Supplementary Information for

How calorie-rich food could help marine calcifiers in a CO_2-rich future

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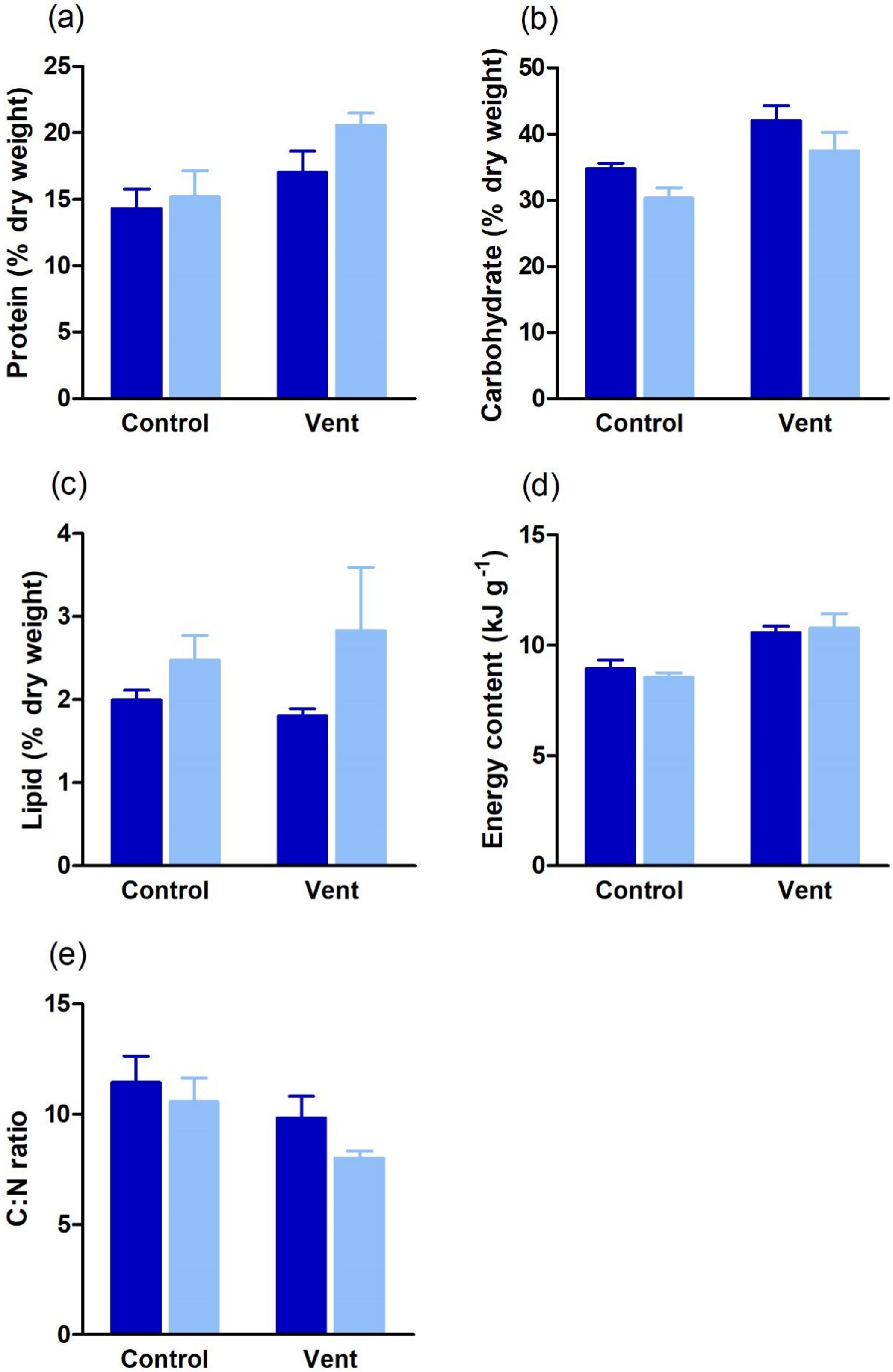

Figure S1 (a) Protein, (b) carbohydrate, (c) lipid, (d) energy content and (e) C:N ratio of turf algae among sites (mean + S.E., *n* = 5 per site). North South

