Paired *Δ*47 and *Δ*48 analyses and model calculations constrain equilibrium, experimentally-manipulated kinetic isotope effects, and mixing effects in calcite

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1   **Paired $Δ_{47}$ and $Δ_{48}$ analyses and model calculations constrain equilibrium, experimentally-**
2   **manipulated kinetic isotope effects, and mixing effects in calcite**

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15

16   **Abstract**
17
18   The high-precision analysis of the abundance of the dominant m/z 47 CO_2 isotopologue derived
19   from acid digestion of carbonate minerals ($^{13}C^{18}O^{16}O$; denoted by $Δ_{47}$) forms the basis for carbonate
20   clumped isotope thermometry. Since the first measurements were published 16 years ago, considerable
21   effort has gone into characterizing the relationship between $Δ_{47}$ and carbonate precipitation temperature,
22   and in identifying carbonates that do not achieve isotopic equilibrium. Mass spectrometry is now capable
23   of the paired measurement of the primary m/z 47 and m/z 48-isotopologues ($Δ_{47}$ and $Δ_{48}$; $^{12}C^{18}O_2$ is
24   denoted by $Δ_{48}$), which has the potential to place additional constraints on kinetic isotope effects in
25   carbonate minerals and trace distinct reaction pathways. Here, we explored factors that contribute to
26   calcite mineral equilibrium and disequilibrium in $Δ_{47}$ and $Δ_{48}$ using a combination of experiments and
27   theoretical calculations with three types of models. We precipitated calcite at pH 8.3 with carbonic
28   anhydrase (CA) to approach quasi-isotopic equilibrium in the dissolved inorganic carbon pool and report
29   values for $Δ_{47}$, $Δ_{48}$, and oxygen isotopes ($δ^{18}O$) for calcite grown over a temperature range from 5 to 25 °C
30   and compare our findings to predictions from an ion-by-ion model that support equilibrium precipitation.
31   We also compare results to the Devils Hole slow-growing cave calcite, and other published temperature
32   calibration data. We report the following combined equilibrium calibration relationships: $Δ_{48\ CDES\ 90}$ =
33   $(0.429 ± 0.010) Δ_{47\ CDES\ 90}$ - $(0.006 ± 0.006)$; $r^2 = 0.98$; $Δ_{47\ I-CDES} = (0.037 ± 0.001) × 10^6 T^{-2} + (0.178 ±$
34   $0.009)$; $r^2 = 0.99$; $Δ_{48\ CDES\ 90} = (0.015 ± 0.0005) × 10^6 T^{-2} + (0.078 ± 0.006)$; $r^2 = 0.98$. We used paired
35   measurements of $Δ_{47}$ and $Δ_{48}$ to constrain kinetic isotope effects in calcite precipitated at pH ranging from
36   8.3-11 and temperatures from 5 to 25 °C, with and without CA present, and observe kinetic enrichments
37   in $Δ_{47}$, negative (hyperstochastic) values for $Δ_{48}$, and depleted values of $δ^{18}O$, compared to equilibrium
38   values. Experimentally constrained kinetic trajectories, when compared with an ion-by-ion model and

39   IsoDIC theoretical predictions, are consistent with CO_2 hydration/hydroxylation. Mixing drives elevated
40   Δ_{47} and Δ_{48} values and was assessed using mixing experiments with endmembers of varying isotopic
41   compositions and compared to a Δ_{47} and Δ_{48} mixing model that constrains nonlinear mixing trajectories
42   for calcite. While mixing may induce artifacts in two-component mixtures when endmember bulk
43   compositions differ by more than 7 ‰, or if endmember Δ_{47} and Δ_{48} differ by more than 0.03 ‰, this
44   should be detectable and potentially correctible using paired clumped isotope measurements and is
45   unlikely to be important for some materials.
46
47
48   **1. Introduction**
49
50
51   Carbonate clumped isotope thermometry is based on the frequency with which rare, heavy
52   isotopes of carbon (^{13}C) or oxygen (^{18}O) in carbonate minerals are bonded to each other relative to a
53   stochastic (random) distribution (Wang et al., 2004; Eiler and Schauble, 2004; Ghosh et al., 2006;
54   Schauble et al., 2006). The dominant clumped isotopologue measured in carbonate minerals from
55   phosphoric acid digestion of carbonates is $^{13}C^{18}O^{16}O$ and has a mass of 47 amu (Δ_{47}), while $^{12}C^{18}O^{18}O$ is
56   the dominant mass-48 isotopologue (Δ_{48}). The use of Δ_{47} and Δ_{48} as paleothermometers rely on
57   equilibrium clumped isotope fractionation in the carbonate mineral lattice. However, recent theoretical
58   and experimental work has shown that some biogenic and abiogenic carbonates yield disequilibrium Δ_{47}
59   (Ghosh et al., 2006; Daëron et al., 2011, Saenger et al, 2012, Tang et al., 2014; Tripati et al., 2015; Guo et
60   al., 2019; Daeron et al., 2019) and Δ_{48} values (Tripati et al., 2015; Guo, 2020; Bajnai et al., 2020;
61   Lucarelli et al., 2021; Fiebig et al., 2021).
62   The paired measurement of Δ_{47} and Δ_{48} has been theoretically (Hill et al., 2014; Tripati et al.,
63   2015; Guo, 2020; Hill et al., 2020) and experimentally (Fiebig et al., 2019; Bajnai et al., 2020; Swart et
64   al., 2021; Lucarelli et al., 2021; Fiebig et al., 2021) shown to have a characteristic equilibrium
65   relationship. The equilibrium Δ_{47}-Δ_{48} relationship along with constrained boundaries of disequilibrium
66   trajectories in dissolved inorganic carbon (DIC) pools and in carbonate minerals may be used to identify
67   the origin of kinetic isotope effects (Tripati et al., 2015; Bajnai et al., 2020; Guo, 2020) and used to
68   correct for such effects and derive expected equilibrium clumped isotope values from samples (Bajnai et
69   al., 2020; Guo, 2020).
70   Through comparison of theoretical predictions with experimental data, a robust framework can be
71   developed to advance the paired carbonate clumped isotope approach as a potential tool for constraining
72   kinetic isotope effects (KIEs), including vital effects in biogenic carbonates. Currently, the paired Δ_{47} and
73   Δ_{48} trajectory of kinetic isotope effects (KIEs) in carbonates is limited to theory with calculations for a
74   DIC pool at equilibrium (Hill et al., 2014; Tripati et al., 2015), hydration/hydroxylation (Guo, 2020), and
75   for growth from a solution of DIC with cations present (Hill et al., 2020). Theory has been used to explain
76   some measurements of disequilibrium Δ_{47} values in corals, speleothems, and high pH travertines (Guo,
77   2020), and disequilibrium Δ_{47} and Δ_{48} values in speleothems, stalagmites, and corals (Bajnai et al., 2020).
78   Here, we adapted experimental approaches used in Tang et al. (2014) and Tripati et al. (2015) to
79   constrain KIEs in Δ_{47}, Δ_{48}, and $\delta^{18}O$ in calcite precipitated under controlled conditions at different pH and
80   temperatures, with and without the addition of the enzyme carbonic anhydrase (CA). We compared data
81   from calcite grown under isotopic disequilibrium conditions at varying pH to calcite formed at quasi-
82   equilibrium conditions. We measured samples to further constrain equilibrium and kinetically governed

83 relationships for clumped isotopes, including Δ_{47}-Δ_{48}, Δ_{47} and T, Δ_{48} and T, and bulk $\delta^{18}O$ and $\delta^{13}C$ and
84 clumped isotopes, and compared experimental results to model calculations. We constructed and used a
85 numerical mixing model and predicted nonlinear effects in Δ_{47} and Δ_{48} and compared results to
86 experimental data.
87
88 **2. Background**
89
90 **2.1 Carbonate clumped isotopes and notation**
91
92 Historically, carbonate clumped isotope thermometry used the deviations in the abundance of the
93 m/z 47 CO_2 isotopologue, ^{16}O-^{13}C-^{18}O, which is liberated from the phosphoric acid digestion of the most
94 abundant (67 ppm) m/z 63 carbonate isotopologue, ^{13}C-^{16}O-^{16}O-^{18}O, relative to a stochastic distribution of
95 isotopologues (Ghosh et al., 2006). Recent developments in mass spectrometry precision, such as the use
96 of secondary electron suppression and 10^{13} Ω resistors, has enabled the accurate measurement of the
97 lower abundance m/z 48 CO_2 isotopologue, ^{18}O-^{12}C-^{18}O, which is liberated from the phosphoric acid
98 digestion of the most abundant (12 ppm) m/z 64 carbonate isotopologue, ^{12}C-^{16}O-^{18}O-^{18}O (Ghosh et al.,
99 2006). The thermodynamic equilibrium relationship between m/z 63 and m/z 64 isotopologues with
100 precipitation temperature is governed by the equilibrium constant, K, in Reactions 1 and 2 (Wang et al.,
101 2004; Ghosh et al., 2006).
102
103 $^{13}C^{16}O^{16}O^{16}O^{2-} + ^{12}C^{18}O^{16}O^{16}O^{2-} \leftrightarrows ^{13}C^{18}O^{16}O^{16}O^{2-} + ^{12}C^{16}O^{16}O^{16}O^{2-}$ 　　　　　Reaction 1
104
105 　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　Reaction 2
106 $^{12}C^{18}O^{16}O^{16}O^{2-} + (^{12}C^{16}O^{18}O^{16}O^{2-}$ or $^{12}C^{16}O^{16}O^{18}O^{2-}) \leftrightarrows (^{12}C^{18}O^{18}O^{16}O^{2-}$ or $^{12}C^{18}O^{16}O^{18}O^{2-}$ or
107 $^{12}C^{16}O^{18}O^{18}O^{2-}) + ^{12}C^{16}O^{16}O^{16}O^{2-}$
108
109 When m/z 63 and 64 carbonate isotopologues are digested in phosphoric acid, one oxygen is cleaved, and
110 the resulting excess m/z 47 and 48 CO_2, respectively, is measured on an isotope ratio mass spectrometer
111 and described as
112 $\Delta_{47} = (R47_{sample}/R47_{stochastic} - 1)$ 　　　　　　　　　　　　　　　　　　　　　　　(1)
113
114 $\Delta_{48} = (R48_{sample}/R48_{stochastic} - 1)$ 　　　　　　　　　　　　　　　　　　　　　　　(2)
115
116 where Ri is the ratio of i/44 CO_2 isotopologues, and Δ_{47} and Δ_{48} are given in parts per thousand (‰)
117 (Ghosh et al., 2006; Eiler et al., 2007).
118
119 **2.2 Disequilibrium processes in carbonates**
120
121 Δ_{47} data for some abiotic and biogenic carbonates have been shown to record precipitation out of
122 isotopic equilibrium (Affek et al., 2008; Daeron et al., 2011; Saenger et al., 2012; Tang et al., 2014;
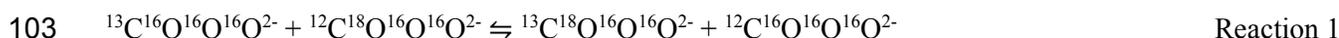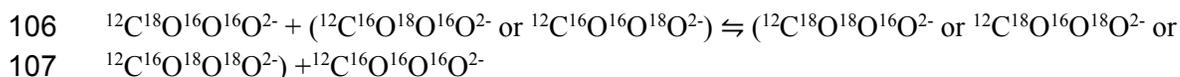
123 Tripati et al., 2015; Kimball et al., 2016; Daëron et al., 2019; Bajnai et al., 2020). This can be correlated
124 with $\delta^{18}O$ disequilibria and observed in experiments at elevated pH (e.g., Tang et al., 2014). Multiple
125 coral species are known to significantly elevate the pH of their calcifying fluid (Ghosh et al., 2006; Affek
126 et al., 2008; Trotter et al., 2011; Anagnostou et al., 2012; Adkins et al., 2003; Saenger et al., 2012;
127 Kimball et al., 2016; Spooner et al., 2016). Strongly alkaline abiotic carbonate precipitation occurs in
128 surface and subsurface aqueous systems, such as alkaline springs with pH > 11 (Christensen et al., 2021).
129   Multiple mechanisms have been proposed to explain Δ_{47} isotope disequilibrium mineral
130 compositions. They have been observed experimentally at elevated pH (Tang et al., 2014). Clumped
131 isotopic disequilibrium can arise from mineral growth from a DIC pool that is at equilibrium with DIC
132 from speciation effects (e.g., Hill et al., 2014; Tripati et al., 2015; Watkins and Hunt, 2015; Hill et al.,
133 2020). Carbonate mineral precipitation can occur before isotopic equilibration of dissolved inorganic
134 carbon (DIC) (Beck et al. 2005; Tripati et al., 2015; Staudigal and Swart, 2018). CO_2 hydration and
135 hydroxylation reactions are another potential source of isotopic disequilibrium (Staudigal and Swart,
136 2018; Guo, 2020; Boettger and Kubicki, 2021), as are dehydration and dehydroxylation reactions, and
137 these can arise from CO_2 absorption and degassing processes (Daeron et al., 2011; Guo, 2020). Aqueous
138 ion and CO_2 gas diffusion (Thiagarajan et al., 2011), and organism-specific vital effects from enzymatic
139 activity and metabolism have also been hypothesized to give rise to clumped isotope disequilibrium.
140 Clumped isotopic disequilibrium in the DIC pool has been hypothesized to be modulated by carbonate
141 precipitation rate (Tripati et al., 2015), as with other geochemical systems such as oxygen isotopes
142 (Dietzel et al., 2009; Watkins et al., 2014).
143   When carbonate minerals precipitate under isotopic disequilibrium conditions, KIEs originating
144 from differing reaction rates of heavy versus light isotopologues can result in clumped isotope values that
145 are higher or lower than equilibrium mineral clumped isotope values, which in turn can yield an over- or
146 underestimation of precipitation temperature. Depending on carbonate mineral growth conditions, there
147 are different trajectories for possible KIEs, and these can potentially be used to identify the process(es)
148 involved through measurement of paired Δ_{47} and Δ_{48}, and bulk isotope values. However, these trajectories
149 are not yet well constrained and are limited to only a few studies. DIC speciation effects that could get
150 recorded in a growing mineral were explored in Tripati et al. (2015). Recent modeling predictions from
151 Guo (2020) has aimed to constrain KIEs in the Δ_{47}, Δ_{48} and $\delta^{18}O$ of the HCO_3^- endmember in hydration
152 and hydroxylation reactions occurring during CO_2 absorption, degassing, and $DIC-H_2O$ exchange. Bajnai
153 et al. (2020) and Fiebig et al. (2021) constructed Δ_{47} and Δ_{48} slopes relative to an equilibrium regression to
154 remove kinetic biases from warm and cold-water coral, brachiopod, belemnite, stalagmite, and
155 speleothem samples.
156
157 *2.2.1 CO_2 hydration and hydroxylation*
158
159   CO_2 hydration (Reaction 3) and hydroxylation (Reaction 4) reactions are responsible for $^{18}O/^{16}O$
160 equilibration, as they are the only direct route for the exchange of O atoms between H_2O and DIC (Zeebe
161 and Wolf-Gladrow, 2001). Clumped and oxygen isotope equilibration of DIC in an aqueous solution are
162 also controlled by three additional key reactions (Reactions 5-7) (Guo, 2020).
163
164 $CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$ (Reaction 3)
165
166 $CO_2 + OH^- \leftrightarrows HCO_3^-$ (Reaction 4)

167
168    $HCO_3^- \leftrightharpoons CO_3^{2-} + H^+$ (Reaction 5)

169
170    $HCO_3^- + OH^- \leftrightharpoons CO_3^{2-} + H_2O$ (Reaction 6)

171
172    $H_2O \leftrightharpoons H^+ + OH^-$ (Reaction 7)
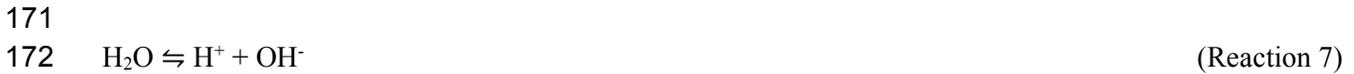
173
174 Equilibrium isotopic compositions are obtained when DIC and H_2O have had sufficient time to
175 isotopically equilibrate. When DIC and H_2O have not been equilibrated, KIEs and disequilibrium isotopic
176 compositions can arise. The parameters governing the time to reach isotopic equilibrium are the forward
177 and reverse CO_2 hydration and hydroxylation kinetic rate constants, which depend on temperature (Zeebe
178 and Wolf-Gladrow, 2001), and DIC speciation, which is function of temperature and pH (Uchikawa and
179 Zeebe, 2012; Tripati et al., 2015). The rate constant for hydration is not sensitive to ionic strength
180 (Johnson, 1982; Miller et al., 1971; Knocke 1980; Zeebe and Wolf-Gladrow, 2001), whereas a subtle
181 dependency has been documented for the hydroxylation rate constant (Johnson, 1982). Higher
182 temperature results in faster reaction kinetics and thus faster isotopic equilibration of the DIC pool. A
183 higher pH causes much longer isotopic equilibration times induced by extreme low molar fraction of
184 aqueous CO_2 in DIC, leading CO_2 to be mostly unavailable for isotope exchange in Reactions 3 and 4
185 (Beck et al., 2005; Weise and Kluge, 2020). For example, at 25 ^oC at pH of 8.7, ^{18}O equilibration is
186 reached at ~17 hours, whereas at pH 12, ^{18}O equilibration is reached at ~35 days (Beck et al., 2005). At
187 40 ^oC and a pH of 12, ^{18}O equilibration time is reduced to ~10 days (Beck et al., 2005).

188
189 **2.2.2 Carbonic anhydrase**

190
191   The addition of the enzyme carbonic anhydrase (CA) catalyzes the forward and reverse hydration
192 reaction and thus significantly decreases the time to reach isotopic equilibrium. The uncatalyzed
193 hydration reaction rate at 25 ^oC and pH 7.4 is ~10^{-1} s^{-1}, while the CA catalyzed rate can reach ~10^6 s^{-1}
194 depending on [CA] (Kernohan, 1964), where highest catalyzation effects are achieved at pH > 8 (Berg et
195 al., 2002). Many marine calcifiers used for climate reconstructions are thought to have CA within their
196 calcifying space, including coccolithophores (Nimer et al., 1994; Soto et al., 2006) and other
197 phytoplankton (Rost et al., 2003), oysters (Miyamoto et al., 1996; Yu et al., 2006), scleractinian coral (Al-
198 Horani et al., 2003; Moya et al., 2008; Bertucci et al, 2011), and benthic foraminifera (de Goeyse et al.,
199 2019). The role that CA plays in calcification is not completely known, including the effectiveness of CA
200 at reducing clumped and bulk KIEs at high pH and varying temperatures.

