Supplemental materials

Net calcification (NC)

Net calcification rates of corals incubated under light and dark conditions were compared to fragments lack of tissue. For this, samples were immersed in sodium hypochlorite (NaOCl) overnight and subsequently rinsed thoroughly with DDW. Vessels containing seawater without fragments under light incubation (hereafter, seawater-only control) were used as a control for changes in carbonate chemistry during incubation not caused by the coral itself. For each treatment, pH was measured and water samples were collected for alkalinity at the beginning and end of the incubation to determine carbonate chemistry. Water samples were stored in the dark at 4°C until analyzed. Calcification rates were calculated from the difference between TA measured at the beginning and the end of each incubation period according to the following equation (Schneider and Erez, 2006):

$$\text{Calcification}(\mu\text{mol } CaCO_3 \text{ cm}^{-2} \text{ h}^{-1} = \frac{\frac{\Delta TA}{2} \times (V_{chamber} - V_{coral}) \times 1000 \times 1.028}{T \times S.A} \quad (1)$$

Where ΔTA is- the difference in TA measured at the beginning and end of each incubation period (milliequivalent per liter); $V_{chamber}$ is- the volume of the experimental vessel (ml); V_{coral} is- the displacement volume of the coral (ml); *1.028* is the density of seawater in the northern Gulf of Eilat (g ml^{-1})**;** *T* is- the incubation duration (hours); *S.A.* is- the surface area of the coral fragment (cm^2). The same equation was used to measure dissolution of bare coral skeleton. Calcification rates as obtained from the intercomparison experiment were also normalized to specimen dry skeleton weight for comparison with the radioactive estimates of calcification.

Gross calcification (GC)

In the present study, an improved ^{45}Ca protocol (Tambutte et al., 1995) was employed using cultured microcolonies (Almoghrabi et al., 1993) entirely covered by coral tissue which prevented non-specific ^{45}Ca binding with the skeleton.

Microcolonies were placed in 40 ml incubation vessels containing FSW with a total activity of 360 kBq (^{45}Ca as CaCl2, 1958.18 MBq ml-1, PerkinElmer Life and Analytical Sciences) taken from a pre-prepared stock solution. The amount of activity was determined in a preliminary experiment. Dead specimens were included in the experiment as a control for isotopic exchange (Al-Horani et al., 2005) and were treated as live specimens. Two dead microcolonies from each pH conditions were immersed in 2% formaldehyde for 30 min, rinsed thoroughly several times with FSW and then, placed in the incubation vessels. Three incubation vessels were left without fragments to serve as a seawater-only control. Aliquots of 100-µl were taken at the beginning and end of each incubation to determine the specific activity. Following the labeling period, specimens were immersed in 600 ml FSW for one minute, then rinsed five times (each rinse lasting one minute) with 10 ml of ice-cold glycine-high calcium medium (50 mM $CaCl_2$, 950mM glycine, pH adjusted to 8.2). Labeled specimens were then incubated for 30 min. in vessels containing 20 ml of ^{45}Ca-free sea water. Water motion was provided by a shaker. Following efflux incubation, microcolony tissue was removed using 2 M NaOH for 20 min at 90°C. A minimum volume of 5 ml NaOH solution was used but an additional volume was added until the specimen was entirely covered by solution (total volume was recorded) for thorough removal of coral tissue. After tissue hydrolysis, the skeleton was first rinsed with 1 ml NaOH (Houlbreque et al., 2003), then thoroughly rinsed with FSW and finally rinsed

