Food ration does not influence the effect of elevated CO_2 on antipredator behaviour of a reef fish

Shannon J. McMahon[1,2,*], Jennifer M. Donelson[2], Philip L. Munday[2]

[1]College of Marine and Environmental Science, and [2]ARC Centre of Excellence for Coral Reef Studies,
James Cook University, Townsville, QLD 4811, Australia

ABSTRACT: The appropriate behavioural response to predation risk is critical to survival; however, behavioural responses can be subjected to trade-offs. For example, individuals may engage in riskier foraging behaviour to secure sufficient energy if resources are limited. Additionally, elevated CO_2 can influence foraging and antipredator behaviour of marine organisms. Yet, how the availability of energetic resources may influence antipredator behaviour in an elevated CO_2 environment is unknown. We tested the effects of food ration (low and high: 4 and 8% of body weight per day, respectively) on antipredator behaviour at ambient (489 µatm) and elevated (1022 µatm) CO_2 in juvenile *Amphiprion percula* at 50 d post-hatching. Juveniles were from parents held at either ambient or elevated CO_2, as parental exposure can influence phenotypic response in offspring. Antipredator behaviour was severely impaired by elevated CO_2, with juveniles reared at elevated CO_2 exhibiting no change in feeding rate in the presence of the predator cue compared with a >67% reduction in feeding rate in ambient CO_2 fish. By contrast, food ration had a minor effect on the change in feeding rate in response to the predator cue, with only a 2.3% difference between high and low food ration fish. The effect of elevated CO_2 on antipredator behaviour of juveniles was not influenced by food ration. Parental exposure to elevated CO_2 influenced the baseline feeding rate and exhibited a small carry-over effect in elevated CO_2 juveniles. These results suggest that reef fish could exhibit riskier behaviour at elevated CO_2 levels, regardless of the energetic resources available.

KEY WORDS: Ocean acidification · Energy budget · Predation · Trade-offs · Parental effects · Climate change

INTRODUCTION

Animals are constantly faced with trade-offs between the activities they perform. One such behavioural trade-off is between avoiding predators and foraging for food (Lima & Dill 1990, Creel & Christianson 2008). Individuals must forage to gather sufficient resources to meet the energetic demands of basic maintenance, growth and reproduction. However, foraging can increase the risk of predation when food resources are located away from shelter sites and due to individuals being less vigilant while feeding (Sih et al. 2000, Brown et al. 2006). Consequently, a trade-off exists where an individual weighs the need to

forage against the threat of predation (Lima & Dill 1990, Brown & Kotler 2004). This trade-off suggests that the availability of energetic resources will also influence foraging and antipredator behaviour, with individuals more likely to avoid risk when food is abundant. Conversely, if food is in limited supply, individuals will likely spend more time foraging or engaging in riskier behaviour to secure enough food (Uiblein et al. 1996, Hammerschlag et al. 2010). Climate change has the potential to alter both the food resources available to individuals and the energy required to sustain essential activities, including the energetic cost of basic cellular maintenance (Pörtner et al. 2004, Melzner et al. 2009, Heuer & Grosell

2016). Consequently, climate change could modify the decisions that individuals make in relation to the time spent between foraging and predator avoidance, with potential consequences for population dynamics.

The amount of carbon dioxide (CO_2) in the atmosphere and surface ocean is increasing at an unprecedented rate due to anthropogenic CO_2 emissions (Doney 2010, Intergovernmental Panel on Climate Change [IPCC] 2014). Recent studies show that projected near-future CO_2 levels (>700 µatm CO_2) can impair sensory performance and alter the behaviour of fish and other marine organisms. Behavioural changes under elevated CO_2 include altered olfactory, auditory and visual preferences, changed activity levels, loss of learning ability, increased anxiety and diminished behavioural lateralization (reviewed in Briffa et al. 2012, Chung et al. 2014, Heuer & Grosell 2014, Clements & Hunt 2015). Of particular concern are impaired antipredator behaviours, because they can increase the risk of mortality (Munday et al. 2010, Ferrari et al. 2011a,b, Chivers et al. 2014, Watson et al. 2014, Jellison et al. 2016). Larval and juvenile fish usually exhibit an aversion to predator odour and chemical alarm cues from injured conspecifics, because they indicate a heightened risk of predation (Brown 2003, Holmes & McCormick 2010), with individuals responding to these chemical cues by reducing activity and seeking shelter (Chivers & Smith 1998, Wisenden et al. 2004, Ferrari et al. 2010). However, the antipredator behaviour of larval and juvenile fish is disrupted or lost when individuals are exposed to elevated CO_2 levels (Dixson et al. 2010, Munday et al. 2010, Ferrari et al. 2011a, 2012, Chivers et al. 2014, Ou et al. 2015). These studies indicate that near-future CO_2 levels could fundamentally alter the outcome of predator–prey interactions in the ocean (Nagelkerken & Munday 2016). One limitation of previous experiments, however, is that all have been conducted on fish that have been fed to satiation in the laboratory. It is unknown if natural variation in food supply, or changes to the energetic cost of living in an elevated CO_2 environment, could influence the effects of elevated CO_2 on antipredator behaviours.

