Parental effects improve escape performance of juvenile reef fish in a high-CO₂ world

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Rising CO₂ levels in the oceans are predicted to have serious consequences for many marine taxa. Recent studies suggest that non-genetic parental effects may reduce the impact of high CO₂ on the growth, survival and routine metabolic rate of marine fishes, but whether the parental environment mitigates behavioural and sensory impairment associated with high CO₂ remains unknown. Here, we tested the acute effects of elevated CO₂ on the escape responses of juvenile fish and whether such effects were altered by exposure of parents to increased CO₂ (transgenerational acclimation). Elevated CO₂ negatively affected the reactivity and locomotor performance of juvenile fish, but parental exposure to high CO₂ reduced the effects in some traits, indicating the potential for acclimation of behavioural impairment across generations. However, acclimation was not complete in some traits, and absent in others, suggesting that transgenerational acclimation does not completely compensate the effects of high CO₂ on escape responses.

1. Introduction

Carbon dioxide (CO₂) levels in the world’s oceans are increasing owing to rising atmospheric CO₂ concentrations [1]. Higher CO₂ levels are predicted to affect the physiology and behaviour of many marine species [2,3]. Exposure to elevated CO₂ can lead to extra- or intracellular acidosis in marine animals, with effects on growth, development and survival [4,5]. This may be particularly relevant for early life stages as the physiological costs of maintaining acid–base balance could be greater owing to the relatively large surface area to volume ratio of early life stages and less developed acid–base regulatory mechanisms [6]. Some of the most dramatic effects of elevated CO₂ levels reported to date are impaired sensory ability and altered behaviours of larval and juvenile fishes [3,7,8]. Behavioural effects of high CO₂ include changes in antipredator responses [9], olfactory [10,11] and auditory preferences [12], activity levels [13,14], behavioural lateralization [15,16] and visual risk assessment [17]. These behavioural changes affect key ecological processes, such as habitat selection [18], the timing of settlement [19] and predator–prey interactions [9,20]. The underlying mechanism linking elevated CO₂ to these diverse changes in behaviour is difficult to assess but appears to be interference of the acid–base regulatory process with the function of major neurotransmitter receptors in the brain [21].

While our understanding of how elevated CO₂ can constrain individual performance is increasing, the potential for adaptation or acclimation over climate change relevant timescales remains largely unresolved [22]. Importantly, the environmental conditions experienced by parents may affect their physiological condition and provide the opportunity for non-genetic effects to be transferred to their offspring [23,24]. Indeed, recent work has demonstrated that some of the negative physiological effects of climate change on marine organisms can be reduced if the parents have been exposed to the same environmental history as
their offspring [25–27]. This indicates that non-genetic parental effects may alter how marine organisms respond to elevated CO$_2$ and provides evidence that the response to environmental stress may depend on the history of previous life stages. However, these studies have focused on physiological acclimation across generations and it is not known whether behaviour is subjected to the same rapid transgenerational acclimation.

A critical stage in the life of reef fishes is the end of the larval phase, when they settle to the benthic environment. Mortality within the first few days of settlement is variable, but uniformly high, averaging 56% for tropical reef fishes [28]. Success at this life stage is predominantly a consequence of the size, growth and escape performance of new recruits [29,30]. Escape responses consist of sudden accelerations in response to a startling stimulus, for example a predator strike, and are observed in many fish species from various taxa [31]. A successful escape response consists of finely tuned responsiveness and locomotor performance. The speed of escape and duration of response may be related to locomotor performance [32]. By contrast, the perception of a threat, response latency and directionality of escape are related to neural and sensory capacity [33–35]. The fast kinematics of the escape responses are usually controlled by the Mauthner cells which are triggered as a reaction to the fast approach of a predator, although other reticulospinal cells may also be involved [33].

