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LETTER

Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions

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Abstract

Successful recruitment in shallow reef ecosystems often involves specific cues that connect planktonic invertebrate larvae with particular crustose coralline algae (CCA) during settlement. While ocean acidification (OA) can reduce larval settlement and the abundance of CCA, the impact of OA on the interactions between planktonic larvae and their preferred settlement substrate are unknown. Here, we demonstrate that CO_2 concentrations (800 and 1300 μatm) predicted to occur by the end of this century significantly reduce coral (*Acropora millepora*) settlement and CCA cover by ≥ 45%. The CCA important for inducing coral settlement (*Titanoderma* spp., *Hydrolithon* spp.) were the most deleteriously affected by OA. Surprisingly, the only preferred settlement substrate (*Titanoderma*) in the experimental controls was avoided by coral larvae as pCO_2 increased, and other substrata selected. Our results suggest OA may reduce coral population recovery by reducing coral settlement rates, disrupting larval settlement behaviour, and reducing the availability of the most desirable coralline algal species for successful coral recruitment.

Keywords

Acropora, coral, crustose coralline algae, electivity, *Hydrolithon*, ocean acidification, recruitment, settlement, *Titanoderma*.

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INTRODUCTION

The effects of ocean acidification (OA) have raised concerns about coral reef ecosystem function by reducing the calcification rates of benthic organisms important to maintaining habitat structure and biodiversity (Hoegh-Guldberg *et al.* 2007; Kroeker *et al.* 2010). Anthropogenic emissions of carbon dioxide (CO_2) have increased atmospheric CO_2 from approximately 280 ppm prior to the year 1750 to > 380 ppm in 2005 (Jansen *et al.* 2007), and these are continuing to rise (Le Quere *et al.* 2009). The absorption of this atmospheric CO_2 by the oceans has reduced global pH by 0.1 units and carbonate saturation state by 20% since 1800 (Orr *et al.* 2005). Numerous laboratory studies have demonstrated that corals (Schneider & Erez 2006; Anthony *et al.* 2008), calcifying algae (Anthony *et al.* 2008; Kuffner *et al.* 2008), and coral reef communities (Langdon *et al.* 2000; Andersson *et al.* 2009) have reduced calcification in seawater with lower pH due to depleted carbonate saturation.

Ecological processes pivotal to coral reef resilience, including coral recruitment, herbivory, trophic integrity, and connectivity (Knowlton 2001; Mumby *et al.* 2007), under high CO_2 levels have hardly been investigated (Doney *et al.* 2009). Yet, growing evidence suggests that interactions between species are altered as CO_2 increases. Under conditions of OA, corals in contact with fleshy macroalgae had higher mortality (Diaz-Pulido *et al.* 2011), and fish mortality increased as OA reduced the ability of juvenile fish to detect their predators (Munday *et al.* 2010). Furthermore, it has been suggested that turf algae can decrease the recruitment of crustose coralline algae (CCA) (Kuffner *et al.* 2008; Russell *et al.* 2009) and kelp (Connell & Russell 2010) because of greater space occupation at elevated pCO_2. While these examples illustrate that ecological interactions can be altered as CO_2 increases, potential interactions of OA on coral recruitment have not been addressed.

Recruitment is critical to community recovery as it represents a crucial process in the development of populations in the post-disturbance period. A key ecological process in the formation of coral reefs is the settlement of coral larvae from the plankton to the reef substrata. Many larvae test benthic substrates for microhabitat suitability prior to settlement (i.e. attachment and metamorphosis), with the selection of optimal microhabitats critical in the post-settlement survival of benthic invertebrates (Raimondi & Keough 1990; Harrington *et al.* 2004). Different benthic algae offer both inductive and inhibitive settlement cues for planktonic invertebrate larvae (Rodriguez *et al.* 1993; Kuffner *et al.* 2006; Diaz-Pulido *et al.* 2010), and larvae often search for appropriate substrata associated with specific CCA and microbial communities for successful settlement (Morse *et al.* 1988; Johnson & Sutton 1994; Heyward & Negri 1999; Negri *et al.* 2001; Webster *et al.* 2004). While recent evidence demonstrates the settlement of coral larvae is reduced as pCO_2 increases (Albright *et al.* 2010; Albright & Langdon 2011; Nakamura *et al.* 2011), the interactions between planktonic larvae and the CCA community under elevated CO_2 levels are unknown.