Elevated CO_2 can also have direct energetic consequences for marine organisms due to the increase costs of acid–base regulation (Pörtner et al. 2004). Fish actively regulate the concentration of acid–base relevant ions in their blood and tissues to prevent acidosis in a high CO_2 environment (Baker et al. 2009, Esbaugh et al. 2012, Heuer & Grosell 2014). This process can be energetically demanding (Ou et al. 2015, Heuer & Grosell 2016), thus energy requirements for basic maintenance are predicted to rise under elevated CO_2 conditions (Ishimatsu et al. 2008, Melzner et al. 2009). Moreover, food resources are predicted to change under projected climate change scenarios (Richardson & Schoeman 2004, Garzke et al. 2015), altering the energetic resources available to individuals. If food is limited, energy partitioning within the animal may be altered, to facilitate some processes over others (Melzner et al. 2009, Pedersen et al. 2014). In order to meet an increased energy demand under elevated CO_2, it is possible that individuals will display altered behaviour(s), such as becoming bolder or traveling further to find food. While these behavioural alterations may be required to secure adequate food, they also increase the risk of predation.

Elevated CO_2 levels pose a serious threat to marine life, but effects need to be considered within a timeframe relevant to which they will occur. CO_2 levels will rise over numerous generations for most marine organisms and consequently some species may be able to acclimate or adapt to the CO_2 levels projected to occur by the end of this century (Sunday et al. 2014). There is increasing evidence that parental exposure to a stressor can influence how well their offspring perform when exposed to the same stressor (Salinas et al. 2013). Importantly, offspring may have improved performance in an otherwise stressful environment when their parents have experienced that same environment (Donelson et al. 2011, Parker et al. 2012, Salinas & Munch 2012). Recent studies have shown that parental exposure to elevated CO_2 can mitigate the negative effects of elevated CO_2 on growth and survival of juvenile fish (Miller et al. 2012, Murray et al. 2014). Whether parental effects can alter the negative impacts of elevated CO_2 on behaviour is less clear. In one of the few studies conducted to date, Welch et al. (2014) found that exposure of parents to elevated CO_2 did not alter the antipredator behavioural response of juvenile spiny chromis *Acanthachromis polyacanthus* to chemical alarm cues in an elevated CO_2 environment. In contrast, Allan et al. (2014) observed partial amelioration of impaired kinematic responses to a startle stimulus in juvenile cinnamon anemonefish *Amphiprion melanopus* when both parents and offspring were reared in the same elevated CO_2 environment. These variable findings suggest that the effects of parental exposure to elevated CO_2 may be species- and/or trait-specific. No studies have yet tested if parental effects could influence the relationship between food supply and antipredator behaviour, in either current-day or elevated CO_2 conditions.

In this study, we experimentally tested if differences in food supply could affect antipredator behaviour of juvenile clownfish *Amphiprion percula* in an elevated CO_2 environment. Specifically, because the trade-off between time spent foraging and the risk of predation may differ between low and high food environments, we tested if feeding rate in the presence and absence of a predator cue was differentially affected by elevated CO_2 in juvenile fish that had been fed either a low or high ration diet. We predicted that food supply would influence antipredator behaviour in juvenile fish in both current-day and elevated CO_2 conditions, with fish reared in elevated CO_2 and on a low ration diet displaying continued feeding behaviour in the presence of a predator, therefore taking a higher amount of risk than juveniles in other treatments. Because parental effects have been shown to alter the response of some physiological traits, and limited behavioural traits, in juvenile fish exposed to elevated CO_2, we further tested if parental exposure to elevated CO_2 influenced the antipredator behaviour of juvenile reef fish that had been fed either a low and high food ration, when reared at predicted future CO_2 levels. This is the first study to test both the effects of food availability and parental CO_2 environment on antipredator behaviour of fishes at projected future CO_2 levels.

MATERIALS AND METHODS

Study species

Amphiprion percula was selected for this study because it has been the focus of previous studies into the effects of elevated CO_2 on fish behaviour (e.g. Munday et al. 2009a, 2010, Dixson et al. 2010, Nilsson et al. 2012) and early life history growth and development (Munday et al. 2009b). *A. percula* is found in Melanesia and on the Great Barrier Reef (Fautin 1992). It forms social groups consisting of a monogamous breeding pair and 0 to 8 subordinate non-breeders living on a host sea-anemone (Buston 2003). Breeding pairs lay egg clutches of >300 eggs throughout the summer on hard substrate near the anemone. The embryonic period lasts 7 to 9 d, during which time the male tends to the eggs (Michael & Allen 2008). Upon hatching, the larvae have a pelagic stage lasting approximately 11 d before they are competent to settle to the reef (Bay et al. 2006).