with DDW (Tambutte et al., 1995). As solution from the first rinse was added to the tissue hydrolysate and the remaining rinsing solution was decanted. Finally, skeletons were dried at 70°C for about 5 h and skeleton dry weight was determined. Skeletons were completely dissolved in 12 M HCl by adding small amounts of the solution (the minimum total volume was 2.4 ml and total volume was recorded), over a period of a day accompanied by gentle shaking. To exclude errors due to adsorption or precipitation of radioisotope on the walls of the vessels, the vessels used in the washing procedure were rinsed with 5 M HCl and FSW between the incubation periods and new vials were used to collect the tissue and the skeleton fractions from each specimen. Samples (500 µl) of skeleton digest and tissue hydrolysate were added to 10 ml Ultima Gold AB (PerkinElmer) scintillation liquid and measured on the scintillation counter (Tri-carb 1600TR, Packard). Prior to counting, 2M HCl were added to tissue fractions until samples were no longer alkaline (acidic or neutralize) to avoid quenching caused by a chemical (NaOH). Counts of both fractions were corrected according to HCl/NaOH total volume to receive the total amount of ^{45}Ca incorporation into the skeleton/tissue during incubation. Calcification rates were then calculated from the activity recorded in seawater control samples and given in µmol Ca^{2+} per skeleton dry weight (Houlbreque et al., 2003;Tambutte et al., 1996) using the formula:

$$\text{Calcification}(\mu\text{mol } CaCO_3\ g^{-1} \text{ dry skeleton}) = \frac{\left(\text{Activity}_{\text{sample}} \times \frac{1.17}{\text{Activity}_{\text{seawater}}}\right)}{W} \quad (2)$$

Where $Activity_{sample}$ is the total DPM in skeleton dissolution sample ($Activity\ in\ 500\ \mu l \times Total\ HCl\ added\ (ml)/500\ \mu l$); $Activity_{seawater}$ is the total DPM in 100µl seawater sample (control); *1.17* is the amount of Ca^{2+} in 100µl ambient

seawater (µmol) and *W* is skeleton dry weight. The amount of ^{45}Ca uptake by dead specimens (covered with tissue) was subtracted from the amount measured in intact (live) specimens.

While we did not discuss ^{45}Ca uptake by the tissue it should be noted that there were no significant differences with time, for both pH treatments (Two-way ANOVA, p=0.728 and p=0.38 at pH_T 8.09 and 7.49 respectively).

Calculation of carbonate system in seawater

Total alkalinity (TA) values were measured using an automatic potentiometric titration (Mettler-Toledo GmbH, DL67 titrator) to the second end point (Almgren et al., 1983) of a 12.3-g accurately weighed seawater sample. It was then computed using the Gran equation (DOE, 1994) with pH values lower than 3.9 for creating the Gran plot. The pH electrodes (Mettler-Toledo DG-111–SC; Stockholm, Sweden) were calibrated daily before starting using the titrator. The acid concentration was 0.049N HCl (JT Baker, Phillipsburg, NJ). In the series of experiments comparing gross and net calcification, a new titrator was utilized: a Metrohm 862 compact titrosampler (autosampler combined with titrator) that uses not less than 35 g seawater samples. Hence, experimental samples containing only 40 ml, were diluted by a factor of 3 and acid concentration was set to 0.025 M. Alkalinity was calculated using the first derivative of the curve for the evaluation of the exact end point. Prior to measurement water samples were filtered (0.2µm membranes). The differences between duplicate samples were less than 6 µEq kg^{-1} (for calibration of the titrator, differences were measured between triplicate samples). Water samples analysis were

stored in darkness at 4°C in brown glass bottles filled up to the top with a gas tight screw and processed within two weeks of collection.

pH Measurements were carried out using a CyberScan pH meter (pH/Ion ,510 Eutech Instruments with automatic temperature compensation) and CyberScan gel-filled pH combination electrode. Prior to experiments, the pH electrode was calibrated against National Bureau of Standards (NBS) scale buffers of 4.01, 7.00 and 10.00 at 25 ^{o}C and was soaked in seawater for at least 1 h before measurement. The manufacturer's technical specifications of the pH meter are 0.01 pH for resolution and ± 0.01 (standard error) for accuracy.

Components of the carbonate system (pCO_2, CO_3^{-2}, HCO_3, DIC concentrations and Ω aragonite) were calculated from total alkalinity along with pH values, temperature and salinity using the CO2SYS program, version 01.03 (Lewis and Wallace 1998; Table 3). The pH_{NBS} were shifted onto the total pH scale (pH_T) by subtracting -0.11 (Zeebe and Wolf-Gladrow, 2001), which includes a minor correction for [SO_4^{2-}] and the stability constant of HSO_4^- at a salinity of 40.7‰. The thermodynamic carbonate dissociation constants for activity scales (K_1 and K_2) were attained from Mehrbach et al. (1973) and the refit by Dickson and Millero (1987).

