1  **The $K_D$ Sr/Ca in cultured massive Porites spp. corals are reduced at low seawater $pCO_2$**

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12

13  **Abstract**

14  Coral skeletal Sr/Ca has valuable potential as a proxy of sea surface temperatures (SSTs). However
15  seawater $pCO_2$ can influence skeletal Sr incorporation and Sr/Ca-SST calibrations derived from present
16  day corals may not be applicable to ancient specimens or older sections of modern corals deposited
17  under lower seawater $pCO_2$ than the present day. In this study we analysed skeletal Sr/Ca in multiple
18  genotypes of massive *Porites* spp. cultured over a range of seawater $pCO_2$ (from 180 to 750 µatm) and
19  temperature (25°C and 28°C). Multiple linear regression analysis indicates that the Sr/Ca aragonite
20  partition coefficient, $K_D$ Sr/Ca is inversely related to seawater temperature and positively related to
21  seawater $pCO_2$ (equivalent to changes in skeletal Sr/Ca of 0.046 mmol mol$^{-1}$ °C$^{-1}$ and
22  0.0002 mmol mol$^{-1}$ µatm$^{-1}$ respectively). Applying present day Sr/Ca-SST equations to older coral
23  skeletons growing at lower $pCO_2$ could underestimate seawater temperatures. However $K_D$ Sr/Ca vary
24  significantly between some coral genotypes cultured at the same seawater $pCO_2$ indicating that other
25  unidentified processes also influence skeletal Sr/Ca and it is unknown how these processes varied when
26  ancient corals were deposited. We do not observe a significant relationship between $K_D$ Sr/Ca and coral
27  calcification rate after combining all coral genotypes to allow identification of the correct $K_D$ Sr/Ca to
28  apply to coral records.

29

30  **1.  Introduction**

31  The skeletal Sr/Ca of massive *Porites* spp. corals is a widely used proxy for sea surface temperatures,
32  SST (D'Olivo et al., 2018). Massive *Porites* spp. are widespread in tropical and sub-tropical Indo
33  Pacific reefs (Veron 1993) and produce aragonite skeletons which are well preserved in modern and
34  ancient environments (Montaggioni and Braithwaite, 2009). Coral skeletons accrete rapidly and high
35  temporal resolution (sub annual) Sr/Ca-SST records can indicate the frequency and magnitude of
36  climate events e.g. El Nino Southern Oscillation (Gagan et al., 2004), Pacific Decadal Variability
37  (Calvo et al., 2007) and interdecadal change (Kawakubo et al., 2017). Most coral records cover the
38  last 3-4 centuries (Kawakubo et al., 2017) but older specimens have also been analysed e.g. 9.5 ka
39  (DeLong et al., 2010), 350 ka (Kilbourne et al., 2004).

40  Sr/Ca-SST calibrations are typically produced by linear regression of SST and skeletal Sr/Ca in
41  modern coral specimens, typically collected after 1980 e.g. (Beck et al. 1992). Recent work shows that
42  seawater $pCO_2$ can significantly affect coral skeletal Sr/Ca (Cole et al., 2016; Tanaka et al., 2015)
43  although this effect is not observed in all corals (Cole et al., 2016; Kuffner et al., 2012). Atmospheric
44  $CO_2$ oscillated from ~ 180 ppm during glacial periods to 260-280 ppm during the interglacials (Luthi et
45  al., 2008) from the middle Pleistocene (~800 ka) to the pre-industrial (~1760). Thereafter atmospheric
46  $CO_2$ increased and currently exceeds 400 ppm (IPCC, 2019). Applying Sr/Ca-SST calibrations derived
47  from present day corals to skeletons that were deposited under lower seawater $pCO_2$ may produce
48  inaccurate SST estimates. This could explain why Sr/Ca based seawater temperatures derived from
49  glacial-age coral skeletons can be significantly cooler than from other marine carbonate proxies see
50  (Gagan et al., 2012).

51  Seawater $pCO_2$ may influence skeletal Sr/Ca by directly affecting Sr incorporation in aragonite at the
52  site of crystallisation or by altering biological processes in the overlying coral tissues that subsequently
53  affect skeletal Sr/Ca. Coral skeleton formation is presumed to occur from an extracellular calcifying
54  medium (ECM) that is semi-isolated from seawater between the base of the coral tissues and the
55  underlying skeleton (Allemand et al., 2011). The Sr/Ca of this ECM ($Sr/Ca_{ECM}$) reflects the relative
56  transport rates of $Ca^{2+}$ and $Sr^{2+}$ across the coral tissue (Allison et al., 2011) and the proportion of the
57  ECM reservoir used for calcification i.e. Rayleigh fractionation (Elderfield et al., 1996). Furthermore
58  aragonite precipitation rate influences Sr/Ca incorporation in synthetic aragonites (Alkhatib and
59  Eisenhauer, 2017). Coral skeletal Sr/Ca can vary significantly between individual corals (equivalent to
60  errors in estimated SST of up to several °C, Alpert et al. 2016, Kuffner et al., 2017). Skeletal Sr/Ca has
61  been inversely correlated with skeletal growth rate measured as skeletal linear extension (De Villiers et
62  al., 1994) and calcification rate (Ferrier-Pages et al., 2002; Kuffner et al., 2012), which is consistent with
63  Rayleigh fractionation and/or mineral precipitation rate exerting significant control. However these
64  relationships are not observed in all corals indicating that our understanding of the processes involved
65  is incomplete (Cole et al., 2016; Hayashi et al., 2013).

66  We have previously cultured massive *Porites* spp. corals over a range of seawater $pCO_2$ and at two
67  temperatures (Cole et al., 2018). Here we present Sr/Ca analyses of these coral skeletons to explore
68  the impact of seawater $pCO_2$ on skeletal Sr/Ca. Individual corals can exhibit varying responses to
69  environmental change (Cole et al., 2016; Tanaka et al., 2015) so we imported large heads from
70  several spatially separate *Porites* spp. colonies (assumed to be different genotypes), splitting each
71  head into multiple pieces to determine the response of each genotype to variations in seawater $pCO_2$
72  and temperature.
73
74  **2.  Methods**

75  **2.1 Culture System**

76  Multiple heads of massive *Porites* spp. were collected from Fiji, imported into the UK and cultured at
77  two temperatures (25 and 28°C) and at seawater $pCO_2$ of ~180, 260, 400 and 750 µatm (simulating
78  conditions in the Last Glacial Maximum, the pre-industrial age, the present day and a potential future
79  $CO_2$ scenario, Barry et al., 2011). Coral heads were assumed to represent different genotypes when

80  they were collected from spatially separate (non-adjoining) colonies. Heads were sawn into multiple
81  pieces (each ~12 cm in diameter) so that at least one piece of each genotype was cultured in each
82  seawater $pCO_2$ treatment. Corals were identified to represent two species (*P. lutea* and *P. murrayensis*)
83  based on corallite morphology (Veron, 1993) and genotypes exhibited a wide range of calcification rates
84  over all the $pCO_2$ conditions (2-38 µmol $CaCO_3$ $cm^{-2}$ $day^{-1}$, Cole et al., 2018).

