

*Supplement of*

**Ocean acidification reduces mechanical properties of the Portuguese oyster shell with impaired microstructure: a hierarchical analysis**

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Treatments/parameter	Control	pH 7.8	pH 7.5	pH 7.2
<b>pH</b>	8.14 ± 0.04	7.88 ± 0.02	7.46 ± 0.01	7.23 ± 0.01
<b>Temperature (°C)</b>	27.04 ± 0.14	27.02 ± 0.08	24.35 ± 0.12	27.50 ± 0.08
<b>Salinity (psu)</b>	31 ± 0.5	31 ± 0.5	31 ± 0.5	31 ± 0.5
<b>TA (μequiv kg<sup>-1</sup>)<sup>a</sup></b>	2053.77 ± 46.51	2032.63 ± 25.60	2061.50 ± 4.56	2091.37 ± 39.37
<b>pCO<sub>2</sub> (μatm)<sup>a</sup></b>	352.93 ± 11.04	861.37 ± 130.34	1997.23 ± 124.42	4091.73 ± 447.85
<b>CO<sub>3</sub><sup>2-</sup> (μmol kg<sup>-1</sup>)<sup>a</sup></b>	175.66 ± 24.96	97.92 ± 16.38	48.82 ± 6.07	26.59 ± 4.72
<b>Ω<sub>Ca</sub><sup>a</sup></b>	4.59 ± 0.25	2.43 ± 0.41	1.21 ± 0.15	0.66 ± 0.12
<b>Ω<sub>Ar</sub><sup>a</sup></b>	3.01 ± 0.18	1.59 ± 0.28	0.79 ± 0.10	0.43 ± 0.08

Data are mean ± s.d. of the replicate culture tanks (n = 4) for the seawater physicochemical parameters measured or calculated during the duration of the experiment: pH (National Bureau of Standards scale), temperature (°C), Salinity (psu), TA (μequiv kg<sup>-1</sup>), carbon dioxide partial pressure (pCO<sub>2</sub>; μatm), carbonate ion concentration (CO<sub>3</sub><sup>2-</sup>; μmol kg<sup>-1</sup>), calcite saturation state (Ω<sub>Ca</sub>), and aragonite saturation state (Ω<sub>Ar</sub>). Value were first averaged within and among days per each of the replicate culture tanks.

25 Afterwards, the treatment mean was computed.

<sup>a</sup> Parameters were calculated using the CO2SYS software program (Pierrot et al., 2006) with equilibrium constants K<sub>1</sub>, K<sub>2</sub> and KSO<sub>4</sub> (Mehrbach et al., 1973; Dickson and Millero, 1987).

## References

- 30 Dickson, A. G., and Millero, F. J.: A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, *Deep Sea Res. (I Oceanogr. Res. Pap.)*, 34, 1733-1743, 10.1016/0198-0149(87)90021-5, 1987.
- Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowicz, R. M.: Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, *Limnol. Oceanogr.*, 18, 897-907, 10.4319/lol.1973.18.6.0897, 1973.
- 35 Pierrot, D., Lewis, E., and Wallace, D.: MS Excel program developed for CO<sub>2</sub> system calculations, ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, 2006.