Supplementary Material

Ocean Warming Amplifies the Effects of Ocean Acidification on Skeletal Mineralogy and Microstructure in the Asterinid Starfish *Aquilonastra yairi*

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Supplementary Materials and Methods:

Animal collection and acclimation:

To prepare the *Aquilonastra yairi* specimens for the experiment, they were cleansed from externally attached materials, thoroughly rinsed with flowing seawater repeatedly and small forceps to lift the attached debris, and allowed to acclimate in a communal tank with a recirculating water system at the ambient temperature of ~27 °C for seven days. Afterwards, they were randomly distributed into 18 experimental aquaria (25 litres), illuminated with LED lamps (Aquaillumination® Hydra 52 HD) at 25–30 µmol photons m^{-2} s^{-1}. After an additional seven-day acclimation phase, the main experiment was started. No visual sign of stress (i.e., discoloration and erratic flipping) was observed during the acclimation period. During acclimation and experimental exposure, the starfish *A. yairi* were able to feed on living diatoms that naturally grow at the aquaria walls and deposited detritus flocs.

Seawater chemistry control and manipulation:

Temperature, salinity, and pH_{NBS} (pH electrodes calibrated in diluted National Bureau of Standards buffers) were measured three times per week using a calibrated multielectrode portable probe (WTW Multiline 3630 IDS, Xylem Analytics, Weilheim, Germany). The $pH_{total\ scale}$ was measured weekly via spectrophotometry (Shimadzu UV-1700 Pharma Spec UV-Vis Spectrophotometer) following SOP 6b [1] using the indicator dye *m*-cresol purple (Sigma-Aldrich). Seawater samples for total alkalinity (A_T) and dissolved inorganic carbon (DIC) analysis were collected weekly in 500-mL borosilicate glass vials, poisoned with 200-µL of saturated mercuric chloride ($HgCl_2$) solution and refrigerated until analysis. A_T and DIC were measured according to the best practice of ocean acidification seawater measurement protocols [1]. A_T was measured by open-cell potentiometric Gran titration (precision, ±10 µmol kg^{-1}), and DIC was determined by the colorimetric analytical method using a Shimadzu DIC analyser (precision, ±10 µmol kg^{-1}).

The seawater carbonate system parameters pCO_2, carbonate ion concentration [CO_3^{-2}], bicarbonate ion concentration [HCO_3^-], aqueous CO_2, calcite saturation state (Ω_{Ca}), aragonite saturation state (Ω_{Ar}) were calculated with the program CO_2SYS for Microsoft Excel [2], using Hansson [3] and Mehrbach*, et al.* [4] refitted by Dickson and Millero [5] for K_1 and K_2 carbonic acid constants; Dickson [6] for K_{HSO4} equilibrium constant; Dickson and Riley [7] for K_{HF} dissociation constant; Uppström [8] for the boric acid constant ($[B]_T$); and Mucci [9] for the stoichiometric calcite solubility constant.

References:

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Supplementary table:

Table S1. Tukey HSD post hoc test for the interactive effects of incubation time (45 and 90 days), pCO_2 (455 µatm, 1052 µatm, 2066 µatm) and temperature (27 °C, 32 °C) on skeletal Mg/Ca ratio of *A. yairi* using the 'agricolae R-package' for multiple comparisons to interrogate the main effects of incubation time, temperature and pCO_2 (incubation time : pCO_2 : temperature = 0.014, Table 2).

