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LETTER

Putting prey and predator into the CO_2 equation – qualitative and quantitative effects of ocean acidification on predator–prey interactions

Maud C. O. Ferrari[1]*, Mark I. McCormick[2], Philip L. Munday[2], Mark G. Meekan[3], Danielle L. Dixson[2], Öona Lonnstedt[2] and Douglas P. Chivers[4]

Abstract

Little is known about the impact of ocean acidification on predator–prey dynamics. Herein, we examined the effect of carbon dioxide (CO_2) on both prey and predator by letting one predatory reef fish interact for 24 h with eight small or large juvenile damselfishes from four congeneric species. Both prey and predator were exposed to control or elevated levels of CO_2. Mortality rate and predator selectivity were compared across CO_2 treatments, prey size and species. Small juveniles of all species sustained greater mortality at high CO_2 levels, while large recruits were not affected. For large prey, the pattern of prey selectivity by predators was reversed under elevated CO_2. Our results demonstrate both quantitative and qualitative consumptive effects of CO_2 on small and larger damselfish recruits respectively, resulting from CO_2-induced behavioural changes likely mediated by impaired neurological function. This study highlights the complexity of predicting the effects of climate change on coral reef ecosystems.

Keywords

Carbon dioxide, coral reef fishes, mesocosm experiment, mortality rate, ocean acidification, predator–prey interaction, selectivity.

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INTRODUCTION

Oceans are acidifying through increased levels of dissolved carbon dioxide (CO_2) from anthropogenic sources (Sabine *et al.* 2004; Doney *et al.* 2009). Elevated CO_2 in the tissues, or hypercapnia, not only impacts calcifying organisms, but also non-calcifying animals such as fishes (Kroeker *et al.* 2010). While we are starting to understand the impacts of increased dissolved CO_2 on the physiology of individuals (Portner & Farrell 2008), we have little understanding of how these impacts may scale up to affect assemblages, communities and ecosystems (e.g. Wootton *et al.* 2008; National Science Fundation 2010). A few studies have investigated the effects of ocean acidification on interspecific interactions involving a variety of taxa (Wootton *et al.* 2008). Both Munday *et al.* (2010) and Ferrari *et al.* (2011) have shown that coral reef fish juveniles exposed in the laboratory to elevated CO_2 and released in their native habitat suffered 5- to 8-fold increases in predation-related mortality compared to controls. CO_2 has also been shown to cause behavioural alterations in antipredator behaviour (Dixson *et al.* 2010; Munday *et al.* 2010; Ferrari *et al.* 2011). These studies provide us with insights into foreseeable effects, but do little to inform us about how predator–prey interactions may be altered, given that so far, predators have been kept out of the equation.

Our goal was to investigate how predator–prey interactions might be affected by elevated CO_2 conditions. While we know from previous studies that prey may suffer increased mortality under elevated CO_2 conditions through elevated activity and boldness (Munday *et al.* 2010), this effect may be negated by a lower foraging performance of predators, for instance. Our study examined this question in the context of one of the world's most species diverse ecosystems, coral reefs of the Great Barrier Reef, Australia. Most coral reef fishes have a pelagic larval stage that resides in the plankton for a period of weeks to months (Leis 2007). At the end of this phase, juvenile fish must locate suitable benthic habitat and in doing so, face a new and abundant array of predatory reef fishes. Predators may remove at least 60% of newly settling fish in a single night (Almany & Webster 2006), creating population bottlenecks. At this critical life phase, predators are often selective for the attributes of prey, such as size (Holmes & McCormick 2009, 2010) and species (Almany & Webster 2006). In the days immediately prior to settlement, juvenile fish can be captured away from the reef in large numbers using light traps (Meekan *et al.* 2001). Although they have juvenile form and colouration, these individuals are naïve to the suite of predators that await them on the reef. Hence, these coral reef fishes provide us with a unique opportunity to examine interactions between predator and prey at a life stage that will likely be under intense selection pressure for CO_2-tolerant phenotypes.