85  Full details on the experimental set-up are included in Cole et al., 2018 (as experiment 2). In brief, corals
86  were housed in a large-volume purpose-built aquarium system constructed of low $CO_2$ permeability
87  materials (Cole et al., 2016), filled with a mixture of natural and artificial seawater and bubbled with
88  different $CO_2$ atmospheres (Cole et al., 2018). The same coral colonies were cultured at both 25°C and
89  28°C for temporally separate periods to explore the effect of temperature. After import, corals were
90  maintained at ambient seawater $pCO_2$ conditions for 2 months, then adjusted to 28°C and altered $pCO_2$
91  conditions over a period of 1 month, and then acclimated at the final seawater $pCO_2$ treatment conditions
92  for a further 4 months. Skeletal material accreted over a further 5 weeks was identified by incubating
93  corals in 10 mg $l^{-1}$ alizarin red for 8 hours at the start and end of this 'experimental period' to create two
94  stain lines in the skeleton. Following this, temperature was adjusted to 25°C (with no changes to $pCO_2$)
95  over a period of 1 month for the second study period. Corals were maintained at these conditions for a
96  further 1 month before incubating with alizarin red, as before, to create a third stain line in the skeleton
97  to identify skeletal material deposited in the subsequent 5 weeks at 25°C (Figure 1a). At the end of this
98  second experimental period the corals were sacrificed and the tissue removed by submerging the
99  colonies in 3-4% sodium hypochlorite for ≥24 h with intermittent agitation. This treatment is effective at
100 removing organic contamination without dissolution of the skeletal structure (Clode and Marshall, 2003).

101 Seawater nutrients, $[NO_3^-+NO_2^-]$ and $[PO_4^-]$, fell to low levels (Table 1) in the first week of the 5 week
102 experimental period in the 400 µatm $pCO_2$ treatment at 28°C and the polyps of these corals appeared
103 retracted. The seawater in this reservoir was discarded and replaced with seawater sourced from the
104 remaining 3 reservoirs bubbled to bring it to seawater $pCO_2$ of 400 µatm before use. The geochemistry
105 of these specimens was only analysed in the skeleton deposited at 25°C. Nutrient levels in the other
106 coral treatments were comparable to natural reef sites (Szmant 2002) and did not vary significantly
107 between treatments.

108

109 **2.2 Monitoring Seawater Composition**

110 Details of seawater temperature, salinity, dissolved inorganic carbon (DIC) parameters (Cole et al.,
111 2018), dissolved nutrients and seawater $[Ca^{2+}]$ and Sr/Ca are summarised in Table 1. Seawater samples
112 were collected weekly during the experimental period for Sr and Ca analysis by quadrupole ICP-MS
113 (Thermo Scientific X Series) at the National Oceanography Centre, Southampton. Samples were diluted
114 1000-fold in 5% $HNO_3$ (with 5 ppb In as an internal standard) and calibrated against matrix-matched
115 synthetic standards prepared from 1000 µg $ml^{-1}$ single-element stock solutions (Inorganic Ventures) in
116 5% $HNO_3$. Seawater Sr/Ca was normalised to IAPSO standard seawater (Sr/Ca = 8.77 mmol $mol^{-1}$).
117 Replicate analyses of this standard (n = 4) yield a Sr/Ca standard deviation of ±0.18% (1σ). Dissolved
118 nutrients were measured in filtered samples from each reservoir using a flow cell spectrophotometer
119 (Lachat 8000) at the Scottish Association of Marine Science, UK.

**Table 1**. Physical and chemical characteristics of the seawater and coral skeletons in each treatment, measured over the 5-week experimental period. Seawater values are mean ± standard deviation (1σ). Skeletal Sr/Ca is mean ± 95% confidence limits (number of analyses, n, in parentheses). na = not analysed. Seawater pH (total scale) is estimated from measured dissolved inorganic carbon (DIC) and $pCO_2$ measurements using $CO_2$sys MATLAB (version 1.1), equilibrium constants for carbonic acid from (Mehrback et al., 1973), refit by (Dickson and Millero, 1987) and for $KHSO_4$ from (Dickson, 1990).

| | Seawater $pCO_2$ | | | |
|---|---|---|---|---|
| | 180 µatm | 260 µatm | 400 µatm | 750 µatm |
| **25°C experiment** | | | | |
| Temperature (°C) | 25.0 ± 0.10 | 25.0 ± 0.16 | 25.2 ± 0.25 | 25.0 ± 0.21 |
| Salinity | 35.1 ± 0.1 | 35.2 ± 0.0 | 35.1 ± 0.1 | 35.1 ± 0.0 |
| Estimated seawater pH | 8.32 | 8.20 | 8.04 | 7.81 |
| Seawater $pCO_2$ µatm | 178 ± 2 | 260 ± 3 | 405 ± 6 | 751 ± 9 |
| Seawater [DIC] µmol $kg^{-1}$ | 1862 ± 7 | 1947 ± 17 | 2025 ± 30 | 2129 ± 20 |
| Estimated $\Omega_{aragonite}$ | 5.49 | 4.51 | 3.44 | 2.21 |
| Seawater nutrients | | | | |
| $NH_4^+$ µM | 0.54 ± 0.00 | 0.57 ± 0.05 | 0.12 ± 0.18 | 0.44 ± 0.22 |
| $PO_4^{3-}$ µM | 0.05 ± 0.04 | 0.02 ± 0.03 | 0.05 ± 0.02 | 0.02 ± 0.02 |
| $Si(OH)_4$ µM | 2.74 ± 1.65 | 0.97 ± 0.31 | 2.44 ± 0.25 | 1.15 ± 0.46 |
| $NO_3^-+NO_2^-$ µM | 2.59 ± 0.06 | 1.54 ± 0.31 | 2.32 ± 0.34 | 1.38 ± 0.09 |
| Seawater Sr/Ca | 12.38 ± 0.02 | 12.41 ± 0.03 | 12.40 ± 0.00 | 12.43 ± 0.02 |
| $[Ca^{2+}]$ mM | 10.3 ± 0.1 | 10.6 ± 0.1 | 10.4 ± 0.5 | 10.7 ± 0.1 |
| Mean skeleton Sr/Ca | | | | |
| Genotype 4 (*P. murrayensis*) | 12.31 ± 0.11 (20) | 12.64 ± 0.09 (20) | 12.37 ± 0.16 (15) | 12.61 ± 0.09 (20) |
| Genotype 5 (*P. lutea*) | 12.28 ± 0.07 (21) | 12.60 ± 0.15 (24) | na | 12.70 ± 0.11 (21) |
| Genotype 6 (*P. murrayensis*) | 12.53 ± 0.08 (54) | 12.67 ± 0.09 (52) | 12.64 ± 0.48 (6) | 12.93 ± 0.09 (47) |
| Genotype 7(*P. lutea*) | 12.28 ± 0.05 (48) | 12.57 ± 0.05 (59) | 12.16 ± 0.09 (21) | 12.72 ± 0.07 (53) |
| | | | | |
| **28°C experiment** | | | | |
| Temperature (°C) | 28.0 ± 0.09 | 28.0 ± 0.18 | 28.0 ± 0.22 | 28.0 ± 0.09 |
| Salinity | 35.1 ± 0.05 | 35.1 ± 0.07 | 35.1 ± 0.09 | 35.1 ± 0.09 |
| Estimated seawater pH | 8.30 | 8.19 | 8.04 | 7.81 |
| Seawater $pCO_2$ µatm | 178 ± 2 | 259 ± 3 | 402 ± 3 | 752 ± 6 |
| Seawater [DIC] µmol $kg^{-1}$ | 1757 ± 39 | 1910 ± 11 | 1999 ± 14 | 2113 ± 23 |
| Estimated $\Omega_{aragonite}$ | 5.50 | 4.86 | 3.76 | 2.46 |
| Seawater nutrients | | | | |
| $NH_4^+$ µM | 0.14 ± 0.03 | 0.08 ± 0.02 | 0.07 ± 0.07 | 0.11 ± 0.09 |
| $PO_4^{3-}$ µM | 0.01 ± 0.02 | 0.01 ± 0.02 | 0.00 ± 0.03 | 0.02 ± 0.03 |
| $Si(OH)_4$ µM | 2.59 ± 1.39 | 2.48 ± 2.64 | 1.60 ± 0.81 | 0.91 ± 0.66 |
| $NO_3^-+NO_2^-$ µM | 2.05 ± 2.69 | 0.72 ± 0.92 | 0.44 ± 0.65 | 0.83 ± 1.06 |
| Seawater Sr/Ca | 12.29 ± 0.06 | 12.44 ± 0.05 | na | 12.44 ± 0.04 |
| $[Ca^{2+}]$ mM | 10.1 ± 0.1 | 10.7 ± 0.0 | | 10.7 ± 0.0 |
| Mean skeleton Sr/Ca | | | | |
| Genotype 4 (*P. murrayensis*) | 11.85 ± 0.09 (21) | 12.48 ± 0.10 (22) | na | 12.34 ± 0.09 (25) |
| Genotype 5 (*P. lutea*) | 11.97 ± 0.08 (35) | 12.42 ± 0.13 (24) | na | 12.36 ± 0.12 (21) |
| Genotype 6 (*P. murrayensis*) | 12.29 ± 0.07 (55) | 12.51 ± 0.07 (50) | na | 12.58 ± 0.10 (55) |
| Genotype 7(*P. lutea*) | 12.00 ± 0.04 (49) | 12.41 ± 0.04 (58) | na | 12.40 ± 0.06 (48) |