Condition	diff.	lwr.	upr.	p_{adj}
90 days:1052 µatm:27 °C vs 45 days:1052 µatm:27 °C	5.4800	−7.3920	18.3520	0.9156
45 days:2066 µatm:27 °C vs 45 days:1052 µatm:27 °C	−1.3833	-14.2553	11.4887	1.0000
90 days:2066 µatm:27 °C vs 45 days:1052 µatm:27 °C	5.3933	−7.4787	18.2653	0.9233
45 days:455 µatm:27 °C vs 45 days:1052 µatm:27 °C	2.3467	−10.5253	15.2187	0.9999
90 days:455 µatm:27 °C vs 45 days:1052 µatm:27 °C	0.0400	−12.8320	12.9120	1.0000
45 days:1052 µatm:32 °C vs 45 days:1052 µatm:27 °C	9.3533	−3.5187	22.2253	0.3220
90 days:1052 µatm:32 °C vs 45 days:1052 µatm:27 °C	5.0967	−7.7753	17.9687	0.9460
45 days:2066 µatm:32 °C vs 45 days:1052 µatm:27 °C	2.0433	−10.8287	14.9153	1.0000
90 days:2066 µatm:32 °C vs 45 days:1052 µatm:27 °C	4.5700	−8.3020	17.4420	0.9741
45 days:455 µatm:32 °C vs 45 days:1052 µatm:27 °C	0.3467	−12.5253	13.2187	1.0000
90 days:455 µatm:32 °C vs 45 days:1052 µatm:27 °C	10.3000	−2.5720	23.1720	0.2077
45 days:2066 µatm:27 °C vs 90 days:1052 µatm:27 °C	−6.8633	−19.7353	6.0087	0.7355
90 days:2066 µatm:27 °C vs 90 days:1052 µatm:27 °C	−0.0867	−12.9587	12.7853	1.0000
45 days:455 µatm:27 °C vs 90 days:1052 µatm:27 °C	−3.1333	−16.0053	9.7387	0.9988
90 days:455 µatm:27 °C vs 90 days:1052 µatm:27 °C	−5.4400	−18.3120	7.4320	0.9192
45 days:1052 µatm:32 °C vs 90 days:1052 µatm:27 °C	3.8733	−8.9987	16.7453	0.9926
90 days:1052 µatm:32 °C vs 90 days:1052 µatm:27 °C	−0.3833	−13.2553	12.4887	1.0000
45 days:2066 µatm:32 °C vs 90 days:1052 µatm:27 °C	−3.4367	−16.3087	9.4353	0.9972
90 days:2066 µatm:32 °C vs 90 days:1052 µatm:27 °C	−0.9100	−13.7820	11.9620	1.0000
45 days:455 µatm:32 °C vs 90 days:1052 µatm:27 °C	−5.1333	−18.0053	7.7387	0.9435
90 days:455 µatm:32 °C vs 90 days:1052 µatm:27 °C	4.8200	−8.0520	17.6920	0.9626
90 days:2066 µatm:27 °C vs 45 days:2066 µatm:27 °C	6.7767	−6.0953	19.6487	0.7494
45 days:455 µatm:27 °C vs 45 days:2066 µatm:27 °C	3.7300	−9.1420	16.6020	0.9945
90 days:455 µatm:27 °C vs 45 days:2066 µatm:27 °C	1.4233	−11.4487	14.2953	1.0000
45 days:1052 µatm:32 °C vs 45 days:2066 µatm:27 °C	10.7367	−2.1353	23.6087	0.1667
90 days:1052 µatm:32 °C vs 45 days:2066 µatm:27 °C	6.4800	−6.3920	19.3520	0.7951
45 days:2066 µatm:32 °C vs 45 days:2066 µatm:27 °C	3.4267	−9.4453	16.2987	0.9973
90 days:2066 µatm:32 °C vs 45 days:2066 µatm:27 °C	5.9533	−6.9187	18.8253	0.8657
45 days:455 µatm:32 °C vs 45 days:2066 µatm:27 °C	1.7300	−11.1420	14.6020	1.0000
90 days:455 µatm:32 °C vs 45 days:2066 µatm:27 °C	11.6833	−1.1887	24.5553	0.1000
45 days:455 µatm:27 °C vs 90 days:2066 µatm:27 °C	−3.0467	−15.9187	9.8253	0.9990
90 days:455 µatm:27 °C vs 90 days:2066 µatm:27 °C	−5.3533	−18.2253	7.5187	0.9266

