

Supplementary Information

Supporting Material 1 - Water conditions and gas mixing:

Ambient air was collected via aquarium pumps and passed through soda-lime columns to remove CO₂. To reach hypoxic conditions, using mass-flow controllers (Aalborg GFC17; L min⁻¹) nitrogen was added to the CO₂ stripped air, mixed in marble-containers, maintaining the dissolved oxygen (DO) between 2.0 - 3.5 mg L⁻¹, which was chosen to represent sublethal O₂ concentrations (Vaquer-Sunyer and Duarte, 2008). The O₂ concentration in the Control and Acidification treatment corresponded to atmospheric equilibrium of 6 - 8 mg L⁻¹ at the experimental temperature (24°C). After that, a precise volume of CO₂ was added to the CO₂-free air/nitrogen mix using mass-flow controllers (Aalborg GFC17; mL min⁻¹) to achieve *p*CO₂ concentrations of 380 ppm (Control, Hypoxia) and 1000 ppm (Acidification and Combined). These values correspond to the annual average atmospheric *p*CO₂ level of 2005 (Lüthi et al., 2008; IPCC, 2014) and to the projected level for 2100 (Canadell et al., 2009; IPCC, 2014), respectively. For a homogeneous mixing, the gases were led through container filled with marbles and added to the aquaria via bubble curtains. Aquaria were pre-bubbled overnight prior to the experiment, to reach the target conditions.

Oxygen concentration of the water was measured daily using oxygen electrodes (Consort, SZ11T) and with the Winkler method on the first and last day of the experiment (after Dickson, 1996, using a Metrohm Titrand 808). pH_{NBS} of the culture water was measured daily using pH_{NBS} electrodes (Consort datalogger D130 and pH electrodes from Metrohm and Hanna Instruments). Additionally, pH_{TOT} was measured at 25°C on the first and last day of the experiment with the spectrophotometrically method using the indicator dye m-cresol purple (total scale, Dickson, 2009). Values in the TOT-scale were converted to the NBS-scale and 24°C, using CO2SYS (Pierrot et al. (2006), using K1 and K2 constants (Mehrbach et al., 1973); refit by Dickson and Millero (1987), and K_HSO₄ dissociation constant (Dickson, 1990)). Punctual samples of alkalinity (60 mL) were taken on the first and last day of the experiment. The samples for alkalinity measurements were fixed with 20 mL HgCl₂ and measured within 3 months (using the Metrohm Titrand 808 after SOP3b (Dickson et al., 2007)). pH_{NBS}, temperature, alkalinity and salinity values were used to calculate *p*CO₂ using CO2SYS CO2SYS (Pierrot et al., 2006), with K1 and K2 constants from Mehrbach et al. (1973), as revised by Dickson and Millero (1987), and the K_HSO₄ constant from Dickson (1990).

References

- Canadell, J.G., Raupach, M.R., Houghton, R.A. (2009). Anthropogenic CO₂ emissions in Africa. *Biogeosciences* 6, 463–468.
- Dickson, A.G. (1990). Thermodynamics of the dissociation of boric-acid in potassium-chloride solutions from 273.15 K to 318.15 K. *J Chem Thermodyn* 22, 113–127.
- Dickson, A.G. (1996). Determination of dissolved oxygen in sea water by Winkler titration: 1–13.
- Dickson, A.G. (2009). Determination of the pH of sea water using the indicator dye m-cresol purple: 1–7.
- Dickson, A.G., Millero, F. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Res* 34, 1733–1743.
- Dickson, A.G., Sabine, C.L., Christian, J.R. (2007). Guide to best practices for ocean CO₂ measurements. PICES Spec Publ 3.
- Lüthi, D., Le Floch, M., Bereiter, B., Blunier, T., Barnola, J.-M., et al. (2008). High-resolution carbon dioxide concentration record 650,000–800,000 years before present. *Nature* 453, 379–382.
- Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol Oceanogr* 18, 897–907.
- Pierrot, D., Lewis, E., Wallace, D. (2006). MS Excel program developed for CO₂ system calculations.
- Vaquier-Sunyer, R., Duarte, C.M. (2008). Thresholds of hypoxia for marine biodiversity. *PNAS* 105, 15452–15457.