± 0.017)		
CIS006 – 3	7.2	± 1.0	2.88 (± 0.006)	–0.16 (± 0.003)	22.82 (± 0.017)	22.63 (± 0.017)		
CIS006 – 4	7.2	± 1.0	2.82 (± 0.005)	–0.39 (± 0.003)	22.49 (± 0.016)	22.30 (± 0.016)		
CIS006 – 5	7.2	± 1.0	2.81 (± 0.007)	–0.36 (± 0.003)	22.54 (± 0.016)	22.33 (± 0.016)		
CIS006 – 6	7.2	± 1.0	2.86 (± 0.008)	–0.45 (± 0.003)	22.52 (± 0.019)	22.27 (± 0.019)		
CIS006 – 7	7.2	± 1.0	2.88 (± 0.008)	–0.16 (± 0.002)	22.85 (± 0.018)	22.61 (± 0.018)		
Mean			2.87	–0.26	22.50	22.72		
1 standard error			0.026	0.068	0.097	0.095		
<i>Mya arenaria</i>								
CIS025 – 1	11.0	± 2.7	1.09 (± 0.010)	1.57 (± 0.003)	22.64 (± 0.024)	22.45 (± 0.016)		
CIS025 – 2	11.0	± 2.7	1.09 (± 0.010)	1.57 (± 0.003)	22.66 (± 0.016)	22.45 (± 0.016)		
Mean			1.09	1.57	22.65	22.45		
1 standard error			0.00	0.00	0.010	0.00		
<i>Perna canaliculus</i>								
CIS023 – 1	14.5	± 3.0	1.42 (± 0.007)	1.06 (± 0.003)	22.48 (± 0.016)	22.29 (± 0.016)		
CIS023 – 2	14.5	± 3.0	1.44 (± 0.007)	1.01 (± 0.003)	22.46 (± 0.018)	22.26 (± 0.018)		
Mean			1.43	1.04	22.47	22.27		
1 standard error			0.012	0.027	0.011	0.015		
<i>Cleidothaerus albidus</i>								
CIS022 – 1	18.8	± 5.0	1.08 (± 0.007)	0.01 (± 0.003)	22.08 (± 0.017)	21.87 (± 0.017)		
CIS022 – 2	18.8	± 5.0	0.94 (± 0.007)	0.93 (± 0.002)	21.86 (± 0.017)	21.68 (± 0.017)		
Mean			1.01	0.97	21.97	21.78		
1 standard error			0.066	0.042	0.107	0.099		
<i>Pharaonella pharaonis</i>								
CIS020 – 1	25.0	± 3.0	–0.37 (± 0.008)	–0.45 (± 0.003)	19.07 (± 0.017)	18.92 (± 0.017)		
CIS020 – 2	25.0	± 3.0	–0.38 (± 0.008)	–0.51 (± 0.003)	19.00 (± 0.023)	18.82 (± 0.023)		
CIS020 – 3	25.0	± 3.0	–0.32 (± 0.007)	–0.52 (± 0.003)	19.06 (± 0.015)	18.89 (± 0.015)		
CIS020 – 4	25.0	± 3.0	–0.34 (± 0.009)	–0.48 (± 0.002)	19.07 (± 0.016)	18.91 (± 0.016)		
CIS020 – 5	25.0	± 3.0	–0.24 (± 0.009)	–0.42 (± 0.003)	19.25 (± 0.014)	19.08 (± 0.014)		
CIS020 – 6	25.0	± 3.0	–0.33 (± 0.010)	–0.41 (± 0.003)	19.18 (± 0.016)	18.99 (± 0.016)		
CIS020 – 7	25.0	± 3.0	–0.36 (± 0.008)	–0.45 (± 0.003)	19.11 (± 0.015)	18.92 (± 0.015)		
Mean			–0.332	–0.463	19.11	18.93		
1 standard error			0.018	0.016	0.031	0.031		
Species (ID – run #)	$\Delta_{47\text{HGL}}$ (‰, ref) ^b		$\Delta_{47\text{BG}}$ (‰, ref) ^c	$\Delta_{47\text{CDES}}$ (‰, sto) ^d		$\Delta_{47\text{BGCDES}}$ (‰, sto) ^c		
<i>Propeamussium dalli</i>								
CIS004 – 1	–0.195	(± 0.016)	–0.151	(± 0.016)	0.707	(± 0.018)	0.731	(± 0.018)
CIS004 – 2	–0.199	(± 0.016)	–0.137	(± 0.016)	0.702	(± 0.018)	0.746	(± 0.017)
CIS004 – 3	–0.211	(± 0.016)	–0.150	(± 0.016)	0.689	(± 0.018)	0.731	(± 0.018)
CIS004 – 4	–0.196	(± 0.015)	–0.155	(± 0.015)	0.705	(± 0.017)	0.726	(± 0.017)
CIS004 – 5	–0.206	(± 0.019)	–0.162	(± 0.019)	0.695	(± 0.021)	0.718	(± 0.021)
Mean	–0.201		–0.151		0.700		0.730	
1 standard error	0.003		0.004		0.003		0.005	
<i>Euciroa pacifica</i>								
CIS006 – 1	–0.195	(± 0.018)	–0.142	(± 0.018)	0.707	(± 0.020)	0.740	(± 0.020)
CIS006 – 2	–0.168	(± 0.017)	–0.117	(± 0.017)	0.736	(± 0.019)	0.768	(± 0.019)
CIS006 – 3	–0.199	(± 0.016)	–0.134	(± 0.017)	0.703	(± 0.018)	0.749	(± 0.019)

Table 1 (continued)

