Supplementary Materials

Mechanisms and seasonal drivers of calcification in the temperate coral
***Turbinaria reniformis* at its latitudinal limits**

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Supplementary Methods

Overview

Measurements for Site 1 began ~3 months after Site 2 due to a shark sighting, which prevented us from diving at Site 1 during the first field trip. Calcification rate and F_v/F_m measurements were made on coral colonies that were mounted onto tiles, while extension rates and geochemical measurements were made on separate colonies growing naturally (i.e. not on tiles) to avoid invasive sampling (i.e. breakage and drilling) on the corals that were being used for calcification measurements. However, colonies used for growth rate and F_v/F_m measurements were located close by (i.e. within a 20-metre radius) to the naturally growing colonies that were measured for linear extension and sampled for skeletal geochemistry.

Environmental measurements

Seawater temperature was measured continuously at each site for the total duration of the study using HOBO temperature loggers (± 0.2°C, Onset Computer Corp.). Down-welling planar photo-synthetically active radiation (PAR in mol m^{-2} d^{-1}) in air was measured using Odyssey light loggers (± 5%, Odyssey Data Recording), which were calibrated against a high-precision LiCor 192A cosine PAR (± 5%, LiCor Scientific) sensor. Additional in-water measurements of light were made for ~3 days during summer and winter to quantify the depth-dependent light attenuation coefficients (k_d). Light (PAR) reaching the benthos was calculated using measurements of in-air surface measurements of PAR according to the following equation:

(1)
$$I_D = I_0 e^{-K_d D}$$

where I_D is the irradiance (PAR) measured at depth, I_0 is the irradiance (PAR) measured at the surface, k_d is the depth-dependent light attenuation coefficient, and D is the depth. In WA, there is a ~2 to 3 month phase lag between the maximum light (i.e. December) and the seasonal maximum in temperature (i.e. February/March) (Fig. 1b-c). Thus, for all data analyses, measurements from 15th April through to 14th October were treated as 'winter' and measurements from 15th October through to 14th April were treated as 'summer', based on trends in both temperature and light (Fig. 1b-c).

Seawater pH was measured on the total scale (pH$_T$, ±0.03 accuracy) using a factory-calibrated SeaFET ocean pH sensor (Satlantic, Canada) that was deployed next to the plated experimental coral colonies at Site 2 in Bremer Bay during the following periods: July to September 2015, March to June 2016, and June to September 2016. Additional measurements of pH$_T$ were made during each field trip at both sites using a Schott Handylab pH metre calibrated as per the methods in Ross et al., (2015). During each field trip, discrete seawater samples were collected from each site and filtered with 0.7-um glass fibre filters (Whatman GF/F; Sigma Aldrich) and taken back to UWA for the analysis of Total Alkalinity (TA) and dissolved inorganic nutrients. Concentrations of dissolved ammonium (± 0.1 µM), nitrate + nitrite (± 0.05 µM), and phosphate (± 0.03 µM) were measured using a QuikChem8500 Series 2 Flow Injection Analysis (FIA) System (Lachat Instrument, USA). TA (±5 µeq kg^{-1}) was measured using a modified version of the spectrophotometric approach [1]. Salinity was measured using a handheld YSI Salinity Meter (Model 85, Taylor Scientific). Dissolved Inorganic Carbon (DIC), the partial pressure of dissolved carbon dioxide (pCO$_2$), and seawater Ω were calculated from measured pH$_T$, TA, and temperature using the CO2SYS program assuming seasonally dependent salinity [2].

Photo-physiology

Due to safety restrictions (i.e. shark attack risk), scuba diving could not be undertaken at night. Therefore, the plated corals were retrieved from the field site just before dusk and transported to the laboratory where they were kept overnight in temperature-controlled aquaria with pumps to provide flow and aeration, and fresh seawater maintained at ambient seawater temperatures.

