

Increased CO₂ stimulates reproduction in a coral reef fish

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Abstract

Ocean acidification is predicted to negatively impact the reproduction of many marine species, either by reducing fertilization success or diverting energy from reproductive effort. While recent studies have demonstrated how ocean acidification will affect larval and juvenile fishes, little is known about how increasing partial pressure of carbon dioxide ($p\text{CO}_2$) and decreasing pH might affect reproduction in adult fishes. We investigated the effects of near-future levels of $p\text{CO}_2$ on the reproductive performance of the cinnamon anemonefish, *Amphiprion melanopus*, from the Great Barrier Reef, Australia. Breeding pairs were held under three CO₂ treatments [Current-day Control (430 μatm), Moderate (584 μatm) and High (1032 μatm)] for a 9-month period that included the summer breeding season. Unexpectedly, increased CO₂ dramatically stimulated breeding activity in this species of fish. Over twice as many pairs bred in the Moderate (67% of pairs) and High (55%) compared to the Control (27%) CO₂ treatment. Pairs in the High CO₂ group produced double the number of clutches per pair and 67% more eggs per clutch compared to the Moderate and Control groups. As a result, reproductive output in the High group was 82% higher than that in the Control group and 50% higher than that in the Moderate group. Despite the increase in reproductive activity, there was no difference in adult body condition among the three treatment groups. There was no significant difference in hatchling length between the treatment groups, but larvae from the High CO₂ group had smaller yolks than Controls. This study provides the first evidence of the potential effects of ocean acidification on key reproductive attributes of marine fishes and, contrary to expectations, demonstrates an initially stimulatory (hormetic) effect in response to increased $p\text{CO}_2$. However, any long-term consequences of increased reproductive effort on individuals or populations remain to be determined.

Keywords: *Amphiprion*, climate change, coral reef fish, hormesis, ocean acidification, reproduction, trade-offs

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Introduction

Successful reproduction is critical to ensure that an individual's genes are carried forward to the next generation and replenish the population with new individuals. However, reproduction can be expensive due to the energy spent provisioning gametes and offspring, or time spent on courting or nest defence behaviours (Stearns, 1992; Cox *et al.*, 2010). In females, egg or offspring provisioning requires the allocation of significant amounts of energy, which often comes from energy stored during nonreproductive periods (Watson *et al.*, 1998; Visser & Lessells, 2001; Grazer & Martin, 2011). For males, energy expenditure usually comes in the form of mate acquisition, nest construction and nest defence (Gillooly & Baylis, 1999; Husak & Swallow, 2011). Due to the costs involved, reproduction often occurs within a narrow range of environmental conditions that favour offspring survival (Cushing, 1969; Visser *et al.*, 2009). Reproduction is also sensitive to

environmental cues (Dawson, 2008), with many species requiring specific environmental conditions to trigger breeding (Pankhurst & Porter, 2003). As reproduction is energetically costly and reliant on specific environmental conditions, if those environmental conditions change or place extra energetic demands on the individual, reproduction might decline or cease altogether with consequences for population sustainability.

Semelparous species reproduce once during their lifetime, putting all available energy into a single reproductive event with the trade-off being the mortality of the adults (Roff, 1992). In contrast, iteroparous species reproduce multiple times throughout their lifetime and are able to adjust their reproductive effort to suit current environmental conditions (Roff, 1992). Iteroparous species can respond in one of two different ways to environmental stressors. Individuals can reduce their investment in current reproduction, thereby saving energy for future reproduction (Clutton-Brock, 1984; Hamel *et al.*, 2011). Alternatively, individuals can invest more energy into current reproduction, in an attempt to offset any negative impacts of environmental stressors on reproductive performance and to potentially

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increase offspring survival (Paul *et al.*, 1993). In this circumstance, there would be additional demand placed on energy acquisition or energy stores to meet the energetic requirements of increased reproductive activity. If environmental conditions are so poor that the adult is unlikely to survive to reproduce again when conditions improve, it may invest all available energy in a final reproductive event, but at the expense of its own survival (terminal investment; Clutton-Brock, 1984).