Experimental design

This study used an orthogonal design to test the effects of juvenile CO_2 treatment, food ration and parental CO_2 treatment on antipredator behaviour of juvenile clownfish (Fig. 1). The CO_2 levels for rearing
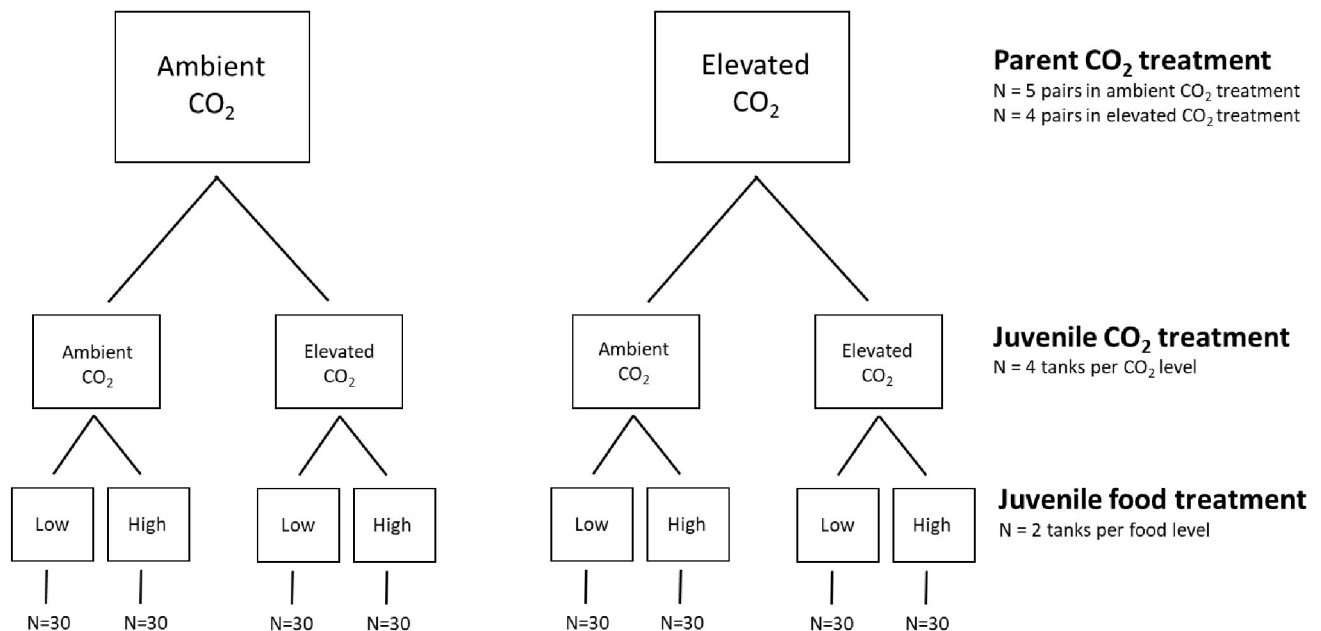

Fig. 1. Experiment design tree. Parental pairs were kept in treatment for 28 wk before the first clutch was laid. Juveniles were moved into treatments at 11 d post hatching (dph) and reared to 50 dph

juvenile fish were a current-day ambient control (mean ± SD: 489 ± 29 µatm) and an elevated CO_2 treatment (1022 ± 111 µatm) consistent with CO_2 levels projected for the end of the century under a business-as-usual emission scenario (IPCC 2014) (Table 1). The 2 food rations for juvenile fish were a high ration diet of 8% of body weight per day and a low ration diet of 4% of body weight per day. These levels were determined from previous research showing that growth rate does not increase above 8% body weight of food per day and 4% body weight per day is sufficient for basic growth (Johnston et al. 2003). Clutches of offspring produced by 5 adult breeding pairs of *A. percula* maintained at ambient CO_2 (489 ± 37 µatm) and 4 adult breeding pairs maintained at elevated CO_2 (1032 ± 95 µatm) for 28 wk before the start of the experiment were used in this study (Table 1). Newly hatched larvae from these pairs were maintained at the same CO_2 treatment as their parents until the end of the larval phase at 11 d post hatching (dph). At 11 dph, juveniles from each clutch were split into 2 duplicate tanks at each of the 4 treatment levels (ambient & elevated CO_2 and high & low food), for a total of 8 tanks per clutch (Fig. 1). Larvae were not divided among treatments until they reached the settlement-stage of development at 11 dph because capture and movement of presettlement larvae can cause significant mortality. Each tank was stocked with 15 settlement-stage juveniles. Juveniles were reared in these treatments until 50 dph.