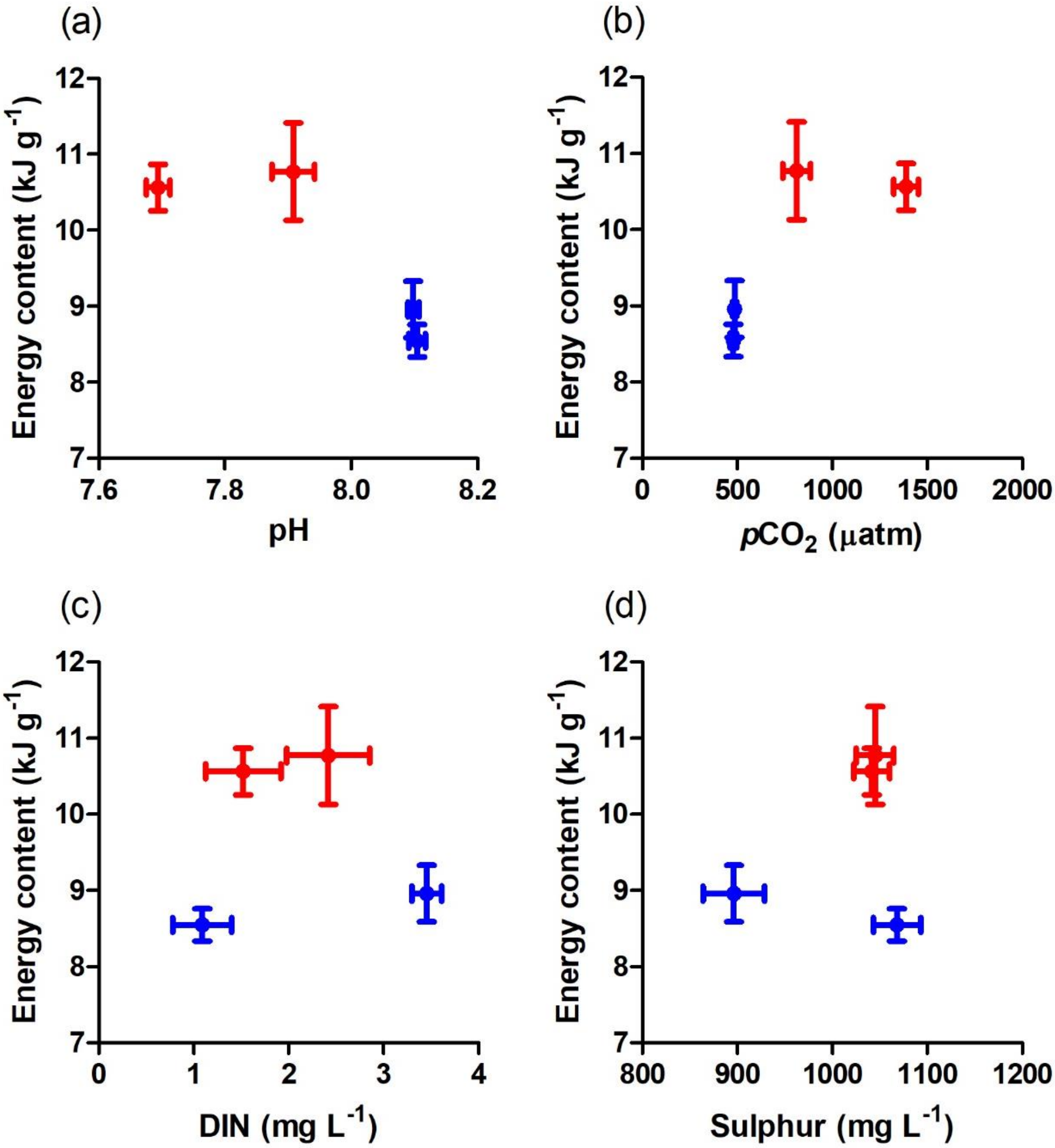

Figure S2 A visual summary of site-level variation in seawater chemistry parameters of notable interest. Whilst energy content of algae tended to increase with (a) reduced pH and (b) elevated pCO_2, this was not observed for (c) dissolved inorganic nitrogen and (d) sulphur, which can have strong effects on algal growth. Each point represents a site (mean ± S.E., n = 5). ● Vent ● Control.

