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Authors

Hamilton, Trevor J
Radke, Nicole Hurst
Bajwa, Jasmin
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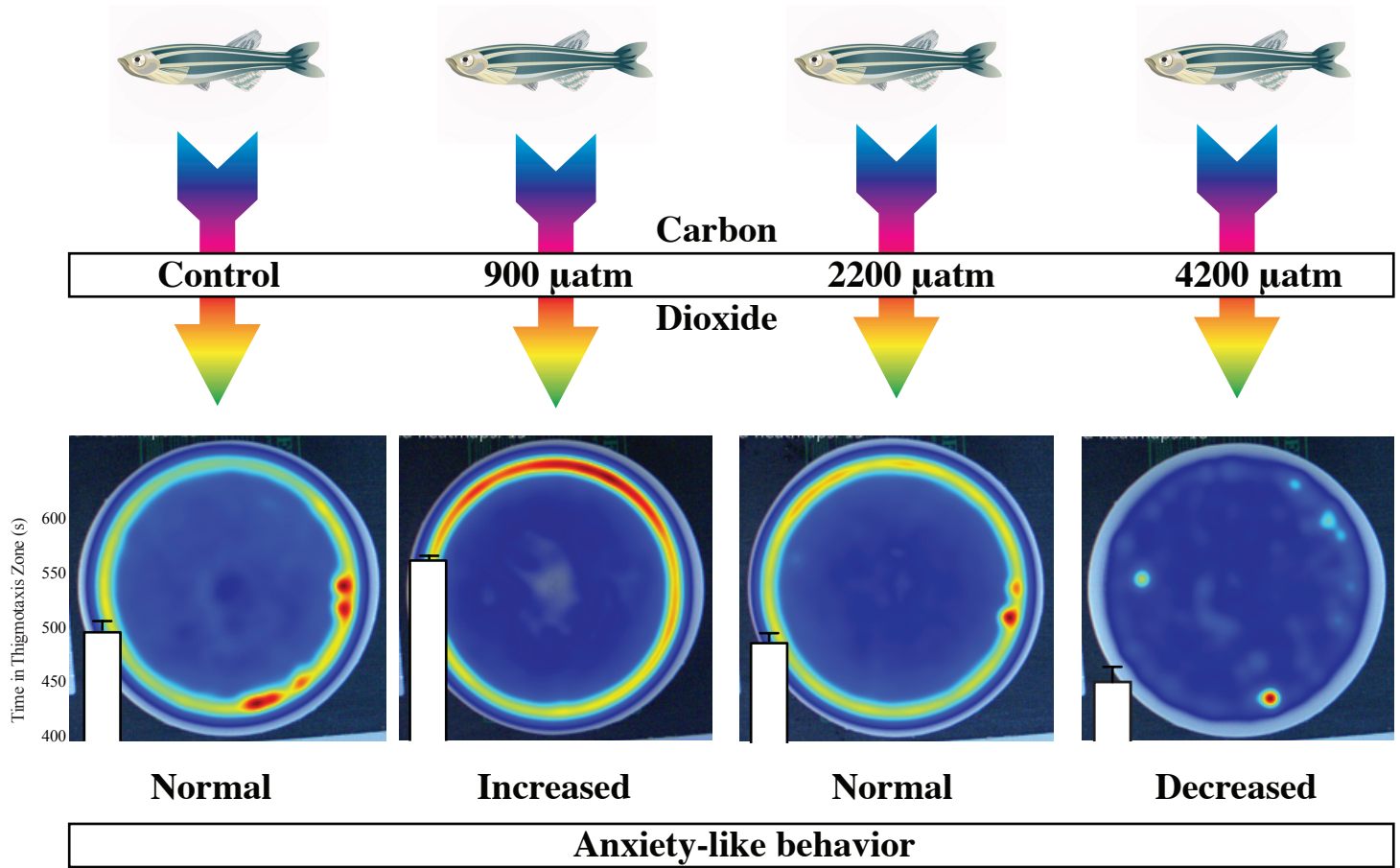
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Highlights

The dose makes the poison: nonlinear behavioural response to CO₂-induced aquatic acidification in fish

Trevor J. Hamilton^{1-2*}, Nicole Hurst Radke¹, Jasmin Bajwa¹, Shayna Chaput¹, Martin Tresguerres^{3*}

- Elevated CO₂ altered behaviour in zebrafish but not in an additive manner
- Acclimations to ~900, 2200, and 4200 μatm cause increased, normal, and decreased anxiety-like behaviour
- Exploratory behaviour was not affected by any CO₂ treatment
- Elevated CO₂ to ~4200 μatm decreased locomotion

1 **The dose makes the poison: non-linear behavioural response to CO₂-induced aquatic acidi-**
2 **fication in zebrafish (*Danio rerio*)**

3 Trevor J. Hamilton^{1-2*}, Nicole Hurst Radke¹, Jasmin Bajwa¹, Shayna Chaput¹, Martin Tres-
4 guerres^{3*}

5
6 ¹Department of Psychology, MacEwan University, Edmonton, AB, Canada

7 ² Neuroscience and Mental Health Institute, University of Alberta, Edmonton, AB, Canada, T6G
8 2H7.

