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1 **Ocean acidification alters predator behaviour and reduces**
2 **predation rate**

3
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12 **Abstract**

13

14 Ocean acidification poses a range of threats to marine invertebrates; however, the emerging
15 and likely widespread effects of rising carbon dioxide (CO₂) levels on marine invertebrate
16 behaviour are still little understood. Here we show that ocean acidification alters and impairs
17 key ecological behaviours of the predatory cone snail *Conus marmoreus*. Projected near-
18 future seawater CO₂ levels (975 μatm) increased activity in this coral reef molluscivore more
19 than 3-fold (from <4 to >12 mm.min⁻¹) and decreased the time spent buried to less than 1/3
20 when compared with present-day control conditions (390 μatm). Despite increasing activity,
21 elevated CO₂ reduced predation rate during predator-prey interactions with control-treated
22 humpbacked conch, *Gibberulus gibberulus gibbosus*; 60% of control predators successfully
23 captured and consumed their prey, compared with only 10% of elevated CO₂ predators. The
24 alteration of key ecological behaviours of predatory invertebrates by near-future ocean
25 acidification could have potentially far-reaching implications for predator-prey interactions
26 and trophic dynamics in marine ecosystems. Combined evidence that the behaviours of both
27 species in this predator-prey relationship are altered by elevated CO₂ suggests food web
28 interactions and ecosystem structure will become increasingly difficult to predict as ocean
29 acidification advances over coming decades.

30

31 **1. Introduction**

32

33 Rising carbon dioxide (CO₂) levels and subsequent ocean acidification affect the survival,
34 growth, calcification and reproduction of marine organisms [1]. However, recent research
35 shows CO₂ levels projected for the end of the century ($p\text{CO}_2 \leq 1000 \mu\text{atm}$, Representative
36 Concentration Pathway, RCP 8.5 [2]) also alter marine invertebrate behaviour, including in
37 tropical [3] and temperate [4, 5] gastropod molluscs, cephalopod molluscs [6] and
38 crustaceans [7, 8]. Notably, behavioural effects are apparent even at modest CO₂ levels where
39 effects on physiology are not yet manifest [3, 9]. Furthermore, evidence that elevated CO₂
40 may alter behaviour by interfering with the function of GABA_A neurotransmitter receptors in
41 fishes [10] and invertebrates [3, 11], suggests behavioural alteration among trophic levels for
42 water-breathing vertebrates and invertebrates [12] is likely to be widespread. For calcifying
43 invertebrates, the rapidly emerging, and likely global reach of behavioural alteration means
44 they now face the dual threats of both impaired calcification and behaviour at projected future
45 CO₂ levels. Invertebrates dominate lower trophic levels, exhibiting diversities at least an
46 order of magnitude greater than vertebrates in marine ecosystems [e.g. 13]. Understanding
47 the behavioural effects of ocean acidification on invertebrates is thus critical to determine the
48 full impacts of anthropogenic CO₂ emissions on the oceans.

49

50 Predator-prey interactions shape the structure of marine communities and ecosystems [14].

51 However, we know little about the potential effects of ocean acidification on predatory

52 behaviours in marine invertebrates, or the potential for ocean acidification to affect

53 invertebrate behaviour across more than one trophic level. Several studies have shown that

54 the response of invertebrate prey species to their predator is affected by high CO₂ [3-5, 15,

55 16], but how elevated CO₂ may affect the predators of these species has not been tested. For a

56 temperate gastropod, one recent study showed that elevated CO₂ affected the avoidance of
57 predator cues, but not cue detection of mussel prey [5]. Here, to determine the potential
58 effects of elevated CO₂ on predator behaviour and predation rate, we assess the effects of
59 near-future CO₂ levels (RCP 8.5 [2]) on the behaviour of the venomous marbled cone snail
60 *Conus marmoreus* Linnaeus, 1758, a molluscivorous predator that inhabits coral reefs and is
61 often an ambush or stealth hunter of other gastropods. Previous work has shown elevated
62 CO₂ affects the antipredator behaviour of its prey, the humpbacked conch *Gibberulus*
63 *gibberulus gibbosus* (Röding, 1798) [3], but how elevated CO₂ might affect the predator, and
64 thus predator-prey interactions between these species, is unknown. Specifically, we
65 investigate whether exposure to elevated CO₂ affects both general behaviour and prey-
66 capture behaviours in this predatory invertebrate.

