Supplementary Information

Diel CO₂ cycles reduce severity of behavioural abnormalities in coral reef fish under ocean acidification

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Experimental systems and CO₂ manipulation

Experiment one

The experimental system used at JCU was an 11,000 L re-circulating system. Briefly, the system consisted of a large external 3,700 L sump tank connected to a bio-filter, protein skimmer, UV steriliser and a 1000 L algal bio-remediation tank. The external sump supplied water to four separate 1,600 L re-circulating systems (one per pCO₂ treatment) made up from one 1000 L sump tank and fifteen 40 L holding tanks, contained within a temperature controlled room. Water was supplied at a rate of approximately 1,600 L per day allowing for a complete exchange with the external sump. Holding tanks were supplied with water at a rate of 1 L min⁻¹. Both the internal sumps and holding tanks were aerated with ambient air.
Elevated $p$CO$_2$ treatments were achieved by dosing the 1000 L internal sumps with CO$_2$. This was controlled by solenoid valves (M-Ventil Standard, Aqua Medic, Germany) connected to a pH control system (Aqua Medic AT Control System, Aqua Medic, Germany) with laboratory grade pH electrodes (Neptune Systems, USA). The Aqua Medic AT Control System has a curve function which allowed us to create fluctuating $p$CO$_2$ profiles. pH profiles in the fluctuating $p$CO$_2$ treatments were recorded every other day using a pH meter (InLab Expert Pro electrode and Seven2Go Pro meter, Mettler Toledo, Switzerland) set to take a reading every 15 min. For the stable $p$CO$_2$ treatments pH$_{NBS}$ was measured twice daily using the same model of pH meter. Seawater pH on the total hydrogen ion concentration scale (total scale, pH$_T$) was measured each week with a spectrophotometer following standard operating procedures$^1$ using the indicator dye meta/m-cresol purple (mCP) ($m$-cresol purple sodium salt 99%, non-purified, Acros Organic). Daily and fluctuating pH$_{NBS}$ measurements were converted to pH$_T$ based on the offset between weekly pH$_T$ and pH$_{NBS}$ measurements. Temperature was recorded daily with a digital thermometer (Comark C26, Norfolk, UK). Salinity readings were taken weekly using a conductivity sensor (HQ15d; Hach, USA). Total alkalinity was also measured weekly using Gran Titration (Metrohm 888 Titrando Titrator Metrohm AG, Switzerland) and certified reference material from Dr. A. G. Dickson (Scripps Institution of Oceanography). All seawater parameters were measured in randomly chosen holding tanks. $p$CO$_2$ values were calculated as a function of pH$_T$, temperature and salinity using CO$_2$SYS$^2$ employing constants from$^3$ refit by$^4$ and the KHSO$_4$ dissociation constant from$^5$. Mean values for each of these seawater parameters are presented in Table 1.
Experiment two

The experimental system used at SeaSim was a flow-through system which comprised of multiple independent lines (duplicate independent lines per $p$CO$_2$ treatment). The system used ultra-filtered seawater (0.04 µm), temperature controlled to 28.5°C. Each seawater line supplied three custom made 50 L tanks at the rate of 50 L h$^{-1}$. The experimental tanks were placed in individual temperature-controlled water baths to ensure temperature stability (± 0.1°C). Treatments and tank replicates were randomly positioned in the experimental room.