9 ³Marine Biology Research Division, Scripps Institution of Oceanography, University of California
10 San Diego, USA

11 *Authors for correspondence: trevorjameshamilton@gmail.com; mtresguerres@ucsd.edu
12

13 **Abstract**

14 CO₂-induced aquatic acidification is predicted to affect fish neuronal GABA_A receptors leading to
15 widespread behavioural alterations. However, the large variability in the magnitude and direction
16 of the responses suggest substantial species-specific CO₂ threshold differences, life history and
17 parental acclimation effects, experimental artifacts, or a combination of these factors. As an estab-
18 lished model organism, zebrafish (*Danio rerio*) can be reared under stable conditions for multiple
19 generations, which may help control for some of the variability observed in wild-caught fishes.
20 Here, we used two standardized tests to investigate the effect of 1-week acclimatization to four
21 pCO₂ levels on zebrafish anxiety-like behaviour, exploratory behaviour, and locomotion. Fish ac-
22 climatized to 900 μatm CO₂ demonstrated increased anxiety compared to control fish (~480 μatm),
23 however, the behaviour of fish exposed to 2,200 μatm CO₂ was indistinguishable from that of
24 controls. In addition, fish acclimatized to 4,200 μatm CO₂ had decreased anxiety-like behaviour;

25 i.e. the opposite response than the 900 μatm CO₂ treatment. On the other hand, exploratory behav-
26 iour did not differ among any of the $p\text{CO}_2$ exposures that were tested. Thus, zebrafish behavioural
27 responses to elevated $p\text{CO}_2$ are not linear; with potential important implications for physiological,
28 environmental, and aquatic acidification studies.

29 **Keywords:**

30 **Novel object approach test, carbon dioxide, open field test, zebrafish, GABA-A receptor**

31 **1. Introduction**

32 The increasing levels of atmospheric CO₂ will invariably result in elevated $p\text{CO}_2$ levels in
33 aquatic environments. Because CO₂ dissolves in water producing H⁺ that lower the pH, this pro-
34 cess is known as “ocean acidification” and “freshwater acidification”; here, we will use “aquatic
35 acidification” to refer to both. When exposed to aquatic acidification conditions in the laboratory,
36 fish have been reported to suffer significant behavioural alterations that have raised concerns about
37 the fate of fish in future aquatic environments. Some of the alterations include impaired olfactory
38 and auditory discrimination, behavioural lateralization, anxiety, locomotion, and boldness (re-
39 viewed in Munday et al., 2019). The proposed underlying cause stems from the mechanism fish
40 use to maintain blood acid-base homeostasis: to counteract the acidosis induced by exposure to
41 elevated $p\text{CO}_2$, fish excrete H⁺ across their gills and accumulate HCO₃⁻ (reviewed in Heuer et al.,
42 2019). Together with putative equimolar reductions in the concentration of chloride ions ([Cl⁻]),
43 the altered blood chemistry is theorized to affect the functioning of GABA_A receptors resulting in
44 neuronal depolarization and altered behaviour (reviewed in Tresguerres and Hamilton, 2017).
45 However, the direction and magnitude of behavioural responses to aquatic acidification that have
46 been reported in different studies are highly variable. Perhaps the highest profile example is the
47 contrasting results in olfactory discrimination reported for coral reef fishes (Munday et al., 2009;

48 Clark et al., 2020a) that has recently sparked what we consider to be an essential debate (Munday
49 et al., 2020, Clark et al., 2020b). Similarly, our group has reported divergent behavioural responses
50 by two congener fish species that inhabit Californian kelp forests: while the splitnose rockfish
51 (*Sebastes diploproa*) demonstrated increased anxiety-like behaviour upon acclimatization to
52 ~1,100 μatm CO₂ (Hamilton et al., 2014), the behaviour of blacksmith (*Chromis punctipinnis*) was
53 not affected (Kwan et al., 2017).

54 The observed variability of behavioural responses is often ascribed to yet unidentified species-
55 specific and life stage-dependent differences in the mechanisms responsible for acid-base regula-
56 tion and neuronal function. In addition, it has been suggested that behavioural responses to ele-
57 vated $p\text{CO}_2$ might not be linear, resulting from complex neuronal responses to intra- and extra-
58 cellular acid-base parameters (Heuer et al., 2016). Finally, many of the discrepancies in the litera-
59 ture may be due to differences in the life history of wild-caught fish and that of their parents, the
60 levels of experimental CO₂ to which the fish are exposed, and inherent variability and limitations
61 of the behavioural tests that are employed.