67

68 **2. Materials and methods**

69

70 We exposed cone snails to control (390 µatm) and elevated (975 µatm) CO₂ conditions (table
71 1) for 2-3 weeks using established methods [e.g. 3]. General behaviours (distance travelled
72 and speed, direction moved, left or right side preference, time taken to bury, time spent
73 buried) were tested on individuals in a large circular arena tank over 15 min. Righting was
74 tested separately over 30 min. Initial predator-prey interactions were conducted in the circular
75 arena over 15 min and longer predator-prey experiments over 32 h were performed in 32 l
76 aquaria. Prey used in experiments were from control CO₂ conditions and a natural sand
77 substrate was provided for all trials. Generalized linear models (GLM) and survival analyses
78 were used to test for the effects of CO₂ on predator behaviour and predation success,
79 respectively. Full details of the experimental system, seawater manipulation and behavioural

80 experiments are provided in the electronic supplementary material. Data are available from
81 the Dryad Digital Repository [17].

82

83 **3. Results**

84

85 **General behaviour**

86 Elevated CO₂ increased the distance travelled by cone snails during the 15 min trial
87 ($t_{20}=2.521$, $p=0.020$, Fig. 1). At 975 μatm CO₂, cone snails moved 3-4 times further
88 ($186.0\pm 59.0\text{mm}$, mean \pm s.e.) than at control conditions ($56.1\pm 6.5\text{mm}$), equivalent to a mean
89 movement speed of $12.4\text{ mm}\cdot\text{min}^{-1}$ at elevated CO₂ conditions, compared with $3.7\text{ mm}\cdot\text{min}^{-1}$
90 at control conditions (figure 1). There was no difference, however, in the direction of
91 movement ($W_2=3.046$, $p=0.218$), with a circular mean of 349.8° for control and 351.8° for
92 elevated CO₂ cone snails. The number of left and right side choices was distributed equally in
93 control predators (54.5%), however, 81.8% of elevated CO₂ predators finished the trial left of
94 the starting line and one elevated CO₂ predator turned in a complete circle, although there
95 was no significant difference in left side preference ($\chi^2=1.932$, $\text{df}=1$, $p=0.165$). In general,
96 control predators tended to travel in a straighter line than elevated CO₂ predators whose
97 trajectory tended to curve.

98

99 During the 15 min trial, 81.8% of control predators buried, however, only 27.3% of elevated
100 CO₂ predators buried. Resultantly, the amount of time spent buried was greater at control
101 ($6.0\pm 1.2\text{ min}$) than at elevated CO₂ conditions ($1.6\pm 0.9\text{ min}$) ($t_{20}=-2.362$, $p=0.028$, figure 1).
102 However, for the predators that chose to bury, there was no difference in time taken to bury
103 between CO₂ treatments ($t_{10}=0.691$, $p=0.505$) with mean time taken to bury being 7.7 ± 0.9
104 min at control and $9.0\pm 1.8\text{ min}$ at elevated CO₂ conditions. In self-righting trials, 9 cone

105 snails righted in each treatment after 30 min. There was no significant difference in righting
106 time with CO₂ ($t_{16}=2.100$, $p=0.052$, Fig. 1), although there was a trend for increased self-
107 righting time at elevated CO₂ (16.4 ± 1.4 min, mean \pm s.e.) compared with control (11.4 ± 1.9
108 min) conditions.

