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Marine mollusc predator-escape behaviour altered by near-future carbon dioxide levels

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Ocean acidification poses a range of threats to marine invertebrates; however, the potential effects of rising carbon dioxide (CO_2) on marine invertebrate behaviour are largely unknown. Marine gastropod conch snails have a modified foot and operculum allowing them to leap backwards rapidly when faced with a predator, such as a venomous cone shell. Here, we show that projected near-future seawater CO_2 levels (961 µatm) impair this escape behaviour during a predator–prey interaction. Elevated-CO_2 halved the number of snails that jumped from the predator, increased their latency to jump and altered their escape trajectory. Physical ability to jump was not affected by elevated-CO_2 indicating instead that decision-making was impaired. Antipredator behaviour was fully restored by treatment with gabazine, a GABA antagonist of some invertebrate nervous systems, indicating potential interference of neurotransmitter receptor function by elevated-CO_2, as previously observed in marine fishes. Altered behaviour of marine invertebrates at projected future CO_2 levels could have potentially far-reaching implications for marine ecosystems.

1. Introduction

The oceans have absorbed about a third of all anthropogenic carbon dioxide (CO_2) emissions released into the atmosphere since the beginning of the Industrial Revolution [1]. As a result, surface oceans are now 0.1 lower in pH and 30% more acidic than that before the Industrial Revolution [2] and the rate of change is approximately 100 times faster than any period in the last 650 000 years [3,4]. Additionally, the partial pressure of CO_2 (pCO_2) in the surface ocean is increasing in line with rising atmospheric CO_2 [5]. Based on current and projected future CO_2 emissions, ocean pH could decline a further 0.3–0.4 units [2] and pCO_2 levels could exceed 900 µatm by the end of this century [6].

Marine ecosystems are threatened by this increasing CO_2 enrichment of the oceans [5,7] with concern focused primarily on the effect of ocean acidification and reduced carbonate saturation state on the growth and development of calcareous marine invertebrates [8,9]. In calcareous invertebrates, elevated-CO_2 and ocean acidification can have a range of negative effects, including disturbance of extracellular ion and acid–base regulation [10], and reductions in growth, calcification and survival (reviewed in [11–14]). In marine fishes, elevated-CO_2 also has dramatic effects on behaviour; including altered olfactory [15,16] and auditory preferences [17], loss of behavioural lateralization [18] and an inability to learn [19]. Juvenile fishes become less risk averse [20,21], even becoming attracted to, rather than repelled from the odour of predators [22]. In fishes, these behavioural effects are caused by interference to the function of type A γ-aminobutyric acid neurotransmitter receptors ($GABA_A$ receptors) [23], possibly as a result of the compensatory changes in transmembrane chloride (Cl^-) and bicarbonate (HCO_3^{2-}) ion gradients that occur during acid–base regulation in fishes exposed to elevated-CO_2 [24,25]. Upon

GABA binding to the GABA-gated ion channel, these changes in ion gradients probably lead to the inappropriate action of the $GABA_A$ receptor resulting in behavioural abnormalities [23].

Marine invertebrate behaviour at elevated-CO_2 has been little studied, except at very high seawater CO_2 (pH 6.6–6.8, equivalent to more than 12 000 µatm pCO_2). In an intertidal snail, these very high CO_2 levels resulted in reduced morphological defences and increased avoidance of seawater containing chemical cues from a predatory crab [26]. In hermit crabs, very high CO_2 decreased either the likelihood or speed that crabs would upgrade from a suboptimal to optimal gastropod shell, decreased resource assessment measured by antennular flicking rates [27] and disrupted chemoreception which resulted in a reduced ability to locate food odour and reduced locomotory activity [28]. Remarkably, the behaviour of marine invertebrates at near-future CO_2 levels projected for the end of this century and any potential mechanism for altered behaviour have been little explored [29]. $GABA_A$ receptors are phylogenetically old with $GABA_A$-like receptors occurring in diverse invertebrate groups, including molluscs [30,31]. This suggests that invertebrate behaviours and nervous systems could be affected by near-future CO_2 levels in a similar way to fishes. Invertebrates are critical for the function of all marine ecosystems [32–34] and their behaviours shape the outcome of key ecological processes [35–37]. Marine invertebrates also sustain fisheries worth over 57 billion US dollars per annum [38]. Consequently, any effects of near-future CO_2 levels on the behaviour of marine invertebrates could have far-reaching implications for marine biodiversity and fisheries productivity [39].

The ability to detect and evade predators is critical to the survival of all organisms. In aquatic systems, chemoreception plays a particularly important role in sensing predators [40]. Eavesdropping prey are able to exploit predator kairomones (chemosensory cues that provide an adaptive benefit for the interspecific receiver, but not the emitter) resulting in a wide range of antipredator behaviours [41]. In molluscs, predator-avoidance behaviours include climbing, crawl-out and general 'move away' behaviours [42,43]. However, some molluscs exhibit dramatic predator-escape behaviours. Marine snails from the family Strombidae (conchs) have a modified foot and operculum used in shell-righting to flick themselves over and to escape predators by 'leaping' or 'jumping' away rapidly with a kicking motion [44,45]. Their typical response to a molluscivorous cone shell predator is to jump quickly out of range of the venomous cone shell dart (see the electronic supplementary material, video S1). This violent escape response occurs upon detection of predator kairomones [40,46,47]. A single leap results in an immediate increase in distance from a potential predator [48] of about one body length (shell height) and field observations demonstrate that this behaviour enhances survival [49].

