

Zebrafish (*Danio rerio*) behaviour is largely unaffected by elevated pCO₂

Laura E. Vossen^{1,*}, Fredrik Jutfelt², Arianna Cocco¹, Per-Ove Thörnqvist¹ and Svante Winberg¹

¹Uppsala University, Department of Neuroscience, Husårgatan 3, 75124 Uppsala, Sweden

²Norwegian University of Science and Technology, Department of Biology, Høgskoleringen 5, Realfagbygget, Trondheim, Norway

*Corresponding author: Uppsala University, Department of Neuroscience, Husårgatan 3, 75124 Uppsala, Sweden. Tel: +46-184714611. Email: laura.vossen@neuro.uu.se

Ocean acidification, the decrease in ocean pH caused by anthropogenic emission of carbon dioxide, can cause behavioural disturbances in marine teleost species. We investigated whether AB-strain zebrafish (*Danio rerio*) show similar behavioural disturbances in the presence of elevated CO₂, because this model species could open up a toolbox to investigate the physiological and neurological mechanisms of CO₂ exposure. We found no effect of elevated CO₂ (~1600 µatm) on the behaviour of zebrafish in the open field test, indicating that zebrafish are largely insensitive to this elevated CO₂ level. In the detour test of lateralization, however, zebrafish exposed to elevated CO₂ swam more often to the right, whereas individuals exposed to control CO₂ (~400 µatm) had no preference for left or right. This may indicate that some behaviours of some freshwater fishes can be altered by elevated CO₂ levels. Given that elevated CO₂ levels often occur in recirculating aquaculture and aquarium systems, we recommend that dissolved CO₂ levels are measured and, if necessary, the aquarium water should be aerated, in order to exclude CO₂ level as a confounding factor in experiments.

Key words: Behavioural lateralization, detour, gabazine, ocean acidification, open field, zebrafish

Editor: Steven Cooke

Received 27 April 2016; Revised 4 November 2016; Editorial Decision 14 November 2016; Accepted 17 November 2016

Cite as: Vossen LE, Jutfelt F, Cocco A, Thörnqvist P-O, Winberg S (2016) Zebrafish (*Danio rerio*) behaviour is largely unaffected by elevated pCO₂. *Conserv Physiol* 4(1): cow065; doi:10.1093/conphys/cow065.

Introduction

Anthropogenic emissions of carbon dioxide are acidifying the world's water bodies. It has been estimated that ocean pH has already dropped 0.1 pH unit since the onset of the industrial revolution, and a further decrease in ocean pH of 0.2–0.3 pH units is expected by the end of the century (Haugan and Drange, 1996; Rhein *et al.*, 2013). Recent experiments on marine teleost fish reveal alarming behavioural impairments upon exposure to near-future CO₂ levels, including the impairment of olfactory, visual and auditory responses, increased activity, increased boldness, reduced learning and changes in behavioural lateralization (reviewed by Munday *et al.*, 2009; Briffa *et al.*, 2012; Clements and Hunt, 2015). Also, 'CO₂-resilient'

species have been reported. In Atlantic cod (*Gadus morhua*), larval swimming kinematics are unaffected and adults strongly avoid CO₂ and predator odour despite long-term CO₂ exposure (Maneja *et al.*, 2012; Jutfelt and Hedgärde, 2013). The hypothesized mechanism causing these behavioural disturbances is that the major inhibitory neurotransmitter, γ -aminobutyric acid (GABA), reverses its function in the presence of an elevated partial pressure of carbon dioxide (pCO₂; Nilsson *et al.*, 2012). To avoid acidosis, fish take up bicarbonate ions (HCO₃⁻) into the gill cells and extrude chloride ions (Cl⁻) into the water (Brauner and Baker, 2009). This alters the plasma ion concentrations, which in turn can affect the electrochemical gradients over the cell membranes. Therefore, when GABA binds to the GABA_A receptor, the reduced extracellular

Cl^- concentrations may reduce the electrochemical gradient for Cl^- influx, while the increased intracellular HCO_3^- concentrations may lead to efflux of these anions and cause depolarization rather than the normal hyperpolarization. In accordance with the ‘GABA hypothesis’, treatment with the GABA_A receptor antagonist gabazine restored the behaviour of CO_2 -exposed fish in the majority of studies (Nilsson *et al.*, 2012; Chivers *et al.*, 2013; Chung *et al.*, 2014; Lai *et al.*, 2015).

Whether freshwater fish are affected by elevated pCO_2 via the same mechanism has not yet been sufficiently investigated (Leduc *et al.*, 2013; Ou *et al.*, 2015). Freshwater pH and pCO_2 vary more over space and time (e.g. because of heavy rainfall or snow melting, biological respiration, lower buffering capacity) and therefore it has been argued that freshwater fish might have evolved greater tolerance to pH fluctuations (Ishimatsu *et al.*, 2005; Leduc *et al.*, 2013; Heuer and Grosell, 2014). In addition, freshwater fish have very robust NaCl uptake mechanisms for ionoregulation, which are linked to H^+ and HCO_3^- secretion. However, even in freshwater fish hypercapnic acidosis may be linked to uptake of water HCO_3^- in exchange for Cl^- , because in freshwater-raised salmon smolts elevated CO_2 results in lower plasma Cl^- concentrations (Fivelstad *et al.*, 2003a). Thus, elevated pCO_2 may potentially impair GABA functioning through the same mechanism as in marine fish. Ultimately, rising freshwater pCO_2 could potentially threaten freshwater fish populations in the same way as in marine ecosystems (Munday *et al.*, 2010); however, much less is known about future changes in freshwater pCO_2 compared with predictions for ocean pCO_2 (Hasler *et al.*, 2016).

