

## **Supplementary information**

### **Triple threat: ocean acidification, warming, and hyposalinity synergistically weaken shell integrity in a Mediterranean calcifying mollusk**

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## **Experimental set-up details**

During the acclimation and the experiments clams were fed daily with the microalgae *Isochrysis galbana*. A short day's photoperiod of 10 h of light and 14 h of darkness was set. During the experiment, 80 clams grew in each mesocosm, with an aeration system and a submersible heater (SICCE SCUBA 50W), on a layer of beach sand replicating the seashore substrate. Two head tanks received seawater: one maintained control pH conditions (Tank 1), while the other served as a CO<sub>2</sub> mixing tank (Tank 4), where pH was lowered to 7.8–7.9 by automatic CO<sub>2</sub> injection, regulated by two independent Aqua Medic pH controllers (Fig. 1). From these two head tanks, four additional reservoir tanks were supplied: two connected to the control pH tank (Tank 2-3) and two to the low pH tank (Tank 5-6; Fig. 1). In two of these reservoir tanks (Tanks 3 and 6), salinity was reduced by adding freshwater, while the other two maintained control salinity conditions (Tanks 2 and 5; Fig. 1). Each reservoir tank fed six experimental mesocosms through a microcomputer-controlled system that delivered approximately 0.5 L of fresh seawater every five minutes to each mesocosm. Thermostats STC 1000 connected to 50 W seawater heaters were used to control temperatures individually at each mesocosms.

## **Determination of Total alkalinity (A<sub>t</sub>) following Sarazin et al., 1999.**

Calibration curves were obtained from a series of NaHCO<sub>3</sub> standard solutions prepared in artificial seawater without calcium (ASW). Water samples (2 mL) were mixed with 2 mL of a

color reagent (formic acid and bromophenol blue) and absorbance was read at 590 nm.  $A_t$  was calculated from the calibration line obtained with the standards. Measurements were performed twice during the experiment, on day 10 and on day 20. Linear interpolation between these two values was then used to estimate daily  $A_t$  values for the last 10 days of exposure.

Suppl. Table 1. Seawater aquarium parameters measured for each tank. Values are expressed as mean  $\pm$  95% confidence intervals.  $n$ , total number of measurements per treatment.

Treatment	Tank	$n$	pH <sub>T</sub>	Temperature (°C)	Salinity
T1 (control)	1	38	8.02 $\pm$ 0.01	18.08 $\pm$ 0.26	34.96 $\pm$ 0.15
T1 (control)	2	38	8.02 $\pm$ 0.01	17.83 $\pm$ 0.18	35.08 $\pm$ 0.18
T1 (control)	3	38	8.02 $\pm$ 0.01	18.08 $\pm$ 0.30	34.98 $\pm$ 0.17
T2 ( $\uparrow$ Temp)	4	38	8.02 $\pm$ 0.01	21.86 $\pm$ 0.13	35.22 $\pm$ 0.17
T2 ( $\uparrow$ Temp)	5	38	8.01 $\pm$ 0.01	22.02 $\pm$ 0.24	35.22 $\pm$ 0.16
T2 ( $\uparrow$ Temp)	6	38	8.01 $\pm$ 0.01	21.92 $\pm$ 0.18	35.21 $\pm$ 0.16
T3 ( $\downarrow$ Sal)	7	38	8.02 $\pm$ 0.01	18.02 $\pm$ 0.16	32.02 $\pm$ 0.17
T3 ( $\downarrow$ Sal)	8	38	8.01 $\pm$ 0.01	17.96 $\pm$ 0.19	32.00 $\pm$ 0.17
T3 ( $\downarrow$ Sal)	9	38	8.02 $\pm$ 0.01	18.03 $\pm$ 0.19	32.00 $\pm$ 0.18
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	10	38	8.03 $\pm$ 0.02	21.93 $\pm$ 0.14	32.17 $\pm$ 0.19
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	11	38	8.01 $\pm$ 0.01	22.01 $\pm$ 0.17	32.09 $\pm$ 0.19
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	12	38	8.02 $\pm$ 0.01	21.94 $\pm$ 0.18	32.20 $\pm$ 0.19
T5 ( $\downarrow$ pH)	13	38	7.88 $\pm$ 0.02	18.08 $\pm$ 0.19	35.10 $\pm$ 0.16
T5 ( $\downarrow$ pH)	14	38	7.90 $\pm$ 0.02	17.90 $\pm$ 0.20	35.06 $\pm$ 0.16
T5 ( $\downarrow$ pH)	15	38	7.89 $\pm$ 0.01	18.09 $\pm$ 0.18	35.12 $\pm$ 0.14
T6 ( $\downarrow$ pH $\uparrow$ Temp)	16	38	7.89 $\pm$ 0.02	22.06 $\pm$ 0.20	35.30 $\pm$ 0.12
T6 ( $\downarrow$ pH $\uparrow$ Temp)	17	38	7.90 $\pm$ 0.02	21.90 $\pm$ 0.22	35.25 $\pm$ 0.15
T6 ( $\downarrow$ pH $\uparrow$ Temp)	18	38	7.89 $\pm$ 0.01	22.05 $\pm$ 0.18	35.17 $\pm$ 0.14
T7 ( $\downarrow$ pH $\downarrow$ Sal)	19	38	7.88 $\pm$ 0.02	18.05 $\pm$ 0.12	31.94 $\pm$ 0.17
T7 ( $\downarrow$ pH $\downarrow$ Sal)	20	38	7.89 $\pm$ 0.02	18.01 $\pm$ 0.14	32.13 $\pm$ 0.19
T7 ( $\downarrow$ pH $\downarrow$ Sal)	21	38	7.89 $\pm$ 0.02	18.00 $\pm$ 0.21	32.01 $\pm$ 0.18
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	22	38	7.88 $\pm$ 0.02	22.00 $\pm$ 0.16	32.05 $\pm$ 0.23
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	23	38	7.87 $\pm$ 0.03	21.87 $\pm$ 0.19	32.22 $\pm$ 0.20
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	24	38	7.91 $\pm$ 0.02	21.91 $\pm$ 0.16	32.15 $\pm$ 0.21