Reproduction in fishes is highly regulated and reliant on specific environmental conditions (Munro *et al.*, 1990; Conover, 1992; Van der Kraak & Pankhurst, 1997). Salinity, temperature, photoperiod, water flow and food availability are all known to influence breeding and to determine reproductive success in fishes (Munro, 1990; Hilder & Pankhurst, 2003; Helfman *et al.* 2007). In many marine fishes, gamete maturation and spawning are dependent on increasing photoperiod and temperatures in spring (Pankhurst & Porter, 2003), which coincide with optimal conditions for larval survival and growth. Many fish species only reproduce within a narrow range of the temperatures they normally experience (Van der Kraak & Pankhurst, 1997). Furthermore, if temperatures exceed their normal breeding conditions, or a temperature increase occurs during a particularly sensitive phase e.g. gamete maturation, reproduction may cease (Donelson *et al.*, 2010; Pankhurst & Munday, 2011). Consequently, reproduction in fish may be particularly sensitive to the impacts of global climate change (Van der Kraak & Pankhurst, 1997; Pankhurst & Munday, 2011).

For marine fishes the impacts of climate change will not be limited to increasing temperature. Marine fish must also cope with the increase in partial pressure of carbon dioxide ($p\text{CO}_2$) in their environment, due to increased uptake of atmospheric CO_2 at the ocean surface (Caldeira & Wickett, 2005; Doney, 2010). For fish, exposure to high $p\text{CO}_2$ in seawater causes an increase in plasma $p\text{CO}_2$, which acts to acidify blood and tissue (Brauner & Baker, 2009). Fish have well-developed acid-base balance regulatory systems and are able to restore their pH, despite the acidifying effects of higher $p\text{CO}_2$, by the exchange of acid-base relevant ions with the external environment (Brauner & Baker, 2009; Esbaugh *et al.*, 2012). However, increased regulation of pH through ion transport is predicted to be energetically expensive (Pörtner *et al.*, 2004; Ishimatsu *et al.*, 2008). An increase in energy used to maintain acid-base balance could reduce the amount of energy available for other activities, including reproduction (Ishimatsu *et al.*, 2008; Sokolova *et al.*, 2012). Increased energy requirements for pH homeostasis could affect female reproductive output, or reduce the provisioning of eggs, with potential consequences for offspring quality.

However, one recent study found that aerobic scope, which is an indicator of individual performance, increased in marine fish exposed to near-future CO_2 levels (Rummer JL, Stecyk JAW, Couturier CS, Watson SA, Nilsson GE, Munday PL, submitted). While the mechanisms responsible for increased aerobic scope at near-future CO_2 remains uncertain, this result suggests that the energy available for reproduction could potentially increase under acidified conditions in some species, and therefore, reproduction could potentially increase as well.

To date, only a handful of studies have examined the effects of ocean acidification on reproduction in fish. Inaba *et al.* (2003) tested the effect of direct CO_2 gas application on the motility of activated sperm from 16 species of fish from a range of different families. CO_2 affected the sperm motility of five species, all of which were flatfishes, with activity arresting within 30 ms of CO_2 being applied; however, there was no effect of CO_2 on sperm motility in the 11 other species tested. Similarly, Frommel *et al.* (2010) found no effect of increased CO_2 on sperm motility of Baltic cod (*Gadhus marhua*) when sperm was activated using acidified seawater. Sundin *et al.* (2012) found no effect of decreased pH on the reproductive propensity of a pipefish (*Syngnathus typhle*), however, animals were exposed to altered pH for a maximum of 5 h. No studies have yet been conducted for extended periods of time to allow for any impacts of increased $p\text{CO}_2$ on gametogenesis or energy provisioning of the gametes to occur, or examined reproductive performance across a breeding season. Given the predicted increased cost of acid-base balance, it might be expected that adults exposed to continuous acidified conditions will exhibit a decline in the reproductive activity through reductions in the number of clutches or eggs produced, or declines in egg provisioning, with subsequent consequences for offspring quality.

We tested the hypothesis that long-term exposure to increased $p\text{CO}_2$ would decrease reproductive performance, either through reductions in gamete production, provisioning or offspring quality. To do this we placed breeding pairs of the cinnamon anemonefish, *Amphiprion melanopus*, into either a current-day control or one of two increased $p\text{CO}_2$ treatments prior to the start of the breeding season and allowed them to reproduce naturally over a 9-month period. These $p\text{CO}_2$ treatments were representative of current-day conditions, predicted mid century (541 ppm) and end of century (936 ppm) $p\text{CO}_2$ levels from the RCP 8.5 scenario (Meinshausen *et al.*, 2011). We monitored reproductive activity on a daily basis and compared the number of clutches produced, the average number of eggs produced, reproductive output, offspring quality and adult body condition.

