

# Selective mortality associated with variation in CO<sub>2</sub> tolerance in a marine fish

## Abstract

Predicted future CO<sub>2</sub> levels can affect reproduction, growth, and behaviour of many marine organisms. However, the capacity of species to adapt to predicted changes in ocean chemistry is largely unknown. We used a unique field-based experiment to test for differential survival associated with variation in CO<sub>2</sub> tolerance in a wild population of coral-reef fishes. Juvenile damselfish exhibited variation in their response to elevated (700 μatm) CO<sub>2</sub> when tested in the laboratory and this influenced their behaviour and risk of mortality in the wild. Individuals that were sensitive to elevated CO<sub>2</sub> were more active and move further from shelter in natural coral reef habitat and, as a result, mortality from predation was significantly higher compared with individuals from the same treatment that were tolerant of elevated CO<sub>2</sub>. If individual variation in CO<sub>2</sub> tolerance is heritable, this selection of phenotypes tolerant to elevated CO<sub>2</sub> could potentially help mitigate the effects of ocean acidification.

## Keywords

Ocean acidification • Climate change adaptation • Phenotypic selection • Coral reef fish • Behavioural impairment

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## Introduction

Ocean acidification, caused by the uptake of additional carbon dioxide (CO<sub>2</sub>) from the atmosphere, is occurring at rate unprecedented in the recent geological past (Doney *et al.* 2009). If CO<sub>2</sub> emissions continue on the current trajectory, atmospheric CO<sub>2</sub> could exceed 900ppm by year 2100 (Meinshausen *et al.* 2011), and the associated uptake of CO<sub>2</sub> at the ocean surface would cause a reduction in average ocean pH of 0.3–0.4 units compared to present-day levels (The Royal Society 2005). An ever-increasing number of experimental studies have demonstrated that changes in ocean chemistry associated with CO<sub>2</sub> levels predicted for the atmosphere and surface ocean by the end of the century can have significant effects on the reproduction, growth, development, behaviour and survival of marine organisms (Fabry *et al.* 2008; Doney *et al.* 2009). However, a critical unanswered question is the capacity of species to adapt to changes in ocean chemistry (Pandolfi *et al.* 2011; Sunday *et al.* 2011). Although rapid by geological standards, ocean acidification will occur over a number of decades, and more importantly, over multiple generations for many marine species. Furthermore, individual variation in the response to elevated CO<sub>2</sub> and reduced pH has been observed in many ocean acidification experiments (e.g. Ries *et al.* 2009; Munday *et al.* 2010; Parker *et al.* 2011; Pistevos *et al.* 2011; Sunday *et al.* 2011), with some individuals appearing more tolerant to CO<sub>2</sub>-induced acidification than others. This suggests there is

potential for the selection of more CO<sub>2</sub> resistant phenotypes over coming decades. Demonstrating that selection of CO<sub>2</sub> resistant phenotypes occurs in nature would be an important step toward understanding the potential for adaptation to ocean acidification in marine organisms.

Recent studies show that the behaviour and sensory capabilities of juvenile reef fish are seriously impaired by exposure to elevated CO<sub>2</sub> during their pelagic larval stage (Munday *et al.* 2009; Munday *et al.* 2010; Ferrari *et al.* 2011; Simpson *et al.* 2011; Domenici *et al.* 2012), leading to higher mortality from predators in natural coral-reef habitat (Munday *et al.* 2010; Ferrari *et al.* 2011). The cause of these behavioural abnormalities appears to be impaired neurotransmitter function due to fish altering ion concentrations to prevent tissue acidosis when exposed to high CO<sub>2</sub> (Nilsson *et al.* 2012). However, considerable variation in individual responses has been observed at some CO<sub>2</sub> levels. For example, behavioural responses of larval fish to predator odour and prey alarm cues is highly variable at ~700 μatm CO<sub>2</sub> (Munday *et al.* 2010; Ferrari *et al.* 2011). In contrast, behaviour of fish is unaffected at ~550 μatm CO<sub>2</sub> and behaviour of all fish is strongly affected at ~850 μatm CO<sub>2</sub>, with little variation among individuals (Munday *et al.* 2010; Ferrari *et al.* 2011). This suggest that selection for CO<sub>2</sub> tolerant individuals could be especially pronounced around 700 μatm CO<sub>2</sub>. The transition of larvae from the pelagic environment to benthic habitat is a severe demographic bottleneck for reef fishes (Almany and Webster 2006), and selection for traits promoting juvenile survivorship is