Suppl. Table 2. Carbonate chemistry parameters calculated for each tank. Values are expressed as mean  $\pm$  95% confidence intervals.  $n$ , total number of measurements per treatment. Abbreviations:  $A_T$  total alkalinity,  $pCO_2$ , partial pressure of  $CO_2$ ; DIC, dissolved inorganic carbon;  $HCO_3^-$ , bicarbonate ion concentration;  $CO_3^{2-}$ , carbonate ion concentration;  $\Omega_{Ar}$ , aragonite saturation state;  $\Omega_{Ca}$ , calcite saturation state.

Treatment	Tank	$n$	$A_T$ ( $\mu\text{mol kg}^{-1}$ )	$pCO_2$ ( $\mu\text{atm}$ )	DIC ( $\mu\text{mol kg}^{-1}$ )	$HCO_3^-$ ( $\mu\text{mol kg}^{-1}$ )	$CO_3^{2-}$ ( $\mu\text{mol kg}^{-1}$ )	$\Omega_A$	$\Omega_C$
T1 (control)	1	19	2526 $\pm$ 190	457 $\pm$ 38	2274 $\pm$ 177	2072 $\pm$ 162	187 $\pm$ 15	2.89 $\pm$ 0.23	4.47 $\pm$ 0.35
T1 (control)	2	19	2505 $\pm$ 175	463 $\pm$ 49	2260 $\pm$ 166	2062 $\pm$ 154	182 $\pm$ 13	2.81 $\pm$ 0.20	4.34 $\pm$ 0.32
T1 (control)	3	19	2663 $\pm$ 212	471 $\pm$ 41	2397 $\pm$ 198	2180 $\pm$ 181	201 $\pm$ 16	3.10 $\pm$ 0.26	4.79 $\pm$ 0.40
T2 ( $\uparrow$ Temp)	4	19	2744 $\pm$ 242	489 $\pm$ 44	2437 $\pm$ 222	2189 $\pm$ 199	233 $\pm$ 22	3.63 $\pm$ 0.34	5.55 $\pm$ 0.53
T2 ( $\uparrow$ Temp)	5	19	2623 $\pm$ 195	482 $\pm$ 38	2334 $\pm$ 179	2102 $\pm$ 160	217 $\pm$ 18	3.39 $\pm$ 0.28	5.18 $\pm$ 0.43
T2 ( $\uparrow$ Temp)	6	19	2491 $\pm$ 184	458 $\pm$ 37	2211 $\pm$ 167	1991 $\pm$ 149	206 $\pm$ 19	3.21 $\pm$ 0.29	4.92 $\pm$ 0.45
T3 ( $\downarrow$ Sal)	7	19	2612 $\pm$ 119	479 $\pm$ 29	2373 $\pm$ 112	2173 $\pm$ 104	184 $\pm$ 9	2.88 $\pm$ 0.14	4.48 $\pm$ 0.23
T3 ( $\downarrow$ Sal)	8	19	2650 $\pm$ 146	499 $\pm$ 23	2417 $\pm$ 133	2217 $\pm$ 120	182 $\pm$ 13	2.84 $\pm$ 0.21	4.43 $\pm$ 0.32
T3 ( $\downarrow$ Sal)	9	19	2601 $\pm$ 137	487 $\pm$ 34	2366 $\pm$ 128	2168 $\pm$ 117	181 $\pm$ 12	2.84 $\pm$ 0.18	4.42 $\pm$ 0.28
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	10	19	2639 $\pm$ 143	481 $\pm$ 48	2360 $\pm$ 131	2131 $\pm$ 122	214 $\pm$ 23	3.40 $\pm$ 0.37	5.24 $\pm$ 0.58
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	11	19	2613 $\pm$ 149	497 $\pm$ 24	2349 $\pm$ 134	2131 $\pm$ 119	203 $\pm$ 16	3.23 $\pm$ 0.25	4.98 $\pm$ 0.38