Species (ID – run #)	$\Delta_{47\text{HGL}}$ (‰, ref) ^b		$\Delta_{47\text{BG}}$ (‰, ref) ^c		$\Delta_{47\text{CDES}}$ (‰, sto) ^d		$\Delta_{47\text{BGDES}}$ (‰, sto) ^e	
CIS006 – 4	–0.234	(±0.016)	–0.184	(±0.016)	0.663	(±0.018)	0.693	(±0.018)
CIS006 – 5	–0.200	(±0.016)	–0.173	(±0.016)	0.701	(±0.018)	0.707	(±0.017)
CIS006 – 6	–0.200	(±0.019)	–0.198	(±0.019)	0.701	(±0.021)	0.678	(±0.021)
CIS006 – 7	–0.168	(±0.018)	–0.164	(±0.018)	0.737	(±0.020)	0.716	(±0.020)
Mean	–0.195		–0.159		0.707		0.722	
1 standard error	0.009		0.011		0.009		0.012	
<i>Mya arenaria</i>								
CIS025 – 1	–0.231	(±0.024)	–0.168	(±0.024)	0.666	(±0.027)	0.712	(±0.027)
CIS025 – 2	–0.212	(±0.016)	–0.169	(±0.015)	0.688	(±0.017)	0.710	(±0.017)
Mean	–0.222		–0.168		0.677		0.711	
1 standard error	0.010		0.001		0.011		0.001	
<i>Perna canaliculus</i>								
CIS023 – 1	–0.222	(±0.016)	–0.171	(±0.016)	0.676	(±0.018)	0.708	(±0.017)
CIS023 – 2	–0.214	(±0.017)	–0.171	(±0.017)	0.685	(±0.019)	0.709	(±0.019)
Mean	–0.218		–0.171		0.681		0.708	
1 standard error	0.004		0.000		0.004		0.000	
<i>Cleidothaerus albidus</i>								
CIS022 – 1	–0.218	(±0.017)	–0.181	(±0.017)	0.681	(±0.018)	0.697	(±0.019)
CIS022 – 2	–0.210	(±0.017)	–0.161	(±0.017)	0.690	(±0.019)	0.720	(±0.019)
Mean	–0.214		–0.171		0.686		0.708	
1 standard error	0.004		0.010		0.004		0.012	
<i>Pharaonella pharaonis</i>								
CIS020 – 1	–0.274	(±0.016)	–0.168	(±0.016)	0.632	(±0.018)	0.683	(±0.018)
CIS020 – 2	–0.243	(±0.023)	–0.219	(±0.023)	0.653	(±0.026)	0.655	(±0.025)
CIS020 – 3	–0.247	(±0.015)	–0.207	(±0.015)	0.649	(±0.017)	0.668	(±0.016)
CIS020 – 4	–0.254	(±0.016)	–0.206	(±0.016)	0.641	(±0.017)	0.669	(±0.017)
CIS020 – 5	–0.239	(±0.014)	–0.197	(±0.014)	0.658	(±0.016)	0.679	(±0.015)
CIS020 – 6	–0.215	(±0.015)	–0.194	(±0.015)	0.684	(±0.017)	0.683	(±0.017)
CIS020 – 7	–0.228	(±0.015)	–0.208	(±0.015)	0.670	(±0.017)	0.667	(±0.016)
Mean	–0.243		–0.200		0.655		0.672	
1 standard error	0.007		0.006		0.007		0.004	

Note: Each measurement represents a single acid digestion of shell material analyzed with 5–10 off-peak background/on-peak analysis/off-peak background blocks. Errors are standard errors of the means ($1\sigma/\sqrt{n}$ where 1σ is the standard deviation of n analyses).

ref = reference gas.

sto = stochastic.

^a $\delta^{47}\text{CO}_2$ is reported against reference gas and is slightly different with background correction.

^b $\Delta_{47\text{HGL}}$ = corrected with HGL of [Huntington et al. \(2009\)](#) only.

^c $\Delta_{47\text{BG}}$ = corrected for backgrounds then HGL method.

^d $\Delta_{47\text{CDES}}$ = corrected with HGL and transferred to CDES scale of [Dennis et al. \(2011\)](#).

^e $\Delta_{47\text{BGDES}}$ = corrected for backgrounds then HGL and transferred to CDES scale.

Shells used in this study come from cold, deep-water sites in the Caribbean and Hawaii and from warmer, shallower sites in Australia and Maine ([Table 1](#)). Four of the species have aragonitic shells, one is predominantly calcitic, and one has a calcitic outer shell layer and an aragonitic inner layer. Samples were obtained by scraping selected regions of the valves with a scalpel or—in the case of delicate shells—small pieces were broken off and ground to a powder in an agate mortar and pestle.

2.2. Bivalve growth temperatures

The temperature calibration for clumped-isotope thermometry requires estimates of water temperatures at the locations where the sampled bivalves grew. In the case of the two deep-water species, *Euciroa pacifica* from Hawaii and *Propeamussium dalli* from Panama, the

sub-thermocline water temperatures in which they lived are thought to have been nearly constant during the lifetimes of the animals. We therefore allocate small error estimates (± 1 °C) to their growth temperatures. Shallow-water temperatures are more seasonably variable than temperatures beneath the thermocline, but we were able to estimate average annual growth temperature for the shallow-water species using data from nearby meteorological stations or, in one case (*Pharaonella pharaonis*), an archival record from associated reef corals. Locality information and evidence for the growth temperature of each species studied is summarized briefly below:

2.2.1. CIS 006 *E. pacifica* Dall 1895

USNM 335528, U.S. Fish Commission Steamer *Albatross* station 3865, 256–283 fm (469–518 m), N of Nakalele Point, Maui, within the Pailolo Channel, Hawaii