109

110 **Predator-prey interactions**

111 Elevated CO₂ increased cone snail activity during the 15 min predator-prey interaction with
112 elevated CO₂ cone snails moving 255.1 ± 93.6 mm (mean \pm s.e.) compared with 32.1 ± 6.4 mm
113 for control cone snails ($t_{15}=2.541$, $p=0.023$, Fig. 1). There was no significant difference,
114 however, in the distance between the predator and the prey at the end of the trial ($t_{15}=0.791$,
115 $p=0.441$, Fig. 1); the mean distance of elevated CO₂ predators from the prey was 370.2 ± 90.8
116 mm compared with 291.0 ± 41.2 mm for control predators, and no predators captured their
117 prey during the 15 min trial, which could have influenced activity. Number of days in
118 treatment had no effect on distance travelled ($t_{15}=-0.602$, $p=0.556$) or distance from prey
119 ($t_{15}=0.022$, $p=0.983$). During the longer 32h predator-prey interactions, there were significant
120 differences in the survival trajectories of prey for control and elevated CO₂ predators
121 ($\chi^2=4.830$, $df=1$, $p=0.028$, Fig. 2), with more than half (60%) of control predators
122 successfully capturing and consuming their prey, compared with only 10% of elevated CO₂
123 predators.

124

125 **4. Discussion**

126

127 Our findings show that projected end-of-century CO₂ conditions [2] affect a range of both
128 general and hunting behaviours of predatory invertebrates, in this case a tropical predatory
129 mollusc. Elevated CO₂ (975 μ atm) increased activity by increasing the distance travelled and

130 thus average speed by more than 3x control levels, and reduced the amount of time spent
131 buried to 1/3 of control levels. Ocean acidification can increase activity in squid [6] and in
132 the current study increased activity and a reduction in the time spent buried may be a result of
133 increased boldness at elevated CO₂, similar to previous findings in fishes [18]. Elevated CO₂
134 did not significantly affect self-righting ability in this predator, nor (as previously found) its
135 prey [3], although elevated CO₂ can alter self-righting time in a temperate mollusc [4].

136

137 While ocean acidification increased activity, predators had reduced foraging and prey-capture
138 success. During predator-prey interactions, distance moved was increased (>3x) at elevated
139 CO₂ levels, but although an increase in distance covered might be expected to increase
140 foraging success, predators were no closer to their prey at the end of the 15 min trial. During
141 the 32 h trial, prey-capture success was reduced markedly from 60% to 10%. Cone snails are
142 often found buried in sandy areas in coral reefs, lying in ambush for their prey. Elevated CO₂
143 could reduce predation rate by: 1) increasing activity and consequently decreasing the time
144 spent buried lying-in-wait for prey – potentially altering prey-capture strategy; 2) reducing
145 the ability to detect or capture prey (but see [5]); 3) reducing motivation to feed; or, 4) a
146 combination of altered decision-making behaviours. Additionally, increased activity levels
147 are likely to result in a greater energetic demand, so reductions in prey-capture rate may have
148 energy budget consequences for these predators. A shift in the balance of energy intake
149 versus expenditure would have a direct effect on individual performance and could
150 potentially impact on optimal foraging strategies [19].

151

152 Our previous research showed that elevated CO₂ levels (961 µatm) alter antipredator
153 behaviours of the prey snail, the humpbacked conch [3], making them more vulnerable to
154 predation. Here we additionally find effects of elevated CO₂ on the behaviour of the predator,

155 providing the first evidence of behavioural alteration caused by ocean acidification across
156 two invertebrate trophic levels. Combinations of behavioural changes such as these are likely
157 to alter trophic interactions in marine food webs, including for keystone species [4, 5]. The
158 prevalence of complex trophic interactions in marine ecosystems often involving multiple
159 species and indirect effects, means that behavioural alterations caused by elevated CO₂ levels
160 are likely to make future ecosystem structure and function more difficult to predict.
161 Increasing evidence for behavioural effects of elevated CO₂ on species at different trophic
162 levels [3-8, 12, 15, 16, 18, 20, 21], including interacting species as demonstrated here,
163 suggests that trait-mediated indirect interactions could be important in predicting the effects
164 of ocean acidification on marine communities and deserves further attention [19].