In the present study, we examined whether or not levels of dissolved CO_2, predicted to occur by 2100, influenced the outcomes of predator–prey interactions of coral reef fishes. Specifically, we explored whether or not elevated CO_2 affected the selectivity of a common predator for prey species, and the extent to which prey size

[1]*Department of Biomedical Sciences, WCVM, 52 Campus Drive, Saskatoon, SK S7N 5B4, Canada*
[2]*ARC Centre of Excellence for Coral Reef Studies, and School of Marine and Tropical Biology, James Cook University, Townsville, Qld 4811, Australia*
[3]*Australian Institute of Marine Science, UWA Ocean Sciences Centre (MO96), Crawley, WA 6009, Australia*
[4]*Department of Biology, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada*
**Correspondence: E-mail: maud.ferrari@usask.ca*

influenced this selection. To achieve this, we performed a mesocosm experiment in which one common adult predator, the dottyback *Pseudochromis fuscus*, was allowed to interact in a semi-natural system for 24 h with eight damselfish juveniles, two from each of four species (*Pomacentrus moluccensis*, *P. amboinensis*, *P. nagasakiensis* and *P. chrysurus*). Trials were run for large and small predator-naive juveniles. Prior to the trial, both prey and predator were exposed to control (440 μatm) or elevated CO_2 levels (700 μatm) for a duration that has been found to result in behavioural responses that mirror individuals reared from embryos under these CO_2 conditions (Munday *et al.* 2010). Mortality rate and predator selectivity were compared across CO_2 treatments, juvenile size-class and species.

METHODS

Fish collection and CO_2 treatment

The experiment took place at Lizard Island Research Station (14°40′ S, 145°28′ E), on the Great Barrier Reef, Australia, in November and December 2010. Juvenile *P. moluccensis, P. amboinensis, P. nagasakiensis* and *P. chrysurus* (16–21 days old) were caught overnight using light traps (Meekan *et al.* 2001) moored *c.* 100 m off the reef at Lizard Island during the recruitment pulses. The core of these pulses lasts about 10 days and coincides with the new moon. These traps collect fish at the end of their pelagic phase, immediately prior to their settlement of the reef (Meekan *et al.* 1993). Every morning during the pulse, fish caught in the traps were brought back to the station, sorted by species, and eight individuals of each of the four species were transferred into 35-L aquaria, with four individuals per species (16 individuals total) placed in 700 μatm CO_2 treatment while the others were placed in 440 μatm CO_2. The fish were fed freshly hatched *Artemia nauplii* three times a day. Towards the end of the pulse, additional fish were stocked in 40-L flow-through bin to be used in our experiment once the recruitment pulse stopped. Previous experiments have demonstrated that the behavioural effects of elevated CO_2 are manifest within 4 days of exposure to relevant CO_2 treatments, and that longer durations of exposure do not further alter behavioural responses (Munday *et al.* 2010), therefore larvae were maintained in the CO_2 treatments for four consecutive days.

The exposure to CO_2 was rather rapid. However, previous studies have shown that Pomacentrid larvae exposed to elevated CO_2 over a few days showed identical behavioural impairment as larvae raised under the same CO_2 levels from birth (Munday *et al.* 2010), indicating that the alterations in behaviour were not due to a sudden CO_2 exposure. Alternative methods of exposure (gradual increases in CO_2 levels over several weeks to months) would prevent us from testing juvenile recruits. Their bipartite life history makes them suitable candidates for these exposures, because: (1) the larvae are coming from the open 'buffered' ocean to the coral reefs, inhabiting microhabitats (inside coral branches) that will naturally show elevated CO_2 concentrations (Gagliano *et al.* 2010) and (2) this life history transition occurs when the population is subject to a severe predation-induced bottleneck. This means this transition is likely to be occurring at the point in time when most of the CO_2-tolerance phenotypic selection will occur (Munday *et al.* 2010). Nevertheless, this experimental setup does not account for the potential adaptation or selection that may occur over generations.

Adult predatory dottybacks were captured from a lagoon using hand nets and dilute clove oil. These were brought back to the research station, kept individually in mesh baskets placed in flow-through tanks and fed daily with squid pieces and fish pellets. Dottybacks underwent the same CO_2 treatment protocol as the damselfishes, but were always kept in separate tanks from the damselfishes during conditioning. While treated with CO_2, each dottyback received six fish food pellets daily for the 4-day duration of the exposure.