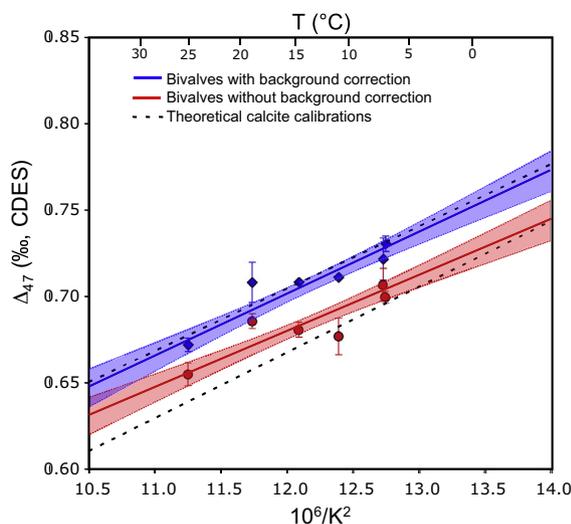


Fig. 2. Calibration of mollusc shells (background corrected = blue diamonds and blue line, without background correction = red circles and red line) and theoretical calibrations for calcite (Schauble et al., 2006; Guo et al., 2009; short-dashed lines: lower = ab initio, upper = recalculated by Henkes et al. (2013) using experimentally determined acid fractionation factor). Diamond and circle symbols represent means of replicate analyses. Error envelopes represent 95% confidence. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(21°09'20"N, 156°35'10"W); collected (dredged) 04/10/1902. Water temperature was 7.2 °C at station when collected (U.S. Fish Commission Report for 1902) and 6 ± 1 °C regionally and 6 ± 1 °C annually during the 1980s (Sansone et al., 1988). We adopted a calibration temperature of 6 ± 1 °C. Sample is of nacreous aragonite from the pulverized left valve.

2.2.2. CIS 004 *P. dalli* Smith 1885

Uncataloged specimens, U.S. Fish and Wildlife Service MV Oregon station 3600, (550 m), W of Colon, Panama (9°03'N, 81°18'W); collected (trawled) 05/31/1962. Water temperature: 7 ± 1 °C (based on nearby Smithsonian Institution MV Hannibal station 241, 9°19'N, 81°22'W, 576 m, 7.04 °C (Wüst, 1964, Table IV, p. 164) and the large-scale hydrodynamic circulation of the Caribbean Sea (Gordon, 1967; Morrison and Nowlin, 1982). Samples of foliated calcite taken from the commissural areas of two left valves.

2.2.3. CIS 025 *Mya arenaria* Linnaeus 1758

Salisbury Cove, ~6 km NW of Bar Harbor, Mount Desert Island, Maine (44.433°N, 68.283°W); collected from the intertidal zone at low tide, 07/23/2003. Average water temperatures for the period July 1, 2002 to June 30, 2003 based on hourly records from NOAA/NOS/CO-OPS meteorological station 8413320 Bar Harbor (44.392°N, 68.205°W) is 7.2 ± 5.6 °C. However, in eastern Maine most shell growth in *M. arenaria* occurs between early April and the beginning of September (Beal, 2002; Beal et al., 2001) so we used that constraint and the earliest available tabulated records for station 8413320 (2010) to obtain a calibration

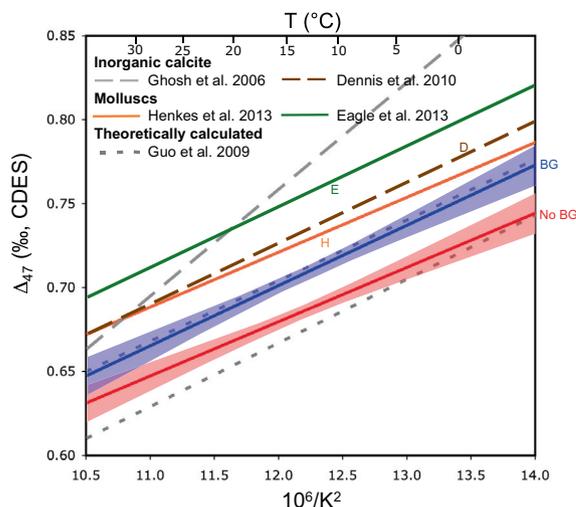


Fig. 3. Comparison of clumped-isotope temperature calibrations. Empirical calibrations derived from acid digestion of: inorganic laboratory-grown calcite (long-dashed gray line = Ghosh et al., 2006; recalculated by Dennis et al., 2011; long-dashed brown line marked with “D” = Dennis and Schrag, 2010; recalculated by Dennis et al., 2011), molluscs and brachiopods (orange line marked with “H” = Henkes et al., 2013; green line marked with “E” = Eagle et al., 2013), bivalves with background correction (blue line marked with “BG” = this study), bivalves without background correction (red line marked with “No BG” = this study), and theoretically calculated calibrations for calcite (short-dashed lines: lower = ab initio calculations of Guo et al. (2009); upper = recalculated by Henkes et al. (2013) using experimentally determined acid fractionation factor). All slopes depicted here are in the common reference frame (CDES of Dennis et al., 2011) and are directly comparable. Calibrations derived from mollusc shells (Eagle et al., 2013; this study) or almost completely from mollusc shells (Henkes et al., 2013) demonstrate general agreement with the Δ_{47} – T relationship reported by the inorganic calibration of Dennis and Schrag (2010). This occurs despite differences in acid digestion temperature, sample preparation methods, and gas handling equipment. Calibrations using corals and foraminifera generally conform to the inorganic calcite calibration of Ghosh et al. (2006; Section 4.2). Error envelopes are 95% confidence level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

temperature of 11 ± 2.7 °C. Samples of crossed-lamellar aragonite from the commissural areas of two valves.