Geochemical analyses

Sections of *Turbinaria reniformis* were collected from ten naturally growing colonies (1 sample per colony) at the two sites (i.e. 5 per site) every 3 to 4 months over the ~2-year study period. We sampled the uppermost section of the growing edge of the skeleton for measurements of δ^{11}B and trace element compositions. Powders derived from the sampled coral skeleton were cleaned using 6% NaOCl solution as per the bleaching procedure outlined by Holcomb et al., (2015). Samples were then acidified in 0.58 N HNO$_3$. Trace element analyses (Sr/Ca, Mg/Ca, Ba/Ca, U/Ca and B/Ca) were performed on aliquots of the acidified samples using an X-Series 2 Quadrupole Inductively Coupled Plasma Mass Spectrometer (Q-ICPMS;

Thermo Fisher Scientific). The extraction of boron from the acidified sample solutions was performed via paired cation-anion resin columns and analysed with a NU Plasma II (Nu Instruments, Wrexham, UK) multi-collector inductively coupled plasma mass spectrometer (MC-ICPMS) [4] at UWA. Sample measurements bracketed by an in-house standard (~19.8‰) and blank measurements. The boron isotopic composition of the skeleton ($\delta^{11}B$) is reported as the per mil deviation of the stable isotopes $^{11}B{:}^{10}B$ relative to NIST SRM-951 boric acid standard.

The pH$_{cf}$ was derived from the measured skeletal $\delta^{11}B$ values according to the following equation [5]:

(1) $$pH_{cf} = pK_B - log\left[\frac{(\delta^{11}B_{sw} - \delta^{11}B_{carb})}{(\alpha_B \delta^{11}B_{carb} - \delta^{11}B_{sw} + 1000\,(\alpha_B - 1))}\right]$$

where pK_B is the dissociation constant of boric acid in seawater [6] at the temperature and salinity of the seawater in Bremer Bay, $\delta^{11}B_{carb}$ and $\delta^{11}B_{SW}$ are the boron isotopic composition of the coral skeleton and average seawater (39.61‰), respectively, and α_B is the isotopic fractionation factor (1.0272) [7].

We estimate the concentration of carbonate ions at the site of calcification ($[CO_3^{2-}]_{cf}$) using molar ratios of boron to calcium (B/Ca) according to the following relationship [8,9]:

(2) $$[CO_3^{2-}]_{cf} = \frac{[B(OH)_4^-]_{cf} K_D^{B/Ca}}{[\frac{B}{Ca}]_{arag}}$$

where $[B(OH)_4^-]_{cf}$ is the concentration of borate in the calcifying fluid, $K_D^{B/Ca}$ is the distribution coefficient for boron between aragonite and seawater [8], and $[B/Ca]_{arag}$ is the elemental ratio of boron to calcium measured in the coral skeleton. To estimate $[B(OH)_4^-]_{cf}$, we assume that the concentration of total inorganic boron in the calcifying fluid is only salinity dependent [10], and equal to that of the surrounding seawater [11]. The relative amounts of borate are determined by the pH$_{cf}$ [6], as determined from the $\delta^{11}B$ isotopic measurements.

The $K_D^{B/Ca}$ is calculated as a function of pH$_{cf}$ according to [8]:

(3) $$K_D^{B/Ca} = 0.00297 * \exp\left(-0.0202 * [H^+]_{cf}\right)$$

where $[H^+]$ in the calcifying fluid is estimated from the coral $\delta^{11}B$ derived pH$_{cf}$ and only varies by less than ±3% over the range in which most coral pH$_{cf}$ are known to occur (8.3 to 8.6) [12].

The concentration of DIC$_{cf}$ is then calculated from pH$_{cf}$ and $[CO_3^{2-}]_{cf}$ using the following relationship [13]:

$$(4) \qquad DIC_{cf} = CO_3^{2-} \left(1 + \frac{\left[H^+ \right]}{K_2^*} + \frac{\left[H^+ \right]^2}{K_1^* K_2^*} \right)$$

where K_1^* and K_2^* are the first and second acidity constants of carbonic acid. Calculations were made using the CO2SYS program [2].

Raman spectroscopy was conducted to determine Ω_{cf} on the same coral skeletal powders used for geochemical analyses. Multiple grains from the powders were dispersed onto a glass slide and measured following the analytical methods of DeCarlo et al., (2017b). Measurements were made on a WITec Alpha 300RA+ with a 785 nm laser, 1200 mm^{-1} grating, Andor iDUS 401 CCD maintained at -60 °C, and a 20x objective with 0.5 numerical aperture. Replication was set to 100 spectra per sample (i.e. per coral individual). Some spectra had poor signal and were removed during data processing to ensure quality control (Supplementary Table 2). Some samples did not have enough sample leftover after boron analysis and were excluded from the Raman analysis. The aragonite v_1 peak of each spectrum was fit with a Gaussian curve and the resulting full width at half maximum intensity (FWHM) converted to Ω as per the methodology described in DeCarlo et al., (2017b). Repeated measurements of the coral geochemical standard JCp-1 (mean Ω_{cf} of 12.3) were used to account for instrument drift between analysis days (corrections all < 0.6 Ω_{cf} units). The replicate FWHM measurements per coral individual were weighted by peak intensity when calculating Ω_{cf} means.

