

Ocean acidification induces biochemical and morphological changes in the calcification process of large benthic foraminifera

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Supplementary material

Figure S1. The relationship between surface area and buoyant weight of (a) *Amphistegina lessonii* and (b) *Marginopora vertebralis* grown under different pH conditions. Ambient: pH 8.1/ 430 $\mu\text{atm CO}_2$; High: pH 7.9/ 855 $\mu\text{atm CO}_2$; Intermediate: pH 7.7/ 1000 $\mu\text{atm CO}_2$; Low: pH 7.6/ 2000 $\mu\text{atm CO}_2$.

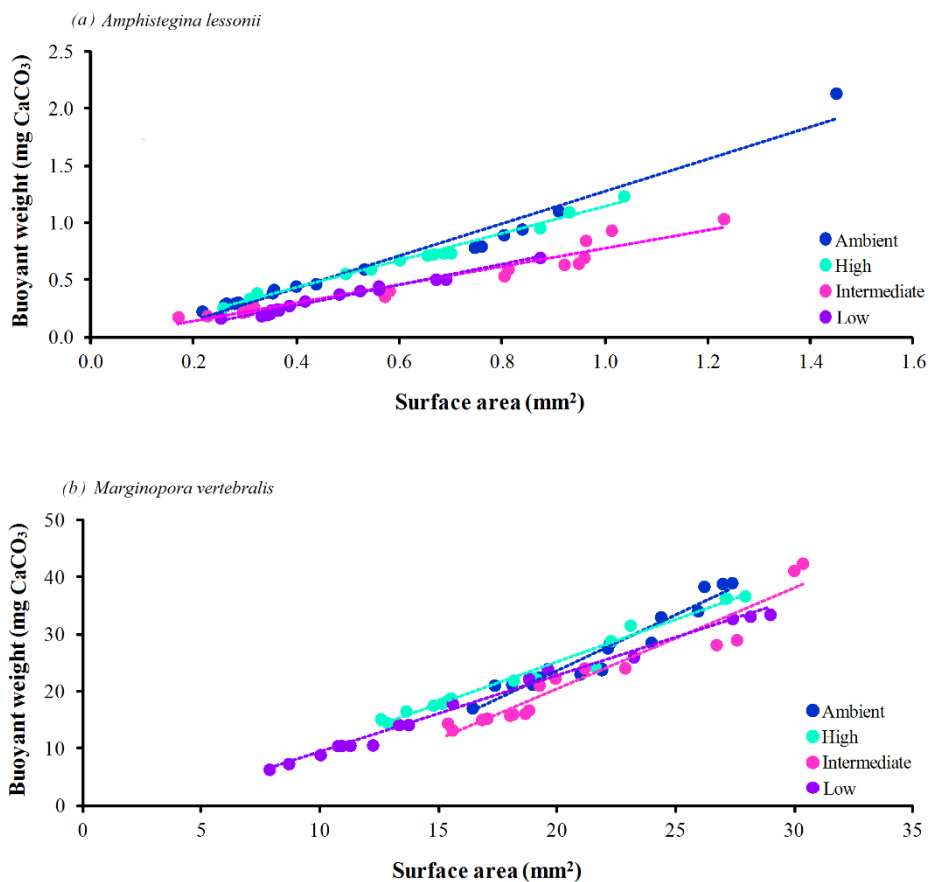


Table S1. Weekly seawater and carbonate system parameters for the 30-day experiment (mean \pm SE; N = 4).