M. arenaria occurs in intertidal mudflats along the western Atlantic coast from the southern United States to Canada, as a Holocene immigrant to the United Kingdom, Ireland and nations bordering the North Sea, and as an invasive species along the U.S. Pacific northwest and adjacent coasts of Canada and Alaska (Petersen et al., 1992; Strasser, 1999; Powers et al., 2006; Crocetta and Turolla, 2011). Its abundance and wide geographic distribution may make it a useful inter-laboratory reference standard for future clumped-isotope paleoenvironmental studies where Δ_{47} – T relationships agree in slope but are displaced in “absolute” Δ_{47} . As a first step in this direction, we compare our results with clumped-isotope data obtained from *M. arenaria* by Henkes et al. (2013) (Fig. 3).

2.2.4. CIS 023 *Perna canaliculus* (Gmelin) 1791

Wellington Harbour, Wellington, New Zealand (41°18'S, 174°47'E); collected from the shoreline, 02/15/1980. Average water temperatures are $\sim 14.5 \pm 3$ °C, based on high-resolution satellite sea surface temperatures (Uddstrom and Oien, 1999) and a mean average annual temperature of 14.5 °C, based on measurements made in Wellington Harbour during 1971–1973 (Hickman, 1979). These temperatures also agree well with measurements of 14.3 ± 2.6 °C given by Booth (1972) for the years 1970–1972. Larger shells, such as the one sampled (190 mm in length) grow at approximately the same rate throughout the year (Hickman, 1979, Fig. 5). Sample of commissural edge of valve comprising both calcitic outer prismatic layer and aragonitic inner nacreous layer.

2.2.5. CIS 022 *Cleidiothaerus albidus* (Lamarck) 1819

Depot Beach, N of Batemans Bay, New South Wales, Australia (35°37.8'S, 150°19.7'E); collected from the intertidal zone, 10/27/2011. *C. albidus* is an anomalodesmatid “oyster” that is cemented by its right valve to hard substrates in subtidal water depths of 10–35 m (Allan, 1950). Average of monthly water temperatures at ~ 70 m water depth from nearby Directional Waverider buoy (35°42.4'S, 150°20.6'E) during the period 09/21/1999–12/31/2011 was 18.8 ± 5.5 °C (Manly Hydraulics Laboratory, N.S.W. Office of Environment and Heritage). Sample of aragonitic nacreous layer of left valve.

2.2.6. CIS 020 *P. pharaonis* (Hanley) 1844

Mission Beach, S of Innisfail, Queensland, Australia (17.89°S, 146.10°E); collected from beach, 08/28/1978. Water temperatures for 1978–1983 based on high-resolution Sr/Ca and $\delta^{18}\text{O}$ sclerochronological records from the coral *Porites lutea* at nearby Pandora Reef (18.81°S, 146.43°E) are given in McCulloch et al. (1994) and average 25 ± 3 °C. Sample is crossed-lamellar aragonite from one pulverized valve.

2.3. Clumped-isotope measurements

All measurements were made at the University of California, Los Angeles in the Department of Earth and Space Sciences on a Thermo-Finnigan 253 gas-source mass spectrometer during February to May of 2012. Due to the unusual arrangement of Faraday cups in this instrument, permitting measurements of either O₂ or CO₂, we used the multi-collector peak hopping (MPH) method of analysis described in Petrizzo and Young (2014). MPH uses sample $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $^{46}\text{CO}_2/^{47}\text{CO}_2$ to calculate Δ_{47} (the ratio $^{18}\text{O}-^{13}\text{C}-^{16}\text{O} + ^{16}\text{O}-^{13}\text{C}-^{18}\text{O}/^{16}\text{O}-^{12}\text{C}-^{16}\text{O}$ relative to the ratio for the stochastic bond ordering). Prior to this study, we found that the $\Delta_{47}/\delta^{47}\text{CO}_2$ non-linearity, described by Huntington et al. (2009) could be eliminated by measuring and subtracting the background signal created by secondary electrons (Petrizzo and Young, 2014). Our strategy is similar to the PBL method of He et al. (2012), but is simpler in that we only need to use the two minor beams for m/z 46 and m/z 47 to make the correction. Internal precision using MPH is similar to that reported in other clumped-isotope

studies (~ 0.022 standard error). Further details of the multi-collector peak hopping (MPH) method are given in Petrizzo and Young (2014).

2.3.1. Temperature-equilibrated CO₂ standards

Stochastic CO₂ standards were created using methods similar to those of Huntington et al. (2009). Initial gas came from several different sources of CO₂, ranging in bulk composition from -55‰ to $+28\text{‰}$ in $\delta^{47}\text{CO}_2$. Aliquots were isolated in quartz ampoules, flame-sealed, and then heated in a muffle furnace to 1000 °C for at least three hours. While it is impossible to confirm a truly stochastic distribution of CO₂ isotopologues following cooling, we sought to minimize potential back-equilibration at intermediate temperature by immediately quenching the ampoules in cold water.

Additional temperature-equilibrated standards were prepared by allowing CO₂ to exchange oxygen with water at 2 and 25 in stopcock-sealed vessels. These vessels may be fitted directly to the extraction line, minimizing handling time and likelihood of back-equilibration during extraction. Standards equilibrated at 25 °C were held in a water bath for 72–192 h. Standards equilibrated at 2 °C were immersed in a beaker of water held at temperature for approximately one month.

2.3.2. Phosphoric acid digestion of carbonates

Approximately 10 mg of powdered sample is loaded into one leg of a two-leg reaction vessel opposite ~ 2 ml of high-density phosphoric acid (specific gravity = 1.92 mg/ml), and then evacuated on the extraction line for 6–10 h (typically overnight). The vessels are sealed off with a baseline pressure of $<1 \times 10^{-5}$ mb then moved into a water bath held at 25 °C and allowed to equilibrate for >30 min. Next, sample vessels were tipped to introduce acid to the carbonate, and then returned to the water bath to react at constant temperature for at least 16 h. Although most reactions were complete by visual inspection after 16 h, inspection under a binocular microscope revealed a few cases where microscopic bubbles were still being produced after CO₂ extraction, indicating less than 100% reaction. However, we do not expect the trivial quantity of CO₂ retained in residual carbonate to have a noticeable effect on the measured isotope ratios (see however, Rosenheim et al., 2013).