The $[Ca^{2+}]_{cf}$ was inferred from Ω_{cf} (Raman) and $[CO_3^{2-}]_{cf}$ (boron systematics: $\delta^{11}B$ and B/Ca) according to the following relationship:

$$(5) \qquad [Ca^{2+}]_{cf} = \frac{\Omega_{cf} \, K_{sp}}{[CO_3^{2-}]_{cf}}$$

where K_{sp}^* is the solubility constant for aragonite as a function of temperature and salinity, Ω_{cf} is the saturation state of the calcifying fluid determined from Raman, and $[CO_3^{2-}]_{cf}$ is the carbonate ion concentration of the calcifying fluid estimated from $\delta^{11}B$ and B/Ca (Eq. 2). Finally, the biological regulation of $[Ca^{2+}]_{cf}$ was calculated as the ratio of $[Ca^{2+}]_{cf}$ and seawater $[Ca^{2+}]$ (i.e. $[Ca^{2+}]_{cf}$ / $[Ca^{2+}]_{sw}$). Values of $[Ca^{2+}]_{sw}$ (mmol kg^{-1}) are calculated as per the equation from Riley and Tongudai (1967):

$$(6) \qquad [Ca^{2+}]_{sw} = \frac{0.02128}{40.078} \left(\frac{S}{1.80655} \right) 1000$$

where S is equal to the salinity of the seawater.

Statistical analyses

A t-test was used to test for the effect of site on temperature and light levels averaged for summer and winter, respectively. Seawater temperature did not differ significantly between the two study sites ($t(1194) = 0.000$, $p = 1.000$); therefore, temperatures were averaged across the two sites. There was a significant difference between the two sites for light ($t(1052) = 5.685$, $p < 0.001$). However, the monthly averaged PAR did not differ between the two sites by more than 4 mol $m^{-2}\,d^{-1}$; therefore, the data for each parameter were averaged across the two sites. Since calcification rates ($F_{5,165} = 0.473$, $p = 0.796$), F_v/F_m ($F_{6,198} = 2.297$, $p = 0.060$), DIC_{cf} ($F_{6,48} = 1.736$, $p = 0.133$), pH_{cf} ($F_{6,48} = 0.831$, $p = 0.552$), $[CO_3^{2-}]_{cf}$ ($F_{6,48} = 0.938$, $p = 0.477$), $[Ca^{2+}]_{cf}$ ($F_{6,37} = 1.138$, $p = 0.315$), and Ω_{cf} ($F_{6,37} = 1.023$, $p = 452$) did not significantly differ between sites, the data for each parameter were pooled across sites for the linear regression analyses. Before conducting statistical analyses, residual values were tested for normality using a Shapiro-Wilk's test, and homogeneity of variance was assessed using Levene's test.

Supplementary Tables

Supplementary Table 1. Average skeletal linear extension rate, skeletal density (g cm^{-3} y^{-1}), and rate of calcification expressed as change in colony weight normalised to colony growing edge surface area (g cm^{-2} y^{-1}) of the coral species *Turbinaria reniformis* at Bremer Bay (mean ± SE).

Season	Winter	Summer	Summer	Winter	Summer
Date	13-Sep-15	20-Jan-16	14-Apr-16	06-Aug-16	25-Nov-16
Extension Rate (mm mo^{-1})	1.28 ± 0.17	0.47 ± 0.04	0.56 ± 0.04	1.03 ± 0.11	0.81± 0.11
Extension Rate (cm^{-1} y^{-1})	1.53 ± 0.21	0.56 ± 0.05	0.67 ± 0.05	1.23 ± 0.13	0.97 ± 0.13
Density (g cm^3)	1.40	1.96	2.02	1.69	nd
Calcification rate normalised to cm^2 of growing edge (g cm^2 y^{-1})	2.14 ± 0.24	1.09 ± 0.26	1.36 ± 0.36	2.08 ± 0.28	nd

Supplementary Table 2. The Ω_{cf}, FWHM (mean and standard error) and number of spectra used from the Raman measurements.