## 2.3 Coral sample processing and analysis

Cleaned skeletons were rinsed repeatedly in deionised water, sawn perpendicular to the growth surface to produce skeletal strips along the maximum growth axes, dried and fixed in 25 mm epoxy resin blocks (Epofix, Struers Ltd.). Blocks were polished using silicon carbide papers (up to 4000 grade, lubricated with water) and polishing alumina (0.05 µm, suspended in water).

Polished, gold coated sections were analysed in the School of Geosciences at the University of Edinburgh, UK by secondary ion mass spectrometry (SIMS). SIMS is a high spatial resolution technique allowing us to confidently analyse skeleton deposited under different conditions. During calcification the coral precipitates aragonite both at the skeleton surface (extending the skeletal trabecula units) and further back in the skeleton (thickening the existing units). Reflecting this, Alizarin Red S stain is incorporated at the tips of trabeculae and, further back in the skeleton, along their edges (Figure 1b). Bulk analyses of drilled coral skeletons can combine aragonite deposited at the skeletal surface with thickening deposits (which may be deposited up to weeks or months later), hampering the accurate identification of palaeoproxy relationships (Gagan et al., 2012). For this study we focused the primary SIMS beam to a diameter of ~25 μm and positioned it approximately midway between the centres of calcification (occurring at the approximate centres of the trabeculae) and the edge of the trabeculae (Figure 1c). Scanning electron micrographs indicate that trabecula are thickened to reach almost their full width within a few days of deposition (Allison et al., 2010) and we are confident that our analyses sample the aragonite deposited during the 28°C period without any contribution of material deposited at 25°C. $K_D$ Sr/Ca did not vary significantly between duplicates of the same coral genotype in each $pCO_2$ treatment in our previous culturing work (Cole et al., 2016) so we analysed one individual of each clone in this study. Multiple SIMS analyses were evenly spaced across the skeleton deposited in 2-5 different corallites of each colony over each experimental period (Figure 1c) and numbers of analyses in each individual are detailed in Table 1. Centres of calcification, which have subtly different Sr/Ca compared to the fasciculi, appear as dark spots or lines in the section surface in reflected light (Allison et al., 2010) and were not analysed in this study. These structures are frequently dissolved in ancient corals (Allison et al., 2007).

SIMS data were collected in two sessions each of 7 days using a Cameca imf-4f ion microprobe ($^{16}O^-$ ion beam accelerated at 10.8 kV; primary beam current = 8 nA; energy offset = 75 eV, energy window = ±20 eV), and a Cameca 1270 ion microprobe ($^{16}O^-$ ion beam accelerated at 12 kV; primary beam current = 8 nA; energy offset = 75 eV; energy window = ±20 eV). Relative ion yields (RIYs) were comparable between the 2 instruments and did not vary significantly within or between days unless substantial refocusing of the primary beam was undertaken. We used a pre-analysis sputter of 1 minute to remove surface contamination. Each analysis is the sum of ten cycles and, for each cycle, we collected secondary singly-charged cations at masses $^{44}Ca$ (2 s) and $^{88}Sr$ (4 s). Count rates were typically ~180,000 and ~80,000 counts per second (cps) respectively using the Cameca 4f and were ~40% higher on the Cameca 1270. The total time per analysis (including other isotopes not reported here) was 8 min and during this time the primary beam sputtered the sample to a depth of 2-3 μm (Allison et al., 2013). We estimate no significant isobaric interference for any of the isotopes studied (Allison et al., 1996). SIMS analyses were normalised to multiple analyses each day of a *Desmophyllum* sp. coral aragonite standard, NaHaxby2 (Sr/Ca = ~2.87 mmol mol$^{-1}$). Internal reproducibility was calculated from ten cycles of a single SIMS analysis (2σ /√10) and was ~0.2% for Sr/Ca. External reproducibility (the precision of ~17 daily analyses on the standard, 2σ /√n) was ~0.4%, or 0.011 mmol mol$^{-1}$, (2σ) for Sr/Ca.