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Here, we test the hypothesis that elevated pCO_2 (400 control, 800 and 1300 μatm) alters the recruitment of a spawning coral (*Acropora millepora*) by affecting the benthic algal community structure, and the interactions between the substrata and larvae during settlement. We used a mechanistic approach with three complementary experiments to investigate how OA reduces larval settlement. First, to investigate whether OA caused a shift in the community structure of the settlement substrata to alter coral settlement, we preconditioned settlement tiles in treatment seawater for 60 days prior to conducting 6 day settlement assays on those tiles in ambient seawater (expt. 1). Second, we conducted the reciprocal experiment by isolating the exposure of elevated pCO_2 seawater to the coral larvae and settlement substrata during the 6 days settlement phase only (expt. 2). Finally, we explored whether there was a combined effect on coral settlement when the settlement substrata and coral larvae were both exposed to elevated pCO_2 for 60 and 6 days respectively (expt. 3). From this series of experiments, we show that OA decreases coral settlement rates by reducing the availability of specific CCA preferred for larval settlement, as well as interfering with the interaction between larvae and CCA by altering the settlement behaviour of the coral larvae, such that previously avoided substrata are preferentially selected as pCO_2 increases in all three conditions.

MATERIAL AND METHODS

CO_2 treatments and general protocol

Coral settlement experiments were conducted from October to December 2009, at Heron Island Research Station, southern Great Barrier Reef (GBR). Settlement substrata and coral larvae were exposed to three treatments, which represented control (pH 8.04, 401 μatm), and two elevated (pH 7.79, 807 μatm; pH 7.60, 1299 μatm) levels of future CO_2 concentrations (Table 1). Treatments were based on the worst-case stabilisation levels V (pCO_2 700–850 μatm) and VI (pCO_2 > 900 μatm) predicted by the Intergovernmental Panel on Climate Change (IPCC) (Meehl *et al.* 2007). These were chosen for the experiment as current CO_2 emissions are tracking the most carbon intensive levels (A1FI) predicted by the IPCC (Le Quere *et al.* 2009).

As pH is reduced in a predictable manner by elevated pCO_2, the CO_2 levels of the experimental seawater were controlled by adjusting the pH of the seawater in 200 L sumps (Table 1) (see Diaz-Pulido *et al.* 2011 for system details). Briefly, the total pH of the seawater was continuously measured with temperature compensated pH electrodes (InPro4501VP; Mettler-Toledo, Melbourne, Victoria, Australia), which maintained the targeted pH levels with a control unit (Aquatronica, AEB technologies, Italy) that opened solenoid valves that injected CO_2 into the seawater when pH exceeded the desired threshold. The calibration of the pH probes was checked daily, and recalibrated with Mettler-Toledo calibration buffers to 0.01 pH units when necessary. Alkalinity was measured on seawater samples taken every 6 h over a spring tidal cycle (2.4 m range) at the end of the study period to capture the largest variation in the seawater alkalinity and consolidate the pH treatments to the CO_2 levels. Alkalinity replicates within a sample were analysed until a maximum 2% error was met, using a Metrohm auto-titrator at Edith Cowan University, WA. The carbonate chemistry of the control and experimental seawater was calculated with CO2SYS (Lewis & Wallace 2006) using pH, total alkalinity, salinity (35.4 ppt ± 0.2 SEM; $n = 8$), and temperature as the inputs, with the constants from Mehrbach *et al.* (1973) refitted by Dickson & Millero (1987).

The settlement tile CO_2 conditioning and settlement assays were conducted on tiles in replicate tanks in the outdoor flow-through aquarium system (details of the CO_2 exposure times, tank and tile replication are described below in the protocols under each experiment and in supplementary Fig. S1). The three treatments were fed from the 200 L sumps into replicate 12 L tanks at a mean flow rate of 2.4 (± 0.2 SEM) L min^{-1}, and each tank had a small powerhead for extra seawater circulation. This flow rate and water movement maintained the target pH levels, which were verified regularly with a portable SG2 SevenGo^{TM} pH meter. Replicate tanks were randomised on the aquarium table under shade-cloth to account for the heterogeneity in light, which averaged 406 (± 18 SEM) μmol m^{-2} s^{-1} between 6 AM to 6 PM.