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known to occur at this time (Gagliano *et al.* 2007). Therefore, this could be an important period for selection of individuals with greater CO<sub>2</sub> tolerance.

The aim of this study was to test if variation in individual sensitivity of juvenile reef fishes to elevated CO<sub>2</sub> could lead to selection of CO<sub>2</sub> tolerant phenotypes in nature. We focused on fish exposed to ~700µatm because this is where the greatest among-individual variation in behavioural responses to elevated CO<sub>2</sub> has been observed, and therefore where the potential for selection for CO<sub>2</sub> tolerant individuals should be greatest. First, we exposed wild-caught larvae of the coral-reef damselfish *Pomacentrus wardi* to either 425µatm pCO<sub>2</sub> (current-day control) or 700µatm pCO<sub>2</sub>. The sensitivity of individual damselfish larvae to elevated CO<sub>2</sub> was then evaluated by testing their response to predator odour using a two-channel flume chamber (Munday *et al.* 2009; Munday *et al.* 2010). Based on the response exhibited in the flume, fish were assigned to affected and unaffected groups. Individuals from both these groups, and current-day controls, were then placed on natural coral-reef habitat in the field where their behaviour was assessed and mortality rate of the different groups monitored over a 70-hour period. This enabled us to both test for selection of CO<sub>2</sub> tolerant phenotypes during a critical life-history transition and to identify the behavioural traits associated with differential mortality.

## Methods

### Fish collection and maintenance

Settlement-stage *Pomacentrus wardi* (16-21 days old) larvae were caught overnight in light traps at Lizard Island, Great Barrier Reef, Australia (14°41'S, 145°27'E), during November 2010. Each morning, *P. wardi* collected in the traps were transferred to replicate 35 l rearing aquariums treated with either control (425µatm) or elevated CO<sub>2</sub> (700µatm). Previous experiments have demonstrated that the behavioural effects of elevated CO<sub>2</sub> are manifest within 4 days of exposure and that longer durations of exposure do not alter behavioural responses (Munday *et al.* 2010; Ferrari *et al.* 2011; Simpson *et al.* 2011; Domenici *et al.* 2012), therefore larvae were maintained in treatments for four consecutive days. Larvae were fed *Artemia* nauplii four times daily.

### CO<sub>2</sub> treatments

Seawater was pumped from the ocean into 60 L sumps where it was diffused with ambient air (control) or CO<sub>2</sub>. A pH-controller (Tunze Aquarientechnik, Germany) maintained pH at the desired level. Equilibrated seawater from each sump supplied four replicate 35L aquariums, each housing a small group of larval fishes. Temperature and pH<sub>NBS</sub> of each aquarium was measured

twice daily with a HQ40d pH meter (Hach, Colorado, USA). Total alkalinity of seawater was estimated by Gran titration from samples taken twice weekly from control and treatment tanks. Alkalinity standardizations achieved accuracy within 1% of certified reference material (Dr. A. Dickson, Scripps Institution of Oceanography). Average seawater pCO<sub>2</sub> was calculated in the program CO2SYS using the constants of Mehrbach *et al.* (1973) refit by Dickson and Millero (1987). Seawater parameters are shown in Table 1.