45 days:1052 µatm:32 °C vs 90 days:2066 µatm:27 °C	3.9600	−8.9120	16.8320	0.9912
90 days:1052 µatm:32 °C vs 90 days:2066 µatm:27 °C	−0.2967	−13.1687	12.5753	1.0000
45 days:2066 µatm:32 °C vs 90 days:2066 µatm:27 °C	−3.3500	−16.2220	9.5220	0.9978
90 days:2066 µatm:32 °C vs 90 days:2066 µatm:27 °C	−0.8233	−13.6953	12.0487	1.0000
45 days:455 µatm:32 °C vs 90 days:2066 µatm:27 °C	−5.0467	−17.9187	7.8253	0.9493
90 days:455 µatm:32 °C vs 90 days:2066 µatm:27 °C	4.9067	−7.9653	17.7787	0.9578
90 days:455 µatm:27 °C vs 45 days:455 µatm:27 °C	−2.3067	−15.1787	10.5653	0.9999
45 days:1052 µatm:32 °C vs 45 days:455 µatm:27 °C	7.0067	−5.8653	19.8787	0.7118
90 days:1052 µatm:32 °C vs 45 days:455 µatm:27 °C	2.7500	−10.1220	15.6220	0.9996
45 days:2066 µatm:32 °C vs 45 days:455 µatm:27 °C	−0.3033	−13.1753	12.5687	1.0000
90 days:2066 µatm:32 °C vs 45 days:455 µatm:27 °C	2.2233	−10.6487	15.0953	1.0000
45 days:455 µatm:32 °C vs 45 days:455 µatm:27 °C	−2.0000	−14.8720	10.8720	1.0000
90 days:455 µatm:32 °C vs 45 days:455 µatm:27 °C	7.9533	−4.9187	20.8253	0.5470
45 days:1052 µatm:32 °C vs 90 days:455 µatm:27 °C	9.3133	−3.5587	22.1853	0.3276
90 days:1052 µatm:32 °C vs 90 days:455 µatm:27 °C	5.0567	−7.8153	17.9287	0.9487
45 days:2066 µatm:32 °C vs 90 days:455 µatm:27 °C	2.0033	−10.8687	14.8753	1.0000
90 days:2066 µatm:32 °C vs 90 days:455 µatm:27 °C	4.5300	−8.3420	17.4020	0.9757
45 days:455 µatm:32 °C vs 90 days:455 µatm:27 °C	0.3067	−12.5653	13.1787	1.0000
90 days:455 µatm:32 °C vs 90 days:455 µatm:27 °C	10.2600	−2.6120	23.1320	0.2119
90 days:1052 µatm:32 °C vs 45 days:1052 µatm:32 °C	−4.2567	−17.1287	8.6153	0.9846
45 days:2066 µatm:32 °C vs 45 days:1052 µatm:32 °C	−7.3100	−20.1820	5.5620	0.6601
90 days:2066 µatm:32 °C vs 45 days:1052 µatm:32 °C	−4.7833	−17.6553	8.0887	0.9645
45 days:455 µatm:32 °C vs 45 days:1052 µatm:32 °C	−9.0067	−21.8787	3.8653	0.3724
90 days:455 µatm:32 °C vs 45 days:1052 µatm:32 °C	0.9467	−11.9253	13.8187	1.0000
45 days:2066 µatm:32 °C vs 90 days:1052 µatm:32 °C	−3.0533	−15.9253	9.8187	0.9990
90 days:2066 µatm:32 °C vs 90 days:1052 µatm:32 °C	−0.5267	−13.3987	12.3453	1.0000
45 days:455 µatm:32 °C vs 90 days:1052 µatm:32 °C	−4.7500	−17.6220	8.1220	0.9661
90 days:455 µatm:32 °C vs 90 days:1052 µatm:32 °C	5.2033	−7.6687	18.0753	0.9384
90 days:2066 µatm:32 °C vs 45 days:2066 µatm:32 °C	2.5267	−10.3453	15.3987	0.9998
45 days:455 µatm:32 °C vs 45 days:2066 µatm:32 °C	−1.6967	−14.5687	11.1753	1.0000
90 days:455 µatm:32 °C vs 45 days:2066 µatm:32 °C	8.2567	−4.6153	21.1287	0.4943
45 days:455 µatm:32 °C vs 90 days:2066 µatm:32 °C	−4.2233	−17.0953	8.6487	0.9855
90 days:455 µatm:32 °C vs 90 days:2066 µatm:32 °C	5.7300	−7.1420	18.6020	0.8910
90 days:455 µatm:32 °C vs 45 days:455 µatm:32 °C	9.9533	−2.9187	22.8253	0.2455