Week	Measured parameters				Calculated parameters*			
	Treatment	A_T ($\mu\text{mol/kg-SW}$)	DIC ($\mu\text{mol/kg-SW}$)	pH (NBS)	DIC ($\mu\text{mol/kg-SW}$)	$p\text{CO}_2$ (μatm)	CO_3^{2-} ($\mu\text{mol/kg-SW}$)	Ω_{Ca}
1	pH 8.1	2249 ± 1.51	1901 ± 15	8.25 ± 0.04	1900 ± 15	321 ± 46	232 ± 18	5.58 ± 0.45
	pH 7.9	2253 ± 0.31	1993 ± 2	7.92 ± 0.02	2019 ± 22	746 ± 35	118 ± 5	2.85 ± 0.13
	pH 7.7	2251 ± 2.15	2052 ± 13	7.74 ± 0.01	2005 ± 14	1180 ± 25	78 ± 2	1.88 ± 0.04
	pH 7.6	2251 ± 0.83	2092 ± 1	7.64 ± 0.006	2006 ± 7	1160 ± 20	79 ± 1	1.91 ± 0.02
2	pH 8.1	2281 ± 0.50	1992 ± 3	8.13 ± 0.01	1991 ± 3	447 ± 5	185 ± 1	4.45 ± 0.03
	pH 7.9	2285 ± 2.11	2059 ± 8	7.84 ± 0.01	2083 ± 8	939 ± 31	103 ± 3	2.48 ± 0.08
	pH 7.7	2292 ± 2.96	2084 ± 15	7.73 ± 0.03	2117 ± 6	1262 ± 78	82 ± 5	1.99 ± 0.12
	pH 7.6	2300 ± 2.13	2115 ± 1	7.68 ± 0.01	2140 ± 5	1418 ± 43	74 ± 2	1.79 ± 0.04
3	pH 8.1	2279 ± 1.24	2032 ± 2	8.15 ± 0.006	2010 ± 11	434 ± 7	197 ± 2	4.74 ± 0.05
	pH 7.9	2291 ± 2.64	2088 ± 6	7.90 ± 0.02	2088 ± 7	821 ± 59	121 ± 7	2.92 ± 0.17
	pH 7.7	2288 ± 2.63	2126 ± 4	7.73 ± 0.01	2124 ± 4	1245 ± 40	83 ± 2	2.00 ± 0.05
	pH 7.6	2288 ± 1.86	2135 ± 1	7.59 ± 0.03	2150 ± 2	1754 ± 139	62 ± 5	1.50 ± 0.11
4	pH 8.1	2259 ± 3.29	1987 ± 3	8.07 ± 0.03	1993 ± 4	517 ± 38	165 ± 10	3.98 ± 0.25
	pH 7.9	2256 ± 0.71	2078 ± 3	7.85 ± 0.02	2042 ± 18	913 ± 51	107 ± 6	2.59 ± 0.15
	pH 7.7	2258 ± 3.60	2136 ± 2	7.83 ± 0.008	2142 ± 4	983 ± 17	105 ± 2	2.54 ± 0.05
	pH 7.6	2260 ± 2.69	2224 ± 7	7.51 ± 0.006	2224 ± 8	2245 ± 32	51 ± 1	1.22 ± 0.02

*Equilibrium constant K1, K2 used according to Mehrbach et al. (1973), modified by Dickson and Millero (1987).

Table S2. Mixed-model ANOVA results for growth rates (surface area and buoyant weight) of *Amphistegina lessonii* and *Marginopora vertebralis*.

Species	Response variable	Source	dF	MS	F	<i>P</i>
<i>A. lessonii</i>	Surface area	Treatment	3	0.0043	3.06	0.07
		Aquarium (Treatment)	12	0.0014	8.55	<0.01
		Residual	16	0.0002		
	Buoyant weight	Treatment	3	0.0247	15.66	<0.01
		Aquarium (Treatment)	12	0.0016	4.97	<0.01
		Residual	16	0.0003		
<i>M. vertebralis</i>	Surface area	Treatment	3	0.0113	3.10	0.07
		Aquarium (Treatment)	12	0.0036	2.10	0.08
		Residual	16	0.0017		
	Buoyant weight	Treatment	3	0.0034	2.62	0.09
		Aquarium (Treatment)	12	0.0013	9.47	<0.01
		Residual	16	0.0001		

Table S3. Tukey's HSD test results for differences in growth rates (surface area and buoyant weight) in *Amphistegina lessonii*.