### Sensitivity to elevated CO<sub>2</sub>

Following CO<sub>2</sub> treatment, the sensitivity of individual damselfish to elevated CO<sub>2</sub> was tested using a two-channel flume chamber (Munday *et al.* 2009). Larval and juvenile reef fishes reared at 700µatm CO<sub>2</sub> exhibit a bimodal response to predator odour; some individuals are strongly repelled from the odour, as are control fish, whereas other individuals exhibit a maladaptive attraction to predator odour (Munday *et al.* 2010). Therefore, testing the response of individuals to predator odour is a simple way to determine their sensitivity to elevated CO<sub>2</sub>. Flume trials were conducted using standard protocols (Munday *et al.* 2009; Munday *et al.* 2010). Briefly, one channel of the flume chamber received seawater containing the chemical cues of *Pseudochromis fuscus*, a common predator of newly-settled fish. The other channel received seawater with no additional chemical cues. Fish were released at the downstream end of the flume where they were free to move to either side or swim toward the preferred water source. After a two-minute acclimation period, the position of the fish was recorded at 5-sec intervals for a 2-min period by an observer positioned behind the flume. The water sources were switched and the test repeated. The same observer (DLD) made all observations. At the end of each trial fish were classified as affected or unaffected by elevated CO<sub>2</sub> depending on their response to the predator odour. An additional 20 fish were retested after 5h and 10h to confirm that individual responses exhibited in the flume were retained.

### Field experiment

Following testing in the flume, fish were released individually onto small coral reefs (18 × 12 × 12 cm) constructed on a shallow sandflat at Lizard Island. A wire cage over the patch allowed fish to adjust to their surroundings while being protected from predators. Cages were removed 30 to 60 min later, between 09:00 and 11:00. Behaviour was assessed for a 3 min period after the cage was removed. Six behaviours were estimated: (1) bite rate, (2) total distance moved, (3) mean distance ventured from shelter, (4) maximum distance ventured from shelter, (5)

Table 1. Mean (±SD) seawater parameters in the experimental system.

pH <sub>NBS</sub>	Temp (°C)	Salinity (ppt)	TA (µmol.kg <sup>-1</sup> SW)	pCO <sub>2</sub>
8.16 (0.04)	27.64 (1.07)	35	2269.66 (15.01)	425.42 (45.28)
7.98 (0.05)	27.57 (1.02)	35	2259.87 (11.55)	703.60 (94.06)

height above the substratum, and (6) boldness scored on a continuous ranking scale from 0-3 (see Munday *et al.* 2010, Ferrari *et al.* 2011 for details). The presence of fish on patch reefs was assessed 2-3 times per day for 70 h. Missing fish were presumed dead as newly recruited juveniles are highly sedentary and previous studies using tagged fish have indicated negligible migration from similar patch reefs (Hoey and McCormick 2004, McCormick 2009). All fieldwork was conducted blind to the CO<sub>2</sub> treatment and the assignment of individuals as affected or unaffected by elevated CO<sub>2</sub>.