To investigate whether elevated CO_2 can also affect the GABA system of a freshwater fish species, we exposed adult zebrafish (*Danio rerio*) to control (~400 μatm) or elevated CO_2

(~1600 μatm). We chose zebrafish for the following three reasons. Firstly, it is a widely used model organism in genetics and neuroscience, and this species would therefore open up a toolbox to investigate the neural, cellular and molecular mechanisms altered by CO_2 (Briggs, 2002; Norton and Bally-Cuif, 2010). Secondly, the natural habitat of the zebrafish consists of slow-moving pools and rice paddies, with reported pH as low as 5.9 and as high as 8.1 (Engeszer *et al.*, 2007), environments where pCO_2 can reach high levels. If this species familiar with high CO_2 concentrations is affected, then this will probably also be the case for many more freshwater fish. Thirdly, laboratory strains of zebrafish are usually housed in recirculating rack systems, in which CO_2 concentrations can quickly rise because of respiration of the fish or microbial activity in tanks and filters. We have included a set of CO_2 measurements taken in seven independent recirculating aquatic housing systems holding zebrafish, one of which measured 1200 μatm (Table 1). The CO_2 level might therefore constitute an unexplained factor in laboratory experiments with zebrafish. In larger recirculating tank systems, such as those used in aquaculture, dissolved CO_2 can even reach levels up to 10 000 μatm (Fivelstad *et al.*, 2003b).

The pCO_2 level used in this study (1600 μatm) was chosen for being higher than what zebrafish are likely to experience in the laboratory or in the field (Engeszer *et al.*, 2007), while not exceeding the maximum of 2000 μatm predicted by current models for long-term ocean pCO_2 (Zickfeld *et al.*, 2013). The open field test used in this study is a widely used test to investigate exploratory behaviour and anxiety as well as activity level (Blaser and Gerlai, 2006; Grossman *et al.*, 2010; Maximino *et al.*, 2010; Stewart *et al.*, 2010), behaviours which have been reported to be affected in the presence of elevated CO_2 levels (Munday *et al.*, 2010, 2013; Cripps *et al.*, 2011). The detour test was chosen because it provides a test of brain function for different decision-making tasks (Vallortigara and Rogers,

Table 1: Survey of fish density, pCO_2 , pH, temperature and conductivity in seven independent zebrafish aquatic housing systems at four different anonymous biomedical facilities in Sweden

Aquatic housing system	Manufacturer	Fish density in tank	pCO_2 (μatm)	pH	Temperature ($^{\circ}\text{C}$)	Conductivity ($\mu\text{S cm}^{-1}$)
1	Aquaneering	12 in 9 litres	470	8.84	27.0	422
2	Tecniplast	30 in 9 litres	450	7.41	27.8	495
3	Aquaneering	30 in 9 litres	720	7.20	28.0	600
4	Aqua medic T 2001 HC	20 in 3 litres	830	7.20	28.0	874
5	Pentair aquatic ecosystems	61 in 10 litres	720	7.8	28.2	n.a.
6	Aquatic habitats (duo system)	5 in 3 litres	730	8.0	27.8	n.a.
7	Aquatic habitats (standalone)	149 in 10 litres	1200	7.5	26.0	n.a.
7	Aquatic habitats (standalone)	3 in 3 litres	820	7.5	26.0	n.a.

Abbreviations: n.a., not assessed; and pCO_2 , partial pressure of carbon dioxide.

2005). Disruptions in behavioural lateralization have already been shown for the coral reef fishes *Neopomacentrus azysron* (Domenici *et al.*, 2012) and *Pomacentrus wardi* (Domenici *et al.*, 2014), as well as for the temperate species the three-spined stickleback (*Gasterosteus aculeatus*; Jutfelt *et al.*, 2013; Lai *et al.*, 2015). Behavioural lateralization was unaffected by CO₂ exposure in temperate wrasse (*Ctenolabrus rupestris*; Sundin and Jutfelt, 2015).

The present study investigated whether zebrafish of the AB strain show abnormal behaviour in open field and detour tests when exposed to elevated pCO₂. Furthermore, we explored whether any behavioural disturbances could be reversed by treatment with the specific GABA_A receptor antagonist, gabazine.