201
202 **2.2.3 Mixing effects**

203
204   Numerical models and experimentation have shown that while $\delta^{13}C$, $\delta^{18}O$, δ^{45}, δ^{46}, and δ^{47} mix
205 linearly, Δ_{47} mixes non-linearly (Eiler and Schauble, 2004; Defliese and Lohmann, 2015). Thus, in
206 addition to KIEs, mixing effects from the homogenization of isotopically heterogeneous samples can
207 result in measured Δ_{47} values that do not accurately reflect precipitation temperature (Defliese and
208 Lohmann, 2015). Some samples that are commonly used for Δ_{47}-based temperature reconstructions
209 display isotopic heterogeneity in isotopically distinct growth bands or multiple cementation stages (Jones

210 et al., 2015), and thus sampling that integrates over growth increments or cements has the potential to
211 give rise to mixing effects.
212
213
214 **3. Methods**
215
216
217 **3.1 Calcite precipitation experiments**
218
219   Calcite was precipitated under controlled conditions at pH 8.3, 9.0, 9.5, 10.0, 10.5, and 11.0 at 5
220 °C, 10 °C, 15 °C, and 25 °C, with and without the addition of CA (molecular weight 29,000 g/mol). CA
221 has been shown to be active in catalyzing CO_2 hydration at all pH values used in our experiment
222 (Kernohan, 1964). Experimental conditions are given in Table 1. Calcite was precipitated by using a
223 method adapted from Dietzel et al. (2004). A schematic of the precipitation apparatus is shown in Figure
224 1. In all experiments, a 0.5 L bottle that is 2 mm thick polyethylene is filled with 0.83 M $NaHCO_3$ and 2
225 M HCl and placed inside a 5 L outer container filled with 4.9 L Milli Q water, 10 mM $CaCl_2$, 0.01 mM
226 $SrCl_2$, 50 mM NaCl, and no DIC. All chemicals are reagent grade from Merck.
227   The pCO_2 gradient between the inner and outer containers causes CO_2 gas to diffuse through the
228 polyethylene membrane into the outer container solution where the CO_2 reacts to form carbonate ions that
229 precipitate as $CaCO_3$. The outer solution was constantly stirred with a large floating stir bar by placing the
230 containers on top of a stir plate at 200 rpm. A piece of Styrofoam was placed in between the stir plate and
231 solution to prevent temperature changes. The pH of the outer container solution was held constant by a
232 Schott TitroLine alpha plus titrator (± 0.03 accuracy) with 2 M NaOH. The entire precipitation apparatus
233 was placed inside an Aqualytic Thermostatic Cabinet (± 0.5 °C accuracy) to hold the precipitation
234 temperature constant. In experiments with CA, 0.25 μM CA is added to the outer solution. A
235 concentration of 0.25 μM CA should be sufficient to maintain oxygen isotopic equilibrium among DIC
236 species up to at least pH = 9.3 (Watkins et al., 2014). Each experiment yielded 30-40 mg of precipitate.
237 The mineralogy of the precipitate was determined by X-ray powder diffraction (XRD) PANalytical
238 X'Pert PRO diffractometer. The mineral phase was determined using PANalytical X'Pert HighScore
239 Plus software.
240   Every 2-3 days a 2 ml sample of the solution was removed from the outer solution via a syringe
241 that was inserted into an airtight port through the lid of the apparatus. The solution was filtered through a
242 0.2 μm cellulose acetate membrane to remove any precipitate and subsequently analyzed for trace
243 elements on a Perkin Elmer Optime 4300DV Inductively Coupled Plasma - Optical Emission
244 Spectrometer (ICP-OES). This analysis yielded the [Ca], allowing for the estimation of the precipitation
245 rate by ($[Ca]_{final}$ - $[Ca]_{initial}$) / (t_{final} - $t_{initial}$), which was converted to mol s^{-1} m^{-2} using the specific surface
246 area of 0.27 m^2/g (Tang et al., 2008). Oxygen isotopes of the precipitation solution were measured at the
247 start and end of most experiments by wavelength-scanned cavity ring-down spectroscopy (WS-CRDS)
248 using a Picarro L2140-i system. Typical analytical precision (1 σ) was ±0.05 ‰ for $\delta^{18}O_{water}$, where the
249 values are referenced relative to the Vienna Standard Mean Ocean Water (VSMOW).
250
251 **3.2 Mixing experiments**
252

253   Two internal carbonate standards, CM Tile and Mallinckrodt, were used as isotopic endmembers
254 to constrain Δ_{48} mixing effects. These standards were selected as endmembers because they have greatly
255 varying bulk and clumped isotope values (see Table 5), with a ~20 ‰ difference in $\delta^{18}O$, ~38 ‰
256 difference in $\delta^{13}C$, 0.164 ‰ difference in Δ_{47}, and 0.062 ‰ difference in Δ_{48}. Endmembers with large
257 differences in isotopic values have been shown to have a greater magnitude of mixing effects than
258 endmembers with smaller isotopic differences (Defliese et al., 2015).
259   The two endmembers were combined into three mixes with varying contributions from each
260 endmember. Mix 1 was 75 % Mallinckrodt and 25 % CM Tile, Mix 2 was 50 % Mallinckrodt and 50 %
261 CM Tile, and Mix 3 was 25 % Mallinckrodt and 75 % CM Tile. The mixes were created by weighing out
262 the carbonate powder from each sample and pouring them into a glass vial, where they were first stirred
263 with a spatula, then shaken by hand for several minutes. The mixtures were then crushed in a mortar and
264 pestle to equalize grain size, then remixed with a spatula and shaken in a glass vial. Each endmember and
265 mix were analyzed for Δ_{47}, Δ_{48}, $\delta^{13}C$, $\delta^{18}O$. The observed change in Δ_{47} and Δ_{48} values due to mixing
266 effects is referred to as Γ, described in Equation 3:

267

268    $\Gamma_i = \Delta_{i\ endmember} - \Delta_{i\ mix}$ (3)

269

270 where $\Delta_{i\ endmember}$ is the Δ_{47} or Δ_{48} of the endmember with the highest value. It represents clumped isotope
271 enrichment beyond what would be observed in linear mixing.

272

273 **3.3 Clumped isotope instrumentation**

274

275   Standards and samples were analyzed on three isotope ratio mass spectrometers (IRMS) during
276 2018-2021. The instruments used in this work were a Thermo Fisher MAT 253, and two Nu Instruments
277 Perspective mass spectrometers, with both Nu Instruments having multiple configurations (Table 2). All
278 instrumental configurations used here have been shown to produce statistically indistinguishable Δ_{47}
279 (Upadhyay et al., 2021; Lucarelli et al., 2021) and the Nu Instruments produce statistically
280 indistinguishable Δ_{48} data (Lucarelli et al, 2021). Only data from the Nu Instruments are used in the Δ_{48}
281 analyses reported here.
282   A typical daily run on all instruments consists of five samples and five standards, with standards
283 bracketing the samples. Raw data was processed and corrected in Easotope 64-bit version from release
284 20201231 (John and Bowen, 2016) using the IUPAC parameter set (Brand et al., 2010; Daëron et al.,
285 2016). Δ_{47} data are presented relative to international standards ETH-1, ETH-2, ETH-3 in the I-CDES
286 reference frame (Bernasconi et al., 2021) at 90 ºC, $\Delta_{47\ I\text{-}CDES}$. Δ_{48} data are presented relative to the same
287 carbonate standards, with the addition of the in-house carbonate standard, Veinstrom (Upadhyay et al.,
288 2021; Lucarelli et al., 2021), in the 90 ºC reference frame, $\Delta_{48\ CDES\ 90}$.
289   The Thermo Fisher MAT 253 used an autosampler similar to what is described in Passey and
290 Henkes (2010) with a 105 weight % phosphoric acid bath held at 90 ºC. After carbonate samples of 4-7
291 mg are digested, CO_2 (g) is cryogenically purified through traps containing dry ice-cooled ethanol and
292 liquid nitrogen, which remove low vapor pressure gases such as H_2O (g). The CO_2 passed through
293 elemental silver wool (Sigma Aldrich), followed by a -20 ºC gas chromatograph (GC) that contains
294 Porapak Type-Q^{TM} 50/80 mesh column pack material with He carrier gas. The m/z 44 beam intensity is
295 16000 mV. There are a total of 80-90 cycles of sample-standard comparison with a total integration time
296 of 720 s.

297   Nu Instruments Perspective configurations 1a, 1b, and 1c (Table 2) consist of the same mass
298   spectrometer with differences in the reaction and digestion system. Thus, the sample purification method,
299   acid digestion temperature, and acid digestion method differ. All configurations on this instrument used a
300   sample size of 0.4-0.6 mg with a total of 60 cycles of sample-standard comparison with a total integration
301   time of 1200 s. This instrument is equipped with two curved plates with a voltage difference in front of
302   the Faraday collectors for m/z 47-49 to perform secondary electron suppression, which makes the linearity
303   correction an order of magnitude smaller than on the Thermo Fisher MAT 253. Nu Instruments
304   Perspective configuration 1a used the same autosampler setup as the MAT 253 with an acid digestion
305   temperature of 90 $^{\circ}$C, common acid bath, GC, He carrier gas, and cryogenic purification traps. New
306   Instruments Perspective 1b is identical to 1a, except for the sample purification method, which used a
307   relatively short GC column called an adsorption trap that is under vacuum (no carrier gas) and contains
308   Poropak Type Q^{TM}. New Instruments Perceptive 1c used an acid digestion temperature of 70 $^{\circ}$C with
309   individual glass reaction vials and an adsorption trap. Nu Instruments Perspective 2a and 2b use the same
310   mass spectrometer with differences in the acid digestion temperature and method. Nu Instruments
311   Perspective 2a used a 90 $^{\circ}$C acid digestion temperature, common acid bath, and adsorption trap. Nu
312   Instruments Perspective Config. 1b used 70 $^{\circ}$C acid digestion, individual glass sample vials, and an
313   adsorption trap. Both configurations used samples from 0.4-0.6 mg, and had 60 cycles of sample-standard
314   comparison with a total integration time of 1200 s.

316   **3.4 Converting Δ_{47} and Δ_{48} to Δ_{63} and Δ_{64}**

318   Measured Δ_{47} and Δ_{48} were converted to theoretical calcite mineral Δ_{63} and Δ_{64}, respectively, for
319   direct comparison to model predictions for kinetic and equilibrium relationships between bulk and
320   clumped isotopes. This conversion was carried out using equations from Lucarelli et al. (2021). In
321   summary, regressions were determined for compositionally dependent acid digestion fractionation factors
322   (AFFs), Δ^{*}_{63-47} and Δ^{*}_{64-48}, by using model predicted Δ_{63} and Δ_{64} values for calcite precipitated at 600 $^{\circ}$C
323   and 33.7 $^{\circ}$C (Hill et al., 2014; Tripati et al., 2015). The difference was calculated between the model-
324   predicted Δ_{63} and Δ_{64} and experimentally determined Δ_{47} and Δ_{48} for ETH-1 and ETH-2, calcite standards
325   equilibrated at 600 $^{\circ}$C (Bernasconi et al., 2018), and Devils Hole, a cave vein calcite precipitated near
326   isotopic equilibrium at 33.7 $^{\circ}$C (Winograd et al., 1988, 1992; Coplen, 2007). These values were used as
327   the AFFs, Δ^{*}_{63-47} and Δ^{*}_{64-48}, for 600 $^{\circ}$C and 33.7 $^{\circ}$C. Regressions were made for Δ^{*}_{63-47} and Δ_{47} and Δ^{*}_{64-48} and Δ_{48}. These regressions (Equations 4 and 5) can be used to calculate acid digestion fractionation
329   factors for unknown samples (Lucarelli et al., 2021), and then applied in Equations 6 and 7 to calculate
330   Δ_{63} and Δ_{64}.

332    $$\Delta^{*}_{63-47} = 0.0190 \times \Delta_{47\text{ I-CDES}} + 0.1842 \tag{4}$$

334    $$\Delta^{*}_{64-48} = 0.0077 \times \Delta_{48\text{ CDES 90}} + 0.1290 \tag{5}$$

336    $$\Delta_{63} = \Delta_{47\text{ I-CDES}} - \Delta^{*}_{63-47} \tag{6}$$

338    $$\Delta_{64} = \Delta_{48\text{ CDES 90}} - \Delta^{*}_{64-48} \tag{7}$$

3.5 Δ_{48} mixing model

For this study, a numerical model was built to estimate Δ_{48} mixing effects in $CaCO_3$ and was compared to experimental data for two carbonate reference materials, Mallinckrodt and CM Tile, which are used as isotopic endmembers. The model was constructed based on methods detailed in Defliese and Lohmann et al. (2015), with the addition of δ^{48} and Δ_{48} in this study. In the model, Δ_{47}, Δ_{48}, $\delta^{18}O$, and $\delta^{13}C$ values are assigned to each endmember. $\delta^{18}O$ and $\delta^{13}C$ values are also given to a hypothetical working gas (WG). The slope and intercept for an empirical transfer function (ETF) are entered into the model. All isotopic values for model samples and the working gas, as well as the transfer function values, were based on experimental data taken from the Nu Perspective instruments. No acid fractionation factor was used, and model calculations were based on values determined in the I-CDES (Bernasconi et al., 2021) reference frame at 90 °C. The model calculates δ^{45}, δ^{46}, δ^{47}, δ^{48}, $\delta^{18}O$, and $\delta^{13}C$ values of CO_2 gas produced by phosphoric acid digestion of carbonate relative to a working gas (WG). The ratio of each endmember is entered into the model and the isotopic values of the mix are calculated. The full set of equations and parameters used and a link to a Github site where the model can be downloaded are detailed in the Appendix (section S.1).

3.6 Parameters used for IsoDIC modelling

To estimate the evolution of the isotopic composition of HCO_3^- and CO_3^{2-} endmembers during CO_2 absorption in an aqueous solution at pH 10.5, in conditions similar to our experiments, we used the IsoDIC model (Guo, 2020). This numerical modeling software predicts kinetic clumped isotope fractionations in the $DIC-H_2O-CO_2$ system. The model simulates five key reactions (Reactions 3-7) that control isotope fractionation, and all related isotopologue reactions involving ^{12}C, ^{13}C, ^{16}O, ^{17}O, and ^{18}O, for a total of 155 reactions. The forward and reverse rate constants were estimated using equation 8,

$$k^* = \alpha_{KIE} \times k \tag{8}$$

where k^* is the rate constant of the isotopically substituted reaction, k is the rate constant of the isotopically non-substituted reactions, and α_{KIE} is the kinetic isotope fractionation factor for the isotopically substituted reaction. Isotopic equilibrium was assumed between HCO_3^- and CO_3^{2-} and H_2O and OH^- due to rapid equilibration via Reactions 5-7 relative to the reaction rate of hydration (Reaction 1) and hydroxylation (Reaction 2). Therefore, the only reactions that contribute to isotopic fractionation in the IsoDIC model are the forward and reverse CO_2 hydration and hydroxylation reactions (Guo, 2020).

To use the IsoDIC model in the CO_2 absorption regime, we assumed the model default values for $\delta^{13}C$ of the CO_2 in air of -10 ‰, $\delta^{18}O_{VSMOW}$ of water to be 0 ‰, and $\Delta'^{17}O_{VSMOW}$ of water to be 0 ‰ (Guo, 2020). The initial oxygen and clumped isotope values of DIC and air were set to be in equilibrium with water. We set the solution temperature to be 5°C in the first simulation and 25°C in the second simulation, both with a solution pH of 10.5. The model has a total evolution time of 12 hours. The full set of equations used are available in Guo (2020).

3.7 Box model for kinetic isotope effects in Δ_{47}, Δ_{48}, and $\delta^{18}O$

384   To predict kinetic isotope effects in calcite during CO_2 absorption for Δ_{47}, Δ_{48}, and $\delta^{18}O$, we used
385 a box model built on the ExClump38 framework (Chen et al., 2018; Uchikawa et al., 2021; Watkins and
386 Devriendt, 2021). The model tracks the isotopic composition of DIC species in a homogeneous (i.e., well-
387 stirred) solution from an initial unequilibrated state. In the box model, the isotopic composition of DIC is
388 affected by two additional fluxes: (1) a specified CO_2 influx and (2) a calculated $CaCO_3$ outflux that is
389 based on the following reactions:

390

391 $Ca^{2+} + HCO_3^- \rightleftharpoons CaCO_3 + H^+$ Reaction 8

392

393 $Ca^{2+} + CO_3^{2-} \rightleftharpoons CaCO_3$ Reaction 9

394

395 These reactions affect the isotopic composition of DIC in two ways: (1) the rate constants for Reactions 8
396 and 9 are mass dependent and can therefore influence the isotopic composition of residual DIC (Watkins
397 and Hunt, 2015) and (2) the rate of calcite precipitation affects the degree of hydration/hydroxylation
398 reaction reversibility at steady state and the extent to which the kinetic isotope effects attending these
399 reactions are expressed in solution.
400   In the model, we begin with $[Ca^{2+}] = 10$ mM and enough DIC to bring the initial degree of
401 supersaturation to 7, as estimated from Dietzel et al. (2009) for spontaneous nucleation of calcite;

402

403 $\Omega = \frac{[CO_3^{2-}][Ca^{2+}]}{K_{sp\,(calcite)}} \sim 7$ (9)

404

405 where Ω is the saturation state and K_{sp} is the solubility product for calcite. The DIC is initially isotopically
406 equilibrated. As CO_2 fluxes into solution it gets converted to isotopically lighter-than-equilibrium HCO_3^-
407 and CO_3^{2-} by hydration (Reaction 3) and hydroxylation (Reaction 4). The rate of calcite growth depends
408 on $[Ca^{2+}]$ and $[CO_3^{2-}]$, which is updated at each time step. Each simulated experiment lasts 1000 hours,
409 which is far longer than the time required to reach steady state, and outputs the steady state composition
410 of each DIC species and calcite. In the default scenario, we treat the reaction rate constants as "known"
411 and the ion-by-ion model as "correct" to assess how well the model predicts the trends observed in the
412 data. This leaves the CO_2 flux (F_{CO2}) as the only adjustable parameter in the default scenario, but even
413 F_{CO2} (which is equal to F_{CaCO3} at steady state) is constrained by the data. A description of all equations
414 and parameters used are provided in Appendix (S.2) that also contains a link to a Github site with the
415 parameters utilized and code used.
416   The steady state Δ_{47}, Δ_{48}, and $1000\ln\alpha_{carb-water}$ at variable pH from 8.3 to 11, at temperatures
417 of 5 °C and 25 °C, and variable FCO2 (related to calcite growth rate) ranging from typical experimental
418 rates to those from extremely slow calcite growth. The oxygen fractionation factor between carbonate and
419 water, $\alpha_{carb-water}$, is expressed by the equation:

420

421 $\alpha_{carb-water} = \frac{^{18}R_{carb}}{^{18}R_{water}} = \frac{\delta^{18}O_{carb}+1000}{\delta^{18}O_{water}+1000}$ (10)

422

423 where ^{18}R is the ratio of ^{18}O to ^{16}O in the carbonate and water.
424   We repeated the same model calculations, but simulated the impact of the addition of 0.25 μM
425 CA. All model calculations had 0 ‰ salinity, while experiments had a starting ionic strength of ~0.045

426 mol/L. This should have a negligible effect on our model calculations given that the reaction rate
427 constants will not be affected by the ionic strength. We compared the results of the model calculations
428 (with and without CA) to our experimental data (with and without CA) and to experimental data from
429 Tang et al. (2014), where calcite was precipitated at pH 8.3, 8.5, 9.0, 10.0, and 10.5 at 5 °C, and pH 8.3 at
430 25 °C (no enzyme was utilized in their experiments).

431
432
433   **4. Results**
434
435 **4.1 Calcite precipitated with and without carbonic anhydrase**
436
437 *4.1.1 $\delta^{13}C$ and $\delta^{18}O$*
438
439   Calcite, verified by XRD, precipitated at increasing pH had an increasing amount of $\delta^{18}O$
440 depletion (Figure 2A-D, Table 3). The largest $\delta^{18}O$ depletion relative to the near equilibrium sample
441 (precipitated at the same temperature at pH 8.3 with CA) was observed in the samples with the lowest
442 temperature and highest pH, at 5 °C and pH 10.5 and 11.0, with depletions of 17.7 ‰ and 18.8 ‰,
443 respectively. All samples precipitated with and without CA at pH 8.3 had statistically identical $\delta^{18}O$. At 5
444 °C, depletions in $\delta^{18}O$ were observed at pH > 9. Calcite precipitated without CA had depleted $\delta^{13}C$ when
445 compared to samples precipitated with CA for all temperatures and pHs (Figure 2E-H, Table 3).