62 With this in mind, we conducted a series of experiments using zebrafish (*Danio rerio*). This
63 fish species is commonly bred under standardized conditions for generations, and therefore pro-
64 vide a set of experimental organisms with a more homogenous life history compared to wild-
65 caught fish. To explore the possibility of a non-linear relation between $p\text{CO}_2$ levels and behav-
66 ioural responses, we examined the effects of four increasing $p\text{CO}_2$: ~480 μatm as the control con-
67 dition, ~900 μatm to simulate aquatic acidification predicted for the end of the century in the sur-
68 face of large water bodies including the ocean, ~2,200 μatm as a more pronounced yet still ocean-
69 acidification relevant scenario, and ~4,200 μatm as a more extreme scenario. To address the po-
70 tential issue of behavioural repeatability, we chose to employ the “open field” and “novel object

71 approach” tests. These are standardized, robust, and relatively simple tests proven to reflect anxi-
72 ety-like and exploratory behaviours that depend, at least in part, to the functioning of the GABA_A
73 receptor (GABA_AR). This has been validated in rodents (Lowery-Gionta et al., 2018), and in
74 zebrafish by our group (Hamilton et al., 2017) and others (Ferreira et al., 2019). Unconscious bias
75 was eliminated by recording and analysing fish behaviour using a camera system and motion track-
76 ing software.

77

78 **2. Methods**

79 **2.1 Animals and Housing**

80 Adult wild-type (short-fin) zebrafish (*Danio rerio*) were obtained from Aquatic Imports (Cal-
81 gary, AB) (n = 150) and were held in the lab for a minimum of 60 days prior to experimentation.
82 All fish were 9-12 months old, approximately equal male:female ratio, and were housed in an
83 Aquatic Habitats (AHAB, Aquatic Ecosystems, Inc. Apopka, FL, USA) three-shelf bench top sys-
84 tem with controlled filtration and aeration, in clear 3L polypropylene tanks. Density was seven to
85 eight fish per tank and fish were fed once per day. Temperature was maintained at 29.8 ± 1.4 °C
86 and water was buffered with NaCl, NaHCO₃, and C₂H₄O₂ to achieve an average pH of 7.1 ± 0.03
87 over the experimental period (see Supplementary data). Weekly water quality measurements in-
88 cluded nitrates, nitrites, alkalinity and conductivity (Hamilton et al., 2017). Zebrafish were fed
89 Gemma Micro 300 pellets (Skretting/BioOregon, ME, USA) daily between 10am and 2pm. All
90 experiments were approved by the Grant MacEwan University Animal Research Ethics Board
91 (AREB) under protocol number 05-12-14, in compliance with the Canadian Council for Animal
92 Care (CCAC) guidelines for the care and use of experimental animals.

93 **2.2 CO₂ acclimatization**

94 Randomly selected zebrafish were moved from the main habitat to experimental tanks (9L)
 95 fitted with an individual filter (Marineland, Blacksburg, VA, USA) and heater (Hydro Aquarium)
 96 in one of four CO₂ conditions (Table 1) (15 zebrafish in each experimental tank) (Supplemental
 97 Material) for 7 days of experimentation. Pure compressed CO₂ (Praxair, Edmonton, AB) was in-
 98 jected to experimental tanks using an Apex control system (Neptune Systems, San Francisco, CA,
 99 USA) allowing for two CO₂ tanks and one control tank to be run in parallel. Fish were placed into
 100 tanks then CO₂ levels were ramped up to their experimental levels over a 24 hour period. Experi-
 101 ments were repeated on four occasions, each time containing at least one control tank. Actual *p*CO₂
 102 levels were monitored with a Vaisala GMP 251 CO₂ probe (Finland) connected to a closed-loop
 103 system made up of a CO₂-impermeable plastic PVC tubing attached to medical grade CO₂ perme-
 104 able silicon tubing (0.8 mm thickness) submerged in the tank with the enclosed air pumped through
 105 the system (Watson et al., 2017). To allow for stable and consistent measurements, the *p*CO₂ read-
 106 ings were made 50 minutes after immersing the probe (Apex double junction lab grade pH probe,
 107 Neptune) in the tank. In addition, pH (NBS) was measured using the Apex system; these readings
 108 were performed twice a day, once in the morning and once in the afternoon. 15% water changes
 109 were done on days 3 and 6. On the day of behavioural testing zebrafish were not until after record-
 110 ing.

111 Table 1. Experimental groups and parameters. Mean ± 1 s.e.m.

	<i>p</i> CO ₂ (µatm)	pH _{NBS}	Number of repli- cates (15 fish each)	Number of fish (total)
Control	481 ± 14	7.86 ± 0.03	4	60

900 $\mu\text{atm CO}_2$	874 ± 29	6.48 ± 0.01	2	30
2,200 $\mu\text{atm CO}_2$	2238 ± 99	6.24 ± 0.01	2	30
4,200 $\mu\text{atm CO}_2$	4220 ± 168	6.07 ± 0.02	2	30