Settlement tile preparation

Unglazed terracotta settlement tiles (~ 5 × 5 × 0.5 cm) were initially preconditioned on the Heron Island reef flat (23° 26′ 42.2″ S, 151° 54′ 47.0″ E) for 5 months to develop a microbial and encrusting community important to coral settlement (Heyward & Negri 1999). Tiles were collected and carefully cleaned of fouling organisms using a toothbrush, tweezers, and a plastic scraper. The tiles were then randomly placed in replicate 12 L aquaria and conditioned in the control and elevated CO_2 treatments for 60 days prior to the settlement assays. During this time the walls of the aquaria were cleaned regularly to minimize any algal growth. Settlement tiles were orientated horizontally at the bottom of the tanks and were stacked in tile pairs with a 0.5 cm spacer, maximising the amount of cryptic surfaces available for settlement, as coral larvae generally settle in cryptic areas in shallow habitats (Wallace 1985).

Coral larvae collection

Gravid adult colonies of *Acropora millepora* were located on the Heron Island reef flat around the time of the predicted spawning (2nd Dec 2009). *Acropora millepora* was chosen as a model organism as it is

Table 1 Summary of the physical and chemical seawater values for CO_2 treatment levels

Treatment	Temp* °C	pH*	TA* μmol kg^{-1}	pCO_2† μatm	HCO_3^-† μmol kg^{-1}	CO_3^{2-}† μmol kg^{-1}	$\Omega_{Aragonite}$†
Control	26.0 (± 0.6)	8.04 (± 0.01)	2355 (± 14)	401 (± 11)	1800 (± 20)	227 (± 4)	3.6 (± 0.07)
Stabilisation level V	26.0 (± 0.6)	7.79 (± 0.01)	2365 (± 20)	807 (± 14)	2019 (± 23)	142 (± 3)	2.3 (± 0.06)
Stabilisation level VI	26.0 (± 0.6)	7.60 (± 0.01)	2363 (± 19)	1299 (± 21)	2125 (± 19)	97 (± 3)	1.6 (± 0.06)

*Temperature, pH, and total alkalinity are means (± SEM) of five replicates.
†pCO_2, bicarbonate, carbonate and aragonite saturation state (Ω) were calculated using CO2 SYS (Lewis & Wallace 2006).

commonly found on GBR and Indo-Pacific shallow reef flats. Five colonies were collected and transported to outdoor aquarium facilities where they were housed in 60 L flow-through aquaria until they released their egg-sperm bundles. The bundles were broken apart by gently stirring and agitating the water, and gametes from the different colonies were collected and cross-fertilized. Fertilization took place for 2 h, after which the embryos were collected and reared in a laboratory at 25 °C in ambient seawater, using 200 L sumps with aeration. At least half the seawater was changed every few hours for the first 24 h and every 6–12 h thereafter. This removed dead larvae and unfertilized gametes, to minimise contamination of the rearing sumps. The larvae developed cilia and began swimming 3 days after spawning, after which they were used for the settlement assays. Swimming *A. millepora* larvae were randomly removed from the rearing sumps, added to the experimental aquaria, and allowed 6 days to settle (i.e. attach and metamorphose). The number of larvae added to each tank during the settlement assays was standardised to 150 (± 10) per tile. After this time, the tiles were removed from the tanks at random and inspected for settlement with a dissecting microscope.

Experiment 1

To isolate whether changes to the benthic community altered coral settlement, settlement tiles were conditioned at 400, 800, and 1300 μatm pCO_2 for 60 days. Following the conditioning period, coral settlement assays were conducted for 6 days on those tiles with control seawater only. Three replicate tanks per treatment, with 8 tiles and 1200 (± 80) larvae per tank, were used for the experiment (Fig. S1).

Experiment 2

A reciprocal experiment was conducted to determine whether settlement was altered by elevated pCO_2 exposure of the coral larvae and the benthic community during the settlement assays only. Settlement assays were conducted for 6 days using the three CO_2 seawater treatments described above with settlement tiles that were conditioned with control seawater only. Two replicate tanks per treatment, with 6 tiles and 900 (± 60) larvae per tank, were used for the experiment (Fig. S1).

Experiment 3

Finally, to investigate the combined effect of prolonged exposure of elevated pCO_2 on the benthic community and the settling larvae, settlement tiles were conditioned in the three CO_2 treatments for 60 days prior to conducting 6 day settlement assays on those tiles in the treatment seawater described above. Three replicate tanks per treatment, with 10 tiles and 1500 (± 150) larvae per tank, were used for the experiment (Fig. S1).