446
447 *4.1.2 Δ_{47} and Δ_{48}*
448
449   For a given temperature, calcite precipitated with and without CA at pH 8.3 had statistically
450 identical Δ_{47} and Δ_{48}. Samples precipitated without CA at pH $\geq$ 9.5 had enriched Δ_{47} and depleted Δ_{48}
451 values relative to calcite precipitated with CA, and these samples also had negative Δ_{48} values (i.e.,
452 $^{12}C^{18}O^{18}O$ occurring less frequently than predicted due to random chance) (Figure 3, Table 3). The
453 enrichment of Δ_{47} and depletion of Δ_{48} increased with increasing pH. The largest Δ_{47} enrichment was 0.198
454 ‰ at 5 °C and pH 10.5. The largest Δ_{48} depletion was -0.628 ‰ at 5 °C and pH 11.0. Despite the
455 relatively large depletions in Δ_{48} compared to enrichments in Δ_{47}, CA appears to be more effective at
456 returning Δ_{48} values to near-equilibrium values than for Δ_{47}.
457   Kinetic slopes for Δ_{47} and $\delta^{18}O$, Δ_{48} and $\delta^{18}O$, and Δ_{48} and Δ_{47} were determined (Table 4). Kinetic
458 slopes for Δ_{47} and $\delta^{18}O$ (Figure 4A-D) at 5 °C, 10 °C, 15 °C, and 25 °C were -0.011 ± 0.001, -0.013 ±
459 0.0001, -0.012 ± 0.0002, and -0.020 ± 0.001, respectively. Kinetic slopes for Δ_{48} and $\delta^{18}O$ (Figure 4E-H)
460 at 5 °C, 10 °C, 15 °C, and 25 °C were 0.033 ± 0.001, 0.038 ± 0.0004, 0.034, and 0.048 ± 0.002,
461 respectively. Kinetic slopes for Δ_{48} and Δ_{47} (Figure 5) at 5 °C, 10 °C, 15 °C, and 25 °C were -2.989 ±
462 0.125, -3.071 ± 0.011, -2.976 ± 0.034, and -2.455 ± 0.024, respectively.
463   Equilibrium regressions were made for Δ_{47} and Δ_{48} relative to precipitation temperature in Kelvin
464 (Figure 6A-B), Δ_{47} and Δ_{48} relative to $\delta^{18}O$ (Figure 6C-D), and Δ_{48} and Δ_{47} (Figure 6E), for calcite
465 precipitated at 5 °C, 10 °C, 15 °C, and 25 °C with CA at pH 8.3, which are expected to achieve quasi-
466 isotopic equilibrium. Devils Hole core DH-2 data previously analyzed in Lucarelli et al. (2021) was
467 included in the equilibrium regressions for Δ_{48} and Δ_{47}, Δ_{47} and precipitation temperature, and Δ_{48} and
468 precipitation temperature. Devils Hole has been shown to be near isotopic equilibrium (Winograd et al.,

469 1998; Coplen, 2007) and has a very precisely known precipitation temperature of 33.7 ± 0.2 ℃ (Plummer
470 et al., 2000). The experimental Δ_i and T relationships were determined to be
471
472 $\Delta_{47\ \text{I-CDES}} = (0.037 \pm 0.002) \times 10^6 T^{-2} + (0.175 \pm 0.026); r^2 = 0.99$ (10)
473 $\Delta_{48\ \text{CDES 90}} = (0.013 \pm 0.001) \times 10^6 T^{-2} + (0.100 \pm 0.012); r^2 = 0.98$ (11)
474
475 where T is the precipitation temperature in Kelvin. The Δ_{48} and Δ_{47} relationship is represented by equation
476 12.
477
478 $\Delta_{48\ \text{CDES 90}} = (0.355 \pm 0.030)\ \Delta_{47\ \text{I-CDES}} + (0.039 \pm 0.018); r^2 = 0.98$ (12)
479
480 The equilibrium relationships for Δ_{47} and Δ_{48} and $\delta^{18}O$ (Figure 6C-D) are represented by equations 13 and
481 14.
482
483 $\Delta_{47\ \text{I-CDES}} = (0.013 \pm 0.001)\ \delta^{18}O_{\text{VPDB}} + (0.743 \pm 0.006); r^2 = 0.99$ (13)
484 $\Delta_{48\ \text{CDES 90}} = (0.004 \pm 0.001)\ \delta^{18}O_{\text{VPDB}} + (0.303 \pm 0.005); r^2 = 0.97$ (14)
485
486 ### 4.1.3 Measured and modeled Δ_{47}, Δ_{48}, $\alpha_{\text{carb-water}}$, and growth rate effects
487
488 We compare our calcite isotopic values to those we predicted using the IsoDIC model (Guo,
489 2020) for different temperatures and pH in Figure 7. We used the model to quantify the temporal
490 evolution of disequilibrium Δ_{63} and Δ_{64} in bicarbonate and carbonate ion endmembers during CO_2
491 absorption in an aqueous solution at pH 10.5 at 5 ℃ and 25 ℃. The experimental kinetic slope for our
492 samples precipitated at 5 ℃ and pH ≥ 9.5 was $m = -3.127 \pm 0.302$ and for samples precipitated at 25 ℃ at
493 pH 10.5, $m = -2.485 \pm 0.025$ (Figure 7). The model trajectories agree with titration data indicating that in
494 experiments with pH ≥ 9.5 at 5 ℃ and 25 ℃, calcite precipitation began during the first day. At both
495 temperatures, the calcite precipitated at pH 10.5 exceeded the model predictions for maximum enrichment
496 of Δ_{63}, and conversely, did not achieve the maximum amount of depletion for Δ_{64} predicted by the model,
497 that could indicate the influence of another factor, such as growth rate.
498 We compare our experimentally constrained mineral isotopic compositions to box model
499 calculations that factor into account modulation of isotopic fractionation by growth rate (Figure 8). We
500 used the model to predict $1000\ln(\alpha_{\text{carb-water}})$, Δ_{47}, and Δ_{48} with and without CA and compared simulated
501 values to our experimental results. We also compared our results to experimental data from Tang et al.
502 (2014) (Figure 8). Tang et al. (2014) performed calcite precipitation experiments at pH 8.3, 8.5, 9.0, 9.5,
503 10.0, and 10.5 at 5 ℃, and pH 8.3 at 25 ℃, with precipitation rates ranging from -5.99 to -7.38. Our
504 precipitation rates are given as the log of R (mol s^{-1} m^{-2}) for direct comparability to previous modeling
505 and measurements (Table 1). The precipitation rates measured here range from -7.02 to -7.48, which are
506 typical growth rates for calcite precipitation experiments or for corals, which range from ~-4.8 to ~-7.6
507 (Dietzel et al., 2009; Ghosh et al., 2006; Saenger et al., 2012).
508 The default model for $1000\ln(\alpha_{\text{carb-water}})$ at 5 ℃ and 25 ℃ underestimates the observed
509 experimental fractionations at high pH, even at the fastest precipitation rate. The largest difference
510 between the model and experimental values is at 5 ℃ and pH 10.5, with a model predicted $1000\ln(\alpha_{\text{carb-}}$
511 $_\text{water})$ value of 22.0, and experimental values of 14.8 and 13.1 for this study and Tang et al. (2014),

512 respectively. The default model-predicted values for Δ_{47} and Δ_{48} had overall good agreement with

513 experimental values at high and low pH. Model calculations that consider the addition of 0.25 μM CA

514 had better agreement with experimental data at pH ≥ 10 at 5 °C and 25 °C.

515   Calcite precipitated at high pH with CA (hollow data points in Figures 4, 5, 7, and 13) followed a

516 different trajectory with a shallower Δ_{47} versus Δ_{48} slope (m = -0.371 ± 0.107 at 5 °C; m = -0.443 at 25 °C)

517 (Figure 5). The shallower slope may be more representative for organisms that have CA in their

518 calcifying fluid. Increased disequilibrium was observed in Δ_{47} relative to Δ_{48} in samples at pH ≥ 9.5 with

519 CA indicating that CA may be better at equilibrating m/z 64 isotopologues than m/z 63 isotopologues.

520 Since m/z 63 isotopologues contain both ^{13}C and ^{18}O it likely takes longer to equilibrate than m/z 64

521 isotopologues, which contain 2 ^{18}O atoms and no ^{13}C, due to CA catalyzing O isotope exchange. It has

522 been shown experimentally that CA discriminates against CO_2 and HCO_3 that contain ^{13}C due to slower

523 rates of diffusion to the enzyme (Paneth and O'Leary, 1985).

524

525 **4.2 Δ_{47} and Δ_{48} mixing experiment and model results**

526

527   Two internal carbonate standards, CM Tile and Mallinckrodt, were used as endmembers to

528 constrain clumped isotope mixing effects because they have large differences in their bulk and clumped

529 isotope values. Mix 1 (75 % Mallinckrodt, 25 % CM Tile) had Γ_{47} = 0.106 ‰ and Γ_{48} = 0.039 ‰, Mix 2

530 (50 % Mallinckrodt, 50 % CM Tile) had Γ_{47} = 0.107 and Γ_{48} = 0.042, Mix 3 (25 % Mallinckrodt, 75 %

531 CM Tile) had Γ_{47} = 0.014 ‰ and Γ_{48} = 0.007 ‰ (Figure 9A, Table 5). The measured Δ_{47} and Δ_{48} were

532 within error of model predicted values, validating the robustness of the model. Table 6 lists all model

533 parameters.

534   Figure 9B shows Δ_{47} and Δ_{48} model predictions for modeled calcite formation with differing

535 clumped and bulk isotopes, differing clumped isotopes and identical bulk isotopes, and identical clumped

536 isotopes with differing bulk isotopes. The model with a difference of 0.141‰ for Δ_{47} and 0.064 ‰ for Δ_{48},

537 and 20 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$ between endmember 1 (E1) and endmember 2 (E2), had Γ_{47} = 0.029

538 ‰ and Γ_{48} = 0.039 ‰. These Γ_{47} and Γ_{48} values were smaller than for the endmembers with the same

539 clumped isotope values and a 20 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$, where Γ_{47} = 0.086 and Γ_{48} = 0.073. The

540 endmembers with differing clumped isotope values and the same bulk isotope values mix linearly. For

541 endmembers with identical clumped isotope values and differing bulk isotope values (Figure 9C-D), the

542 largest Γ_{47} and Γ_{48} were 0.769 ‰ and 0.660 ‰, respectively, for endmembers with a 30 ‰ difference in

543 $\delta^{18}O$ and $\delta^{13}C$. The smallest Γ_{47} and Γ_{48} predicted were 0.021 ‰ and 0.018 ‰, respectively, for

544 endmembers with a 10 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$.

545

546

547   **5. Discussion**

548

549 **5.1 Comparison of kinetic effects observed in isotopic data with theory**

550

551 *5.1.1 $\delta^{18}O$ and $\delta^{13}C$*

552

553   There are multiple factors that may contribute to the observed clumped and bulk kinetic isotope

554 effects in this study, including CO_2 hydration and hydroxylation, pH dependent DIC speciation, growth

555 rate effects, diffusive isotope effects, and precipitation occurring before temperature and pH dependent

556   equilibrium is achieved in the DIC pool. Modeling predicts that hydration should discriminate against ^{18}O
557   by 14-15 ‰ and ^{13}C by 19-23 ‰ at 25 °C, while hydroxylation would discriminate against ^{18}O by 27-30
558   ‰ and ^{13}C by 26-31 ‰ at 25 °C (Boettger and Kubicki, 2021). At a pH > ~8.4 hydroxylation starts to
559   dominate, and at a pH of 10, hydroxylation represents 95% of reactions (McConnaughey, 1989).
560   Additionally, at equilibrium, δ^{18}O decreases in DIC species in the following order: $CO_2 > H_2CO_3 > HCO_3^-$
561    $> CO_3^{2-}$ (Usdowski et al., 1991; Beck et al., 2005), and at pH > 10, CO_3^{2-} dominates the DIC composition
562   (Hill et al., 2014; Tripati et al., 2015). This is consistent with what we experimentally observed, with
563   increasing depletion in oxygen isotopes starting in samples precipitated at pH > 9 without CA (Table 3,
564   Figure 2).
565   The trend observed in δ^{13}C also indicates subtle depletion starting at pH ≥ 9.5 for samples
566   precipitated without CA (Figure 2). For calcite precipitated with CA, there was depletion starting at pH ≥
567   9. When calcite precipitated with and without CA at the same pH and temperature were directly
568   compared, there was an offset observed in δ^{13}C, including for samples precipitated at pH 8.3 (Table 3,
569   Figure 3). This offset may be due to diffusion and the effects of CA on the CO_2 gradient between the
570   inner and outer solutions. The enzyme CA makes the hydration reaction more reversible, and thus, the
571   [CO_2] in the outer solution could be higher in experiments with CA. If so, this would decrease the
572   CO_2 gradient between the inner and outer chambers and lead to less diffusive isotopic fractionation. For
573   samples precipitated without CA in the outer solutions, the CO_2 diffusing into the outer chamber would
574   be isotopically lighter, which is consistent with observations. The offset in δ^{13}C between experiments with
575   and without CA decreases with increasing pH, consistent with the decreasing influence of the CA-
576   catalyzed hydration reaction with increasing pH.
577   This same effect is not evident in δ^{18}O, with catalyzed and uncatalyzed reactions precipitated at
578   pH 8.3 and the same temperature having statistically indistinguishable δ^{18}O values (Figure 2). Since CA
579   catalyzes the pathway for O exchange among DIC to a nearly instantaneous reaction, does not exchange
580   C among DIC species, and changes the transition state to a more hydroxylation-type reaction, this
581   potentially explains why the use of CA may alter the δ^{13}C, but not the δ^{18}O, of inorganically precipitated
582   carbonates.

583

584   *5.1.2 Paired clumped isotopes (Δ_{47}-Δ_{48} and Δ_{63}-Δ_{64}) and clumped isotopes with δ^{18}O*

585

586   Samples grown at elevated pH without CA exhibit enriched Δ_{47} and depleted δ^{18}O (Figure 4)
587   consistent with CO_2 hydrolysis (Tripati et al., 2015; Guo, 2020). These samples also exhibit
588   "hyperstochastic" values for Δ_{48} that we hypothesized resulted from a depletion in $^{12}C^{18}O^{18}O$ produced by
589   the relative rate kinetics for different CO_2 isotopologues during hydration and hydroxylation, which we
590   test by comparing experimental data to model calculations. Our experimental data yield slopes that range
591   between -2.455 to -3.071 for Δ_{47} and Δ_{48} (Figure 5), and -2.485 to -3.127 for Δ_{63} and Δ_{64}. These
592   experimental data at high pH fall between model-predicted slopes for CO_2 absorption and high pH
593   travertines (Figure 10). The IsoDIC model (Guo, 2020) yields predicted slopes for Δ_{63} and Δ_{64} of HCO_3^-
594   produced from hydration and hydroxylation reactions during CO_2 absorption, CO_2 outgassing, DIC
595   speciation, DIC diffusion, thermodynamic equilibrium, and in pH 11.5 travertines. We calculated model-
596   predicted slopes using the clumped isotope composition of the bicarbonate endmember for times ranging
597   from 0-5 minutes after the initial reaction occurs. The model predicted slope for Δ_{63} and Δ_{64} during CO_2
598   absorption at a pH 9 was -1.72, while the predicted slope for pH 11.5 travertines was -8.33.
599   We were able to simulate observed hyperstochastic Δ_{64} values that are consistent with our
600   experimental observations of Δ_{48}. Our measured kinetic slopes at 25 °C for Δ_{47} and δ^{18}O of -0.020 ± 0.001,
601   and Δ_{48} and δ^{18}O of 0.048 ± 0.002 (Figure 5, Table 4) are in good agreement with model predicted slopes

602 for hydration/hydroxylation during CO_2 absorption of -0.024 for Δ_{47} and $\delta^{18}O$ and 0.041 for Δ_{48} and $\delta^{18}O$
603 (Guo, 2020). This agreement indicates that hydration and hydroxylation KIEs occurring following CO_2
604 absorption are likely the most significant KIEs observed in our samples precipitated at elevated pH.
605   In our 5 °C sample at pH 9, there was a depletion in $\delta^{18}O$ of 4 ‰ relative to the calcite
606 precipitated with CA under the same conditions, and no statistically significant change in Δ_{47} (Table 3).
607 This may indicate that carbonates precipitated at pH ≤ 9 are more likely to exhibit a decoupling of
608 disequilibrium in $\delta^{18}O$ and Δ_{47}, but calcite precipitated at pH ≥ 9.5 exhibit disequilibrium in $\delta^{18}O$ and Δ_{47}
609 values. It should also be noted that our 5 °C sample at pH 9 had a depletion of 0.042 ‰ relative to
610 expected equilibrium in Δ_{48}, possibly indicating a greater sensitivity to pH and non-equilibrium oxygen
611 isotope values than for Δ_{47}, potentially due to the presence of two ^{18}O substitutions in *m/z* 64
612 isotopologues. It is possible this may represent the same type of decoupling between oxygen isotopes and
613 Δ_{47} that were observed in deep-sea corals (Thiagarajan et al., 2011).
614
615 *5.1.3 The effect of precipitation rate on bulk and clumped isotopes*
616
617   KIEs resulting in non-equilibrium clumped isotope compositions of DIC species have the
618 potential to be recorded in carbonate minerals, modulated by growth rate (Tripati et al., 2015; Watkins
619 and Hunt, 2015). It has been suggested that at rapid growth rates, a kinetic limit may be reached while at
620 slow growth, an equilibrium limit may be attained (e.g., DePaolo, 2011; Tripati et al., 2015; Watkins and
621 Hunt, 2015). For samples precipitated at pH 8-9 in a solution with a fully equilibrated DIC pool at 25 °C,
622 Watkins and Hunt (2015) used an ion-by-ion growth model to predict that the Δ_{63} of calcite precipitated at
623 typical experimental growth rates ($\log_{10}R$ = -5 to -7 $mol/m^2/s$) should be at or near the kinetic limit, and
624 that given equilibrium isotopic compositions for DIC endmembers (Hill et al., 2014; Tripati et al, 2015),
625 calcite Δ_{63} would be ~0.01 ‰ higher than expected equilibrium value at 25 °C, while for samples
626 precipitated at pH > 10, calcite would be ~0.01 ‰ to ~0.02 ‰ lower than expected equilibrium Δ_{63}
627 values. The kinetic limit should be substantially different if the DIC pool has not achieved equilibrium
628 (Tripati et al., 2015; Guo, 2020), and we find that our measured Δ_{47} values for samples precipitated at pH
629 10.5 have Δ_{47} that are elevated by 0.168-0.198 from expected mineral equilibrium values (Figure 3).
630 While these observations are consistent with relatively large kinetic effects associated with CO_2
631 absorption, including hydration, hydroxylation, and a DIC pool that is not equilibrated, it is also likely
632 they include a kinetic contribution to the isotopic signatures from growth rate effects.
633   For samples from this study and most from Tang et al. (2014) at pH 8.3, we find that Δ_{47} and Δ_{48}
634 values are indistinguishable from equilibrium values irrespective of precipitation rate. This is the case for
635 calcite precipitation at temperatures of 5 °C and 25 °C, both with and without CA. This suggests that the
636 intrinsic kinetic fractionation factor for clumped isotopes is close to unity (Guo, 2020) and that accurate
637 temperatures can be retrieved even when there are large kinetic oxygen isotope effects.
638   At higher pH, over the growth rates observed here, modeling and experimental data suggests that
639 mineral disequilibrium occurs. Specifically, our calculations (Figure 8) suggest that samples precipitated
640 at the same pH and temperature but at different growth rates, in a system where CO_2 absorption is
641 dominant, and a DIC pool that has not reached equilibrium, may yield calcite with mineral disequilibrium
642 of up to ~8.4 for 1000ln(carb-water), ~0.23 ‰ for Δ_{47}, and ~0.51 ‰ for Δ_{48}. These effects are the largest
643 for calcite growing at pH ≥ 9.5 and low temperature.
644   We note this model underestimates oxygen isotope fractionations observed in the experimental
645 samples synthesized in both this study and Tang et al. (2014) at multiple temperature and pH. This may

reflect the presence of an additional KIE influencing oxygen isotopes in DIC. In both this work and in Tang et al. (2014), CO_2 diffused through a polyethylene membrane, which may enrich the solution in ^{12}C and ^{16}O. Thus, results may reflect a kinetic oxygen isotope effect from membrane diffusion, as Tang et al. (2014) hypothesized in their work. Since the time to reach isotopic equilibrium is much longer at high pH and low temperature (Beck et al., 2005), a diffusive signal in oxygen isotopes would be more apparent in samples precipitated at high pH and low temperature, which is what we observe. The other possibility is that the hydroxylation KFFs from the literature are underestimated. When the hydroxylation KFF for oxygen is increased to match the $1000\ln(\alpha_{carb\text{-}water})$ data (Figure 8Q-X), however, there is significantly less agreement between measured and modeled Δ_{48}. This suggests either that diffusion is responsible for the low $1000\ln(\alpha_{carb\text{-}water})$ values or that the model is over-predicting the sensitivity of Δ_{48} to kinetic oxygen isotope fractionations. Additionally, the model may underestimate the catalytic ability of CA to equilibrate Δ_{47} and Δ_{48} for calcite formed at fast growth rates and at high pH.

