

*Supplemental materials*

# **Cuttlefish buoyancy in response to food availability and ocean acidification**

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**Table S1.** Seawater physicochemical parameters in all experimental setups.

	Control	High CO <sub>2</sub>
<i>Measured</i>		
Temperature (°C)	18.3 ± 0.2	18.4 ± 0.2
pH	8.059 ± 0.005	7.743 ± 0.033
TA (μmol/kgSW)	2620.4 ± 13.5	2640.1 ± 29.8
Salinity (ppm)	36.0 ± 0.1	36.0 ± 0.1
<i>Calculated</i>		
TC (μmol/kgSW)	2331.4 ± 10.8	2508.6 ± 27.5
pCO <sub>2</sub> (μatm)	426.7 ± 6.0	994.8 ± 79.6
Ω Arg	3.3 ± 0.1	1.8 ± 0.1

Salinity, pH and temperature were measured daily and averaged over the whole experimental period. The combination of total alkalinity (TA) and pHT (pH total scale) was used to calculate carbonate system parameters [ $p\text{CO}_2$  (carbon dioxide partial pressure), TC (total inorganic carbon) and Ω Arg (aragonite saturation state)]. Values are represented as mean ± standard deviation

**Figure S1.** Experimental design and timeline according to Sampling Endpoints.

Recently spawned egg masses were collected in October 2019 in Sado estuary (Portugal). The eggs were separated by hand and individually placed in vials.

**Acclimation time:**

The eggs stayed in control water during **6 days** upon arrival. They were then randomly affected to a CO<sub>2</sub> treatment.

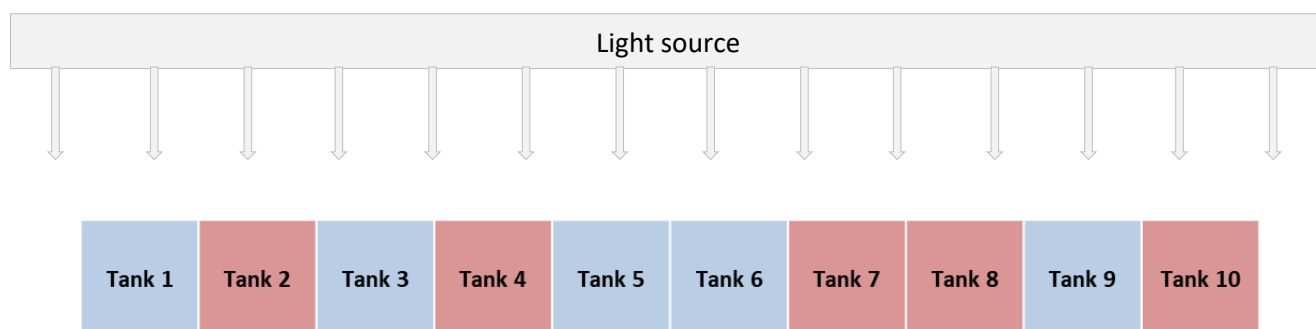
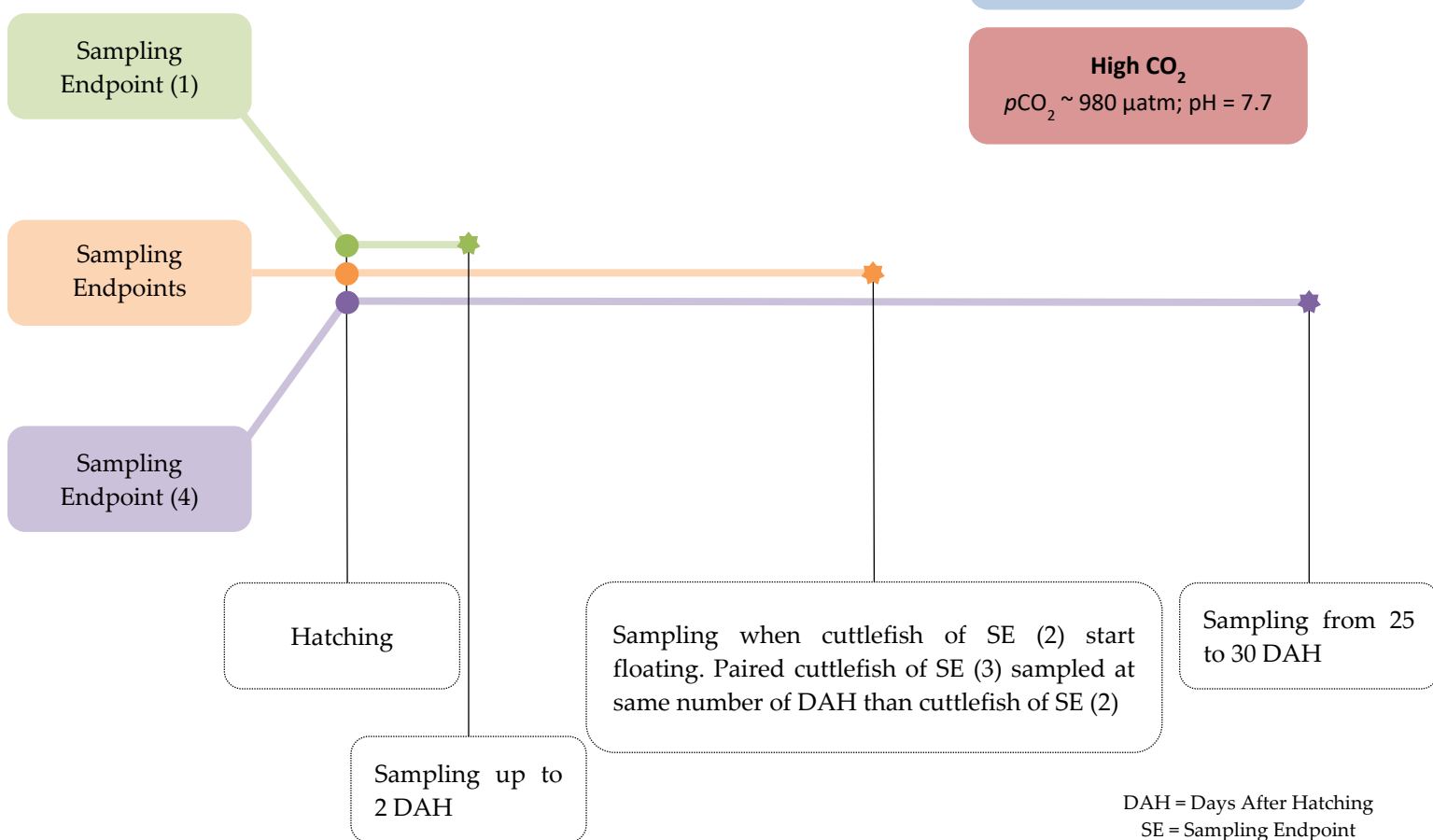
**Exposure treatments, until sampling:**