112

113 **2.3 Behavioural testing**

114 The testing arena was a circular, opaque white cylinder 34 cm in diameter and 15 cm deep (Supp
115 Fig. 1), which was filled with water to a height of 6 cm. Because constantly switching water
116 $p\text{CO}_2$ between trials was unfeasible, all testing took place in control water. In any case, previous
117 research has indicated that short term exposure of high $p\text{CO}_2$ acclimatized fish to control $p\text{CO}_2$
118 does not affect fish behaviour (Munday et al., 2016). An enclosure of white corrugated plastic
119 was mounted around the arena to minimize external visual stimuli. An individual fish was trans-
120 ferred in a small net from the CO₂-dosing tank and placed into the centre of the testing arena;
121 transfer took a maximum of 5 seconds. The “open field test” started immediately after the fish
122 was placed in the center of the arena and lasted 10 minutes (Dean et al., 2020). Shortly after the
123 open field test ended, a Lego® figurine was placed in the center of the arena and the “novel ob-
124 ject approach test” commenced (Hamilton et al., 2017; Johnson and Hamilton, 2017; Leighton et
125 al., 2018). To prevent zebrafish preference to single colours (Avdesh et al., 2012), the figurine
126 was multi-coloured (Supp. Fig. 1). Fish movement was tracked for 10 minutes. Water was
127 changed after every five fish to maintain temperature between 26-29°C and prevent the build-up
128 of waste products. Fish behavioural responses were not significantly different among fish tested
129 in the same arena water (see Suppl. Fig. 2), ruling out potentially confounding effects of conspe-
130 cific or ‘alarm cues’. Light levels in the testing room were 28 cd/m² (measured with a cal Spot

131 photometer; Cooke Corp. CA, USA) lighting was provided with incandescent lamps above and
132 beside the arena.

133

134 **2.4 Quantification of Behaviour**

135 Zebrafish movement was recorded using the differencing method in EthoVision XT motion
136 tracking software (version 11, Noldus, Leesburg, VA, USA) (Hamilton et al., 2017b; Pham et al.,
137 2009). For both the open field and novel object approach test, the arena was divided into three
138 zones: inner (the centremost zone 0-12 cm in diameter), transition (in between the other two zones,
139 12-23 cm in diameter), and thigmotaxis (the outermost zone, 23-34 cm in diameter). Time in inner,
140 transition, and thigmotaxis zones, average velocity, and immobility were quantified for all fish
141 using EthoVision (Hamilton et al., 2017b). Heatmaps represent a coloured representation of the
142 location of the fish (n=15) during the full trial and were generated by combining all trials from an
143 experimental tank in the EthoVision program.

144

145 **2.5 Statistical Analysis**

146 Data were analysed using Graphpad Prism 9.0 (CA, USA). An alpha level of $p < 0.05$ and
147 95% confidence intervals were used for assessing statistical significance in all tests. All datasets
148 were assessed for normality using the D'Agostino and Pearson omnibus normality test. Data sets
149 that failed the normality test were normalized. Datasets with normal distribution were analyzed
150 using a nested one-way ANOVA with Tukey's multiple comparison post-hoc test. If the nested
151 ANOVA indicated that subgroups were significantly different the data sets were shown graphically
152 but removed from the analysis (Riley & Edwards, 1998)(only one subgroup fit this criteria, for

153 two parameters; velocity in the open field test and time immobile in the open field test 4,200A vs.
154 4,200B). Data for all subgroups is in Supplementary data.

155

156

157 **3 Results and Discussion**

158 **3.1 Open Field Test**

159 The open field test is used to assay general locomotion activity and anxiety levels (Prut et
160 al., 2003). Under water quality parameters common in zebrafish husbandry (see Supplementary
161 Data) and at ambient $p\text{CO}_2$, zebrafish in the open field test spend the majority of the time near
162 the wall of the arena (i.e. the thigmotaxic zone) and are considered to have intrinsically high anx-
163 iety levels, but are still amenable to modulation in both directions by extrinsic factors (Hamilton
164 et al., 2017a). In the current study, zebrafish acclimatized to control $p\text{CO}_2$ ($\sim 480 \mu\text{atm CO}_2$)
165 demonstrate the expected high anxiety level (Fig. 1a, and note the different y-axes range in 1b-
166 d). However, zebrafish acclimatized to $\sim 900 \mu\text{atm CO}_2$ spent significantly more time in the thig-
167 motaxis zone compared to controls (Fig. 1b; $F(3,6) = 10.40$, $P = 0.009$) which indicates increased
168 anxiety-like behaviour. Although the time in the transition zone and inner zone were not signifi-
169 cantly different from control (Fig. 1c; $F(3, 6) = 8.35$, $P = 0.015$, and Fig. 1d; $F(3, 6) = 4.87$, $P =$
170 0.048 , respectively) the trend was consistent with decreased anxiety. According to the GABA_AR
171 hypothesis, acclimatization to further elevated CO₂ levels should further alter the reversal poten-
172 tial of GABA_A receptors, giving these receptors less of a hyperpolarizing influence on neuronal
173 circuits (Heuer et al., 2016, Tresguerres and Hamilton, 2017). The behavioural outcome on anxi-
174 ety would be a further increase. However, zebrafish acclimatized to $\sim 2,200 \mu\text{atm CO}_2$ spent the
175 same amount of time in the inner, transition, and thigmotaxic zones (Fig. 1a-d), which implies
176 similar anxiety across groups. Moreover, zebrafish acclimatized to the highest $p\text{CO}_2$ of $\sim 4,200$

177 μatm spent the most time in the transition and inner zone and the least time in the thigmotaxic
178 zone amongst all $p\text{CO}_2$ levels (Fig. 1a-d), and therefore had the lowest levels of anxiety. These
179 responses are readily evident in the heat maps shown in Fig. 1a, which that denote the averaged
180 movement patterns of all fish from a given trial. The continuous movement of fish along the
181 edge of the arena appeared as a bright halo in warm colours, while fish immobility resulted in
182 bright red spots.