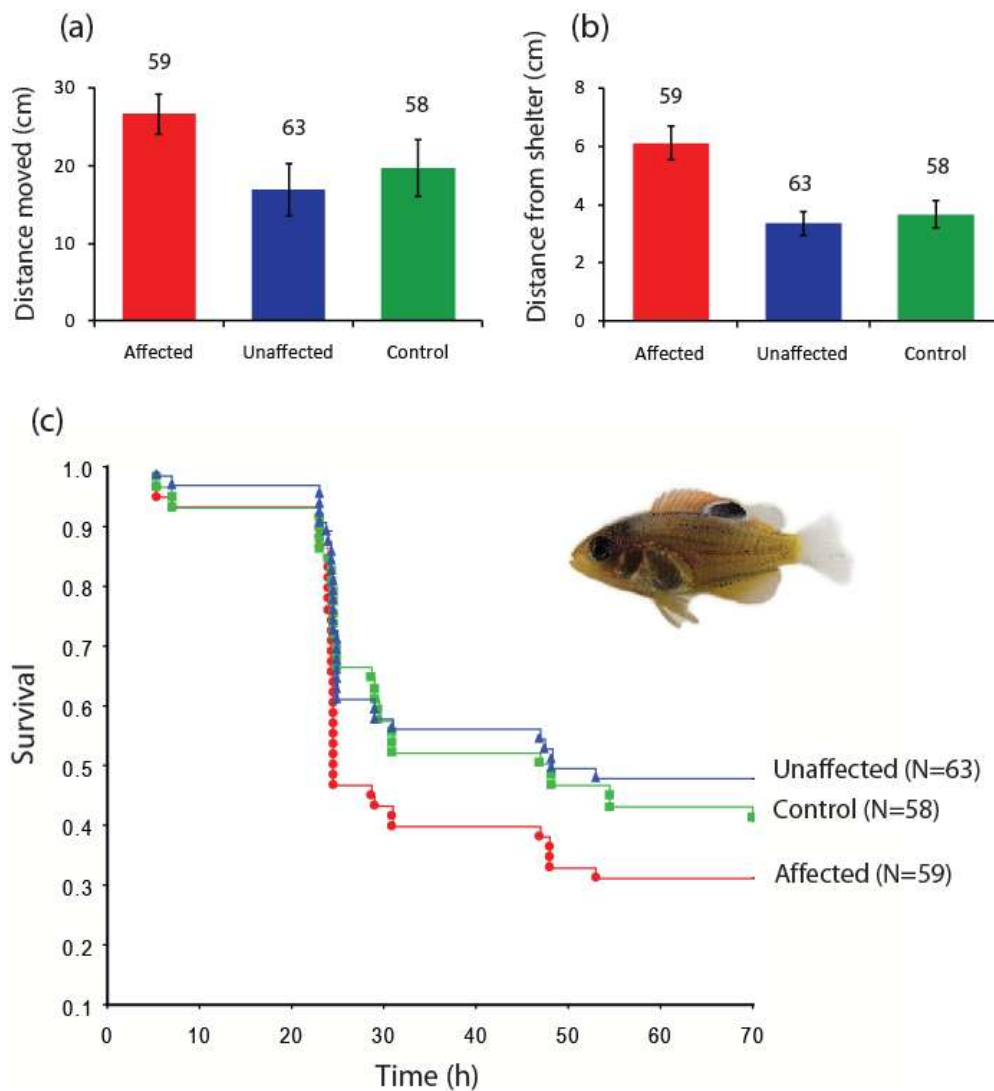
### Analysis

The affect of CO<sub>2</sub> treatment on behaviour was tested with MANOVA, using log<sub>10</sub>(x+1) transformed data. ANOVA and

Tukey's HSD post-hoc tests were then used to determine the nature of the significant difference found by MANOVA. Multiple sample survival analysis was used to compare survival rates among treatment groups. A Cox F-test then compared survival between CO<sub>2</sub> affected and unaffected fish. Survival trajectories were displayed with a Kaplan-Meier plot.

### Results and discussion

All control larvae (N=58) strongly avoided the predator odour in the flume (<2% time in cue water on average). Approximately half (N = 63) of the 700 $\mu$ atm CO<sub>2</sub> treated larvae also exhibited a strong aversion to the predator odour (i.e. were unaffected by CO<sub>2</sub> treatment), whereas the other half (N= 59) exhibited a strong



**Figure 1.** Selective mortality in coral-reef habitat favours juvenile fish tolerant to elevated CO<sub>2</sub>. Total distance moved (a), maximum distance ventured from shelter (b), and survival (c) of juvenile damselfish placed on patch reefs following exposure to elevated (700  $\mu$ atm) or control (425  $\mu$ atm) CO<sub>2</sub> for 4 days. Fish exposed to elevated CO<sub>2</sub> were classified as either affected (red) or unaffected (blue) by high CO<sub>2</sub> prior to being placed on patch reefs. Fish exposed to control CO<sub>2</sub> are shown in green. Values are mean  $\pm$  SE.