To determine whether near-future CO_2 levels affected this vital escape behaviour, we assessed the antipredator-escape response of a jumping conch snail *Gibberulus* (previously *Strombus*) *gibberulus gibbosus* to its cone shell predator *Conus marmoreus* under current-day 'control' and near-future 'elevated-CO_2' conditions (405 and 961 µatm pCO_2, respectively). We used a series of six experiments to test in detail the effects of elevated-CO_2 on escape behaviour of the snail and the mechanisms involved. (i) First, we used a self-righting experiment to test whether elevated-CO_2 affected fundamental exercise behaviour, which might cause snails to jump less. (ii) Next, we placed snails in a test arena with a venomous cone shell predator to test whether elevated-CO_2 affected predator-escape behaviour. (iii) We measured snail oxygen consumption rate at rest and during jumping to determine whether elevated-CO_2 altered the metabolic cost of jumping. (iv) We then treated snails with the GABA antagonist gabazine (SR 95531) to assess the potential involvement of the nervous system in the responses observed at elevated-CO_2, as recently demonstrated in marine fishes [23]. (v) Additionally, we tested whether elevated-CO_2 could affect the predator cue directly. (vi) Finally, we tested the duration of exposure to elevated-CO_2 required to impair snail behaviour in order to understand whether short-term fluctuations in CO_2, for example diel cycles on coral reefs, could induce impaired predator-escape behaviour.

2. Material and methods

(a) Experimental system and seawater manipulation

The herbivorous gastropod mollusc *G.* (previously *Strombus*) *gibberulus gibbosus* and its specialist mollusc-eating predator *C. marmoreus* occur in sandy subtidal areas around tropical coral reefs. Prey snails and cone shell predators were collected throughout October and November from the Lizard Island Lagoon, Great Barrier Reef, Australia (14°41′ S, 145°28′ E) and transferred to an environmentally controlled aquarium facility at Lizard Island Research Station. Prey snails were assigned randomly to four replicate control (405 µatm pCO_2) or four replicate elevated-CO_2 (961 µatm pCO_2) aquaria. Twenty snails were housed in each 32 l (380 L × 280 W × 300 H mm) aquarium. These snails feed on algal film which was abundant on the surfaces of each aquarium. Snails were kept for 5–7 days in captivity after which they were tested. Fresh snails were collected for each experiment, handled and housed identically. Each aquarium was supplied with control or elevated-CO_2 seawater at 720 ml min^{-1}. Elevated-CO_2 seawater was achieved by dosing with CO_2 to a set pH, following standard techniques [50]. Seawater was pumped from the ocean into 2 × 60 l header tanks where it was diffused with ambient air (control) or 100% CO_2 to achieve the desired pH (elevated-CO_2 treatment). A pH-controller (Aqua Medic, Germany) attached to the CO_2 treatment header tank maintained pH at the desired level. Seawater pH_{NBS} (HQ40d, Hach, Loveland, CO, USA) and temperature (C22, Comark, Norwich, UK) were recorded daily in each aquarium and seawater CO_2 confirmed with a portable CO_2 equilibrator and infrared sensor (GMP343, Vaisala, Helsinki, Finland). Water samples were analysed for total alkalinity by Gran titration (888 Titrando, Metrohm, Switzerland) to within 1% of certified reference material (Prof. A. Dickson, Scripps Institution of Oceanography). Carbonate chemistry parameters (table 1) were calculated in CO2SYS [51] using the constants K1, K2 from Mehrbach *et al.* [52] refit by Dickson & Millero [53], and Dickson for $KHSO_4$.

(b) Behavioural experiments

After 5–7 days in control or elevated-CO_2 treatment aquaria, snail behaviour was tested in six separate experiments (see the electronic supplementary material, table S1). All trials were videographed with a Panasonic Lumix DMC-FT3 or Canon Powershot G15 digital camera and behaviour was quantified subsequently from videos. All behavioural and respirometry trials were conducted in seawater at the same CO_2 level as the experimental treatment of the snail tested (i.e. control or elevated-CO_2). Mean shell height (±s.e.) was 35.95 ± 0.20 mm, shell width 17.85 ± 0.12 mm and total animal wet mass 5.74 ± 0.09 g (wet mass on shell height $F_{1,107} = 273.80$, $p < 0.0001$, $r^2 = 0.7164$, total animal

Table 1. Seawater carbonate chemistry for each treatment. (Values are means ± s.e. to nearest integer, one or two decimal places as appropriate.)

treatment	temperature (°C)	salinity	pH_{NBS}	total alkalinity (μmol kg^{-1} SW)	pCO_2 (μatm)	Ω_{Ca}	Ω_{Ar}
control	27.0 (±0.2)	35.2	8.17 (±0.01)	2275 (±7)	405 (±11)	5.27 (±0.09)	3.50 (±0.06)
elevated-CO_2	27.0 (±0.2)	35.2	7.85 (±0.01)	2257 (±2)	961 (±13)	2.85 (±0.04)	1.89 (±0.02)

wet mass = 0.440 (shell height) − 10.1 (3 s.f.)). Shell mass comprised 80% of the whole animal wet mass.