Response variable	Comparison groups	Mean*	Lower 95% C.I.	Mean difference	Upper 95% C.I.	P-value
Surface area	pH 8.1	0.4226				
	-pH 7.9	0.4775	-0.1107	-0.0549	0.0008	0.05
	-pH 7.7	0.4611	-0.0943	-0.0385	0.0172	0.22
	-pH 7.6	0.4482	-0.0814	-0.0256	0.0301	0.54
	pH 7.9	0.4775				
	-pH 7.7	0.4611	-0.0393	0.0164	0.0721	0.81
	-pH 7.6	0.4482	-0.0264	0.0293	0.0850	0.43
	pH 7.7	0.4611				
	-pH 7.6	0.4482	-0.0428	0.0128	0.0686	0.89
	Buoyant weight	pH 8.1	0.4477			
-pH 7.9		0.4729	-0.0843	-0.0252	0.0338	0.60
-pH 7.7		0.4122	-0.0236	0.0354	0.0945	0.32
-pH 7.6		0.3448	0.0437	0.1028	0.1619	<0.01
pH 7.9		0.4729				
-pH 7.7		0.4122	0.0015	0.0606	0.1197	0.04
-pH 7.6		0.3448	0.0689	0.1280	0.1871	<0.01
pH 7.7		0.4122				
-pH 7.6		0.3448	0.0083	0.0674	0.1264	0.02

*Data were arc sine-square root transformed, and values of sample mean refer to transformed values.

Table S4. Tukey's HSD test results for differences in growth rates (surface area and buoyant weight) in *Marginopora vertebralis*.

Response variable	Comparison groups	Mean*	Lower 95% C.I.	Mean difference	Upper 95% C.I.	P-value
Surface area	pH 8.1	0.1249				
	-pH 7.9	0.1234	-0.0192	0.0014	0.0220	0.08
	-pH 7.7	0.1485	-0.0443	-0.0236	-0.0030	0.15
	-pH 7.6	0.1455	-0.0413	-0.0206	-1.37E-05	0.82
	pH 7.9	0.1234				
	-pH 7.7	0.1485	-0.0457	-0.0250	-0.0044	0.97
	-pH 7.6	0.1455	-0.0427	-0.0221	-0.0014	0.31
	pH 7.7	0.1485				
	-pH 7.6	0.1455	-0.0176	0.0029	0.0236	0.51
	Buoyant weight	pH 8.1	0.1698			
-pH 7.9		0.2099	-0.0935	-0.0401	0.0131	0.16
-pH 7.7		0.2096	-0.0932	-0.0399	0.0134	0.17
-pH 7.6		0.1803	-0.0638	-0.0105	0.0428	0.93
pH 7.9		0.2099				
-pH 7.7		0.2096	-0.0530	0.0003	0.0536	1.00
-pH 7.6		0.1803	-0.0237	0.0296	0.0829	0.39
pH 7.7		0.2096				
-pH 7.6		0.1803	-0.0240	0.0293	0.0826	0.40

*Data were arc sine-square root transformed, and values of sample mean refer to transformed values.

Table S5. Comparison between slopes among pH treatments for *Amphistegina lessonii* and *Marginopora vertebralis*. SDD stands for the standard error of the difference between slopes of the linearised function defining the relationship between BW and surface area.

Comparison between slopes	<i>A. lessonii</i>			<i>M. vertebralis</i>		
	SSD	t-test	<i>P</i>	SDD	t-test	<i>P</i>
pH 8.1 (Ambient) x pH 7.9	0.04	0.62	0.53	0.12	2.95	<0.01
pH 8.1 (Ambient) x pH 7.7	0.06	2.51	0.02	0.13	-1.17	<0.01
pH 8.1 (Ambient) x pH 7.6	0.07	-2.26	0.03	0.08	1.66	0.10
pH 7.9 x pH 7.7	0.06	2.17	0.04	0.06	6.13	<0.01
pH 7.9 x pH 7.6	0.07	-2.72	0.01	0.04	7.32	<0.01
pH 7.7 x pH 7.6	0.08	-3.84	<0.01	0.06	-1.27	0.21

Table S6. One-way ANOVA results for shell density of *Amphistegina lessonii* and *Marginopora vertebralis*.

Species	Source	dF	MS	F	<i>P</i>
<i>A. lessonii</i>	Treatment	3	0.03	10.32	<0.001
	Residual	19	0.00		
<i>M. vertebralis</i>	Treatment	3	0.00	2.25	0.12
	Residual	19	0.00		

Table S7. Tukey's HSD test results for density of *Amphistegina lessonii* and *Marginopora vertebralis*.