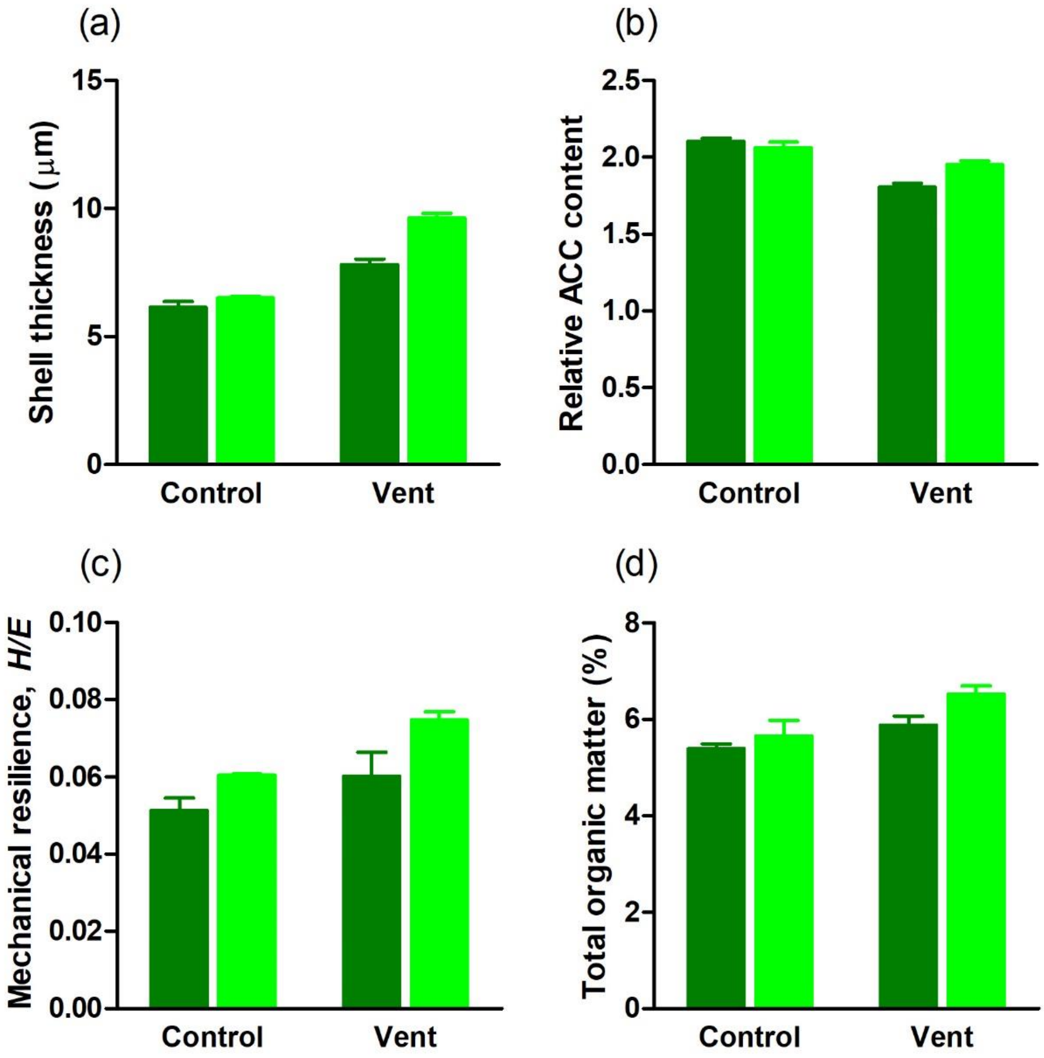

Figure S3 (a) Shell thickness, (b) relative ACC content, (c) mechanical resilience and (d) total organic matter of shells among sites (mean + S.E., n = 5 per site). North South