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	12	19	2727 $\pm$ 146	509 $\pm$ 26	2451 $\pm$ 133	2221 $\pm$ 119	214 $\pm$ 14	3.40 $\pm$ 0.23	5.25 $\pm$ 0.35
T5 ( $\downarrow$ pH)	13	19	2447 $\pm$ 207	650 $\pm$ 69	2270 $\pm$ 196	2109 $\pm$ 182	138 $\pm$ 15	2.13 $\pm$ 0.24	3.30 $\pm$ 0.36
T5 ( $\downarrow$ pH)	14	19	2622 $\pm$ 172	704 $\pm$ 57	2441 $\pm$ 163	2271 $\pm$ 151	146 $\pm$ 12	2.24 $\pm$ 0.19	3.47 $\pm$ 0.29
T5 ( $\downarrow$ pH)	15	19	2467 $\pm$ 178	649 $\pm$ 53	2287 $\pm$ 167	2125 $\pm$ 154	140 $\pm$ 13	2.16 $\pm$ 0.21	3.34 $\pm$ 0.32
T6 ( $\downarrow$ pH $\uparrow$ Temp)	16	19	2654 $\pm$ 224	705 $\pm$ 81	2435 $\pm$ 213	2242 $\pm$ 198	172 $\pm$ 16	2.68 $\pm$ 0.25	4.11 $\pm$ 0.38
T6 ( $\downarrow$ pH $\uparrow$ Temp)	17	19	2542 $\pm$ 184	617 $\pm$ 52	2318 $\pm$ 172	2127 $\pm$ 157	172 $\pm$ 15	2.69 $\pm$ 0.23	4.12 $\pm$ 0.35
T6 ( $\downarrow$ pH $\uparrow$ Temp)	18	19	2756 $\pm$ 232	710 $\pm$ 78	2525 $\pm$ 220	2321 $\pm$ 204	183 $\pm$ 16	2.86 $\pm$ 0.25	4.37 $\pm$ 0.39
T7 ( $\downarrow$ pH $\downarrow$ Sal)	19	19	2455 $\pm$ 199	689 $\pm$ 79	2300 $\pm$ 193	2150 $\pm$ 182	127 $\pm$ 11	1.98 $\pm$ 0.17	3.09 $\pm$ 0.26
T7 ( $\downarrow$ pH $\downarrow$ Sal)	20	19	2541 $\pm$ 196	663 $\pm$ 53	2370 $\pm$ 182	2207 $\pm$ 167	139 $\pm$ 16	2.18 $\pm$ 0.25	3.39 $\pm$ 0.38
T7 ( $\downarrow$ pH $\downarrow$ Sal)	21	19	2610 $\pm$ 228	721 $\pm$ 76	2446 $\pm$ 217	2284 $\pm$ 202	137 $\pm$ 15	2.14 $\pm$ 0.24	3.34 $\pm$ 0.37
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	22	19	2458 $\pm$ 182	702 $\pm$ 78	2278 $\pm$ 173	2112 $\pm$ 161	144 $\pm$ 15	2.29 $\pm$ 0.23	3.53 $\pm$ 0.36
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	23	19	2577 $\pm$ 205	623 $\pm$ 75	2360 $\pm$ 197	2171 $\pm$ 185	170 $\pm$ 12	2.71 $\pm$ 0.20	4.17 $\pm$ 0.31
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	24	19	2481 $\pm$ 202	575 $\pm$ 62	2264 $\pm$ 192	2078 $\pm$ 178	168 $\pm$ 14	2.67 $\pm$ 0.22	4.11 $\pm$ 0.34

Suppl. Table 3. Shell biometric parameters measured for each tank. Values are expressed as mean  $\pm$  95% confidence intervals. *n*, total number of measurements per treatment.