The accuracy of our SIMS estimates is affected by uncertainty in the composition of the standard (e.g. reflecting geochemical heterogeneity between grains used for SIMS and bulk characterisation). To

175  overcome this, skeletal Sr/Ca was also determined in bulk samples microdrilled from the outermost
176  sections (deposited at 25°C) of 3 coral heads of G7 cultured at 180, 260 and 750 µatm. These colonies
177  exhibited high calcification rates (Cole et al., 2018) and we were able to drill sufficient material for bulk
178  analyses. This was the final seawater temperature tested so all aragonite was deposited at 25°C
179  regardless of any skeletal thickening. The bulk samples were dissolved by submerging the powder in
180  18.2 MΩ·cm water (Milli-Q) and adding drops of 1M acetic acid with brief sonication until no bubbles
181  could be seen around the aragonite. Following centrifugation, the supernatants were transferred into
182  pre-cleaned Savillex$^{TM}$ vials and converted to $NO_3^-$ form via concentrated $HNO_3$ refluxing. The Sr/Ca
183  analysis was carried out on an Agilent 7500ce ICP-MS at GEOMAR, Kiel. A known amount of dissolved
184  sample corresponding to 10 µg g$^{-1}$ of Ca was taken for measurements using isotope dilution method
185  with simultaneous addition of known amounts of $^{42}$Ca and $^{84}$Sr isotope spikes. The well-characterised
186  JCp-1 coral was used as primary standard and analysed as unknowns to assess long-term
187  reproducibility of our results. The mean value of replicate analyses of JCp-1 during the course of this
188  study yield 8.86 mmol mol$^{-1}$ for Sr/Ca, which is within uncertainty of the mean of Sr/Ca among twenty
189  one different labs intercalibration (Hathorne et al., 2013). The long-term external reproducibility (1 σ) of
190  replicate analyses of JCp-1 during the course of this study was 0.24% or 0.021 mmol mol$^{-1}$ for Sr/Ca.
191  The mean SIMS Sr/Ca of the 2 fastest growing corals (cultured at 180 and 260 µatm) are 96.8 and
192  97.2% of the bulk values (Figure 2). For the third coral the mean SIMS Sr/Ca is 95.5% of the bulk
193  estimate. This coral grew the slowest and the bulk drilled powder had a pink colouration indicating that
194  some of the alizarin stain was sampled during drilling. Alizarin red suppresses coral calcification and
195  photosynthesis rates (Allison et al., 2011) and the Sr/Ca of the bulk sample of this skeleton may be
196  affected by this. We normalised all SIMS data to the mean of bulk analyses of the two fast growing
197  skeletons (i.e. by multiplying the data by 1.03).

198  To reduce the effect of calibration difference between and within SIMS sessions, analyses on the
199  skeleton deposited at 25 and 28°C in each coral were made on the same day and analyses comparing
200  genotypes were made within the same week. 3 corals were analysed in both SIMS sessions and skeletal
201  Sr/Ca estimates agree within 0.8% between sessions. Due to beamtime limitations the G5 coral head
202  cultured at 400 µatm at 25°C was not analysed.

203

204  **Results**

205  Skeletal and seawater Sr/Ca data are summarised in Table 1 (full details of individual SIMS analyses
206  are included in the supplementary data) and we use these data to estimate Sr/Ca partition coefficients
207  as $K_D$ Sr/Ca = (skeletal Sr/Ca)/(seawater Sr/Ca) for each coral (Figure 3). These are best described as
208  apparent $K_D$ as the Sr/Ca of the coral ECM used to build the skeleton is unknown. Genotypes are
209  numbered from 4 to 7 (abbreviated to G4, G5 etc) to facilitate comparison with the published calcification
210  data for the same corals (Cole et al., 2018). We compared the $K_D$ between colonies of the same
211  genotype in different seawater $pCO_2$ and between different coral genotypes cultured in the same
212  seawater $pCO_2$ treatments (Table 2). Seawater $pCO_2$ affected Sr/Ca partitioning with $K_D$ significantly
213  lower at 180 µatm than at 750 µatm in all genotypes at 25°C and in 3 of the 4 genotypes at 28°C (Table
214  2). Similarly $K_D$ Sr/Ca at 180 µatm were significantly lower than at 260 µatm in 3 of the 4 genotypes at
215  both temperatures. Some significant variations in $K_D$ Sr/Ca were observed between genotypes within a

216  pCO$_2$ treatment and temperature (Table 2). K$_D$ G6 were significantly higher than for all other coral
217  genotypes at both 25 and 28°C in 180 µatm and at 28°C in 750 µatm. K$_D$ G6 was significantly higher
218  than G4 at 25°C and 28°C in 750 µatm and K$_D$ G4 was also lower than K$_D$ G7 at 28°C in 180 µatm
219  (Table 2). Temperature significantly affected Sr incorporation (paired 2 tailed t test comparing mean K$_D$
220  Sr/Ca for each head at 25 and 28$^0$C, p=0.000016) with K$_D$ Sr/Ca higher at 25°C than at 28°C in all corals
221  analysed. K$_D$ Sr/Ca did not vary significantly between duplicate colonies of the same coral genotype in
222  the same pCO$_2$ treatment in our previous culturing work (Cole et al., 2016) and we attribute differences
223  in K$_D$ Sr/Ca between coral colonies here to genotype or coral responses to seawater temperature/pCO$_2$.

224  To explore the origin of variations in skeletal Sr/Ca incorporation we plot linear regressions of K$_D$ Sr/Ca
225  as a function of seawater pCO$_2$ and coral calcification rate for each temperature. We produce these
226  plots for individual coral genotypes (Figure 4) and for all corals combined (Figure 5), including specimens
227  previously cultured at 180, 400 and 750 µatm and  25°C in a previous study in our laboratory (Cole et
228  al., 2016). We observe inverse correlations (r$^2$>0.5) between K$_D$ Sr/Ca and seawater pCO$_2$ and
229  calcification rate within most genotypes at 25°C but not at 28°C (Table 3) but these datasets are too
230  small (n=3-4) for meaningful statistical analysis. In the combined datasets we observe weak significant
231  positive correlations between K$_D$ Sr/Ca and seawater pCO$_2$ at 25°C but other relationships are
232  insignificant (Table 3). Finally, a multiple linear regression analysis indicates that a significant degree
233  (p=0.00096) of variance in K$_D$ Sr/Ca across the combined dataset is dependent on temperature and
234  seawater pCO$_2$ but independent of calcification rate (Table 4).

235
236

237  **Table 2**. Summary of significant differences (p≤0.05) comparing K$_D$ Sr/Ca between colonies of the same
238  genotype in different seawater pCO$_2$ (µatm) and between different coral genotypes cultured in the same
239  seawater pCO$_2$ treatments e.g. '400<260,750' shows that K$_D$ Sr/Ca in the 400 µatm treatment was
240  significantly lower than in both the 260 and 750 µatm treatments. Significant differences were identified
241  by one way ANOVA followed by Tukey's pairwise comparisons. ns = not significant, nd= not determined.

| Parameter | 25°C | 28°C |
|---|---|---|
| Comparing corals of the same genotype between seawater pCO2 treatments | | |
| Genotype 4 | 400<260, 750<br>180<260, 750 | 180<260,750 |
| Genotype 5 | 180<260, 750 | 180<260,750 |
| Genotype 6 | 180<750 | ns |
| Genotype 7 | 180<260,400,750<br>260<750<br>400<260 | 180<260,750 |
| Comparing corals of different genotype in the same seawater pCO2 treatment | | |
| Seawater pCO$_2$ = 180 µatm | G4, G5, G7<G6 | G4, G5, G7<G6<br>G4<G7 |
| Seawater pCO$_2$ = 260 µatm | ns | ns |
| Seawater pCO$_2$ = 400 µatm | ns | nd |
| Seawater pCO$_2$ = 750 µatm | G4<G6 | G4,G5,G7<G6 |