**Control**

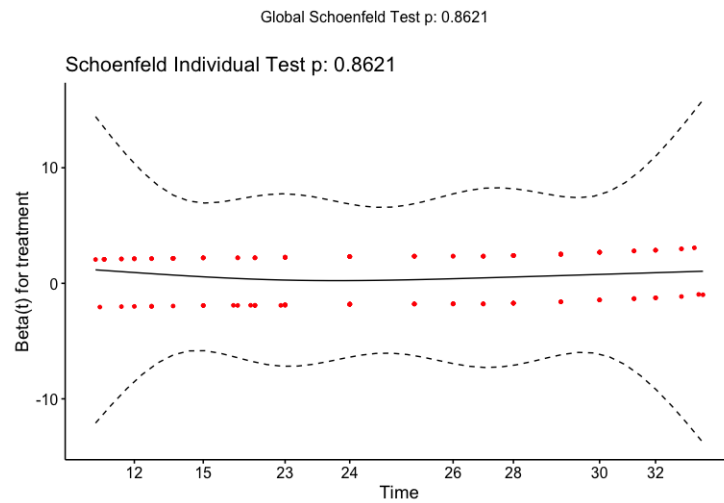
$p\text{CO}_2 \sim 400 \mu\text{atm}$ ; pH = 8.1

