Selective mortality associated with variation in CO$_2$ tolerance in a marine fish

Abstract
Predicted future CO$_2$ 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$_2$ tolerance in a wild population of coral-reef fishes. Juvenile damselfish exhibited variation in their response to elevated (700 µatm) CO$_2$ when tested in the laboratory and this influenced their behaviour and risk of mortality in the wild. Individuals that were sensitive to elevated CO$_2$ 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$_2$. If individual variation in CO$_2$ tolerance is heritable, this selection of phenotypes tolerant to elevated CO$_2$ 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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Received 02 May 2012
Accepted 27 August 2012

Introduction
Ocean acidification, caused by the uptake of additional carbon dioxide (CO$_2$) from the atmosphere, is occurring at rate unprecedented in the recent geological past (Doney et al. 2009). If CO$_2$ emissions continue on the current trajectory, atmospheric CO$_2$ could exceed 900ppm by year 2100 (Meinshausen et al. 2011), and the associated uptake of CO$_2$ 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$_2$ 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$_2$ 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$_2$-induced acidification than others. This suggests there is potential for the selection of more CO$_2$ resistant phenotypes over coming decades. Demonstrating that selection of CO$_2$ 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$_2$ 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$_2$ (Nilsson et al. 2012). However, considerable variation in individual responses has been observed at some CO$_2$ levels. For example, behavioural responses of larval fish to predator odour and prey alarm cues is highly variable at ~700µatm CO$_2$ (Munday et al. 2010; Ferrari et al. 2011). In contrast, behaviour of fish is unaffected at ~550µatm CO$_2$ and behaviour of all fish is strongly affected at ~850µatm CO$_2$, with little variation among individuals (Munday et al. 2010; Ferrari et al. 2011). This suggest that selection for CO$_2$ tolerant individuals could be especially pronounced around 700µatm CO$_2$. 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...
The aim of this study was to test if variation in individual sensitivity of juvenile reef fishes to elevated CO₂ could lead to selection of CO₂ 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 CO₂ has been observed, and therefore where the potential for selection for CO₂ tolerant individuals should be greatest. First, we exposed wild-caught larvae of the coral-reef damselfish Pomacentrus wardi to either 425µatm pCO₂ (current-day control) or 700µatm pCO₂. The sensitivity of individual damselfish larvae to elevated CO₂ 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 CO₂ 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 CO₂ (700µatm). Previous experiments have demonstrated that the behavioural effects of elevated CO₂ 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.

CO₂ treatments
Seawater was pumped from the ocean into 60 L sumps where it was diffused with ambient air (control) or CO₂. 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 were 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 pCO₂ 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 CO₂
Following CO₂ treatment, the sensitivity of individual damselfish to elevated CO₂ was tested using a two-channel flume chamber (Munday et al. 2009). Larval and juvenile reef fishes reared at 700µatm CO₂ 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 CO₂. 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 CO₂ 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>
<thead>
<tr>
<th>pH</th>
<th>Temp (ºC)</th>
<th>Salinity (ppt)</th>
<th>TA (μmol.kg⁻¹SW)</th>
<th>pCO₂ (µatm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.16 (0.04)</td>
<td>27.64 (1.07)</td>
<td>35</td>
<td>2269.66 (15.01)</td>
<td>425.42 (45.28)</td>
</tr>
<tr>
<td>7.98 (0.05)</td>
<td>27.57 (1.02)</td>
<td>35</td>
<td>2259.87 (11.55)</td>
<td>703.60 (94.06)</td>
</tr>
</tbody>
</table>

Table 1. Mean (±SD) seawater parameters in the experimental system.
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 CO₂ treatment and the assignment of individuals as affected or unaffected by elevated CO₂.

**Analysis**
The affect of CO₂ treatment on behaviour was tested with MANOVA, using log10(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 CO₂ 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 700µatm CO₂ treated larvae also exhibited a strong aversion to the predator odour (i.e. were unaffected by CO₂ treatment), whereas the other half (N= 59) exhibited a strong

![Figure 1. Selective mortality in coral-reef habitat favours juvenile fish tolerant to elevated CO₂. 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 µatm) or control (425 µatm) CO₂ for 4 days. Fish exposed to elevated CO₂ were classified as either affected (red) or unaffected (blue) by high CO₂ prior to being placed on patch reefs. Fish exposed to control CO₂ are shown in green. Values are mean ± SE.](image)
attraction to the odour (i.e. were affected by CO₂ 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_{0,34}=4.37, p<0.001). Affected fish were more active (ANOVA: F_{2,176}=9.19, p<0.001) and moved a greater distance from shelter (ANOVA: F_{2,176}=9.86, p<0.001) than non-affected fish (Figure 1a-b). No other behaviours differed between affected and unaffected fish in the 700µatm CO₂ treatment. There were no behavioural differences between fish unaffected by 700µatm CO₂ 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_{66,86}=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 CO₂-tolerant phenotypes can occur in nature. If the individual variation in CO₂ 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 CO₂ 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 CO₂ 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 CO₂-tolerance occurs in nature is an important step towards understanding the capacity for adaptation to rising CO₂ in the ocean. A necessary next step would be to show that the variation in CO₂ sensitivity observed here in juvenile fish is heritable. The variation in response to elevated CO₂ 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 CO₂ 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 CO₂ 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 CO₂-favourable individuals in the population, even if heritability for the trait is low.

Variation in individual responses to elevated CO₂ 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 CO₂ levels. However, the heritability of phenotypic variation in CO₂ 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 CO₂ declines dramatically at higher CO₂ levels (>800 µatm CO₂) (Munday et al. 2010; Ferrari et al. 2011), consequently the strength of selection may also decline markedly at more extreme CO₂ levels. Understanding the potential for species to adapt to rapidly rising CO₂ levels is critical for predicting the biological impacts of ocean acidification and should be a priority area for future research.

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