183 The non-linear response whereby anxiety-like behaviour was maximal at ~ 900 and mini-
184 mal at $\sim 4,200 \mu\text{atm CO}_2$ (Fig. 1b-d) suggests the involvement of other mechanisms in addition
185 to GABA_A receptors; for instance, K⁺ channels, or dopamine, glycine, or glutamate receptors. It
186 seems possible that neurons from zebrafish acclimatized to $\sim 900 \mu\text{atm CO}_2$ experience a combi-
187 nation of [HCO₃⁻], [Cl⁻], and V_m resulting in an excitatory depolarization reflected as increased
188 anxiety-like behaviour. Alternatively, compensation of altered excitability of GABA_{AR} during
189 exposure to $\sim 2,200 \mu\text{atm CO}_2$ could drive the system back to equilibrium, and overcompensation
190 during exposure to $\sim 4,200 \mu\text{atm CO}_2$ could drive the system beyond equilibrium and into an an-
191 xiolytic state.

192 Velocity was not different across treatment groups (Fig. 1e; $F(2,5) = 1.67$, $P = 0.279$), nor
193 was immobility (Fig. 1f; $F(2,5) = 1.152$, $P = 0.402$). This suggests that the difference in zone
194 preference (ie. more time in the thigmotaxic zone for $900 \mu\text{atm CO}_2$ and less for $4,200 \mu\text{atm}$
195 CO_2) was not due locomotion-induced causes. Furthermore, fish from the two replicate $4,200$
196 μatm tanks displayed statistically significant different velocity and immobility, yet they spent
197 comparable amount of time in the different areas of the arena. In addition, the differences in
198 these parameters disappeared during the novel object test (see next section).

199

200 3.2 Novel Object Approach Test

201 The novel object approach test is used to quantify “boldness behaviour” based on the ani-
202 mal’s tendency to explore a never-before-seen object placed into an open field arena (Toms et
203 al., 2010) (i.e. time spent in the inner zone where the object is located, Fig. 2a). This test has
204 been validated for zebrafish with drugs known to increase boldness in mammals, like ethanol
205 (Hamilton et al., 2017; Dean et al., 2021) and nicotine (Dean et al., 2020).

206 In the current study, CO₂ acclimatization did not induce any significant differences in
207 time spent in the thigmotaxis (Fig. 2b; $F(3,6) = 1.25$, $P = 0.372$), transition (Fig. 2c; $F(3,6) =$
208 1.31 , $P = 0.356$) or inner (Fig. 2d; $F(3,6) = 1.54$, $P = 0.207$) zones, but note that the variability is
209 high in the ~4,200 μatm group in Fig. 2d. Interestingly, zebrafish in the novel object approach
210 test moved at slower average velocity and their time spent immobile roughly doubled relative to
211 the open field test, regardless of the CO₂ acclimatizations (Fig. 2e, f). In addition, zebrafish ex-
212 posed to ~4,200 μatm tended to move slower than control zebrafish (Fig. 2e; $F(3,6) = 1.25$, $P =$
213 0.372), and there was a significantly greater time immobile for the 4,200 μatm compared to 900
214 μatm group (Fig. 2d; $F(3,6) = 5.06$, $P = 0.044$). These trends are qualitatively similar to those
215 from the open field test (compare Fig. 1e,f and Fig 2e,f). These behaviours during the novel ob-
216 ject approach test are readily evident in the heat maps shown in Fig. 2a, especially the bright red
217 spots that result from fish remaining immobile for long periods of time.

218 In summary, introduction of the novel object significantly affected zebrafish immobility
219 compared to the open field test, but location preference in the arena was not altered by the CO₂
220 acclimations. Interestingly, the mere action of placing a novel object in the centre of the arena
221 reversed all CO₂ acclimatization-dependent effects on zebrafish anxiety behaviour (based on the

222 open field test, Fig. 1a-d). Thus, we highlight how one change in a behavioural test can dramati-
223 cally alter the inferred outcome, and are therefore cautious about predicting how and if aquatic
224 acidification will affect the behaviour of fish in their natural environment, which is infinitely
225 more complex than a testing arena with a novel object in it.