**High CO<sub>2</sub>**

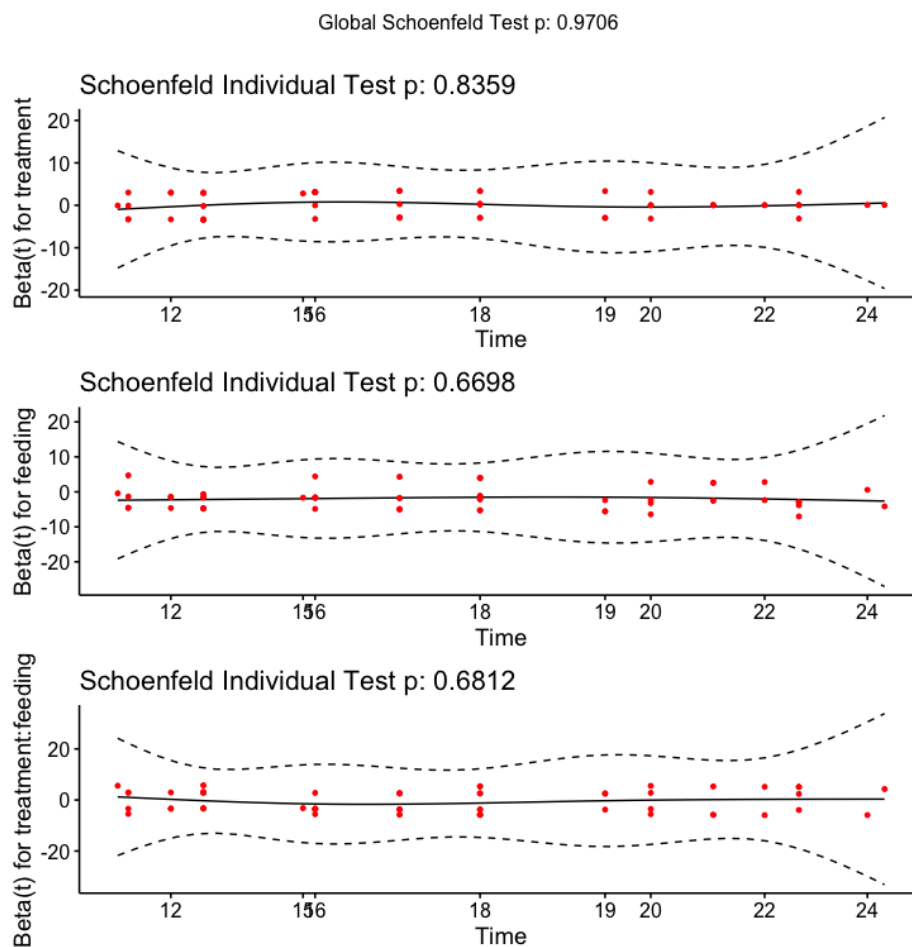
$p\text{CO}_2 \sim 980 \mu\text{atm}$ ; pH = 7.7



**Figure S2.** Smoothed spline plots of Schoenfeld residuals of the hatching success Cox mixed effects model relative to time.



**Figure S3.** Smoothed spline plots of Schoenfeld residuals of the floating Cox mixed effects model relative to time.



**Table S2.** Summary output for Cox mixed effects model of hatching success among CO<sub>2</sub> treatments.

	coef	exp(coef)	se(coef)	z	<i>p</i>
CO <sub>2</sub> treatment <sub>control</sub>	0.542	1.719	0.264	2.050	<b>0.040</b>

**Table S3.** Analysis of deviance table (Type II tests) for the Cox mixed effect model on the hatching success among CO<sub>2</sub> treatments. Values of  $p < 0.05$  are shown in bold.

	df	$\chi^2$	<i>p</i>
CO <sub>2</sub> treatment	1	4.220	<b>0.040</b>

**Table S4.** Summary output for mixed effects model of cuttlebone weight/area ratio of hatchlings among CO<sub>2</sub> treatments and days in treatment until hatching.

	Estimates	Std. error	t-value	<i>p</i>
(Intercept)	0.079	0.015	5.011	<b>&lt;0.001</b>
CO <sub>2</sub> treatment <sub>control</sub>	0.024	0.021	1.117	0.264
Days until hatching	0.001	0.001	1.345	0.179
CO <sub>2</sub> treatment <sub>control</sub> × days until hatching	-0.001	0.001	-1.242	0.214

**Table S5.** Analysis of deviance table (Type II tests) for the mixed effects model of cuttlebone weight/area ratio of hatchlings among CO<sub>2</sub> treatments and days in treatment until hatching. Values of  $p < 0.05$  are shown in bold.

	df	$\chi^2$	<i>p</i>
CO <sub>2</sub> treatment	1	0.135	0.713
Days until hatching	1	0.369	0.544
CO <sub>2</sub> treatment × days until hatching	1	1.543	0.214

**Table S6.** Summary output for mixed effects model of cuttlebone weight/area ratio of newborns among CO<sub>2</sub> treatment, feeding treatment and days in treatment after hatching.

