

Effects of elevated CO₂ on fish behaviour undiminished by transgenerational acclimation

Megan J. Welch^{1,2*}, Sue-Ann Watson¹, Justin Q. Welsh², Mark I. McCormick^{1,2} and Philip L. Munday^{1,2}

Behaviour and sensory performance of marine fishes are impaired at CO₂ levels projected to occur in the ocean in the next 50–100 years^{1–5}, and there is limited potential for within-generation acclimation to elevated CO₂ (refs 6,7). However, whether fish behaviour can acclimate or adapt to elevated CO₂ over multiple generations remains unanswered. We tested for transgenerational acclimation of reef fish olfactory preferences and behavioural lateralization at moderate (656 μ atm) and high (912 μ atm) end-of-century CO₂ projections. Juvenile spiny damselfish, *Acanthochromis polyacanthus*, from control parents (446 μ atm) exhibited an innate avoidance to chemical alarm cue (CAC) when reared in control conditions. In contrast, juveniles lost their innate avoidance of CAC and even became strongly attracted to CAC when reared at elevated CO₂ levels. Juveniles from parents maintained at mid-CO₂ and high-CO₂ levels also lost their innate avoidance of CAC when reared in elevated CO₂, demonstrating no capacity for transgenerational acclimation of olfactory responses. Behavioural lateralization was also disrupted for juveniles reared under elevated CO₂, regardless of parental conditioning. Our results show minimal potential for transgenerational acclimation in this fish, suggesting that genetic adaptation will be necessary to overcome the effects of ocean acidification on behaviour.

Experiments and field studies have shown that the sensory performance and behaviours of marine fishes are impaired when they are exposed to CO₂ levels projected to occur in the ocean by the end of this century^{1,2,4,5,7}. Changes include altered activity levels, loss of behavioural lateralization, inability to learn, slowed visual acuity, and altered auditory and olfactory preferences^{3,8–10}. These behavioural alterations have significant effects on ecological processes, such as predator–prey^{2,11} and competitive interactions¹², that may have far-reaching implications for population replenishment, community structure and ecosystem function. Of particular concern are behavioural changes that can increase the risk of predation in juvenile fishes, such as reduced response to chemical alarm cue (CAC) and predator odour^{11,13}, and impaired cognitive function^{14,15}. The underlying mechanisms of these behavioural changes appear to be the interference with neurotransmitter function in the fish brain caused by elevated CO₂ levels^{5,15,16}. A major unanswered question, however, is whether fish behaviour can acclimate or adapt¹⁷ to higher CO₂ over coming decades.

Long-term experiments and studies at natural CO₂ seeps indicate that behavioural impairment persists even when fish are permanently exposed to elevated CO₂ for weeks to months^{6,7}. This suggests that there is limited potential for within-generation acclimation of impaired behavioural responses. Furthermore, the greatest risk of predation for coral reef fishes occurs during the

first few days of settlement to the reef¹⁸, and there is no evidence that acclimation of behaviour to elevated CO₂ occurs during this crucial life phase^{2,6}. More promising, however, is new evidence of transgenerational acclimation to ocean acidification, where exposure of parents to higher CO₂ levels confers benefits to their offspring in a high-CO₂ environment^{19–21}. For example, effects of elevated CO₂ on the metabolic rate, growth and survival of juvenile anemonefish *Amphiprion melanopus* were absent when their parents were also reared under high-CO₂ (ref. 19). Furthermore, the effects of elevated CO₂ on the kinematics of predator-escape responses in juvenile anemonefish were less pronounced when parents experienced the same high-CO₂ environment as the offspring²². These studies indicate that transgenerational acclimation can be a powerful mechanism by which some organisms may be able to adjust to projected future environmental changes, such as ocean acidification. Yet, the potential for transgenerational acclimation to overcome the negative consequences of rising CO₂ on olfactory and cognitive impairment in reef fishes has not been tested.