However, no such signal is observed for clumped isotopes, with all measured sample Δ_{47} and Δ_{48} values residing within the model predicted range, with the exception of one calcite sample precipitated with CA at pH 11 at 25°C. It may be possible that a diffusive signal, which is expected to lower $\delta^{18}O$ and increase Δ_{47} (Thagarajan et al., 2011), is smaller or absent in the experimental Δ_{47} and Δ_{48}. Currently, we are unable to resolve the diffusive contribution to our experimental isotope signatures, but an upper bound of a 22 ‰ decrease for $\delta^{18}O$ and 0.5 ‰ increase for Δ_{47} for Knudsen diffusion was calculated by Eiler and Schauble (2004). An additional possibility is that the oxygen isotope kinetic fractionation factors used in the model calculations are underestimated. However, when oxygen isotope kinetic fractionation factors are increased to match experimental oxygen isotope data (Figure 8Q-X) the agreement between model and experimental Δ_{48} is decreased. This may indicate than another kinetic effect is the source of the mismatch between modeled and measured $1000\ln(\alpha_{carb\text{-}water})$ or that Δ_{48} is less sensitive to oxygen isotope fractionation factors than is predicted by the model.

Future work could further constrain growth rate effects by precipitating calcite under controlled conditions over an even broader range of growth rates than what was done in this work and in Tang et al. (2014). This would allow for experimental data to be compared to model predictions for the slow and fast growth limits. Diffusion effects could also be explored by precipitating calcite at fast growth rates from CO_2 diffused at different rates through membranes of varying pore sizes.

5.2 Paired clumped isotope data: Near equilibrium mineral compositions

Samples grown at pH 8.3 and at variable temperatures with CA yield Δ_{47} and Δ_{48} values that are consistent with mineral equilibrium as constrained using multiple independent approaches. The calcite growth rates achieved are ones that our modeling indicates should attain the equilibrium limit, and experimental data and model calculations converge (Figure 8). Theory from Hill et al. (2014) and Tripati et al. (2015) combined with experimental AFF values (Lucarelli et al., 2021) show that measured Δ_{47} and Δ_{48} from this work are consistent with calcite equilibrium (Figure 11A-C).

These results lend further support to experimental and field-derived calibration datasets being in near equilibrium. An F test (performed in PRISM 7) was used for a statistical comparison between experimentally determined regressions for Δ_{47} and Δ_{48} values for near-equilibrium samples with known precipitation temperatures from Swart et al. (2021), Fiebig et al. (2021), with results from this study (Table 7). The test determined that the regressions were not statistically different ($p = 0.99$, Table 8) and a combined regression is represented by equation 15 (Figure 11).

690

691 $\Delta_{48\,CDES\,90} = (0.407 \pm 0.016)\,\Delta_{47\,CDES\,90} + (0.006 \pm 0.009);\ r^2 = 0.98$ (15)

692

693 The data from Fiebig et al. (2021) includes lake and cave calcites, inorganic precipitations, and samples
694 equilibrated at high temperatures, with samples having crystallization temperatures from 7.9 ºC to 1100
695 ºC. Sample data from Swart et al. (2021) includes 7 inorganic calcite precipitations from 5 ºC to 65 ºC.
696 The experimentally determined regressions from Swart et al. (2021), Fiebig et al. (2021), and this study
697 are bracketed by regressions from Bajnai et al. (2020) and Lucarelli et al. (2021) determined using a
698 combination of theory from Hill et al. (2014) and Tripati et al. (2015) and experimental AFF values.
699 These regressions are statistically indistinguishable from the experimental regressions ($p = 0.99$).
700   It was also determined that the experimental regressions based on samples with known
701 precipitation temperatures from Swart et al. (2021), Fiebig et al. (2021), and this study are statistically
702 indistinguishable ($p > 0.99$) from the Δ_{47} and Δ_{48} regression from Lucarelli et al. (2021) based on
703 measurements of 20 carbonates, most of which are used as standards and had unknown precipitation
704 temperatures but were shown to have clumped isotope values near clumped isotopic equilibrium. The
705 combined regression for these experimental datasets is represented by equation 16.

706

707 $\Delta_{48\,CDES\,90} = (0.429 \pm 0.010)\,\Delta_{47\,CDES\,90} - (0.006 \pm 0.006);\ r^2 = 0.98$ (16)

708

709   An F test was used to test for statistical differences between experimentally determined
710 regressions for Δ_{47} and $10^6 T^{-2}$ and Δ_{48} and $10^6 T^{-2}$ from Swart et al. (2021), Fiebig et al. (2021), and this
711 study (Figure 11). It was determined that these regressions were not statistically different ($p = 0.99$ for Δ_{47}
712 and $10^6 T^{-2}$; $p = 0.99$ for Δ_{48} and $10^6 T^{-2}$). The combined experimentally based regressions are presented in
713 equations 17 and 18 with temperature in Kelvin.

714

715 $\Delta_{47\,I\text{-}CDES} = (0.037 \pm 0.001) \times 10^6 T^{-2} + (0.178 \pm 0.009);\ r^2 = 0.99$ (17)

716 $\Delta_{48\,CDES\,90} = (0.015 \pm 0.0005) \times 10^6 T^{-2} + (0.078 \pm 0.006);\ r^2 = 0.98$ (18)

717

718 The experimental regressions were also determined to be statistically indistinguishable from the
719 regressions based on theory and experimental AFFs from Bajnai et al. (2020) and Lucarelli et al. (2021)
720 ($p = 0.99$).
721   Offsets in Δ_{48} are likely from differences in standardization. This study and Lucarelli et al. (2021)
722 used carbonate-based standardization, Swart et al. (2021) used gas-based standardization, and Bajnai et al.
723 (2020) and Fiebig et al. (2021) used a combination of carbonate and gas-based standardization. For Δ_{47},
724 this study and Fiebig et al. (2021) use the I-CDES reference frame, while Bajnai et al. (2020) and Fiebig
725 et al. (2021) use the CDES 90 reference frame. While I-CDES and CDES 90 are very similar reference
726 frames being that they are both present data at 90 ºC, the main difference is that the I-CDES reference
727 frame uses multi-lab determined values presented in Bernasconi et al. (2021) for the carbonate standards
728 ETH-1, ETH-2, and ETH-3 for standardization, while CDES 90 may use slightly different values these
729 carbonate standards and other carbonate standards or may also present data relative to equilibrated gas
730 standards. All data reported thus far use the CDES 90 reference frame for Δ_{48}, however, differences in
731 values used for carbonate standards and the use of carbonate and/or gas-based standardization vary
732 between labs, as discussed in Lucarelli et al. (2021). Additionally, Δ_{48} is relatively sensitive compared to
733 Δ_{47} to subtle changes in transfer functions and nonlinearity slopes used in data corrections. For example, a

734 shift of ~0.05 in the slope of the Δ_{48} transfer function can shift Δ_{48} values by ~0.029 ‰, while the same
735 magnitude shift in the Δ_{47} transfer function will shift Δ_{47} values by ~0.013 ‰ (Supplementary Table S5).
736
737 **5.3 Mixing effects in paired clumped isotopes**
738
739   Biominerals and abiotic natural systems often have fluxes of isotopically distinct fluids associated
740 with carbonate mineral precipitation, which in turn can yield products with distinct bulk isotopic
741 compositions. This can occur from the precipitation processes themselves, or arise from temperature
742 fluctuations over the course of precipitation, or be associated with the integration of seawater into
743 calcifying spaces. Additionally, diagenetic processes can give rise to multiple phases of carbonates being
744 present, which in turn can be sampled. Thus, it is possible that carbonates with different bulk isotopic
745 compositions, and clumped isotope compositions, can be mixed physically within a single clumped
746 isotope sample. Mixing effects have been explored for Δ_{47} in CO_2 (Eiler and Schauble, 2004) and in
747 carbonate minerals (Defliese and Lohmann, 2015). For carbonate minerals, this can potentially lead to the
748 underestimation of temperature from enriched Δ_{47} values (Defliese and Lohmann, 2015).
749   Both experimental data and theoretical calculations from our non-linear mixing model indicate
750 that artifacts in both Δ_{47} and Δ_{48} can arise from mixing (Figures 9-10; Table 6). Values for Δ_{47} and Δ_{48} in
751 mixtures are enriched compared to equilibrium values. The trajectories for Δ_{47} and Δ_{48} mixing effects in
752 this study have slopes that vary depending on endmember compositions and the fraction of the mixture.
753 Values for slopes range from 0.397-0.872.
754   For the calculations performed here, the minimum non-linear mixing-related biases occur when
755 calcite endmembers have the same clumped isotope compositions but a 10 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$.
756 This could represent several scenarios, such as correspond to mixing of carbonate from growth bands in a
757 coral that formed at the same temperature but with variable bulk compositions, or mixing of different
758 mollusk specimens that formed at similar temperatures and are within the same sediment sample, or of
759 adjacent foraminiferal samples during times when temperatures were relatively similar.
760   Maximum mixing-related biases are predicted when calcites with the same clumped isotope
761 values is mixed that has a 60 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$ (Figure 9C-D). This type of difference is
762 likely to rarely be important for most natural settings, though this suggests that caution should be taken
763 when working with methane-derived carbonates, or when sampling rocks containing cements formed
764 from brine and freshwater settings.
765   Mixing of samples with different clumped and bulk isotope endmember values predicted from
766 our model fall between the minimum and maximum trajectories (Figure 9A-B). Our measured values
767 from mixing experiments fall in this category and agree with model predictions. The minimum trajectory
768 observed here is slightly higher than the equilibrium slope for Δ_{47} and Δ_{48}.
769   If endmembers vary by less than 5 ‰ in $\delta^{13}C$ and $\delta^{18}O$, mixing effects of 0.005 ‰ could be
770 observed in Δ_{47} and 0.004 ‰ in Δ_{48}, which are within the range of instrumental precision. Thus, mixing
771 could introduce ~1.9 °C of potential bias in Δ_{47}-derived temperatures (as has previously been discussed in
772 Defliese and Lohmann, 2015), and ~3.5 °C in Δ_{48} -derived temperatures, at Earth surface temperatures. If
773 endmembers have the same bulk composition but varying Δ_{47} and Δ_{48}, the clumped isotope values mix
774 linearly as a weighted sum of the contribution of each endmember. Additionally, Δ_{47} values below
775 equilibrium values are predicted when an endmember has $\delta^{18}O$ that is higher and $\delta^{13}C$ that is lower than
776 the other endmember. For example, if two endmembers have identical Δ_{47}, and one end member has a
777 $\delta^{18}O$ of 5 ‰ higher and $\delta^{13}C$ of 5 ‰ lower than the other endmember, the resultant Δ_{47} value will be

778   0.005 ‰ below the Δ_{47} value of each individual endmember. In this scenario, the Δ_{47} value continues to
779   decrease with increasing $\delta^{18}O$ and decreasing $\delta^{13}C$.
780   This work indicates that paired clumped isotopes (Δ_{47} and Δ_{48}) could be used to identify mixing
781   effects that would otherwise bias paleothermometry, but also suggests that endmember compositions in
782   clumped or bulk compositions, or both, would need to be substantially different for biases to be
783   expressed. Δ_{47} and Δ_{48} values for unknown calcite samples that do not conform to temperature-dependent
784   equilibrium Δ_{47} - Δ_{48} relationship (Figure 11), or kinetically governed disequilibrium mineral signatures
785   (Figure 10), but that do fall within the mixing effect bounds shown in Figure 10 for calcite samples, could
786   be indicative of enriched clumped isotope values from non-linear mixing effects. If mixing fractions and
787   endmembers could be constrained, paired Δ_{47} and Δ_{48} data could potentially be used to correct for artifacts
788   and extract primary temperatures.
789
790   **6. Conclusions**
791
792   Utilizing a combination of experimental data and multiple modeling frameworks, we show that
793   paired Δ_{47} and Δ_{48} can be used to identify when disequilibrium effects are negligible, and in cases where
794   disequilibrium is non-negligible, constrain kinetic isotope effects and mixing effects. By precipitating
795   calcite at five temperatures at pH 8.3 with the enzyme carbonic anhydrase (CA), we reduce kinetic
796   isotope effects in the DIC pool, with Δ_{47} and Δ_{48} values consistent with model predictions for equilibrium
797   precipitation. Experimental data provide an independent set of constraints on the equilibrium relationship
798   between Δ_{47} and Δ_{48}, Δ_{47} and T, Δ_{48} and T, Δ_{47} and $\delta^{18}O$, and Δ_{48} and $\delta^{18}O$. The experimentally determined
799   Δ_{47} and Δ_{48} relationship from this study is statistically indistinguishable from published regressions. We
800   report a new combined regression fit through all experimental data from Swart et al. (2021), Fiebig et al.
801   (2021), Lucarelli et al. (2021), and this study:
802
803    $\Delta_{48\ CDES\ 90} = (0.429 \pm 0.010)\ \Delta_{47\ CDES\ 90} - (0.006 \pm 0.006);\ r^2 = 0.98$
804
805   which further constrains the equilibrium relationships between Δ_{47} and Δ_{48}. We also report combined
806   temperature dependent relationships using data from Swart et al. (2021), Fiebig et al. (2021), and this
807   study:
808
809    $\Delta_{47\ I\text{-}CDES} = (0.037 \pm 0.001) \times 10^6 T^{-2} + (0.178 \pm 0.009);\ r^2 = 0.99$
810    $\Delta_{48\ CDES\ 90} = (0.015 \pm 0.0005) \times 10^6 T^{-2} + (0.078 \pm 0.006);\ r^2 = 0.98$
811
812   which further constrains equilibrium relationships between Δ_{47} and T, and Δ_{48} and T.
813   At elevated pH, we observe Δ_{47} values that are enriched relative to expected isotope equilibrium,
814   while Δ_{48}, $\delta^{18}O$, and $\delta^{13}C$ are depleted. The largest enrichment relative to quasi-equilibrium observed here
815   for Δ_{47} was 0.198 ‰ at pH 10.5 and 5 $^\circ$C. The largest depletion relative to quasi-equilibrium for Δ_{48} was -
816   0.628 ‰ at pH 11 and 5 $^\circ$C. All calcite precipitated here with pH ≥ 9.5 had Δ_{48} values less than 0 ‰,
817   indicating values below a stochastic distribution. Calcite precipitated with CA in the solution returned to
818   Δ_{47} and Δ_{48} values expected at quasi-equilibrium, where CA was more successful at catalyzing Δ_{48} than
819   Δ_{47}.

820   The kinetic slopes for hydration and hydroxylation for paired Δ_{47} and Δ_{48} measurements
821 determined here may be used to return calcite samples to near equilibrium clumped isotope values.
822 Calcite precipitated at pH $\geq$ 9.5 followed a steep kinetic slope, while calcite precipitated at pH $\geq$ 9.5 with
823 CA followed a shallower kinetic slope. Calcite precipitated at pH $\leq$ 9.0 had negligible clumped isotope
824 kinetic effects, while kinetic oxygen isotope effects may have been present.
825   Samples grown at pH 8.3 and with growth rates of $\log_{10}R$ that are -7.3 to 7.5 mol/m^2/s have Δ_{47}
826 and Δ_{48} values that overlap the equilibrium limit, while samples at higher pH have clumped isotope
827 compositions that record kinetic isotope effects. The observed paired clumped isotope values in calcite
828 samples grown at elevated pH without CA can be explained by hydration/hydroxylation during CO_2
829 absorption. Calcite precipitated at fast growth rates may inherit larger kinetic isotopic effects from CO_2
830 hydration/hydroxylation and a disequilibrium DIC pool compared to calcite precipitated at slower growth
831 rates. Kinetic isotope effects associated with fast growth rate and CO_2 hydration/hydroxylation may be as
832 large as ~0.23 ‰ for Δ_{47} and ~0.51 ‰ for Δ_{48}.
833   Mixing experiments and non-linear mixing model calculations show that mixing results in
834 enrichments in both Δ_{47} and Δ_{48} compared to equilibrium values. There are a range of characteristic
835 trajectories for mixing effects in Δ_{47} and Δ_{48} for calcites with varying bulk and clumped isotopes. Paired
836 measurements of Δ_{47} and Δ_{48} may provide a mechanism by which mixing effects can be identified in
837 biological and abiogenic carbonate samples. Such effects are likely to be $\geq$ 0.009 ‰ for Δ_{47} if there is
838 more than 30 % of a secondary component in a two-component system if bulk compositions vary by more
839 than 7 ‰, or if Δ_{47} and Δ_{48} vary by more than 0.03 ‰.
840

852

853 **Author Contributions**

854 AT initiated and supported the work. AT, MD, BP, and JL designed the research. BP carried out the
855 precipitation experiments with input from MD and JL. JL performed the isotope analyses and calculations
856 with input from AT. JL performed the mixing experiments and associated calculations with input from
857 AT. ZP contributed to the box model and IsoDIC calculations. JW provided the box model script. JL, BP,
858 RE, MD, and AT contributed insights to data analyses and interpretations. JL wrote the manuscript with
859 guidance from AT and input from all coauthors. AT and RE advised JL and ZP.

860

861 **References**

Adkins, J.F., Boyle, E.A., Curry, W.B. and Lutringer, A., 2003. Stable isotopes in deep-sea corals and a new mechanism for "vital effects". *Geochimica et Cosmochimica Acta, 67*(6), pp.1129-1143. https://doi.org/10.1016/S0016-7037(02)01203-6

Affek, H.P., Bar-Matthews, M., Ayalon, A., Matthews, A., Eiler, J.M., 2008. Glacial/interglacial temperature variations in Soreq cave speleothems as recorded by 'clumped isotope' thermometry. Geochimica et Cosmochimica Acta 72, 5351–5360. https://doi.org/10.1016/j.gca.2008.06.031

Al-Horani, F.A., Al-Moghrabi, S.M., de Beer, D., 2003. Microsensor study of photosynthesis and calcification in the scleractinian coral, Galaxea fascicularis: active internal carbon cycle. *Journal of Experimental Marine Biology and Ecology 288*, 1–15. https://doi.org/10.1016/S0022-0981(02)00578-6

Anagnostou, E., K-F. Huang, C-F. You, E. L. Sikes, and R. M. Sherrell. "Evaluation of boron isotope ratio as a pH proxy in the deep sea coral *Desmophyllum dianthus*: Evidence of physiological pH adjustment." *Earth and Planetary Science Letters 349* (2012): 251-260. https://doi.org/10.1016/j.epsl.2012.07.006

Bajnai, D., Guo, W., Spötl, C., Coplen, T.B., Methner, K., Löffler, N., Krsnik, E., Gischler, E., Hansen, M., Henkel, D. and Price, G.D., 2020. Dual clumped isotope thermometry resolves kinetic biases in carbonate formation temperatures. *Nature communications*, *11*(1), pp.1-9. https://doi.org/10.1038/s41467-020-17501-0

Beck, W.C., Grossman, E.L., Morse, J.W., 2005. Experimental studies of oxygen isotope fractionation in the carbonic acid system at 15°, 25°, and 40°C. *Geochimica et Cosmochimica Acta 69,* 3493–3503. https://doi.org/10.1016/j.gca.2005.02.003

Berg, J., Tymoczko, J., Stryer, L., 2002. Biochemistry, 5th ed. W H Freeman, New York.