2.3.3. CO₂ purification

We use a glass vacuum extraction line with on-line gas chromatograph to remove contaminants from CO₂ samples prior to analysis. The CO₂ liberated from carbonate by reaction with H₃PO₄ at 25 °C is separated from H₂O cryogenically, and then entrained in ~ 12 –15 ml/min helium flow through a 2 m porapak Q column chilled to -30 °C with an internal diameter of 2.0 mm. After passing through the column, CO₂ samples are recaptured by freezing in a multi-loop, stainless steel u-trap and helium is pumped away. Finally, the CO₂ is transferred cryogenically to stopcock-sealed borosilicate sample vessels for analysis. Each standard and sample is analyzed within five hours of passing through this system in order to minimize the possibility of re-equilibration of CO₂ at room temperature.

2.3.4. Mass spectrometric analysis

Each sample is introduced to the mass spectrometer through a dual-inlet system using nickel capillaries that were evacuated and then cleaned by heating until red-hot between samples. Bellows pressure is adjusted to produce a mass 44 signal of ~ 14 volts (amplifier resistor = $10^8 \Omega$) for determining $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, and then is re-adjusted to produce a mass 46 signal of ~ 6 volts (amplifier resistor = $10^{10} \Omega$) in order to measure $^{47}\text{CO}_2/^{46}\text{CO}_2$. The measurement sequence consists of: (1) two ten-cycle blocks measuring m/z 44, 45 and 46; (2) reducing the accelerating voltage to direct mass 46 and 47 beams into the Faraday cups normally used for masses 45 and 46; (3) running a three-cycle pre-analysis background measurement; (4) running the ten-cycle sample analysis; and (5) running another three-cycle background analysis after the sample analysis. Steps 3–5 are almost always repeated ten times (resulting in 10 blocks of background-sample-standard-background sequences), and almost always result in standard errors of the mean of 0.02 or less (Table 1).

2.3.5. Reference gas

All measurements in this study were made against the same reference gas purchased from OzTech (Dallas, TX, USA). The composition of this reference gas is $\delta^{13}\text{C} = 3.58\text{‰}$ PDB, $\delta^{18}\text{O} = +24.96$ SMOW ‰ . The reference gas has Δ_{47} values reflective of near room-temperature equilibration.

2.3.6. Data reduction and analysis

In order to provide both a clear comparison of the effects of different measurement procedures and to produce a temperature calibration comparable to calibrations reported in other laboratories, we wrote a Fortran program to reduce the data using the “heated gas line” (HGL) method of Huntington et al. (2009) and our own background correction method (Fortran code and further description are provided in Petrizzo and Young, 2014). Additionally, we report data in the CDES scale, for both HGL-only corrected and background corrected data, using a $\Delta_{47}^{\text{theoretical}}/\Delta_{47}^{\text{measured}}$ calibration line as described by Dennis et al. (2011). This results in four different Δ_{47} values for each sample (Table 1): (1) HGL correction only [$\Delta_{47,\text{HGL}}$]; (2) HGL correction after measured background correction [$\Delta_{47,\text{BG}}$]; (3) HGL correction, and conversion to CDES [$\Delta_{47,\text{CDES}}$]; (4) HGL correction after background correction, followed by conversion to CDES [$\Delta_{47,\text{BG,CDES}}$].

3. RESULTS

Table 1 reports isotopic compositions ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^{47}\text{CO}_2$ and Δ_{47}) and growth temperatures inferred or measured (Section 2.2) for all bivalves sampled. Bulk composition ($\delta^{47}\text{CO}_2$) is needed for heated gas lines with and without background correction, necessitating the reporting of $\delta^{47}\text{CO}_2$ values with and without background corrections in Table 1. Fig. 1 is a plot of the two distinct calibrations resulting from CDES-normalized data with (blue) and without (red) background corrections.

Internal precision using standard error for twenty-three of twenty-five analyses of Δ_{47} comprising the temperature

calibration in this study are $< \pm 0.02\text{‰}$ (1 standard error), similar to that reported in previous studies of methods employed in measuring Δ_{47} by gas-source mass spectrometry (Huntington et al., 2009). The two exceptions are one analysis of *M. arenaria* and one analysis of *P. pharaonis* (internal standard error = $\pm 0.027\text{‰}$ and $\pm 0.024\text{‰}$, respectively). External standard error in the mean is typically $\sim \pm 0.01$ based on 2–7 replicates, and varied only slightly with measurement/data reduction method.

Table 1 demonstrates that bivalves believed to have grown in the coldest waters (*E. pacifica* and *P. dalli*) are the most enriched in Δ_{47} , while the tropical surface bivalve species (*P. pharaonis*) is the least enriched in Δ_{47} . We find no significant outlier in Δ_{47} as it relates to temperature among the species we examined. Least squares linear regression (York regression with errors in both x and y) of the Δ_{47} data vs. $1/T^2$ results in two distinct calibration lines. For background-corrected data we obtain

$$\Delta_{47} = 0.0358 (\pm 0.0060) \times 10^6 / T^2 + 0.2717 (\pm 0.0734). \quad (1)$$

Without background correction we obtain

$$\Delta_{47} = 0.0323 (\pm 0.0060)[10^6 / \text{K}^2] + 0.2918 (\pm 0.0729). \quad (2)$$

The R^2 and reduced chi squared values for these equations are 0.856, 0.929 and 0.722, 0.823, respectively, and the uncertainties are 1σ .