226 The novel object approach test has been used to examine the effects of aquatic acidifica-
227 tion on fish boldness in only a few previous studies, which have all reported different outcomes:
228 decreased boldness (Jutfelt et al., 2013), no effect (Tix et al., 2016), or increased boldness (Ou et
229 al., 2015). These differences might be due to the specific $p\text{CO}_2$ levels that were tested, the rela-
230 tion between the preferred $p\text{CO}_2$ level of each fish species and the experimental $p\text{CO}_2$ levels, or
231 unknown stochastic variables. Placing our results in the broader field of aquatic acidification is
232 even less straightforward because most other previous studies have used diverse tests to estimate
233 boldness. These include the tendency of fish to swim away from their home reef (Munday et al.,
234 2010), the time spent in the inner circle within an open field test (Jarrold et al., 2018, Lau-
235 benstein et al., 2018), and the time that takes to leave a shelter (Jutfelt et al., 2013). In addition to
236 changes to boldness behaviour, these methods and parameters may reflect interactive and con-
237 founding effects of changes in locomotion, anxiety, and other unknown variables.

238 There has yet to be a consensus on the ecological consequences that will result from
239 aquatic acidification. Many studies on freshwater fish species have shown negative effects in-
240 cluding impaired growth, altered olfaction and feeding (reviewed in Hasler et al., 2018), and be-
241 haviour (Ou et al., 2015; Ikuta et al., 2003). However, other studies have found minimal or no
242 behavioural alterations (Vossen et al., 2016; Tix et al., 2017; Midway et al., 2017). As pointed
243 out by our study, these discrepancies might be explained by the different $p\text{CO}_2$ levels used in
244 each study and putative ensuing non-linear effects.

245 It is interesting to note that wild zebrafish inhabit streams with relatively high $p\text{CO}_2$ lev-
246 els (~4,000 μatm ; Sundin et al., 2019). Therefore, while our protocol allowed us to identify non-
247 linear effects of $p\text{CO}_2$ acclimation on zebrafish behaviour under controlled laboratory conditions,
248 they might not represent the responses of zebrafish in their natural hypercapnic environment. Im-
249 portantly, this situation applies to most other studies, and realizing these limitations will lead to
250 better design of experiments and downstream will further our understanding of the ecological
251 consequences of aquatic acidification. In addition, these results cast new questions about the ef-
252 fects of acid-base homeostasis and highlight the need for multiple levels of CO₂ exposures to
253 capture the potential non-linear effects.

254

255 **4 Conclusion**

256 Acclimatization to increasing $p\text{CO}_2$ levels had a non-linear effect on zebrafish anxiety-like behav-
257 iour but not on exploratory behaviour. The implication of this research is at least threefold: (1)
258 increased $p\text{CO}_2$ levels do not necessarily affect fish behaviour in an additive manner. This might
259 help explain some of the reported diverse responses of fish to aquatic acidification, and guide meta-
260 analyses that attempt to identify universal responses. (2) The type and design of behavioural test
261 is paramount to unravelling the impacts of aquatic acidification on fish behaviour, stressing the
262 need for standardizing behavioural tests in the field of aquatic acidification. (3) While behavioural
263 studies might detect effects of aquatic acidification on fish behaviour, establishing their relevance
264 to the real world is greatly complicated by habitat complexity, rate of $p\text{CO}_2$ increase, species-
265 specific $p\text{CO}_2$ adaptations, amongst multiple other parameters. Thus, we recommend a cautious
266 approach when extrapolating experimental results to predictions of environmental outcomes.

267

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270 bandry and aquarium maintenance. Thanks to Garfield Kwan for his design of the zebrafish in
271 the graphical abstract.

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276

277

278 **Figure Legends.**

279 **Fig 1.** Acclimatization to increasing $p\text{CO}_2$ causes non-linear behavioural alterations in the open
280 field test. (a) Group heatmaps showing a coloured representation of the location of the average of
281 the group of fish ($n=15$) over the trial (proportional to the time spent in each pixel). Average time
282 the fish spent in the thigmotaxis (b), transition (c), and inner (d) zones during the open field test.
283 Mobility was quantified by measuring velocity (e) and time spent immobile (f). Data are mean \pm
284 1 s.e.m. Significant differences between controls and aquatic acidification groups are indicated
285 by *($P<0.05$), **($P<0.01$). # denotes a significant difference between subgroups, identified by
286 nested one-way ANOVA.

287

288 **Fig 2.** Acclimatization to increasing $p\text{CO}_2$ only alters mobility in the novel object approach test.
289 (a) Group heatmaps showing a coloured representation of the location of the average of the group
290 of fish ($n=15$) over the trial (proportional to the time spent in each pixel), with an image of the
291 novel object where it was placed into the centre of the arena (standing vertically). Average time
292 the fish spent in the thigmotaxis (b), transition (c), and inner/object (d) zones during the novel
293 object approach test. Mobility was quantified by measuring velocity (e) and time spent immobile
294 (f). Data are mean \pm 1 s.e.m. Significant differences between controls and aquatic acidification
295 groups are indicated by *($P<0.05$).