We tested for transgenerational acclimation of coral reef fish behaviour at CO₂ levels projected to occur in the ocean during this century under mid-CO₂ (656 μ atm) and high-CO₂ (912 μ atm) CO₂ emissions scenarios²³. We used a fully factorial design, where breeding pairs of the spiny damselfish, *Acanthochromis polyacanthus*, were reared in present-day control conditions (446 μ atm) or one of the two elevated CO₂ treatments (mid and high) (Supplementary Fig. 1 and Table 1). Clutches of newly hatched offspring from these breeding pairs were then divided equally into control, mid-CO₂ and high-CO₂ treatments (Supplementary Fig. 2), and reared for 40–45 days before testing (Supplementary Fig. 1 and Table 1). This design enabled us to test for acute effects of elevated CO₂ on the behaviour of juvenile fish and the potential for mitigation of these effects when parents were maintained in elevated CO₂ conditions. This design also allowed us to distinguish if cross-generation CO₂ conditioning had additional negative influences on fish behaviour, or if there were carry-over effects when offspring from mid- and high-CO₂ parents were hatched into control conditions. Multiple clutches from each breeding pair were tested to examine the consistency in behavioural responses (Supplementary Table 2). We tested olfactory preferences and behavioural lateralization in juveniles, as these are two of the most commonly affected behaviours in marine fishes exposed to elevated CO₂ (refs 4,6,13–15), and are key to predator avoidance and survival of juvenile fishes.

As expected, juveniles from control parents that were reared in present-day control conditions strongly avoided chemical alarm cue (CAC) when presented in a two-channel choice flume, spending less than 10% of their time in the water stream containing CAC (Fig. 1). In contrast, juveniles from control parents that were reared

¹ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland 4811, Australia. ²School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia. *e-mail: meg.welch@my.jcu.edu.au

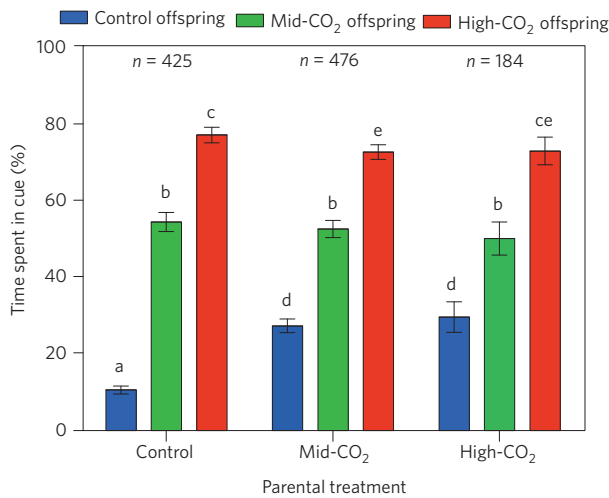


Figure 1 | Preference of juvenile fish for water streams containing chemical alarm cues presented in a two-channel flume chamber. Juvenile fish from control (446 μatm), mid-CO₂ (656 μatm) and high-CO₂ (912 μatm) parental treatments, that had been reared in the same three CO₂ treatments (Supplementary Fig. 2), were given the choice of untreated water or water with conspecific chemical alarm cues (CAC). The graph shows mean percentage time (\pm s.e.m.) spent in the CAC water stream. Letters above bars represent Tukey's HSD groups. Less breeding occurred at high-CO₂, resulting in a smaller sample size of juveniles for this group (Supplementary Table 2).

in elevated CO₂ treatments exhibited strikingly different responses ($F_{2,1077} = 291.17$, $p < 0.01$). Control-bred juveniles reared under mid-CO₂ spent 50% of their time on average in CAC water, while control-bred juveniles reared at high-CO₂ became strongly attracted to CAC, spending nearly 80% of their time in the cue (Fig. 1). These results are consistent with previous studies testing the effect of elevated CO₂ on olfactory preferences in juvenile fishes^{1,13,24}.

Maintaining parents in elevated CO₂ did not affect the response of their offspring to elevated CO₂, regardless of parent treatment ($F_{2,1077} = 2.18$, $p > 0.05$, Fig. 1). Consequently, there was no transgenerational acclimation of olfactory behavioural responses. On average, juveniles reared at mid-CO₂ spent approximately 50% of their time in CAC water, and juveniles reared at high-CO₂ spent 75–80% of their time in CAC water, regardless of the parental CO₂ treatment. The attraction to CAC exhibited by juveniles from mid-CO₂-treated parents that were reared at high-CO₂ was slightly less than the juveniles from control parents reared at high-CO₂ (Fig. 1); however, the magnitude of the difference was minor in comparison with the marked change in response to CAC exhibited by all juveniles reared in the elevated CO₂ treatments versus juveniles reared in control conditions (Fig. 1). Offspring from mid- and high-CO₂ parents that were reared in control conditions had a significantly greater attraction to CAC than offspring from control parents reared in control conditions (Fig. 1), demonstrating a carry-over effect of parental mid- and high-CO₂ on offspring behaviour. There was no significant effect of parent pair ($F_{14,1044} = 1.41$, $p > 0.05$) or clutch number ($F_{3,1044} = 1.65$, $p > 0.05$) on the olfactory responses.