Treatment	Tank	<i>n</i>	Length (mm)	Height (mm)	Width (mm)	Mass (g)
T1 (control)	1	43	24.85 $\pm$ 0.47	21.33 $\pm$ 0.38	5.87 $\pm$ 0.12	1.32 $\pm$ 0.07
T1 (control)	2	42	24.78 $\pm$ 0.50	21.12 $\pm$ 0.34	5.85 $\pm$ 0.10	1.33 $\pm$ 0.07
T1 (control)	3	42	24.90 $\pm$ 0.44	21.29 $\pm$ 0.33	5.91 $\pm$ 0.10	1.34 $\pm$ 0.07
T2 ( $\uparrow$ Temp)	4	15	26.11 $\pm$ 1.02	22.15 $\pm$ 0.69	6.04 $\pm$ 0.25	1.48 $\pm$ 0.16
T2 ( $\uparrow$ Temp)	5	15	25.05 $\pm$ 0.80	21.42 $\pm$ 0.57	5.97 $\pm$ 0.15	1.37 $\pm$ 0.14
T2 ( $\uparrow$ Temp)	6	23	24.09 $\pm$ 0.48	20.89 $\pm$ 0.41	5.73 $\pm$ 0.13	1.23 $\pm$ 0.08
T3 ( $\downarrow$ Sal)	7	40	25.35 $\pm$ 0.42	21.70 $\pm$ 0.33	5.89 $\pm$ 0.12	1.39 $\pm$ 0.07
T3 ( $\downarrow$ Sal)	8	39	24.79 $\pm$ 0.57	21.33 $\pm$ 0.42	5.77 $\pm$ 0.13	1.34 $\pm$ 0.07
T3 ( $\downarrow$ Sal)	9	49	24.86 $\pm$ 0.43	21.61 $\pm$ 0.32	5.86 $\pm$ 0.10	1.34 $\pm$ 0.05
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	10	27	25.56 $\pm$ 0.72	21.91 $\pm$ 0.59	6.08 $\pm$ 0.15	1.46 $\pm$ 0.12
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	11	15	25.31 $\pm$ 0.76	21.38 $\pm$ 0.48	5.97 $\pm$ 0.20	1.35 $\pm$ 0.10
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	12	29	25.42 $\pm$ 0.60	21.60 $\pm$ 0.48	5.91 $\pm$ 0.13	1.39 $\pm$ 0.09
T5 ( $\downarrow$ pH)	13	23	24.45 $\pm$ 0.41	21.29 $\pm$ 0.34	6.00 $\pm$ 0.16	1.33 $\pm$ 0.06
T5 ( $\downarrow$ pH)	14	30	25.19 $\pm$ 0.54	21.95 $\pm$ 0.44	5.87 $\pm$ 0.13	1.37 $\pm$ 0.07
T5 ( $\downarrow$ pH)	15	42	24.84 $\pm$ 0.44	21.37 $\pm$ 0.32	5.88 $\pm$ 0.11	1.31 $\pm$ 0.06
T6 ( $\downarrow$ pH $\uparrow$ Temp)	16	8	24.93 $\pm$ 1.16	21.73 $\pm$ 0.93	5.95 $\pm$ 0.32	1.33 $\pm$ 0.15
T6 ( $\downarrow$ pH $\uparrow$ Temp)	17	6	24.38 $\pm$ 1.33	21.04 $\pm$ 0.84	5.82 $\pm$ 0.23	1.22 $\pm$ 0.13
T6 ( $\downarrow$ pH $\uparrow$ Temp)	18	11	24.65 $\pm$ 0.82	21.46 $\pm$ 0.68	5.84 $\pm$ 0.20	1.32 $\pm$ 0.11
T7 ( $\downarrow$ pH $\downarrow$ Sal)	19	21	25.29 $\pm$ 0.98	21.79 $\pm$ 0.67	5.98 $\pm$ 0.18	1.38 $\pm$ 0.13
T7 ( $\downarrow$ pH $\downarrow$ Sal)	20	12	25.42 $\pm$ 1.25	21.77 $\pm$ 0.90	5.99 $\pm$ 0.30	1.45 $\pm$ 0.21
T7 ( $\downarrow$ pH $\downarrow$ Sal)	21	21	23.98 $\pm$ 0.62	21.04 $\pm$ 0.32	5.82 $\pm$ 0.16	1.24 $\pm$ 0.07
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	22	13	24.99 $\pm$ 0.97	21.74 $\pm$ 0.64	5.93 $\pm$ 0.20	1.27 $\pm$ 0.12
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	23	6	25.01 $\pm$ 1.52	21.42 $\pm$ 1.30	5.74 $\pm$ 0.44	1.25 $\pm$ 0.18
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	24	8	23.83 $\pm$ 1.29	20.81 $\pm$ 0.69	5.78 $\pm$ 0.39	1.23 $\pm$ 0.19

Suppl. Table 4. Shell skeletal parameters measured for each tank. Values are expressed as mean  $\pm$  95% confidence intervals. *n*, total number of measurements per treatment.