Supplementary figure:

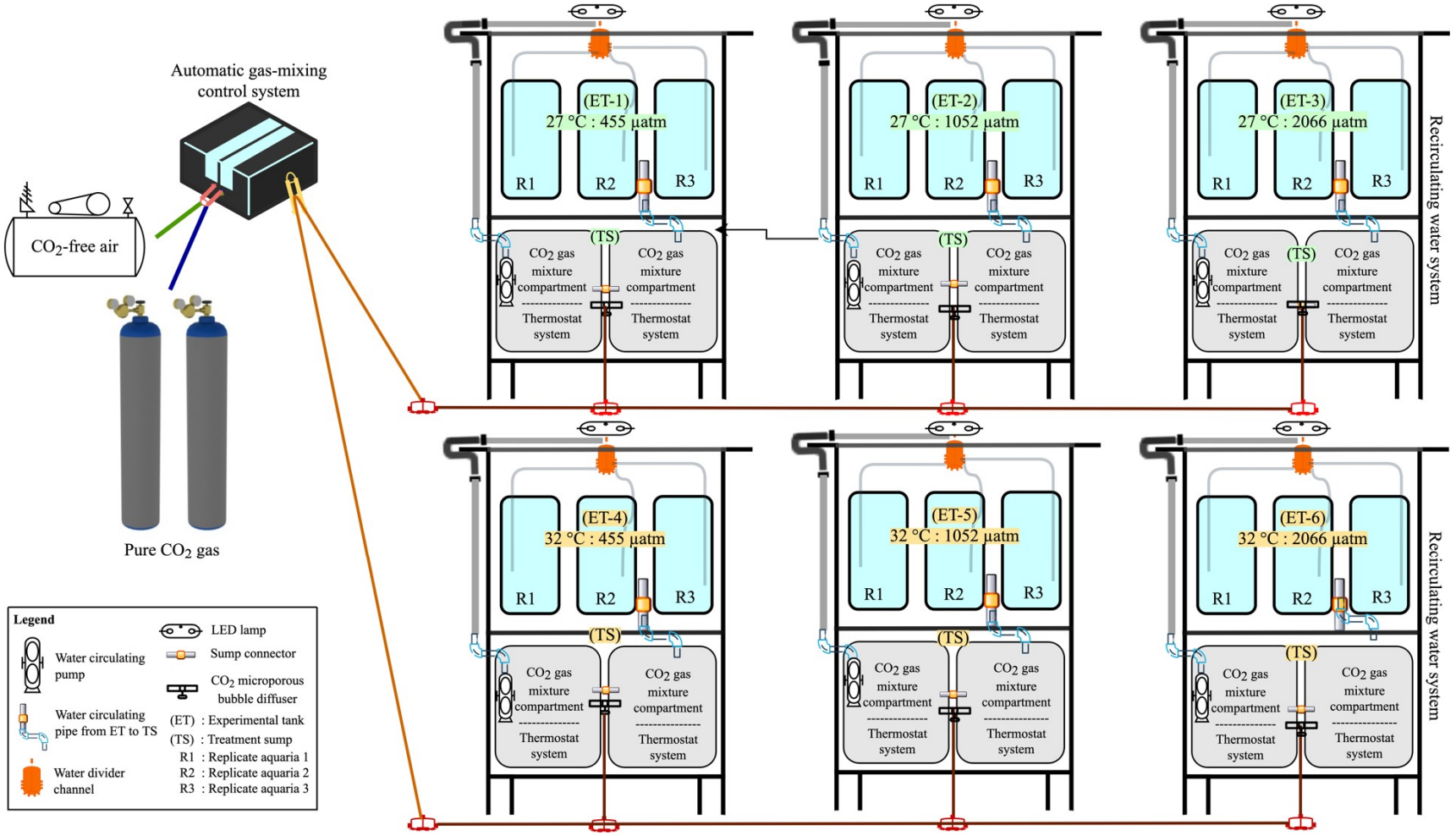

Figure S1. Schematic of ocean acidification and ocean warming experimental design with a fully factorial combination of low pCO_2 (455 µatm), moderate pCO_2 (1052 µatm), and high pCO_2 (2066 µatm) treatments with ambient temperature (27 °C) and high temperature (32 °C) treatments.