**Table 3**. Coefficients of determination ($r^2$) and p values for linear regressions between coral $K_D$ Sr/Ca and seawater $pCO_2$ and calcification rate (Cole et al., 2018) for individual coral genotypes and for the entire dataset combined with additional data for corals cultured at 25°C and seawater pCO2 of ~180, 400 and 750 µatm from Cole et al.,, 2016. $p < 0.05$ are highlighted in bold.

| Parameter | 25°C | | 28°C | |
|---|---|---|---|---|
| | $r^2$ | p | $r^2$ | p |
| Seawater $pCO_2$ | | | | |
| Genotype 4 | 0.20 | 0.55 | 0.14 | 0.76 |
| Genotype 5 | 0.62 | 0.42 | 0.20 | 0.71 |
| Genotype 6 | **0.93** | **0.035** | 0.88 | 0.22 |
| Genotype 7 | 0.37 | 0.43 | 0.35 | 0.60 |
| Coral calcification rate | | | | |
| Genotype 4 | 0.52 | 0.28 | 0.48 | 0.51 |
| Genotype 5 | 0.74 | 0.34 | 0.31 | 0.62 |
| Genotype 6 | 0.80 | 0.10 | **1.00** | **0.011** |
| Genotype 7 | 0.00 | 0.99 | 0.09 | 0.81 |
| Entire dataset combined with Cole et al., 2016 | | | | |
| Seawater $pCO_2$ | 0.20 | **0.022** | 0.15 | 0.22 |
| Coral calcification rate | 0.055 | 0.25 | 0.051 | 0.48 |

**Table 4**. Intercept, gradients and goodness of fit of a multiple linear regression model to predict skeletal $K_D$ Sr/Ca from seawater temperature, $pCO_2$ and calcification rate. This analysis combined data from the present study with that of Cole et al., 2016 (normalised to the bulk skeletal Sr/Ca determination analysed here to enable direct comparison with the present study). The coefficient of determination of the model ($r^2$) = 0.38.

| | Coefficients ± standard error | P value |
|---|---|---|
| Intercept | 1.14 ± 0.043 | $4.22 \times 10^{-24}$ |
| Seawater $pCO_2$ | $2.35 \times 10^{-5} \pm 0.98 \times 10^{-5}$ | 0.021 |
| Temperature | -0.00533 ± 0.0017 | 0.0032 |
| Calcification | -0.00015 ± 0.00026 | 0.57 |

## **Discussion**

Both temperature and seawater $pCO_2$ significantly affect $K_D$ Sr/Ca in this study (Table 4). Increasing seawater temperature is associated with a reduction in $K_D$ Sr/Ca while seawater $pCO_2$ and $K_D$ Sr/Ca are positively related. Similar increases in skeletal Sr/Ca have been reported in some corals cultured at high seawater $pCO_2$ compared to ambient (Tanaka et al., 2015; Cole et al., 2016) but not in others (Kuffner et al., 2012; Cole et al., 2016). Given these disparities it is likely that $pCO_2$ driven variations in $K_D$ Sr/Ca are mediated by responses of coral biomineralisation to seawater $pCO_2$ rather than the direct influence of seawater DIC chemistry.

**4.1 Palaeoenvironmental implications of changes in $K_D$ Sr/Ca**

Modern day seawater $pCO_2$ has increased dramatically in the last 200 years. Atmospheric $CO_2$ oscillated from ~ 180 µatm during the ice ages to ~260-280 µatm during the interglacials (Luthi et al., 2008) from ~800 ky to the pre-industrial (~1760) but today atmospheric $CO_2$ exceeds 400 µatm. Our finding that coral $K_D$ Sr/Ca is reduced at low seawater $pCO_2$ suggests that applying Sr/Ca-SST calibrations derived from modern day corals (growing at relatively high seawater $pCO_2$) to both pre-industrial specimens and older carbonate records in modern corals will result in overestimation of seawater temperatures.

We temper this finding with our conclusion that skeletal Sr/Ca probably does not directly reflect seawater $pCO_2$ but rather the response of the biomineralisation process to seawater $pCO_2$. The calcification rates of corals cultured at 180 and 260 µatm in our laboratory are usually higher than their clones cultured at 400 µatm (Cole et al., 2018). However skeletal extension rates of corals which grew during periods of lower atmospheric $CO_2$ can be lower than their modern day counterparts. For example, skeletal extension of a 9.5 ky Tahiti Porites (growing under a $CO_2$ atmosphere of ~ 260 ppm) is about 75% that of a modern day specimen from a nearby location (DeLong et al., 2010; Boiseau et al., 1998). Coral calcification is affected by seawater temperature (Lough and Barnes 2000), light (Chalker, 1981) and photosynthesis (Gattuso et al., 1999) as well as genotype (Cole et al., 2018) and changes in these environmental parameters could also influence skeletal Sr/Ca (see section 4.2).

The regression analysis (Table 4) indicates that the influence of temperature on $K_D$ Sr/Ca is -0.0053 °C$^{-1}$, equivalent to a change in skeletal Sr/Ca of 0.046 mmol mol$^{-1}$ °C$^{-1}$, assuming a reefwater Sr/Ca of 8.541 mmol mol$^{-1}$ (de Villiers et al., 1994). This temperature dependence is similar to that reported in some inorganic aragonites (Kinsman and Holland, 1969, Dietzel et al., 2004, but lower than that reported in Gaetani and Cohen, 2006). The temperature sensitivity of skeletal Sr/Ca in massive *Porites* spp. corals collected in modern reef environments is highly variable (from 0.041 to 0.082 mmol mol$^{-1}$, Correge 2006). Skeletal thickening (the deposition of new aragonite onto existing trabeculae) can compromise the identification of the exact skeletal Sr/Ca temperature dependence (Gagan et al., 2012) as sampling can combine material deposited under varying tempertaures. Gagan et al (2012) calculate a temperature sensitivity of 0.084 mmol mol$^{-1}$ °C$^{-1}$ in *Porites* spp. after correcting for this thickening. This is in good agreement with other estimates derived by comparing mean annual Sr/Ca and SST of multiple *Porites* spp. from a broad range of localities and temperatures (0.089 and 0.081 mmol mol$^{-1}$ °C$^{-1}$ (DeLong et al., 2010; Murty et al., 2018) and from SIMS analysis (avoiding thickening deposits) of a Hawaiian *P. lobata* (0.080 mmol mol$^{-1}$ °C$^{-1}$, Allison and Finch, 2004). However these calibrations encompass the effects of all seasonal influences, not just temperature, on skeletal Sr/Ca. Our observation that coral Sr/Ca temperature sensitivity is comparable to that of inorganic aragonite in a culture system suggests that seasonal changes besides temperature may also play an important role in controlling skeletal Sr/Ca.

