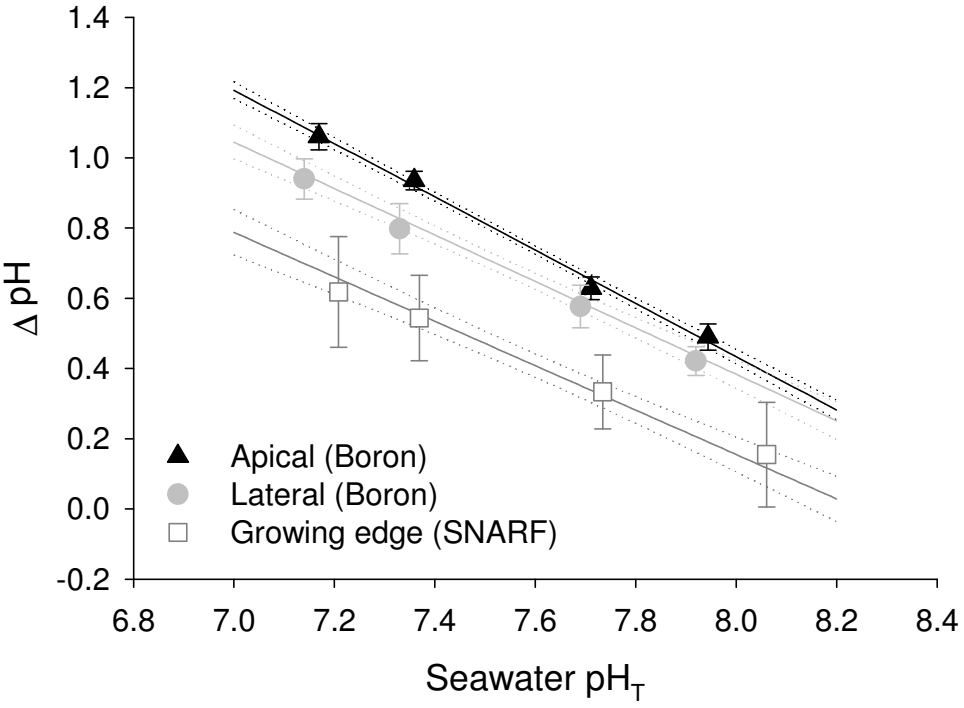
Supplemental Materials for: Coral calcifying fluid pH dictates response to ocean acidification

Authors: M. Holcomb, A. A. Venn, E. Tambutté, S. Tambutté, D. Allemand, J. Trotter, M. McCulloch



Supplementary Figure S1. Data from Fig 2b plotted as ΔpH, the difference between pH_{cf} and seawater pH_T, versus average seawater pH_T. Symbols are weighted means, error bars are standard deviation, with n=3 time-points, symbols for apical and lateral measurements are offset on the x-axis for clarity.

Supplementary Table S1

Average seawater chemistry for each tank. Data are presented as mean and standard deviation. Alkalinity and pH were measured, other values were calculated using CO2SYS[38-40], salinity was 38, temperature 25 °C. Units are µmol kg sw^{-1} for alkalinity, C_T, and CO_3^{2-}, µatm for fCO_2.

pH_T	Alkalinity	C_T	fCO_2	CO_3^{2-}	$\Omega_{Aragonite}$
7.17 ± 0.07	2480 ± 50	2540 ± 50	4120 ± 810	41 ± 7	0.63 ± 0.11
7.36 ± 0.08	2450 ± 40	2430 ± 30	2490 ± 510	63 ± 11	0.97 ± 0.17
7.71 ± 0.08	2450 ± 40	2300 ± 40	1090 ± 230	124 ± 20	1.93 ± 0.31
7.94 ± 0.04	2430 ± 40	2160 ± 40	550 ± 60	198 ± 16	3.07 ± 0.25

Supplementary Table S2

ANOVA results for $\delta^{11}B$ based pH_{cf} estimates, showing the significance for effects of CO_2, sampling location (apical versus lateral growth), and sampling date.

Source	Df	SS	Mean square	F	p
Model	6	0.551	0.092	31.54	<0.0001
Error	53	0.154	0.003		
CO_2	3	0.409	0.136	46.87	<0.0001
Location	1	0.103	0.103	35.28	<0.0001
Date	2	0.086	0.043	14.70	<0.0001

Supplementary Table S3

Linear regression coefficients (± standard error) for dependence of measured values on external pH_T (measured value = slope × seawater pH_T + intercept). For boron measurements, the average seawater pH_T for the given tank was used for regressions. For measurements at the growing edge, the pH_T value measured in seawater 100 μm from the growing edge at the time of pH_{cf} measurements was used for regression. All regressions were significant ($p<0.01$).