Response variables and data analyses

We analysed total larval settlement, benthic community and CCA community cover of the settlement tiles, and coral settlement substrate preferences for each of the three experiments. The number of settled (i.e. attached and metamorphosed) coral larvae was initially quantified for all orientations of each tile. However, we only analysed the undersides of each tile (for this and all other variables) as the number of corals settled in this orientation accounted for ≥ 95% of the total settlement.

The benthic community of the settlement tiles was quantified by placing a grid on a tile, and evaluating the dominant substrate in a square (7.5 mm^2) using a dissecting microscope, with 224–377 squares per tile. The substrata were characterised into eight major benthic groups which were: bare tile, CCA, dead crustose coralline algae (DCCA), endolithic algae found in dead crustose coralline algae (EDCCA), turf algae found on dead crustose coralline algae (TDCCA), turf algae (Turf), encrusting fleshy algae (EFA), and other organisms which included biofilm, bryozoans, foraminifera, and other encrusting organisms (Other). CCA specimens were identified to the finest taxonomic resolution where possible and included nine CCA taxa (see Appendix S1 in Supporting Information for details on CCA identification). When CCA specimens could not be identified to genus or species, they were placed in to an Unknown CCA group, which represented ~ 6% of the total CCA community. See supplementary Fig. S2 for images of the dominant benthic groups and CCA taxa.

The substrate settled on by each individual was quantified to investigate larval settlement behaviour using Vanderploeg and Scavia's electivity index (E^*). This index is analogous to Ivlev's E, but incorporates a selectivity coefficient and the number of substrata available for settlement (Lechowicz 1982). Therefore: $E^* = [W_a - (1/n)]/[W_a + (1/n)]$, where n is the total number of substrate types available on each tile and W is the selectivity coefficient for substrate 'a' determined by: $W_a = [r_a/p_a]/\sum(r_a/p_a),(r_b/p_b)\ldots(r_z/p_z)$, r is the proportion of coral larvae settled on substrata a to z on each tile, and p is the proportion of substrata a to z available for settlement on each tile. A substrate was selected at random for larval settlement when E^* was ~ 0, preferably settled on when E^* was > 0, and avoided for settlement when E^* was < 0.

The number of coral larvae settled per tile was analysed with a generalised linear mixed effects model using Poisson distribution. We tested the effects of elevated pCO_2 on counts of coral settlement amongst CO_2 treatment (3 levels, fixed) with replicate tanks as a random effect and nested in CO_2 treatment. The effect of elevated pCO_2 on the percent cover of the broad benthic community and CCA community composition were tested using a mixed effects permutational MANOVA (PERMANOVA), with the same fixed and random factors described for the previous model. When significant differences were detected ($P < 0.05$), pair-wise comparisons were performed to investigate treatment effects. In multivariate analyses, SIMPER analysis was used to determine the variables that characterised the dissimilarity amongst treatments. Univariate ANOVA was conducted within CCA cover to determine any significant treatment effects. All percentage cover data were $\sin^{-1}\sqrt{x}$ transformed to meet requirements of homogeneity (permDISP) prior to analysis. Finally, we tested the effect of CO_2 treatment (3 levels, fixed) on coral settlement behaviour with tanks as replicates using PERMANOVA. In all PERMANOVA main effect and pair-wise tests, we used the P-values generated by 99 999 permutations when the number of unique permutations were large, and the Monte Carlo asymptotic P-value otherwise (Anderson 2005).

RESULTS

We report the results of each of the three settlement experiments in turn, describing the impacts of OA on overall coral settlement density,

the structure of benthic substrata, and settlement behaviour of the larvae. Results are summarised in Table 2.

Experiment 1

A reduction in the cover of CCA and shift in the CCA community from elevated CO_2 decreased coral settlement in the OA treatments. The reduction in settlement decreased significantly from an average of 11.0 individuals per 25 cm^2 in the control, to 1.6 and 5.5 individuals at 800 and 1300 μatm, respectively (Table 2; Fig. 1a). The cover of CCAs changed dramatically in the elevated CO_2 treatments with a significant decline of ~ 50% (ANOVA: $F_{2,6} = 13.283$; $P = 0.014$; Table 2; supplementary Fig. S3a). The CCA community structure also changed as pCO_2 increased, with three out of ten coralline algal taxa declining with increasing CO_2 concentrations (MANOVA: $F_{2,6} = 3.286$; $P = 0.017$; Table 2). *Titanoderma* spp., *Hydrolithon boreale*, and *H. farinosum* were the species that characterised the loss of CCA cover in both the elevated CO_2 treatments (supplementary Fig. S3b).