Treatment	Tanks	<i>n</i>	Micro-density (g/cm <sup>3</sup> )	Apparent porosity (%)	Bulk density (g/cm <sup>3</sup> )
T1 (control)	1	43	2.80 $\pm$ 0.01	4.56 $\pm$ 0.59	2.64 $\pm$ 0.03
T1 (control)	2	42	2.82 $\pm$ 0.02	5.35 $\pm$ 0.85	2.67 $\pm$ 0.01
T1 (control)	3	42	2.84 $\pm$ 0.02	5.92 $\pm$ 1.00	2.67 $\pm$ 0.01
T2 ( $\uparrow$ Temp)	4	15	2.81 $\pm$ 0.01	5.42 $\pm$ 0.72	2.67 $\pm$ 0.03
T2 ( $\uparrow$ Temp)	5	15	2.78 $\pm$ 0.01	4.28 $\pm$ 0.58	2.59 $\pm$ 0.09
T2 ( $\uparrow$ Temp)	6	23	2.80 $\pm$ 0.04	4.29 $\pm$ 1.11	2.68 $\pm$ 0.05
T3 ( $\downarrow$ Sal)	7	40	2.81 $\pm$ 0.01	4.63 $\pm$ 0.89	2.68 $\pm$ 0.01
T3 ( $\downarrow$ Sal)	8	39	2.84 $\pm$ 0.03	7.77 $\pm$ 1.05	2.63 $\pm$ 0.03
T3 ( $\downarrow$ Sal)	9	49	2.80 $\pm$ 0.01	4.84 $\pm$ 0.42	2.66 $\pm$ 0.01
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	10	27	2.85 $\pm$ 0.06	5.22 $\pm$ 0.81	2.70 $\pm$ 0.05
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	11	15	2.83 $\pm$ 0.02	6.28 $\pm$ 1.25	2.65 $\pm$ 0.02
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	12	29	2.81 $\pm$ 0.01	4.67 $\pm$ 0.75	2.67 $\pm$ 0.01
T5 ( $\downarrow$ pH)	13	23	2.81 $\pm$ 0.02	5.98 $\pm$ 0.88	2.64 $\pm$ 0.02
T5 ( $\downarrow$ pH)	14	30	2.79 $\pm$ 0.03	5.52 $\pm$ 0.64	2.62 $\pm$ 0.03
T5 ( $\downarrow$ pH)	15	42	2.84 $\pm$ 0.08	7.55 $\pm$ 1.63	2.64 $\pm$ 0.04
T6 ( $\downarrow$ pH $\uparrow$ Temp)	16	8	2.80 $\pm$ 0.02	4.77 $\pm$ 1.66	2.69 $\pm$ 0.05
T6 ( $\downarrow$ pH $\uparrow$ Temp)	17	6	2.84 $\pm$ 0.03	8.95 $\pm$ 2.93	2.59 $\pm$ 0.06
T6 ( $\downarrow$ pH $\uparrow$ Temp)	18	11	2.79 $\pm$ 0.02	4.07 $\pm$ 1.32	2.68 $\pm$ 0.02
T7 ( $\downarrow$ pH $\downarrow$ Sal)	19	21	2.77 $\pm$ 0.05	6.52 $\pm$ 1.41	2.68 $\pm$ 0.06
T7 ( $\downarrow$ pH $\downarrow$ Sal)	20	12	2.78 $\pm$ 0.01	5.24 $\pm$ 1.24	2.70 $\pm$ 0.08
T7 ( $\downarrow$ pH $\downarrow$ Sal)	21	21	2.81 $\pm$ 0.03	7.60 $\pm$ 1.29	2.59 $\pm$ 0.02
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	22	13	2.86 $\pm$ 0.04	11.20 $\pm$ 3.06	2.51 $\pm$ 0.06
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	23	6	2.82 $\pm$ 0.06	7.40 $\pm$ 3.82	2.61 $\pm$ 0.08
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	24	8	2.87 $\pm$ 0.05	10.44 $\pm$ 2.33	2.52 $\pm$ 0.06

Suppl. Table 5. Skeletal properties of *Chamelea gallina* across treatment (T1 to T8; data from the three replicated tanks were pooled). Values are expressed as mean  $\pm$  95% confidence intervals. *n*, total number of measurements per treatment. Statistical significance of Kruskal–Wallis test across treatments is reported in the last row as *p*-values.