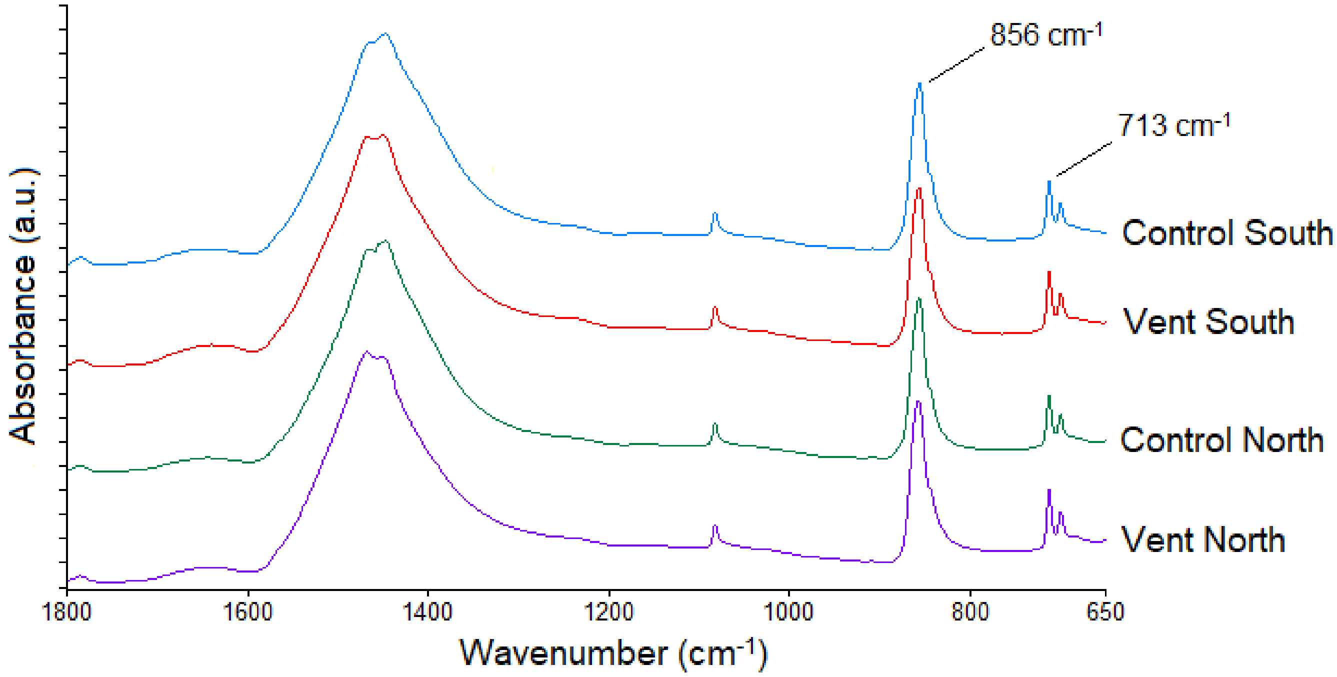

Figure S4 FTIR spectrum showing the relative ACC content, which is indicated by the intensity ratio of peaks between 856 cm^{-1} and 713 cm^{-1}. This ratio was greater at controls than vents, meaning that the shells were more crystalline at vents.