Material and methods

Study species and collection

The anemonefish, *A. melanopus*, is common on coral reefs throughout the Indo-Pacific where it occurs in colonies containing multiple breeding pairs (Drew *et al.*, 2008). *A. melanopus* is a serial benthic spawner, laying multiple clutches of oblong shaped eggs during the summer breeding season. Embryonic duration is usually between 7 and 9 days, during which time the male tends the eggs (Michael, 2008). The larvae have a pelagic phase of approximately 11 days, after which they are competent to settle to the reef.

Breeding pairs of *A. melanopus* were collected in the austral winter (late May–early July) from four adjacent reefs within the Orpheus Island region of the Central Great Barrier Reef: Orpheus Island (18.62°S, 146.49°E), Bramble Reef (18.42°S, 146.7°E), Davies Reef (18.8°S, 147.63°E) and Slasher's Reef (18.47°S, 147.08°E). Anemonefish populations on these reefs are expected to be genetically panmictic because of their close proximity (Bay *et al.*, 2006; Jones *et al.*, 2010). These reefs experience approximately the same mean summer water temperature of 28.5 °C. Pairs were collected using hand nets and dilute clove oil (Munday & Wilson, 1997). Breeding pairs were transported to the Marine and Aquaculture Research Facility at James Cook University where each pair was housed in an individual 45 l aquarium.

Experimental systems and CO₂ manipulation

The experiment used three 8000 l recirculating aquarium systems, each set to a different CO₂ and corresponding pH level. The CO₂ treatments were a current-day Control (430 µatm), a mid century Moderate CO₂ (584 µatm) and an end of century High CO₂ (1032 µatm). The Moderate and High treatment levels are consistent with the RCP 8.5 (Meinshausen *et al.*, 2011) scenario for predicted atmospheric CO₂ levels for the middle and end of this century. An Aqua Medic AT Control system (Loveland, CO, USA) was used to maintain the desired pH level of each system, by dosing CO₂ into a 3000 l sump. The equilibrated seawater was then delivered to the individual aquaria at a rate of ca. 1.5 l min⁻¹. pH_{NBS} and temperature in the aquaria were recorded daily using a Hach pH (HQ40d; Hach, Loveland, CO, USA) probe and a Comark C26 (Norfolk, UK) temperature probe. Total alkalinity was estimated weekly by Gran Titration (Metrohm 888 Titrande Titrator Metrohm AG, Switzerland) and using certified reference material from Dr. A.G. Dickson (Scripps Institute of Oceanography). Salinity was measured weekly using a Hach multimeter (HQ15d; Hach, Loveland, CO, USA). Aqua Medic dosing set points were adjusted as needed to maintain the desired pCO₂ concen-

trations. Average pCO₂ for the experimental period was determined in CO2SYS v2.1 (<http://cdiac.ornl.gov/oceans/co2rprt.html>) using the daily temperature and pH_{NBS} readings and weekly total alkalinity and salinity measurements (Table 1).

Experimental design

Eighteen pairs of *A. melanopus* were placed into each of the three CO₂ treatment groups (Control, Moderate and High). All individuals were weighed (wet weight; g) and standard length (mm) was measured immediately before being placed into treatment in August 2010 and again at the end of the breeding season (ensuring that there was an even distribution of weights among treatment groups). Fulton's K condition factor (body condition) was calculated at the end of the breeding season using the formula $K = 100 \cdot (W/L^3)$ where W is the wet weight in grams and L is the standard length in centimetres. At the start of the experiment, August 2010, pairs were placed into individual 45 l tubs with continuous water flow at winter nonbreeding temperatures (22.5 °C) and at ambient pCO₂. pCO₂ was slowly adjusted over a 2-week period to the desired levels and maintained at those levels from late August 2010 to the end of the breeding season in May 2011. This allowed for an acclimation to CO₂ treatment for 2 months prior to the start of the breeding season. Temperature was increased by 0.5 °C weekly until the average summer breeding temperature (28.5 °C) was reached in the first week of November 2010. Each pair was provided with a half terracotta pot as a shelter and breeding substrate. Pairs were fed 0.1 g of commercial fish feed pellet (INVE NRD 12/24) three times a day (1.21% of average body weight; Donelson *et al.*, 2010) during the breeding season (November 2010–May 2011).