Animal husbandry

Parental pairs were maintained in 70 l tanks at either ambient or elevated CO_2 (Table 1). Each pair was provided with suitable substrate for laying (terracotta pot and tiles) and were able to both spawn and nest tend naturally. Egg clutches were removed from parental tanks in the evening 8 to 9 d after being laid and placed into a 100 l recirculating larval rearing tank at the same CO_2 treatment water as their parents. Larvae were fed a stage-specific mixture of rotifers (15 to 20 ml^{-1}) and *Artemia* spp. nauplii (5 to 10 ml^{-1}) before transitioning to commercial aquaculture feed (Primo Wean-L 0.3–5 mm) at 8 dph (10 g per tank, once daily). All larvae received food to satiation twice per day during the larval stage. Following transfer to the 4 experimental treatments, juveniles were fed commercial aquaculture pellets (Primo Wean-L 0.3–5 mm), at 8% of body weight (high ration) or 4% of body weight (low ration). An initial clutch was used to test the 8% ration and determine the mean growth rate throughout the 50 dph testing period. The weight of this pilot clutch was monitored weekly and used to calculate the amount of food to be given to each fish group throughout the experiment. Specifically, food levels increased each week of the treatment (18, 25, 32, 39 and 46 dph). A water temperature of 28.5°C was maintained throughout the experiment (Table 1). Salinity was measured daily (Hach HQ40d meter, IntelliCAL CDC401 probe) and maintained at 35 ppt by adding dechlorinated freshwater as required.

CO_2 manipulation

Experimental tanks received water from a 20 000 l recirculating seawater system. One 3000 l sump attached to the system was maintained at ambient CO_2 and another 3000 l sump was maintained at an elevated CO_2 level consistent with projected future CO_2 levels under Representative Concentration Pathway (RCP) 8.5 (Table 1). To achieve elevated CO_2 conditions, the sump was dosed with CO_2 to reach a set pH following standard techniques (Gattuso et al. 2010). The pH was regulated by a pH computer (Aquamedic AT-Control) connected to a pH probe and a solenoid valve, which maintained the

Table 1. Mean (±SD) seawater chemistry parameters for *Amphiprion percula*, adults and juveniles, held under ambient control and elevated CO_2. Juvenile measurements were taken from the day the first clutch was laid until the last clutch reached 50 d post hatching. Parental measurements were taken from the start of parental CO_2 treatment until the last clutch was removed from parental care. Parents were maintained in treatments for 28 wk before the first clutch was laid. SW: seawater

Treatment	Salinity (ppt)	Temperature (°C)	Total alkalinity (µmol kg^{-1} SW)	pH$_{total}$	pCO$_2$ (µatm)
Juvenile ambient CO_2	35.55 ± 0.70	28.38 ± 0.32	2121 ± 95	7.93 ± 0.03	489 ± 29
Juvenile elevated CO_2	35.83 ± 0.58	28.41 ± 0.40	2166 ± 104	7.66 ± 0.04	1022 ± 111
Parental ambient CO_2	35.05 ± 1.29	28.52 ± 0.77	2348 ± 279	7.97 ± 0.05	489 ± 37
Parental elevated CO_2	35.84 ± 1.02	28.35 ± 0.87	2415 ± 321	7.71 ± 0.05	1032 ± 95

desired pH by slowly dosing CO$_2$ when pH deviated above the set point. The pH was cross-checked daily with a Mettler Toledo SG9 pH meter and temperature was measured daily with a Comark-22 thermometer. Total alkalinity of each system was measured weekly by Gran titration (Metrohm 888 titrando) to within 1% of certified reference material (Prof. A. G. Dickson, Scripps Oceanographic Institute). The pH$_{total}$ of each weekly sample was measured by spectrophotometry (Shimadzu, UV mini 1240) and salinity was measured with a conductivity probe (Hach HQ40d meter, IntelliCAL CDC401 probe). The pCO$_2$ of each seawater sample was then calculated in CO2SYS (Pierrot et al. 2006) from the measured values of total alkalinity, pH$_{total}$, temperature and salinity, and using the constants of Mehrbach et al. (1973), refit by Dickson & Millero (1987).

Predator species

The predator selected for this study was the coral cod *Cephalopholis miniata* (Serranidae). This is a piscivorous species that occurs throughout the Indo-Pacific region and is a known predator of juvenile reef fish (Blaber et al. 1990, Kulbicki et al. 2005). Fish were collected by a professional aquarium collector (Cairns Marine Pty) in December, 2015, from the Northern Cairns region of the Great Barrier Reef. Three adult cod were housed in 80 l aquaria within a 10 000 l recirculating system that was separate to the *A. percula* experimental system to ensure there was no contamination of chemical cues. Seawater was maintained at 28.44°C (±0.23) and CO$_2$ was at ambient conditions of 487 ± 16 µatm CO$_2$ with an average pH of 7.94 (±0.02). Predators were fed 2 cubes of prepared piscivorous diet (Fish Dinner, Marine Food, Fish Fuel), every second day, and were not fed the day before they were used in a trial.