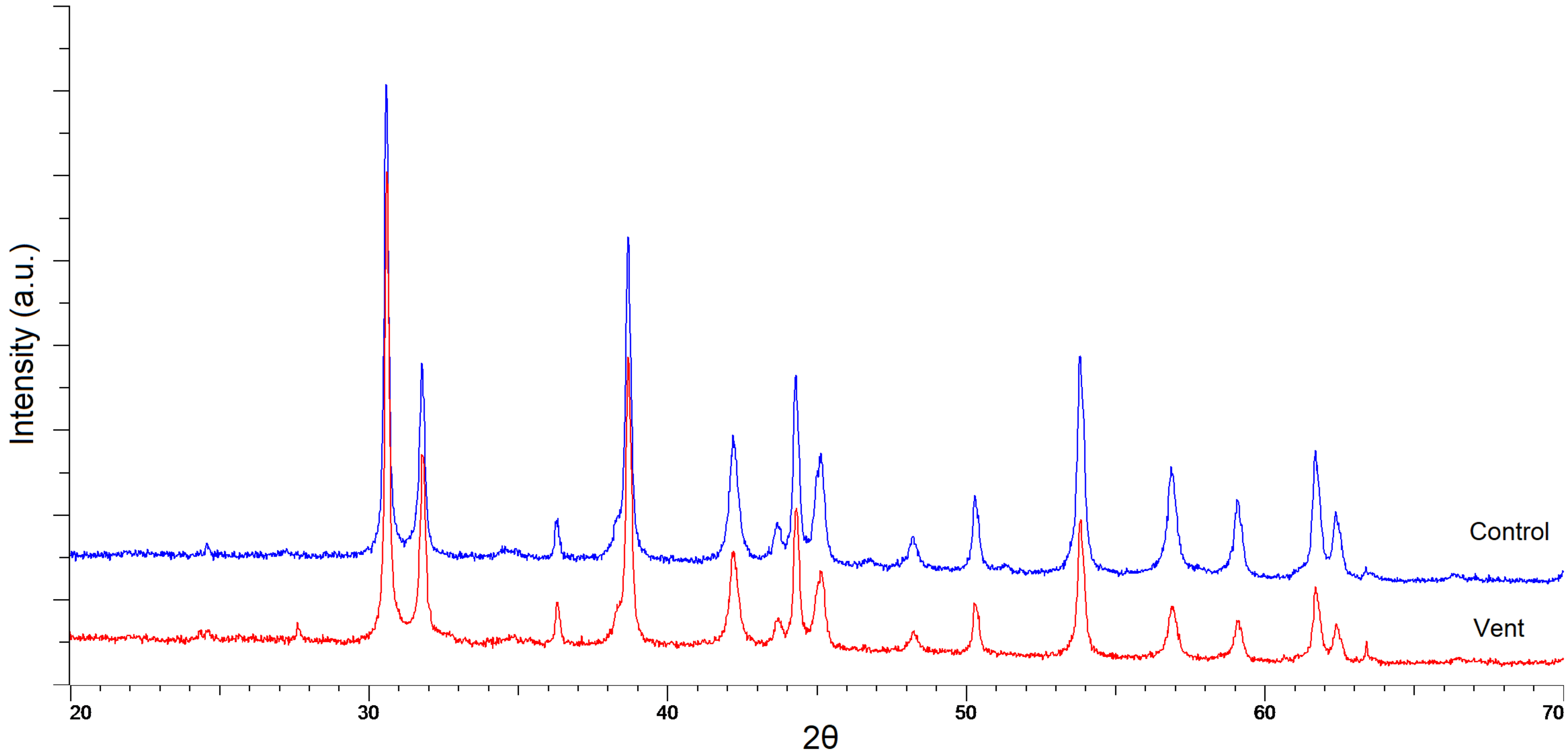

Figure S5 X-ray diffraction spectrum showing the presence of aragonite as the only carbonate mineral in gastropod shells at vents and controls. Carbonate mineral composition was determined using an X-ray diffractometer (D4 Endeavour, Bruker, Germany) with Co Kα radiation (35 kV and 30 mA) from 20° to 70° 2θ (step size: 0.018° and step time: 1 s), and identified using the EVA XRD analysis software.