In our study $K_D$ Sr/Ca were significantly lower at 180 µatm compared to 260 µatm in 3 of the 4 coral genotypes at both temperatures. These data suggest that even the relatively minor changes in seawater $pCO_2$ observed over glacial-interglacial cycles from 800 ky to ~1760 before present (Luthi et al., 2008) may have influenced coral skeletal Sr/Ca. Our regression analysis (Table 4) indicates that the influence

305 of seawater $pCO_2$ on $K_D$ Sr/Ca is 0.000024 µatm$^{-1}$, equivalent to a change in skeletal Sr/Ca of +0.020
306 mmol mol$^{-1}$ per 100 µatm $CO_2$ increase. This change in skeletal Sr/Ca is equivalent to 0.44°C per 100
307 µatm CO2 increase using the Sr/Ca temperature dependence derived in this study and 0.33°C using
308 the mean Sr/Ca-SST *Porites* spp. calibration of Correge (2006). Atmospheric $CO_2$ varies by up to ~80
309 ppm between glacial and interglacial periods and oscillations of this magnitude would have a minimal
310 effect on coral derived seawater temperatures. No significant trend in skeletal Sr/Ca is observed in a
311 modern *Porites* sp. skeleton record spanning ~1880 to 1998 (Nurhati et al., 2011), although atmospheric
312 $CO_2$ increased by ~100 ppm over the duration of this record Luthi et al., 2008). This change in skeletal
313 Sr/Ca is equivalent to <0.25°C on the palaeothermometer equation used in the study (Nurhati et al.,
314 2011) and it is unlikely to be detected in the record. Atmospheric $CO_2$ changes between the present and
315 glacial periods are more significant (>200 ppm) and could lead to larger reconstructed seawater
316 temperature errors.

317 Given the variability between coral genotypes in this study and others (Cole et al., 2016), it is likely that
318 variations in $K_D$ Sr/Ca between seawater $pCO_2$ treatments reflect responses of coral biomineralisation
319 rather than the direct influence of seawater DIC chemistry. During our study, corals were acclimated to
320 altered seawater $pCO_2$ over periods of months and it is not certain that their geochemistry responses
321 duplicate those of corals which grew naturally at low seawater $pCO_2$. We find no evidence that $K_D$ Sr/Ca
322 are increased in corals at low seawater $pCO_2$, a potential explanation for the cold bias observed in some
323 older coral skeletons (reviewed in Gagan et al., 2012). The higher skeletal Sr/Ca observed in these older
324 specimens may indicate deposition of secondary cements in the coral skeleton (McGregor and Gagan,
325 2003), post-depositional re-equilibration processes, variations in the seawater Sr/Ca at the time of coral
326 deposition (Stoll and Schrag, 1998) or changes in coral physiological processes that influence skeletal
327 Sr/Ca e.g. rate of skeletogenesis.

328

329 **4.2 Origin of $K_D$ Sr/Ca variations in coral aragonite**

330 Multiple observations indicate that coral skeletal Sr/Ca is affected by other factors besides seawater
331 temperature. Large variations in Sr/Ca-SST relationships are observed between modern *Porites* spp.
332 corals collected from the same reef location or cultured in the same laboratory (e.g. this study, Alpert et
333 al. 2016). Large ~monthly Sr/Ca cycles are resolved in coral skeletons by high resolution microanalysis
334 methods that are not related to seawater temperature (Meibom et al., 2003). Skeletal Sr/Ca is lower
335 than synthetic aragonites precipitated at comparable temperatures (Gaetani and Cohen, 2006) and in
336 symbiotic versus asymbiotic (non-algal bearing) polyps of the same coral species raised in the same
337 conditions (Inoue et al., 2018). We discuss three popular hypotheses that are invoked to explain Sr/Ca
338 variability in coral aragonite and consider how they relate to the data in this study.

339

340 **4.2.1 Variations in Sr/Ca$_{ECM}$ dependent on Rayleigh fractionation**

341 Rayleigh fractionation occurs when a proportion of a fluid reservoir is utilised during $CaCO_3$ precipitation
342 (Elderfield et al., 1996) and the $K_D$ of the element of interest does not equal 1. In the case of elements
343 (Me) with $K_D$ Me/Ca > 1, the Me/Ca of the fluid decreases as precipitation proceeds and Me is

344 preferentially removed from the solution compared to Ca. Precipitation of a relatively large proportion of
345 the reservoir then produces a $CaCO_3$ with a lower bulk Me/Ca composition than when a smaller portion
346 of the reservoir is utilised. Estimates of $K_D$ Sr/Ca at 25°C in inorganic aragonite precipitations range from
347 ~1.06 to 1.14 (AlKhatib and Eisenhauer, 2017; Gaetani and Cohen, 2006; Dietzel et al., 2004). These
348 $K_D$ are higher than observed in coral (Giri et al., 2018; Cole et al., 2016; this study). $K_D$ Sr/Ca for coral
349 aragonite should be viewed as apparent distribution coefficients, calculated by comparing skeletal and
350 seawater Sr/Ca. The coral aragonite forms from the ECM and the Sr/Ca of this fluid is unknown.

351 If we assume that the actual $K_D$ Sr/Ca in coral aragonite are >1 i.e. describing partitioning between the
352 ECM and skeleton, as suggested in inorganic aragonite precipitations, then aragonite Sr/Ca will be high
353 when a small proportion of the reservoir is used for calcification and will decrease as more of the ions
354 in the ECM are utilised. Aragonite precipitation rates from seawater are largely controlled by $[CO_3^{2-}]$
355 (Burton and Walter, 1987), itself dependent on [DIC] and seawater pH. *Porites* spp. corals cultured at
356 high seawater $pCO_2$ typically have lower $pH_{ECM}$ and reduced calcification rates (Allison et al, 2018a and
357 Cole et al., 2018 respectively), which are both suggestive of limited $CaCO_3$ precipitation. In this respect
358 our observation of higher $K_D$ Sr/Ca in corals cultured at high seawater $pCO_2$ (Figures 4 and 5) supports
359 the Rayleigh fractionation hypothesis. Despite this we do not observe consistent inverse correlations
360 between calcification rate and $K_D$ Sr/Ca (Figure 4) or identify calcification as a control on $K_D$ Sr/Ca (Table
361 4). We note that calcification per se may not provide a good indication of the proportion of the ECM
362 reservoir used during calcification. As the skeletal aragonite crystals are deposited approximately
363 perpendicular to the maximum growth axis of the coral skeleton in *Porites* spp., variations in coral
364 calcification may reflect an increase in the surface area over which aragonite is deposited instead of the
365 extension rates of individual crystals. We also do not observe a significant relationship between $K_D$
366 Sr/Ca and the pH of the ECM (Figure 6a) in a suite of corals cultured over varying seawater $pCO_2$ at
367 25°C in our laboratory (Cole et al., 2016; Allison et al., 2018a). $pH_{ECM}$ is a potential indicator of the
368 saturation state of the ECM and thereby a proxy for the proportion of the ECM reservoir utilised during
369 calcification.