CO_2 treatments were maintained by CO_2 dosing to a set pH_{NBS}, following standard techniques for ocean acidification research, as set out in the Best Practices Guides for Ocean Acidification Research (Gattuso *et al.* 2010). Seawater was pumped from the ocean into 2 × 60 L sumps where it was diffused with ambient air (control) or CO_2 to achieve a pH of *c.* 8.15 (control) and 7.97. The reduced pH values were selected to achieve the approximate CO_2 conditions required, based on preliminary observations of total alkalinity, salinity and temperature of seawater at Lizard Island. A pH-controller (Tunze Aquarientechnik, Penzberg, Germany) was attached to each of the CO_2 treated sumps to maintain pH at the desired level. A solenoid injected a slow stream of CO_2 into a powerhead at the bottom of the sump whenever the pH of the seawater rose above the set point. The powerhead rapidly dissolved CO_2 into the seawater and also served as a vigorous stirrer. Equilibrated seawater from each sump was supplied at a rate of *c.* 500 mL s^{-1} to four replicate 35-L aquariums, each housing a group of larval fishes or predators. To maintain oxygen levels and the required *p*CO2 levels, aquariums were individually aerated with air (control *c.* 440 μatm) or CO_2-enriched air (*c.* 700 μatm). The concentration of CO_2-enriched air was controlled by a scientific-grade pressure regulator and precision needle valve and measured continuously with an infrared CO_2 probe (Vaisala GM70, Vaisala, Helsinki, Finland). Temperature and pH_{NBS} of each aquarium was measured each morning and afternoon using an HQ40d pH metre (Hach, Loveland, CO, USA) calibrated with fresh buffers. Total alkalinity of seawater was estimated by Gran titration from water samples taken twice weekly from each CO_2 treatment. Alkalinity standardisations performed before processing each batch achieved accuracy within 1% of certified reference material from Dr. A. Dickson (Scripps Oceanographic Institute). Average seawater *p*CO2 was calculated using these parameters in the programme CO2SYS and using the constants of Mehrbach *et al.* (1973) refit by Dickson & Millero (1987). Estimated seawater parameters are shown in Table 1.

Experimental setup

Following the 4-day CO_2 conditioning, eight randomly chosen individuals (two of each species) from each of the two CO_2 treatments were placed in separate flow-through mesocosm pools (111 cm diameter, 45 cm high, 368 L) containing a 1-cm deep sand substrate, two air-stones, and two pieces of live bushy hard coral (*Pocillopora damicornis*) placed beside each other. These two pieces formed a coral patch of *c.* 90 cm in circumference and *c.* 20 cm in height. The water was pumped directly from the ocean so it followed natural temperature fluctuations. One hour after the introduction of the damselfish, we introduced a dottyback of matching CO_2 treatment. Hence, the pool contained prey and predator that were all exposed to 440 μatm CO_2 or all exposed to 700 μatm CO_2. The next day, all the fish were removed from the pool and we recorded the number and species of the surviving damselfishes. The water was drained, the water flow increased, and the pool reset for the next trial. Each day, a 440 and a 700 μatm trial were conducted simultaneously

Table 1 Mean (± SD) seawater parameters in the experimental system. Temperature, pH, salinity and total alkalinity (TA) were measured directly. pCO_2 was estimated from these parameters using CO2SYS.

pH_{NBS}	Temperature (°C)	Salinity (p.p.t.)	TA (μmol kg^{-1}SW)	pCO_2
8.15 (0.04)	27.66 (0.98)	35	2269.66 (15.01)	440.53 (44.46)
7.97 (0.06)	27.59 (0.97)	35	2259.87 (11.55)	718.37 (110.82)

and the location of the CO_2 treatment was switched between days to avoid a pool effect, except for the first 2 days for which only 440 μatm trials were run. The fish were fed twice daily (1100 and 1700 h) with 60 mL of a solution of freshly hatched *Artemia* sp. (*c.* 250 mL^{-1}). *Pocillopora* colonies were replaced every 4–5 days with freshly collected ones. The total number of replicates were 38 (440 μatm) and 34 (700 μatm).