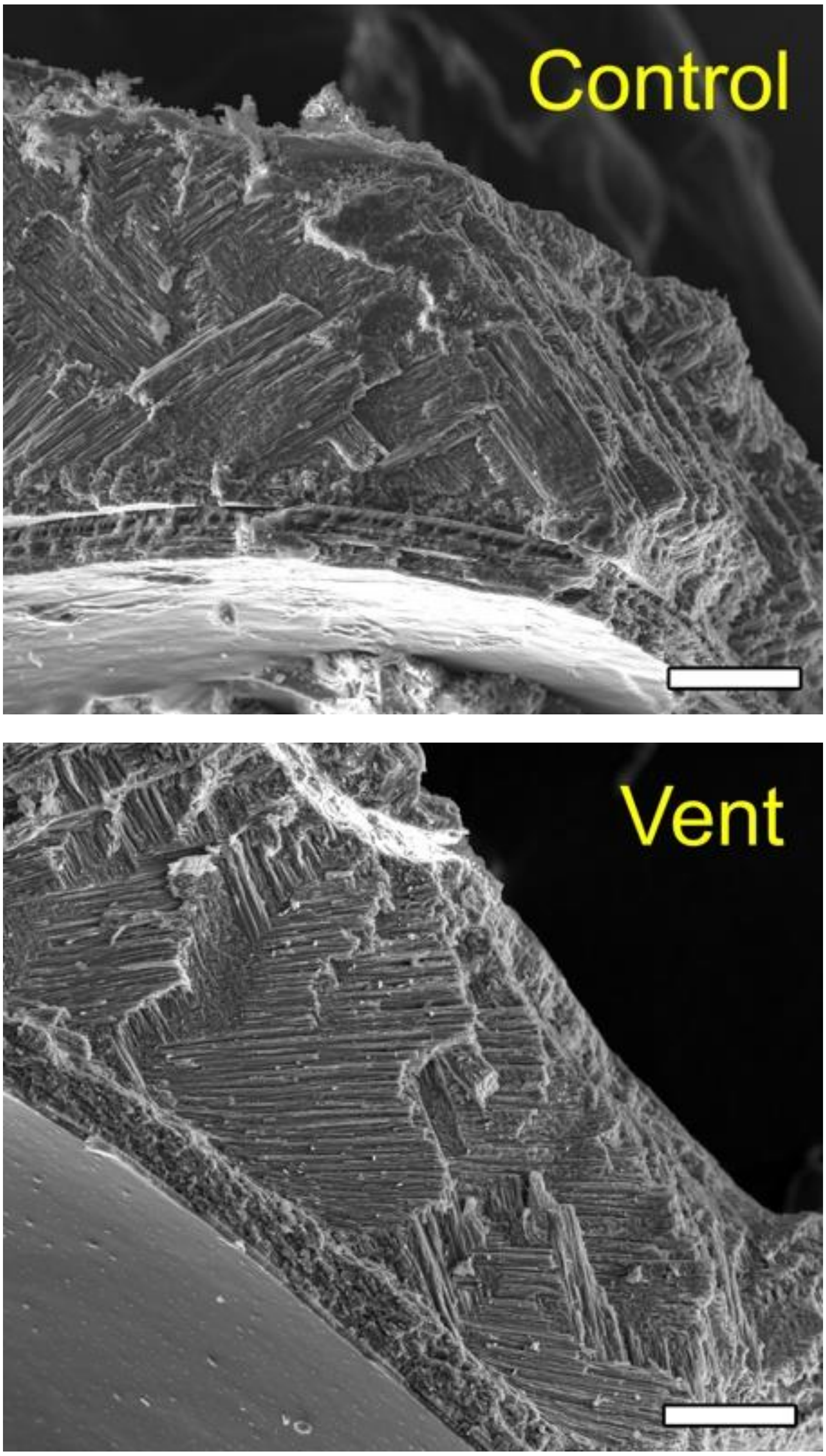

Figure S6 SEM images of *Eatoniella mortoni* shells in the outer lip region using a Philips XL 30 field emission scanning electron microscope, showing similar shell integrity between vents and controls. Bar: 20 µm

Table S1 Carbonate chemistry parameters, concentrations of nutrients and minerals (mg L^{-1}) in seawater among sites (mean ± S.E., *n* = 5 per site). Temperature and pH were measured using a pH/temperature meter (HI 98128, HANNA Instruments, Germany), calibrated using NBS buffers. Salinity and total alkalinity were measured using a hand-held refractometer and potentiometric titrator (888 Titrando, Metrohm, Switzerland), respectively. The pCO_2, bicarbonate ions (HCO_3^-), carbonate ions (CO_3^{2-}) and saturation state of aragonite (Ω_{ara}) were calculated using the CO2SYS program [1], with dissociation constants from Mehrbach et al. [2] refitted by Dickson and Millerro [3]. Nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) ions were measured by a flow injection analyser (QuikChem 8500, Lachat Instruments, USA). Dissolved inorganic nitrogen (DIN) is the sum of NO_3^-, NO_2^- and NH_4^+ concentrations. Minerals were measured by an inductively coupled plasma mass spectrometer (Agilent 7500cs, Agilent Technologies Inc., USA). The concentrations of Cu and Zn are below detection limit.

	Control North	Vent North	Control South	Vent South
Seawater carbonate chemistry				
Temperature (°C)	21.2 ± 0.02	21.5 ± 0.12	21.3 ± 0.20	21.1 ± 0.21
pH (NBS scale)	8.10 ± 0.01	7.69 ± 0.02	8.10 ± 0.01	7.91 ± 0.03
Salinity (psu)	36.0 ± 0.00	36.0 ± 0.00	36.0 ± 0.00	36.0 ± 0.00
Total alkalinity (μmol kg^{-1})	2289 ± 0.61	2287 ± 1.26	2290 ± 0.99	2288 ± 1.35
pCO_2 (μatm)	485 ± 12	1390 ± 65	478 ± 18	813 ± 72
HCO_3^- (μmol kg^{-1})	1871 ± 7.4	2100 ± 6.0	1866 ± 9.6	1999 ± 18
CO_3^{2-} (μmol kg^{-1})	169 ± 2.7	75.8 ± 2.9	171 ± 3.8	117 ± 7.2
Ω_{ara}	2.63 ± 0.04	1.18 ± 0.04	2.66 ± 0.06	1.81 ± 0.11
Nutrients				
NO_3^- + NO_2^-	0.003 ± 0.000	0.029 ± 0.008	0.044 ± 0.097	0.014 ± 0.005
NH_4^+	3.54 ± 0.16	1.49 ± 0.40	1.16 ± 0.32	2.40 ± 0.44
DIN	3.55 ± 0.16	1.52 ± 0.40	1.21 ± 0.31	2.42 ± 0.44
PO_4^{3-}	0.0003 ± 0.0000	0.0008 ± 0.0003	0.0024 ± 0.0004	0.0012 ± 0.0003
Minerals				
S	896 ± 32.5	1042 ± 18.7	1068 ± 24.9	1045 ± 19.8
Mg	876 ± 39.5	1014 ± 10.9	962 ± 19.1	1027 ± 23.4
Ca	318 ± 10.9	414 ± 22.0	381 ± 9.41	369 ± 7.05
Sr	6.10 ± 0.21	7.09 ± 0.15	7.03 ± 0.12	6.96 ± 0.13
K	289 ± 14.6	268 ± 7.36	299 ± 10.1	257 ± 6.68
Rb	0.089 ± 0.003	0.105 ± 0.002	0.106 ± 0.002	0.104 ± 0.002

Mn	0.0004 ± 0.0002	0.005 ± 0.002	0.008 ± 0.002	0.004 ± 0.0005
Fe	0.038 ± 0.017	0.040 ± 0.018	0.291 ± 0.094	0.173 ± 0.078
Cu	< 0.0004	< 0.0004	< 0.0004	< 0.0004
Zn	< 0.0006	< 0.0006	< 0.0006	< 0.0006

Table S2 Two-way ANOVA testing the effects of Vent (vent vs. control) and Site (north vs. south) on the seawater chemistry, nutritional quality of turf algae and shell quality of gastropods. See footnote for the protocol used for analysis. Nomenclature used to report the results of SNK test: V_N (Vent North), V_S (Vent South), C_N (Control North) and C_S (Control South).