Materials and methods

Experimental animals

Rearing, handling and experimental procedures were approved by the ethical committee on animal experiments of Uppsala, Sweden (ethical permit: C 55/13 to S.W.). All fish used in this experiment were adult wild-type (AB) zebrafish. They were ordered as eggs from ZIRC (University of Oregon, USA), bred at the Evolutionary Biology Center (Uppsala University, Uppsala, Sweden) and held at the Department of Neuroscience (Uppsala University) for at least 6 months before the start of the experiment. Fish were kept at 28°C with a 14 h–10 h light–dark photoperiod. Water quality was monitored for alkalinity (mean ± SD, 2.03 ± 0.09 mequiv l⁻¹), total hardness (80 ppm), conductivity (mean ± SD, 426.01 ± 2.73 μS cm⁻¹), nitrite (<1 mg l⁻¹), nitrate (<10 mg l⁻¹) and ammonium (mean ± SD, 0.15 ± 0.13 mg l⁻¹). Fish were fed *ad libitum* once a day in the morning with flakes (Serasan flakes for tropical fish) and live *Artemia* nauplii.

Experimental treatments

The experiment took place from February to April 2014. At the start of the experiment, a total of 120 adult fish were equally distributed over four 25 litre aquaria; 15 males and 15 females per tank. Two aquaria were connected to a header tank that was bubbled with air (control exposure), while the other two aquaria received water from a header tank that was bubbled with 100% CO₂ gas using a solenoid valve controlled by a pH stat computer such that pCO₂ was maintained at a target value of 1600 (Aqua Medic, Bissendorf, Germany). We realize that our experimental set-up would have benefited from the use of several header tanks per CO₂ treatment (Riebesell *et al.*, 2010; Moran, 2014). In all four aquaria, pCO₂ was measured daily using an infrared dissolved CO₂ meter (Qubit, Kingston, ON, Canada). The pCO₂ of the control aquaria was 420.5 ± 57.3 μatm (mean ± SD) and the pCO₂ of the elevated-CO₂ aquaria was 1610.7 ± 277.8 μatm (mean ± SD). The pH was 8.31 ± 0.04 (mean ± SD; control pCO₂) and 7.58 ± 0.09 (mean ± SD;

elevated pCO₂). The fish were kept in these exposure aquaria for 26–48 days to allow for potential acclimation. This is a longer duration than the exposure used for fish in many other studies, and larval clownfish (*Amphiprion percula*) show impairments already after CO₂ exposure for 1 day (Munday *et al.*, 2010).

On the day of behavioural testing, zebrafish from each exposure tank were randomly assigned to either an individual immersion treatment with the specific GABA_A receptor antagonist gabazine (4 mg l⁻¹ in 4 litres of tank water for 30 min at 27°C) or control treatment (50 ml tap water in 4 litres of tank water for 30 min at 27°C). Immersion treatment tanks were prepared fresh on the morning of every testing day from cooled (4°C) stock solutions of gabazine and tap water (the ‘control stock’, cooled and thawed in the same way as the gabazine stock solution). Each immersion treatment tank was used by two or three individual fish during the testing day. After the behavioural tests (see next subsection), animals were euthanized in a benzocaine solution (500 mg l⁻¹ buffered to pH 7.5) with ice, the spine was cut at the neck, and the gonads were dissected to confirm the sex of the individual.

Behavioural tests

Fish were first tested in the open field test (118 individuals, 30 min) and subsequently in the detour test (68 individuals). Testing aquaria contained water of control pCO₂, and tests were carried out within 3 h after catching from the home tank, long before fish recover from elevated pCO₂ (Munday *et al.*, 2010). The open field arenas were rectangular plastic tanks (30 cm width × 35 cm length) with white opaque walls, filled with 4 litres of water, resulting in 4 cm of water depth. An infrared light board (Noldus, Wageningen, The Netherlands) was placed under the arenas, and an overhead infrared camera (JVC SuperLoLux, Yokohoma, Japan) attached to a computer recorded fish activity. The testing room was sound and light proof and had ambient symmetrical lighting. Films were analysed with the automatic tracking software EthoVision XT10 (Noldus) at 25 frames s⁻¹, collecting the following activity data: swimming speed; total distance moved; movement (duration moving/not moving); and mobility state (duration mobile, highly mobile and immobile; Grieco *et al.*, 2010). The swimming speeds were recalculated into relative speeds by dividing the speed (in centimetres per second) by the individual’s total length measurement. In order to quantify thigmotaxis (‘wall-hugging’), the arena was divided into a wall zone (defined as the outermost 4 cm of the arena) and an inner zone (the rest, or centre of the arena; Gerlai *et al.*, 2000; Maximino *et al.*, 2010). To quantify the number of home bases, the function ‘Heatmap Visualization’ inside Ethovision XT10 was applied to each individual trial, and the number of home bases were counted as the number of red and/or dark red areas in the arena, which are indicative of a long presence of the subject’s centre point in that area (Stewart *et al.*, 2010).