Treatment	<i>n</i>	Micro-density (g cm <sup>-3</sup> )	Bulk density (g cm <sup>-3</sup> )	Apparent Porosity (%)
T1 (control)	127	2.82 $\pm$ 0.01	2.66 $\pm$ 0.01	5.27 $\pm$ 0.47
T2 ( $\uparrow$ Temp)	53	2.80 $\pm$ 0.02	2.65 $\pm$ 0.03	4.61 $\pm$ 0.54
T3 ( $\downarrow$ Sal)	128	2.82 $\pm$ 0.01	2.66 $\pm$ 0.01	5.67 $\pm$ 0.50
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	71	2.83 $\pm$ 0.02	2.68 $\pm$ 0.02	5.22 $\pm$ 0.50
T5 ( $\downarrow$ pH)	95	2.82 $\pm$ 0.04	2.63 $\pm$ 0.02	6.53 $\pm$ 0.78
T6 ( $\downarrow$ pH $\uparrow$ Temp)	25	2.80 $\pm$ 0.02	2.66 $\pm$ 0.03	5.47 $\pm$ 1.20
T7 ( $\downarrow$ pH $\downarrow$ Sal)	54	2.79 $\pm$ 0.02	2.65 $\pm$ 0.03	6.65 $\pm$ 0.78
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	27	2.85 $\pm$ 0.02	2.53 $\pm$ 0.04	10.13 $\pm$ 1.71
<i>Kruskal Wallis test</i>		<i>0.000</i>	<i>0.000</i>	<i>0.000</i>

Suppl. Table 6. Mechanical properties of *Chamelea gallina* across treatment (T1 to T8; data from the three replicated tanks were pooled). Values are expressed as mean  $\pm$  95% confidence intervals. *n*, total number of measurements per treatment. Statistical significance across treatments (Kruskal–Wallis test or one-way ANOVA) is reported in the last row as *p*-values.

Treatment	<i>n</i>	Maximum load (kN)	Structural stiffness (kN mm <sup>-1</sup> )
T1 (control)	12	0.09 $\pm$ 0.02	1.77 $\pm$ 0.19
T2 ( $\uparrow$ Temp)	12	0.08 $\pm$ 0.01	1.59 $\pm$ 0.24
T3 ( $\downarrow$ Sal)	12	0.07 $\pm$ 0.02	1.38 $\pm$ 0.31
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	12	0.09 $\pm$ 0.01	1.68 $\pm$ 0.31
T5 ( $\downarrow$ pH)	12	0.07 $\pm$ 0.02	1.37 $\pm$ 0.20
T6 ( $\downarrow$ pH $\uparrow$ Temp)	12	0.07 $\pm$ 0.01	1.36 $\pm$ 0.24
T7 ( $\downarrow$ pH $\downarrow$ Sal)	12	0.07 $\pm$ 0.02	1.28 $\pm$ 0.29
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	12	0.06 $\pm$ 0.01	1.21 $\pm$ 0.33
<i>K-W/ANOVA</i>		<i>0.031</i>	<i>0.013</i>

Suppl. Table 7. Shell microstructural properties for each treatment for the whole shell (T1 to T8; data from the three replicated tanks were pooled). Values are expressed as mean  $\pm$  95% confidence intervals.  $n = 6$  represents the total number of measurements per treatment. Statistical significance across treatments (Kruskal–Wallis test or one-way ANOVA) is reported in the last row as  $p$ -values. Different letters beside calcium values indicate significant differences among treatments (Tukey’s post-hoc test,  $p \leq 0.05$ ).