Feeding trial and data collection

At 50 dph, all juveniles were used in a feeding experiment designed to quantify feeding rate in the absence and presence of a predator cue. The approach used was a modification of the methods used to test the effect of chemical alarm cues on feeding rate (e.g. Ferrari et al. 2011a, 2012), adjusted to suit the use of predator cue. The experiment consisted of a 2 min observation period of feeding rate without predator cue, followed by another 2 min observation period of feeding rate once the test arena had been flushed with seawater containing predator cue, or a seawater control. Flushing with predator cue enabled us to determine the effect of this cue on feeding rate. Flushing with seawater without predator cue controlled for any change in feeding rate between the pre- and post-stimulus period. At the start of the trial, an individual fish was placed into the test arena (150 × 90 × 90 mm) with a steady flow of water (1.5 l min^{-1}) from their respective rearing treatment. Each individual was allowed to habituate to the arena for 10 min. As per standard protocol (Mitchell et al. 2011, Ferrari et al. 2012), a small amount of food (100 *Artemia* spp. nauplii l^{-1}) was added at the start of the habituation period to help mitigate a possible hyperphagia effect at the start of the trial. After the habituation period the flowing water was turned off and approximately 500 *Artemia* spp. (330 l^{-1}) added by gentle injection through a silicon tube attached to the rim of each arena. Feeding strikes were video recorded (Canon G9X) during a 2 min pre-stimulus feeding period. At the end of the first 2 min feeding period, the arena was flushed for 3 min with seawater containing either predator cue or seawater without predator cue (seawater control) at a flow rate of 1.5 l min^{-1}. The water was then turned off again and approximately 500 *Artemia* spp. (330 l^{-1}) added and the individual's feeding strikes recorded with video for 2 min.

Seawater containing predator cue and control seawater without predator cue was supplied from two 60 l header tanks adjacent to the test arenas. To obtain the predator cue water, a single coral cod was placed in 1 of the 60 l tanks containing seawater at the same CO$_2$ level as the treatment of the juvenile fish being tested. The predator remained in the tank for approximately 20 min before the water was used. The second tank was filled with the seawater at the same CO$_2$ level as the juvenile's treatment level and left for 20 min prior to usage. Four arenas were set up to run concurrently, with 2 receiving predator cue and 2 receiving the seawater control. The predator and seawater control tanks were connected to the test arenas with plastic pipes and valves that enabled either arena to receive either water type. New cue water was prepared for each run and the water delivered to each arena (predator cue or seawater control) was alternated after every second run. Between each run the arenas were flushed completely with seawater for 10 min at a flow of 1.5 l min^{-1}, equivalent to 15 full volume exchanges. The video recordings were later analysed to count the number of feeding strikes in each 2 min period. The observer was blinded to the treatment when viewing the video.

Statistical analysis

The pre-stimulus feeding rate of juveniles was compared among treatments using a linear mixed effects (LME) model fitted by restricted maximum likelihood (REML). Juvenile CO_2, food level and parental CO_2 were fixed effects in the model. To determine which random effects to include in the LME model, we first ran a generalized least squares (GLS) model that included only the fixed effects. We then compared this model with an additional 3 LME models that included either parent ID, rearing tank ID, or both factors as random effects. The model with the lowest Akaike's information criterion (AIC) was selected as the most suitable model (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m586p155_supp.pdf). Parent ID was shown to be a significant random effect and was included in the final LME.

The proportional change in feeding strikes following introduction of the predator cue was calculated by subtracting the number of strikes during the pre-stimulus period from the number of strikes by the same fish during the post-stimulus period and dividing by the number of strikes during the pre-stimulus period ([post − pre]/pre) (Ferrari et al. 2011a). The proportional change in feeding strikes during the stimulus period was compared among treatments using a LME fitted by REML. Juvenile CO_2, food level, parental CO_2 and trial cue were included as fixed effects. As above, model selection was used to determine if parent ID, rearing tank ID, or both should be included as random effects. The LME model including parent ID was superior to the other models (see Table S2 in the Supplement). All assumptions of the LMEs were met except for normal distribution of residuals in the proportional change in feeding strikes LME. Therefore, an identifying variable (juvenile CO_2-trial cue) was included as a covariate to account for the variation within each of these groups and restore normality. Where significant interactions were observed between factors, Tukey's contrasts were completed (in R version 8.0). Statistical analysis was conducted in Splus 8.0 and statistical significance was assessed using α = 0.05.

RESULTS

Pre-stimulus feeding rate

The average feeding strikes made by *Amphiprion percula* juveniles during the pre-stimulus phase was significantly affected by the food ration ($F_{1,1001}$ = 15.04, p < 0.001; see Table S3 in the Supplement). Fish from the low food ration treatment made 12% more feeding strikes on average than fish from the high food ration treatment (Fig. 2). Parental treatment also influenced the number of feeding strikes during the pre-stimulus period, with significantly more strikes made by offspring from elevated CO_2 parents compared with those from ambient CO_2 parents ($F_{1,7}$ = 9.22, p = 0.019). Juveniles from elevated CO_2 parents made 6 to 12% more feeding strikes regardless of juvenile food or CO_2 treatment compared with juveniles from ambient CO_2 parents. There was no effect of juvenile CO_2 treatment on pre-stimulus feeding strikes ($F_{1,1001}$ = 3.25, p = 0.072) and no significant interactions between the treatments (all 2-way and 3-way interactions p > 0.05; see Table S3 in the Supplement).