Bernasconi, S.M., Daëron, M., Bergmann, K.D., Bonifacie, M., Meckler, A.N., Affek, H.P., Anderson, N., Bajnai, D., Barkan, E., Beverly, E. and Blamart, D., 2021. InterCarb: A community effort to improve interlaboratory standardization of the carbonate clumped isotope thermometer using carbonate standards. *Geochemistry, Geophysics, Geosystems, 22*(5), p.e2020GC009588. https://doi.org/10.1029/2020GC009588

Bernasconi, S.M., Müller, I.A., Bergmann, K.D., Breitenbach, S.F., Fernandez, A., Hodell, D.A., Jaggi, M., Meckler, A.N., Millan, I. and Ziegler, M., 2018. Reducing uncertainties in carbonate clumped isotope analysis through consistent carbonate-based standardization. *Geochemistry, Geophysics, Geosystems, 19*(9), pp.2895-2914. https://doi.org/10.1029/2017GC007385

Bertucci, A., Innocenti, A., Scozzafava, A., Tambutté, S., Zoccola, D., Supuran, C.T., 2011. Carbonic anhydrase inhibitors. Inhibition studies with anions and sulfonamides of a new cytosolic enzyme from the scleractinian coral Stylophora pistillata. *Bioorganic & Medicinal Chemistry Letters 21*, 710–714. https://doi.org/10.1016/j.bmcl.2010.11.124

Boettger, J.D., Kubicki, J.D., 2021. Equilibrium and kinetic isotopic fractionation in the CO2 hydration and hydroxylation reactions: Analysis of the role of hydrogen-bonding via quantum mechanical calculations. *Geochimica et Cosmochimica Acta 292*, 37–63. https://doi.org/10.1016/j.gca.2020.09.019

Brand, W.A., Assonov, S.S., Coplen, T.B., 2010. Correction for the 17O interference in δ(13C) measurements when analyzing CO2 with stable isotope mass spectrometry (IUPAC Technical Report). *Pure and Applied Chemistry 82*, 1719–1733. https://doi.org/10.1351/PAC-REP-09-01-05

Christensen, J.N., Watkins, J.M., Devriendt, L.S., DePaolo, D.J., Conrad, M.E., Voltolini, M., Yang, W., Dong, W., 2021. Isotopic fractionation accompanying CO2 hydroxylation and carbonate

precipitation from high pH waters at The Cedars, California, USA. *Geochimica et Cosmochimica Acta 301*, 91–115. https://doi.org/10.1016/j.gca.2021.01.003

Coplen, T.B., 2007. Calibration of the calcite–water oxygen-isotope geothermometer at Devils Hole, Nevada, a natural laboratory. *Geochimica et Cosmochimica Acta 71*, 3948–3957. https://doi.org/10.1016/j.gca.2007.05.028

Daëron, M., Blamart, D., Peral, M., Affek, H.P., 2016. Absolute isotopic abundance ratios and the accuracy of $\Delta 47$ measurements. Chemical Geology 442, 83–96. https://doi.org/10.1016/j.chemgeo.2016.08.014

Daëron, M., Drysdale, R.N., Peral, M., Huyghe, D., Blamart, D., Coplen, T.B., Lartaud, F. and Zanchetta, G., 2019. Most Earth-surface calcites precipitate out of isotopic equilibrium. *Nature communications*, *10*(1), pp.1-7. https://doi.org/10.1038/s41467-019-08336-5

Daëron, M., Guo, W., Eiler, J., Genty, D., Blamart, D., Boch, R., Drysdale, R., Maire, R., Wainer, K., Zanchetta, G., 2011. 13C18O clumping in speleothems: Observations from natural caves and precipitation experiments. *Geochimica et Cosmochimica Acta 75*, 3303–3317. https://doi.org/10.1016/j.gca.2010.10.032

de Goeyse, S., Webb, A.E., Reichart, G.-J., de Nooijer, L.J., 2021. Carbonic anhydrase is involved in calcification by the benthic foraminifer *Amphistegina lessonii*; *Biogeosciences 18*, 393–401. https://doi.org/10.5194/bg-18-393-2021

Defliese, W.F., Lohmann, K.C., 2015. Non-linear mixing effects on mass-47 CO_2 clumped isotope thermometry: Patterns and implications: Non-linear mixing effects on mass-47 clumped isotopes. Rapid Commun. Mass Spectrom. 29, 901–909. https://doi.org/10.1002/rcm.7175

Dennis, K.J., Affek, H.P., Passey, B.H., Schrag, D.P., Eiler, J.M., 2011. Defining an absolute reference frame for 'clumped' isotope studies of CO2. *Geochimica et Cosmochimica Acta 75*, 7117–7131. https://doi.org/10.1016/j.gca.2011.09.025

Dietzel, M., Gussone, N., Eisenhauer, A., 2004. Co-precipitation of Sr2+ and Ba2+ with aragonite by membrane diffusion of CO2 between 10 and 50 °C. *Chemical Geology 203*, 139–151. https://doi.org/10.1016/j.chemgeo.2003.09.008

Dietzel, M., Tang, J., Leis, A., & Köhler, S. J., 2009. Oxygen isotopic fractionation during inorganic calcite precipitation—Effects of temperature, precipitation rate and pH. *Chemical Geology 268*, 107-115. https://doi.org/10.1016/j.chemgeo.2009.07.015

Eiler, J.M., Schauble, E., 2004. 18O13C16O in Earth's atmosphere. *Geochimica et Cosmochimica Acta 68*, 4767–4777. https://doi.org/10.1016/j.gca.2004.05.035

Fiebig, J., Bajnai, D., Löffler, N., Methner, K., Krsnik, E., Mulch, A., Hofmann, S., 2019. Combined high-precision $\Delta 48$ and $\Delta 47$ analysis of carbonates. *Chemical Geology 522*, 186–191. https://doi.org/10.1016/j.chemgeo.2019.05.019

Fiebig, J., Daëron, M., Bernecker, M., Guo, W., Schneider, G., Boch, R., Bernasconi, S.M., Jautzy, J., Dietzel, M., 2021. Calibration of the dual clumped isotope thermometer for carbonates. *Geochimica et Cosmochimica Acta S0016703721004208*. https://doi.org/10.1016/j.gca.2021.07.012

Ghosh, P., Adkins, J., Affek, H., Balta, B., Guo, W., Schauble, E.A., Schrag, D., Eiler, J.M., 2006. 13C–18O bonds in carbonate minerals: A new kind of paleothermometer. *Geochimica et Cosmochimica Acta 70*, 1439–1456. https://doi.org/10.1016/j.gca.2005.11.014

949   Gonfiantini, R., Stichler, W., Rozanski, K., 1995. Standards and intercomparison materials distributed by
950   the International Atomic Energy Agency for stable isotope measurements (No. IAEA-TECDOC--
951   825).

952   Guo, W., Mosenfelder, J.L., Goddard, W.A., Eiler, J.M., 2009. Isotopic fractionations associated with
953   phosphoric acid digestion of carbonate minerals: Insights from first-principles theoretical
954   modeling and clumped isotope measurements. *Geochimica et Cosmochimica Acta 73*, 7203–
955   7225. https://doi.org/10.1016/j.gca.2009.05.071

956   Guo, W., 2020. Kinetic clumped isotope fractionation in the DIC-H2O-CO2 system: patterns, controls,
957   and implications. *Geochimica et Cosmochimica Acta*, *268*, pp.230-257.
958   https://doi.org/10.1016/j.gca.2019.07.055

959   Hill, P.S., Schauble, E.A., Tripati, A., 2014. Theoretical constraints on the effects of pH, salinity, and
960   temperature on clumped isotope signatures of dissolved inorganic carbon species and
961   precipitating carbonate minerals. Geochimica et Cosmochimica Acta 125, 610–652.
962   https://doi.org/10.1016/j.gca.2013.06.018

963   Hill, P.S., Schauble, E.A. and Tripati, A., 2020. Theoretical constraints on the effects of added
964   cations on clumped, oxygen, and carbon isotope signatures of dissolved inorganic carbon species
965   and minerals. Geochimica et Cosmochimica Acta, 269, pp.496-539.
966   https://doi.org/10.1016/j.gca.2019.10.016

967   Johnson, K.S., 1982. Carbon dioxide hydration and dehydration kinetics in seawater1: CO$_2$ reaction
968   kinetics. Limnol. Oceanogr. 27, 849–855. https://doi.org/10.4319/lo.1982.27.5.0849

969   Jones, J.P., Carricart-Ganivet, J.P., Prieto, R.I., Enríquez, S., Ackerson, M. and Gabitov, R.I., 2015.
970   Microstructural variation in oxygen isotopes and elemental calcium ratios in the coral skeleton of
971   Orbicella annularis. *Chemical Geology*, 419, pp.192-199.
972   https://doi.org/10.1016/j.chemgeo.2015.10.044

973   Kernohan, J.C., 1964. The activity of bovine carbonic anhydrase in imidazole buffers. *Biochimica et*
974   *Biophysica Acta (BBA) - Specialized Section on Enzymological Subjects 81*, 346–356.
975   https://doi.org/10.1016/0926-6569(64)90050-1

976   Kimball, J., Eagle, R., Dunbar, R., 2016. Carbonate "clumped" isotope signatures in aragonitic
977   scleractinian and calcitic gorgonian deep-sea corals. *Biogeosciences 13*, 6487–6505.
978   https://doi.org/10.5194/bg-13-6487-2016

979   Knoche, W., 1980. Chemical Reactions of CO2 in Water, in: Bauer, C., Gros, G., Bartels, H. (Eds.),
980   Biophysics and Physiology of Carbon Dioxide, *Proceedings in Life Sciences*. Springer Berlin
981   Heidelberg, Berlin, Heidelberg, pp. 3–11. https://doi.org/10.1007/978-3-642-67572-0_1

982   Lindskog, S., 1997. Structure and mechanism of carbonic anhydrase. *Pharmacology & Therapeutics 74*,
983   1–20. https://doi.org/10.1016/S0163-7258(96)00198-2

984   Lucarelli, J., Carroll, H., Elliott, B., Eagle, R., Tripati, A., 2021. Equilibrated gas and carbonate standard-
985   derived paired clumped isotope (Δ47 and Δ48) values on the absolute reference frame (preprint).
986   Physical Sciences and Mathematics. https://doi.org/10.31223/X5T318

987   McConnaughey, T., 1989. 13C and 18O isotopic disequilibrium in biological carbonates: II. In vitro
988   simulation of kinetic isotope effects. *Geochimica et Cosmochimica Acta 53*, 163–171.
989   https://doi.org/10.1016/0016-7037(89)90283-4

990   Miller, R.F., Berkshire, D.C., Kelley, J.J. and Hood, D.W., 1971. Method for determination of reaction
991   rates of carbon dioxide with water and hydroxyl ion in seawater. *Environmental Science &*
992   *Technology*, *5*(2), pp.127-133. https://doi.org/10.1021/es60049a006

993 Miyamoto, H., Miyashita, T., Okushima, M., Nakano, S., Morita, T., Matsushiro, A., 1996. A carbonic
994   anhydrase from the nacreous layer in oyster pearls. *Proceedings of the National Academy of*
995   *Sciences 93*, 9657–9660. https://doi.org/10.1073/pnas.93.18.9657
996 Moya, A., Tambutté, S., Bertucci, A., Tambutté, E., Lotto, S., Vullo, D., Supuran, C.T., Allemand, D.,
997   Zoccola, D., 2008. Carbonic Anhydrase in the Scleractinian Coral Stylophora pistillata. *Journal*
998   *of Biological Chemistry 283*, 25475–25484. https://doi.org/10.1074/jbc.M804726200
999 Nimer, N.A., Guan, Q., Merrett, M.J., 1994. Extra- and intra-cellular carbonic anhydrase in relation to
1000   culture age in a high-calcifying strain of Emiliania huxleyi Lohmann. *New Phytologist 126*, 601–
1001   607. https://doi.org/10.1111/j.1469-8137.1994.tb02954.x
1002 Passey, B.H., Henkes, G.A., 2012. Carbonate clumped isotope bond reordering and geospeedometry.
1003   *Earth and Planetary Science Letters 351–352*, 223–236.
1004   https://doi.org/10.1016/j.epsl.2012.07.021
1005 Rost, B., Riebesell, U., Burkhardt, S. and Sültemeyer, D., 2003. Carbon acquisition of bloom-forming
1006   marine phytoplankton. *Limnology and oceanography*, *48*(1), pp.55-67.
1007   https://doi.org/10.4319/lo.2003.48.1.0055
1008 Saenger, C., Affek, H.P., Felis, T., Thiagarajan, N., Lough, J.M., Holcomb, M., 2012. Carbonate clumped
1009   isotope variability in shallow water corals: Temperature dependence and growth-related vital
1010   effects. *Geochimica et Cosmochimica Acta 99*, 224–242.
1011   https://doi.org/10.1016/j.gca.2012.09.035
1012 Schauble, E.A., Ghosh, P., Eiler, J.M., 2006. Preferential formation of 13C–18O bonds in carbonate
1013   minerals, estimated using first-principles lattice dynamics. *Geochimica et Cosmochimica Acta 70*,
1014   2510–2529. https://doi.org/10.1016/j.gca.2006.02.011
1015 Spooner, Peter T., Weifu Guo, Laura F. Robinson, Nivedita Thiagarajan, Katharine R. Hendry, Brad E.
1016   Rosenheim, and Melanie J. Leng. "Clumped isotope composition of cold-water corals: A role for
1017   vital effects?." *Geochimica et Cosmochimica Acta 179* (2016): 123-141.
1018   https://doi.org/10.1016/j.gca.2016.01.023
1019 Staudigel, P.T. and Swart, P.K., 2018. A kinetic difference between 12C-and 13C-bound oxygen
1020   exchange rates results in decoupled δ18O and Δ47 values of equilibrating DIC solutions.
1021   *Geochemistry, Geophysics, Geosystems, 19*(8), pp.2371-2383.
1022   https://doi.org/10.1029/2018GC007500
1023 Swart, Peter K., Chaojin Lu, Evan W. Moore, Megan E. Smith, Sean T. Murray, and Philip T. Staudigel.
1024   "A calibration equation between Δ48 values of carbonate and temperature." *Rapid*
1025   *Communications in Mass Spectrometry 35*, no. 17 (2021): e9147.
1026   https://doi.org/10.1002/rcm.9147
1027 Tang, J., Dietzel, M., Böhm, F., Köhler, S.J. and Eisenhauer, A., 2008. Sr2+/Ca2+ and 44Ca/40Ca
1028   fractionation during inorganic calcite formation: II. Ca isotopes. *Geochimica et Cosmochimica*
1029   *Acta, 72(15),* pp.3733-3745. https://doi.org/10.1016/j.gca.2008.05.033
1030 Tang, J., Dietzel, M., Fernandez, A., Tripati, A.K., Rosenheim, B.E., 2014. Evaluation of kinetic effects
1031   on clumped isotope fractionation (Δ47) during inorganic calcite precipitation. *Geochimica et*
1032   *Cosmochimica Acta 134*, 120–136. https://doi.org/10.1016/j.gca.2014.03.005
1033 Thiagarajan, N., Adkins, J., Eiler, J., 2011. Carbonate clumped isotope thermometry of deep-sea corals
1034   and implications for vital effects. *Geochimica et Cosmochimica Acta 75*, 4416–4425.
1035   https://doi.org/10.1016/j.gca.2011.05.004

1036   Tripati, A.K., Hill, P.S., Eagle, R.A., Mosenfelder, J.L., Tang, J., Schauble, E.A., Eiler, J.M., Zeebe, R.E.,
1037   Uchikawa, J., Coplen, T.B., Ries, J.B., Henry, D., 2015. Beyond temperature: Clumped isotope
1038   signatures in dissolved inorganic carbon species and the influence of solution chemistry on
1039   carbonate mineral composition. *Geochimica et Cosmochimica Acta 166*, 344–371.
1040   https://doi.org/10.1016/j.gca.2015.06.021

1041   Trotter, J., Montagna, P., McCulloch, M., Silenzi, S., Reynaud, S., Mortimer, G., Martin, S., Ferrier-
1042   Pagès, C., Gattuso, J.-P., Rodolfo-Metalpa, R., 2011. Quantifying the pH 'vital effect' in the
1043   temperate zooxanthellate coral Cladocora caespitosa: Validation of the boron seawater pH proxy.
1044   *Earth and Planetary Science Letters 303*, 163–173. https://doi.org/10.1016/j.epsl.2011.01.030

1045   Uchikawa, J., Zeebe, R.E., 2012. The effect of carbonic anhydrase on the kinetics and equilibrium of the
1046   oxygen isotope exchange in the CO2–H2O system: Implications for δ18O vital effects in biogenic
1047   carbonates. *Geochimica et Cosmochimica Acta 95*, 15–34.
1048   https://doi.org/10.1016/j.gca.2012.07.022

1049   Uchikawa, J., Chen, S., Eiler, J.M., Adkins, J.F. and Zeebe, R.E., 2021. Trajectory and timescale of
1050   oxygen and clumped isotope equilibration in the dissolved carbonate system under normal and
1051   enzymatically-catalyzed conditions at 25° C. *Geochimica et Cosmochimica Acta*, *314*, pp.313-
1052   333. https://doi.org/10.1016/j.gca.2021.08.014

1053   Upadhyay, D., Lucarelli, J., Arnold, A., Flores, R., Bricker, H., Ulrich, R.N., Jesmok, G., Santi, L.,
1054   Defliese, W., Eagle, R.A. and Carroll, H.M., 2021. Carbonate clumped isotope analysis (Δ47) of
1055   21 carbonate standards determined via gas-source isotope-ratio mass spectrometry on four
1056   instrumental configurations using carbonate-based standardization and multiyear data sets. *Rapid
1057   Communications in Mass Spectrometry*, *35*(17), p.e9143.https://doi.org/10.1002/rcm.9143

1058   Usdowski, E., Michaelis, J., Boettcher, M., Hoefs, J., 1991. Factors for the oxygen isotope equilibrium
1059   fractionation between aqueous and gaseous CO2, carbonic acid, bicarbonate, carbonate, and
1060   water (19°C). *Zeitschrift fur physikalische chemie-international journal of research in physical
1061   chemistry & Chemical Physics 237–249.*

1062   Wang, Z., Schauble, E.A., Eiler, J.M., 2004. Equilibrium thermodynamics of multiply substituted
1063   isotopologues of molecular gases. *Geochimica et Cosmochimica Acta 68*, 4779–4797.
1064   https://doi.org/10.1016/j.gca.2004.05.039

1065   Watkins, J.M., Hunt, J.D., Ryerson, F.J., DePaolo, D.J., 2014. The influence of temperature, pH, and
1066   growth rate on the δ18O composition of inorganically precipitated calcite. *Earth and Planetary
1067   Science Letters 404*, 332–343. https://doi.org/10.1016/j.epsl.2014.07.036

1068   Watkins, J.M. and Hunt, J.D., 2015. A process-based model for non-equilibrium clumped isotope effects
1069   in carbonates. *Earth and Planetary Science Letters*, *432*, pp.152-165.
1070   https://doi.org/10.1016/j.epsl.2015.09.042

1071   Weise, A. and Kluge, T., 2020. Isotope exchange rates in dissolved inorganic carbon between 40° C and
1072   90° C. *Geochimica et Cosmochimica Acta*, *268*, pp.56-72.
1073   https://doi.org/10.1016/j.gca.2019.09.032

1074   Winograd, I.J., Coplen, T.B., Landwehr, J.M., Riggs, A.C., Ludwig, K.R., Szabo, B.J., Kolesar, P.T.,
1075   Revesz, K.M., 1992. Continuous 500,000-Year Climate Record from Vein Calcite in Devils Hole,
1076   Nevada. *Science 258*, 255–260. https://doi.org/10.1126/science.258.5080.255

1077   Winograd, I.J., Coplen, T.B., Szabo, B.J., Riggs, A.C., 1988. A 250,000-Year Climatic Record from
1078   Great Basin Vein Calcite: Implications for Milankovitch Theory. *Science 242*, 1275–1280.
1079   https://doi.org/10.1126/science.242.4883.1275

1080 Wolthers, M., Nehrke, G., Gustafsson, J.P. and Van Cappellen, P., 2012. Calcite growth kinetics:
1081   Modeling the effect of solution stoichiometry. *Geochimica et Cosmochimica Acta*, *77*, pp.121-
1082   134. https://doi.org/10.1016/j.gca.2011.11.003
1083 Yu, Z., Xie, L., Lee, S., Zhang, R., 2006. A novel carbonic anhydrase from the mantle of the pearl oyster
1084   (Pinctada fucata). *Comparative Biochemistry and Physiology Part B: Biochemistry and*
1085   *Molecular Biology 143*, 190–194. https://doi.org/10.1016/j.cbpb.2005.11.006
1086 Zeebe, R.E., Wolf-Gladrow, D.A., 2001. CO_2 in seawater: equilibrium, kinetics, isotopes, *Elsevier*
1087   *oceanography series*. Elsevier, Amsterdam; New York.
1088 Zeebe, R.E., 2020. Oxygen isotope fractionation between water and the aqueous hydroxide ion.
1089   *Geochimica et Cosmochimica Acta*, *289*, pp.182-195. https://doi.org/10.1016/j.gca.2020.08.025
1090 Zeebe, R.E., 2014. Kinetic fractionation of carbon and oxygen isotopes during hydration of carbon
1091   dioxide. *Geochimica et Cosmochimica Acta*, *139*, pp.540-552.
1092   https://doi.org/10.1016/j.gca.2014.05.005
1093