Measured value	Slope	Intercept	R^2
Apical $\delta^{11}B$	3.47 ± 0.47	-3.94 ± 3.55	0.62
Lateral $\delta^{11}B$	4.49 ± 0.70	-12.9 ± 5.3	0.64
Apical pH_{cf}	0.24 ± 0.03	6.51 ± 0.25	0.62
Lateral pH_{cf}	0.34 ± 0.05	5.68 ± 0.40	0.64
Growing edge pH_{cf}	0.37 ± 0.07	5.22 ± 0.54	0.49
Apical ΔpH	-0.76 ± 0.03	6.5 ± 0.25	0.94
Lateral ΔpH	-0.66 ± 0.05	5.68 ± 0.40	0.87
Growing edge ΔpH	-0.63 ± 0.07	5.22 ± 0.54	0.74

Supplementary Table S4.

$\delta^{11}B$ values measured for each sample. Coral indicates a number assigned to individual samples taken from a given tank and cultured for the same time to allow apical measurements to be compared with lateral for the same coral, a value of 'pooled' indicates all corals were pooled to provide enough material for measurement.

Coral	Culture time (months)	pH_T	Apical / Lateral	$\delta^{11}B$	Standard error
1	1.5	7.169	apical	20.08	0.25
1	1.5	7.359	apical	20.34	0.40
1	1.5	7.711	apical	21.24	0.27
1	1.5	7.944	apical	23.83	0.19
2	1.5	7.169	apical	20.23	0.23
2	1.5	7.359	apical	21.59	0.25
2	1.5	7.711	apical	22.28	0.22
2	1.5	7.944	apical	22.93	0.27
3	1.5	7.169	apical	19.93	0.33
3	1.5	7.359	apical	22.35	0.14
3	1.5	7.711	apical	21.50	0.20
3	1.5	7.944	apical	22.80	0.29
1	2	7.169	apical	21.92	0.08
1	2	7.359	apical	21.68	0.04
1	2	7.711	apical	23.54	0.02
1	2	7.944	apical	25.16	0.06
2	2	7.169	apical	20.00	0.02
2	2	7.359	apical	22.04	0.14
2	2	7.711	apical	22.70	0.15
2	2	7.944	apical	25.00	0.08
3	2	7.169	apical	22.05	0.11
3	2	7.359	apical	22.23	0.09
3	2	7.711	apical	22.48	0.08
3	2	7.944	apical	24.84	0.08
1	3	7.169	apical	21.18	0.18
1	3	7.359	apical	22.23	0.01

1	3	7.711	apical	22.07	0.03
1	3	7.944	apical	23.01	0.13
2	3	7.169	apical	21.74	0.18
2	3	7.359	apical	21.34	0.06
2	3	7.711	apical	23.20	0.07
2	3	7.944	apical	22.81	0.09
3	3	7.169	apical	20.77	0.09
3	3	7.359	apical	22.07	0.04
3	3	7.944	apical	23.96	0.03
pooled	1.5	7.169	lateral	18.72	0.11
pooled	1.5	7.359	lateral	18.62	0.14
pooled	1.5	7.711	lateral	20.51	0.07
pooled	1.5	7.944	lateral	22.02	0.08
1	2	7.711	lateral	20.62	0.04
1	2	7.944	lateral	22.34	0.04
2	2	7.169	lateral	19.08	0.04
2	2	7.359	lateral	20.01	0.05
2	2	7.711	lateral	21.87	0.11
2	2	7.944	lateral	23.01	0.15
3	2	7.711	lateral	22.04	0.25
3	2	7.944	lateral	21.91	0.11
1 and 3	2	7.169	lateral	18.52	0.13
1 and 3	2	7.359	lateral	19.81	0.42
1	3	7.169	lateral	20.60	0.03
1	3	7.359	lateral	21.37	0.03
1	3	7.711	lateral	23.10	0.10
1	3	7.944	lateral	23.88	0.00
2	3	7.169	lateral	20.63	0.01
2	3	7.359	lateral	21.23	0.10
2	3	7.711	lateral	22.96	0.14
2	3	7.944	lateral	24.15	0.03

3	3	7.169	lateral	20.64	0.23
3	3	7.359	lateral	21.68	0.05
3	3	7.944	lateral	23.77	0.04

Materials and Methods

Corals and coral growth rates

Colonies of *Stylophora pistillata* were originally collected from the Gulf of Aqaba (Jordan) at 5 m depth in 1990 and subsequently propagated clonally at the Centre Scientifique de Monaco. Fragments from these colonies were maintained in Mediterranean seawater (S=38, T=25^{o}C) at various CO_2 levels (maintained by injecting CO_2) and an irradiance of ~170 μmol photons m^{-2} s^{-1}. Branch tips were attached to glass coverslips (see Fig. 1 for an example colony) for use in lateral growth assays[32,34]. All corals had been previously maintained at the given pCO_2 for at least a year, thus the material attached to the coverslip as well as all subsequent growth formed under the desired pCO_2 treatment. Aquarium chemistries are summarized in Table S1, details on aquarium setup, pH and alkalinity measurement, and aquarium maintenance are provided in[28]. One aquarium was used per treatment condition, however experiments were repeated at least three times over the course of a year with at least three replicate corals used each time.