Species	Comparison groups	Mean	Lower 95% C.I.	Mean difference	Upper 95% C.I.	P-value
<i>A. lessonii</i>	pH 8.1	1.542				
	-pH 7.9	1.474	-0.0380	0.068	0.1740	0.295
	-pH 7.7	1.504	-0.0680	0.038	0.1440	0.739
	-pH 7.6	1.348	0.0879	0.194	0.3000	<0.01
	pH 7.9	1.474				
	-pH 7.7	1.504	-0.1360	-0.03	0.0760	0.085
	-pH 7.6	1.348	0.0199	0.126	0.2320	0.017
	pH 7.7	1.504				
	-pH 7.6	1.348	0.0499	0.156	0.2620	0.017
	<i>M. vertebralis</i>	pH 8.1	2.104			
-pH 7.9		2.116	-0.0928	-0.012	0.0689	0.973
-pH 7.7		2.056	-0.0328	0.048	0.1288	0.357
-pH 7.6		2.062	-0.0388	0.042	0.1228	0.469
pH 7.9		2.116				
-pH 7.7		2.056	-0.0208	0.06	0.1408	0.189
-pH 7.6		2.062	-0.0268	0.054	0.1348	0.263
pH 7.7		2.056				
-pH 7.6		2.062	-0.0868	-0.006	0.0748	0.996

Table S8. Two-way ANOVA results for Ca-ATPase and Mg-ATPase enzymatic activity of *Amphistegina lessonii* and *Marginopora vertebralis*.

Species	Response variable	Source	dF	MS	F	P
<i>A. lessonii</i>	Ca-ATPase	Treatment	3	0.6000	37.58	<0.01
		Time	2	9.6143	708.6	<0.01
		Treatment*Time	6	0.2733	20.15	<0.01
		Residual	84	0.0136		
	Mg-ATPase	Treatment	3	0.3529	24.60	<0.01
		Time	2	4.2036	293.11	<0.01
		Treatment*Time	6	0.3015	21.02	<0.01
		Residual	84	0.0143		
<i>M. vertebralis</i>	Ca-ATPase	Treatment	3	0.0895	7.78	<0.01
		Time	2	0.2347	20.41	<0.01
		Treatment*Time	6	0.02400	2.08	0.064
		Residual	84	0.0115		
	Mg-ATPase	Treatment	3	0.1659	11.56	<0.01
		Time	2	0.3979	27.72	<0.01
		Treatment*Time	6	0.2815	14.50	<0.01
		Residual	84	0.0143		

Table S9. Tukey's HSD test results for Ca-ATPase and Mg-ATPase in *Amphistegina lessonii*.

Response variable	Comparison groups	Mean*	Lower 95% C.I.	Mean difference	Upper 95% C.I.	P-value
Ca-ATPase	pH 8.1	0.694				
	-pH 7.9	0.963	-0.3579	-0.2695	-0.1813	<0.01
	-pH 7.7	0.940	-0.3349	-0.2466	-0.1584	<0.01
	-pH 7.6	1.025	-0.4197	-0.3314	-0.2432	<0.01
	pH 7.9	0.963				
	-pH 7.7	0.940	-0.0654	0.0229	0.1112	0.90
	-pH 7.6	1.025	-0.1502	-0.0618	0.0264	0.26
	pH 7.7	0.940				
	-pH 7.6	1.025	-0.1731	-0.0847	0.0035	0.06
	Mg-ATPase	pH 8.1	0.821			
-pH 7.9		1.017	-0.2862	-0.1954	-0.1046	<0.01
-pH 7.7		1.111	-0.3807	-0.2899	-0.1991	<0.01
-pH 7.6		0.961	-0.2304	-0.1396	-0.0488	<0.01
pH 7.9		1.017				
-pH 7.7		1.111	-0.1853	-0.0945	-0.0037	0.04
-pH 7.6		0.961	-0.0349	0.0557	0.1465	0.38
pH 7.7		1.111				
-pH 7.6		0.961	0.0595	0.1503	0.2411	<0.01

*Data were log transformed, and values of sample mean refer to transformed values.

Table S10. Tukey's HSD test results for Ca-ATPase and Mg-ATPase in *Marginopora vertebralis*.