The management of $p$CO$_2$ and temperature was achieved through the design and implementation of a custom Model Predictive Control logic running on a micro-PLC (Series S7-1500, Siemens, Australia). The micro-PLC was integrated with the general SeaSim control system, to provided SCADA (Siemens WinCC) accessibility and data archiving. The $p$CO$_2$ feedback for each of the replication lines was provided via non-dispersive infrared measurements$^1$. Tank water was delivered to the equilibrator (Seasim, AIMS design, custom built) by an in-tank submersible pump (Universal Pump 1260, EHEIM, Deizisau, Germany) where the $p$CO$_2$ of the air in the chamber reaches and maintains equilibrium with the $p$CO$_2$ of the experimental water. The air was constantly delivered to a NDIR CO$_2$ analyser (Telair T6613, Amphenol, Australia) that provided live feedback to the PLC. The CO$_2$ analysers were calibrated monthly using certified calibration gas mixtures at 0, 600 and 2000 ppm. The control system delivered CO$_2$ though Gas Mass Flow Controllers (GFC17 series, Aalborg, Orangeburg, USA) according to the profiling schedule designed for the $p$CO$_2$ treatment and the feedback signal coming from the experimental tanks. CO$_2$ was dissolved in the flow-through water by mean of membrane contactors (Membrana Liqui-Cel 2.5x8 Extra-Flow. 3M, USA). Total alkalinity was also measured weekly as described above. Mean values for seawater parameters are presented in Table 2.
Throughout the experiment incoming coastal water had a $p$CO$_2$ ranging between 500-550 µatm. Thus, to achieve a control $p$CO$_2$ level closer to 460 µatm, membrane contactors (Membrana Liqui-Cel 4x28 Extra-Flow) were used to remove CO$_2$, using CO$_2$– depleted air as sweep gas. This was only possible for the control treatments and consequently the lower $p$CO$_2$ levels in the 750 ± 300 µatm treatment matched the $p$CO$_2$ of the incoming seawater (500-550 µatm).
Figure S1. Relative laterization ($L_R$) for juvenile *Acanthochromis polyacanthus* presented with a T-maze choice chamber in experiment one. Juvenile fish from each $p$CO$_2$ treatment ($n = 60$ per treatment) were allowed to choose to turn left or right for a total of 10 turns. Graphs show $L_R$ with positive and negative values indicating right and left turns, respectively. The extreme values of $|100|$ indicate fish that turned in the same direction for all 10 turns.
Figure S2. Relative lateralization ($L_R$) for juvenile *Acanthochromis polyacanthus* presented with a T-maze choice chamber in experiment two. Juvenile fish from each $pCO_2$ treatment ($n = 60$ per treatment) were allowed to choose to turn left or right for a total of 10 turns. Graphs show $L_R$ with positive and negative values indicating right and left turns, respectively. The extreme values of $\mid 100 \mid$ indicate fish that turned in the same direction for all 10 turns.
**Figure S3. Mean daily $p\text{CO}_2$ profiles for experiment one.** Coloured sections are ± 1 SD. Profiles for the stable $p\text{CO}_2$ treatments were based on measurements taken twice per day. In reality some minor daily variation would have likely occurred.

![Mean daily $p\text{CO}_2$ profiles for experiment one.](image)

**Figure S4. Mean daily (a) stable and (b) cycling $p\text{CO}_2$ profiles for experiment two.** Coloured sections are ± 1 SD.

![Mean daily (a) stable and (b) cycling $p\text{CO}_2$ profiles for experiment two.](image)
References

1. Dickson, A., Sabine, C. L. & Christian, J. Guide to best practices for ocean CO₂ measurements. PICES special publication 3. IOCCP report No. 8. (2007).

2. Pierrot, D., Lewis, E. & Wallace, D. CO₂SYS DOS program developed for CO₂ system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory. US Department of Energy, Oak Ridge, Tennessee. (2006).

3. Mehrbach, C., Culberson, C. H., Hawley, J. E. & Pytkowicz, R. M. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907 (1973).

4. Dickson, A. G. & Millero, F. J. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Res. Part A. Oceanogr. Res. Pap. 34, 1733–1743 (1987).

5. Dickson, A. G. Standard potential of the reaction: AgCl(s) + 1/2H₂(g) = Ag(s) + HCl(aq), and and the standard acidity constant of the ion HSO₄⁻ in synthetic sea water from 273.15 to 318.15 K. J. Chem. Thermodyn. 22, 113–127 (1990).