To assess behavioural lateralization we measured turning preference in a custom-built maze¹⁴. Absolute lateralization (L_A) is an index of lateralization strength irrespective of direction, where an L_A of zero represents an equal proportion of turns to the right and to the left, and an L_A of 100 represents all turns in the same direction. Mean L_A among individuals decreased in elevated CO₂ treatments compared with controls (Fig. 2). Juveniles from control parents that were reared in control conditions were over 60% lateralized. In contrast,

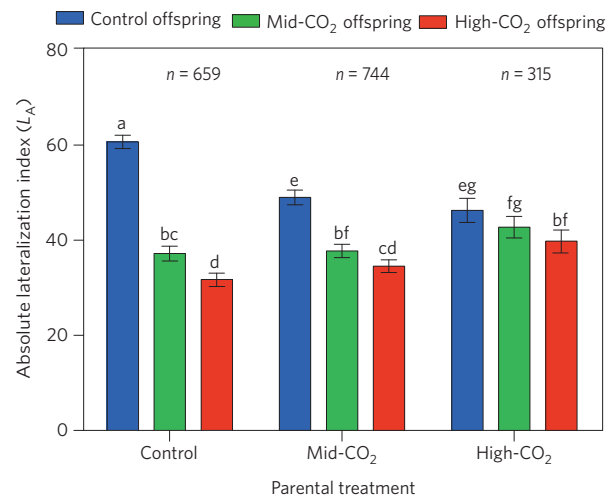


Figure 2 | Absolute lateralization (L_A) for juvenile fish presented with a T-maze choice chamber. Juvenile fish from control (446 μatm), mid-CO₂ (656 μatm) and high-CO₂ (912 μatm) parental treatments, that had been reared in the same three CO₂ treatments (Supplementary Fig. 2), were allowed to choose to turn left or right for a total of 20 turns. The graph shows mean L_A (\pm s.e.m.) calculated from relative lateralization (L_R) values for each individual. Letters above bars represent Tukey's HSD groups. Less breeding occurred at high-CO₂ level, resulting in a smaller sample size of juveniles for this group (Supplementary Table 2).

lateralization for juveniles from control parents dropped below 40% when reared at mid-CO₂ and below 35% when reared at high-CO₂ (Fig. 2). Offspring from mid-CO₂ parents showed a similar trend to those from control parents, with offspring less lateralized at higher CO₂ levels (Pearson Chi-square test, $p < 0.001$). Offspring from high-CO₂ parents exhibited a slight increase in L_A compared with juveniles from control parents reared at high-CO₂, but their overall lateralization was still significantly lower than present-day controls (Fig. 2). These results indicate that transgenerational acclimation does not ameliorate the effects of elevated CO₂ on turning preferences in juvenile fishes, although juveniles from parents exposed to the highest CO₂ level (912 μatm) were slightly less affected by elevated CO₂ than juveniles from control parents.

As observed for the olfactory preferences, there was a carry-over effect of parental elevated CO₂ for offspring reared in control conditions (Fig. 2), where L_A was reduced compared with control-bred offspring reared in control conditions. There was no effect of breeding pair on L_A , indicating that all pairs produced similar behaving offspring amongst CO₂ treatment groups ($F_{14,26} = 2.076$, $p > 0.5$). Clutch order nested within breeding pair was significant ($F_{26,1677} = 2.97$, $p < 0.001$), with individuals reared under high-CO₂ conditions showing a slight increase in lateralization in later clutches. Juveniles reared under control and mid-CO₂ conditions showed no consistent trend in relation to clutch order.

Further examination of turning preference using relative lateralization (L_R) revealed a left-turn bias was prominent in control-reared juveniles (Supplementary Fig. 3a). At higher CO₂, L_R shifted right, towards a value of zero, indicating a less pronounced left-turning preference (Supplementary Fig. 3b,c). As observed for L_A , parental exposure to high-CO₂ reduced the magnitude of the effect of elevated CO₂ on L_R in juveniles; nevertheless, there was still a shift in mean L_R towards zero (Supplementary Fig. 3g–i), demonstrating that transgenerational acclimation did not restore the bias in turning direction found in present-day conditions. There was also a clear carry-over effect of elevated CO₂, with control-reared juveniles exhibiting a reduced left-turn bias if their parents were exposed to elevated CO₂ (Supplementary Fig. 3d,g).