Though recent studies have shown that elevated CO$_2$ can increase the mortality rate of prey when exposed to predators [13,14], the extent to which fish larvae can acclimate their escape responses across generations exposed to elevated CO$_2$ is unknown. Rearing of adults and juveniles under current-day CO$_2$ levels (400 $\mu$atm) or CO$_2$ levels projected to be encountered by the end of the century (1087 $\mu$atm) [36] allowed us to test the hypothesis that transgenerational acclimation may reduce the impact of elevated CO$_2$ on the outcome of predator–prey interactions. Comparisons between treatments allowed us to determine the acute (within-generation) effects of increased CO$_2$ on juvenile escape performance and to test whether such effects were mediated by exposure of parents to increased CO$_2$. Furthermore, by investigating a range of behavioural and locomotor variables that are critical to a successful escape response [37] we were able to test whether the capacity for acclimation to elevated CO$_2$ may differ between escape processes linked to locomotor performance and those more closely linked to cognitive processes.

2. Material and methods

(a) Study species and brood-stock maintenance
To examine the effect of elevated CO$_2$ on the escape responses of juvenile reef fish, adult breeding pairs of the cinnamon anemone fish, Amphiprion melanopus, were collected from the Palm Island Region of the central Great Barrier Reef, Australia (18°37’S, 146°30’E). Breeding pairs were housed in individual 45 l aquaria and maintained at long-term average temperatures for the collection location (22.5°C winter and 28.5°C summer). Pairs were randomly assigned to the control (400 $\mu$atm) and high (1087 $\mu$atm) CO$_2$ treatments in June 2011, and CO$_2$ was slowly adjusted to the desired level over a two-week period. This allowed pairs to be conditioned in their CO$_2$ treatments for five months before the start of the breeding season in November 2011. Temperatures were increased from winter temperatures of 22.5°C at a rate of 0.5°C per week until the summer breeding temperature of 28.5°C was reached in the first week of November 2011. Breeding pairs were maintained in their CO$_2$ treatments until May 2012. Breeding pairs were provided with half of a terracotta pot as a shelter and a spawning site. Pairs were allowed to spawn naturally during the breeding season (November 2011–May 2012) and spawning sites were checked daily for the presence of a new egg clutch.

(b) Juvenile rearing
To examine the difference between acute exposure to CO$_2$ and parental effects of CO$_2$ on juvenile reef fish, three juvenile treatment groups were used: (i) juveniles from control parents were hatched into control CO$_2$ levels (control–control), (ii) juveniles from high CO$_2$ parents were hatched into high CO$_2$ (high–high) and (iii) juveniles from control parents were hatched into high CO$_2$ (control–high). On the night of hatching, terracotta pots with clutches were moved to 60 l larval rearing tanks and juveniles were reared in their designated CO$_2$ level from hatching. Tanks were filled with treated system water and aerated with premixed air to the desired CO$_2$ level. Juveniles were held under 13 L:11 D regime. Aquaria were slowly flushed for 11 h overnight with fresh treatment water at the relevant CO$_2$ level to remove any excess food and to maintain water quality. Larvae were fed live rotifers (10 individuals ml$^{-1}$ of tank water) and then weaned to max of 5 individuals ml$^{-1}$ of Artenia spp. Fish were allowed 13 h to feed as food was administered in the morning. Fish were reared in clutch groups until 11 days posthatching at which point the juveniles are considered competent to settle. Clutches from three to four different parents per CO$_2$ treatment were used in the experiment to ensure genetic diversity. Offspring used in the experiments were approximately equally distributed among the three to four breeding pairs in each treatment: 29–40% per pair from three pairs in control–control, 29–37% per pair from three pairs in high–high and 21–30% per pair from four pairs in control–high. At the end of their pelagic larval phase (10–11 days posthatching), juveniles (mean SL 13.2 mm ± 0.06 s.d.) were used for escape performance analysis. We found no significant difference among the three groups with respect to standard length (SL), body depth, caudal peduncle length and tail width.