The settlement behaviour of the larvae, as measured by their substrate selectivity, was significantly affected by the exposure of settlement tiles to elevated pCO_2 prior to the settlement assays (MANOVA: $F_{2,6} = 4.291$; $P = 0.004$; Table 2). *Titanoderma* spp. was the only preferred settlement substrate in the control treatment ($E^* = 0.8$) and there were lower rates of settlement on all other substrata than would be expected by chance (supplementary Fig. S4a). At 800 μatm, larvae did not show any clear settlement preferences and most substrata were avoided (supplementary Fig. S4b), while the larvae showed a weak preference for *H. farinosum* ($E^* = 0.2$) at 1300 μatm (supplementary Fig. S4c).

Experiment 2

As expected, there were no differences between the broad community composition, the CCA percent cover, or the CCA community amongst the settlement tiles that were allocated for use in these settlement assays (Table 2). Yet, exposure of coral larvae and the settlement tiles to elevated pCO_2 during the 6 day settlement assays significantly reduced coral settlement, as it declined from an average of 11.9 individuals per 25 cm^2 in the control, to 4.7 and 2.8 individuals at 800 and 1300 μatm, respectively (Table 2; Fig. 1b). A similar disruption to larval settlement behaviour occurred to that found when only the tiles were pre-exposed to elevated pCO_2 for a prolonged period of time (exp. 1). Again, coral larvae preferred to settle on *Titanoderma* spp. ($E^* = 0.75$) in controls (supplementary Fig. S5a), most substrata were avoided at 800 μatm (supplementary Fig. S5b), and a weak preference for *H. farinosum* ($E^* = 0.2$) was found at 1300 μatm (supplementary Fig. S5c).

Experiment 3

Again, settlement was reduced when the tiles were conditioned in the CO_2 treatments for 60 days, and 6 day settlement assays were conducted on those tiles under elevated pCO_2. The magnitude of the effect was similar to whether the tiles were conditioned in the CO_2 treatments for 60 days prior to the 6 day settlement assays with control seawater only (exp.1), or whether the larvae and tiles were exposed to the CO_2 treatments during the 6 day settlement assays only (exp. 2) (Table 2). Increased CO_2 reduced the settlement of *A. millepora* from an average of 9.7 individuals per 25 cm^2 in the control, to 5.2 and 4.2 individuals at 800 and 1300 μatm, respectively (Fig. 1c). The reduction in settlement was significant between the control and highest CO_2 treatment ($P = 0.046$) and marginally significant between the control and intermediate treatment ($P = 0.060$).

The changes in tile community structure were similar to those in experiment 1, but the effects of OA appeared to be less variable in this experiment. As a result, the wider benthic community structure on the tile undersides was found to differ significantly amongst the CO_2 treatments (MANOVA: $F_{2,6} = 2.612$; $P = 0.003$; Table 2; Fig. 2a). The loss of coralline algae was partly replaced by an increase of 8% in the cover of 'bare tile' (Fig. 2a). As in experiment 1, OA led to a significant reduction in the cover of CCAs on the tiles (ANOVA: $F_{2,6} = 40.538$; $P = 0.002$; Table 2), characterised by declines in *Titanoderma* spp., *H. boreale*, and *H. farinosum* (Fig. 2b).

Coral settlement behaviour was again altered significantly by elevated pCO_2 (MANOVA: $F_{2,6} = 4.224$; $P = 0.004$; Table 2; Fig. 3). Of the 19 substrata available, *Titanoderma* spp. was again the only preferred settlement substrate in the control ($E^* = 0.6$), while all other substrata were avoided (Fig. 3a). At 800 μatm, *Hydrolithon reinboldii* was the only preferred coral settlement substrate ($E^* = 0.3$), and all other settlement substrata were either randomly settled on or avoided (Fig 3b). No substrate was preferred for settlement at 1300 μatm, with random settlement on bare tile ($E^* = -0.05$), and all other substrata were avoided (Fig 3c).