Date of sample	Raman-derived Ω_{cf}	FWHM (1/cm)	FWHM standard error	Number of spectra
10-Feb-15	10.57	3.40	0.05	110
10-Feb-15	9.93	3.36	0.07	103
10-Feb-15	10.40	3.39	0.06	98
10-Feb-15	10.17	3.38	0.06	63
10-Feb-15	10.52	3.40	0.09	103
17-Jun-15	11.13	3.43	0.05	85
17-Jun-15	10.72	3.41	0.06	111
17-Jun-15	10.70	3.41	0.06	112
17-Jun-15	10.67	3.41	0.06	99
17-Jun-15	10.83	3.42	0.06	108
17-Jun-15	11.26	3.44	0.05	99
17-Jun-15	10.48	3.40	0.07	106
17-Jun-15	11.21	3.44	0.06	99
16-Sep-15	10.77	3.41	0.05	108
16-Sep-15	11.12	3.43	0.07	110
16-Sep-15	10.56	3.40	0.07	96
16-Sep-15	10.87	3.42	0.06	112
10-Dec-15	10.88	3.42	0.06	95
10-Dec-15	11.26	3.44	0.09	70
10-Dec-15	10.90	3.42	0.06	80
10-Dec-15	10.67	3.41	0.05	100
10-Dec-15	11.12	3.43	0.07	87
10-Dec-15	10.71	3.41	0.06	106
3-Mar-16	10.89	3.42	0.06	103
3-Mar-16	11.39	3.45	0.06	94
3-Mar-16	10.62	3.40	0.05	88
3-Mar-16	11.34	3.44	0.07	81
3-Mar-16	10.51	3.40	0.06	91
3-Mar-16	11.12	3.43	0.08	101
3-Mar-16	10.56	3.40	0.07	107
3-Mar-16	10.94	3.42	0.05	111
07-Jun-16	11.03	3.43	0.07	102
07-Jun-16	11.09	3.43	0.07	112
07-Jun-16	10.84	3.42	0.07	85
07-Jun-16	10.98	3.42	0.06	101
07-Jun-16	10.76	3.41	0.06	105
07-Jun-16	11.10	3.43	0.06	109
07-Jun-16	11.38	3.45	0.07	103
07-Jun-16	11.17	3.43	0.06	103
07-Jun-16	10.94	3.42	0.06	123
07-Jun-16	10.68	3.41	0.06	109
5-Oct-16	11.14	3.43	0.09	58
5-Oct-16	11.44	3.45	0.10	22
5-Oct-16	11.32	3.44	0.07	17

| 5-Oct-16	11.12	3.43	0.09	23
5-Oct-16	11.04	3.43	0.08	30
5-Oct-16	12.19	3.49	0.13	39
5-Oct-16	11.97	3.48	0.10	27
5-Oct-16	11.43	3.45	0.08	68
5-Oct-16	10.65	3.41	0.07	96
5-Oct-16	11.16	3.43	0.08	82

Supplementary Table 3. Seasonal averages of light, temperature and biogeochemical seawater (sw) data measured at Site 1 and Site 2 in Bremer Bay between 2014 and 2016. pH_T is the pH on the total hydrogen ion concentration scale, Ω_{sw} is aragonite saturation state, pCO_2 is the partial pressure of dissolved carbon dioxide, and DIC is dissolved inorganic carbon. Measurements of individual discrete samples of seawater are presented as mean ± SE. Continuous measurements (i.e. sampled every 10 minutes) of temperature, light and pH are presented as the mean ± variance. Summer (15-October to 14-April) and winter (15-April to 14-October) are defined based on seasonal changes in light and temperature.

| Parameter	Unit		n	Summer	n	Winter
Light (PAR)	mol m^{-2} d^{-1}	Site 1	-	18.0 ± 2.18	-	13.5 ± 2.03
		Site 2	-	20.9 ± 2.36	-	15.0 ± 2.15
K_d (PAR)	m^{-1}	Site 1	-	0.070 ± 0.005	-	0.057 ± 0.004
		Site 2	-	0.071 ± 0.005	-	0.060 ± 0.004
Temperature	°C	Site 1	-	19.76 ± 1.95	-	17.24 ± 1.79
		Site 2	-	19.80 ± 1.90	-	17.51 ± 1.85
pH_T	-	Site 1	-	-	-	-
		Site 2	-	8.05	-	8.10
Total alkalinity	µmol kg^{-1}	Site 1	7	2333.68 ± 3.40	9	2340.22 ± 3.32
		Site 2	9	2331.42 ± 3.69	10	2335.32 ± 3.79
Ω_{sw}	-	Site 1	7	3.05 ± 0.02	9	2.94 ± 0.02
		Site 2	9	3.16 ± 0.02	10	2.89 ± 0.01
pCO_2	µatm	Site 1	7	388.97 ± 1.35	9	357.26 ± 2.02
		Site 2	9	386.57 ± 1.54	10	358.30 ± 3.35
DIC_{sw}	µmol kg^{-1}	Site 1	7	2052.92 ± 5.14	9	2065.15 ± 2.18
		Site 2	9	2040.53 ± 1.77	10	2064.68 ± 4.37
Ammonium	mmol m^{-3}	Site 1	4	0.23 ± 0.04	3	0.26 ± 0.04
		Site 2	6	0.49 ± 0.03	6	0.45 ± 0.13
Nitrate	mmol m^{-3}	Site 1	5	0.49 ± 0.11	3	0.28 ± 0.05
		Site 2	6	0.18 ± 0.06	6	0.54 ± 0.19
Phosphate	mmol m^{-3}	Site 1	5	0.003 ± 0.002	3	0.02 ± 0.01
		Site 2	4	0.02 ± 0.03	6	0.05 ± 0.02