1094

1095
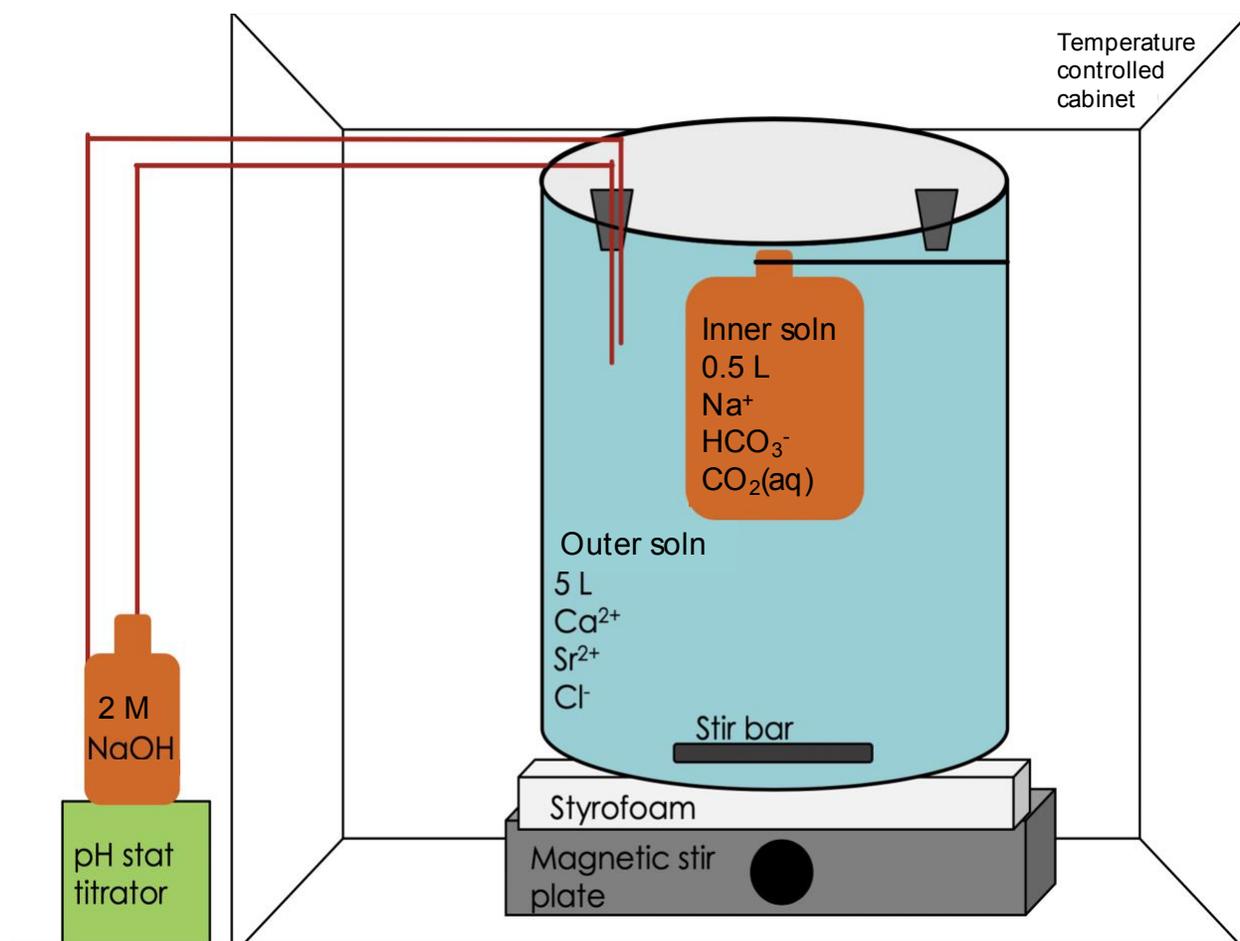
Figure 1. Schematic of the precipitation apparatus used for this work. The inner bottle is a 0.5 L, 2 mm thick polyethylene bottle that serves as a membrane through which CO_2 diffuses into the outer solution. The outer solution is inside a 5 L container that contains Ca^{2+} from CaCl. A pH-stat titrator holds the pH of the outer solution constant by titrating NaOH. The temperature is held constant by placing the entire apparatus inside a temperature-controlled cabinet. Adapted from Dietzel and Usdowski (1996), Dietzel et al. (2004), Tang et al., (2008), and Tang et al. (2014).
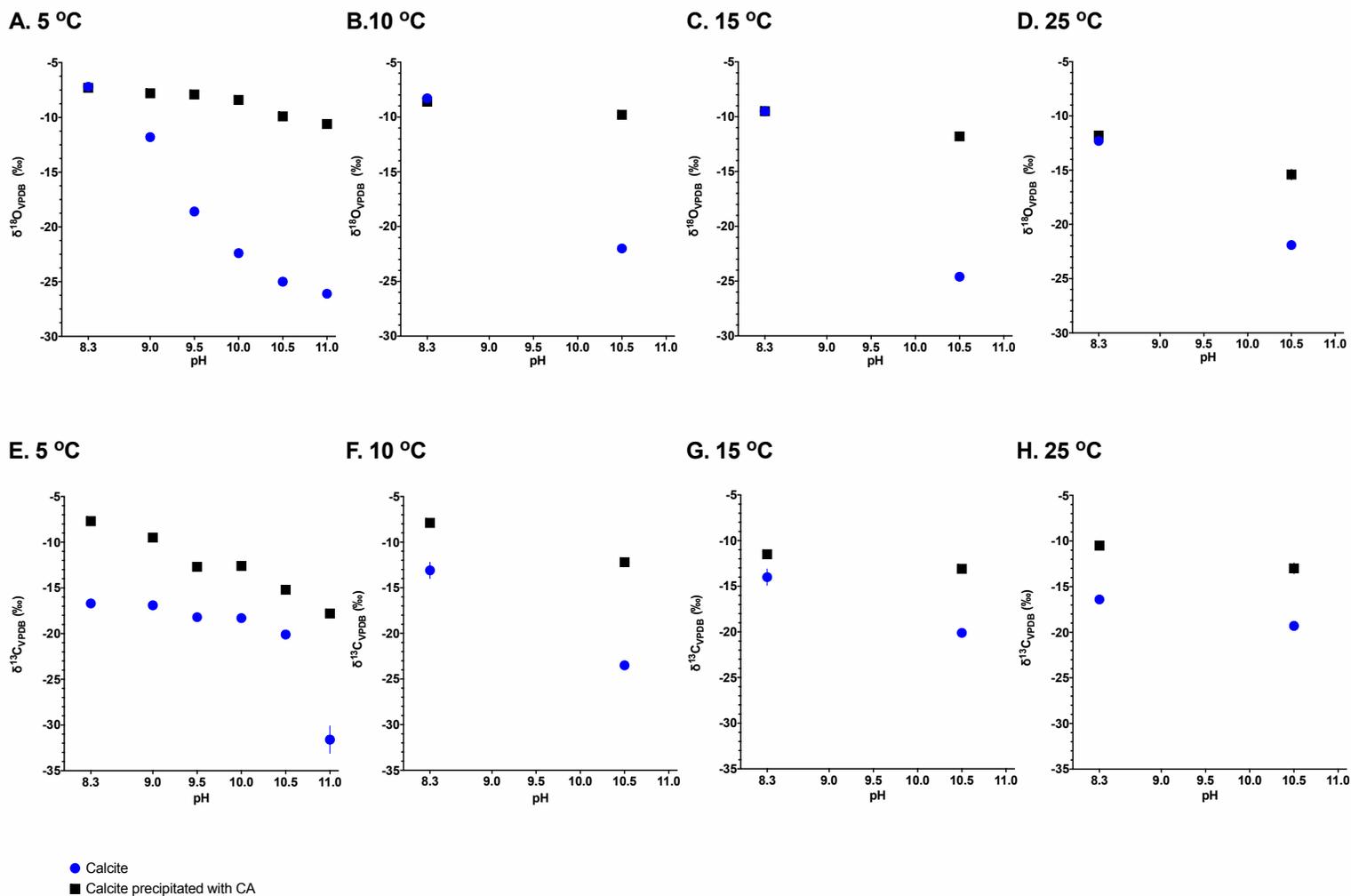
Figure 2. $\delta^{18}O$ and $\delta^{13}C$ with precipitation pH for calcite precipitated with and without carbonic anhydrase (CA) at variable temperature. $\delta^{18}O$ versus precipitation pH for A) 5 ºC, B) 10 ºC, C) 15 ºC, and D) 25 ºC. $\delta^{13}C$ versus precipitation pH for E) 5 ºC, F) 10 ºC, G) 15 ºC, and H) 25 ºC.

Figure 3. Δ_{47} relative to pH at variable temperature for for calcite precipitated with and without carbonic anhydrase (CA). A) 5 °C, B) 10 °C, C) 15 °C, and D) 25 °C, and Δ_{48} relative to pH at E) 5 °C, F) 10 °C, G) 15 °C, and H) 25 °C. Enrichments in Δ_{47} and depletions in Δ_{48} in samples precipitated at pH > 9.5. The gray bar is the range of expected equilibrium values derived from Swart et al. (2021), Fiebig et al. (2021), Lucarelli et al. (2021), and this study.
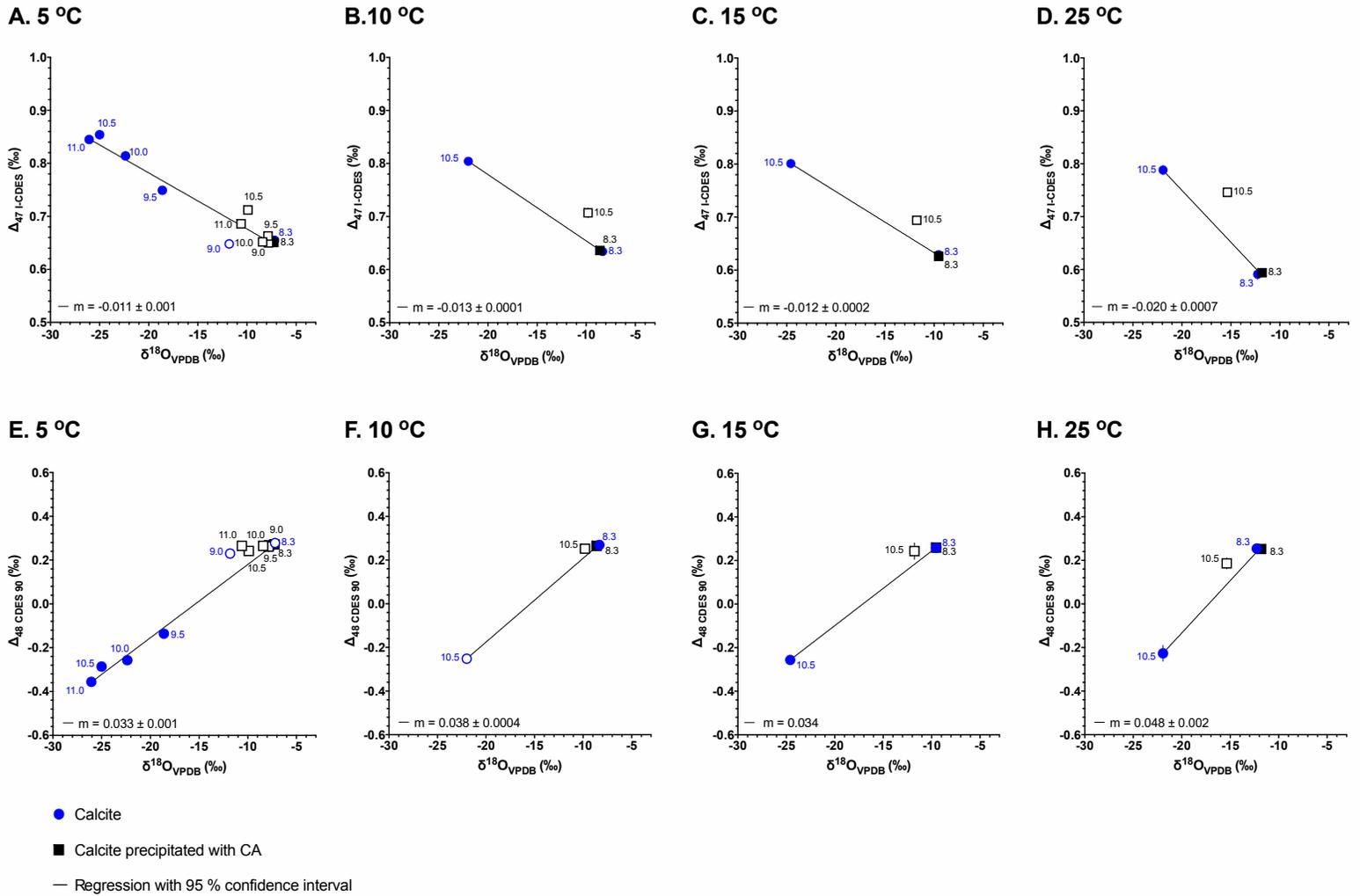
Figure 4. Δ_{47} and $\delta^{18}O$ at variable temperature for calcite precipitated with and without carbonic anhydrase (CA). A) 5 °C ($r^2 = 0.96$), B) 10 °C ($r^2 = 0.99$), C) 15 °C ($r^2 = 0.99$), and D) 25 °C ($r^2 = 0.99$), and Δ_{48} and $\delta^{18}O$ at E) 5 °C ($r^2 = 0.99$), F) 10 °C ($r^2 = 0.99$), G) 15 °C ($r^2 = 0.99$), and H) 25 °C ($r^2 = 0.99$). Numbers next to each data point indicate precipitation pH. Samples at elevated pH are depleted in $\delta^{18}O$ and Δ_{47}, and enriched in Δ_{48} relative to expected equilibrium values. Regressions are between quasi-equilibrium samples synthesized at pH 8.3 and samples synthesized at elevated pHs without CA, with negative slopes for Δ_{47} relative to $\delta^{18}O$, and positive slopes for Δ_{48} relative to $\delta^{18}O$. Data with open symbols are not included in regression.
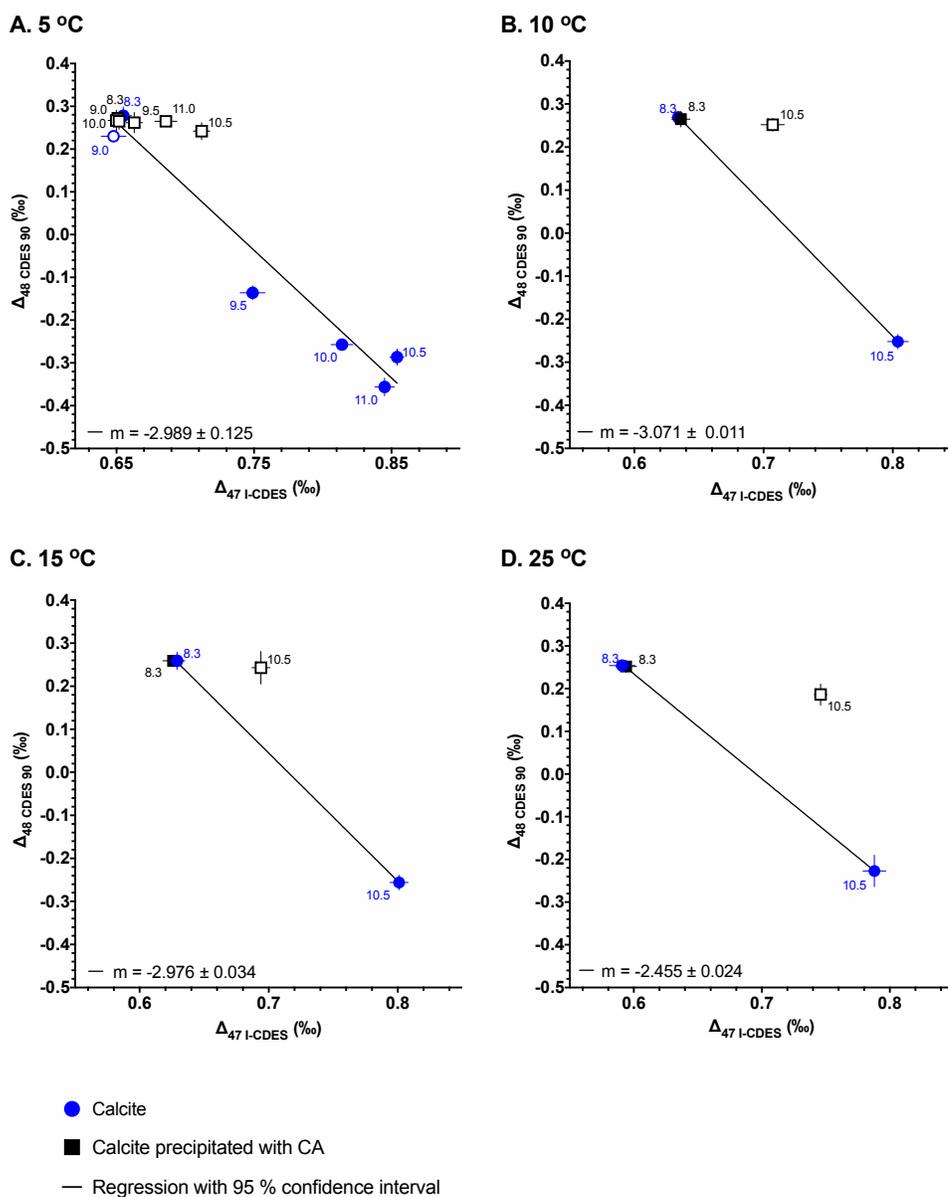
Figure 5. $\mathit{\Delta}_{47}$ and $\mathit{\Delta}_{48}$ for calcite synthesized at variable pH with and without CA at different temperatures. A) 5 °C ($r^2 = 0.95$), B) 10 °C ($r^2 = 0.99$), C) 15 °C ($r^2 = 0.99$), and D) 25 °C ($r^2 = 0.99$). Numbers next to each data point indicate precipitation pH. Regressions are between quasi-equilibrium samples synthesized at pH 8.3 and samples synthesized at elevated pHs without CA, with negative slopes observed for all experiments. Data with open symbols are not included in the regressions.
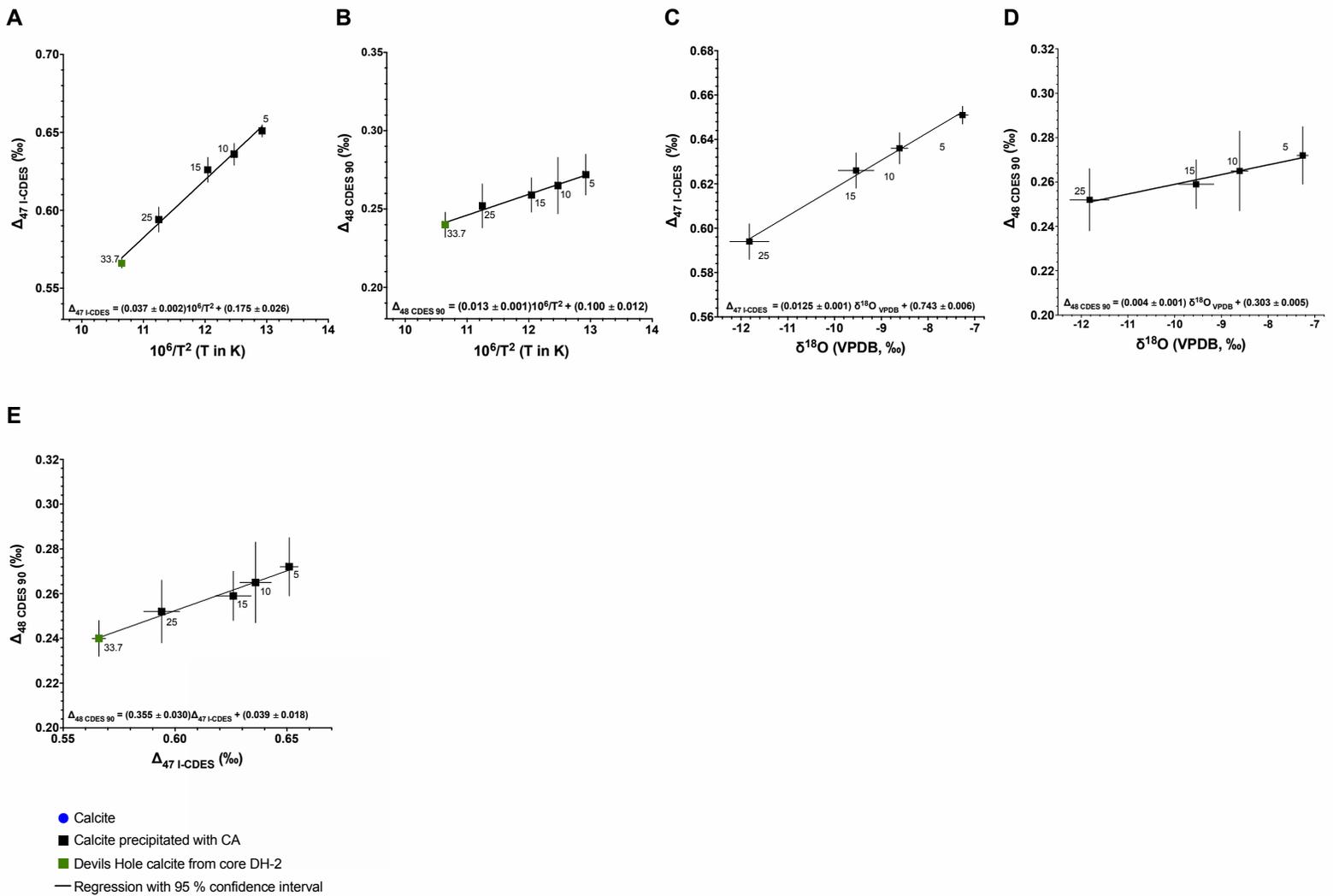
Figure 6. Quasi-equilibrium regressions for clumped isotope data and precipitation temperature, A) Δ_{47} and 10^6T^{-2} ($r^2 = 0.99$) and B) Δ_{48} and 10^6T^{-2} ($r^2 = 0.98$) with temperature in Kelvin. Quasi-equilibrium regressions for C) Δ_{47} and $\delta^{18}O$ ($r^2 = 0.99$) and D) Δ_{48} and $\delta^{18}O$ ($r^2 = 0.97$). Quasi-equilibrium regression for Δ_{47} and Δ_{48} ($r^2 = 0.98$). Data on all panels is for samples precipitated at pH 8.3 with CA and for Devils Hole calcite (DH-2), which is thought to have formed in near isotopic equilibrium at a temperature of 33.7 °C (Winograd et al., 1988; Winograd et al., 1992; Coplen, 2007; Kluge et al., 2014; Tripati et al., 2015; Daëron et al., 2019; Bajani et al., 2021).
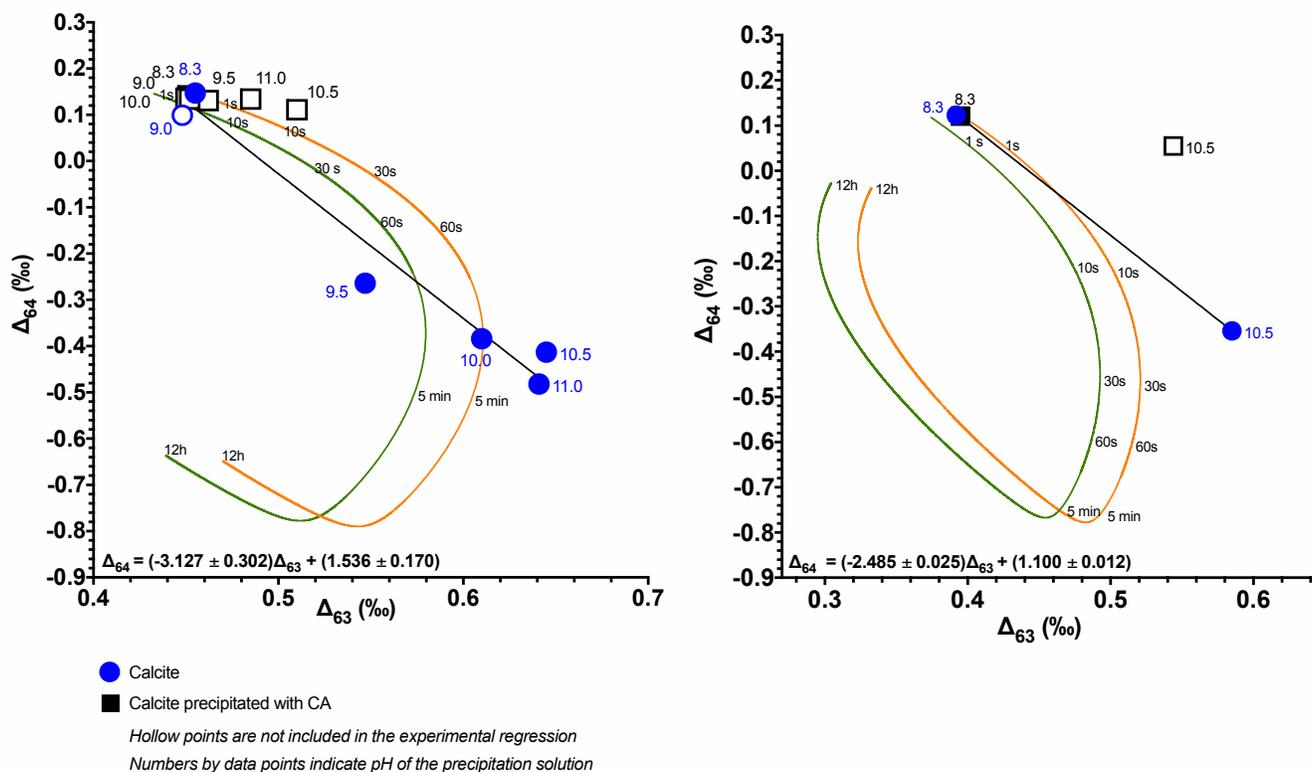
Figure 7. Experimental results compared to IsoDIC model predictions for the evolution of HCO_3^- and CO_3^{2-} isotopic composition at different temperatures and as a function of time. Experimental data are for calcite precipitated with (blue circles) and without CA (black squares) at varying pH. Numbers by data points indicate pH of precipitation solution. Regression through data (black line) and 95% confidence interval also shown. Calculations are for end member compositions at A) 5 °C and pH 10.5 and B) 25 °C and pH 10.5. Green trajectory shows modelled values for CO_3^{2-} and orange trajectory shows modelled values for HCO_3^-. Numbers on model trajectories indicate evolution time after the start of calcite precipitation. IsoDIC model software we used for our calculations was from Guo (2020).