Our results show that impaired sensory and cognitive functions in juvenile reef fish caused by a high- CO_2 environment are not reversed or ameliorated by parental exposure to the same high- CO_2 levels as the offspring. Like many reef fish, *A. polyacanthus*, have an innate ability to recognize chemical alarm cues, as well as a tendency to be behaviourally lateralized, but these capabilities are lost when juveniles are exposed to CO_2 levels projected to occur over the next 50–100 years, as previously demonstrated in other reef fish species^{1,13,14,24}. These behaviours are essential for daily survival of a coral reef fish, especially in early life-history stages when predation risk is greatest¹⁸. Impaired behaviours under elevated CO_2 levels suggest increased mortality that could affect population sustainability². Transgenerational acclimation can restore metabolic and growth rates in reef fish exposed to elevated CO_2 (ref. 19), but our results show that transgenerational acclimation does not restore key sensory and behavioural traits.

Appropriate predator avoidance behaviour in response to alarm cues is essential for the survival of reef fish, especially young juveniles who are more prone to predation²⁵. Alarm cues are chemical compounds released into the water when the epidermal tissue is damaged, such as during a predator attack^{26,27}. Juvenile fish have an innate response to the presence of conspecific CAC, rapidly reducing activity levels and seeking shelter in the presence of even minute traces of CAC. The attraction to conspecific alarm cues observed in CO_2 -reared offspring, regardless of parental treatment, could lead to significantly higher mortality among juvenile fishes as CO_2 levels rise in the ocean. Critically, our results indicate that exposure of parents to higher CO_2 levels does not improve the behavioural responses of juveniles to CAC when reared at elevated CO_2 . The carry-over effects from high- CO_2 parents for juveniles reared in control conditions further indicate that parental exposure to high- CO_2 does not have a beneficial effect on the behaviour of their offspring. There was no effect of parental breeding pair or clutch number on olfactory responses, suggesting that the responses observed here are widespread and not confined to some breeding pairs or parts of the reproductive season.

Lateralization is another fundamental behaviour for many reef fishes. Gregarious individuals depend on lateralization for group coordination²⁸, especially in defence or escape from a predator. Furthermore, individuals show a higher tendency for lateralization in high-predation locations than those in low-predation locations²⁸. Behavioural lateralization is believed to reduce decision-making time, which is especially important in a life-threatening situation such as a predator encounter. Behavioural lateralization is also closely linked to visual assessment²⁹ and cognitive function³⁰, both of which are affected by elevated CO_2 (refs 10,15). We observed a loss of lateralization at projected future CO_2 levels that was not ameliorated across generations, which suggests that predator avoidance might be impaired in a future high- CO_2 environment. *A. polyacanthus* forms loose schools and is not as gregarious as some other marine fishes; consequently, the effects of changed lateralized behaviour may be less for this species than for highly gregarious species. The partial improvement in lateralization for juveniles from high- CO_2 parents that were reared at high- CO_2 is consistent with partial acclimation of some kinematic traits involved in predator-escape responses observed in the only other study²² to examine transgenerational acclimation of behavioural traits to high- CO_2 . Our results show that while there may be some partial improvement in behavioural traits linked to cognitive performance, such as lateralization, negative effects are not fully ameliorated by parental exposure to elevated CO_2 in this species. Moreover, other highly maladaptive behavioural effects, such as attraction to conspecific alarm cues, exhibit no transgenerational acclimation.

Miller *et al.*¹⁹ observed complete restoration of metabolic traits in juvenile reef fishes when their parents were exposed to the same high- CO_2 levels. In contrast, we observed no improvement

in olfactory-mediated behaviour and only limited improvement in behavioural lateralization when both parents and offspring were exposed to elevated CO_2 . This suggests that metabolic traits may have greater potential than behavioural traits for transgenerational acclimation to elevated CO_2 . Behavioural changes at elevated CO_2 appear to be a result of the interference with the function of GABA_A neurotransmitter receptors^{5,15,16}. It is possible that the functioning of GABA_A receptors is less plastic¹⁶ than metabolic pathways in reef fish¹⁹, thus limiting the potential for transgenerational acclimation; however, this hypothesis requires further investigation. A further intriguing result was the carry-over effect observed in juveniles from mid- and high- CO_2 parents when reared in control conditions. This could suggest an epigenetic effect due to prior CO_2 exposure, either directly on GABA_A receptor function, or on acid–base regulatory process that control ion gradients across receptor membranes. Because eggs developed in the same CO_2 conditions as parents before juveniles were transferred to control conditions, an epigenetic effect could be developmental or transgenerational. Further studies are needed to determine the mechanisms involved.