Although calcite shells are predicted to be $\sim 0.05\text{‰}$ depleted in Δ_{47} as compared to aragonite shells (Guo et al., 2009), we are unable to discern a difference in Δ_{47} related to the CaCO_3 phase of the shells. Mean values for both *P. dalli*, composed solely of calcite and *P. canaliculus*, composed of both calcite and aragonite are $< 0.01\text{‰}$ from the calibration line.

4. DISCUSSION

The clumped-isotope temperature calibrations reported here result from employing different methods to analyze individuals of several different species of bivalved molluscs that grew in a wide range of water temperatures (7–25 °C). In Section 4.1 we seek to explain the differences we observed between measurements made with and without background correction, and the potential for effects on clumped-isotope thermometry. In Section 4.2 we explore differences between our calibrations and previous calibrations from measurements of shells from other marine organisms and laboratory-grown inorganic calcite. We do not compare our findings to Δ_{47} data from non-marine environments (soil carbonate nodules and speleothems) as they introduce additional uncertainties that might affect measured Δ_{47} .

4.1. Effects of background correction

4.1.1. Absolute value of Δ_{47}

It is clear from this study that correcting measurements for backgrounds significantly changes Δ_{47} values measured in CO_2 derived from the acid digestion of carbonates

(Fig. 1). As we are dealing with the same gas sample in each case, the difference is not attributable to variability in gas handling during extraction and purification. It also cannot be due to the small differences in $\Delta_{47}^{\text{theoretical}}/\Delta_{47}^{\text{measured}}$ calibration lines required by the two data reduction schemes for conversion to the CDES scale, as differences in Δ_{47} ($\sim 0.045\text{‰}$) between background-corrected and non-background-corrected data is apparent prior to conversion to CDES (Table 1). Negative background signals (voltages in this case) registered by Faraday cups during measurements reduce the apparent intensities of the ion beams. Because the Faraday amplifier that measures mass 47 has a higher resistor (e.g., $10^{12}\ \Omega$), it is more sensitive to secondary electrons and therefore displays a more negative background voltage. Subtracting the background voltages increases the measured 47 beam intensity significantly while the 46 and 44 beam intensities are relatively unaffected. The result is correction for spuriously low Δ_{47} values. In this study, without correction for background voltage, sample measurements were spuriously low in m/z 47 compared to reference gas because of more negative backgrounds in the former. One explanation could be an undetected leak in the reference-side plumbing of the dual inlet that would add to the background by scattering. Regardless of the cause, incomplete nulling of these effects when no correction is made for background differences between sample and standard is a likely explanation for the relative positions of our background corrected and uncorrected calibration lines.

The observation that absolute Δ_{47} values vary with backgrounds in our laboratory may go a ways towards explaining discrepancies between other temperature calibrations based on bivalve shells (or calibrations derived mostly from bivalve shells). For example, it is possible that the offset in absolute Δ_{47} seen in Henkes et al. (2013) and Eagle et al. (2013) is due to differing instrument backgrounds that were not accounted for. However, while the background correction changed the absolute Δ_{47} at a given temperature (intercept), the Δ_{47} vs. temperature relationship (slope) for the calibration was largely unaffected.

4.1.2. Magnitude of effect on thermometry

The difference in derived growth temperature is typically small ($\pm 2\ ^\circ\text{C}$) between the background-corrected and the non-background corrected calibrations. A relatively extreme example in this study is *M. arenaria*. This sample yields $\Delta_{47} = 0.712\text{‰} \pm 0.027$ and $0.710\ \text{‰} \pm 0.017$ in two separate analyses when background corrected and $\Delta_{47} = 0.666\text{‰} \pm 0.027$ and $0.688\text{‰} \pm 0.017$ when not corrected for background. If the growth temperature for this shell ($11.0 \pm 2.7\ ^\circ\text{C}$) were not known (an unconstrained sample), the background correction method (Eq. (1)) would suggest an average temperature of $12.3\ ^\circ\text{C}$, while omitting the background correction (Eq. (2)) would indicate in average temperature of $16.5\ ^\circ\text{C}$. In other words, correcting for backgrounds shifted the result $4.2\ ^\circ\text{C}$ and gave an accurate result within errors while omitting the background correction resulted in a temperature several degrees too high. This has serious implications for studies where clumped-isotope (paleo)temperatures are obtained from comparison with independent measures of temperature.

4.2. Comparison to previous calibrations

Figs. 2 and 3 include lines representing previous clumped-isotope temperature calibrations generated from acid-digestion of modern shells and inorganic carbonates, as well as theoretical slopes for Δ_{47} vs. temperature, all of which are reported in the CDES scale and should therefore be directly comparable. A comparison between the different calibrations follows.

4.2.1. Clumped-isotopes in mollusc shells

Both background-corrected and non-background-corrected temperature calibrations are in reasonable agreement with the calibration of Henkes et al. (2013) and Eagle et al. (2013; Fig. 2 in this study). This provides evidence against the hypothesis that differing acid digestion temperature is the primary cause of disagreements in Δ_{47} vs. T relationships. These studies together include 25 extant species of molluscs: 23 bivalves, one species of *Nautilus* (Henkes et al., 2013), and one species of gastropod (Henkes et al., 2013). This suggests that biomineralization of either calcite or aragonite by molluscs consistently produces shell carbonate with similar Δ_{47} vs. T relationships. However, our calibration lines differ from the previously mentioned calibration in the absolute magnitude of clumped-isotope enrichment (Δ_{47}) for mollusc shells that grew at any given temperature. The difference in Δ_{47} between this study and that of Henkes et al. is $\sim 0.02\text{‰}$ with background correction and $\sim 0.05\text{‰}$ without background correction. The Δ_{47} – T relationships (slopes) in both this study and those of Henkes et al. (2013) and Eagle et al. (2013) differ significantly from the original slope reported by Came et al. (2007). The latter found three species of mollusc (and three species of brachiopod) conforming to the inorganic calcite Δ_{47} – T calibration of Ghosh et al. (2006; described further in Section 4.2.3). Additionally, the Δ_{47} – T slopes obtained here and by Henkes et al. (2013) and Eagle et al. (2013) from mollusc shells are all within error of the theoretical Δ_{47} – T relationship for phosphoric acid digestion of carbonate minerals (Guo et al., 2009).