(i) Experiment 1: effect of elevated-CO_2 on exercise ability

To test whether elevated-CO_2 affected fundamental exercise behaviour, we placed control and CO_2 snails upside down and recorded the time taken and number of foot flicks required for the animal to self-right. The test arena consisted of a large circular tank (diameter 1040 mm), with a 50 mm deep sand substrate, filled with seawater to a depth of 200 mm above the sand. A total of 40 control and 40 CO_2 snails were tested individually for self-righting.

(ii) Experiment 2: effect of elevated-CO_2 on predator-escape behaviour

To test the predator-escape response of control and CO_2 snails, we placed a single snail in the centre of the test arena described above after recording its self-righting behaviour. The snail was placed 10 mm in front of a thin transparent plastic barrier (100 L × 80 H mm) with a cone shell predator 10 mm behind the barrier. Predator and prey anterior ends faced each other and behaviour was recorded for 5 min. The barrier functioned to prevent a successful predatory attack should the snail fail to escape the predator. A total of 40 control and 40 CO_2 snails were tested. The following traits were measured by video analysis: number of jumps during 5 min, latency to first jump, final distance from the predator and angle of escape trajectory [54] after 5 min. Only two individuals (both controls) out of the 40 control and 40 CO_2 snails reached the wall of the test arena during experimentation, demonstrating that the test arena was big enough to capture the complete predator-escape response of 97.5% of all individuals. Difficulties with videographing meant not all traits could be measured for all snails and sample sizes for each trait are shown in the electronic supplementary material, table S1. We noted that the cone shell predator successfully captured and consumed the prey snail when kept in an aquarium together overnight, but only after the prey snail stopped jumping. To compare the jumping behaviour in the absence of a predator, another 20 control and 20 CO_2 snails were tested using the procedure described above, but without a cone shell predator in the arena.

(iii) Experiment 3: effect of elevated-CO_2 on oxygen consumption

Snail oxygen consumption rate ($\dot{M}O_2$) was measured by closed respirometry to determine whether exposure to elevated-CO_2 had an effect on the metabolic cost of jumping. Snails held for 5–7 days in control or elevated-CO_2 were transferred to individual 250 ml respirometers (80 Ø × 50 L mm). Jumping was then induced by the injection of 50 ml of seawater containing chemical cues from a predatory cone shell. Predator-conditioned seawater 'predator cue' was made by placing one cone shell (length *ca* 60 mm, wet mass *ca* 45–50 g) in 2 l of seawater for 10–20 min. The cone shell was then removed and the predator cue was mixed well before a 50 ml cue subsample was taken. Pilot measurements determined the volume of predator cue required for the experiments given the concentration of cue and the subsequent dilution in seawater. The 50 ml of predator-cued seawater was injected through a small hole in the respirometer using a syringe and fine tube. The tube extended the full internal length of the respirometer so that the predator cue was released at the far side of the chamber. Any excess seawater was extruded through the same hole so that the final volume remained at 250 ml. The hole was then sealed. The number of jumps made by the snail and duration of jumping was recorded. If jumping did not begin within 2 min of predator cue injection, the trial was terminated, and a new snail was introduced to the respirometer. Seawater oxygen concentration was measured with a galvanometric oxygen probe (OXI 340i, WTW, Germany) and recorded with PowerLab 4/20 using Chart v. 5.4.2 software (ADInstruments). Mixing of seawater within the respirometer was achieved by a small propeller attached to the tip of the probe and powered by a magnetic plate placed near the respirometer. Blank respirometers with no snails were run to measure the background oxygen consumption (microbial $\dot{M}O_2$) both before and after the actual experiment, using new treatment seawater and no snail before the trial, and after taking out the snail at the end of the trial. $\dot{M}O_2$ data are reported per unit wet tissue mass and all measures were corrected for the average microbial $\dot{M}O_2$ in the respirometer before and after the trial (less than or equal to 10%).