(c) Experimental system and seawater chemistry
Adult and juvenile anemone fish were reared in an environmentally controlled aquarium facility at James Cook University, Townsville, Australia. Two 8000 l recirculating seawater systems, one maintained at control CO$_2$ (400 $\mu$atm) and the other at elevated CO$_2$ (1087 $\mu$atm), supplied seawater to individual aquariums. CO$_2$ treatments were achieved by CO$_2$ dosing to a set pH following standard techniques. A pH computer (Aquamedic AT-Control, Germany) regulated CO$_2$ dosing in a 3000 l temperature-controlled sump within each system. Temperature (Comark C22) and pH$_{KHS}$ (Hach HQ40d) were recorded daily in the rearing tanks. Salinity and total alkalinity were measured weekly. Total alkalinity was measured by Gran titration from water samples of replicate tanks in each system. Accuracy of titrations was within 1% of certified reference material (Prof. A. Dickson, Scripps Oceanographic Institute). Average seawater pCO$_2$ was calculated in the program CO2SYS using the Mehrbach constants refit by [38]. Seawater parameters are presented in table 1.

(d) Experimental protocol
Juvenile fish were individually introduced into a grey circular experimental arena (diameter 250 mm; water level 100 mm) filled with water at the same CO$_2$ treatment as they were reared in. Shallow water depth (100 mm) was used in the experimental tank in order to minimize displacement in the vertical dimension. Fish were introduced to the arena via a water-filled
sample jar. This minimized any stress associated with the movement from the tank to arena. Water temperature in the experimental arena was 28.5°C. The arena was illuminated with four 150 W spotlights, placed above the water surface, by the side of the tank. One minute after being released into the experimental tank, an escape response was elicited by the release of a clear plastic cylinder with a tapered end (38.3 g, 25 mm diameter, 120 mm length) from 350 mm above the water surface. This was accomplished by turning off an electromagnetic to which the cylinder was attached via a metal disc (15 mm in diameter) applied to the top end of the cylinder. In order to provide a sudden stimulation and allow calculation of the escape latency [39,40], the stimulus was released through a white PVC tube (diameter 6 cm, length 30 cm) suspended above the experimental tank, with the bottom edge at a distance of 10 mm above the water level. In order to record the frame during which the stimulus hit the water surface (i.e. stimulus onset), a small mirror (5 × 5 cm) was placed at 45° near the cylinder, above the water surface. Fish were startled more than two body lengths from the water surface. Fish that displayed no reaction to the stimulus were omitted from the directionality analysis leading to the escape of the animal.

(f) Statistical analyses
The effects of CO2 exposure on the escape responses of juvenile fish were examined using a one-way multivariate analysis of variance (MANOVA). The effect of CO2 exposure on each locomotor variable (response distance, mean response speed, maximum response speed, response duration and latency) was further explored using an analysis of variance (ANOVA). Residual analysis was used to determine whether the data met the assumptions of normality and homogeneity of variance. Tukey’s HSD post hoc tests were used to examine the differences detected by the ANOVA. The effect of CO2 exposure on responsiveness was analysed with a χ² test on the count data (number of responding and non-responding fish at each treatment). Two-tailed binomial tests were performed to determine whether CO2 exposure had an effect on directionality of response, comparing the proportion of individuals that moved away from or towards the stimulus and assuming a 0.5 probability of turning either way.

3. Results
The MANOVA revealed that escape responses were influenced by the CO2 treatment (Pillai’s trace(12,212) = 2.93, p < 0.0001). ANOVA detected significant differences in four out of the six variables tested (distance travelled, mean response speed, maximum response speed and response duration).

(a) Locomotor variables
The response distance following stimulation was significantly shorter in the control–high group compared with the control–control group demonstrating an acute effect of high CO2 on distance travelled (figure 1a; F2,122 = 4.135, p = 0.018). By contrast, there was no significant difference between the high–high group and the control–control group (Tukey’s test, p = 0.6), demonstrating a parental effect on distance travelled in the high–high group. However, the distance travelled by the high–high group was intermediate to the control–control group and control–high group (figure 1a), indicating that exposing parents to high CO2 did not completely reduce the effect of high CO2 on response distance.