Default model

A. 5 °C, no CA
B.
C. F_{CO_2} = 0.1 mmol/h, 0.01 mmol/h, 0.001 mmol/h, 0.0001 mmol/h
D.

B. calcite equilibrium; 2/3 CO_2 + 1/3 H_2O; 2/3 CO_2 + 1/3 OH^-; model fails to explain low $\delta^{18}O_c$ values at high pH

E. 5 °C with CA — model can't explain variable $\delta^{13}C$ with pH
F. calcite equilibrium; 2/3 CO_2 + 1/3 H_2O; 2/3 CO_2 + 1/3 OH^-
G.
H.

I. 25 °C, no CA
J. calcite equilibrium; 2/3 CO_2 + 1/3 H_2O; 2/3 CO_2 + 1/3 OH^-
K.
L.

M. 25 °C with CA
N. calcite equilibrium; 2/3 CO_2 + 1/3 H_2O; 2/3 CO_2 + 1/3 OH^-
O.
P.

Model with larger hydroxylation KFF for oxygen

Q. 5 °C, no CA
R. calcite equilibrium; 2/3 CO_2 + 1/3 H_2O; 2/3 CO_2 + 1/3 OH^-
S. F_{CO_2} = 0.1 mmol/h, 0.01 mmol/h, 0.001 mmol/h, 0.0001 mmol/h
T. Note the change in y-axis scale

U. 5 °C with CA
V. calcite equilibrium; 2/3 CO_2 + 1/3 H_2O; 2/3 CO_2 + 1/3 OH^-
W.
X.

Axis labels: $\delta^{13}C$; $1000 \ln \alpha$; Δ_{47} I-CDES (‰); Δ_{48} CDES 90 (‰); pH

Legend: $\log_{10}R$ (moles/m²/s); Fast; Slow; −6.5; −7.0; −7.5; −8.0; −8.5; −9.0; −9.5; −10.0

Figure 8. Experimental results from this work compared to data from Tang et al. (2014) and box model predictions. Symbol color indicates precipitation rate with color scale shown. Black points have precipitation faster than a log R (mol/m^2/s) value of -6.5. Colored curves show $\alpha_{\text{carb-water}}$, Δ_{47}, and Δ_{48} of calcite predicted by box model for different precipitation rates, with kinetic limit reflecting CO_2 hydration and hydroxylation and presence or absence of carbonic anhydrase (CA; calculations utilize values of 0 or 0.25 μM CA). Panels A-P show default model conditions with and with and without CA, and panels Q-X show the model with larger KFFs for oxygen. Clumped isotopic compositions of calcite are predicted to be sensitive to temperature, growth rate, and pH (Watkins and Hunt, 2015; Tripati et al., 2015, Guo, 2020; Uchikawa et al., 2021).
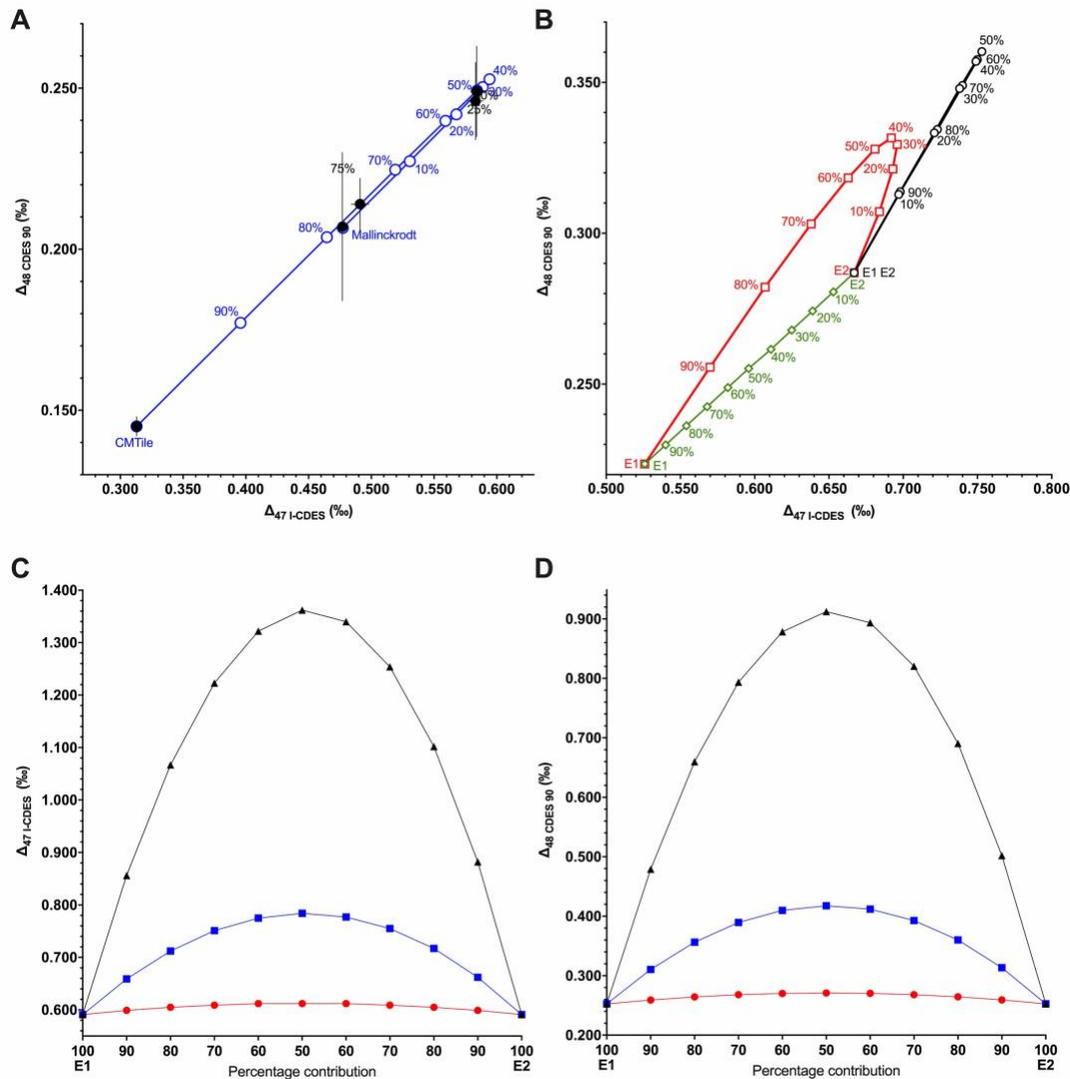
Figure 9. Predicted and observed clumped isotope composition for mixing experiments. A) $\varDelta_{47}$ and $\varDelta_{48}$ for non-linear mixing model predictions compared to experimental results. Two internal standards, CM Tile and Mallinckrodt, were used as endmembers. B) Mixing model predictions with different endmembers. Red line is for end members with a 0.141‰ and 0.063 ‰ difference in $\varDelta_{47}$ and $\varDelta_{48}$, and a 20 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$. Green line endmembers with a 0.141‰ and 0.063 ‰ difference in $\varDelta_{47}$ and $\varDelta_{48}$, and identical $\delta^{18}O$ and $\delta^{13}C$. The difference in the clumped isotope values of the endmembers in the green and red lines is approximately equivalent to a 50 °C temperature difference. Black line endmembers with identical clumped isotope values and a 20 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$. C) $\varDelta_{47}$ and percentage contribution of each endmember for mixing model predictions where the endmembers have identical Δ_{47} values and a 60 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$ (black line), 30 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$ (blue line), and 10 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$ (red line). D) $\varDelta_{48}$ and percentage contribution of each endmember for mixing model predictions where the endmembers have identical $\varDelta_{48}$ values and a 60 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$ (black line), 30 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$ (blue line), and 10 ‰ difference in $\delta^{18}O$ and $\delta^{13}C$ (red line).
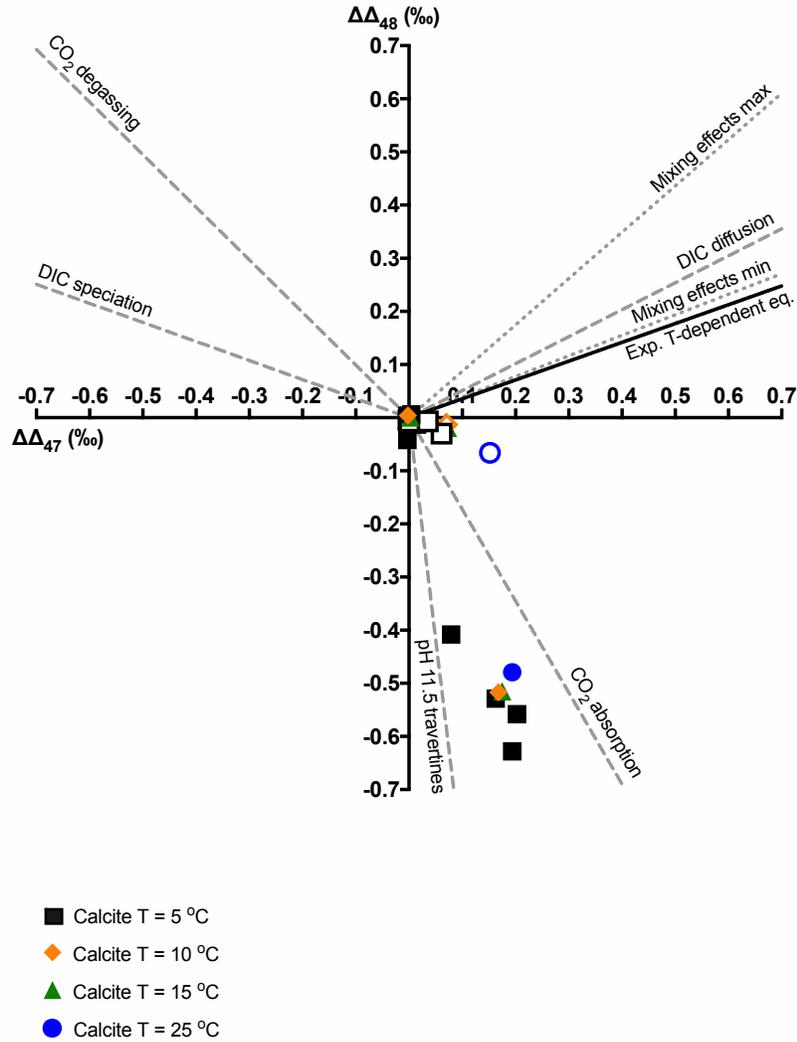
Figure 10. $\Delta\Delta_{47}$ and $\Delta\Delta_{48}$ data from experiments compared to theoretical predictions. Colored symbols are for calcite from this study synthesized at different temperatures. Open symbols are for samples grown with carbonic anhydrase (CA). Kinetic slopes include Δ_{63} and Δ_{64} values for HCO_3^- endmembers produced through varying processes (Guo, 2020) that we use to predict Δ_{47} and Δ_{48}, and mixing model predictions for calcite (this study). Experimental T-dependent equilibrium slope = 0.387. Slopes published in Guo (2020) using IsoDIC modeling software and our mixing model predicted non-equilibrium slopes for different processes. DIC speciation =- -0.358; DIC diffusion = 0.506, CO_2 degassing at pH 8 = -0.990; CO_2 absorption at pH 9 = -1.72; travertine precipitation at pH 11.5 = -8.33; mixing values = 0.387 to 0.872.
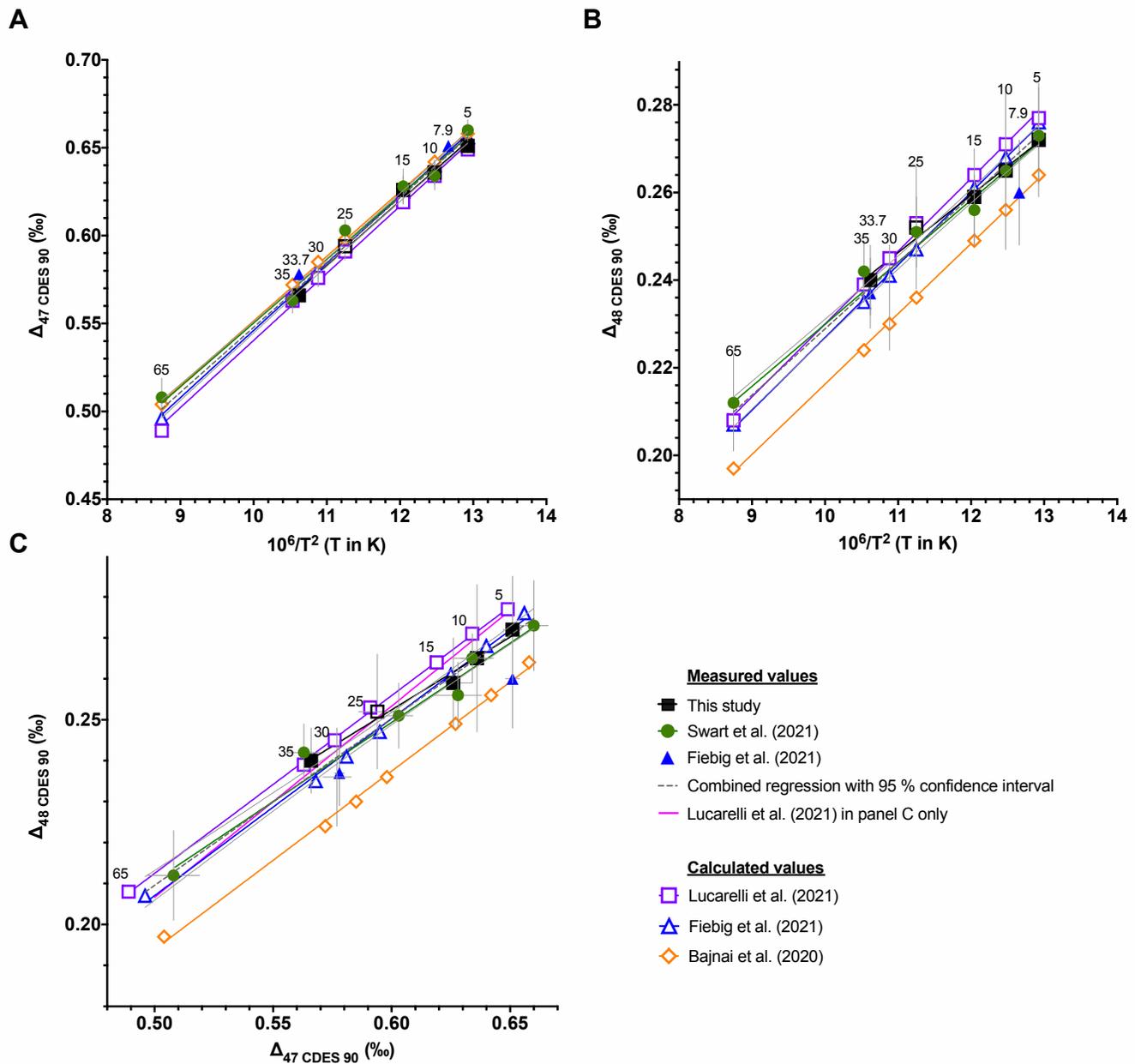
Figure 11. Comparison of Δ_{47} and Δ_{48} regressions from this work to published results. A) Δ_{47} and 10^6 T^{-2} with T in K, B) Δ_{48} and 10^6 T^{-2} with T in K, and C) is Δ_{47} and Δ_{48}. Results for samples grown at pH 8.3 with carbonic anhydrase (CA) from this work are compared to experimentally-based regressions (Swart et al., 2021; Fiebig et al., 2021; Bajnai et al., 2020; Lucarelli et al., 2021). Bajnai et al. (2020) and Lucarelli et al. (2021) are based on theory from Hill et al. (2014) and Tripati et al. (2015) with experimental AFFs. All regressions were determined to be statistically indistinguishable, and a combined regression was determined for the experimentally based regressions. Numbers by symbols indicate precipitation temperature in Celsius.