Change in feeding with predator cue

The effect of predator cue on feeding rate differed markedly between fish reared in ambient conditions and in elevated CO_2 (Fig. 3) (juvenile CO_2 × trial cue interaction $F_{1,1001}$ = 1049.62, p < 0.001; see Table S4 in the Supplement). Fish reared under ambient CO_2
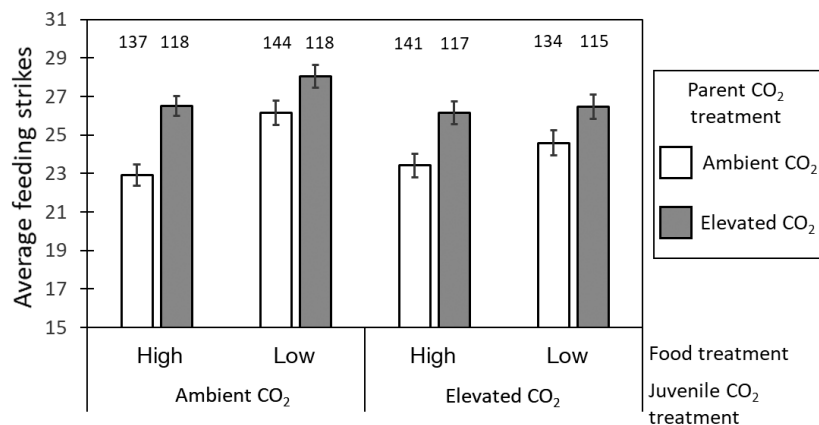

Fig. 2. Average number of feeding strikes (±SE) of *Amphiprion percula* in the pre-stimulus period. Juveniles were reared at either high or low food ration cross factored with ambient control (489 µatm) or elevated CO_2 (1022 µatm). Juveniles were from parents exposed to either ambient control (489 µatm) or elevated CO_2 (1032 µatm). The number of individuals tested in each treatment is shown above the bars
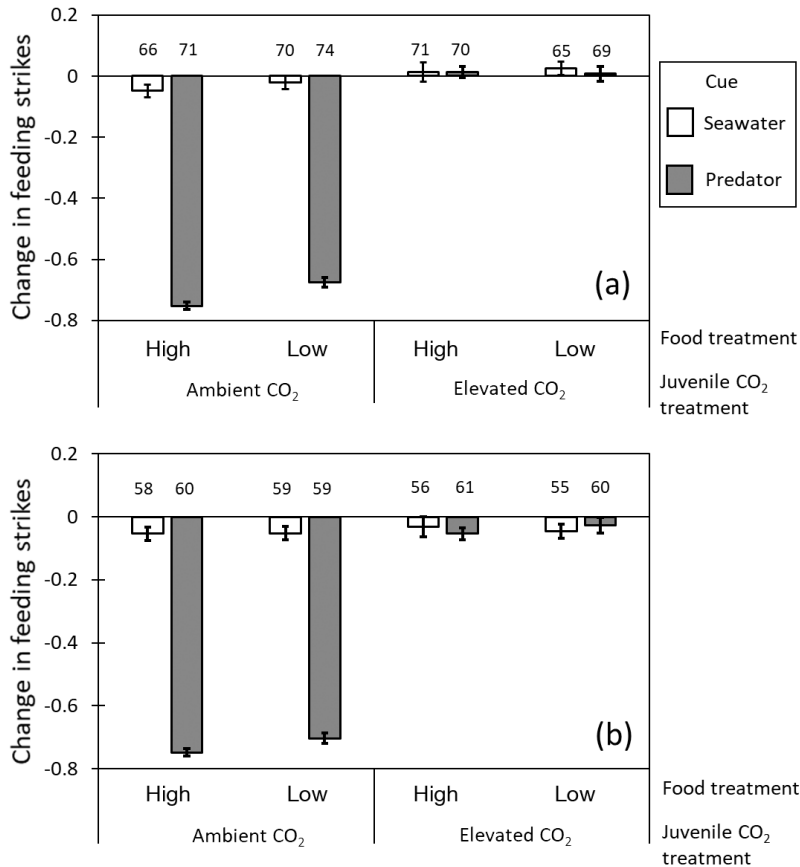
Fig. 3. Mean proportional change in feeding strikes of *Amphiprion percula* when exposed to predator cue or seawater control. Juveniles were reared in either high or low food ration cross factored with ambient control (489 µatm) or elevated CO$_2$ (1022 µatm). Juveniles were from parents exposed to either control (489 µatm) or elevated CO$_2$ (1032 µatm). Graphs are split to show juveniles from (a) ambient CO$_2$ treated parents and (b) elevated CO$_2$ treatment parents. The number of individuals in each treatment is shown above the bars

CO$_2$ treatment (no change in feeding rate) compared with that of fish in the ambient control (>67% reduction in feeding rate). There was no effect of parental CO$_2$ treatment on the proportional change in feeding strikes as a main effect (see Table S4 in the Supplement); however, there was a significant interaction between parental CO$_2$ treatment and juvenile CO$_2$ treatment ($F_{1,1001}$ = 5.76, p = 0.017). This interaction was driven by juveniles from elevated CO$_2$ parents that displayed a 3.5 to 7.2% reduction in feeding rate in elevated CO$_2$ compared to juveniles from ambient CO$_2$ parents in the same elevated juvenile CO$_2$ (Fig. 3). However, further analysis revealed that this difference was marginal (Tukey's p = 0.421).