The mean response speed during an escape response differed significantly among the groups (figure 1b; F2,122 = 4.13, p = 0.01). Response speed was significantly less in the control–high group when compared with the control–control group demonstrating an acute effect of elevated CO2 on escape speed (Tukey’s test, p = 0.03). There was no significant difference in response speed between the high–high group and the control–control group (Tukey’s test p = 0.3). Nevertheless, the response speed exhibited by the high–high group was intermediate to the control–control group and control–high group (figure 1b), indicating that parental exposure to

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<table>
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<th>treatment</th>
<th>temp. (°C)</th>
<th>salinity (ppt)</th>
<th>total alkalinity (µmol kg⁻¹ SW)</th>
<th>pH (_{\text{HIS}})</th>
<th>pCO₂ (µatm)</th>
</tr>
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<tbody>
<tr>
<td>control</td>
<td>28.4 ± 0.01</td>
<td>33.3 ± 0.12</td>
<td>2058.32 ± 15.81</td>
<td>8.15 ± 0.005</td>
<td>400 ± 6.33</td>
</tr>
<tr>
<td>high</td>
<td>28.5 ± 0.01</td>
<td>33.6 ± 0.09</td>
<td>2168.24 ± 6.70</td>
<td>7.81 ± 0.008</td>
<td>1087 ± 24.67</td>
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high CO₂ was not sufficient enough to fully reduce the negative effects of CO₂ exposure on response speed in juveniles.

The maximum response speed was significantly less in the control–high group compared with the control–control and high–high groups (figure 1c; $F_{2,122} = 6.995, p = 0.001$) demonstrating an acute effect of CO₂ treatment on the maximum speed achieved. However, there was no difference in maximum response speed between the control–control group and the high–high group (Tukey’s test $p = 0.7$, figure 1c), indicating that parental effects had completely reduced the negative effects of high CO₂ on maximum response speed in juveniles.

The duration of the burst response following stimulation (response duration) was significantly shorter in the control–high group compared with the control–control group demonstrating an acute effect of high CO₂ on response duration (figure 1d; $F_{2,122} = 7.78, p < 0.001$). By contrast, there was no significant difference between the high–high group and the control–control group, suggesting a parental influence on response duration in the high–high group (Tukey’s test $p = 0.09$). However, the response duration exhibited by the high–high group was intermediate to the control–control group and control–high group (figure 1d), indicating that parental effects did not completely reduce the acute effect of high CO₂ on distance travelled.

(b) Non-locomotor variables

The proportion of juveniles that displayed no reaction to the stimulus was significantly greater in the control–high group compared with the control–control group (χ² = 5.16, d.f. = 1, $p = 0.023$) and the high–high group (χ² = 4.09, d.f. = 1, $p = 0.044$).
Elevated CO₂ can lead to changes in growth, survival, routine metabolic rate [26,43], behaviour [10–15] and predator–prey interactions [9,20] of reef fish. Here, we demonstrate that CO₂ concentrations that are predicted for the end of this century adversely affected the escape-response performance of juvenile fish. In the control–high treatment, there was a significant increase in the number of fish that did not initiate an escape response compared with control–control group, and negative effects on distance travelled, mean response speed, maximum response speed and response duration were observed. This demonstrates that exposure of juvenile fish to elevated CO₂ has a highly significant effect on the kinematics of the escape response that is likely to impact on the ability of prey to avoid predators [37]. However, negative effects of high CO₂ exposure on escape responses were greatly reduced or absent in the high–high group, with fish showing similar performance levels found in the control–control group for some traits. This result is important because it indicates that transgenerational acclimation can help to overcome behavioural impairment observed in fishes exposed to high CO₂. As CO₂ levels rise over coming decades, both parental and offspring generations will experience similar elevated CO₂ levels; thus our results indicate that this parental exposure will help to reduce some of the negative effects of high CO₂ on behaviour.

While parental effects moderated the impacts of high CO₂ on most of the escape-response traits measured, performance was intermediate between control–control and control–high treatment groups for over half the traits examined. This indicates that although parental effects may be beneficial, they may not fully restore performance in many instances and some negative effects of high CO₂ on escape performance will remain. Furthermore, while some responses by juveniles, for example proportion of non-reactors, were completely reduced by exposing parents to high CO₂, other responses, for example the direction of response, were not improved. Consequently, our results demonstrate that transgenerational acclimation of behaviour to high CO₂ can be complete, partial or absent and that the magnitude of acclimation can vary markedly among behavioural responses.