Supplementary Figures
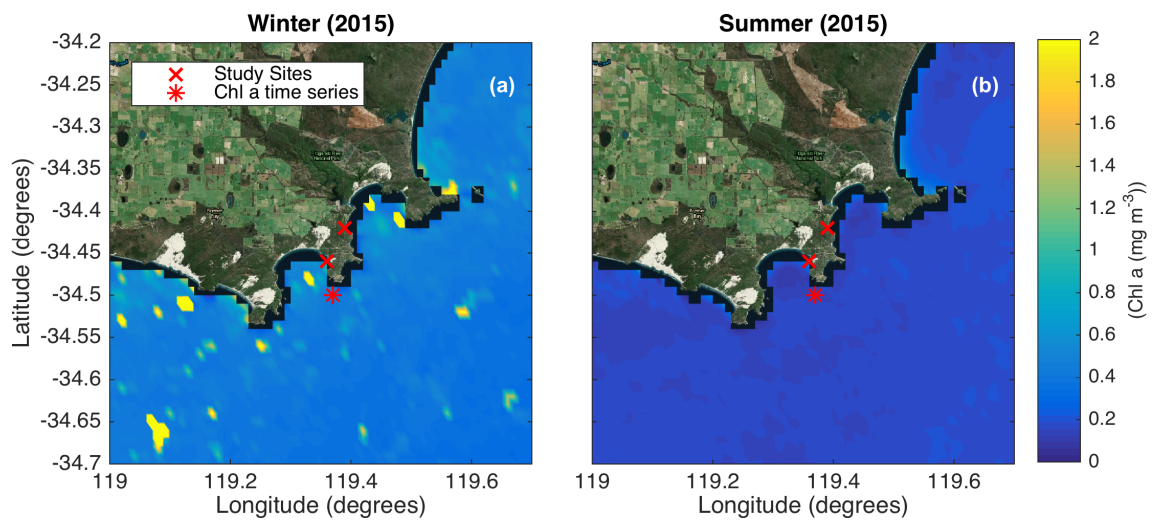
Supplementary Figure S1. Monthly satellite-derived chlorophyll *a* for Bremer Bay during (**a**) winter 2015, and (**b**) summer 2015/16. Red crosses denote the two field sites and the asterisk denotes the site of the satellite-derived chlorophyll *a*.
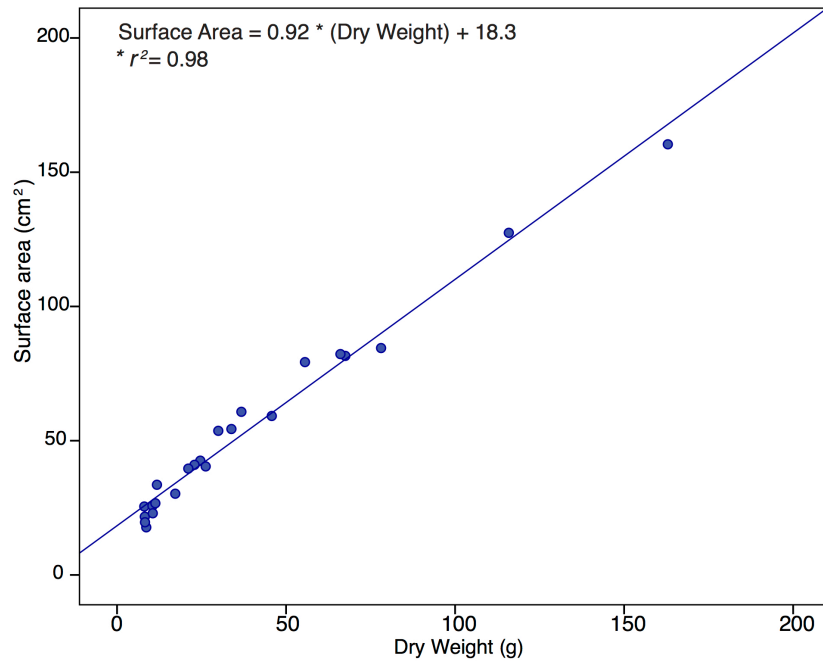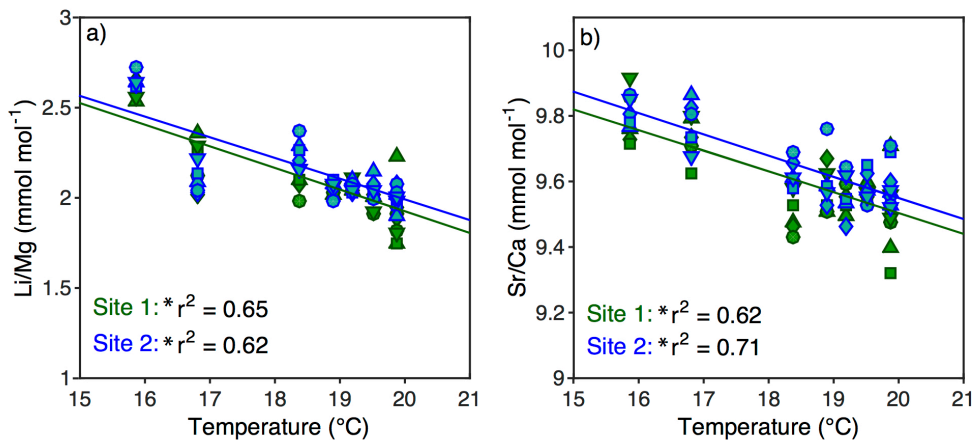
Supplementary Figure S2. Surface area to weight regressions for colonies of *Turbinaria reniformis* (y = 0.92x + 18.3, *n* = 23). Asterisks denote statistical significance.
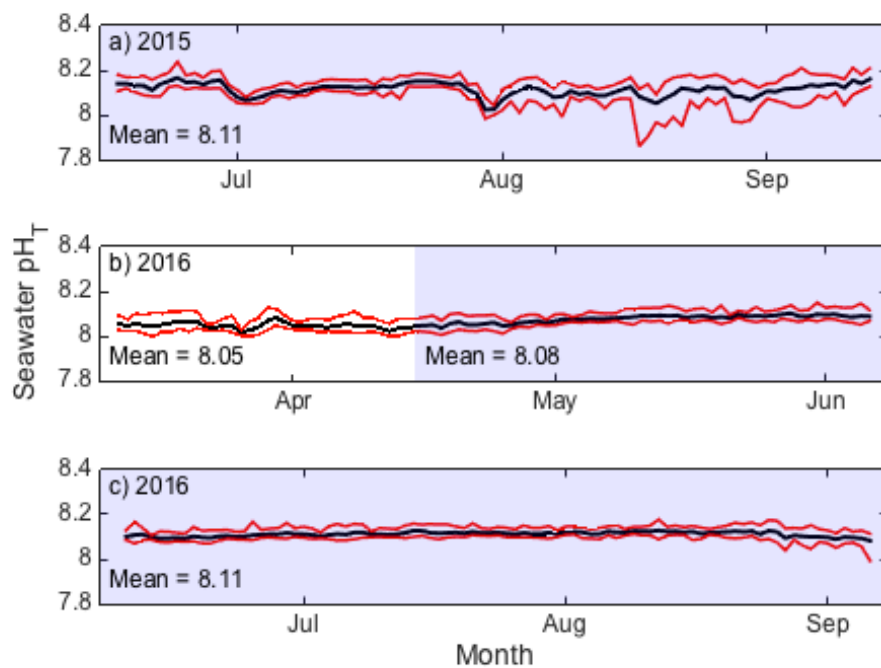
Supplementary Figure S3. Seawater pH$_T$ (pH on the total scale) measured during the three separate SeaFET pH sensor deployment in (a) July–September 2015, (b) March–June 2016, and (c) June–September 2016 in Little Boat Harbour (site 2), Bremer Bay, Western