Seawater carbonate chemistry varied among sites ($V_N > V_S > C_N = C_S$) for pH, carbonate ion and aragonite saturation, while a reverse pattern was found for pCO_2 and bicarbonate ion. No difference was detected for temperature and total alkalinity.

Seawater nutrients and minerals did not vary among sites, except that higher ammonium concentration was detected at C_N and higher phosphate concentration was detected at the northern sites.

Algal nutritional quality was greater at vents than controls for all parameters (i.e. protein, carbohydrate, energy content and the relative content of nitrogen to carbon), except lipid which did not differ among sites. The northern sites had greater carbohydrate content.

Shell quality of gastropods varied among sites, where mechanical resilience and total organic matter at vents were greater than those at controls. Shell thickness ($V_S > V_N > C_N = C_S$) and relative ACC content ($V_N < V_S < C_N = C_S$) also varied among sites.

	df	MS	*F*	*p*	SNK test
Seawater carbonate chemistry					
pH					
Vent	1	0.450	8.32	0.212	
Site	1	0.061	27.8	**< 0.001**	North < South
Vent × Site	1	0.054	24.9	**< 0.001**	$V_N < V_S < C_N = C_S$
Residual	16	2.18×10^{-3}			
pCO_2					
Vent	1	1.91×10^{6}	4.74	0.274	
Site	1	4.25×10^{5}	34.2	**< 0.001**	North > South
Site × Location	1	4.05×10^{6}	32.6	**< 0.001**	$V_N > V_S > C_N = C_S$
Residual	16	1.24×10^{4}			
Bicarbonate ion					
Vent	1	0.042	15.5	0.158	
Site	1	3.38×10^{-3}	21.0	**< 0.001**	North > South
Vent × Site	1	2.73×10^{-3}	17.0	**< 0.001**	$V_N > V_S > C_N = C_S$

Residual	16	1.61×10^{-4}			
Carbonate ion					
Vent	1	2.73×10^{4}	14.3	0.165	
Site	1	2.34×10^{3}	22.7	**< 0.001**	North < South
Vent × Site	1	1.91×10^{3}	18.6	**< 0.001**	$V_N < V_S < C_N = C_S$
Residual	16	103			
Aragonite saturation					
Vent	1	6.57	14.4	0.164	
Site	1	0.56	23.1	**< 0.001**	North < South
Vent × Site	1	0.46	18.8	**< 0.001**	$V_N < V_S < C_N = C_S$
Residual	16	0.024			
Total alkalinity					
Vent	1	18.2	3.22	0.091	
Site	1	2.81	0.50	0.491	
Vent × Site	1	0.761	0.13	0.726	
Residual	16	5.98			
Temperature					
Vent	1	0.013	0.05	0.864	
Site	1	0.221	1.82	0.196	
Vent × Site	1	0.265	2.18	0.159	
Residual	16	0.121			
Seawater nutrients and minerals					
Nitrate + Nitrite					
Vent	1	6.62×10^{-6}	1.63×10^{-3}	0.974	
Site	1	9.19×10^{-4}	0.87	0.365	
Vent × Site	1	4.08×10^{-3}	3.86	0.067	
Residual	16	1.06×10^{-3}			
Ammonium					
Vent	1	0.824	0.06	0.846	
Site	1	2.69	0.89	0.358	
Vent × Site	1	13.5	4.49	**0.050**	$C_N > V_S = V_N = C_S$
Residual	16	3.01			
DIN					
Vent	1	0.83	0.06	0.843	
Site	1	2.59	0.88	0.362	
Vent × Site	1	13.1	4.45	0.051	