In the detour test, a double T-chamber was used to evaluate the effect of elevated CO₂ on behavioural lateralization (see Fig. 1; after Jutfelt *et al.*, 2013). Most zebrafish readily swim through a double T-chamber from side to side, i.e. without any encouragement from researchers. If an individual had not made the first five choices within 5 min, the trial was discarded (an equal number of trials was discarded from control and elevated pCO₂; binomial test for equality of proportions, $\chi^2 = 0.605$, $P = 0.437$). Individuals were introduced into the chamber in the middle of one long arm, and the first left or right turn was recorded as soon as they went more than half-way through the runway and then chose a left or right arm. Each individual's turning preference was measured 20 times post testing from video recordings. For easier comparison with previous studies, we calculated the relative lateralization index (L_R), which reflects the preference to turn either left or right, for each fish as follows: [(number of right turns – number of left turns)/(number of right turns + number of left turns)] × 100. The absolute lateralization index (L_A), which reflects the strength of any possible side bias regardless of this bias being to the left or the right, was calculated as the absolute value of the relative lateralization index (Bisazza *et al.*, 1998).

Measurements of pCO₂ in zebrafish recirculating tank systems

The pCO₂ was measured using an infrared dissolved CO₂ meter (Qubit Biology Inc., Canada) inside holding tanks in seven independent zebrafish housing systems in four different biomedical laboratories (Table 1). Temperature, pH and conductivity were read off from housing system displays. For one housing system (number 7), two pCO₂ measurements were performed in two different holding tanks, one with low and one with high fish density.

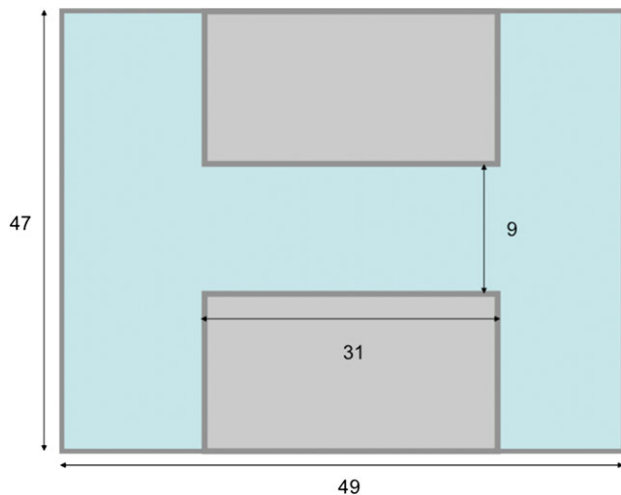


Figure 1: Schematic representation of the double T-chamber used for the detour tests of lateralization. Measurements are in centimetres. The figure is not drawn to scale.

Statistical analyses

Statistical analyses were performed using R language and environment for statistical computing and graphics, version 3.2.3 (R Development Core Team, 2013) and the R package ‘lme4’ (Bates *et al.*, 2015; see also the Supplementary material).

Results

Open field test

Carbon dioxide exposure did not affect any of the activity variables [generalized linear mixed effects models (GLMMs), $P > 0.05$; Table S1]. There was a significant effect of sex [linear mixed effects model (LMM), $F_{1,113} = 17.686$, $P < 0.001$] and gabazine treatment (LMM, $F_{1,105} = 5.763$, $P = 0.018$), as well as a sex-by-treatment interaction (LMM, $F_{1,108} = 5.561$, $P = 0.020$) on the swimming speed (in fish lengths per second; Fig. 2 and Table S3) and distance moved in the open field test. Males swam faster than females in the gabazine treatments (gabazine/control pCO₂ and gabazine/elevated pCO₂; *post hoc t-test*, $t = -4.6$, $P < 0.001$) than in the drug control treatments (no gabazine/control pCO₂ and no gabazine/elevated pCO₂; *post hoc t-test*, $t = -1.38$, $P = 0.169$). Males were immobile for a shorter duration of time in the gabazine treatments (*post hoc t-test*, $t = 3.44$, $P < 0.001$). The durations of time moving, not moving, mobile and highly mobile were not affected by any of the explanatory variables (GLMMs, $P > 0.05$). Gabazine treatment increased thigmotaxis (GLMM with binomial errors, Wald $\chi^2 = 5.288$, $P = 0.021$; Table S4), whereas sex and CO₂ exposure level had no effect on thigmotaxis ($P > 0.2$). The number of home bases was not influenced by CO₂ exposure, drug treatment or sex (generalized linear model with Poisson error distribution, $P > 0.05$).

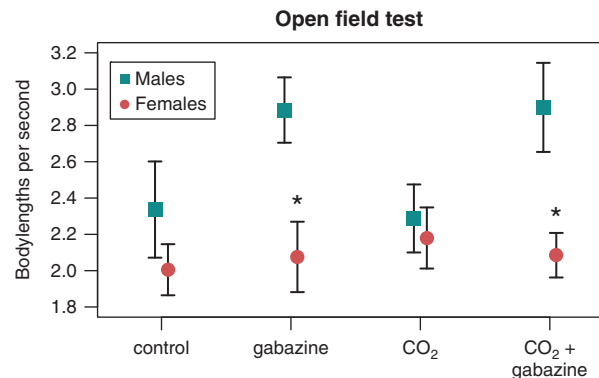


Figure 2: Relative swimming speed (mean ± SEM; in body lengths per second) in the open field test during the 30 min trial of male (blue squares) and female (red circles) zebrafish exposed to control pCO₂ (~400 μatm; ‘control’), control pCO₂ and gabazine (‘gabazine’), elevated pCO₂ (~1600 μatm; ‘CO₂’) and elevated pCO₂ and gabazine (‘CO₂ + gabazine’) for an average of 37 days. Asterisks indicate the significant increase in male swimming speed in response to gabazine treatment ($P < 0.001$).