The jumping escape response, elicited by injection of predator cue, caused an immediate and large increase in $\dot{M}O_2$, which remained elevated until jumping ceased. Active oxygen consumption ($\dot{M}O_{2\,active}$) was determined as the $\dot{M}O_2$ measured during jumping. When jumping stopped for at least 3 min, the respirometer seawater was replaced with fresh treatment seawater containing no predator cue, so that jumping was no longer stimulated and oxygen consumption was recorded for a further 3–5 h. Pilot measurements showed that this duration was sufficient for $\dot{M}O_2$ to return to resting levels. Resting oxygen consumption ($\dot{M}O_{2\,rest}$) was therefore determined from the lowest $\dot{M}O_2$ during this period. $\dot{M}O_{2\,rest}$ was subtracted from $\dot{M}O_{2\,active}$ to give aerobic scope. The aerobic cost per jump was calculated as

$$\text{cost per jump} = \frac{(\dot{M}O_{2\,active} - \dot{M}O_{2\,rest}) \times \text{time spent jumping}}{\text{total number of jumps}}.$$

(iv) Experiment 4: effect of the GABA antagonist gabazine on behavioural responses at elevated-CO_2

To examine the possible involvement of neurotransmitter receptors in altering escape behaviour in elevated-CO_2-exposed snails, we treated conch snails with gabazine and then tested their response to a predator cue. Gabazine (SR 95531) is a $GABA_A$ neurotransmitter receptor antagonist known to inhibit GABA binding to $GABA_A$ receptors in vertebrates [55], and has also been found to inhibit GABA-induced ion currents or GABA binding to receptors in some invertebrates, including insects [56,57] and a hydrozoan [58,59]. Although the pharmacology of gabazine has not been studied in molluscs, gabazine has been shown to restore normal behaviour in fishes exposed to elevated-CO_2 and, therefore, provides a useful starting point for testing the possible mechanisms responsible for altered behaviour in marine organisms exposed to elevated-CO_2. If

gabazine restores predator-escape behaviour in the conch snail, it may suggest that a similar mechanism could potentially be responsible for impaired escape behaviour at elevated-CO_2 in both fishes and molluscs. Using a fully crossed design, we first individually placed 30 control and 30 CO_2 snails for 30 min in 100 ml seawater containing 4 mg l^{-1} of gabazine or 100 ml seawater without gabazine (sham treatment). Snails were then removed from the treatment containers and placed individually in 2 l of seawater in small plastic aquaria (200 L x 130 W × 150 H mm). After 2 min acclimatization, 70 ml of seawater was added to control for the physical addition of water. No snails jumped during acclimatization or with the addition of plain seawater. At each subsequent 2 min interval, 70 ml of predator cue was added to stimulate the initial and continual presence of a molluscivorous cone shell predator and to compensate for any degradation of the cue, and any snail jumps were counted. A total of six predator cue additions were made over 12 min. For this experiment, predator cue was made by placing one cone shell (length *ca* 60 mm, wet mass *ca* 45–50 g) in 3 l of seawater for 10 min. The cone shell was then removed and the predator cue was mixed well before each 70 ml cue subsample was taken.

(v) Experiment 5: effect of elevated-CO_2 on predator cue

To test whether elevated-CO_2 seawater could have affected the predator cue directly, and thus altered the jumping response, the jumping response of control snails presented with the predator cue made by placing a cone shell predator in control seawater was compared to the jumping response of control snails given a predator cue made by placing a cone shell predator in elevated-CO_2 seawater. This experiment was performed using the method described in experiment 4, except no gabazine was used. For comparison with the previous experiment, snails were placed in 100 ml seawater without gabazine (sham treatment), and then placed individually in 2 l of seawater, where 70 ml aliquots of first plain seawater, and then predator cue were added. Of 30 control snails, 18 were exposed to predator cue made in control seawater and 12 were exposed to predator cue made in elevated-CO_2 seawater. If elevated-CO_2 (low pH) seawater affected the chemical cue, we predicted there would be a difference in the jumping behaviour of the two groups.

(vi) Experiment 6: effect of exposure time to elevated-CO_2 on behaviour

Finally, to examine the exposure time to elevated-CO_2 required to alter behaviour, snails were exposed to control or elevated-CO_2 for different time periods. For this experiment, snails were held for a total of 5 days in experimental aquaria in one of four treatments: (i) control seawater ($n = 18$); (ii) control seawater switched to elevated-CO_2 in the final 12 h ($n = 12$); (iii) control seawater switched to elevated-CO_2 in the final 2 days ($n = 12$); and (iv) elevated-CO_2 seawater ($n = 18$). After 5 days, the individual jumping response of each snail to the predator cue was then measured, as described in experiment 4 and using a sham treatment for comparison as described in experiment 5.

(c) Statistical analysis

Parametric tests (*t*-tests and ANOVA) were used to test latency to first jump, the number of jumps and distance moved (for jumpers only), oxygen consumption and for the predator cue experiments. A Mardia–Watson–Wheeler test was used to compare the circular distributions of escape trajectories. Mann–Whitney *U*-tests were used where data did not fit parametric assumptions, including for self-righting behaviour, and the number of jumpers and distance moved from the predator for all snails, because data included non-jumpers. Snails were used once for either: (i) self-righting and then the predator–prey interaction, (ii) one of the predator cue response trials, or (iii) respirometry (see the electronic supplementary material, table S1). All reported *p*-values are two-tailed.