	Estimates	Std. error	t-value	<i>p</i>
(Intercept)	0.102	0.012	8.436	<0.001
Feeding	-0.028	0.014	-1.971	<b>0.049</b>
CO <sub>2</sub> treatment <sub>control</sub>	-0.026	0.018	-1.473	0.141
days_until_sampling	-0.001	0.001	-1.014	0.311
Feeding × CO <sub>2</sub> treatment <sub>control</sub>	0.045	0.020	2.222	<b>0.026</b>
Feeding × days until sampling	0.002	0.001	1.948	0.051
CO <sub>2</sub> treatment <sub>control</sub> × days until sampling	0.001	0.001	1.290	0.197
Feeding × CO <sub>2</sub> treatment <sub>control</sub> × days until sampling	-0.002	0.001	-2.009	<b>0.045</b>

**Table S7.** Analysis of deviance table (Type II tests) for the mixed effects model of cuttlebone weight/area ratio of newborns among CO<sub>2</sub> treatments and days in treatment until hatching. Values of  $p < 0.05$  are shown in bold.

	df	$\chi^2$	<i>p</i>
Feeding	1	0.622	0.430
CO <sub>2</sub> treatment <sub>control</sub>	1	0.830	0.362
days_until_sampling	1	0.858	0.354
Feeding × CO <sub>2</sub> treatment <sub>control</sub>	1	1.445	0.229
Feeding × days until sampling	1	0.579	0.447
CO <sub>2</sub> treatment <sub>control</sub> × days until sampling	1	2.164	0.141
Feeding × CO <sub>2</sub> treatment <sub>control</sub> × days until sampling	1	4.034	<b>0.045</b>

**Table S8.** Summary output for Cox mixed effects model on floating among CO<sub>2</sub> and feeding treatments.

	coef	exp(coef)	se(coef)	z	p
CO <sub>2</sub> treatment <sub>control</sub>	0.024	1.025	0.346	0.070	0.940
Feeding	-1.996	0.136	0.426	-4.680	<b>&lt;0.001</b>
Feeding × CO <sub>2</sub> treatment <sub>control</sub>	-0.562	0.570	0.570	-0.980	0.320

**Table S9.** Analysis of deviance table (Type II tests) for the Cox mixed effect model on floating among CO<sub>2</sub> and feeding treatments. Values of p<0.05 are shown in bold.

	df	$\chi^2$	p
CO <sub>2</sub> treatment	1	0.430	0.512
Feeding	1	47.085	<b>&lt;0.001</b>
Feeding × CO <sub>2</sub> treatment	1	0.970	0.325

**Table S10.** Summary output for mixed effects model of cuttlebone weight/area ratio of newborns among CO<sub>2</sub> treatment, floating and days in treatment after hatching.

	Estimates	Std. error	t-value	p
(Intercept)	0.084	0.008	10.949	<b>&lt;0.001</b>
Floating	-0.022	0.017	-1.283	0.200
CO <sub>2</sub> treatment <sub>control</sub>	0.012	0.010	1.252	0.211
Days until sampling	0.001	0.000	1.587	0.112
Floating × CO <sub>2</sub> treatment <sub>control</sub>	0.006	0.021	0.278	0.781
Floating × days until sampling	0.001	0.001	0.616	0.538
CO <sub>2</sub> treatment <sub>control</sub> × days until sampling	-0.001	0.000	-1.798	0.072
Floating × CO <sub>2</sub> treatment <sub>control</sub> × days until sampling	0.000	0.001	0.053	0.958

**Table S11.** Analysis of deviance table (Type II tests) for the mixed effects model of cuttlebone weight/area ratio of newborns among CO<sub>2</sub> treatments, floating and days in treatment after hatching. Values of  $p < 0.05$  are shown in bold.

	<b>df</b>	<b><math>\chi^2</math></b>	<b><math>p</math></b>
Floating	1	11.460	<b>0.001</b>
CO <sub>2</sub> treatment <sub>control</sub>	1	0.459	0.498
Days until sampling	1	0.648	0.421
Floating $\times$ CO <sub>2</sub> treatment <sub>control</sub>	1	2.351	0.125
Floating $\times$ days until sampling	1	1.240	0.266
CO <sub>2</sub> treatment <sub>control</sub> $\times$ days until sampling	1	3.928	<b>0.047</b>
Floating $\times$ CO <sub>2</sub> treatment <sub>control</sub> $\times$ days until sampling	1	0.003	0.958