Australia. The mean daily pH$_T$ is denoted in black while the daily minimum and maximum are shown in red. Blue shading denotes winter months while no shading denotes summer months.
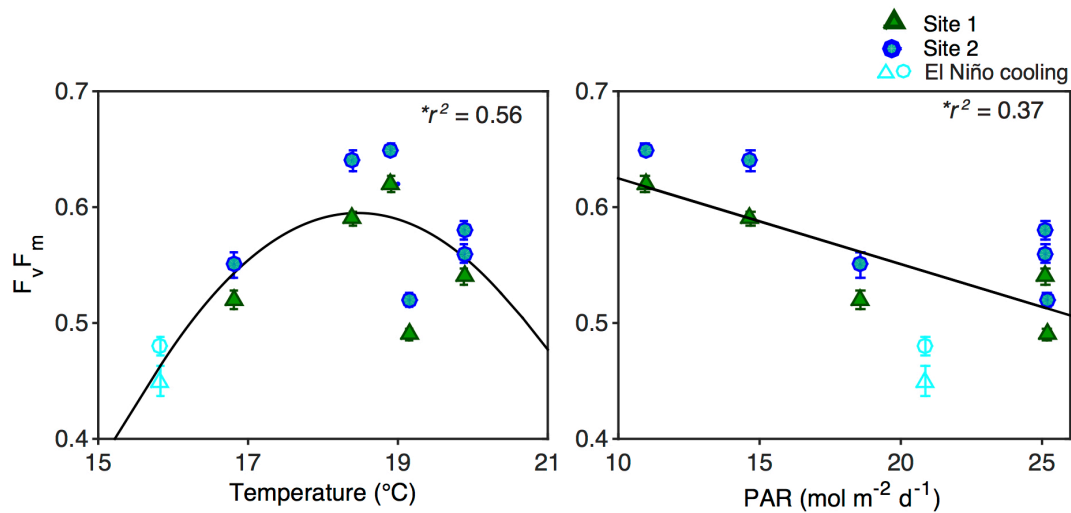
Supplementary Figure S4. Sensitivity of photochemical efficiency (F_v/F_m) to (**a**) temperature, and (**b**) photosynthetically active radiation (PAR) for the coral species *Turbinaria reniformis* in Bremer Bay, WA. Values represent the mean ± 1 SE (*n* = 14 at Site 1, *n* = 21 at site 2). Black line in (**a**) denote the fit of the non-linear regression ($y = -0.020x^2 + 0.729x - 6.136$) and in (b) black line denotes the fit of the linear regression ($y = -0.0059x + 0.660$). Turquoise symbols denote values at time of -1°C temperature deviation from average winter monthly minimum (El Niño cooling). Asterisks denote statistical significance.