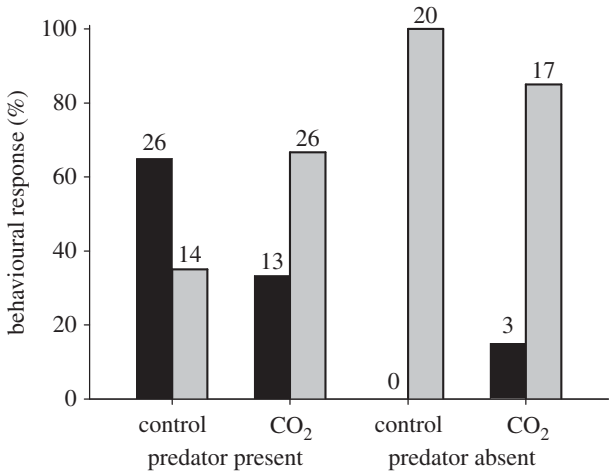

Figure 1. Percentage of jumping (black) and non-jumping (grey) snails in the presence and absence of a cone shell predator during 5 min trials. The number of snails that jumped away from the predator was reduced in snails treated with elevated-CO_2. Numbers of replicates are given above the bars.

3. Results

Elevated-CO_2 did not affect self-righting ability, and therefore did not affect fundamental exercise behaviour in this marine mollusc. There was no difference in the time taken for upturned snails to right (mean ± s.e. control 39.2 ± 3.4 s, elevated-CO_2 33.8 ± 3.4 s, $U = 638.0$, $n = 40,40$, $p = 0.119$) or the number of foot flicks required to right (mean ± s.e. control 2.7 ± 0.3, elevated-CO_2 2.3 ± 0.2, $U = 736.0$, $n = 40,40$, $p = 0.525$) between control and elevated-CO_2-treated snails. By contrast, antipredator-escape behaviour was altered by elevated-CO_2. When snails were placed in a circular test arena in front of a cone shell predator, the majority of control snails (65%) jumped, compared with only 33% of elevated-CO_2 snails ($\chi^2 = 7.922$, $n = 79$, $p = 0.005$; figure 1). For snails that did jump, elevated-CO_2 nearly doubled the latency to first jump from 60 ± 9 s (mean ± s.e.) in control to 100 ± 21 s in elevated-CO_2 snails ($t_{37} = -2.032$, $p = 0.049$). Among jumpers, the escape trajectory also changed such that elevated-CO_2 snails moved on an angle closer to the predator (84 ± 6° circular mean ± s.e.) compared with control snails (109 ± 10°) ($W = 6.207$, $N = 22,13$, $p = 0.045$; figure 2). Snail size (wet mass) had no effect either on self-righting time ($F_{1,78} = 0.267$, $p = 0.607$, $r^2 < 0.001$) or on the number of jumps from a predator ($F_{1,74} = 0.0316$, $p = 0.859$, $r^2 < 0.001$).

As fewer elevated-CO_2 snails jumped when faced with a predator, the average number of jumps per snail ($U = 478.5$, $n = 38,39$, $p = 0.004$) and the average distance moved from the predator ($U = 370.0$, $n = 38,37$, $p < 0.001$) were reduced for all elevated-CO_2 snails compared with all control snails (figure 3). However, the elevated-CO_2 snails that did jump, jumped as many times ($t_{35} = 1.499$, $p = 0.143$) and as far ($t_{35} = 1.673$, $p = 0.103$) as control snails (figure 3). As a result, there was no difference in the mean (±s.e.) distance moved per jump between control (30.4 ± 1.4 mm) and elevated-CO_2 (31.7 ± 2.3 mm) snails ($t_{35} = -0.511$, $p = 0.612$), which was equivalent to just less than one body length (shell height). On

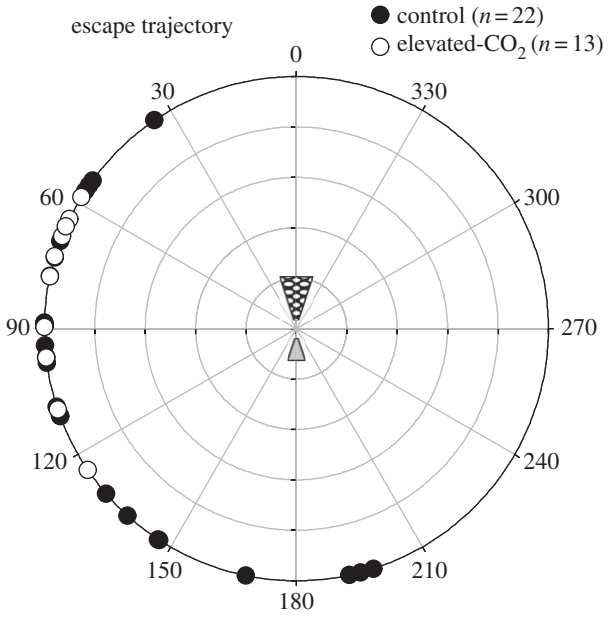

Figure 2. The escape trajectory of control and elevated-CO_2 snails that jumped in response to a cone shell predator. The starting position and orientation of the cone shell predator is shown by the black- and white-spotted triangle and the position of the prey snail is shown by the light grey triangle (not to scale). The asymmetrical nature of prey snail foot and shell morphology resulted in snails jumping generally in the opposite direction to the shell outer lip (left and backwards). Elevated-CO_2 snails jumped on an acute angle closer to the predator compared with control snails.

average, jumpers moved a total distance of more than 20 cm away from the predator, equivalent to over five times their body length (shell height) and beyond immediate reach of the cone shell.