Response variable	Comparison groups	Mean	Lower 95% C.I.	Mean difference	Upper 95% C.I.	P-value
Ca-ATPase	pH 8.1	0.6290				
	-pH 7.9	0.5498	-0.0019	0.0793	0.1606	0.05
	-pH 7.7	0.5938	-0.0460	0.0352	0.1165	0.67
	-pH 7.6	0.6943	-0.1465	-0.0652	0.0160	0.16
	pH 7.9	0.5498				
	-pH 7.7	0.5938	-0.1253	-0.0440	0.0372	0.49
	-pH 7.6	0.6943	-0.2258	-0.1445	-0.0633	<0.01
	pH 7.7	0.5938				
	-pH 7.6	0.6943	-0.1818	-0.1005	-0.0192	<0.01
	Mg-ATPase	pH 8.1	1.111			
-pH 7.9		0.9393	0.0808	0.1717	0.2625	<0.01
-pH 7.7		0.9477	0.0725	0.1633	0.2541	<0.01
-pH 7.6		1.0510	-0.0308	0.0600	0.1508	0.31
pH 7.9		0.9393				
-pH 7.7		0.9477	-0.0991	-0.0084	0.0824	0.99
-pH 7.6		1.0510	-0.2025	-0.1117	-0.0208	<0.01
pH 7.7		0.9477				
-pH 7.6		1.0510	-0.1941	-0.1033	-0.0124	0.02

Table S11. Shapiro-Wilk's normality and Levene's homogeneity of variance tests results of all variables tested for *Amphistegina lessonii*.

Species	Response variable	Test	Test value	Probability
<i>A. lessonii</i>	Surface area	Normality	0.99	0.99
		Variance	0.70	0.55
	Buoyant weight	Normality	0.95	0.15
		Variance	1.04	0.38
	Density	Normality	0.96	0.56
		Variance	0.26	0.84
	Ca-ATPase*	Normality	0.97	0.06
		Variance	1.66	0.17
	Mg-ATPase*	Normality	0.96	0.06
		Variance	2.19	0.09

*Data were log transformed.

Table S12. Results summary of variables analysed of *Amphistegina lessonii* and *Marginopora vertebralis*.

Species	Treatment	Response variable				
		Surface area ^a	Buoyant weight ^a	Density ^b	Ca-ATPase ^c	Mg-ATPase ^c
<i>A. lessonii</i>	pH 8.1	0.42 ± 0.01	0.48 ± 0.02	1.54 ± 0.03	1.03 ± 0.06	0.91 ± 0.05
	pH 7.9	0.43 ± 0.01	0.46 ± 0.01	1.47 ± 0.01	1.64 ± 0.03	1.47 ± 0.03
	pH 7.7	0.36 ± 0.01	0.37 ± 0.00	1.50 ± 0.02	1.58 ± 0.03	1.71 ± 0.03
	pH 7.6	0.33 ± 0.01	0.31 ± 0.01	1.34 ± 0.02	1.86 ± 0.03	1.42 ± 0.05
<i>M. vertebralis</i>	pH 8.1	0.12 ± 0.00	0.16 ± 0.00	2.10 ± 0.01	0.62 ± 0.04	1.24 ± 0.06
	pH 7.9	0.16 ± 0.01	0.22 ± 0.01	2.12 ± 0.01	0.45 ± 0.02	0.67 ± 0.02
	pH 7.7	0.14 ± 0.01	0.23 ± 0.02	2.06 ± 0.02	0.49 ± 0.03	0.72 ± 0.04
	pH 7.6	0.14 ± 0.00	0.17 ± 0.01	2.06 ± 0.02	0.57 ± 0.03	0.89 ± 0.02

^aGrowth rates expressed as % increase day⁻¹ (mean ±SE; N = 8)

^bDensity after 30 days expressed as mean g CaCO₃ cm⁻³ (mean ±SE; N = 5)

^cCa-ATPase and Mg-ATPase activities after 30 days expressed as mean μmol P_i mg protein⁻¹ h⁻¹ (mean ± SE; N = 8)

Table S13. Shapiro-Wilk's normality and Levene's homogeneity of variance tests results of all variables tested for *Marginopora vertebralis*.

Species	Response variable	Test	Test value	Probability
<i>M. vertebralis</i>	Surface area*	Normality	0.96	0.41
		Variance	1.80	0.16
	Buoyant weight	Normality	0.95	0.15
		Variance	1.11	0.36
	Density	Normality	0.94	0.25
		Variance	0.56	0.64
	Ca-ATPase	Normality	0.99	0.39
		Variance	1.77	0.16
	Mg-ATPase	Normality	0.98	0.62
		Variance	0.60	0.61

*Data were log transformed.