Dennis et al. (2013) found the temperature range inhabited by *Nautilus* to be too narrow to define a viable calibration, but noted that measured Δ_{47} was much lower than expectations based on the inorganic calcite calibration of Ghosh et al. (2006), and concluded therefore, that there must be unidentified vital effects specific to cephalopods that led to real depletions ($\sim 0.05\text{‰}$, and in some cases as much as 0.1‰) of Δ_{47} in sampled shell material. Regression of the Dennis et al. (2013) data provides little clarity, as the slope of the calibration line (0.0463 ± 0.0135) is highly uncertain, and falls between the steeper slope of Ghosh et al. and shallower slopes of Henkes et al., Eagle et al., and this study.

We emphasize that the shallower slopes such as that shown in Fig. 2 from this study, agree with the theoretical Δ_{47} vs. T prediction. What is more, the difference between the Ghosh et al./Came et al. calibrations and the shallower calibrations that match theory cannot be attributed solely to species-specific sensitivities to temperature because molluscs and brachiopods were at one time seen to conform to

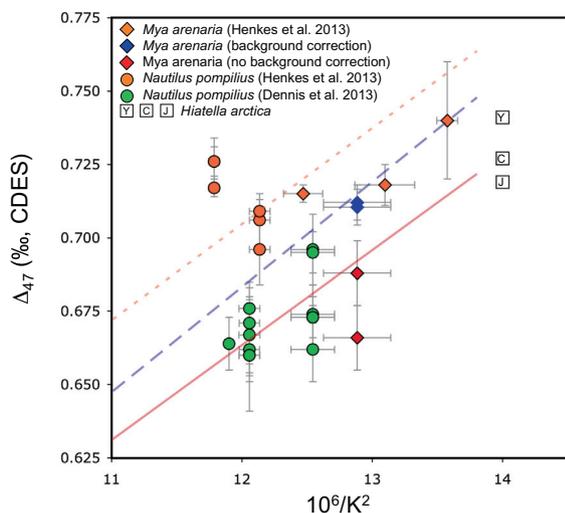


Fig. 4. Comparison of mollusc species common to multiple published empirical calibrations (all measurements reported in CDES). *Mya arenaria* appears in both Henkes et al. (2013; open diamonds) and this study (background corrected = filled diamonds, without background correction = gray diamonds). *N. pompilius* is common to both Henkes et al. (2013; open circles) and Dennis et al. (2013; filled circles). Square symbols represent measurements of the same *Hiatella arctica* shell measured in three different laboratories (Y = Yale University, C = California Institute of Technology, J = Johns Hopkins University) and corrected by comparison to stochastic gas standards, but not converted to the universal reference frame (Henkes et al., 2013). Although these measurements are not directly comparable to the calibrations pictured here, the scale of difference in these measurements is similar to that of CDES data, indicating that inter-laboratory bias remains significant. Calibrations: short-dashed and orange = Henkes et al., 2013, long-dashed and blue = with background correction, solid and red = without background correction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the steeper calibration (see Section 4.3). We conclude that the mollusc calibration given here, which agrees with theoretical Δ_{47} - T relationship, may apply to other species of mollusc as well, including *Nautilus* (Fig. 4).

4.2.2. Direct comparison of species in common

Henkes et al. (2013) included six individuals of *Nautilus pompilius* and three individuals of *M. arenaria* in their calibration, allowing a direct comparison to be made with *N. pompilius* measured by Dennis et al. (2013) and *M. arenaria* used in this study. Fig. 4 shows Δ_{47} values measured in these mollusc shells, demonstrating the current state of inter-laboratory variability. In addition, Henkes et al. (2013; symbol “J” in Fig. 4) report an inter-laboratory comparison of Δ_{47} values measured in the same *Hiatella arctica* shell, by distributing aliquots of shell to laboratories at Yale University (“Y” in Fig. 4) and the California Institute of Technology (“C” in Fig. 4). These are plotted in Fig. 4 as squares labeled with a single letter to identify their sources.

Measurements of *H. arctica* demonstrate minor pre-CDES inter-laboratory disagreement. Measurements of Δ_{47} in the same shell distributed to Yale University, Johns Hopkins University and the California Institute of Technology differ by as much as 0.02‰, corresponding to a temperature difference of ~ 7 degrees based on a Δ_{47} - T slope of ~ 0.032 all else equal. The CDES scale is intended to nullify the effects of instrument-specific source fragmentation or recombination (scrambling) reactions during analysis as well as “scale compression” of Δ_{47} in order to bring all laboratories into a “common reference frame”. The objective of a common reference frame is to produce statistically indistinguishable Δ_{47} values from shells of the same species grown at the same temperature, independent of laboratory. However this was not achieved in two examinations of *N. pompilius*, as after CDES scaling the inter-laboratory differences in Δ_{47} remain (Fig. 4). Six individuals used in the Henkes et al. (2013) calibration appear to be enriched by approximately 0.025‰ in Δ_{47} (a temperature difference of ~ 9 degrees all else equal) compared with the twelve specimens sampled by Dennis et al. (2013). Because this difference is similar in magnitude to the difference between our background-corrected and uncorrected calibration lines, we speculate that inter-laboratory differences in backgrounds are responsible for the different values of Δ_{47} measured in *N. pompilius* shells of similar temperature.

However, *M. arenaria* shells measured by Henkes et al. (2013), exhibit a range of Δ_{47} values similar to the background-corrected measurements of *M. arenaria* presented in this study (Fig 4). Uncorrected measurements of *M. are-*

Table 2

Comparison of several carbonate clumped-isotope thermometer calibrations in CDES.