attraction to the odour (i.e. were affected by CO<sub>2</sub> treatment), as has been observed previously (Dixon *et al.* 2010, Munday *et al.* 2010). Individual preferences were always retained when larvae were retested 5h and 10h later (N= 20). When placed in natural coral-reef habitat, the behaviour of affected fish differed significantly from unaffected fish (MANOVA: Pillai's trace =0.22, F<sub>10,346</sub>=4.37, p<0.001). Affected fish were more active (ANOVA: F<sub>2,176</sub>=9.19, p<0.001) and moved a greater distance from shelter (ANOVA: F<sub>2,176</sub>=9.86, p<0.001) than non-affected fish (Figure 1a-b). No other behaviours differed between affected and unaffected fish in the 700µatm CO<sub>2</sub> treatment. There were no behavioural differences between fish unaffected by 700µatm CO<sub>2</sub> and control fish (Figure 1a-b). Mortality in the first 70 hours on the reef was significantly higher in affected fish compared with unaffected fish (Cox-F test: F<sub>66,86</sub>=1.81, p=0.005). 44% of control and 49% of unaffected fish survived, whereas only 32% of affected fish survived (Figure 1c). Mortality patterns were established in the first 24hrs and maintained for the duration of the experiment. A suite of resident fishes that prey on newly-recruited juveniles were the agent of mortality.

These results demonstrate that rapid selection of CO<sub>2</sub>-tolerant phenotypes can occur in nature. If the individual variation in CO<sub>2</sub> tolerance in reef fish observed here, and in previous studies (Munday *et al.* 2010; Ferrari *et al.* 2011), is heritable then we might expect that fish populations will exhibit adaptation to elevated CO<sub>2</sub> through time. Like most marine species, coral-reef fishes are highly fecund, spawn repeatedly, reproduce over many seasons, and only a small fraction of offspring survive to become juveniles. Consequently, there is considerable potential for selection to favour populations dominated by CO<sub>2</sub> tolerant individuals over coming decades. Furthermore, most reef fishes have very large populations, which increases genetic variation and the number of breeding individuals likely to produce favourable genotypes

Establishing that rapid selection for CO<sub>2</sub>-tolerance occurs in nature is an important step towards understanding the capacity

for adaptation to rising CO<sub>2</sub> in the ocean. A necessary next step would be to show that the variation in CO<sub>2</sub> sensitivity observed here in juvenile fish is heritable. The variation in response to elevated CO<sub>2</sub> we observed in the flume does appear to have at least some genetic basis, as full-sibs reared under identical conditions in the laboratory exhibit the same variation in CO<sub>2</sub> sensitivity (Munday *et al.* 2010). Nevertheless, quantitative genetic analyses, such as comparisons of parent-offspring or half-sib variation will be required to estimate heritability (Pandolfi *et al.* 2011; Sunday *et al.* 2011). Importantly, we demonstrate that differential mortality associated with CO<sub>2</sub> tolerance occurs at a critical demographic bottleneck for reef fishes, when fewer than 50% of individuals survive for more than 48 hours (Almany and Webster 2006; this study). The correlation of enhanced survivorship of some phenotypes with a strong demographic bottleneck should enhance the establishment of CO<sub>2</sub>-favourable individuals in the population, even if heritability for the trait is low.

Variation in individual responses to elevated CO<sub>2</sub> and reduced pH has been observed in experiments with other fish species (Munday *et al.* 2010; Ferrari *et al.* 2011) and other marine organisms (Ries *et al.* 2009; Parker *et al.* 2011; Pistevos *et al.* 2011; Sunday *et al.* 2011). Our study shows that this variability provides the raw material for selection to act upon, with the potential to mitigate some biological effects of rapidly rising CO<sub>2</sub> levels. However, the heritability of phenotypic variation in CO<sub>2</sub> responses requires testing, and genetic trade-offs may limit the scope for adaptation, or make species more susceptible to other stresses (Pandolfi *et al.* 2011). Furthermore, variation in individual response to elevated CO<sub>2</sub> declines dramatically at higher CO<sub>2</sub> levels (>800 µatm CO<sub>2</sub>) (Munday *et al.* 2010; Ferrari *et al.* 2011), consequently the strength of selection may also decline markedly at more extreme CO<sub>2</sub> levels. Understanding the potential for species to adapt to rapidly rising CO<sub>2</sub> levels is critical for predicting the biological impacts of ocean acidification and should be a priority area for future research.

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