DISCUSSION

Displaying the appropriate behavioural response to the threat of predation is vital to survival, especially during the juvenile stage where risk of predation is highest. However, the behavioural response to predation risk will also trade-off with other essential activities, such as foraging to satisfy energetic demands. This study found that fish reared at projected future CO$_2$ levels failed to respond normally to the presence of predator cue, as has been observed previously (e.g. Dixson et 2010, Munday et al. 2010, 2016, Chivers et al. 2014), and this impaired behaviour was not significantly affected by differences in the food ration they had received. Fish reared under current-day ambient CO$_2$ conditions reduced the number of feeding strikes by approximately 75% in the presence of predator cue, but sibling fish reared at 1000 µatm CO$_2$ did not reduce their feeding rate in the presence of predator cue. When food is abundant, animals are predicted to take fewer risks to obtain sufficient nutrition. This response was observed in the pre-stimulus period, where fish from the low food ration made 12% more feeding strikes on average than fish from the high food ration. There was also an overall effect of food ration on the response to predator cues in the post-stimulus period. However, this effect was small

exhibited a >67% reduction in feeding strikes in the presence of a predator cue, compared to the seawater control, irrespective of food ration or parental CO$_2$ treatment (Fig. 3; Tukey's all p < 0.001). By contrast, juveniles reared in elevated CO$_2$ conditions exhibited no change in feeding strikes in the presence of predator cue (Fig. 3).

There was an overall effect of food ration on the response to predator cue ($F_{1,1001}$ = 6.66, p = 0.01), but no significant interactions between food ration and other factors (see Table S4 in the Supplement). Feeding rate was, on average, 2.3% lower in the high food versus low food treatment, although the difference ranged from 1 to 7.6% between treatments (Fig. 3; $F_{1,1001}$ = 6.66, p = 0.01). Nevertheless, the effect of food ration on feeding rate in the presence of predator cue was small compared with the fundamentally different response exhibited by fish in the elevated

(2.3%) compared with the effect of elevated CO_2 on the change in feeding rate (>67%) and there was no interaction with juvenile CO_2 treatment. Parental CO_2 treatment caused a small (3.5 to 7.2%), marginally significant, improvement in the behavioural response of elevated CO_2 juveniles. These results indicate that under projected future CO_2 scenarios, reef fish could exhibit an impaired behavioural response to predators, regardless of food availability.

There was a stark difference in behavioural response to predator cue between juveniles from ambient and elevated CO_2 treatments. Consistent with previous studies (e.g. Ferrari et al. 2011a, 2012, Chivers et al. 2014), juveniles reared at current-day ambient CO_2 levels decreased their feeding rate by between 67 and 75% in the presence of predator chemical cues, indicating an antipredator response to the enhanced risk of predation. By contrast, juveniles in the elevated CO_2 treatment did not alter their feeding rate in the presence of the predator cue, and their impaired response to predator cue was not affected by food ration. The similarity in the behavioural response between the food rations at elevated CO_2 further supports previous findings that the underlying mechanism of behavioural changes in elevated CO_2 is likely to be neurological impairment to the sensory perception of predation risk (Nilsson et al. 2012, Chivers et al. 2014, Ou et al. 2015, Heuer et al. 2016), rather than a choice to exhibit more risky behaviour. Prey fish typically exhibit a suite of behavioural changes when they encounter a reliable risk signal such as predator odour, including reduced feeding rate, remaining close to shelter and becoming more vigilant (Lima & Dill 1990, Ferrari et al. 2010). In this study, we were only able to measure changes in feeding rate as an indicator of antipredator behaviour. Further studies could also measure swimming activity and proximity to shelter in fish from different food treatments. The complete absence of an antipredator response exhibited by juvenile fish reared under elevated CO_2 conditions would have significant implications for survival and population viability in the future (Munday et al. 2010, Nagelkerken & Munday 2016).

As predicted by theory (Brown & Kotler 2004, Creel & Christianson 2008), the availability of energetic resources (food ration) influenced the behavioural response to predation risk. Juveniles exhibited a different behavioural response to predator cue depending on food ration, with fish reared on the low food ration exhibiting a proportional change in feeding strikes 1 to 7% less than their high food counterparts. This difference in behavioural response between the food rations is likely due to lower energetic reserves in fish on the low ration diet, which in turn can cause them to become bolder in the presence of a predator to secure more food (Uiblein et al. 1996, Hammerschlag et al. 2010). While there was an overall effect of food ration on feeding rate in the presence of predator cue, this change was minor compared with the overwhelming effect of elevated CO_2, where there was a 67 to 75% difference in feeding rate between ambient and elevated CO_2 treatments.