370

371 **4.2.2 The growth entrapment model**

372 The growth entrapment model (Watson, 1996) describes the growth dependent enrichment or depletion
373 of trace/minor elements in the surface of crystals relative to the bulk crystal. The chemical composition
374 of the surface zone of a crystal may not reach equilibrium with the aqueous solution if crystal growth is
375 rapid and the diffusivity of the element in the crystal is slow. Instead a non-equilibrium composition can
376 become buried (or entrapped) in the crystal by subsequent crystal growth.

377 $K_D$ Sr/Ca at 25°C have been both positively and negatively correlated with aragonite precipitation rate
378 (AlKhatib and Eisenhauer, 2017). This inconsistency could reflect the adsorption of $Mg^{2+}$ to the crystal
379 surface which reduces uptake of $Sr^{2+}$ (AlKhatib and Eisenhauer, 2017). Given this uncertainty it's
380 unclear how growth entrapment will influence Sr/Ca in coral aragonite. Our observation of higher $K_D$
381 Sr/Ca in corals cultured at high seawater $pCO_2$ could reflect growth entrapment, assuming aragonite
382 Sr/Ca increases at low crystal growth rates. However as noted above we do not observe significant
383 relationships between calcification rate and Sr/Ca to support a growth entrapment mechanism although,

384    once again we note that calcification rates may not provide a reliable indicator of the extension rates of
385    individual skeletal crystals.

386

387    **4.2.3 Variations in Sr/Ca$_{ECM}$ dependent on ion transport**

388    The Sr/Ca of the ECM, used to build the skeleton may vary between corals. Ca$^{2+}$ is transported across
389    the coral tissues by a L type Ca$^{2+}$ channel (Zoccola et al., 1999) and by a plasma membrane Ca-ATPase
390    (Zoccola et al., 2004). It is uncertain if Sr$^{2+}$ is also transported by these processes (Carafoli, 1987; Ip
391    and Lim, 1991; Yu and Inesi, 1995) but any discrimination against Sr will reduce both Sr/Ca$_{ECM}$ and K$_D$
392    Sr/Ca. Corals at high seawater pCO$_2$ increase the H$^+_{sw}$-H$^+_{ECM}$ gradient more than corals at ambient
393    seawater pCO$_2$ (Venn et al., 2013) indicating that Ca-ATPase activity may be enhanced in these corals
394    as the enzyme is a H$^+$/Ca$^{2+}$ antiporter (Allemand et al., 2011). However we do not observe significant
395    relationships between the K$_D$ Sr/Ca reported in Cole et al 2016 and the H$^+$ gradient between the culture
396    seawater and the calcification media (termed H$^+_{sw}$-H$^+_{ECM}$) for the same corals (Figure 6b) to support this
397    hypothesis. We calculate the gradient from the pH$_{seawater}$ and pH$_{ECM}$ reported for the same corals (Allison
398    et al., 2018a). These observations are not consistent with the hypothesis that Ca-ATPase activity is
399    reduced at high seawater pCO$_2$ resulting in less dilution of Sr/Ca$_{ECM}$, assuming the enzyme discriminates
400    against Sr$^{2+}$ (Tanaka et al., 2015). Inhibiting Ca-ATPase and Ca$^{2+}$ channels in the branching coral
401    *Pocillopora damicornis* did not affect skeletal Sr/Ca suggesting these transport processes do not
402    fractionate Sr/Ca (Allison et al., 2011).

403    After considering these three hypotheses we are unable to resolve the source of K$_D$ Sr/Ca variations
404    between seawater pCO$_2$ treatments. Variations in K$_D$ Sr/Ca between genotypes are relatively small in
405    the 260 µatm treatment compared to other seawater pCO$_2$ conditions (Figure 5). It is noteworthy that
406    these smallest variations in skeletal geochemistry are observed in the conditions under which corals
407    naturally grew before the rapid addition of anthropogenic CO$_2$ to seawater in the last 2 centuries.

408

409    **4.3 Absolute K$_D$ Sr/Ca values in the present study**

410    The K$_D$ Sr/Ca determined here (0.980 to 1.019) are substantially lower than estimated from Sr/Ca-SST
411    calibrations of *Porites* spp. collected from natural habitats. Combining the mean Sr/Ca-SST calibration
412    for *Porites* spp. determined by Correge (2006) i.e. Sr/Ca = -0.0607 x SST + 10.553, with a reefwater
413    Sr/Ca of 8.540 mmol mol$^{-1}$ (the mean of measurements by de Villiers et al., 1994 and Sun et al., 2005)
414    yields a K$_D$ Sr/Ca of 1.058 at 25°C. Surface seawater Sr/Ca may vary by up to 2% (summarised in Sun
415    et al., 2005) but changes of this magnitude are still insufficient to account for the offset between the K$_D$
416    Sr/Ca in this study and previous estimates. Our K$_D$ Sr/Ca are also lower than in culturing studies of the
417    branching coral *Pocillopora damicornis* at typical seawater [Ca$^{2+}$] and present day atmospheric CO$_2$ (K$_D$
418    = 1.03-1.04, Giri et al., 2018). We consider it most likely that the low K$_D$ Sr/Ca observed in this study
419    reflect a decrease in the Sr/Ca of the ECM used to build the skeleton. This could reflect enhanced
420    transport of Ca$^{2+}$ relative to Sr$^{2+}$ into the ECM (ion transport) or could indicate that a relatively large
421    proportion of the ions in the ECM reservoir are precipitated (Rayleigh fractionation) in the corals in our
422    culture system.

423   We consider it unlikely that $K_D$ Sr/Ca reflect large differences in the physiological behaviour of corals in
424   this culture study compared to field specimens i.e. growing in the natural environment. Calcification rates
425   in this study range from 5-40 µmol $CaCO_3$ $cm^{-2}$ $day^{-1}$ (Cole et al., 2018) and are in good agreement with
426   estimates from modern field *Porites* spp.  Massive *Porites* spp. typically extend their skeletons by 8-20
427   mm $year^{-1}$ with a typical skeletal density of 0.9-0.7 g $cm^{-3}$ (Allison et al., 1996) equivalent to calcification
428   rates of 19-38 µmol $CaCO_3$ $cm^{-2}$ $day^{-1}$. Lower calcification rates in the culture study reported here are
429   usually associated with the artificial increase of seawater $pCO_2$ (Cole et al., 2018). Respiration rates in
430   the corals cultured here ranged from 0.8-1.4 µmol $O_2$ $cm^{-2}$ $h^{-1}$ (Cole et al., 2018), in good agreement
431   with estimates from field corals (0.6-1 µmol $O_2$ $cm^{-2}$ $h^{-1}$ Hennige et al., 2010). Gross photosynthesis
432   rates in the cultured corals ranged from 2-6 µmol $O_2$ $cm^{-2}$ $h^{-1}$ (Cole et al., 2018) and are equivalent to or
433   higher than other estimates from field corals (15-28 µmol $O_2$ $cm^{-2}$ $12h^{-1}$ i.e. 1.3-2.3 µmol $O_2$ $cm^{--2}$ $h^{-1}$
434   (Hennige et al., 2010). Skeletal Sr/Ca is lower in symbiotic *Acropora digitifera* compared to
435   azooxanthellate specimens cultured under the same conditions (Inoue et al., 2018) and variations in
436   colony photosynthesis between our study and field specimens could influence $K_D$ Sr/Ca. However light
437   conditions were the same between seawater $pCO_2$ treatments and between temperatures in the present
438   study so this does not affect $K_D$ Sr/Ca between treatments.