Detour test

Carbon dioxide exposure significantly increased the proportion of right turns in the detour test (GLMM with binomial errors, Wald $\chi^2 = 8.1697$, $P = 0.0043$; Tables S2 and S5), whereas gabazine treatment and sex did not influence the proportion of right turns. The relative lateralization index (L_R) was 5.44 ± 4.08 in control CO_2 and 22.94 ± 4.81 in elevated CO_2 (mean \pm SEM; Fig. 3). The absolute lateralization index (L_A) was 16.91 ± 2.99 in control CO_2 and 28.24 ± 3.86 in elevated CO_2 (mean \pm SEM; Fig. 4).

Discussion

Exposure to elevated levels of carbon dioxide ($1611 \mu\text{atm}$) did not alter the behaviour of zebrafish in the open field test, which may indicate that adult zebrafish are mostly insensitive to this pCO_2 . Nonetheless, zebrafish behaviour might be altered by even higher levels of CO_2 or in other life stages, warranting further investigation. Gabazine treatment affected male and female behaviour differently in the open field test. Males became significantly more active after gabazine treatment, whereas no effect of gabazine on the activity of female zebrafish was detected. Sex differences in the dynamic pattern of hyperactivity have also been observed in response to the partial inverse GABA_A receptor agonist FG-7142 (López-Patiño *et al.*, 2008). Ou *et al.* (2015) studied the effects of CO_2 -induced acidification in freshwater-reared pink salmon and found that fish reared at $2000 \mu\text{atm}$ CO_2 displayed less thigmotaxis than control fish and that gabazine treatment increased thigmotaxis. The present study verified the positive effect of gabazine on thigmotaxis; however, we did not detect an effect of $1611 \mu\text{atm}$ pCO_2 on

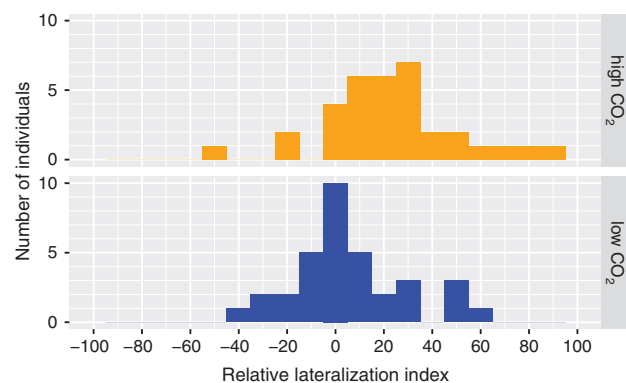


Figure 3: The relative lateralization index (L_R , a measure that reflects the preference to turn either left or right) of zebrafish exposed to control pCO_2 ($\sim 400 \mu\text{atm}$) and elevated pCO_2 ($\sim 1600 \mu\text{atm}$). Frequency distributions of the number of fish with each L_R are shown. An L_R of -100 indicates all left turns and no right turns, an L_R of 0 corresponds to an equal number of left and right turns, and an L_R of 100 indicates that all turns were to the right. Carbon dioxide exposure significantly increased the proportion of right turns.

thigmotaxis despite reasonable sample size, which suggests that this behaviour is robust to this particular pCO_2 in zebrafish.

Elevated pCO_2 did have an effect on the behaviour of both male and female zebrafish in the detour test. Zebrafish exposed to elevated pCO_2 turned more often to the right compared with zebrafish from control pCO_2 , which did not have a turning bias. Zebrafish possess a pattern of lateralization typical for tetrapods, where right frontal retinal fixation is associated with a period in which response (in particular