Animals that possess lower energetic reserves, or high metabolic costs, would also be expected to exhibit higher feeding rates in the absence of a predator (Abrams 1991, Metcalfe et al. 1999). Support for this hypothesis was found in the baseline feeding rate in the pre-stimulus period, which was higher in fish fed the low food ration compared with fish fed the high food ration. In contrast, juvenile CO_2 treatment did not influence pre-stimulus feeding rates. This was surprising since the physiological mechanisms required for living in elevated CO_2, such as acid–base regulation, are expected to incur some energetic costs (Claiborne et al. 2002, Melzner et al. 2009, Heuer & Grosell 2016) and therefore also require increased energy intake (Pansch et al. 2014, Pedersen et al. 2014). It is possible that the additional physiological costs of acid–base regulation processes were not large enough to warrant an increase in feeding and the enhanced risk associated, or instead, the required energy was covered through internal reallocation and trade-offs. Interestingly, offspring of parents exposed to elevated CO_2 had significantly higher feeding rates than juveniles from ambient CO_2 parents, regardless of the juvenile food ration. This suggests that parental exposure to elevated CO_2 may have affected metabolic processes in juvenile fish, increasing their energetic demands (Miller et al. 2012). Alternatively, parental exposure may have caused the fish to be bolder and more willing to feed in conditions they perceive to be relatively low risk (Munday et al. 2010, Ferrari et al. 2011a, McCormick et al. 2013). Future studies could investigate this further by incorporating high and low feeding ration treatments for parents. The implications of higher feeding rates and potentially increased energetic demands are unknown, but could flow on to a range of life history traits.

Parental exposure to elevated CO_2 affected the baseline feeding rate of juvenile *Amphiprion percula* and there was also a significant interaction between parental CO_2 exposure and juvenile CO_2 exposure in the post-exposure period. Juveniles reared in elevated CO_2 and whose parents were also exposed to

elevated CO$_2$ exhibited a reduction in post stimulus feeding rate (3.5 to 7.2%) in response to predator odour compared with no change in juveniles whose parents were exposed to ambient CO$_2$. This reduction in feeding rate suggests that parental exposure to elevated CO$_2$ may partly offset the effects of elevated CO$_2$ on the antipredator behaviour of juvenile fish. However, this reduction was also observed in juveniles given the blank cue, which indicates that this apparently beneficial effect is a carry-over effect of parental exposure to elevated CO$_2$ rather than an actual improvement in the ability of elevated CO$_2$ juveniles to respond to predator cues. However, this improvement is still small in magnitude (not significant in post-hoc analysis), being 90% less than the normal antipredator response to predator odour exhibited by fish in ambient control conditions. This means that under elevated CO$_2$ juveniles lose their normal antipredator response regardless of parental exposure. This is consistent with the absence of significant beneficial effects of parental exposure to elevated CO$_2$ on behavioural lateralization and response to conspecific alarm cue in juvenile spiny chromis (Welch et al. 2014). These results suggest that, with the limited evidence for plasticity, negative effects of elevated CO$_2$ on fish behaviours are likely to occur once the critical CO$_2$ level for the induction of behavioural effects is reached in the ocean. However, it remains to be investigated whether beneficial parental effects could occur with longer exposure times in parents as we know modification of some offspring traits require parental exposure from hatching (Donelson & Munday 2015). There is also the possibility that fish might adapt to rising CO$_2$ levels over coming decades (Sunday et al. 2014). Previous studies have reported substantial individual variation in the behavioural response to predator cue at moderate CO$_2$ levels around 700 µatm CO$_2$, and increased survivorship in the presence of predators has been observed in those that are more behaviourally tolerant to elevated CO$_2$ (Munday et al. 2012). These results hold promise that adaptive evolution of behavioural responses to projected CO$_2$ levels could occur in fish populations. Yet, in the only study of its kind to date, Welch & Munday (2017) found that behavioural tolerance to elevated CO$_2$ was not heritable when juveniles were chronically exposed to elevated CO$_2$ levels. While there was strong heritability in behavioural tolerance to elevated CO$_2$ when fish were acutely exposed to elevated CO$_2$ for just a few days, this heritability was lost in sibling fish that had been chronically exposed to elevated CO$_2$ for many weeks. This casts doubt on

the potential for adaption of antipredator responses in an ocean where CO$_2$ levels will be permanently elevated in the future. More studies on the plastic and adaptive potential of fish behaviours to elevated CO$_2$ are urgently required.

The deficit of vital antipredator behaviour at elevated CO$_2$ reported here, and in other studies, has the potential to impact fish populations in the future. Alongside these changes in ocean CO$_2$, the marine food web and energetic resources in the world's oceans are expected to shift, and be reduced in many locations (Frederiksen et al. 2006, Kortsch et al. 2015). Our results showing that feeding is higher in fish reared with low food supply, suggests that there is the potential for fish to increase risky foraging behaviour prior to the neurological tipping point for impaired behaviour at elevated CO$_2$. Furthermore, the beneficial parental effects observed do not seem to be of sufficient magnitude to offset the overwhelmingly negative behavioural response associated with elevated CO$_2$. With this in mind, our results indicate that projected future CO$_2$ levels could have impacts on reef fish populations through impaired antipredator behaviours, unless these populations are able to adapt over the same timeframe that CO$_2$ levels are rising in the ocean.

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