Trials were split into two groups based on larvae size, one group containing trials where damselfish larvae were placed directly into the CO_2 system after capture [mean total length (TL) of the damselfish in the mesocosm < 14.5 mm; $N = 18$ and 14 for 440 and 700 μatm CO_2 groups respectively] and the other containing the trials with larger fish that had been kept in the laboratory storage tanks for 2–10 days after capture (mean TL ≥ 14.5 mm; $N = 20$ for both 440 and 700 μatm CO_2 groups respectively). The size of the fish did not differ between CO_2 treatments; although fish from different species differed in size, this difference was consistent across size-classes (see Figure S1 for details).

Statistical analyses

Predation rate

We computed species-specific predation rates [No. individual eaten over 24 h/total no. of individuals (2)], which were arcsine-transformed to normalise the data. We performed a two-way repeated-measures MANOVA to test the effect of CO_2 (440 vs. 700 μatm) and prey size-class (small vs. large recruits) on the mortality of each of the four prey species. Individual dottyback were treated as test subjects, making each pool our replicate unit. The repeated-measures approach accounted for the dependency of the mortality rates among species, while still allowing us to compare mortality among species (as per Shoup & Wahl 2009).

Predator selectivity

We also computed a prey selectivity index for *P. fuscus* following Chesson (1983):

$$\hat{\alpha}_i = \frac{r_i/n_i}{\sum_{j=1}^{m}(r_j/n_j)}, \quad i = 1, \ldots, m$$

where n_i is the number of prey type i at the beginning of the experiment, r_i is the number of prey type i consumed by the predator, and j is the number of different prey types. This selectivity can be interpreted as the preference of the predator for a prey type relative to the average preference for alternative prey types. The selectivity value ranges from 0 (total avoidance of prey type) to 1 (only prey type selected). If all four prey species are selected equally by the predator, the selectivity for each prey species is 0.25. Trials where predators ate none of the prey species ($N = 17$ across all treatments, 13 of which were in the large class recruits) were removed, given that no selectivity could be computed. Selectivity values were arcsine-transformed to normalise the data. Similarly to the approach for mortality, we performed a two-way repeated-measures MANOVA to test the effect of CO_2 and prey size-class on the selectivity of the predator for each of the four prey species.

RESULTS

Predation rate

Predation rate was not influenced by CO_2 treatment (two-way repeated measures MANOVA: $F_{1,68} = 3.7$, $P = 0.059$), but was significantly affected by prey size-class ($F_{1,68} = 10.9$, $P = 0.002$) and a significant $CO_2 \times$ prey size-class interaction ($F_{1,68} = 7.1$, $P = 0.009$, Fig. 1) was found. Within-subject effects revealed no effects of species ($F_{3,204} = 0.3$, $P = 0.80$), no species × prey size-class interaction ($F_{3,204} = 0.9$, $P = 0.97$), no species × CO_2 interaction ($F_{3,204} = 0.4$, $P = 0.77$) and no species × prey size-class × CO_2 interaction ($F_{3,204} = 1.1$, $P = 0.34$).

To investigate the interaction between $CO_2 \times$ prey size-class, we performed a similar analysis on each size-class separately. For small recruits, mortality rate was significant affected by CO_2 treatment ($F_{1,30} = 8.9$, $P = 0.006$, Fig. 1), but there was no difference among species ($F_{3,90} = 0.3$, $P = 0.86$), and no species × CO_2 interaction ($F_{3,90} = 0.3$, $P = 0.86$). The smaller prey size-classes suffered increased mortality rate under high CO_2 concentrations, and this pattern was similar for all four species (Fig. 1).

For larger recruits, we found no significant effect of CO_2 ($F_{1,38} = 0.3$, $P = 0.57$), no effect of species ($F_{3,114} = 0.1$, $P = 0.97$), and no species × CO_2 interaction ($F_{3,114} = 1.7$, $P = 0.18$, Fig. 1). Large recruits did not suffer differential mortality due to the CO_2 treatment and this pattern was not different among species.