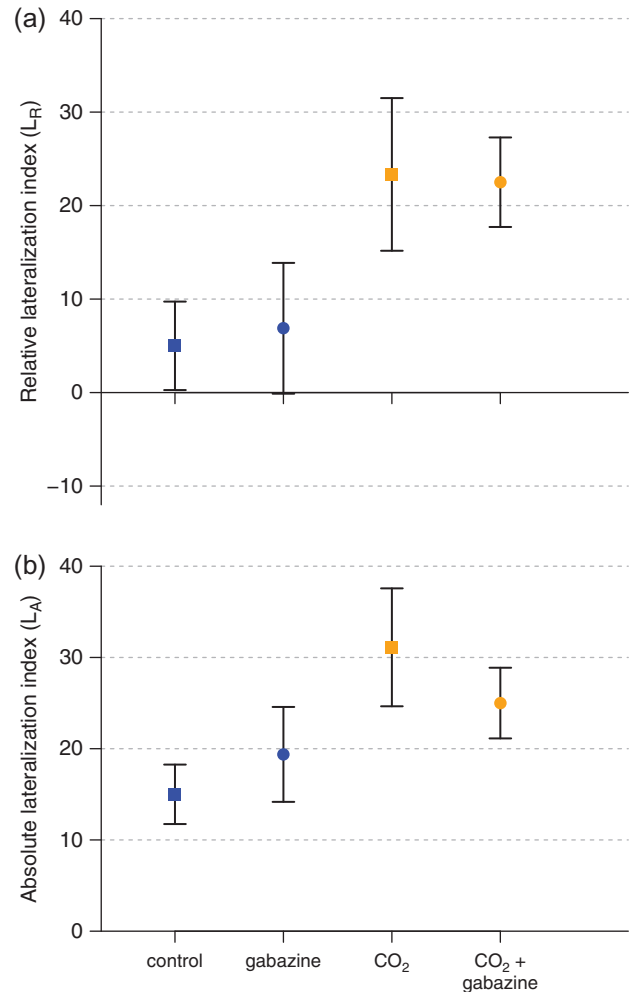


Figure 4: Mean (\pm SEM) relative (a) and absolute (b) lateralization index in the detour test for adult zebrafish exposed to control pCO_2 ($\sim 400 \mu\text{atm}$; 'control', blue squares), control pCO_2 and gabazine ('gabazine', blue circles), elevated pCO_2 ($\sim 1600 \mu\text{atm}$; ' CO_2 ', orange squares) and elevated pCO_2 and gabazine (' CO_2 + gabazine', orange circles) for an average of 37 days. The relative lateralization index (L_R) reflects the preference to turn either left (negative L_R) or right (positive L_R), whereas a higher absolute lateralization index indicates a stronger side preference (irrespective of the left or the right side). Carbon dioxide exposure significantly increased the proportion of right turns.

escape) has to be inhibited until a decision has been taken about the nature of what is being viewed, such as when searching for a hidden predator (Miklósi *et al.*, 1997; Facchin *et al.*, 1999; Miklósi and Andrew, 2006). The left eye system is used to assess whether an object is novel or not in low-risk situations (Miklósi and Andrew, 2006). In our experimental chamber, the CO₂-exposed zebrafish that swam more often to the right might have been driven more by stimuli from their right frontal field, because there was no stimulus in front of the runway. After they inspected the right side with the right eye and established that it did not contain a threat, they swam into the right arm. However, the fish from control CO₂ showed no side preference and were therefore probably using both eyes to an equal extent. The greater use of the right eye system could therefore mean that CO₂-exposed fish were in a higher state of alertness than control zebrafish.

Elevated pCO₂ can be an indicator of poor water quality, which is stressful to fish (Huntingford *et al.*, 2006). Zebrafish can sense small changes in pCO₂ in the environment via neuro-epithelial cells located in the gills (Qin *et al.*, 2010), and adult zebrafish also increase their ventilation amplitude in response to a pCO₂ of 1 mmHg (~1300 µatm; Vulesevic, 2006). In future experiments, it would be interesting to measure cortisol concentrations in response to CO₂ exposure. The response cannot be explained by altered GABA function from the chronic effects of CO₂, as suggested in marine fishes (Nilsson *et al.*, 2012), because gabazine treatment did not affect lateralization, and the elevated CO₂/gabazine group did not show restored lateralization behaviour.

Disturbances in behavioural lateralization might hamper an individual's schooling ability, which is important for survival in the wild (Bisazza and Dadda, 2005). Several studies have investigated behavioural lateralization in the context of ocean acidification, with somewhat differing results, as discussed by Sundin and Jutfelt (2015). Our results perhaps best resemble those of Welch *et al.* (2014), who also reported an increase in the number of right turns. However, their juvenile damselfish shifted from a left bias in control pCO₂ to equal numbers of left- and right-biased fish in elevated pCO₂, rather than from no bias to a right bias in the presence of elevated pCO₂ (our study).

In recirculating tank systems, CO₂ levels can quickly rise as a result of respiration by fish and micro-organisms and the high solubility of this gas in water. We performed pCO₂ measurements in seven independent zebrafish housing systems (Table 1) and found that five had a pCO₂ >700 µatm. The tank with the highest pCO₂ level (1200 µatm) also had the highest density of fish. We therefore advise researchers to measure dissolved CO₂ levels in their tank systems and to aerate tanks when the relative biomass is high. Some species might be more tolerant to CO₂ than others; therefore, more research is needed to establish the exact upper limit of pCO₂ in the zebrafish.

Owing to a behavioural shift in lateralization in high pCO₂, zebrafish could be considered as a model species in the

context of aquatic acidification research. We do not yet know whether the mechanisms behind the behavioural effects seen here are similar to the mechanisms in marine fishes. Other behavioural tests could be performed on zebrafish in elevated pCO₂ to verify the results of the detour test. In addition, a better understanding is needed of the mechanisms regulating freshwater pCO₂ and how freshwater pCO₂ responds to climate change (Hasler *et al.*, 2016).

Supplementary material

Supplementary material is available at *Conservation Physiology* online.

Acknowledgements

The authors would like to thank Professor Bryndis Birnir, Professor Erik Petersson, Abdul Alim and the anonymous caretakers at the zebrafish facilities.