Treatment	Crystallite Size Aragonite (nm)			Mineral composition (wt%)		
	111	021	200	Aragonite	Calcite	Ca (wt%)
T1 (control)	110.0 $\pm$ 9.4	94.0 $\pm$ 7.6	108.7 $\pm$ 11.5	99.50 $\pm$ 0.21	0.50 $\pm$ 0.21	37.20 $\pm$ 4.57 <sup>a</sup>
T2 ( $\uparrow$ Temp)	72.7 $\pm$ 6.4	63.1 $\pm$ 6.7	77.5 $\pm$ 9.3	98.24 $\pm$ 0.62	1.76 $\pm$ 0.62	35.18 $\pm$ 2.28 <sup>ab</sup>
T3 ( $\downarrow$ Sal)	87.4 $\pm$ 14.5	75.6 $\pm$ 11.5	89.6 $\pm$ 23.0	98.72 $\pm$ 0.53	1.28 $\pm$ 0.53	37.64 $\pm$ 2.17 <sup>a</sup>
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	108.4 $\pm$ 5.5	93.3 $\pm$ 4.5	104.8 $\pm$ 9.2	99.62 $\pm$ 0.62	0.38 $\pm$ 0.62	36.81 $\pm$ 0.54 <sup>ab</sup>
T5 ( $\downarrow$ pH)	75.0 $\pm$ 11.4	64.7 $\pm$ 9.0	79.7 $\pm$ 9.4	98.30 $\pm$ 0.88	1.70 $\pm$ 0.89	35.20 $\pm$ 0.86 <sup>ab</sup>
T6 ( $\downarrow$ pH $\uparrow$ Temp)	73.6 $\pm$ 20.1	63.1 $\pm$ 17.1	76.9 $\pm$ 13.3	98.26 $\pm$ 0.85	1.74 $\pm$ 0.85	37.21 $\pm$ 1.62 <sup>a</sup>
T7 ( $\downarrow$ pH $\downarrow$ Sal)	71.5 $\pm$ 19.3	62.5 $\pm$ 14.1	74.4 $\pm$ 21.1	98.17 $\pm$ 0.79	1.83 $\pm$ 0.79	34.17 $\pm$ 1.16 <sup>b</sup>
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	68.9 $\pm$ 9.8	59.5 $\pm$ 3.9	72.1 $\pm$ 14.9	98.02 $\pm$ 0.46	1.98 $\pm$ 0.46	35.95 $\pm$ 3.83 <sup>ab</sup>
<i>K-W/ANOVA</i>	<i>0.016</i>	<i>0.012</i>	<i>0.017</i>	<i>0.020</i>	<i>0.020</i>	<i>0.006</i>

Suppl. Table 8. Crystallite size and mineral composition measurements of the shell edge region for each treatment. Values are expressed as mean  $\pm$  95% confidence intervals.  $n = 3$  represents the total number of measurements per treatment.

Treatment	Crystallite Size Aragonite (nm)			Mineral composition (wt%)	
	A (111)	A (021)	A (200)	Aragonite	Calcite
T1 (control)	64.55 $\pm$ 15.34	55.17 $\pm$ 14.67	72.33 $\pm$ 8.80	92.54 $\pm$ 7.42	7.49 $\pm$ 7.29
T2 ( $\uparrow$ Temp)	60.89 $\pm$ 13.24	51.16 $\pm$ 12.32	71.30 $\pm$ 14.09	93.55 $\pm$ 6.83	6.45 $\pm$ 6.83
T3 ( $\downarrow$ Sal)	64.11 $\pm$ 4.89	53.50 $\pm$ 7.73	74.37 $\pm$ 8.85	95.49 $\pm$ 1.20	4.51 $\pm$ 1.20
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	65.91 $\pm$ 3.58	55.81 $\pm$ 6.09	74.80 $\pm$ 5.98	95.89 $\pm$ 0.50	4.11 $\pm$ 0.50
T5 ( $\downarrow$ pH)	55.60 $\pm$ 6.62	46.37 $\pm$ 2.79	64.57 $\pm$ 15.82	95.11 $\pm$ 1.71	4.89 $\pm$ 1.71
T6 ( $\downarrow$ pH $\uparrow$ Temp)	56.79 $\pm$ 12.68	47.27 $\pm$ 12.40	67.37 $\pm$ 7.97	93.16 $\pm$ 9.95	6.84 $\pm$ 9.95
T7 ( $\downarrow$ pH $\downarrow$ Sal)	57.18 $\pm$ 18.18	47.22 $\pm$ 14.06	70.60 $\pm$ 22.37	94.60 $\pm$ 2.53	5.40 $\pm$ 2.53
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	58.15 $\pm$ 13.03	48.66 $\pm$ 13.07	69.20 $\pm$ 10.96	94.81 $\pm$ 1.77	5.19 $\pm$ 1.77

Suppl. Table 9. Crystallite size and mineral composition measurements of the shell central region for each treatment. Values are expressed as mean  $\pm$  95% confidence intervals.  $n = 3$  represents the total number of measurements per treatment.