Statistical analysis

Data from the tissue fixation, anesthesia and coral freezing experiments, as well as the lesioned corals experiment were analyzed by one- or two-way factorial analysis of variance (ANOVA) using the statistical software SPSS 15. If necessary, logarithmic or reciprocal transformations were performed to satisfy the assumptions of normality. Comparison of light and dark calcification with the dissolution of coral skeleton were performed using an ANOVA permutation test (e.g. Fisher 1935, Manly

1997) as the data did not meet with assumptions of normality and homogeneity of variance even with transformation. Permutation tests provide superior Type I error control when assumptions of traditional parametric tests are violated (Good, 1994). We then used the Akaike's Information Criterion (AIC_c), corrected for small sample size (Burnham and Anderson, 2002), to select the model (combination of factors) that best explained calcification rates patterns (minimum AIC_c score). Akaike weights were computed to evaluate the probability that a specific model is the best model for the observed data (Burnham and Anderson 2002; Johnson and Omland, 2004). In the results we refer only to the selected model. Where significant effects existed, we used Tukey's HSD multiple comparison to identify differences between subgroups.

To assess the compatibility of TA depletion and the ^{45}Ca-labling techniques we examined: (1) the strength of the relationship between methods using the Reduced Major Axis regression (RMA, Model 2 regression; Ricker, 1973;Jacques and Pilson, 1980). The regression was calculated with the geometric mean estimate described by Ricker (1973) and Ricker (1975); the slope of the functional regression is computed by dividing the slope of the least squares predictive regression by the correlation coefficient; and (2) the similarity between mean values and precision level (the difference of calcification value from the average, at each pH and time interval) of both methods using the permutation test for repeated measure ANOVA. The latter can evaluate whether both methods produce similar values for all individuals (degree of agreement between methods; Bland and Altman, 1986).

To detect differences between subgroups we used Tukey's HSD-adjusted for repeated measure ANOVA using the statistical program R (Maxwell and Delaney 2003). R, version 2.13.2 (R Development Core Team, 2006) was used to perform the

permutational ANOVA and the RMA analyses. The level of statistical significance was set at $p<0.05$.

Supplemental references

Al-Horani, F. A., Al-Rousan, S. A., Manasrah, R. S., and Rasheed, M. Y.: Coral calcification: Use of radioactive isotopes and metabolic inhibitors to study the interactions with photosynthesis and respiration, J. Chem. Ecol., 21, 325-335, 10.1080/02757540500258724, 2005.

Almgren, T., Dyrssen, D., and Fonselius, S.: Determination of alkalinity and total carbonate, in: Methods of seawater analysis, edited by: Grasshoff, K., Ehrhadt, M., and Kremling, K., Verlag Chemie GmbH, 99-123, 1983.

Almoghrabi, S., Allemand, D., and Jaubert, J.: Valine Uptake by the Scleractinian Coral Galaxea-Fascicularis - Characterization and Effect of Light and Nutritional-Status, J. Comp. Physiol. B.,, 163, 355-362, 1993.

Bland, J. M., and Altman, D. G.: STATISTICAL-METHODS FOR ASSESSING AGREEMENT BETWEEN 2 METHODS OF CLINICAL MEASUREMENT, Lancet, 1, 307-310, 1986.

Burnham, K.P., and Anderson, D.R. (2002). Model Selection and Multimodel Inference: A Practical Information-Theoretic App roach (NewYork: Springer), 488 pp.

Dickson, A. G., and Millero, F. J.: A Comparison of the Equilibrium-Constants for the Dissociation of Carbonic-Acid in Seawater Media, Deep-Sea Research Part a-Oceanographic Research Papers, 34, 1733-1743, 1987.

DOE: Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water version 2 ed., edited by: Dickson, A. G., and Goyet, C., ORNL/CDIAC-74, 1994.