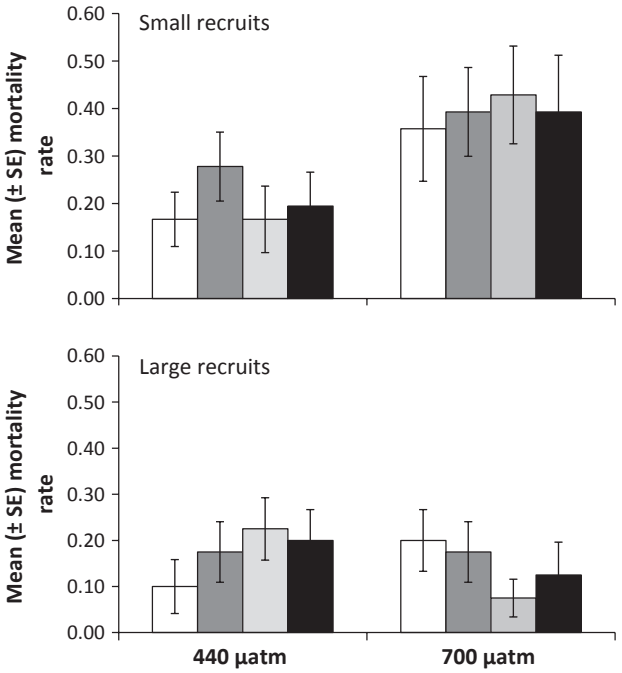

Figure 1 Mean (± SE) mortality rate (proportion consumed over 24 h) suffered by *Pomacentrus moluccensis* (white bars), *P. amboinensis* (dark grey bars), *P. nagasakiensis* (light grey bars) and *P. chrysurus* (black bars), according to size-classes (small and large damselfishes) and CO_2 conditions (440 and 700 μatm).

Predator selectivity

We found no effect of species (two-way repeated measure ANOVA: $F_{3,49} = 0.2$, $P = 0.92$), no species × CO_2 interaction ($F_{3,49} = 2.0$, $P = 0.13$), no species × prey size-class interaction ($F_{3,49} = 1.0$, $P = 0.96$), and no species × CO_2 × prey size-class interaction ($F_{3,49} = 2.3$, $P = 0.086$, Fig. 2) on selectivity. Selectivity was also not affected by CO_2 ($F_{3,51} = 1.1$, $P = 0.31$), or prey size-class ($F_{3,51} = 2.2$, $P = 0.15$), but we found a significant interaction between CO_2 and prey size-class ($F_{3,51} = 4.9$, $P = 0.031$).

Following this interaction, we looked at the effect of CO_2 on the selectivity of species for each size-class separately. For small-size class recruits, all species were equally selected by *P. fuscus* (species × CO_2: $F_{3,24} = 0.4$, $P = 0.75$, Fig. 2). In contrast, selectivity by *P. fuscus* on large damselfish larvae switched between the CO_2 treatments (species × CO_2: $F_{3,2} = 3.6$, $P = 0.028$; Fig. 2).

DISCUSSION

Predation rates and prey selectivity were impacted by exposure to elevated levels of dissolved CO_2, but the outcome of the interaction was dependent on the size of juvenile prey. Elevated dissolved CO_2 had a numerical (i.e. quantitative) effect on the predator–prey interactions involving small juvenile damselfishes; predation rates were higher under elevated CO_2 than under control conditions and predators did not show species-specific preference, consuming roughly equal numbers of the four species. These results are supported by Almany & Webster (2006) who demonstrated that *P. fuscus* did not show any species-specific selectivity for this size of damselfishes. This non-selective predation occurred under both CO_2 conditions. In contrast, we saw a qualitative effect of elevated CO_2 when the prey damselfishes were slightly larger. CO_2 did not affect the number of prey consumed, but rather, elevated CO_2 affected the composition of the prey assemblage; *P. fuscus* preferentially consumed two species of damselfishes (*P. nagasakiensis* and *P. chrysurus*) under present day CO_2 conditions, but this preference was reversed with elevated CO_2. This study suggests that the outcome of predator–prey interactions will change in fundamental ways, which will influence the relative abundance of species within communities.

