

## Resilience to ocean acidification: decreased carbonic anhydrase activity in sea anemones under high $p\text{CO}_2$ conditions

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### Seawater chemistry for long-term *in situ* exposure

During the one week fieldwork, seawater pH (NBS scale) was measured each day ( $n=7$ ) using a pH-meter and an electrode (Metrohm pH mobile). Seawater samples were filtered with a Whatman GF/F, treated with 0.05 ml of 50 %  $\text{HgCl}_2$  (Merck, Analar) and stored in the dark at 4°C pending analysis. Three replicate were analysed at 25°C. Titration of TA standards provided by A.G. Dickson was within  $0.5 \mu\text{mol kg}^{-1}$  of the nominal value. The other parameters of the carbonate system ( $p\text{CO}_2$ ,  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ , and  $\Omega_{\text{ara}}$ ) were calculated from pH, mean TA, temperature, pressure and mean salinity using the free-access  $\text{CO}_2\text{SYS}$  (Pierrot *et al.* 2006) package. Data were in the range reported by Suggett *et al.* (2012).

### Experimental $\text{CO}_2$ re-circulating seawater system

Briefly, the system comprised of two large holding trays (vol. 300 L; one *per*  $p\text{CO}_2$  treatment). The trays fed into one sump in which sea water was filtered, heavily aerated, and recirculated, *via* a submersible pump (1262; EHEIM GmbH and Co. KG, Deizisau, Germany). The experimental design involved a certain level of pseudoreplication since one common sump supplied the two trays. However, this allowed the standardization of sea water quality before conducting experimental exposures.  $\text{CO}_2$ -enriched air was supplied to the corresponding holding tray *via* two large air stones and monitored using a  $\text{CO}_2$  analyzer (280; LI-COR, Lincoln, NE, USA). Each holding tray contained two  $900 \text{ L h}^{-1}$  circulation pumps (Koralia nano 900, Hydor, Sacramento, USA) to provide the anemones with ample flow rate. Temperature of the experimental system was maintained at 15 °C *via* the use of chiller units (L-500, Boyu, Raoping Guangdong, China). Finally, each tray contained 12 small baskets ( $L = 10 \text{ cm} \times W = 10 \text{ cm} \times H = 11 \text{ cm}$ ) to house anemones individually, and which were illuminated by three LED light strips (Reef White Aquabeam 600 Ultra Strips, Tropical Marine Centre, Bristol, UK). Approximately 10 % of the sea water in the system was replaced weekly, and deionized water was added as needed to maintain stable salinity levels.

### Effect of repeated cutting of tentacles

In order to determine whether the repeated cutting of tentacles throughout the experiment had a significant effect on anemones performance we kept another two sets of eight anemones incubated at control conditions that were only sampled once, one set on day 5 and the other on day 21. We performed a Mann-Whitney U-Tests to compare the different responses (Table S1).

Table S1. Comparison of the responses of anemones in control condition with tentacles removed at each time point versus anemones from which we remove tentacles only on a specific sampling point. Data are expressed as means  $\pm$  SE. No significant differences were found by the using Mann-Whitney U-Tests. d = days.

Trait	Time point (d)	Control	Time point 5d	Time point 21 d
<i>Symbiodinium</i> density ( $10^7$ cells. $\text{mg}^{-1}$ protein)	5 d	1.77 $\pm$ 0.15	1.57 $\pm$ 0.126	-
	21 d	1.97 $\pm$ 0.153	-	1.81 $\pm$ 0.06
<i>Symbiodinium</i> chlorophyll <i>a</i> content (pg. cell $^{-1}$ )	5 d	0.851 $\pm$ 0.08	0.918 $\pm$ 0.094	-
	21 d	0.914 $\pm$ 0.107	-	1.091 $\pm$ 0.092
Net Photosynthesis (mmol O <sub>2</sub> min $^{-1}$ . g $^{-1}$ protein)	5 d	8.087 $\pm$ 0.701	8.39 $\pm$ 1.318	-
	21 d	11.26 $\pm$ 1.48	-	15.82 $\pm$ 5.476
CA activity (Units s $^{-1}$ . mg $^{-1}$ protein)	5 d	0.09 $\pm$ 0.01	0.07 $\pm$ 0.01	-
	21 d	0.093 $\pm$ 0.02	-	0.083 $\pm$ 0.001

Table S2. Repeated measures ANOVA (RMANOVA) analysis on response variables measured in *Anemonia viridis* under laboratory short-term exposure to control and high  $p\text{CO}_2$ . Post-hoc tests were performed with Tukey's HSD tests. Statistical significant differences are highlighted in bold.

		pCO <sub>2</sub> condition					
		Control			High		
Trait	RMANOVA	0/5 d	0/21 d	5/21 d	0/5 d	0/21 d	5/21 d
<i>Symbiodinium</i> density	F(2,44) = 16.015 p = 0.0001	p = 0.3681	p = 0.0522	p = 0.7261	<b>p = 0.00369</b>	<b>p = 0.008032</b>	p = 0.8630
Chlorophyll <i>a</i>	$\chi^2(2) = 0.750$ p = 0.687	-	-	-	-	-	-
Net Photosynthesis	F(2,38) = 0.684 p = 0.510	-	-	-	-	-	-
CA activity	F(6,38) = 3.975 p = 0.003	p = 1.000	p = 0.4368	p = 0.5609	p = 0.6677	<b>p = 0.000371</b>	<b>p = 0.0802</b>

Note: Chlorophyll *a* data did not follow a Normal distribution and the non-parametric Friedman test was used for the analysis.