In conjunction with the lack of transgenerational acclimation for abnormal olfactory and lateralization responses seen in this study, there is no evidence for within-generation acclimation of behaviour to high- CO_2 in fish⁶. Therefore, genetic adaptation will be necessary to overcome these behavioural impairments as CO_2 levels rise in the ocean. Previous studies have reported a bimodal distribution of individual responses to predator odour at intermediate CO_2 levels, with some individuals strongly attracted to the cue whereas others remain repelled²⁷. We did not observe a bimodal response of individuals to CAC in either of the elevated CO_2 treatments as found by previous studies; however, there was still some variation among individuals in all the experimental conditions that could be important for future adaptive responses. Selection of CO_2 -tolerant behavioural phenotypes has been observed in field-based experiments³¹, yet whether the phenotypic variation is heritable remains unknown. Determining the heritability of individual variation in CO_2 sensitivity is a priority for future research, as it would provide the opportunity for genetic adaptation to higher CO_2 levels. If reef fish behaviour does not adapt to rising CO_2 levels there could be serious implications for population dynamics and the function of marine ecosystems.

Methods

Olfaction. Response to chemical alarm cue (CAC) was tested in a two-channel choice flume (13 cm × 4 cm) as used in previous studies^{1,13}. The flume combination was conspecific chemical alarm cue versus untreated water. Water from the two different sources was gravity fed into the choice flume, which is divided down half of its length. A constant flow rate of 100 ml min⁻¹ was maintained and monitored using a flow meter and dye test after every water change. Water chemistry in the flume matched the rearing condition for each juvenile and was consistent with seawater parameters during the breeding season (Supplementary Table 1).

To produce CAC, control donor fish were euthanized with a quick blow to the head. Superficial cuts were made along the sides of the donor fish and then rinsed with 15 ml of treatment water²⁴. This water was collected and immediately mixed with 10 l of treatment water in the tank used to supply CAC to the flume. This ensured a consistent concentration of fresh CAC for the duration of each trial. A ratio of one donor fish to one test fish was used.

For each trial, a single test fish was placed in the centre of a downstream end of the choice flume (Supplementary Information) and given a 2 min acclimation period. The position of the fish was then recorded every five seconds for a total of 2 min. A rest period of 3 min followed, during which time the water sources were switched to eliminate potential side preferences. The entire acclimation and trial process was then repeated. Fish from the first three clutches from each breeding pair were tested, except for one breeding pair in high- CO_2 that produced only one clutch (Supplementary Table 2). Ten fish from each clutch were tested per CO_2 treatment, for a total of thirty fish per clutch. Each fish was only used once.

Lateralization. A T-maze was used to test lateralization. Dimensions were based on those used by Domenici *et al.*¹⁴ (60 cm × 30 cm × 20 cm), with a runway in the middle (25 cm × 3 cm, length × width), and at both ends of the runway

(3 cm ahead of the runway) an opaque barrier (12 cm × 12 cm × 1 cm) was positioned perpendicular to the runway. Water depth in the maze was 4 cm and matched the CO₂ chemistry in which the fish were reared (Supplementary Table 1).

A single fish was placed at one end of the T-maze (Supplementary Information) and given a 5 min habituation period, during which time it could explore the apparatus. At the end of the habituation period the fish was gently guided to the beginning of the runway and left to swim along the runway until reaching the perpendicular barrier. Direction choice was recorded, with the criterion for choice being the first turning direction when the fish exited the runway. Twenty runs were recorded per fish. The first three clutches from each breeding pair were tested, except for one breeding pair in high-CO₂ that produced only one clutch (Supplementary Table 2). Fifteen fish from each clutch were tested per CO₂ treatment, for a total of 45 fish per clutch. Each fish was only used once. To account for any possible asymmetry in the maze, turns were recorded alternately on the two ends of the runway¹⁴.

