Electronic supplementary material

Ocean acidification alters predator behaviour and reduces predation rate

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Supplementary methods

(a) Experimental system and seawater manipulation

Marbled cone snails were collected from coral reefs within the Lizard Island group, Great Barrier Reef, Australia (S 14° 41’, E 145° 28’) and transferred to flow-through aquaria at Lizard Island Research Station. Cone snails are cryptic and can be hard to find on coral reefs. Three and two days of intensive field collections yielded 22 and 18 marbled cone snails, group A and B respectively, that were assigned randomly to 3 replicate control CO_2 (385 µatm pCO_2) or 3 replicate elevated CO_2 (975 µatm pCO_2) 32 l (38L x 28W x 30H cm) aquaria each containing 3-4 cone snails. Cone snails were fed gastropods from the family Strombidae: the strawberry conch *Conomurex luhuanus* (Linnaeus, 1758) synonym *Strombus luhuanus* (Linnaeus, 1758) and the humpbacked conch. Control and elevated CO_2 predators were fed prey of both species at equal rates, and predator and prey sizes were randomised across treatments. Prey snails were housed separately from predators at control conditions and the humpbacked conch was used as the prey species in experimental trials as its behaviours have been quantified previously [1].

Elevated CO_2 seawater was achieved by dosing with CO_2 to a set pH, following standard techniques [2]. Seawater was pumped from the ocean into large aquarium facility header tanks and flowed under gravity into 60 l header tanks where it was diffused through a powerhead with ambient air (control CO_2 treatment) or ambient air and 100% CO_2 to achieve the desired pH (elevated CO_2 treatment) using a pH-stat controller (pH Computer, Aqua Medic, Germany). Each aquarium was supplied with control or elevated CO_2 seawater at 500 $ml.min^{-1}$. Seawater pH_{NBS} (Seven2Go pH/ion meter S8, Mettler Toledo, Ohio, US) and temperature (C22, Comark, Norwich, UK) were recorded daily in each aquarium and seawater CO_2 confirmed with a portable CO_2 equilibrator and non-dispersive infrared (NDIR) gas analyser (GMP-343, Vaisala, Helsinki, Finland) (for method details see [3]). Water samples were analysed for total alkalinity (A_T) by Gran titration (888 Titrando, Metrohm, Switzerland) to within 1% of certified reference material (Prof. A. Dickson, Scripps Institution of Oceanography, batch numbers 118 and 135). Salinity data were obtained from moorings within the Lizard Island group (Australian Institute of Marine Science). Carbonate chemistry parameters (Table 1) were calculated in CO2SYS [4] using the constants K1, K2 from Mehrbach *et al*. [1973] refit by Dickson & Millero [1987], and Dickson for $KHSO_4^-$.

(b) Behavioural experiments

After exposure to control or elevated CO_2 seawater conditions, cone snail behaviour was tested in a series of experiments. General behaviours, self-righting trials and initial predator-prey interactions were video-recorded from directly above with digital cameras (Powershot G15 or G16, Canon, Japan) and behaviour quantified subsequently from videos using ImageJ. Prey-capture success was recorded by visual observation of predator-prey interactions over 32 h. All trials were conducted in seawater at the same CO_2 level used during the treatment period (i.e. control or elevated CO_2). Cone snail mean shell height (±s.e.) was 69.4 ± 2.8 mm, shell width 39.9 ± 1.4 mm and total animal wet mass 79.35 ± 7.93 g (wet mass on shell height $F_{1,20} = 549.08$, $p < 0.0001$, $r^2 = 0.965$, total animal wet mass = 0.0023 * shell height^2.4481 (4 d.p.)) for group A. There was no difference in the size of cone snails (total animal wet mass) between control and elevated CO_2 treatments (group A $t_{20}=1.262$, $p=0.221$, group B $t_{16}=-0.341$, $p=0.738$).

(i) General behaviour

To test if elevated CO_2 affected activity, after 15 d in treatment conditions we placed control and elevated CO_2 cone snails (from group A) individually in the centre of a test arena consisting of a large circular tank (Ø1040 mm), with a 50 mm deep natural sand substrate, filled with seawater to a depth of 200 mm above the sand. Behaviour was video-recorded for 15 min. Distance travelled and average speed, direction of travel (angle), the side of the arena the individual was in the end of the trial (left or right side), whether the individual chose to bury or not, and the time taken to start to bury were quantified by video analysis. Cone snails were then carefully returned to their treatment tanks. To test if elevated CO_2 affected self-righting behaviour, after a total of 16 d in treatment conditions we placed control and elevated CO_2 cone snails individually in plastic aquaria (200Lx130Wx150H mm), with a 30 mm deep sand substrate, containing seawater to a depth of 100 mm above the sand. Cone snails were gently placed upside down and the time taken for the animal to self-right recorded. Trials lasted for 30 min and cone snails were then carefully returned to their treatment tanks.

(ii) Predator-prey interactions

To examine the initial predator responses during a predator-prey interaction with the humpbacked conch snail, we used another set of cone snails (group B) that were exposed for 14-18 d to control or elevated CO_2 conditions in an identical way to the first set of cone snails. We placed cone snails individually in the centre of the test arena described above. After a 15 min habituation period, we carefully placed the predator in the centre of the arena and then placed a control prey snail 20 mm in front of the predator. Predator and prey anterior ends faced each other [1] and behaviour was recorded for 15 min. Since cone snails are slow moving and often ambush predators, we conducted a longer experiment to determine the effect of elevated CO_2 on predator foraging and prey-capture success. After a total of 20 d in treatment conditions, we placed cone snails (from group A) individually in 32 l (38Lx28Wx30H cm) aquaria with a 40 mm deep sand substrate and flow-through seawater at control or elevated CO_2 conditions to maintain CO_2 treatment conditions during the experiment. After 30 min habituation, the cone snail was gently repositioned in the centre of the tank and a control prey snail was placed 20 mm in front of the predator. Predator and prey anterior ends faced each other and subsequent predator-prey interactions were recorded by visual observation at the following time points: 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 22, 24, 26, 28, 29, 30 and 32 hours.

(c) Statistical analysis

Generalized linear models (GLM) were used to test for the effects of CO_2 on general behaviours (distance travelled, left side preference, time taken to bury, time spent buried and self-righting time) and initial predator-prey interactions (distance travelled during the interaction and distance from prey with time in treatment as a fixed effect). A Gaussian distribution was used to compare time taken to bury and time taken to self-right between CO_2 treatment levels, while a quasipoisson distribution and the log link function was used to compare distance travelled and initial predator-prey interactions (distance travelled during the interaction, distance from prey), and a zero-inflated negative binomial distribution with logit link function was used to compare time spent buried. A Mardia-Watson-Wheeler test was used to compare the circular distributions of directional trajectories and a logistic regression was performed to compare side preference (left or right) during general behaviour trials. Predator foraging success was analysed by comparing prey survival trajectories during interactions with control and elevated CO_2 predators using Kaplan-Meier log-rank survival analysis. Analyses were performed in R [5] and SigmaPlot 11.0.

References in supplementary methods

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