165
166 This study tested the potential effect of ocean acidification on predator behaviour. Although it
167 was not possible to determine the effect of elevated CO₂ on both the predator and the prey
168 simultaneously due to limited predator sample sizes, future research should examine how
169 predator-prey interactions may be influenced if both the predator and prey are treated with
170 elevated CO₂, and any potential interaction with rising ocean temperatures. In fish, predator-
171 prey interactions can be altered when both predator and prey are exposed to elevated CO₂
172 [20] and with elevated temperature [21]. Additionally, the precise effects on prey mortality
173 rates and predator-prey dynamics will depend on how predators and prey interact together
174 under wild conditions.

175
176 For some of the behaviours tested here, we found greater variation at elevated CO₂ compared
177 with control conditions, suggesting that individuals differ in their tolerance to increased CO₂.
178 Indeed in fish, variation in CO₂ tolerance results in selective mortality in the wild [22]. Since
179 selection acts upon variation within a population, the potential for adaptation through

180 selection should be a focus of future research, especially for key ecological behaviours that
181 have the potential to reorganise trophic dynamics in marine ecosystems.

182

183 **Ethics.** Research adhered to local guidelines and appropriate permissions were obtained.

184 **Data accessibility.** Data are available from the Dryad Digital Repository

185 (<http://dx.doi.org/10.5061/dryad.jc77j>) [17].

186 **Competing interests.** We have no competing interests.

187 **Authors’ contributions.** S.-A.W. & P.L.M. conceived the study, S.-A.W. designed the
188 experiment, S.-A.W. & J.B.F. performed the experiments, S.-A.W. analysed the data and
189 wrote the first draft of the manuscript. All authors discussed the results and contributed to the
190 final version of the manuscript. All authors agree to be held accountable for the content
191 therein and approve the final version of the manuscript.

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197

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199

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271

272 **Table**

273

274 **Table 1.** Seawater carbonate chemistry for each treatment.

275

Group	Treatment	Temperature (°C)	Salinity	pH_{NBS}	Total alkalinity ($\mu\text{mol.kg}^{-1}$ SW)	<i>p</i>CO₂ (μatm)	Ω_{Ca}	Ω_{Ar}
A	Control	28.2 (± 0.2)	35.5	8.19 (± 0.01)	2297.9 (± 3.3)	389.3 (± 7.1)	5.70 (± 0.08)	3.80 (± 0.05)
A	Elevated CO ₂	28.4 (± 0.1)	35.5	7.86 (± 0.00)	2288.3 (± 4.3)	973.9 (± 9.0)	3.04 (± 0.02)	2.03 (± 0.02)
B	Control	27.8 (± 0.3)	35.5	8.19 (± 0.01)	2278.6 (± 13.9)	387.1 (± 8.8)	5.58 (± 0.10)	3.71 (± 0.07)
B	Elevated CO ₂	27.8 (± 0.3)	35.5	7.85 (± 0.00)	2266.6 (± 16.5)	975.5 (± 7.4)	2.93 (± 0.03)	1.95 (± 0.02)