Data analysis. A total of 1,085 individuals were tested for changes in olfactory behaviour. A linear mixed effects model (LME) was used to test for transgenerational acclimation in offspring's responses to elevated CO₂. Percentage time in the CAC-water was arcsine-transformed before analysis to improve the distribution of the proportional data. Parental pair was included as a fixed factor in the model, while clutch number was a random factor nested within parental pair. A two-factor univariate LME was used to test for an effect of parental treatment, offspring treatment, and an interaction effect. Parental and offspring treatments were fixed factors in the models. A Tukey's HSD post hoc test was used to compare treatment means and identify homogeneous grouping among treatments.

A total of 1,728 individuals were tested in the lateralization experiments. Both absolute and relative lateralization were calculated and used to analyse potential for transgenerational acclimation¹⁴. The absolute lateralization index (L_A) of each fish was calculated to determine the strength of lateralization amongst individuals, regardless of a turning bias.

The absolute lateralization (L_A) of individuals was compared among treatments using LME models. Parental pair was included as a fixed factor in the model, with clutch number as a random factor nested within parental pair. A two-factor univariate LME was used to test for an effect of parental treatment, offspring treatment, and an interaction effect. Parental and offspring treatments were fixed factors in the models. A Tukey's HSD post hoc test was used to compare treatment means and identify homogeneous grouping among treatments. Transformations were not able to rectify the heterogeneity of variances in the lateralization data. Therefore, a more conservative alpha value of 0.01 was adopted when interpreting the results of the statistical tests on this dataset³².

The relative lateralization index (L_R) (Supplementary Information) was used to compare turning bias (that is, left versus right) among the nine groups. A Pearson's Chi-square test for independence was conducted on each CO₂ grouping (three parental groups × three offspring groups) using L_R data.

Received 27 May 2014; accepted 5 September 2014;
published online 5 October 2014

References

- Munday, P. L. *et al.* Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl Acad. Sci. USA* **106**, 1948–1852 (2009).
- Munday, P. L. *et al.* Replenishment of fish populations is threatened by ocean acidification. *Proc. Natl Acad. Sci. USA* **107**, 12930–12934 (2010).
- Briffa, M., de la Haye, K. & Munday, P. L. High CO₂ and marine animal behaviour: Potential mechanisms and ecological consequences. *Mar. Pollut. Bull.* **64**, 1519–1528 (2012).
- Jutfelt, F., Bresolin de Souza, K., Vuylsteke, A. & Sturve, J. Behavioural disturbances in a temperate fish exposed to sustained high-CO₂ levels. *PLoS ONE* **8**, e65825 (2013).
- Hamilton, T. J., Holcombe, A. & Tresguerres, M. CO₂-induced ocean acidification increases anxiety in rockfish via alteration of GABA_A receptor functioning. *Proc. R. Soc. B* **281**, 20132509 (2014).
- Munday, P. L., Cheal, A. J., Dixon, D. L., Rummer, J. L. & Fabricius, K. E. Behavioural impairment in reef fishes caused by ocean acidification at CO₂ seeps. *Nature Clim. Change* **4**, 487–492 (2014).
- Munday, P. L. *et al.* Elevated CO₂ affects the behavior of an ecologically and economically important coral reef fish. *Mar. Biol.* **160**, 2137–2144 (2013).
- Branch, T. A., DeJoseph, B. M., Ray, L. J. & Wagner, C. A. Impacts of ocean acidification on marine seafood. *Trends Ecol. Evol.* **28**, 178–186 (2013).
- Leduc, A. O. H. C., Munday, P. L., Brown, G. E. & Ferrari, M. C. O. Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: A synthesis. *Phil. Trans. R. Soc. B* **368**, 20120447 (2013).