Data collection

From October 2010 through to the end of May 2011, representative of a single breeding season, the terracotta nesting pots were checked daily between 9:00 hours and 10:00 hours for the presence of a new egg clutch. As *A. melanopus* lay clutches after dark, this ensured that digital photographs of the clutch were taken within 12 h of being laid. The total number of eggs in each clutch was counted using image analysis software (ImageJ, National Institute of Health, Bethesda, MD, USA). A random sample of 10–20 eggs (1–2% of the total number of eggs) was taken from each clutch and preserved in 4% phosphate buffered formaldehyde. Digital photographs of the sampled eggs were taken within 3 days of preserving using a Leica camera attached to a stereo microscope. The eggs were placed in a horizontal position, with the longest axis visible on

Table 1 Experimental system seawater parameters for the reproductive adults held under Control, Moderate and High CO₂ concentrations

Treatment	Salinity	Temp. (°C)	Total alkalinity (µmol. kg ⁻¹ SW)	pH _{NBS}	pCO ₂ (µatm)
Control	36.4	28.5 ± 0.3	2049 ± 79	8.11 ± 0.04	430 ± 54
Moderate	36.4	28.5 ± 0.3	2089 ± 143	8.01 ± 0.05	584 ± 77
High	36.2	28.5 ± 0.3	1945 ± 23	7.77 ± 0.05	1032 ± 137

a 5 mm grid. The individual area of five eggs from the sample was then determined using ImageJ by tracing around the egg and estimating area to the nearest 0.01 mm². Reproductive output, per clutch, was calculated by multiplying the total number of eggs in the clutch by the average individual egg area for that clutch, to give an overall area (mm²) of eggs, and therefore an estimate of maternal investment per clutch. On the night of hatching egg clutches were transferred into 70 l hatching tanks containing the same treatment water as their parents. A sample of 10–20 hatchlings (between 2% and 5% of the hatchlings) was collected within an hour of hatching (ca. 9.30 pm, or 1 h after dark). Hatchlings were euthanized using an overdose of clove oil before being preserved in 4% phosphate buffered formaldehyde. The hatchlings were photographed on a 5 mm grid using a stereomicroscope. Hatchling standard length was determined to the nearest 0.01 mm. Yolk area was determined by tracing the yolk sac and estimating the area to the nearest 0.1 mm² in ImageJ.

Data analysis

Female weight at the start and end of the experiment and Fulton's K body condition factor at the end of the breeding season were compared among treatment groups using ANOVA. The proportion of pairs that reproduced was compared among treatments using a χ^2 test of homogeneity. The average number of clutches produced per pair was analysed using an ANCOVA with the number of clutches as the dependent variable, the treatment groups as the predictor variable and female weight as the covariate. All other reproductive characteristics were analysed using Linear Mixed Effects (LME) models (Pinheiro & Bates, 2000) with the reproductive characteristic (number of eggs, egg area, reproductive output, hatchling size and yolk area) being the response variable, CO₂ treatment being a fixed explanatory variable and female weight included as a random explanatory variable. Data were grouped by breeding pair (number of clutches, eggs per clutch and reproductive output per clutch) or egg clutch (egg area, hatchling length and yolk area) according to the level at which replication occurred for the measure of interest and then heterogeneous variance was allowed to occur at this level. Linear mixed effect models control for inherent variation between

female reproductive levels by estimating error terms for variation among individuals as well as for estimating residual error terms for variation within individuals. Akaike information criteria (AIC) were used to determine which model best fit the data, whereby the model with the smallest AIC value was the best fit. All statistical analyses were conducted using S-Plus v.8.0.4 (Tibco Pty Ltd, Palo Alto, CA, USA).

Results

Parental condition and timing of breeding

Female weight at the start of the experiment (mean 24.82 ± 1.1 g SE) did not differ among the three CO₂ treatment groups (ANOVA; $F_{2, 50} = 0.042$, $P = 0.387$). Increased CO₂ significantly stimulated reproduction, with approximately twice the number of reproductive pairs breeding in the Moderate and High CO₂ treatment groups (21 of 36 pairs in the two groups) compared to the Control (5 of 18 pairs; $\chi^2_1 = 3.741$, $P < 0.05$). There was no difference in the proportion of pairs that reproduced in the Moderate and High groups. In the Moderate group 61% (11 of 18) of pairs reproduced and in the High group 55% (10 of 18) of pairs reproduced, compared with only 27% (5 of 18) pairs that reproduced in the Control group. Despite the increase in reproductive activity in the High CO₂ group, the onset of breeding was the same for all treatments, with the first clutch of the season from each treatment group being laid during the last week of October 2010 (Fig. 1). The length of the breeding season was also unaffected by CO₂, with all groups breeding until the end of April 2011. All treatments displayed two peaks in reproductive activity: one between December and January, the other in April.