276

277 Values are means \pm s.e.

278 **Figure legends**

279

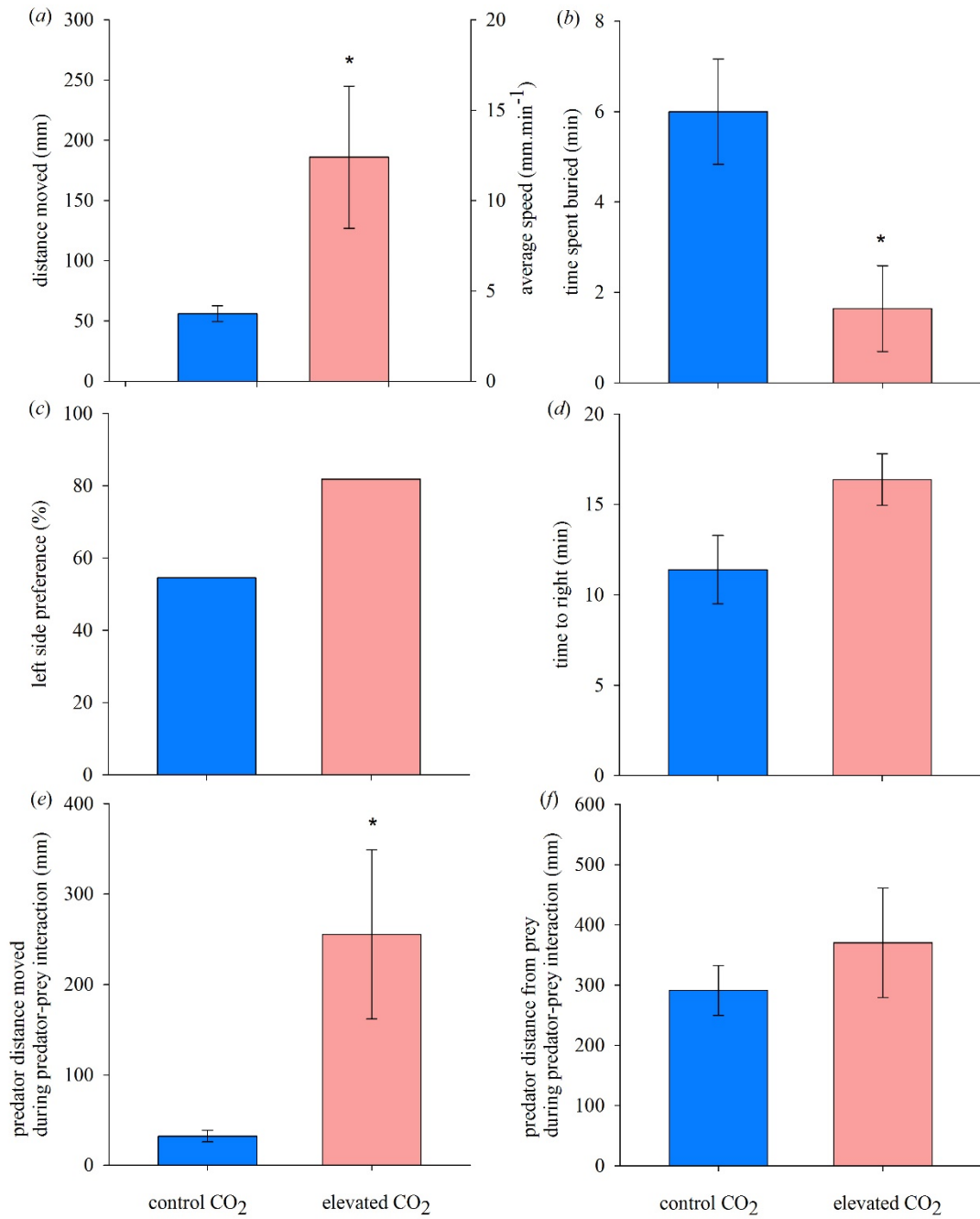
280 **Figure 1.** Effect of ocean acidification on key ecological behaviours of the predatory marbled
281 cone snail: a) distance travelled and speed, b) time spent buried, c) percentage of individuals
282 that finished the trial on the left side of the test arena, d) time to right; and during the 15 min
283 predator-prey interaction in the arena: e) distance travelled by the predator, and f) predator
284 distance from prey. Blue and red bars show control and elevated CO₂ treated predators,
285 respectively. Values are means \pm s.e. *Denotes a significant difference.

286

287 **Figure 2.** Effect of ocean acidification on prey-capture success. Survival trajectories of
288 humpbacked conch prey during predator-prey interactions. Blue and red lines show control
289 and elevated CO₂ treated predators, respectively.