Elevated-CO_2 did not affect the metabolic cost of jumping. Snail oxygen consumption measured by respirometry was unaffected by elevated-CO_2. Resting oxygen consumption did not differ between control and elevated-CO_2 snails (see the electronic supplementary material, figure S1A; $t_{38} = -0.537$, $p = 0.594$), and more importantly, the aerobic scope of jumping snails (i.e. the difference in oxygen consumed during rest and during jumping) was not affected by elevated-CO_2 exposure (see the electronic supplementary material, figure S1B; $t_{23} = 1.045$, $p = 0.307$). As a result, the amount of oxygen used per jump (a proxy for the energy used or cost per jump) was not altered in snails exposed to elevated-CO_2 (see the electronic supplementary material, figure S1C; $t_{16} = 0.639$, $p = 0.532$).

Jumping was restored to control levels by treatment with gabazine. The total number of jumps per snail in the elevated-CO_2 group was less than half that of control snails ($F_{3,56} = 3.237$, $p = 0.029$, post hoc $p = 0.012$) over the 12 min period (figure 4). By contrast, there was no difference in the total number of jumps for elevated-CO_2 snails treated with gabazine when compared with controls (post hoc $p = 0.852$; figure 4). Gabazine did not stimulate jumping *per se* because control snails treated with gabazine showed no statistical difference in jumping when compared with control snails treated with a seawater sham (post hoc $p = 0.130$).

The results of the gabazine experiment indicate that there was no effect of elevated-CO_2 directly on the odour cues from the predator, because elevated-CO_2 snails treated with gabazine jumped in response to predator cue presented in elevated-CO_2 seawater (figure 4), indicating an ability to detect and respond to the predator cue in this treatment group.

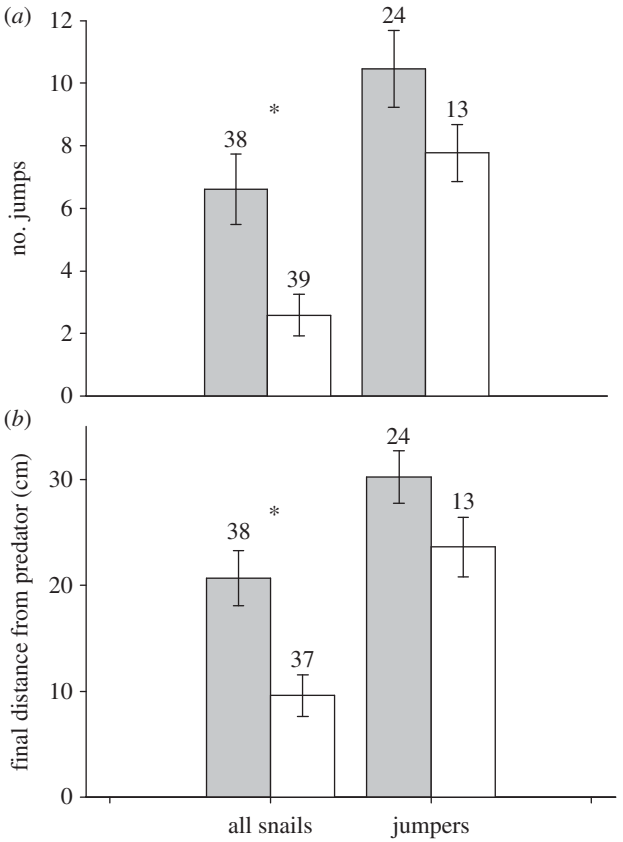

Figure 3. Behavioural responses of snails during 5 min trials in the presence of a predator. (*a*) The number of jumps per snail and (*b*) distance moved from the predator recorded for control (light grey) and elevated-CO_2 (white) snails. Snails treated with elevated-CO_2 jumped fewer times and had a shorter escape distance from the predator, but for jumpers alone there were no significant differences in the number of jumps or the escape distance from the predator. Values are means ± s.e. Numbers of replicates are given above the bars. Asterisk (*) denotes a significant difference.

