Supplement of Biogeosciences, 15, 3267–3276, 2018
https://doi.org/10.5194/bg-15-3267-2018-supplement

Biogeosciences Open Access EGU

Supplement of

Effects of hypoxia and non-lethal shell damage on shell mechanical and geochemical properties of a calcifying polychaete

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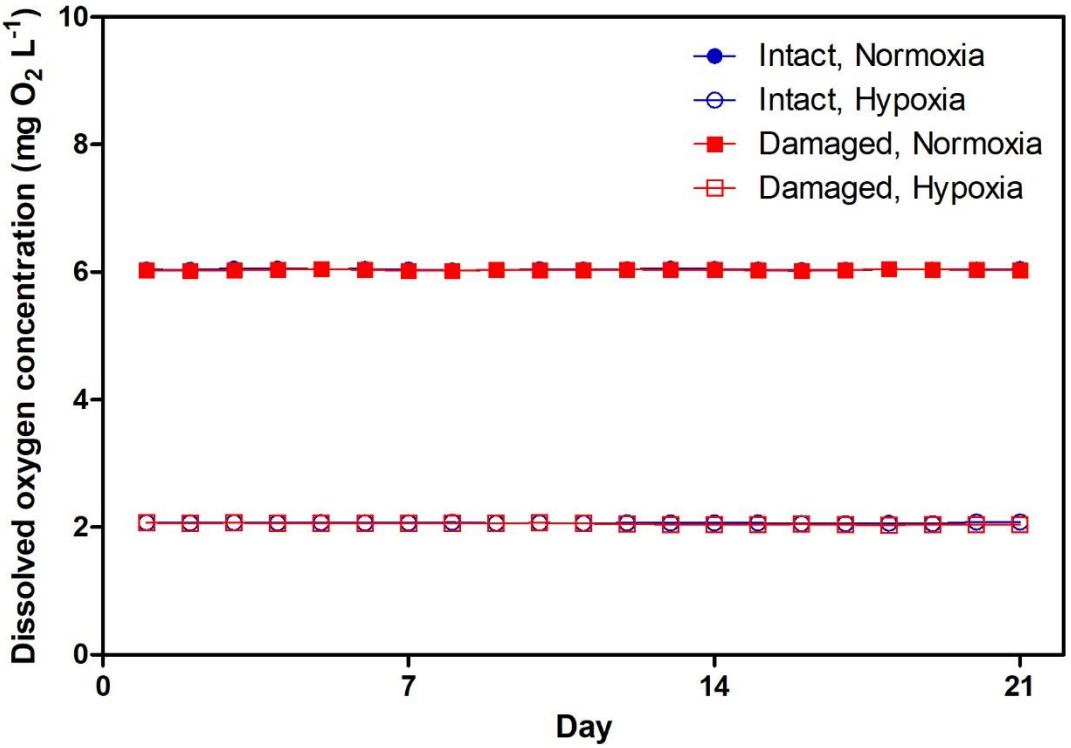

Figure S1 Dissolved oxygen concentration of seawater in different treatments across the 3-week experimental period (mean ± S.D., *n* = 3).

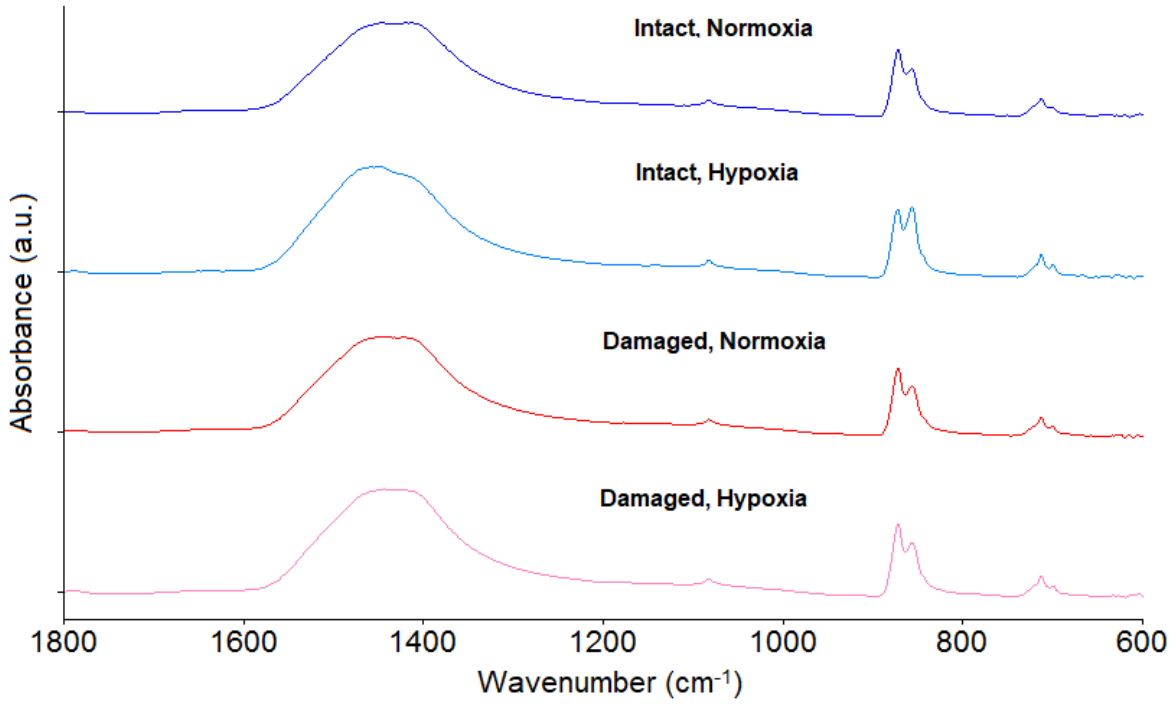

Figure S2 Infrared spectra for the newly produced shells of *H. diramphus* growing under different treatment conditions.