Reproductive frequency and egg production

Elevated CO₂ affected the frequency of reproduction and the number of clutches produced. Overall, the

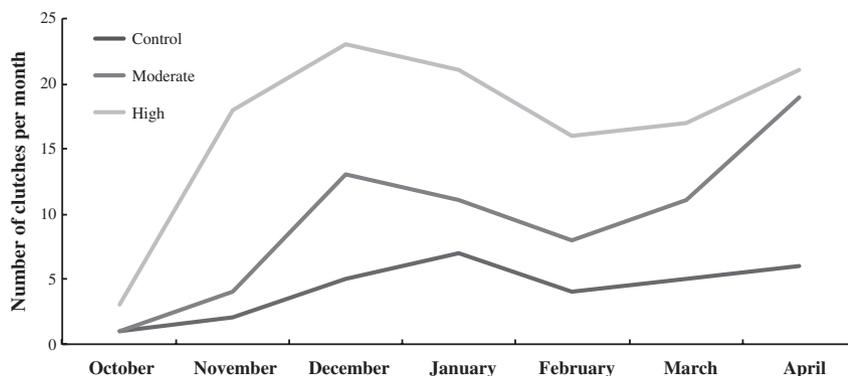


Fig. 1 The number of clutches laid for each month from breeding pairs of *Amphiprion melanopus* held under Control, Moderate or High CO₂. Total numbers of clutches produced for each treatment group were Control N = 30, Moderate N = 67 and High N = 119.

Control group produced a total of 30 clutches, the Moderate group produced 67 and the High group produced 119 clutches (Fig. 1). On average, pairs in the Control and Moderate groups produced 6.6 ± 2.1 and 6.45 ± 1.2 (mean \pm SE) clutches, respectively, during the breeding season. In contrast, pairs in the High group produced on average 12.6 ± 0.9 clutches throughout the breeding season, approximately double the number from the Control and Moderate groups (ANCOVA: $F_{2,22} = 7.33$, $P = 0.003$; Fig. 2a). Embryonic duration was not affected by CO₂, with all successful clutches hatching in 7–8 days.

The number of eggs per clutch increased with increasing CO₂ (Fig. 2b). The Control group produced 504 ± 92 eggs per clutch, the Moderate produced 559 ± 123 and the High group 847 ± 121 eggs per clutch (LME model: Intercept $P < 0.001$; Table 2). The High group produced 67.16% more eggs compared to Control (LME model: High $P < 0.01$; Table 2). There was no significant difference between the Moderate and Control group (LME model: Moderate $P = 0.654$; Table 2). There was no evidence of a decline in male reproductive capacity, with few unfertilized eggs observed in any of the clutches.

Reproductive characteristics

CO₂ treatment group had a significant effect on egg size (LME model: Intercept $P < 0.001$; Table 2) with the

Moderate treatment producing significantly smaller eggs (1.96 ± 0.08 mm²) compared to the Control (2.15 ± 0.06 mm²; Moderate $P = 0.02$, Table 2; Fig. 2c). However, eggs from fish in the High CO₂ treatment were not significantly different in area (2.12 ± 0.08 mm²; High $P = 0.708$) from the Control CO₂ treatment (2.15 ± 0.06 mm²). CO₂ treatment also had a significant effect on reproductive output (LME model: Intercept $P < 0.0001$). Reproductive output was significantly higher in the High CO₂ group compared to the Control (High $P = 0.003$, Table 2), but there was no difference between the Moderate and Control CO₂ groups (Moderate $P = 0.437$, Table 2; Fig. 2d). There was no significant difference in hatchling length between the Control and either of the elevated CO₂ groups (Moderate $P = 0.664$; High $P = 0.9162$; Table 2; Fig. 3a). However, CO₂ did have a significant influence on yolk provisioning (LME model: Intercept $P < 0.0001$, Fig. 3b). The Moderate parents produced larvae with the largest yolks (0.559 ± 0.02 mm²; Moderate $P = 0.338$; Table 2), though not significantly different from Control (0.054 ± 0.17 mm²). Larvae from the High CO₂ parents had significantly smaller yolks compared to the Control group (0.5 ± 0.01 mm²; High $P = 0.02$; Table 2).