- Chung, W. S., Marshall, N. J., Watson, S. A., Munday, P. L. & Nilsson, G. E. Ocean acidification slows retinal function in a damselfish through interference with GABA_A receptors. *J. Exp. Biol.* **217**, 323–326 (2014).
- Ferrari, M. C. O. *et al.* Putting prey and predator into the CO₂ equation — Qualitative and quantitative effects of ocean acidification on predator–prey interactions. *Ecol. Lett.* **14**, 1143–1148 (2011).
- McCormick, M. I., Watson, S. A. & Munday, P. L. Ocean acidification reverses competition for space as habitats degrade. *Sci. Rep.* **3**, 3280 (2013).
- Dixon, D. L., Munday, P. L. & Jones, G. P. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecol. Lett.* **13**, 68–75 (2010).
- Domenici, P., Allan, B., McCormick, M. I. & Munday, P. L. Elevated carbon dioxide affects lateralization in a coral reef fish. *Biol. Lett.* **8**, 78–81 (2012).
- Chivers, D. P. *et al.* Impaired learning of predators and lower prey survival under elevated CO₂: A consequence of neurotransmitter interference. *Glob. Change Biol.* **20**, 515–522 (2014).
- Nilsson, G. E. *et al.* Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Clim. Change* **2**, 201–204 (2012).
- Sunday, J. M. *et al.* Evolution in an acidifying ocean. *Trends Ecol. Evol.* **29**, 117–125 (2014).
- Almany, G. R. & Webster, M. S. The predation gauntlet: Early post-settlement mortality in reef fishes. *Coral Reefs* **25**, 19–22 (2006).
- Miller, G. M., Watson, S. A., Donelson, J. M., McCormick, M. I. & Munday, P. L. Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. *Nature Clim. Change* **2**, 858–861 (2012).
- Parker, L. M. *et al.* Adult exposure influences offspring response to ocean acidification in oysters. *Glob. Change Biol.* **18**, 82–92 (2012).
- Murray, C. S., Malvezzi, A., Gobler, C. J. & Baumann, H. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Mar. Ecol. Prog. Ser.* **504**, 1–11 (2014).
- Allan, B. J. M., Miller, G. M., McCormick, M. I., Domenici, P. & Munday, P. L. Parental effects improve escape performance of juvenile reef fish in a high-CO₂ world. *Proc. R. Soc. B* **281**, 20132179 (2014).
- Collins, M. *et al.* in *IPCC Climate Change 2013: The Physical Science Basis* (eds Stocker, T. F. *et al.*) 1096–1097 (Cambridge Univ. Press, 2013).
- Ferrari, M. C. O. *et al.* Intragenerational variation in antipredator responses of coral reef fishes affected by ocean acidification: Implications for climate change projections on marine communities. *Glob. Change Biol.* **17**, 2980–2986 (2011).
- Lönstedt, O. M., McCormick, M. I., Meekan, M. G., Ferrari, M. C. O. & Chivers, D. P. Learn and live: The role of predator experience in influencing prey behaviour and survival. *Proc. R. Soc. B* **279**, 2091–2098 (2012).
- Brown, G. E. Learning about danger: Chemical alarm cues and local risk assessment in prey fishes. *Fish Fish.* **4**, 227–234 (2003).
- Chivers, D. P. & Smith, R. J. F. Chemical alarm signaling in aquatic predator–prey systems: A review and prospectus. *Ecoscience* **5**, 338–352 (1998).
- Brown, C., Western, J. & Braithwaite, V. A. The influence of early experience on and inheritance of cerebral lateralization. *Anim. Behav.* **74**, 231–238 (2007).
- Sovrano, V. A., Rainoldi, C., Bisazza, A. & Vallortigara, G. Roots of brain specializations: Preferential left-eye use during mirror-image inspection of six species of teleost fish. *Behav. Brain Res.* **106**, 175–180 (1999).
- Dadda, M. & Bisazza, A. Lateralized female topminnows can forage and attend to a harassing male simultaneously. *Behav. Ecol.* **17**, 358–363 (2006).
- Munday, P. L. *et al.* Selective mortality associated with variation in CO₂ tolerance in a marine fish. *Ocean Acidification* **1**, 1–5 (2012).
- Quinn, G. P. & Keough, M. J. *Experimental Design and Data Analysis for Biologists* (Cambridge Univ. Press, 2002).

Acknowledgements

We thank B. Lawes, S. Wever and A. Thompson for continued support of laboratory set-up and maintenance. Special thanks to J. Donelson, M. Mitchell and J. White for assistance with collections and to K. Corkill for assistance with experiments. This study was funded by the ARC Centre of Excellence for Coral Reef Studies and the School of Marine and Tropical Biology at James Cook University.

Author contributions

P.L.M., M.J.W. and M.I.M. designed the study; M.J.W. performed the experiments; J.Q.W. analysed the data; S.A.W. and M.J.W. controlled the CO₂ system; M.J.W. and P.L.M. co-wrote the paper; all authors provided important comments and approved the paper.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.J.W.

Competing financial interests

The authors declare no competing financial interests.