Fisher R.A.: The Design Of Experiments. Hafner, New York, N.Y, 1935

Good, P.: Springer Series in Statistics: Permutation tests: A practical guide to resampling methods for testing hypotheses, Springer Series in Statistics; Permutation tests: A practical guide to resampling methods for testing hypotheses, 1994.

Houlbreque, F., Tambutte, E., and Ferrier-Pages, C.: Effect of zooplankton availability on the rates of photosynthesis, and tissue and skeletal growth in the scleractinian coral Stylophora pistillata, J. Exp. Mar. Biol. Ecol., 296, 145-166, 2003.

Jacques, T. G., and Pilson, M. E. Q.: Experimental ecology of the temperate scleractinian coral Astrangia danae 1. Partition of respiration, photosynthesis and calcification between host and symbionts, Mar. Biol., 60, 167-178, 1980.

Johnson, J. B., and Omland, K. S.: Model selection in ecology and evolution, Trends in Ecology & Evolution, 19, 101-108, 10.1016/j.tree.2003.10.013, 2004.

Manly B. F. J.: Randomization, Bootstrap and Monte Carlo, 1997.

Maxwell, S. E., & Delaney, H. D.: Designing experiments and analyzing data: A model comparison perspective. Mahwah, NJ: Lawrence Erlbaum Associates. Methods in Biology, 2nd edn. Chapman & Hall, London, 2003.

Mehrbach, C., Culberso.Ch, Hawley, J. E., and Pytkowic.Rm: Measurement of Apparent Dissociation-Constants of Carbonic-Acid in Seawater at Atmospheric-Pressure, Limnol. and Oceanogr., 18, 897-907, 1973.

Ricker, W. E.: Linear Regressions in Fishery Research, J. Fish. Res. Bd., 30, 409-434, 1973.

Ricker, W. E.: Linear Regressions in Fishery Research - Note, J. Fish. Res. Bd., 32, 1494-1498, 1975.

Schneider, K., and Erez, J.: The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral Acropora eurystoma, Limnol. and Oceanogr., 51, 1284-1293, 2006.

Tambutte, E., Allemand, D., Bourge, I., Gattuso, J. P., and Jaubert, J.: An Improved Ca-45 Protocol For Investigating Physiological-Mechanisms In Coral Calcification, Mar. Biol., 122, 453-459, 1995.

Tambutte, E., Allemand, D., Mueller, E., and Jaubert, J.: A compartmental approach to the mechanism of calcification in hermatypic corals, J. Exp. Biol., 199, 1029-1041, 1996.

Zeebe, R. E., and Wolf-Gladrow, D.: CO2 in Seawater: Equlibrium, Kinetics, Isotopes, Elsevier Science, B.V, Amsterdam, 346 pp., 2001.