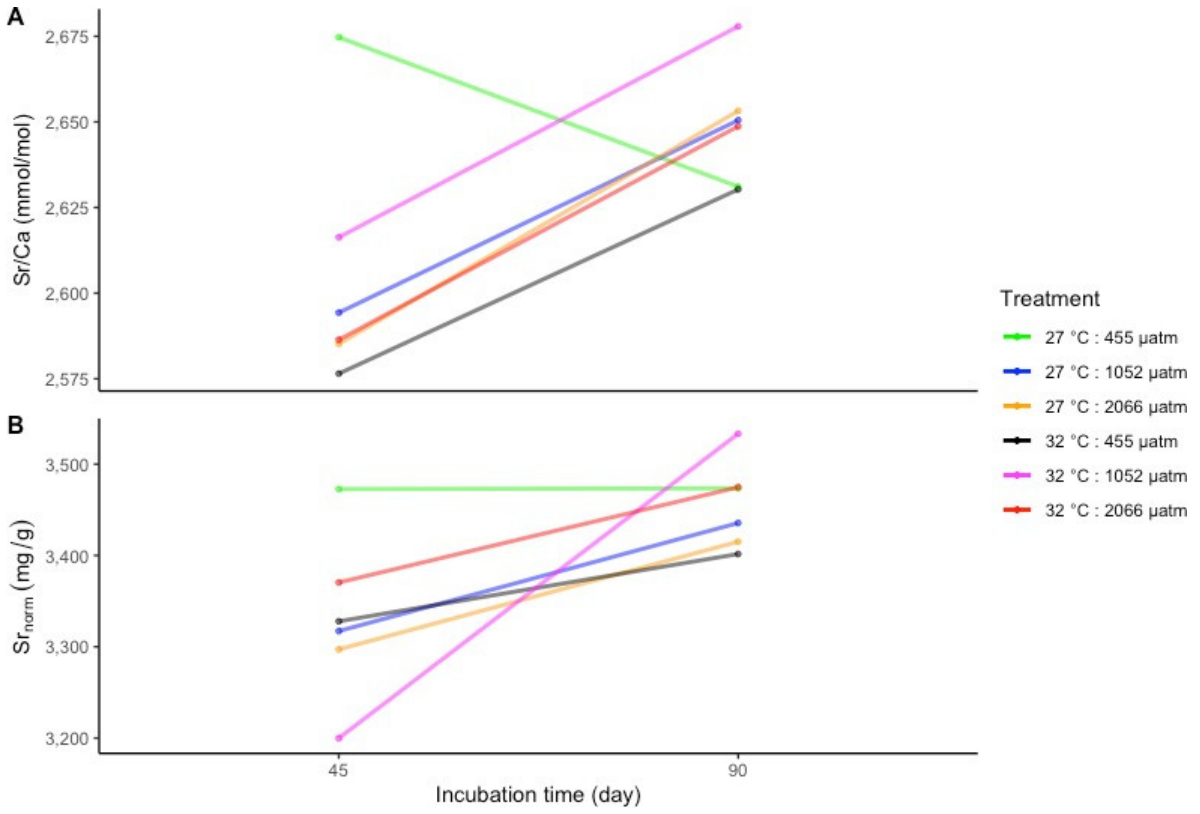

Figure S2. Skeletal Sr values over incubation time in *A. yairi* exposed to elevated temperatures levels (27 °C and 32 °C) crossed with increased pCO_2 concentrations (455 µatm, 1052 µatm, and 2066 µatm). (A) Sr/Ca ratio (mmol/mol), (B) Sr_{norm} ratio (mg/g). Data were presented as mean (n = 36).