Supplementary Figure S5. (a) Regressions of lithium to magnesium (Li/Mg) ratios with mean temperature at study site 1 (Li/Mg = -0.12T + 4.32, *n* = 5) and study site 2 (Li/Mg = -0.12T + 4.41, *n* = 5), and (**b**) strontium to calcium (Sr/Ca) ratios with mean temperature for *Turbinaria reniformis* at study site 1 (Sr/Ca = -0.064T + 10.78, *n* = 5) and study site 2 (Sr/Ca = -0.069T + 10.91, *n* = 5) in Bremer Bay, Western Australia. Symbols represent each of the five replicate colonies at each site. Asterisks denote statistical significance.
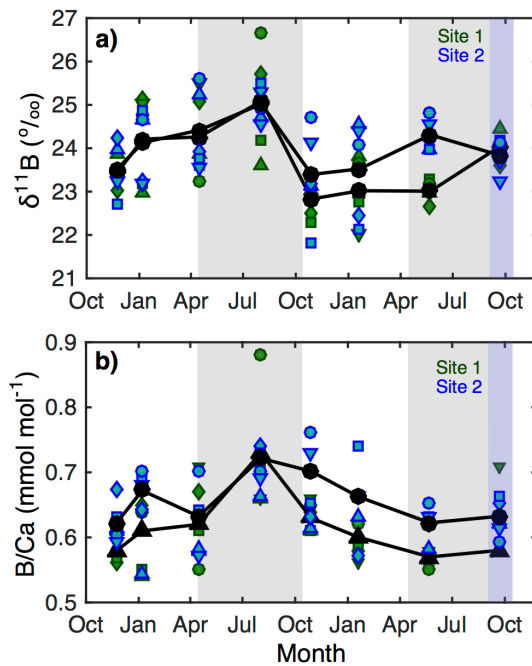
Supplementary Figure S6. (a) Boron isotope values and (**b**) boron to calcium (B/Ca) ratios for *Turbinaria reniformis* at study sites 1 and 2 in Bremer Bay, Western Australia. Symbols represent different colonies, while the black symbols represent the mean. Light grey shading