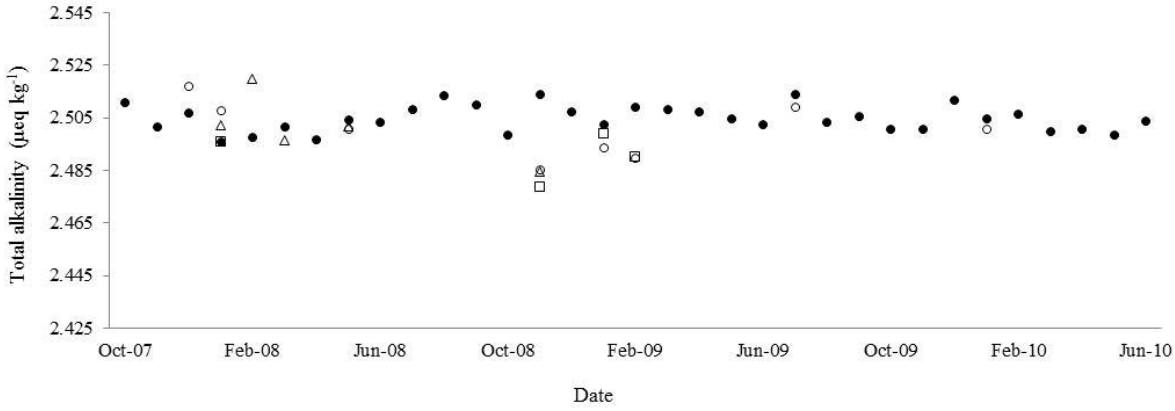

Fig .S1. Total alkalinity (TA) as measured from the recirculating seawater system in which corals were maintained during the time frame of experiments: 7.19 (open triangle), 7.49 (open square) and 8.09 (open diamond) pH_T treatments as compared with monthly measurements of ambient seawater (closed diamond) as received from the Israel National Monitoring Program (NMP) of the Gulf of Eilat database (sampling depth was 20-40 m, similar to the depth from which seawater was supplied to the system). Additions of CO_2 to seawater did not alter total alkalinity (Zeebe and Wolf-Gladrow 2003). Indeed, results of the CO_2 enriched treatments, pH_T 7.19 and 7.49 are in the range of the pH_T 8.09 (as recorded from our system). The small differences in TA found between the ambient pH treatments in our system and the monthly measurements by the NMP, can derive from secreting organisms that alter alkalinity in the seawater before entering the tanks.