The increase in reproduction at elevated CO₂ did not appear to come at a cost to adult body condition, with no significant difference in Fulton's K body condition

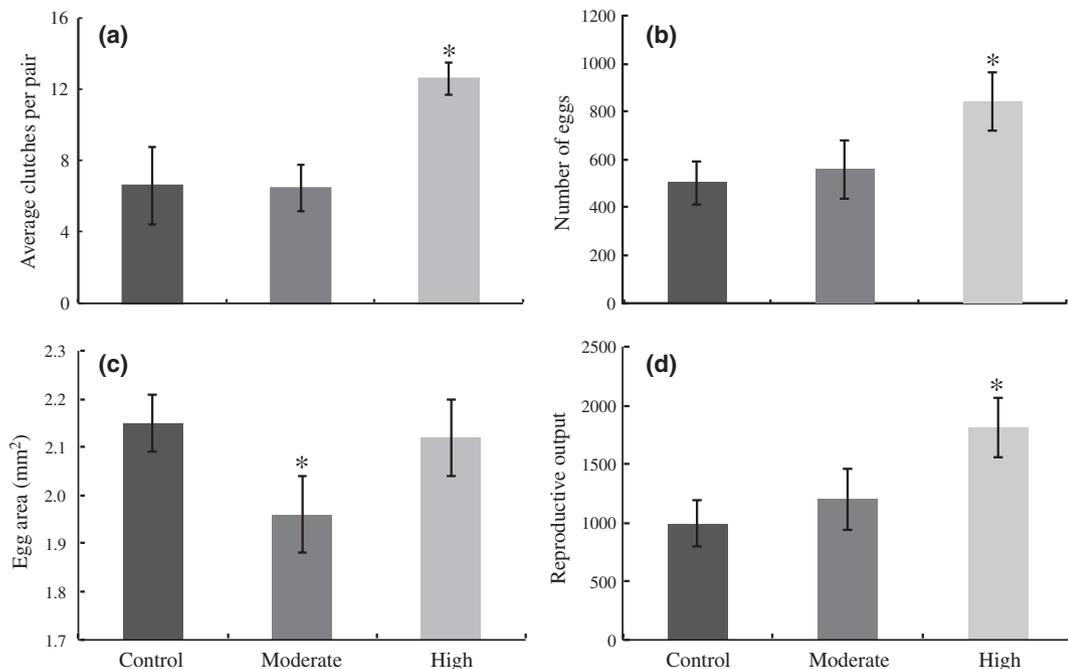


Fig. 2 Effect of increased CO₂ on reproductive traits of a coral reef fish. The average number of clutches produced per pair (a), the average number of eggs produced per clutch (b), the average area of the eggs produced (c) and the reproductive output per clutch (d) of adult breeding pairs of *Amphiprion melanopus* held under three different CO₂ conditions. *Indicates a significant difference from the Control group. Data are means \pm SE.

Table 2 Linear mixed effects tables for reproductive characteristics. Significant effects ($P < 0.05$) are indicated by *. Intercept is the Control group; Moderate and High are compared to the control

Reproductive variable	Treatment	Variable value	SE	DF	<i>t</i> -value	<i>P</i> -value	AIC
Number of eggs	(Intercept)	503.74	91.65	196	5.49	<0.001*	3026.35
	Moderate	55.67	122.71	23	0.45	0.654	
	High	338.35	121.45	23	2.78	0.01*	
Egg area	(Intercept)	2.15	0.06	1084	30.86	<0.0001*	-1278.33
	Moderate	-0.19	0.08	23	-2.33	0.02*	
	High	-0.03	0.08	23	-0.37	0.708	
Reproductive output	(Intercept)	991.31	196.54	189	5.04	<0.0001*	3318
	Moderate	207.97	263.19	23	0.79	0.437	
	High	816.51	253.12	23	3.22	0.003*	
Hatchling length	(Intercept)	3.66	0.02	718	122.52	<0.0001*	-690.06
	Moderate	-0.015	0.03	82	-0.43	0.664	
	High	-0.003	0.03	82	-0.11	0.9162	
Yolk area	(Intercept)	0.54	0.01	707	31.13	<0.0001*	-2251.04
	Moderate	0.019	0.02	81	0.96	0.338	
	High	-0.04	0.01	81	-2.23	0.02*	

AIC, Akaike information criteria.

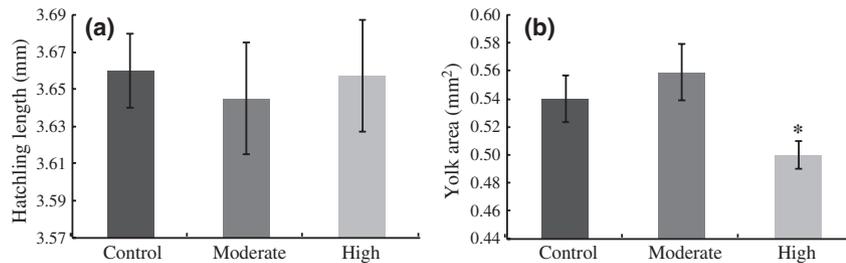


Fig. 3 The average length of hatchlings (a) and the average area of the yolk (b) of newly hatched larvae of *Amphiprion melanopus* from reproductive adults, held under different $p\text{CO}_2$ treatments. *Indicates where the treatment value is significantly different from the Control CO_2 group. Data are means \pm SE.