Funding

The authors were supported by grants from the Swedish Research Council (VR 621-2012-4679 to S.W.) and the Swedish Research Council Formas (2009-596 to F.J.).

References

- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67: 1–48.
- Bisazza A, Dadda M (2005) Enhanced schooling performance in lateralized fishes. *Proc Biol Sci* 272: 1677–1681.
- Bisazza A, Facchin L, Pignatti R, Vallortigara G (1998) Lateralization of detour behaviour in poeciliid fish: the effect of species, gender and sexual motivation. *Behav Brain Res* 91: 157–164.
- Blaser R, Gerlai R (2006) Behavioral phenotyping in zebrafish: comparison of three behavioral quantification methods. *Behav Res Methods* 38: 456–469.
- Brauner CJ, Baker DW (2009) Patterns of acid–base regulation during exposure to hypercarbia in fishes. In ML Glass, SC Wood, eds, *Cardio-respiratory Control in Vertebrates*. Springer, Berlin, Heidelberg, pp 1–21.
- Briffa M, de la Haye K, Munday PL (2012) High CO₂ and marine animal behaviour: potential mechanisms and ecological consequences. *Mar Pollut Bull* 64: 1519–1528.
- Briggs JP (2002) The zebrafish: a new model organism for integrative physiology. *Am J Physiol Regul Integr Comp Physiol* 282: R3–R9.
- Chivers DP, McCormick MI, Nilsson GE, Munday PL, Watson SA, Meekan MG, Mitchell MD, Corkill KC, Ferrari MC (2013) Impaired learning of predators and lower prey survival under elevated CO₂: a consequence of neurotransmitter interference. *Glob Chang Biol* 20: 515–522.

- Chung WS, Marshall NJ, Watson SA, Munday PL, Nilsson GE (2014) Ocean acidification slows retinal function in a damselfish through interference with GABA_A receptors. *J Exp Biol* 217: 323–326.
- Clements JC, Hunt HL (2015) Marine animal behaviour in a high CO₂ ocean. *Mar Ecol Prog Ser* 536: 259–279.
- Cripps IL, Munday PL, McCormick MI (2011) Ocean acidification affects prey detection by a predatory reef fish. *PLoS One* 6: e22736.
- Domenici P, Allan B, McCormick MI, Munday PL (2012) Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. *Biol Lett* 8: 78–81.
- Domenici P, Allan B, Watson SA, McCormick MI, Munday PL, Osorio D (2014) Shifting from right to left: the combined effect of elevated CO₂ and temperature on behavioural lateralization in a coral reef fish. *PLoS One* 9: e87969.
- Engeszer RE, Patterson LB, Rao AA, Parichy DM (2007) Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish* 4: 21–40.
- Facchin L, Bisazza A, Vallortigara G (1999) What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behav Brain Res* 103: 229–234.
- Fivelstad S, Olsen AB, Åsgård T, Baeverfjord G, Rasmussen T, Vindheim T, Stefansson S (2003a) Long-term sublethal effects of carbon dioxide on Atlantic salmon smolts (*Salmo salar* L.): ion regulation, haematology, element composition, nephrocalcinosis and growth parameters. *Aquaculture* 215: 301–319.
- Fivelstad S, Waagbø R, Zeitz SF, Hosfeld A, Olsen AB, Stefansson S (2003b) A major water quality problem in smolt farms: combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (*Salmo salar* L.) smolts: physiology and growth. *Aquaculture* 215: 339–357.
- Gerlai R, Lahav M, Guo S, Rosenthal A (2000) Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacol Biochem Behav* 67: 773–782.
- Grieco F, Loijens L, Krips OE, Smit G, Spink AJ, Zimmerman P (2010) *EthoVision XT Reference Manual*. Noldus Information Technology, Wageningen.
- Grossman L, Utterback E, Stewart A, Gaikwad S, Chung KM, Suci C, Wong K, Elegante M, Elkhayat S, Tan J *et al.* (2010) Characterization of behavioral and endocrine effects of LSD on zebrafish. *Behav Brain Res* 214: 277–284.
- Hasler CT, Butman D, Jeffrey JD, Suski CD. (2016) Freshwater biota and rising pCO₂? *Ecol Lett* 19: 98–108.
- Haugan PM, Drange H (1996) Effects of CO₂ on the ocean environment. *Energy Convers Manag* 37: 1019–1022.
- Heuer RM, Grosell M (2014) Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *Am J Physiol Regul Integr Comp Physiol* 307: R1061–R1084.
- Huntingford FA, Adams C, Braithwaite VA, Kadri S, Pottinger TG, Sandoe P, Turnbull JF (2006) Current issues in fish welfare. *J Fish Biol* 68: 332–372.
- Ishimatsu A, Hayashi M, Lee K-S (2005) Physiological effects on fishes in a high-CO₂ world. *J Geophys Res* 110: C09S09.
- Jutfelt F, Hedgärde M (2013) Atlantic cod actively avoid CO₂ and predator odour, even after long-term CO₂ exposure. *Front Zool* 10: 81–88.
- Jutfelt F, Bresolin de Souza K, Vuylsteke A, Sturve J (2013) Behavioural disturbances in a temperate fish exposed to sustained high-CO₂ levels. *PLoS One* 8: e65825.
- Lai F, Jutfelt F, Nilsson GE (2015) Altered neurotransmitter function in CO₂-exposed stickleback (*Gasterosteus aculeatus*): a temperate model species for ocean acidification research. *Conserv Physiol* 3: cov018; doi:10.1093/conphys/cov018.
- Leduc AO, Munday PL, Brown GE, Ferrari MC (2013) Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. *Philos Trans R Soc Lond B Biol Sci* 368: 20120447.
- López-Patiño MA, Yu L, Cabral H, Zhdanova IV (2008) Anxiogenic effects of cocaine withdrawal in zebrafish. *Physiol Behav* 93: 160–171.
- Maneja RH, Frommel AY, Browman HI, Clemmesen C, Geffen AJ, Folkvord A, Piatkowski U, Durif C, Bjelland R, Skiftesvik AB (2012) The swimming kinematics of larval Atlantic cod, *Gadus morhua* L., are resilient to elevated seawater pCO₂. *Mar Biol* 160: 1963–1972.
- Maximino C, de Brito TM, da Silva Batista AW, Herculano AM, Morato S, Gouveia A Jr (2010) Measuring anxiety in zebrafish: a critical review. *Behav Brain Res* 214: 157–171.
- Miklósi A, Andrew RJ (2006) The zebrafish as a model for behavioral studies. *Zebrafish* 3: 227–234.
- Miklósi A, Andrew RJ, Savage H (1997) Behavioural lateralisation of the tetrapod type in the zebrafish (*Branchydanio rerio*). *Physiol Behav* 63: 127–135.
- Moran D (2014) The importance of accurate CO₂ dosing and measurement in ocean acidification studies. *J Exp Biol* 217: 1827–1828.
- Munday PL, Dixon DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV, Døving KB (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc Natl Acad Sci USA* 106: 1848–1852.
- Munday PL, Dixon DL, McCormick MI, Meekan M, Ferrari M, Chivers DP (2010) Replenishment of fish populations is threatened by ocean acidification. *Proc Natl Acad Sci USA* 107: 12930–12934.
- Munday PL, Pratchett MS, Dixon DL, Donelson JM, Endo G, Reynolds AD, Knuckey R (2013) Elevated CO₂ affects the behavior of an ecologically and economically important coral reef fish. *Mar Biol* 160: 2137–2144.
- Nilsson GE, Dixon DL, Domenici P, McCormick MI, Sørensen C, Watson SA, Munday PL (2012) Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nat Clim Chang* 2: 201–204.