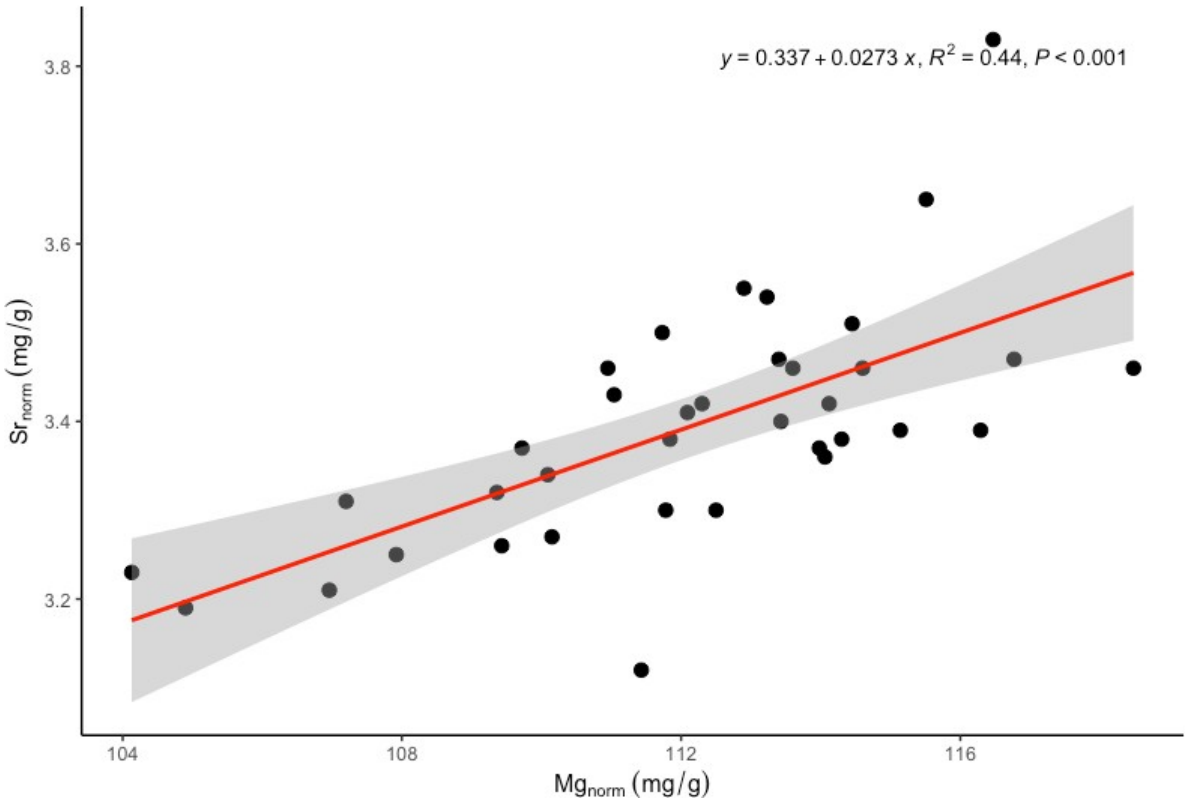

Figure S3. Correlation of Sr_{norm} ratios (mg/g) against Mg_{norm} ratios (mg/g) ($R^2 = 0.44$, $F_{1, 34} = 26.78$, $p = 1.019 \times 10^{-5}$) in *A. yairi* exposed to elevated temperatures levels (27 °C and 32 °C) crossed with increased pCO_2 concentrations (455 µatm, 1052 µatm, and 2066 µatm). (n = 36).