Apical growth (extension) was assessed by measuring the increase in height of branch tips attached to glass slides. Branch tips ~1 cm high were attached to glass slides and allowed to recover for at least a week. Calipers were then used to measure the height of

the colony, and measurements repeated 2-3 months later. Descriptions of lateral growth measurements are given in[28]. Briefly lateral surface area was measured from photographs at the beginning and end of a 2 month incubation period and the change in slide area covered with tissue calculated.

Tissue imaging

Measurements of pH_{cf} at the growing edge were made on fluid between the calcifying tissue layer (calicoblastic epithelium) of the coral and the glass coverslip adjacent to newly forming crystals using confocal microscopy and the ratiometric pH indicator SNARF. Corals were placed in perfusion chambers, illuminated, and continuously supplied with treatment seawater during pH measurements, details are provided in[28,35]. A subset of the SNARF measurements was made on the total pH scale by calibrating the electrode used for SNARF calibration with seawater buffers (Tris and AMP[36]). The offset from NBS to total scale was assumed to remain constant with an average measured offset of 0.08, which was used to shift measurements done on the NBS scale to the total scale.

Boron Isotopes

Measurements of boron isotopes in the deposited skeletal material were carried out after killing the corals. Following the completion of pH_{cf} measurements at the growing edge, corals were allowed to grow for an additional 0.5-2 months, after which they were rinsed with fresh water, bleached (~1% NaOCl), rinsed with deionized water and air dried. Corals were harvested at three different time points, n=3 for each of 4 CO_2 treatments at

each time point, but not all samples could be used for boron measurements. Coverslips were scraped with a razor blade to remove residual detritus and then laterally growing skeleton scraped off and weighed. Apical skeleton was cut using a dental drill with a diamond embedded disk blade (Komet Dental). Apical fragments were sonicated in H_2O (Milli Q) to remove debris from cutting, then crushed in an agate mortar and pestle. Samples were rinsed then bleached to remove any residual organic material, dissolved in HNO_3, boron purified using ion exchange resins, and boron isotopes measured relative to the SRM 951 standard via multicollector inductively coupled plasma mass spectrometry (Neptune ThermoFisher Scientific). All measurements were repeated, and when feasible, multiple boron extractions were carried out on a given sample to check reproducibility. Measurements of $\delta^{11}B$ were generally reproducible to within 0.21‰ (average 2 standard error). Calculations of pH based on $\delta^{11}B$ were made using the measured isotopic composition of seawater from the incubation tanks (40.1± 0.2) following the calculations of[6] with a boron dissociation constant calculated per[37].

Statistics

The effects of CO_2 treatments on apical growth rates were evaluated using one-way ANOVA. The effects of CO_2 treatments and sampling locations (lateral versus apical) on pH_{cf} estimated from boron isotopes were evaluated with ANOVA (Table S2) blocked by the date samples were harvested using the SAS 7.2 software package. ANOVA residuals were plotted against predicted values, measured values, and on normal probability plots to assess violations of ANOVA assumptions. Linear regression was performed using Matlab to compare seawater pH with estimates of internal pH and $\delta^{11}B$. Different

sampling time-points were significantly different based on the ANOVA results and the number of samples measured was not the same at each time point, thus to avoid biasing the regressions and calculations of averages, values were weighted to give each sampling time-point equal weight.

References

34. Reynaud-Vaganay, S., Gattuso, J.-P., Cuif, J.-P., Jaubert, J. & Juillet-Leclerc, A. A novel culture technique for scleractinian corals: application to investigate changes in skeletal d18O as a function of temperature. *Mar. Ecol. Prog. Ser.* **180**, 121-130 (1999).
35. Venn, A., Tambutte, E., Holcomb, M., Allemand, D. & Tambutte, S. Live tissue imaging shows reef corals elevate pH under their calcifying tissue relative to seawater. *PloS one* **6**, e20013, doi:10.1371/journal.pone.0020013 (2011).
36. DOE, *Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water version 2*. A. G. Dickson, C. Goyet, Eds., ORNL/CDIAC-74 (1994).
37. Dickson, A. G. Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K. *Deep-Sea Res* **37**, 755-766 (1990).
38. van Heuven, S., Pierrot, D., Lewis, E. & Wallace, D.W.R. MATLAB Program Developed for CO_2 System Calculations. ORNL/CDIAC-105b. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee (2009).

39. Mehrbach, C., Culberson, C. H., Hawley, J. E. & Pytkowicz, R. M. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol Oceanogr* **18**, 897-907 (1973).

40. Dickson, A. G. & Millero, F. J. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Res* **34**, 1733-1743 (1987).