	Temperature range (°C)	Slope	Intercept	R^2
Ghosh et al. (2006 and 2011 ^a)	2 to 50	0.0636 ± 0.0049	0.0047 ± 0.0520	0.8877
Dennis and Schrag (2010 and 2011 ^b)	7.5 to 77	0.0340 ± 0.0038	0.3155 ± 0.0408	0.8600
Henkes et al. (2013)	-1 to 29	0.0327 ± 0.0022	0.3286 ± 0.0278	0.84
Dennis et al. (2013) ^c	15 to 23	0.0463 ± 0.0153	0.1345 ± 0.1570	0.158
Eagle et al. (2013)	-1 to 29	0.0362 ± 0.0044	0.3140 ± 0.0527	0.7258
This study (background correction)	7 to 25	0.0358 ± 0.0060	0.2717 ± 0.0734	0.856
This study (no background correction)	7 to 25	0.0323 ± 0.0060	0.2918 ± 0.0729	0.722

^a Original empirical calibration of clumped-isotope thermometer using lab-grown calcite ($\Delta_{47} = 0.05927 \times 10^6/T^2$) was converted to CDES by Dennis et al. (2011). This calibration fits several others derived from corals, foraminiferans and coccoliths, and bivalves and molluscs (Section 4.2).

^b Original published calibration of Dennis and Schrag (2010) was converted to CDES by Dennis et al. (2011).

^c Data regressed for comparison purposes in this study only.

naria from this study are lower in Δ_{47} by $\sim 0.05\%$ relative to the background-corrected measurements, as expected from Fig. 2. One difference between our *M. arenaria* and those of Henkes et al. (2013) is the bulk oxygen isotope ratio. It is conceivable, but to us seems unlikely, that a small difference in $\delta^{18}\text{O}$ influences measurements of Δ_{47} at the same temperature at the 0.01‰ level (however see Rosenheim et al., 2013).

4.2.3. Laboratory-grown calcite, corals, foraminiferans

Both versions of the calibration reported in this study differ significantly in slope from the Δ_{47} – T relationships determined in studies using inorganically precipitated carbonates (Ghosh et al., 2006), foraminiferans and coccoliths (Tripathi et al., 2010; Grauel et al., 2013), and corals (Thiagarajan et al., 2011), and the earliest measurement of clumped-isotopes in bivalved molluscs and brachiopods (Came et al., 2007) all of which found greater temperature sensitivity (slopes of ~ 0.060 in CDES vs. ~ 0.035 ; Table 2). Henkes et al. (2013) also found a lower Δ_{47} – T slope of 0.0327 in a multi-phyletic study of mostly molluscs. The issue of whether this lower slope is specific to molluscs is addressed below.

4.3. Comparing Δ_{47}/T^2 among molluscs, theory and other phyla

While the slopes of bivalve calibrations in Henkes et al. (2013), Eagle et al. (2013), and this study agree closely with each other (Table 2), and with the inorganic calibrations of Dennis and Schrag (2010), Fernandez et al. (2014), Tang et al. (2014), and the theoretical calibrations of Schauble et al. (2006) and Guo et al. (2009), these are clearly different from the steeper slopes reported in Ghosh et al. (2006; inorganic calcite and corals), Tripathi et al. (2010; foraminiferans and coccoliths), Thiagarajan et al. (2011; corals), Schmid (2011; inorganic carbonates), and Bernasconi et al. (2013; foraminiferans). There are three observations that suggest to us that the differences between the bivalve calibrations and the other calibrations are not due to a vital effect in the former. Firstly, the original bivalve and brachiopod calibration of Came et al. (2007) agreed with the steeper calibration of Ghosh et al. (2006) and both were measured on the same instrument using the same procedures. Secondly, inorganic calibrations of Ghosh et al. (2006) and Schmid (2011), each measured on different instruments, do not agree with the calibration of Dennis and Schrag (2010). Thirdly, the slopes of the inorganic calibration of Dennis and Schrag (2010), and the recent bivalve calibrations agree with theoretically calculated calibrations of Guo et al. (2009) and Schauble et al. (2006), and this seems unlikely to be by chance. We hypothesize that the different Δ_{47} – T relationships (calibration slopes) are not a reflection of species-specific Δ_{47} – T relationships but rather may be analytical artifacts. One component might be various degrees of trace contaminants and the resulting effects on backgrounds in the mass spectrometer.

5. CONCLUSIONS AND IMPLICATIONS

We show agreement with two recent mollusc-based calibrations of the clumped-isotope thermometer, but

significant disagreement with those made from measurements of lab-grown calcite, corals, foraminiferans and coccoliths and bivalves in one earlier study. Because acid digestion of shell carbonate in the two recent studies that agree with this one were done at significantly higher temperature (90 °C), we suggest that differences in acid digestion temperature are not the cause of inter-laboratory disagreements in Δ_{47} – T as has been suggested (Fernandez et al., 2014). It appears that the temperature-dependence of clumped-isotopes in mollusc shells is established, but agreement on the absolute magnitude of Δ_{47} for any specific temperature of shell formation is not yet established. If that is the case, then clumped-isotope temperatures of unknown shells obtained from machine-specific and time-specific (same instrument state and same study period) calibration curves should be widely applicable, even though the absolute magnitude of Δ_{47} , which is not important for carbonate thermometry, may remain uncertain.

Measurements of CO_2 liberated from natural carbonates in this study approximate the theoretically calculated Δ_{47} –temperature relationship for carbonate minerals better than others to date, but this means that temperatures standardized by comparison to theoretical isotopologue distributions or empirical calibrations from other laboratories may be susceptible to significant error. However, by using background corrections, and therefore proper measures of ion beam intensities, the need for in-house temperature calibrations might be lessened.

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