denotes winter and no shading denotes summer. Dark blue shading denotes time of -1°C deviation from average winter monthly minimum (El Niño driven cooling).
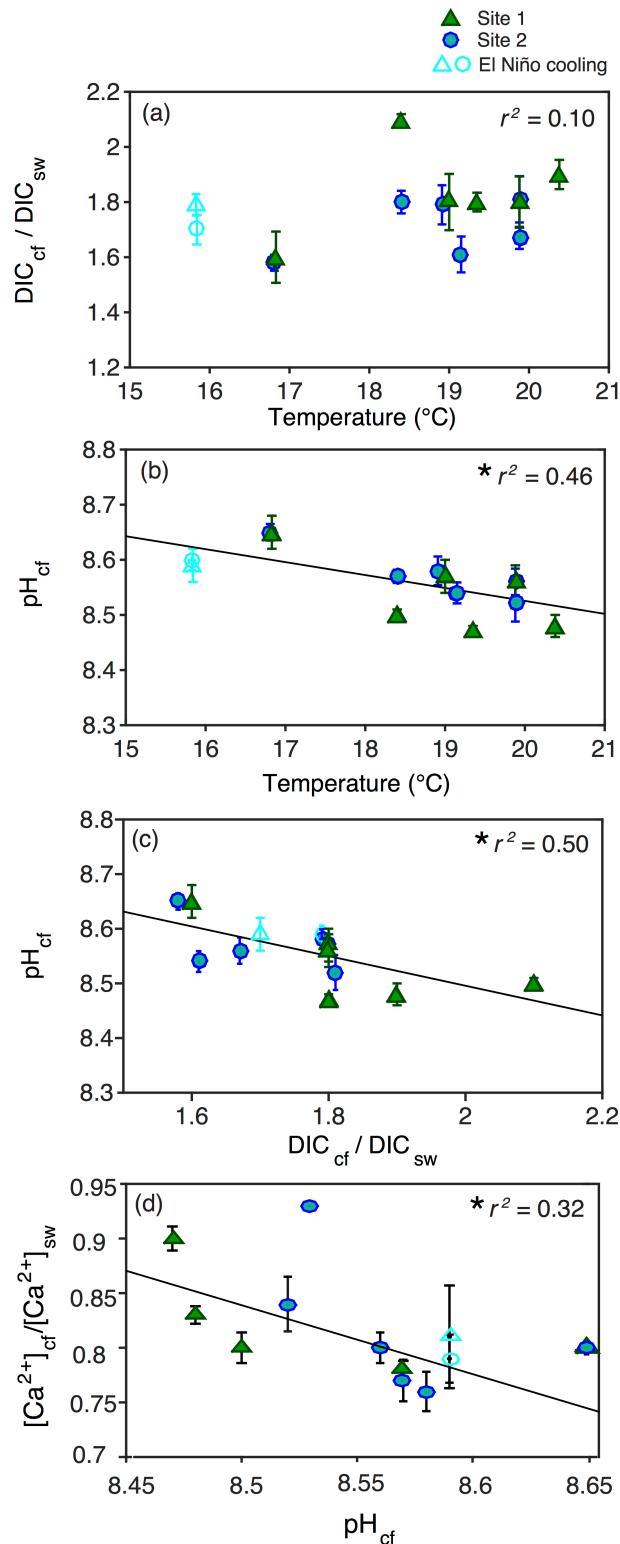
Supplementary Figure S7. (**a**) DIC_{cf}/DIC_{sw} response to seasonally varying temperature, (**b**) pH_{cf} response to seasonally varying temperature ($pH_{cf} = -0.025T + 9.018$), (**c**) relationship between pH_{cf} and DIC_{cf}/DIC_{sw} ($pH_{cf} = -0.269\ DIC_{cf}/DIC_{sw} + 9.036$), and (**d**) response of $[Ca^{2+}]_{cf}/[Ca^{2+}]_{sw}$ to pH_{cf} ($[Ca^{2+}]_{cf}/[Ca^{2+}]_{sw} = -0.53 + 5.32$) for *Turbinaria reniformis* in Bremer Bay, WA. Values represent the mean ± 1 SE (*n* = 5 per site). Turquoise symbols denote values at time of -1°C El Niño winter cooling. Asterisks denote statistical significance.

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