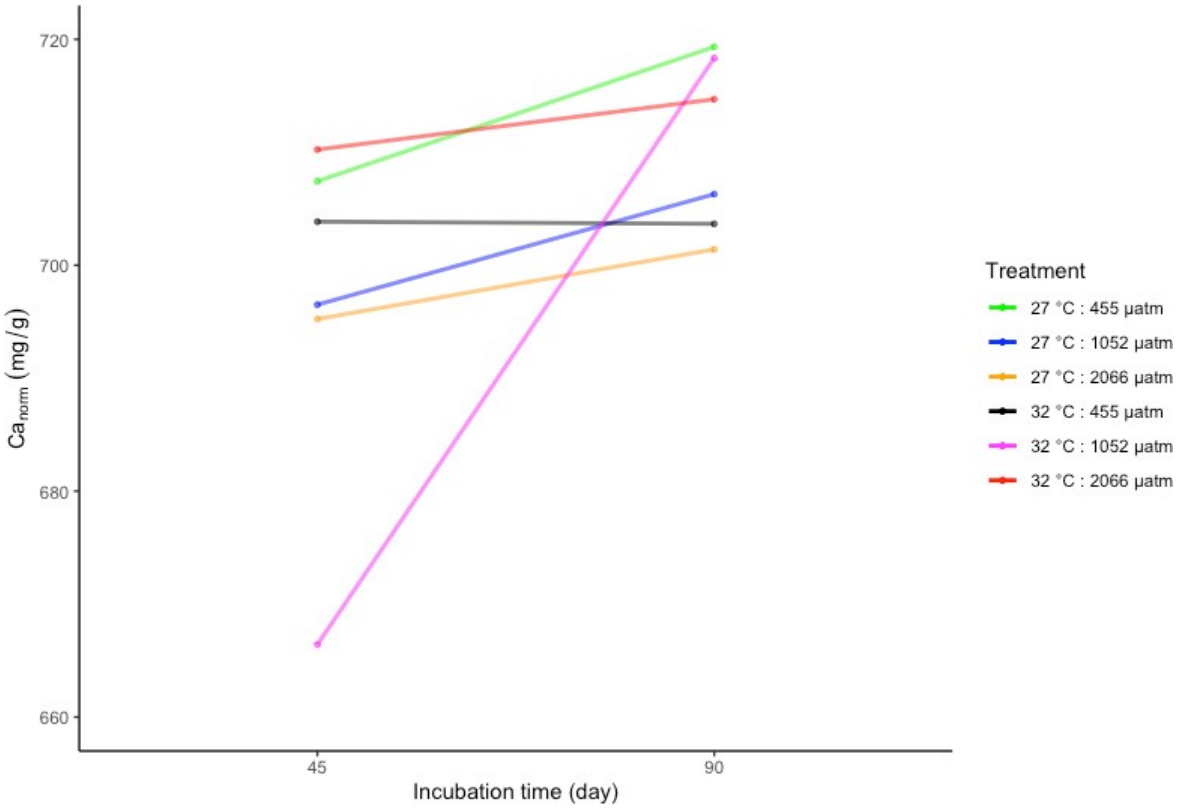

Figure S4. Skeletal Ca_{norm} (mg/g) ratio over incubation time in *A. yairi* exposed to elevated temperatures levels (27 °C and 32 °C) crossed with increased pCO_2 concentrations (455 µatm, 1052 µatm, and 2066 µatm). Data were presented as mean (n = 36).