factor among the treatment groups (All females: mean \pm SE Control = 4.89 ± 0.08 , Moderate = 5.02 ± 0.08 , High = 5.00 ± 0.09 , ANOVA: $F_{2, 47} = 0.67$, $P = 0.517$; Reproductive females only: Control = 4.86 ± 0.15 , Moderate = 4.95 ± 0.11 , High = 4.86 ± 0.11 , ANOVA: $F_{2, 23} = 0.234$, $P = 0.79$). During the breeding season, treatment groups on average gained mass (Control = 2.06 ± 0.72 g; Moderate = 1.05 ± 0.69 g; High = 1.49 ± 0.65 g, ANOVA: $F_{2, 47} = 0.499$, $P = 0.609$). Similarly, when only females that had reproduced were considered all treatment groups on average gained weight (weight gain: Control = 0.61 ± 0.76 g, Moderate = 1.79 ± 0.51 g, High = 0.88 ± 0.54 g, ANOVA: $F_{2, 23} = 1.125$, $P = 0.341$). There were no deaths in any group during the experiment.

Discussion

Previous studies in a range of invertebrates (copepods: Fitzer *et al.*, 2012; sea urchins: Havenhand *et al.*, 2008;

oysters: Parker *et al.*, 2009) have found that reproduction is negatively impacted by elevated $p\text{CO}_2$ and predictions were that fishes would be similarly affected. Contrary to expectations, this study showed a distinct stimulation of reproductive activity with increasing CO_2 . To our knowledge this is the first study to show an increase in reproduction in response to ocean acidification in any marine organism. The increase in reproduction was most marked in the High treatment group with a doubling in the number of clutches produced, 67% more eggs per clutch and an increase of 82% in reproductive output per clutch compared to Control pairs. The number of pairs breeding also doubled in the Moderate group, however, these pairs did not increase reproductive effort to the same extent of the High CO_2 group, producing 11% more eggs per clutch and with a 21% increase in reproductive output compared to Control pairs. This increase did not appear to come at a cost to adult body condition in either elevated CO_2 group. These results contradict the

hypothesis that increased CO₂ will negatively impact reproduction due to the increased costs associated with acid–base regulation.

Increases in reproduction have been documented in birds (Velando *et al.*, 2006; Hall *et al.*, 2009; Bowers *et al.*, 2012), insects (Copeland & Fedorka, 2012; Nielsen & Holman, 2012) and mammals (Hoffman *et al.*, 2010) that are senescing and/or facing a lethal stressor. This response is known as terminal investment. Individuals that display terminal investment use all available energy for reproduction rather than homeostasis or growth. As such, terminal investment has two clear outcomes: adults do not survive and there is often a decline in the quality of the offspring as the adults are already in poor condition (Bonneaud *et al.*, 2003). In this experiment, we saw a dramatic increase in reproduction, however, this increase was not associated with adult mortality or a decline in adult body condition. While there was a decline in yolk sac area of the offspring in the highest CO₂ group, there was no significant difference in hatchling length, which is a key fitness-associated trait (Miller *et al.*, 1998). Consequently, the increase in reproduction seen in response to elevated CO₂ is not consistent with the occurrence of terminal investment.

The increase in reproduction observed here could be a hormetic response to an increase in pCO₂. A hormetic response occurs where an organism's response to an environmental stressor varies with the dose of the toxicant, specifically a mild dose of a stressor results in an increase in a given performance measure (Constantini *et al.* (2010)). This initial increase in performance could occur either through speeding up physiological reactions or by stimulating the organism to perform activities at a higher rate (Costantini *et al.*, 2010; Schreck, 2010). Our results show that a mild increase in pCO₂ appears to be beneficial for reproduction with no readily apparent cost to the organism. We detected no negative impacts of increased reproduction or pCO₂ on adult body condition over a period of 9 months, suggesting that the elevated CO₂ treatments were not harmful in the short term. This is consistent with previous findings where growth and survival of adult fish are not affected until CO₂ levels reach at least an order of magnitude higher than those used here (Ishimatsu *et al.*, 2008; Melzner *et al.*, 2009a, b). The exact mechanisms that allow this dramatic increase in reproduction to occur with no apparent costs are not known, however, it is plausible that there could be alterations to the endocrine pathways leading to a stimulation of reproductive activity (Pankhurst & Munday, 2011) or this species may have increased energetic efficiency under these levels of CO₂, and could therefore have more energy available for reproduction.