Table 1. Experimental conditions for precipitation experiments with and without carbonic anhydrase (CA). Mineralogy as determined by XRD indicated.

Temperature of precipitation (°C)	pH of precipitation solution	CA (yes or no)	Time from beginning of experiment until start of precipitation (days)	Total experiment time (days)	Precipitation rate ($\log_{10}R$ with R in mol s^{-1} m^{-2})	Initial water $\delta^{18}O_{VSMOW}$ (‰)	Final water $\delta^{18}O_{VSMOW}$ (‰)	Calcite (weight %)	Vaterite (weight %)	Aragonite (weight %)
5	8.3	Y	23	34	-7.11	n/a	-9.52	100		
5	8.3	N	29	52	-7.43	n/a	-9.44	100		
5	9.0	Y	3	27	-7.45	-9.22	-9.14	100		
5	9.0	N	5	27	-7.41	-9.40	-9.31	100		
5	9.5	Y	1	26	-7.47	-9.37	-9.26	100		
5	9.5	N	<1	26	-7.48	-9.44	-9.33	100		
5	10.0	Y	<1	9	-7.02	-9.47	-9.13	100		
5	10.0	N	<1	9	-7.02	-9.64	-9.56	100		
5	10.5	Y	<1	14	-7.21	n/a	-9.07	100		
5	10.5	N	<1	14	-7.21	-9.37	-9.25	100		
5	11.0	Y	<1	12	-7.15	n/a	-9.44	100		
5	11.0	N	<1	12	-7.15	n/a	n/a	100		
10	8.3	Y	14	26	-7.15	-9.26	-9.40	100		
10	8.3	N	10	26	-7.27	-9.18	-9.35	100		
10	10.5	Y	1	15	-7.21	-8.83	-8.86	21	75	4
10	10.5	N	1	15	-7.21	-8.96	-9.01	100		
15	8.3	Y	9	20	-7.11	-9.03	-9.15	100		
15	8.3	N	9	20	-7.11	-9.16	-9.10	100		
15	10.5	Y	<1	14	-7.21	-9.22	-9.02	54	43	3
15	10.5	N	<1	14	-7.21	-9.34	-9.06	100		
25	8.3	Y	2	14	-7.15	-9.30	-9.29	100		
25	8.3	N	3	14	-7.11	-9.32	-9.33	100		
25	10.5	Y	<1	12	-7.15	-9.53	-9.38	72	2	26
25	10.5	N	<1	12	-7.15	-9.33	-9.29	60		40

Table 2. Mass spectrometer configurations used in this study.

Mass Spectrometer	Acid digestion T (°C)	Acid digestion method	Sample size (mg)	GC column with He carrier gas	Adsorption trap	m/z 44 ion beam intensity	Integration time (s)
Thermo Fisher MAT 253	90	common acid bath	5	x		16 V	720
Nu Instruments Perspective-1a	90	common acid bath	0.5	x		80-30 nA	1600
Nu Instruments Perspective -1b	70	individual vials	0.5		x	80-30 nA	1600
Nu Instruments Perspective-1c	90	common acid bath	0.5		x	80-30 nA	1600
Nu Instruments Perspective-2a	70	individual vials	0.5		x	80-30 nA	1200
Nu Instruments Perspective-2b	90	common acid bath	0.5		x	80-30 nA	1200

Table 3. Clumped and stable isotope data for all samples measured in this study. Calcite mineral Δ_{63} and Δ_{64} was calculated using equations from Lucarelli et al. (2021), which were based on theory from Hill et al. (2014) and Tripati et al. (2015) and experimentally determined acid digestions fractionation fractors (AFFs).

Temp. (°C)	pH	CA (yes or no)	$\Delta_{47\,I\text{-}CDES}$ (‰)	SE	N	$\Delta_{48\,CDES\,90}$ (‰)	SE	N	Δ_{63} (‰)	Δ_{64} (‰)	$\delta^{18}O_{VPDB}$ (‰)	SD	1000ln(αCaCO3-H2O)	$\delta^{13}C_{VPDB}$ (‰)	SD
5	8.3	Y	0.651	0.004	15	0.272	0.013	10	0.451	0.141	-7.3	0.1	32.8	-7.7	0.0
5	8.3	N	0.655	0.006	5	0.278	0.021	4	0.455	0.147	-7.2	0.1	32.7	-16.7	0.1
5	9.0	Y	0.650	0.006	13	0.267	0.024	5	0.450	0.136	-7.8	0.1	32.0	-9.5	0.1
5	9.0	N	0.648	0.009	10	0.230	0.009	7	0.448	0.099	-11.8	0.1	28.2	-16.9	0.1
5	9.5	Y	0.663	0.005	12	0.262	0.024	7	0.462	0.131	-7.9	0.1	32.0	-12.7	0.1
5	9.5	N	0.749	0.009	6	-0.136	0.015	4	0.547	-0.264	-18.6	0.1	21.1	-18.2	0.1
5	10.0	Y	0.652	0.003	7	0.265	0.019	4	0.452	0.134	-8.4	0.1	31.2	-12.6	0.0
5	10.0	N	0.814	0.008	10	-0.257	0.007	4	0.610	-0.384	-22.4	0.2	18.6	-18.3	0.0
5	10.5	Y	0.712	0.006	7	0.242	0.020	5	0.510	0.111	-9.9	0.2	30.3	-15.2	0.1
5	10.5	N	0.849	0.005	8	-0.286	0.019	7	0.645	-0.413	-25.0	0.1	14.8	-20.1	0.0
5	11.0	Y	0.686	0.008	9	0.265	0.011	5	0.485	0.134	-10.6	0.1	29.6	-17.8	0.0
5	11.0	N	0.845	0.007	4	-0.356	0.021	4	0.641	-0.482	-26.1	0.1	n/a	-31.6	1.5
10	8.3	Y	0.636	0.007	8	0.265	0.018	8	0.436	0.134	-8.6	0.2	31.4	-7.9	0.2
10	8.3	N	0.634	0.005	9	0.269	0.014	8	0.434	0.138	-8.3	0.4	31.4	-13.1	0.9
10	10.5	Y	0.707	0.009	9	0.252	0.015	4	0.506	0.121	-9.8	0.0	29.7	-12.2	0.3
10	10.5	N	0.804	0.008	11	-0.252	0.017	4	0.601	-0.379	-22.0	0.2	17.3	-23.5	0.1
15	8.3	Y	0.626	0.008	12	0.259	0.011	7	0.426	0.128	-9.5	0.4	30.3	-11.5	0.1
15	8.3	N	0.629	0.006	10	0.259	0.020	6	0.429	0.128	-9.5	0.3	29.9	-14.0	0.9
15	10.5	Y	0.694	0.007	10	0.243	0.038	2	0.493	0.112	-11.8	0.4	28.1	-13.1	0.3
15	10.5	N	0.801	0.007	13	-0.256	0.017	5	0.598	-0.383	-24.6	0.3	14.9	-20.1	0.1
25	8.3	Y	0.594	0.008	17	0.252	0.015	9	0.395	0.121	-11.8	0.4	28.1	-10.5	0.2
25	8.3	N	0.591	0.010	12	0.254	0.009	7	0.392	0.123	-12.3	0.4	27.5	-16.4	0.2
25	10.5	Y	0.746	0.005	7	0.185	0.025	5	0.544	0.055	-15.4	0.5	24.8	-13.0	0.6
25	10.5	N	0.788	0.009	11	-0.227	0.037	4	0.585	-0.354	-21.9	0.2	18.1	-19.3	0.3

Table 4. Equilibrium and kinetic trajectories for clumped and bulk isotopes for calcite precipitated with varying temperature and pH with and without CA. Near equilibrium slopes were determined by precipiating calcite at pH 8.3 with CA at 5 $^\circ$C, 10 $^\circ$C, 15 $^\circ$C, and 25 $^\circ$C. Kinetic slopes at 5 $^\circ$C were determined with a regression through samples precipitated at pH 8.3 with and without CA, and 9.5, 10.0, 10.5, and 11.0 without CA. Kinetic slopes at 10 $^\circ$C, 15 $^\circ$C, and 25 $^\circ$C were determined by regressions through samples precipitated at pH 8.3 with and without CA, and at pH 10.5 without CA. Calcite mineral Δ_{63} and Δ_{64} were calculated using equations from Lucarelli et al. (2021), which use theory from Hill et al. (2014) and Tripati et al. (2015) with experimentally-determined compositionally-dependant acid digestion fractionation factors.

Temp. ($^\circ$C)	Quasi-equilibrium or kinetic	Slope: $\Delta_{47\,I\text{-}CDES}$ versus $\delta^{18}O_{VPDB}$	SE	Slope: $\Delta_{48\,CDES90}$ versus $\delta^{18}O_{VPDB}$	SE	Slope: $\Delta_{47\,I\text{-}CDES}$ versus $\Delta_{48\,CDES90}$	SE	Slope: Δ_{64} versus Δ_{63}
5 to 25	quasi-equilibrium	0.013	0.001	0.004	0.001	0.355	0.030	0.373
5	kinetic	-0.011	0.001	-0.033	0.001	-2.989	0.125	-3.127
10	kinetic	-0.013	0.000	-0.038	0.000	-3.071	0.011	-3.102
15	kinetic	-0.012	0.000	-0.034	n/a	-2.976	0.034	-2.996
25	kinetic	-0.020	0.001	-0.048	0.002	-2.455	0.024	-2.485

Table 5. Measured and model predicted clumped and bulk isotope values from a mixing experiment using two endmembers with varying clumped and bulk isotope compositions. Mix 1 is 75% Mallinckrodt and 25 % CM Tile. Mix 2 is 50 % Mallinckrodt and 50 % CM Tile. Mix 3 is 25 % Mallinckrodt and 75 % CM Tile.

Sample	Measured $\Delta_{47\,\text{I-CDES}}$ (‰)	SE	N	Modeled $\Delta_{47\,\text{I-CDES}}$ (‰)	Difference	Measured $\Delta_{48\,\text{CDES90}}$ (‰)	SE	N	Modeled $\Delta_{48\,\text{CDES90}}$ (‰)	Difference	Measured $\delta^{18}O_{VPDB}$ (‰)	SD	Modeled $\delta^{18}O_{VPDB}$ (‰)	Measured $\delta^{13}C_{VPDB}$ (‰)	SD	Measured $\delta^{13}C_{VPDB}$ (‰)
Mallinckrodt	0.477	0.003	9	0.477	0.000	0.207	0.023	5	0.207	0.000	-22.00	0.05	-22.01	-40.60	0.20	-40.56
Mix 1	0.583	0.006	17	0.580	0.003	0.246	0.012	7	0.247	0.001	-16.30	0.50	-16.88	-29.10	0.90	-29.92
Mix 2	0.584	0.005	18	0.584	0.000	0.249	0.014	10	0.249	0.000	-11.30	0.30	-11.76	-18.60	0.70	-19.28
Mix 3	0.491	0.007	21	0.494	-0.003	0.214	0.008	11	0.215	0.001	-6.40	0.30	-6.63	-8.20	0.50	-8.64
CM Tile	0.313	0.001	463	0.313	0.000	0.145	0.003	309	0.145	0.000	-1.50	0.00	-1.50	2.00	0.00	2.00

Table 6. Mixing model conditions for model data plotted in Figure 9, including experimental data using the internal carbonate standards Mallinckrodt and CM Tile as endmembers, and modeled endmembers with varying clumped and bulk isotope compositions. For the experimental data in Figure 9a, the largest Γ_{47} and Γ_{48} were observed when the mix contained 60% Mallinckrodt and 40 % CM Tile, where $\Gamma_i = \Delta_{i\,endmember} - \Delta_{i\,mix}$.

Figure	Line color, symbol	Endmember	Initial $\Delta_{47\,\text{I-CDES}}$ (‰)	Initial $\Delta_{48\,\text{CDES90}}$ (‰)	Initial $\delta^{18}O$ VPDB (‰)	Initial $\delta^{13}C$ VPDB (‰)	Γ_{47} (‰)	Γ_{48} (‰)
9A	blue, circle	Mallinckrodt	0.477	0.202	-21.9	-40.5	0.117	0.045
		CM Tile	0.313	0.576	-1.5	2.0		
9B	red, square	E1	0.667	0.287	-10.0	-10.0	0.029	0.045
		E2	0.526	0.223	10.0	10.0		
	black, circle	E1	0.667	0.287	-10.0	-10.0	0.086	0.073
		E2	0.667	0.287	10.0	10.0		
	green, diamond	E1	0.667	0.287	1.0	1.0	0.000	0.000
		E2	0.526	0.223	1.0	1.0		
9C	black, triangle	E1	0.591	0.253	-30.0	-30.0	0.769	
		E2	0.591	0.253	30.0	30.0		
	blue, square	E1	0.591	0.253	-15.0	-15.0	0.193	
		E2	0.591	0.253	15.0	15.0		
	red, circle	E1	0.591	0.253	-5.0	-5.0	0.021	
		E2	0.591	0.253	5.0	5.0		
9D	black, triangle	E1	0.591	0.253	-30.0	-30.0		0.660
		E2	0.591	0.253	30.0	30.0		
	blue, square	E1	0.591	0.253	-15.0	-15.0		0.164
		E2	0.591	0.253	15.0	15.0		
	red, circle	E1	0.591	0.253	-5.0	-5.0		0.018
		E2	0.591	0.253	5.0	5.0		

Table 7. Comparison of measured and calculated Δ_{47} and Δ_{48} values. Experimental data from Swart et al. (2021) is from inorganic calcite precipitations. Fiebig et al. (2021) calculated values are based on a regression determined from experimental measurments of carbonates, including lake and cave calcite, inorganic precipitations, and samples equilibrated at high temperature, with samples having crystallization temperatrues from 7.9 °C to 1100 °C. Lucareli et al. (2021) and Bajnai et al. (2020) calculated values are based on regressions determined using a combination of theory from Hill et al. (2014) and Tripati et al. (2015) with experimentally determined acid digestion fractionation factors.

T (°C)	Measured values				Calculated values			Measured values				Calculated values		
	$\Delta_{47\,\text{I-CDES}}$ (this study) (‰)	SE	$\Delta_{47\,\text{CDES90}}$ (Swart et al., 2021) (‰)	SE	$\Delta_{47\,\text{I-CDES}}$ (Lucarelli et al., 2021) (‰)	$\Delta_{47\,\text{CDES90}}$ (Bajnai et al., 2020) (‰)	$\Delta_{47\,\text{CDES90}}$ (Fiebig et al., 2021) (‰)	$\Delta_{48\,\text{CDES90}}$ (this study) (‰)	SE	$\Delta_{48\,\text{CDES90}}$ (Swart et al., 2021) (‰)	SE	$\Delta_{48\,\text{CDES90}}$ (Lucarelli et al., 2021) (‰)	$\Delta_{48\,\text{CDES90}}$ (Bajnai et al., 2020) (‰)	$\Delta_{48\,\text{CDES90}}$ (Fiebig et al., 2021) (‰)
5	0.651	0.004	0.660	0.006	0.649	0.658	0.656	0.272	0.013	0.273	0.011	0.277	0.264	0.276
10	0.636	0.007	0.634	0.008	0.634	0.642	0.640	0.265	0.018	0.265	0.006	0.271	0.256	0.268
15	0.626	0.008	0.628	0.01	0.619	0.627	0.625	0.259	0.011	0.256	0.008	0.264	0.249	0.261
25	0.594	0.008	0.603	0.006	0.591	0.598	0.595	0.252	0.014	0.251	0.008	0.253	0.236	0.247
30			0.577	0.006	0.576	0.585	0.581			0.236	0.012	0.245	0.230	0.241
35			0.563	0.007	0.563	0.572	0.568			0.242	0.007	0.239	0.224	0.235
65			0.508	0.011	0.489	0.504	0.496			0.212	0.011	0.208	0.197	0.207

Table 8. Extra-sum-of-squares F test results which determines if regressions have significant differences between them and if all datasets can be represented by one combined regression. Experimental regressions for samples with known precipitation temperature were used from Swart et al. (2021), Fiebig et al. (2021), and this study. An additional comparison was performed using an experimental Δ_{47} and Δ_{48} regression with samples of unknown precipitation temperature, including standards, from Lucarelli et al. (2021). The regressions from Bajnai et al. (2020) and Lucarelli et al. (2021) based on theory from Hill et al. (2014) and Tripati et al. (2015) with experimental AFFs were then compared to the experimental regressions.

	Comparison of Fits	Source of regression	Null hypothesis	Alternative hypothesis	P value	Conclusion (alpha = 0.05)	Preferred model	F (DFn, DFd)
Experimental regressions	Δ_{47} and Δ_{48}	Swart et al., 2021; Fiebig et al., 2021; this study	One curve for all data sets	Different curve for each data set	0.99	Do not reject null hypothesis	One curve for all data sets	0.03 (6, 43)
	Δ_{47} and Δ_{48}	Swart et al., 2021; Fiebig et al., 2021; Lucarelli et al., 2021; this study	One curve for all data sets	Different curve for each data set	>0.99	Do not reject null hypothesis	One curve for all data sets	0.05 (8, 57)
	Δ_{47} and $10^6\,T^{-2}$	Swart et al., 2021; Fiebig et al., 2021; this study	One curve for all data sets	Different curve for each data set	0.99	Do not reject null hypothesis	One curve for all data sets	0.13 (6, 43)
	$\Delta_{48}\ 10^6\,T^{-2}$	Swart et al., 2021; Fiebig et al., 2021; this study	One curve for all data sets	Different curve for each data set	0.99	Do not reject null hypothesis	One curve for all data sets	0.03 (6, 43)
Experimental regressions with regressions based on theory with experimental AFFs	Δ_{47} and Δ_{48}	Bajnai et al., 2020; Swart et al., 2021; Fiebig et al., 2021; Lucarelli et al., 2021; this study	One curve for all data sets	Different curve for each data set	0.99	Do not reject null hypothesis	One curve for all data sets	0.18 (10, 62)
	Δ_{47} and $10^6\,T^{-2}$	Bajnai et al., 2020; Swart et al., 2021; Fiebig et al., 2021; Lucarelli et al., 2021; this study	One curve for all data sets	Different curve for each data set	0.99	Do not reject null hypothesis	One curve for all data sets	0.26 (10, 53)
	$\Delta_{48}\ 10^6\,T^{-2}$	Bajnai et al., 2020; Swart et al., 2021; Fiebig et al., 2021; Lucarelli et al., 2021; this study	One curve for all data sets	Different curve for each data set	0.99	Do not reject null hypothesis	One curve for all data sets	0.12 (10, 53)