439   Seawater [Sr] in the present study is higher than in reefwaters although [$Ca^{2+}$] is comparable, resulting
440   in increased seawater Sr/Ca (12.29-12.44 mmol $mol^{-1}$, Table 1) compared to natural reefwaters
441   (8.5 mmol $mol^{-1}$, De Villiers et al., 1994; Sun et al., 2005). Skeletal Sr/Ca in the branching coral
442   *Pocillopora damicornis* is affected by the [$Ca^{2+}$] of seawater and reducing [$Ca^{2+}$] by ~40% (thereby
443   increasing Sr/Ca) reduced coral $K_D$ Sr/Ca by ~15% (Giri et al., 2018). $Ca^{2+}$ is a key substrate in the
444   production of $CaCO_3$ and increased transcellular $Ca^{2+}$ transport at low seawater [$Ca^{2+}$] will reduce
445   Sr/$Ca_{ECM}$ if transport processes discriminate against $Sr^{2+}$. It is unclear how changes in seawater Sr/Ca
446   driven by variations in $Sr^{2+}$ will impact $K_D$ Sr/Ca. We cannot rule out the possibility that the change in
447   seawater [$Sr^{2+}$] affects $K_D$ however variations in Sr/Ca and [$Ca^{2+}$] between treatments are small (Table
448   1) and are unlikely to drive the variations in coral $K_D$ observed between treatments.

449   SIMS analyses in this study were focused midway between centres of calcification and the outside
450   edges of the trabeculae. Centres of calcification are relatively enriched in Sr/Ca (Allison and Finch,
451   2004) and the exclusion of this material from the analyses could bias the SIMS estimates to lower Sr/Ca
452   values. However the SIMS analyses were normalised to bulk Sr/Ca estimates (combining fasciculi and
453   centres of calcification) from 2 coral samples so this is unlikely to be a significant factor.

454   We observe large variations in $K_D$ between corals of different genotype from the same treatment. Similar
455   variations in skeletal Sr/Ca are reported in other culturing (Cole et al., 2016; Tanaka et al., 2015) and
456   field studies (Alpert et al., 2016). We considered whether any other physical or chemical skeletal
457   parameter could be used to predict the $K_D$ Sr/Ca to apply to a coral specimen. We are unable to identify
458   any single factor (calcification rate, $pH_{ECM}$ or $H^+_{sw}$-$H^+_{ECM}$) that correlates consistently with $K_D$ Sr/Ca to
459   enable us to estimate the correct $K_D$ Sr/Ca to apply to coral records. Similarly we did not observe
460   consistent relationships between $K_D$ Sr/Ca and $K_D$ Mg/Ca or $K_D$ Ba/Ca in our first study culturing massive
461   *Porites* spp. over a range of seawater $pCO_2$ (Allison et al. 2018b). We conclude that it is not yet possible
462   to identify the correct $K_D$ Sr/Ca to apply to older coral skeletons for accurate seawater temperature
463   reconstruction.

464

465    All seawater and skeletal Sr/Ca data are included in an electronic annex.

466

475

**Figure legends**

**Figure 1**. Images of coral skeletons cultured at seawater $pCO_2$ of 260 µatm.. A: Alizarin Red S stain lines indicate 1 = the start of the experimental period at 28°C, 2 = the end of the 28°C period and 3 = the start of the experimental period at 25°C. The coral was sacrificed at the end of the 25°C period. B: A higher resolution image indicating the incorporation of stain over skeletal structures (see arrow). C: A polished block of skeleton after SIMS analysis. The black circles are SIMS pits and the arrow indicates the growth direction of the skeleton. A trabecula is annotated.

**Figure 2**. Comparison of skeletal Sr/Ca (mmol mol$^{-1}$) in 3 coral samples (all genotype 7 at 25°C) analysed by SIMS (normalised to NAHaxby2) and ICP-MS (isotope dilution method). The $pCO_2$ conditions under which each coral was cultured are indicated. The dotted line indicates where SIMS and ICP-MS analyses are equal. Error bars are 95% confidence limits for SIMS Sr/Ca and 1 standard deviation of repeat analyses of the standard by ICP-MS.

**Figure 3.** Influence of seawater $pCO_2$ and temperature on $K_D$ Sr/Ca in 4 genotypes of *Porites* spp. Bars represent the mean of multiple analyses (n = 15-59) across 2 or more corallites within colonies except for G6, 400 µatm, 25°C where n=6. Bars indicate mean $K_D$ Sr/Ca and error bars indicate 95% confidence limits (propagating 95% confidence limits in the determinations of skeletal and seawater Sr/Ca as $K_D$ Sr/Ca error = $\sqrt{((\text{skeletal Sr/Ca error})^2 + (\text{seawater Sr/Ca error})^2)}$.

**Figure 4**. Relationships between $K_D$ Sr/Ca and calcification rate (Cole et al., 2018) and seawater $pCO_2$ in each coral genotype. Coefficients of determination ($r^2$) and p values for linear regressions are summarised in Table 3. Note the difference in scale between 25 ºC and 28ºC.

**Figure 5**. Relationships between $K_D$ Sr/Ca and seawater $pCO_2$ and calcification rate (Cole et al., 2018) for the combined dataset (all corals) at each temperature. Linear regressions at 25°C combine the data from this study with that of Cole et al 2016, normalised to the bulk skeletal Sr/Ca determination analysed here to enable direct comparison with the present study.

**Figure 6**. Relationships between $K_D$ Sr/Ca and a) $pH_{ECM}$ and b) $H^+_{sw}$-$H^+_{ECM}$ in a series of corals cultured over varying seawater $pCO_2$ in our laboratory in a previous experiment. $K_D$ Sr/Ca are from Cole et al.,

502 2016, $pH_{ECM}$ are from Allison et al., 2018a and $H^+_{sw}$-$H^+_{ECM}$ are calculated from the $pH_{seawater}$ and $pH_{ECM}$
503 data in Allison et al., 2018a. Coefficients of determination ($r^2$) and p values for linear regressions are
504 overlain on each graph.

505

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A

3

2

1

1 mm

B

200 µm

C

trabecula

200 µm

Panel A: x-axis $pH_{ECM}$ total scale (8.3 to 8.6); y-axis $K_D$ Sr/Ca (0.97 to 1.05). Annotation: $r^2 = 0.00$, $p = 0.05$.

Panel B: x-axis $H^+_{SW} - H^+_{ECM}$ ($\times 10^{-9}$ mol) (0 to 15); y-axis $K_D$ Sr/Ca (0.97 to 1.05). Annotation: $r^2 = 0.13$, $p = 0.28$.