290

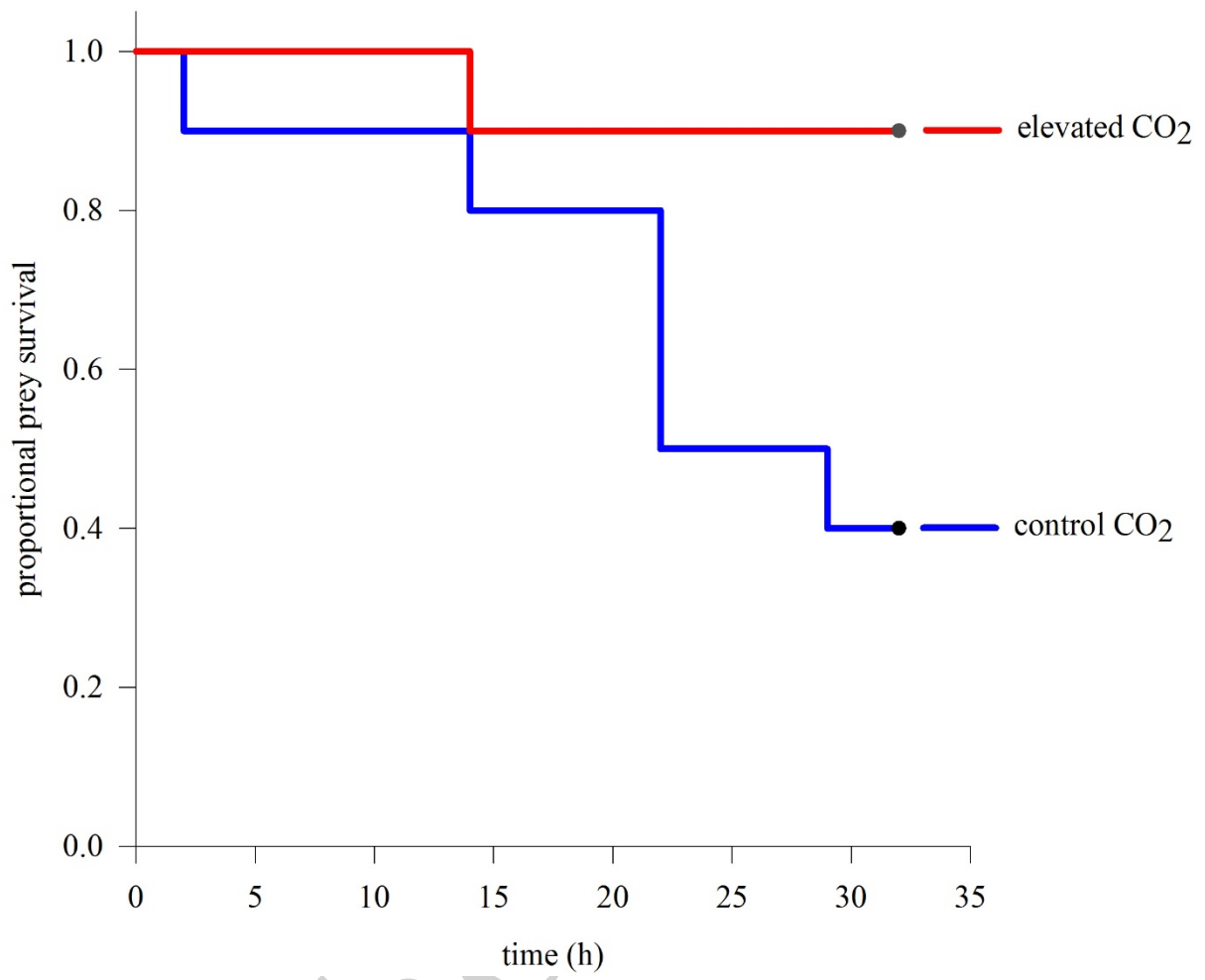
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291

292 **Figure 1.**

293



294

295 **Figure 2.**

Auth