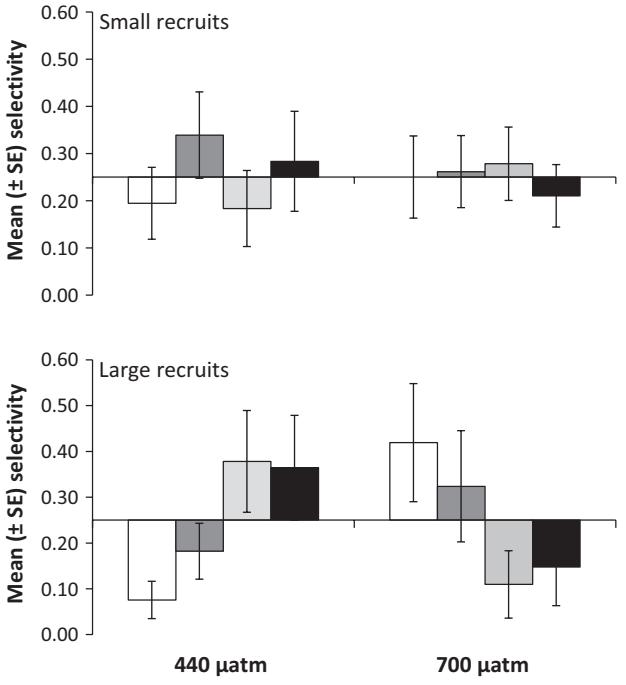

Figure 2 Mean (± SE) selectivity of the predatory dottyback on *Pomacentrus moluccensis* (white bars), *P. amboinensis* (dark grey bars), *P. nagasakiensis* (light grey bars) and *P. chrysurus* (black bars) according to size-classes (small and large damselfishes) and CO_2 conditions (440 and 700 µatm).

Given that predator and prey were matched for their CO_2 treatments, it is not known whether or not changes in predation stem from CO_2 influences on predator, prey or both. Munday *et al.* (2010) showed that exposure to 700 p.p.m. (*c.* 700 µatm) CO_2 led to a fivefold increase in juvenile damselfish mortality in the field. Thus, we know that such CO_2 exposures make damselfishes much more vulnerable to wild (i.e. unaffected) predators. If the negative effects of CO_2 were balanced between prey and predators, we would not expect any change in overall mortality rate. As the present study found that predation rate increased for small damselfishes but not for the larger ones, it is reasonable to suggest that, on average, larger fish are less affected than smaller ones, and that they may be at least as affected as the predators by the CO_2 treatment. Size and/or speed may also compensate for their CO_2 vulnerability.

Evidence suggests that the changes in predator–prey dynamics are due to CO_2-induced changes in behaviour. Munday *et al.* (2009) placed clownfish larvae in a y-maze and let them choose between either the odour of a host anemone or a control odour. They found that larvae exposed to control levels of CO_2 always chose the arm containing the anemone odour, while larvae exposed to *c.* 1000 p.p.m. CO_2 systematically chose the other arm. This phenomenon of preference reversal in a homing context was also found in a predation context. Munday *et al.* (2010) provided juvenile damselfish with a choice between an arm containing the odour of a predator and another arm containing a control odour. Once again, larvae maintained under current day conditions systematically avoided the arm containing the predator odour, while larvae exposed to elevated CO_2 levels displayed reversed preferences. Could this CO_2-induced preference switch explain the qualitative CO_2 effects observed in our study? A recent study using dottybacks in a y-maze supported our findings that CO_2 altered their use of foraging cues (Cripps *et al.* in press). While this hypothesis is consistent with our results, another more likely possibility is that different species may be differently affected by CO_2.