Treatment	Crystallite Size Aragonite (nm)			Mineral composition (wt%)	
	A (111)	A (021)	A (200)	Aragonite	Calcite
T1 (control)	75.99 $\pm$ 12.57	65.67 $\pm$ 9.46	80.37 $\pm$ 11.58	98.24 $\pm$ 0.98	7.49 $\pm$ 0.98
T2 ( $\uparrow$ Temp)	75.58 $\pm$ 22.93	65.13 $\pm$ 20.15	79.00 $\pm$ 17.16	98.15 $\pm$ 2.15	6.45 $\pm$ 2.15
T3 ( $\downarrow$ Sal)	72.33 $\pm$ 11.98	61.83 $\pm$ 10.97	76.07 $\pm$ 11.54	98.01 $\pm$ 0.70	4.51 $\pm$ 0.70
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	72.10 $\pm$ 8.55	64.82 $\pm$ 11.60	74.27 $\pm$ 6.05	98.29 $\pm$ 1.76	4.11 $\pm$ 1.76
T5 ( $\downarrow$ pH)	77.11 $\pm$ 19.67	66.64 $\pm$ 19.46	80.40 $\pm$ 19.40	98.00 $\pm$ 0.53	4.89 $\pm$ 0.53
T6 ( $\downarrow$ pH $\uparrow$ Temp)	74.14 $\pm$ 2.76	64.91 $\pm$ 3.43	76.77 $\pm$ 7.23	97.96 $\pm$ 0.15	6.84 $\pm$ 0.15
T7 ( $\downarrow$ pH $\downarrow$ Sal)	72.76 $\pm$ 11.34	61.77 $\pm$ 7.48	77.63 $\pm$ 17.38	97.70 $\pm$ 0.50	5.40 $\pm$ 0.50
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	67.38 $\pm$ 9.76	57.56 $\pm$ 12.60	71.30 $\pm$ 6.34	97.35 $\pm$ 0.47	5.19 $\pm$ 0.47

Suppl. Table 10. Crystallite size and mineral composition measurements of the shell umbo region for each treatment. Values are expressed as mean  $\pm$  95% confidence intervals.  $n = 3$  represents the total number of measurements per treatment.

Treatment	Crystallite Size Aragonite (nm)			Mineral composition (wt%)	
	A (111)	A (021)	A (200)	Aragonite	Calcite
T1 (control)	62.14 $\pm$ 6.96	52.26 $\pm$ 4.83	65.93 $\pm$ 7.14	94.24 $\pm$ 2.23	5.76 $\pm$ 2.23
T2 ( $\uparrow$ Temp)	60.34 $\pm$ 10.63	52.32 $\pm$ 6.68	64.43 $\pm$ 15.55	93.82 $\pm$ 1.75	6.18 $\pm$ 1.75
T3 ( $\downarrow$ Sal)	53.49 $\pm$ 17.25	47.47 $\pm$ 13.31	55.13 $\pm$ 20.00	93.81 $\pm$ 2.46	6.19 $\pm$ 2.46
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	61.32 $\pm$ 13.14	52.28 $\pm$ 7.92	68.03 $\pm$ 25.05	93.38 $\pm$ 2.40	6.53 $\pm$ 2.75
T5 ( $\downarrow$ pH)	64.54 $\pm$ 29.71	53.94 $\pm$ 20.57	71.90 $\pm$ 42.49	94.91 $\pm$ 2.86	5.09 $\pm$ 2.86
T6 ( $\downarrow$ pH $\uparrow$ Temp)	58.60 $\pm$ 20.14	51.35 $\pm$ 17.12	61.97 $\pm$ 22.42	94.61 $\pm$ 3.92	5.39 $\pm$ 3.92
T7 ( $\downarrow$ pH $\downarrow$ Sal)	57.92 $\pm$ 14.68	48.95 $\pm$ 11.51	64.03 $\pm$ 15.29	93.67 $\pm$ 2.08	6.33 $\pm$ 2.08
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	66.71 $\pm$ 13.66	56.13 $\pm$ 12.10	74.40 $\pm$ 19.93	96.89 $\pm$ 2.65	3.11 $\pm$ 2.65

Suppl. Table 11. Intracrystalline material and shell decarboxylation percentage weight loss for each treatment. Values are expressed as mean  $\pm$  95% confidence intervals.  $n$ , total number of measurements per treatment.

Treatment	$n$	Intracrystalline material + H <sub>2</sub> O (wt%; 150 - 500°C)	Decarboxylation (wt%; 500 - 900°C)
T1 (control)	5	1.84 $\pm$ 0.20	42.68 $\pm$ 0.34
T2 ( $\uparrow$ Temp)	5	1.91 $\pm$ 0.30	42.55 $\pm$ 0.24
T3 ( $\downarrow$ Sal)	5	1.91 $\pm$ 0.02	42.02 $\pm$ 0.64
T4 ( $\uparrow$ Temp $\downarrow$ Sal)	5	1.81 $\pm$ 0.07	42.63 $\pm$ 1.18
T5 ( $\downarrow$ pH)	5	1.75 $\pm$ 0.27	42.38 $\pm$ 0.35
T6 ( $\downarrow$ pH $\uparrow$ Temp)	5	1.82 $\pm$ 0.23	42.57 $\pm$ 0.18
T7 ( $\downarrow$ pH $\downarrow$ Sal)	5	1.79 $\pm$ 0.23	42.38 $\pm$ 0.33
T8 ( $\downarrow$ pH $\uparrow$ Temp $\downarrow$ Sal)	5	1.83 $\pm$ 0.16	42.43 $\pm$ 0.38