Residual	16	2.94			
Phosphate					
Vent	1	2.88×10^{-4}	0.06	0.842	
Site	1	8.51×10^{-3}	5.49	**0.032**	North < South
Vent × Site	1	4.51×10^{-3}	2.91	0.107	
Residual	16	1.55×10^{-3}			
S					
Vent	1	1.86×10^{4}	0.53	0.601	
Site	1	3.83×10^{4}	2.54	0.131	
Vent × Site	1	3.54×10^{4}	2.34	0.145	
Residual	16	1.51×10^{4}			
Mg					
Vent	1	5.12×10^{4}	3.16[a]	0.093	
Site	1	1.24×10^{4}	0.76	0.394	
Vent × Site	1	6.75×10^{3}	0.40	0.535	
Residual	16	1.68×10^{4}			
Ca					
Vent	1	8.89	0.61	0.579	
Site	1	448	0.10	0.760	
Vent × Site	1	1.47×10^{4}	3.16	0.095	
Residual	16	4.65×10^{3}			
Sr					
Vent	1	1.05	0.75	0.546	
Site	1	0.809	1.29	0.274	
Vent × Site	1	1.40	2.22	0.155	
Residual	16	0.630			
K					
Vent	1	4.98×10^{3}	2.02[a]	0.173	
Site	1	1.54	0.00	0.980	
Vent × Site	1	543	0.21	0.653	
Residual	16	2.58×10^{3}			
Rb					
Vent	1	2.18×10^{-4}	0.57	0.589	
Site	1	3.18×10^{-4}	2.54	0.131	
Vent × Site	1	3.85×10^{-4}	3.07	0.099	
Residual	16	1.25×10^{-4}			
Mn					

Vent	1	1.15×10^{-7}	1.46×10^{-3}	0.976	
Site	1	6.51×10^{-5}	1.38	0.257	
Vent × Site	1	7.87×10^{-5}	1.67	0.214	
Residual	16	4.70×10^{-5}			
<u>Fe</u>					
Vent	1	0.017	0.18[a]	0.673	
Site	1	0.188	2.04	0.171	
Vent × Site	1	0.018	0.19	0.671	
Residual	16	0.096			
Nutritional quality of algae					
<u>Protein</u>					
Vent	1	82.3	7.18[a]	**0.016**	Vent > Control
Site	1	24.8	2.17	0.159	
Vent × Site	1	8.64	0.74	0.402	
Residual	16	11.6			
<u>Carbohydrate</u>					
Vent	1	258	9.53×10^{3}	**0.007**	Vent > Control
Site	1	103	5.00	**0.040**	North > South
Vent × Site	1	0.027	1.32×10^{-3}	0.972	
Residual	16	20.6			
<u>Lipid</u>					
Vent	1	7.75×10^{-3}	0.07[a]	0.843	
Site	1	0.318	2.89	0.081	
Vent × Site	1	0.018	0.16	0.697	
Residual	16	0.116			
<u>Energy content</u>					
Vent	1	18.3	21.8[a]	**< 0.001**	Vent > Control
Site	1	0.052	0.06	0.806	
Vent × Site	1	0.484	0.56	0.464	
Residual	16	0.861			
<u>C:N ratio</u>					
Vent	1	21.7	4.96[a]	**0.040**	Vent < Control
Site	1	9.09	2.08	0.168	
Vent × Site	1	1.09	0.24	0.633	
Residual	16	4.59			

Shell quality of gastropods					
Shell thickness					
Vent	1	28.8	10.8	0.188	
Site	1	5.98	35.6	**< 0.001**	South > North
Vent × Site	1	2.68	16.0	**0.001**	$V_S > V_N > C_N = C_S$
Residual	16	0.168			
Relative ACC content					
Vent	1	0.207	4.64	0.277	
Site	1	0.013	3.61	0.076	
Vent × Site	1	0.045	12.5	**0.003**	$V_N < V_S < C_N = C_S$
Residual	16	3.57×10^{-3}			
Mechanical resilience					
Vent	1	0.161	9.40[a]	**0.007**	Vent > Control
Site	1	0.203	11.9	**0.003**	South > North
Vent × Site	1	5.27×10^{-3}	0.30	0.594	
Residual	16	0.018			
Total organic matter					
Vent	1	2.32	10.5[a]	**0.005**	Vent > Control
Site	1	1.04	4.72	**0.044**	South > North
Vent × Site	1	0.180	0.80	0.383	
Residual	16	0.224			

Two-way ANOVA tests the effects of Vent as a fixed factor (vent vs. control) and Site as a random factor (north vs. south). Post-hoc pooling of the interaction term with the residual enables a more powerful test of the main factor 'Vent' ($p > 0.25$) [4]. [a] *F*-ratios and *p*-values affected by pooling and resultant values are given. The critical value of 'α' was adjusted to allow significant heterogeneity of variances (Cochran's *C*-test, $p < 0.05$). Transformation: Ln(x) for lipid and mechanical resilience; Ln(x+1) for protein, Mn and bicarbonate ion; x × 33 for phosphate. Bold letters indicate significant difference ($p < 0.05$).

References

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4. Winer BJ, Brown DR, Michels KM. 1991 *Statistical Principles in Experimental Design, 3rd edition*. New York: McGraw-Hill.