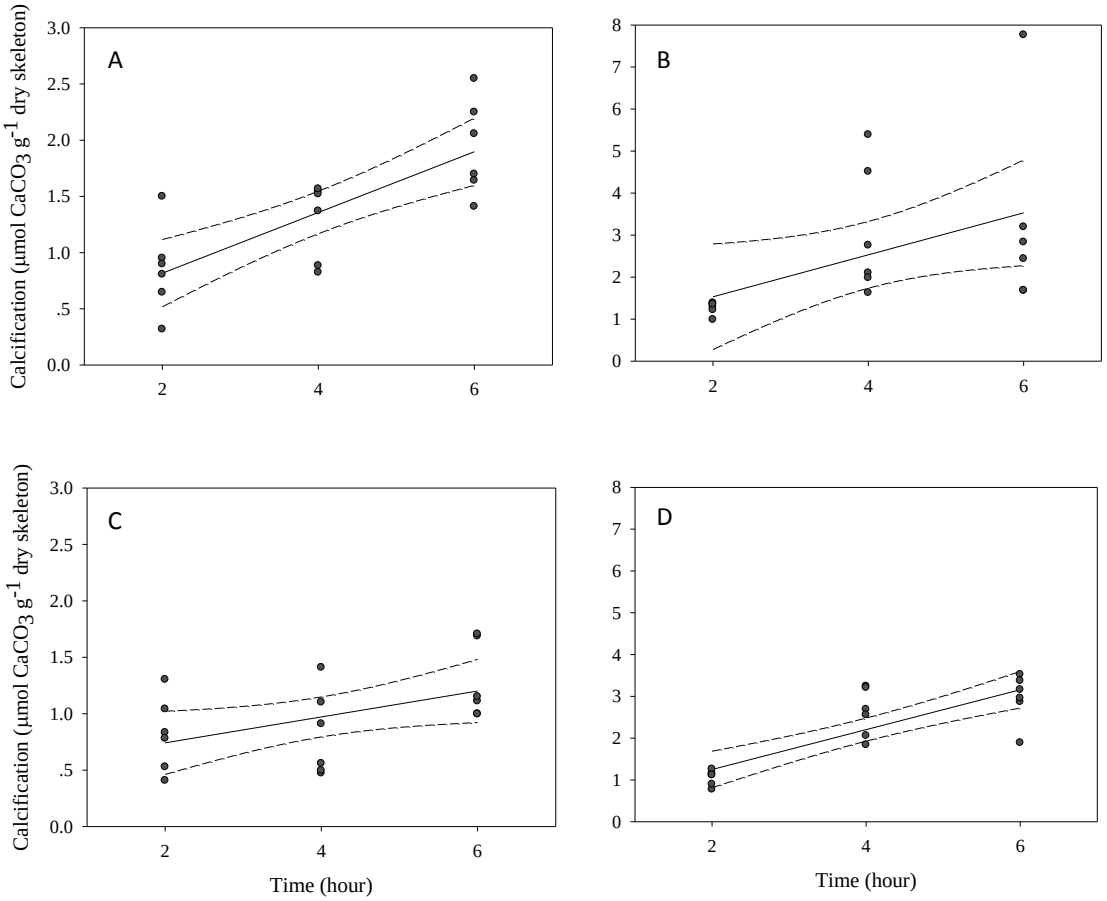

Fig. S2. Calcification values of *S. pistillata* micorcolonies as obtained from the (A), (B) alkalinity-depletion technique and (C), (D) ^{45}Ca incorporation method. Each point represents a calcification rate of a given fragment. Microcolonies were incubated under normal (8.09; plots A, C) and reduced (7.49; plot B, D) initial pH_T and in the presence of light, over three time points, 2, 4 and 6 h; n=6 for time points. Calcification rates of intact specimens, derived from the ^{45}Ca method, were corrected by subtracting ^{45}Ca uptake of the dead fragments –control. Microcolonies used in the experiment were cultured in the pH system for a period of 14 months before the experiment was conducted. Linear regression (solid line) is plotted along with the 95% confidence interval (dashed line). All plotted regressions are statistically significant ($p<0.05$).

Table S1. Calcification and final pH values of *S. pistillata* microcolonies resulting from alkalinity-depletion and ^{45}Ca incorporation methods (pH values measured at the end of the alkalinity experiment). Data are presen ted as mean±STDV.

pH_T treatment	Time (h)	Total calcification ($\mu mol\ CaCO_3\ g^{-1}$ dry skeleton)		Calcification rates ($\mu mol\ CaCO_3\ g^{-1}$ dry skeleton h^{-1})		Final pH_T
		Total alkalinity	^{45}Ca uptake	Total alkalinity	^{45}Ca uptake	
8.09	2	0.853±0.39	0.815±0.328	0.426±0.195	0.408±0.164	8.26±0.04
	4	1.285±0.342	0.824±0.382	0.321±0.085	0.206±0.095	8.5±0.06
	6	1.933±0.426	1.275±0.332	0.322±0.071	0.212±0.055	8.55±0.1
7.49	2	1.268±0.15	1.053±0.182	0.634±0.075	0.527±0.091	7.77±0.11
	4	3.061±1.533	2.598±0.577	0.765±0.383	0.65±0.144	8±0.09
	6	3.265±2.287	2.962±0.58	0.544±0.381	0.494±0.097	8.3±0.06

Table S2. Results of permutational ANOVA design for the effect of pH (8.09 and 7.49, on the pH_T scale), time (2, 4 and 6 h) and method (^{45}Ca and total alkalinity) on the total calcification values of the coral *S. pistillata*.

Source of variation	df	SS	MS	*P* (perm)
pH	1	44.933	44.933	0.0327
Time	2	1.447	0.724	0.9216
Method	2	2.284	2.284	0.2015
pH × Time	1	7.134	3.567	0.0048
pH × Method	1	0.015	0.015	0.2015
Time × Method	2	0.479	0.239	1
pH × Time × Method	2	0.221	0.11	0.5701
Residuals	59	44.516	0.755	0.9804

Table S3. Seawater carbonate chemistry in each of the incubation vessels in the alkalinity experiment of the long-term acclimation experiment. TA and pH were measured, while all other parameters were calculated using the CO2SYS program.