- Norton W, Bally-Cuif L (2010) Adult zebrafish as a model organism for behavioural genetics. *BMC Neurosci* 11: 90.
- Ou M, Hamilton TJ, Eom J, Lyall EM, Gallup J, Jiang A, Lee J, Close DA, Yun S-S, Brauner CJ (2015) Responses of pink salmon to CO₂-induced aquatic acidification. *Nat Clim Chang* 5: 950–955.
- Qin Z, Lewis JE, Perry SF (2010) Zebrafish (*Danio rerio*) gill neuroepithelial cells are sensitive chemoreceptors for environmental CO₂. *J Physiol* 588: 861–872.
- R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rhein M, Rintoul SR, Aoki S, Campos E, Chambers D, Feely RA, Gulev S, Johnson GC, Josey SA, Kostianoy A *et al.* (2013) Observations: ocean. In TF Stocker *et al.*, eds, *Climate Change 2013: The Physical Science Basis*. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp 255–297.
- Riebesell U, Fabry VJ, Hansson L, Gattuso J-P (Eds.) (2010) Guide to best practices for ocean acidification research and data reporting, 260 p. Luxembourg: Publications Office of the European Union.
- Stewart A, Cachat J, Wong K, Gaikwad S, Gilder T, DiLeo J, Chang K, Utterback E, Kalueff AV (2010) Homebase behavior of zebrafish in novelty-based paradigms. *Behav Processes* 85: 198–203.
- Sundin J, Jutfelt F (2015) 9–28 d of exposure to elevated pCO₂ reduces avoidance of predator odour but had no effect on behavioural lateralization or swimming activity in a temperate wrasse (*Ctenolabrus rupestris*). *ICES J Mar Sci* 73: 620–632.
- Vallortigara G, Rogers LJ (2005) Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav Brain Sci* 28: 575–633.
- Vulesevic B (2006) Chemoreceptor plasticity and respiratory acclimation in the zebrafish *Danio rerio*. *J Exp Biol* 209: 1261–1273.
- Welch MJ, Watson SA, Welsh JQ, McCormick MI, Munday PL (2014) Effects of elevated CO₂ on fish behaviour undiminished by transgenerational acclimation. *Nat Clim Chang* 4: 1086–1089.
- Zickfeld K, Eby M, Weaver AJ, Alexander K, Crespin E, Edwards NR, Eliseev AV, Feulner G, Fichet T, Forest CE *et al.* (2013) Long-term climate change commitment and reversibility: an EMIC intercomparison. *J Clim* 26: 5782–5809.