Table S1 The seawater parameters under different treatment conditions throughout the exposure period (mean ± S.D.). Dissolved oxygen concentration was measured daily using an optical dissolved oxygen probe (SOO-100, TauTheta Instruments, USA). pH was measured daily using a pH meter (HI 9025, HANNA Instruments, USA). Temperature and salinity were measured daily using a thermometer and refractometer, respectively. Total alkalinity was measured weekly using a titrator (HI 84431, HANNA Instruments, USA). Saturation states (Ω) of calcite and aragonite were calculated using the CO2SYS program (Pierrot et al., 2006), with dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

	Intact, Normoxia	Intact, Hypoxia	Damaged, Normoxia	Damaged, Hypoxia
Measured parameters				
Dissolved oxygen (mg O_2 L^{-1})	6.04 ± 0.02	2.07 ± 0.03	6.03 ± 0.01	2.05 ± 0.03
pH (NBS scale)	8.10 ± 0.05	8.26 ± 0.04	8.09 ± 0.05	8.26 ± 0.04
Temperature (°C)	28.2 ± 0.08	28.2 ± 0.09	28.2 ± 0.08	28.2 ± 0.10
Salinity (psu)	32.9 ± 0.35	33.0 ± 0.25	33.0 ± 0.42	33.1 ± 0.25
Total alkalinity (μmol kg^{-1})	2241 ± 8.96	2231 ± 12.2	2241 ± 9.11	2243 ± 9.44
Calculated parameters				
C_T (μmol kg^{-1})	1984 ± 26.1	1885 ± 27.9	1988 ± 29.0	1895 ± 24.3
HCO_3^- (μmol kg^{-1})	1784 ± 40.2	1632 ± 44.3	1790 ± 45.0	1641 ± 38.5
CO_3^{2-} (μmol kg^{-1})	187 ± 16.1	244 ± 17.6	184 ± 18.3	246 ± 15.4
$\Omega_{calcite}$	4.61 ± 0.40	6.01 ± 0.43	4.54 ± 0.45	6.05 ± 0.38
$\Omega_{aragonite}$	3.04 ± 0.26	3.98 ± 0.29	3.01 ± 0.30	4.01 ± 0.25

See Hanna Instruments (2008) and TauTheta Instruments LLC (2007) for the operation manuals of the titrator for total alkalinity and the optical dissolved oxygen probe for dissolved oxygen concentration, respectively.

Table S2 PERMANOVA table showing the effects of dissolved oxygen (DO) and context on shell growth, mechanical strength, organic matter content, calcite/aragonite, Mg/Ca in calcite, relative ACC content, respiration rate and clearance rate. The bold letters indicate significant difference ($p < 0.05$).

	df	Mean square	Pseudo-F	*p*	Comparison of means
Shell growth (Day 21)					
DO	1	21.8	5.66	**0.019**	Normoxia > Hypoxia
Context	1	676	175	**0.001**	Damaged > Intact
DO × Context	1	0.163	0.042	0.838	
Mechanical strength					
DO	1	2.00×10^{-7}	0.119	0.734	
Context	1	1.97×10^{-5}	11.7	**0.004**	Damaged > Intact
DO × Context	1	1.69×10^{-7}	0.101	0.755	
Organic matter content					
DO	1	1.04	1.10	0.309	
Context	1	9.83	10.5	**0.005**	Damaged > Intact
DO × Context	1	0.022	0.024	0.880	
Calcite/Aragonite					
DO	1	0.070	11.0	**0.017**	Hypoxia > Normoxia
Context	1	1.14×10^{-3}	0.178	0.623	
DO × Context	1	8.84×10^{-3}	1.39	0.249	
Mg/Ca in calcite					
DO	1	0.018	16.0	**0.004**	Hypoxia > Normoxia
Context	1	6.92×10^{-4}	0.618	0.455	
DO × Context	1	4.83×10^{-4}	0.431	0.530	
Relative ACC content					
DO	1	0.355	6.02	**0.047**	Hypoxia > Normoxia
Context	1	0.199	3.37	0.104	
DO × Context	1	0.116	1.97	0.206	
Respiration rate					
DO	1	2.15×10^{-3}	4.36×10^{3}	**0.001**	Normoxia > Hypoxia
Context	1	8.52×10^{-6}	14.2	**0.001**	Intact > Damaged
DO × Context	1	7.40×10^{-8}	0.150	0.715	
Clearance rate					
DO	1	84.0	140	**0.001**	Within Intact: Normoxia > Hypoxia Within Damaged: Normoxia > Hypoxia
Context	1	89.1	148	**0.001**	Within Normoxia: Intact > Damaged Within Hypoxia: N.S.
DO × Context	1	57.2	95.5	**0.001**	

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