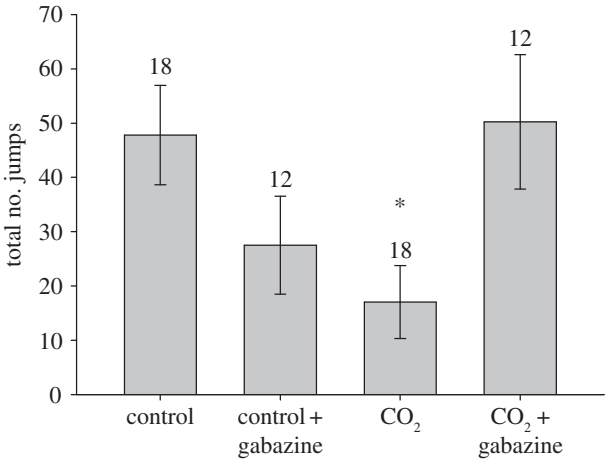

Figure 4. The total number of jumps per individual in control or elevated-CO_2 snails treated with a seawater sham or gabazine, and then exposed to predator cue. In elevated-CO_2 snails, jumping behaviour was restored by gabazine. Values are means ± s.e. Numbers of replicates are given above the bars. Asterisk (*) denotes a significant difference from control.

Furthermore, when control snails were tested, the percentage of jumpers was no different when predator cue was presented in either control or elevated-CO_2 seawater ($t_{10} = -0.212$, $p = 0.837$; electronic supplementary material, figure S2). These results demonstrate that the predator cue was not altered by

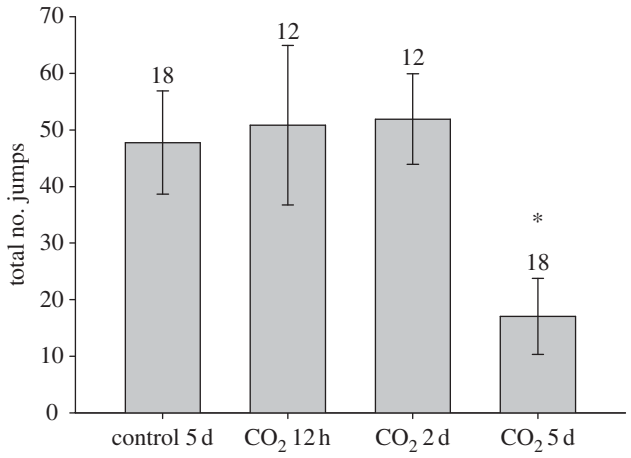

Figure 5. The total number of jumps per individual in response to predator cue according to snail CO_2 exposure time. The jumping escape response was impaired after 2–5 days exposure to elevated-CO_2. Values are means ± s.e. Numbers of replicates are given above the bars. Asterisk (*) denotes a significant difference from control.

exposure to mildly acidified seawater. Finally, it took between 2 and 5 days exposure to elevated-CO_2 to elicit the behavioural effects on the snails (figure 5). Snails exposed to elevated-CO_2 for 12 h or 2 days exhibited a similar total number of jumps to controls, whereas snails exposed to elevated-CO_2 for 5 days exhibited a significant decrease in the total number of jumps ($F_{3,56} = 3.450$, $p = 0.022$, post hoc $p = 0.014$).

4. Discussion

Our findings show that CO_2 concentrations projected to occur in the oceans by the end of this century [6] may have important effects on the behaviour of a marine mollusc. In this case, 961 µatm pCO_2 altered the behavioural decisions of a coral reef conch snail when faced with a predator. Elevated-CO_2 impaired the predator-escape response in this jumping snail by potentially affecting decision-making, while the physical ability to jump, and therefore capacity to escape, was retained. Elevated-CO_2 reduced the number of snails that jumped from the predator, and also altered behaviour in snails that did decide to jump by increasing the time taken to jump, thus increasing the exposure time to the predator, and by changing the escape trajectory such that the snail moved on an angle closer to the predator. Combinations of behavioural changes such as these are likely to alter complex trophic interactions in marine food webs.

While previous studies have reported altered behaviour in crabs and a mollusc at extremely high CO_2 levels (more than 12 000 µatm) [26–28], our results show that CO_2 levels projected to occur in the surface ocean by 2100 can significantly impair predator-escape behaviour, with implications for the outcome of predator–prey interactions. Our findings of behavioural modifications in a marine mollusc at near-future CO_2 levels are significant because invertebrates, such as molluscs and crustaceans, are fundamental to marine ecosystems; they dominate lower trophic levels that support marine food webs [60], they are ecosystem engineers [61] and they are keystone species in ecological interactions that shape the structure of marine communities [36]. Altered behaviour of marine invertebrates caused by elevated-CO_2 has the potential to modify the outcome of key ecological interactions, with potentially far-reaching consequences for ecosystem function. Nevertheless, the effects of elevated-CO_2 on ecological interactions may vary among species or with CO_2 levels. In hermit crabs, decision-making, resource allocation and locomotion are impaired at more than 12 000 µatm CO_2 [27,28] and these results are consistent with our findings of impaired behaviour in the conch snail at 961 µatm CO_2. By contrast, Bibby *et al*. [26] found snails exhibited increased predator-avoidance (crawl-out) behaviour in response to predator cue at more than 12 000 µatm CO_2.