pH_T treatment	Time (h)	Number of repeat	TA ($\mu eqv\ kg^{-1}$)	Final pH_T	DIC ($\mu mol\ kg^{-1}$)	$p\,CO_2$ (μatm)	$CO_{2(aq)}$ ($\mu mol\ kg^{-1}$)	HCO_3^- ($\mu mol\ kg^{-1}$)	CO_3^{2-} ($\mu mol\ kg^{-1}$)	Ω_{arg}
8.09	Control	1	2506.6	8.09	2133	390.9	10.8	1859.2	263.1	4.01
	2	1	2380.5	8.23	1920.2	243.8	6.7	1600.8	312.7	4.76
		2	2462.9	8.26	1967.7	230.5	6.4	1621.9	339.4	5.17
		3	2391.2	8.25	1914.1	230.4	6.4	1583.8	323.9	4.93
		4	2377.2	8.29	1871	202.1	5.6	1523.7	341.7	5.2
		5	2422.5	8.3	1901.3	199.9	5.5	1542	353.8	5.39
		6	2367.2	8.2	1931.1	265.6	7.3	1627.2	296.6	4.52
	4	1	2403.5	8.48	1734.4	110.1	3	1285.1	446.3	6.8
		2	2401.5	8.5	1715.5	102.7	2.8	1255.9	456.8	6.96
		3	2288.0	8.55	1583.9	81.9	2.3	1123.2	458.4	6.98
		4	2363.0	8.54	1650.7	87.9	2.4	1178.4	469.9	7.16
		5	2305.5	8.4	1722.6	137.3	3.8	1333.6	385.3	5.87
		6	2360.7	8.55	1640.3	84.8	2.3	1163.3	474.7	7.23
	6	1	2344.4	8.56	1619.1	81.3	2.2	1140.6	476.3	7.25
		2	2336.6	8.71	1485.7	47.1	1.3	933.7	550.7	8.39
		3	2302.9	8.58	1570.1	74.2	2	1091	477	7.27
		4	2267.1	8.57	1550.9	75.6	2.1	1085.2	463.7	7.06
		5	2280.5	8.5	1619.8	97	2.7	1185.9	431.3	6.57
		6	2314.8	8.4	1730.3	137.9	3.8	1339.5	387	5.89
7.49	Control	1	2509.9	7.41	2474.8	2355.5	65	2340.6	69.2	1.05
	2	1	2357.6	7.7	2211	1064.6	29.4	2062.8	118.9	1.81
		2	2398.6	7.48	2338.1	1892.3	52.2	2209.2	76.7	1.17
		3	2365.0	7.76	2191.2	913.2	25.2	2031.6	134.5	2.05
		4	2343.0	7.73	2183.8	978.4	27	2031.4	125.5	1.91
		5	2311.8	7.76	2140.6	892.1	24.6	1984.6	131.3	2
		6	2296.3	7.77	2121.3	862.9	23.8	1964.4	133	2.03
	4	1	2317.0	8.02	2007.8	440	12.1	1781.2	214.5	3.27
		2	2235.3	7.9	2000.4	592	16.3	1818	166.1	2.53
		3	2111.0	8.15	1741.4	273.1	7.5	1491.6	242.3	3.69
		4	2239.3	8.03	1931.3	412.6	11.4	1709.2	210.6	3.21
		5	2013.9	7.95	1769.6	462.5	12.8	1593.5	163.3	2.49
		6	2282.7	7.96	2011.7	512.8	14.1	1808	189.6	2.89
	6	1	2049.6	8.29	1593.2	172.1	4.7	1297.5	291	4.43
		2	2188.2	8.34	1673.1	157.6	4.3	1333.3	335.5	5.11
		3	2306.3	8.31	1795.3	183.7	5.1	1449.8	340.4	5.19
		4	2053.6	8.37	1538.7	133.4	3.7	1209.1	326	4.97
		5	2208.3	8.3	1720.3	180.9	5	1395.1	320.1	4.88
		6	2180.3	8.18	1782.3	258.4	7.1	1512	263.2	4.01

Table S4. Changes in seawater carbonate chemistry from the initial conditions (T0) as obtained from the alkalinity experiment of the long-term acclimation experiment. Changes, expressed in percentage, were calculated between the beginning and end of each incubation (2, 4 and 6 h).

Carbonate parameter	% Change from initial (T0)					
Time (h)	2		4		6	
pH_T	8.09	7.49	8.09	7.49	8.09	7.49
Alkalinity	4.2	6.4	6.1	12.2	7.9	13.6
DIC	10	11.1	21.5	22.8	25.1	31.9
pH final	2.1	3.9	5.0	7.9	5.7	11.8
HCO_3^-	14.8	12.5	34.2	27.3	39.2	41.6
CO_3^{2-}	-24.6	-73.3	70.0	185	76.4	351