Although we did not detect any effects of increased reproduction on adult body condition or offspring standard length, it is possible that there will be trade-offs with other life history traits, or over longer time scales (Creighton *et al.*, 2009). For example, longer term exposure to increased pCO₂ could result in declines in longevity and lifetime fecundity (Pörtner & Peck, 2010) that could not be measured in our experiment. It is also possible that the adults used in this experiment may show reduced reproduction in subsequent breeding seasons. Research on reproduction in invertebrates and birds has suggested that increased reproduction can lead to reduced reproduction or survival of the parents, potentially due to increased oxidative stress (Costantini, 2008). Furthermore, there could be effects on offspring condition. While the size at hatching of offspring did not differ among treatments, the average yolk area of larvae in the highest CO₂ treatment was smaller than larvae from current-day controls. This suggests that high CO₂ may have had some energetic cost to the larvae. Reduced yolk size could potentially affect juvenile growth or survival in the wild (Hoey & McCormick, 2004; Grorud-Colvert & Sponaugle, 2006). However, while there was a decline in yolk area at the highest CO₂, there was no significant difference in hatchling length, which may be equally important to larval survival (Meekan & Fortier, 1996; Miller *et al.*, 1998). Furthermore, Miller *et al.* (2012) showed that, rather than displaying negative impacts, juveniles from parents held under high pCO₂ were better acclimated to increased pCO₂. When reared at high CO₂ these offspring displayed similar growth and survival compared to offspring reared under control conditions. Consequently, any differences in yolk area do not appear to have negative effects on growth and survival, presumably because the offspring are better acclimated to the high CO₂ conditions.

Interestingly, different levels of increased CO₂ appeared to lead to differing levels of provisioning for the offspring. The Moderate group had significantly smaller eggs compared to the High group and also appeared to gain more weight across the breeding season. This suggests that different levels of increased CO₂ may lead to different investment strategies, with fish exposed to moderate levels of CO₂ choosing to invest more energy into adult body condition at the expense of high rates of reproduction.

The dramatic increase in reproductive effort, with no apparent cost to adult body condition, suggests that there may be more energy available for reproduction in fish exposed to the near-future CO₂ levels used in this study. Fish were fed the same amount in each treatment, so there was no difference in nutritional availability among treatments that could explain the similar

increase in mass of breeding females across treatments despite the vastly differing levels of energy allocated to reproduction. Although activity levels were not quantified, there was no apparent difference in activity among treatments that could explain the different levels of energy allocation. Previous studies have shown that juvenile *A. melanopus* exposed to high CO₂ do not increase their foraging rate (Nowicki *et al.*, 2012). Our results suggest that near-future levels of CO₂ may not be as costly for adult reef fish as expected. Reef fish experience large variations in pCO₂ and pH levels in their environment on a diurnal basis (Gagliano *et al.*, 2010; Shaw *et al.*, 2013) and may be preconditioned or better adapted to increased CO₂ than would otherwise be expected. Indeed, Rummer JL, Stecyk JAW, Couturier CS, Watson SA, Nilsson GE, Munday PL (submitted) found that the routine metabolic rate decreased and maximum oxygen uptake increased, in a common reef fish under levels of CO₂ similar to levels used in our experiment. The changes in metabolic rates suggest that some reef fish require less energy for routine activities, such as homeostasis, when CO₂ levels are slightly elevated, and could therefore put more energy into reproduction.

In direct contrast to the prediction that the energetically costly phase of reproduction would be particularly sensitive to increased CO₂, here we show that increased pCO₂ stimulates reproduction in a marine fish. Furthermore, we found limited evidence of negative impacts of increased reproductive effort on either adult body condition or offspring standard length. Although yolk area of hatchlings was reduced, previous experiments suggest this might not have significant effects on offspring performance in a high CO₂ environment. These results suggest that some species may have a much greater capacity to tolerate increased pCO₂ than has been predicted. This is the first study to examine the impacts of ocean acidification by exposing reproductive adults to increased CO₂ prior to and during a breeding season, allowing for any impacts on gametogenesis to occur. We also investigated the effects of ocean acidification on multiple stages of the reproductive cycle, from potential effects on the adult condition through to the condition of the resulting offspring. Given the unexpected results found here, future experiments on the impacts of ocean acidification will need to examine the potential impacts on all stages of reproduction, including on gametogenesis and adult condition, to provide a more realistic indication of the effects of future levels of ocean acidification on marine populations.

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