From a prey viewpoint, we know that CO_2 has a detrimental effect by influencing the way they respond to predator cues (Munday *et al.* 2010). Ferrari *et al.* (2011) compared the CO_2 tolerance of the four species used in this experiment. They found that, although phylogenetically very similar, the four congeneric species showed dramatic differences in tolerance. *Pomacentrus amboinensis* was the most affected by CO_2, losing 95% of its antipredator response at 700 p.p.m. while *P. nagasakiensis* was the least affected by CO_2 losing 30% of its antipredator response. These interspecific differences may also explain the switch observed in dottyback foraging, which may have selected the easy prey. Indeed, under elevated CO_2, the preference of *P. nagasakiensis* went down while the preference for *P. amboinensis* went up. Consequently, we are left with two hypotheses explaining the qualitative effect of CO_2 on predator–prey interactions: CO_2 may affect the predator by switching their foraging preference and/or the prey by creating species-specific alterations in antipredator responses. While these hypotheses are not mutually exclusive, additional factorial experiments are needed to tease apart the relative importance of these alternatives.

Recent research showed that exposure to elevated CO_2 affects both olfactory (Munday *et al.* 2009; Dixson *et al.* 2010) and auditory (Simpson *et al.* in press) senses and a diverse range of behavioural activities in larval (Munday *et al.* 2010; Ferrari *et al.* 2011) and adult fishes (Cripps *et al.* in press). Furthermore, a new study by Domenici *et al.* (in press) provides compelling evidence that elevated CO_2 directly affects brain function in larval fishes, because behavioural lateralisation (the propensity for individuals to turn left or right) is impaired by elevated CO_2. The accumulating experimental evidence shows that impaired and altered behaviour following exposure to elevated CO_2 is caused by a systemic effect at the neurological level. Although the precise mechanism is yet to be elucidated, it seems likely associated with ionic changes associated with acid-base regulation (Munday *et al.* 2010; Simpson *et al.* in press). We encourage researchers examining other environmental stressors to consider systemic neurological effects rather than focussing their attention on impaired sensory perception.

To our knowledge, this is the first study to simultaneously compare the effect of CO_2 on both prey and predators. Our results indicate that CO_2 effects may first accentuate the predation-induced bottleneck occurring at the time of settlement in damselfish populations. This increase in consumptive effect may increase the amount of energy going up the food chain, which may alter ecological interactions between upper level species. As the recruits grow, the consumptive effect of predators may return to current day levels, however, the pattern of prey species may be shifted due to the shifts in foraging preference by predators. Prey species showing a lower tolerance for CO_2 effects may be subject to selection pressure much earlier than other species, which in the long term, may result in a shift in community composition. All four species of damselfishes tested herein are considered to have a very similar ecology and life history, and although species composition may change, we do not know whether or not this shift will affect ecosystem functioning. In coral reef ecosystems, large numbers of recruits can be found during the recruitment pulses, and these recruits are subject to intense predation selection (Munday *et al.* 2010), with at least 60% of newly settling fish being killed by predators in a single night (Almany & Webster 2006). Recruits come to the reef with large variability in body condition and escapes speeds (Holmes & McCormick 2009). Adding a high phenotypic variability in CO_2 tolerance both within and among species (Ferrari *et al.* 2011), this system may provide a large potential for predation-related selection imposed by elevated CO_2. Thus, the predictive value of our results has to be taken together with the caveat that adaptation and selection occurring over multiple generations have been ignored herein. Phenotypic plasticity and/or genetic evolution will likely play a key role in the ability of species to adapt to ocean acidification. Traits associated with CO_2 tolerance may be selected in future cohorts, hence decreasing the amplitude of the effects illustrated in our study. Nevertheless, like all studies focusing on future climate change scenarios, our results provide a working hypothesis on the nature of ecological change that may be observed in the near future.

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AUTHORS CONTRIBUTIONS

MF, DC, MIM, PM conceived the project; MF, DC collected and analysed data; MF, DC, MIM, PM contributed to the writing of the manuscript; PM and DD provided water chemistry parameters and technical assistance with the CO_2 system; MGM and OL provided assistance with the implementation of the experiment.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Mean (± SE) total length in centimetre of *Pomacentrus moluccensis* (white bars), *P. amboinensis* (dark grey bars), *P. nagasakiensis* (light grey bars) and *P. chrysurus* (black bars) according to size-classes (small and large damselfishes) and CO_2 conditions (440 and 700 μatm).

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