Recent research suggests that cognitive impairment in fishes exposed to elevated CO₂ is owing to the interference of acid–base regulatory process with the functioning of brain neuroreceptors [21]. Given this, it is not surprising that directionality was affected in the fish exposed to elevated CO₂. Directionality is an important trait and an early mistake, as turning towards a predator, may increase the risk of capture [35,44]. Although transgenerational acclimation improved performance in this particular trait, individuals whose parents were exposed to elevated CO₂ exhibit a directionality that does not differ from random, likely owing to an impairment of the sensory-motor system. Consequently, exposure to elevated CO₂ could have a significant effect on the outcome of the predator–prey interactions, even if parents have been exposed to similar CO₂ levels. This contrasts with the behavioural acclimation seen with responsiveness, which highlights the specific nature of behavioural acclimation. Interestingly, elevated CO₂ had no effect on escape latency, which has also been observed in fish exposed to hypoxia suggesting that decreased oxygen may impair the sensory mechanisms involved in the detection of the mechanico-acoustic signal. However, as long as the response threshold is reached, the time course to initiate the response does not appear to be affected [34].

We found that almost all locomotory variables were affected following acute CO₂ exposure. More specifically, there were reductions in the distance travelled, mean response speed, maximum response speed and response durations. The indirect mechanism underlying this response could be a decline in aerobic scope caused by an increase in resting metabolic rate after short-term exposure to elevated CO₂ [43]. This effect may be similar to the observed reduction in escape swimming performance observed in hypoxia, which has been attributed to a trade-off between physiological exhaustion requiring energy saving, and the need to escape from a predatory attack [34]. Because the anaerobic burst is eventually paid off by excess post-exercise oxygen consumption, fish with reduced metabolic scope under elevated CO₂ conditions may therefore show low-performance escapes as an energy-saving strategy. In addition, reduced aerobic scope may have caused a decrease in burst swimming performance indirectly, i.e. as a result of reduced condition [26]. Notably, the negative effects of CO₂ exposure on all locomotory variables were less apparent if the parents had been exposed similarly. This suggests that transgenerational acclimation may play a major role not only in juvenile development and metabolism [26], but also in important locomotory behaviour, for example escape performance. Previous work has demonstrated that various physiological parameters (i.e. routine metabolic rate, growth and survival) are improved in juvenile fish that have been exposed to...
elevated CO2, providing the parents have been exposed to a similar environment [26]. The restoration of routine metabolic rate seen in Miller et al. [26] could explain why we see an improvement in locomotory capabilities. Energy saved through improved cellular processes simply means more energy is available to escape predation.

Transgenerational plasticity (acclimation) occurs in a diverse group of taxa, such as plants [45], insects [46,47], bryozoans [48], shellfish [49] and fish [27], leading to changes in morphology [46,47], dispersal potential [48] and growth rates [27,49]. Recently, it has been observed to occur in response to environmental stressors, such as hypoxia [50], elevated temperature [25,27] and elevated CO2 [26,49], suggesting that transgenerational acclimation may be an important mechanism in helping taxa to cope with climate change. For example, Salinas & Munch [27] found that sheephead minnows (Cyprinodon variegatus) reared under high or low temperatures had optimal growth provided their parents had been exposed to similar temperatures. While our results are consistent with growing evidence for the potential importance of transgenerational acclimation in enabling organisms to cope with rapid climate change, they also demonstrate that such acclimation has limits and that not all traits will be beneficially influenced by parents being exposed to the same environmental conditions as their offspring.

We have demonstrated that there is a potential for rapid acclimation for many variables that influence escape performance, if the parents have been exposed to the same environmental history. However, parental effects did not fully restore performance in most traits. Thus, negative effects of higher CO2 levels on behaviour remain, although at reduced levels. Whether behaviours could be fully acclimated in future generations and the potential costs of such acclimation to other processes is not yet known. This highlights the need to exercise caution when making statements about whole-organism acclimation. It remains to be seen whether these non-genetically inherited parental effects will be extended into future generations, thus potentially reducing the effects of rising CO2 levels on populations, communities and ecosystems.

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References