An additional challenge for organisms inhabiting coastal and coral reefs ecosystems are the marked diel fluctuations in carbonate chemistry parameters, including pH and CO_2, that can occur [62,63]. Organisms in some coral reef habitats may already experience CO_2 levels for several hours each day that are at least as high as those projected for the open ocean at the end of the century. However, we found that an exposure time between 2 and 5 days to elevated-CO_2 was required to impair behaviour, suggesting that shorter term exposure to elevated-CO_2, for example during diel fluctuations, would not affect behaviour or increase vulnerability to predation at night. Nevertheless, the interaction between the magnitude of CO_2 variation and the exposure time to induce behavioural effects is important to consider when predicting future impacts on marine systems [64]. As absorption of anthropogenic CO_2 continues, marine habitats with naturally variable carbonate chemistry conditions will experience an amplification of pCO_2 relative to open-ocean conditions [65] and this could potentially accelerate the onset of predicted responses of marine organisms to increasing CO_2 [64].

Our results indicate that interference with the function of neurotransmitter receptors might be responsible for the compromised predator-escape behaviour of snails exposed to elevated-CO_2. Gabazine, a drug known to block $GABA_A$ receptors in vertebrates [55] and $GABA_A$-like receptors in some invertebrates [56–59], has previously been found to restore normal behaviour in fishes exposed to elevated-CO_2 [23]. We found that gabazine was also effective in restoring the antipredator jumping behaviour in elevated-CO_2-exposed snails. This suggests that molluscan $GABA_A$-like receptors [30,31] could be involved in the behavioural effects of elevated-CO_2 seen, although other mechanisms may be involved because the pharmacology of gabazine has not been studied in molluscs. If a similar mechanism is responsible for the behavioural effects of elevated-CO_2 observed here in a marine mollusc and in previous studies with fishes, then we might expect elevated-CO_2 could cause behavioural impairment in a broad suite of marine animals, potentially including commercially important groups such as molluscan and crustacean shellfish, cephalopods and echinoderms. If the behavioural effects of elevated-CO_2 in marine invertebrates function in a similar way to fishes, then there may also be marked differences among invertebrate species and individuals in how they respond to elevated-CO_2 [21,66], and this should be a focus of future research.

This study highlights the potential for near-future ocean acidification to alter behaviour in a marine mollusc; however, the potential for organisms to adapt to this problem is unknown. New studies have detected genetic variation in the effect of ocean acidification on growth and development of some marine invertebrates [67,68], and there is, therefore, potential for selection of more tolerant genotypes over coming

decades in these species. Whether similar evolutionary potential exists for the behavioural traits tested here is unknown. Selection for adaptive behaviours, particularly those involved in life or death decisions, will be strong. Predator–prey interactions and subsequent avoidance and escape behaviours create a strong selective advantage for prey individuals that respond appropriately. Escape responses can also be modified through learning as demonstrated in the shell-less marine mollusc *Tritonia diomedea* [69]. There was considerable variation in behaviour among individual prey snails in our experiments, with some elevated-CO_2 snails jumping in response to predator presence, while most did not. Obviously, appropriate escape behaviour will confer an immediate survival advantage. Additionally, more subtle differences including increased time to first jump and escape angle were subject to variation among individuals. These differences among individuals could be owing to phenotypic plasticity or they may indicate genetic variation in CO_2 sensitivities. Many escape responses in the scallop *Argopecten purpuratus* have significant heritabilities [70] but whether variation in behavioural responses to elevated-CO_2 is heritable is currently unknown. Further research is required to determine whether variation in escape responses in the conch snail caused by elevated-CO_2 is heritable and whether the spread of tolerant genotypes could possibly occur quickly enough for evolution to rescue populations from any negative effects of altered behaviour, such as potential increased rates of predation.

In this study, we only tested the predator-escape behaviour of the prey snail. Further studies are required to determine whether elevated-CO_2 alters the behaviour of predators, such as cone shells, or their ability to capture prey including their ability to produce toxic venom. Studies with fishes show that the dynamics of predator–prey interactions can be altered in different ways when only the prey, only the predator or both are exposed to elevated-CO_2 [71]. While we have demonstrated a clear effect of elevated-CO_2 on mollusc predator-escape behaviour, the precise effect this will have on mortality rates will depend on how CO_2-treated predators and prey interact together under natural conditions.

We conclude that CO_2 impairs decision-making in a marine mollusc, and consequently alters key ecological behaviours associated with trophic interactions. As near-future CO_2 levels alter behavioural strategies and can cause a reduction in wariness, predator avoidance, or escape behaviour, this could mean marine organisms become easier prey for predators, including humans, to catch in the future. Altered trophic interactions with rising CO_2 may have implications not only for marine ecosystem dynamics and shellfish industries but also for future food security. Determining the extent of behavioural disturbance as well as estimating evolutionary potential in behaviour will now be critical for predicting the future consequences of rising CO_2 in both marine fishes and invertebrates.

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