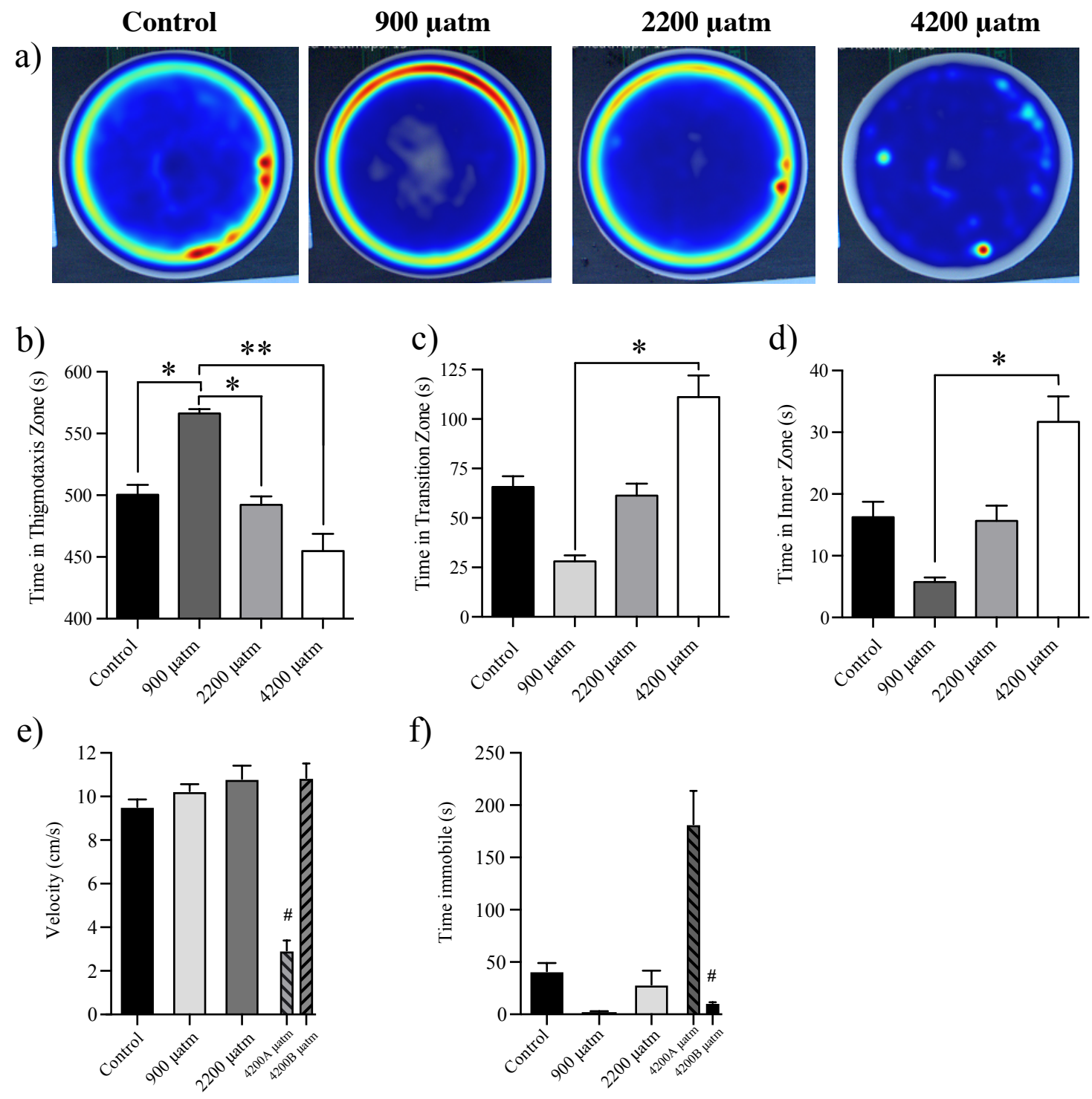
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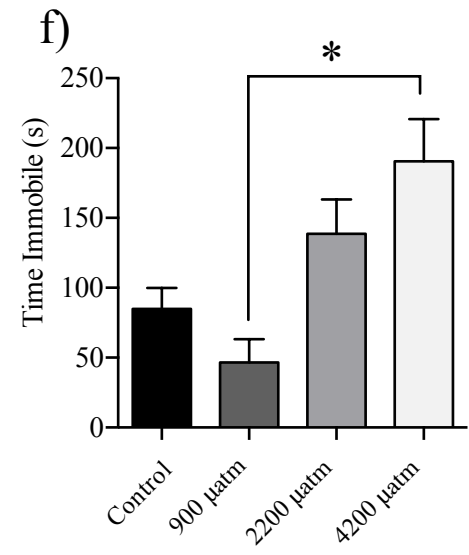
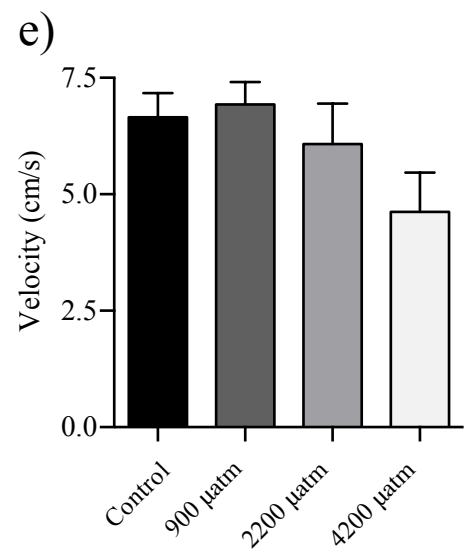
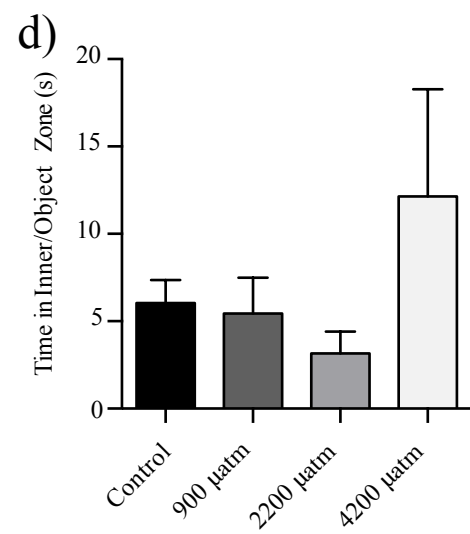
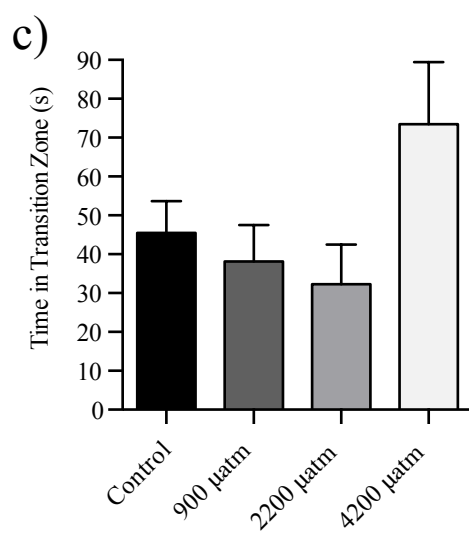
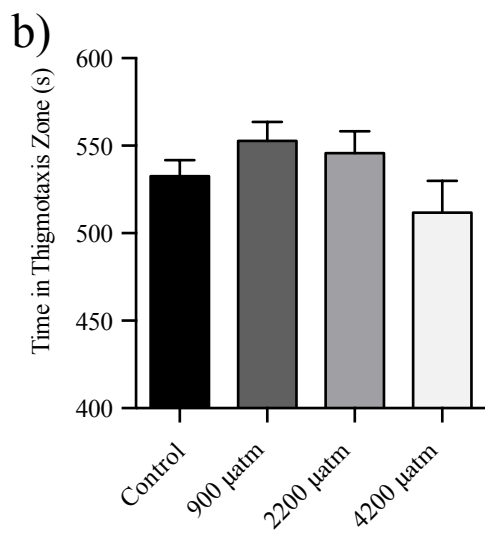
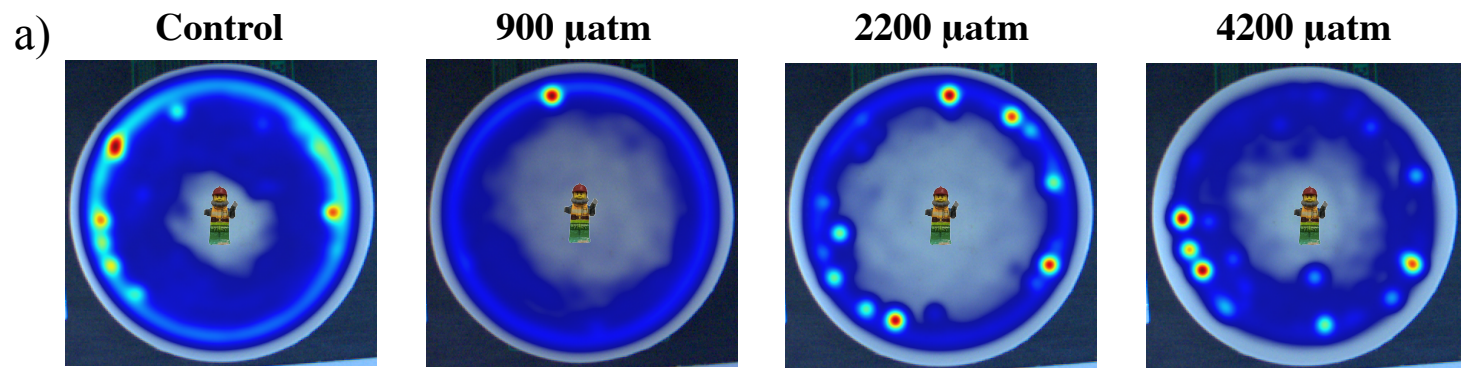
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Author Contributions Statement

Trevor J. Hamilton: Conceptualization, Methodology, Formal Analysis, Writing, Visualization, Supervision, Resources, Project administration, Funding acquisition. **Nicole Hurst Radke:** Methodology, Investigation, Formal Analysis, Writing – Original draft. **Jasmin Bajwa:** Methodology, Investigation, Formal Analysis. **Shayna Chaput:** Methodology, Investigation, Formal Analysis. **Martin Tresguerres:** Conceptualization, Methodology, Writing, Project administration.