DISCUSSION

In our study, the settlement density of coral larvae decreased by ≥ 45% as pCO_2 increased from 400 to 800 and 1300 μatm in all three

Table 2 Changes to the response variables in Experiments 1, 2, and 3, comparing elevated CO_2 treatments (800 and 1300 μatm) to the controls (400 μatm)

	Experiment 1		Experiment 2		Experiment 3	
Response variable	800 μatm	1300 μatm	800 μatm	1300 μatm	800 μatm	1300 μatm
1. Total settlement	↓ 82%***	↓ 45%	↓ 58%***	↓ 75%***	↓ 50%	↓ 60%*
2. Benthic community structure	NS	NS	NS	NS	CCA	CCA**
3. CCA cover	↓ 47%*	↓ 52%*	NS	NS	↓ 42%*	↓ 63%***
4. CCA community structure	*Titanoderma***	*Titanoderma**	NS	NS	NS	*Titanoderma***
5a). Overall settlement behaviour	*Titanoderma**	*Titanoderma**	NS	NS	*Sporolithon**	*Titanoderma***
5b). Selectivity from *Titanoderma*	↓ 72%	↓ 74%	↓ 69%	↓ 65%	↓ 35%	↓60%

SIMPER analysis determined the variable that characterised the difference between the control and elevated CO_2 treatments in multivariate analyses. Coral behaviour (5) is divided into the change in settlement preferences of the larvae (5a) among the substrate community, and (5b) from *Titanoderma* spp., the only preferred settlement substrate in the controls. Significance values are indicated by: NS = non-significant, * = < 0.05, ** = < 0.01, *** = < 0.001.

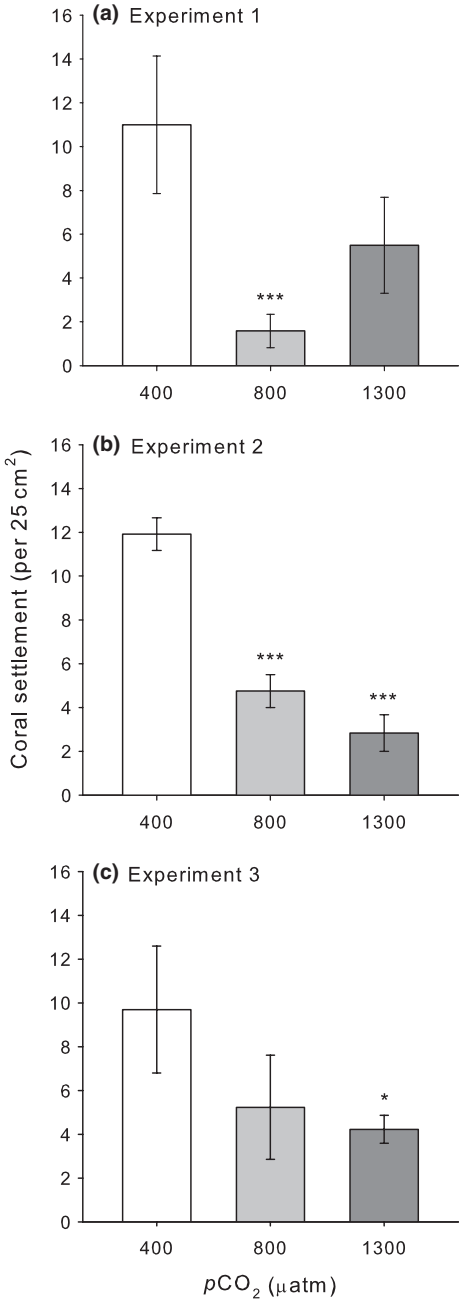

Figure 1 Coral (*Acropora millepora*) settlement rates on experimental tiles (25 cm^2) in response to increasing pCO_2. Assays occurred on (a) settlement tiles conditioned in treatment seawater for 60 days prior to 6 day larval settlement assays on those tiles with control seawater (n = 3); (b) settlement tiles and larvae exposed to treatment seawater for 6 days during the settlement assays on tiles conditioned in control seawater only (n = 2); and (c) settlement tiles and larvae exposed to treatment seawater for 60 and 6 days, respectively (n = 3). Data are means ± SEM. Significance values comparing elevated CO_2 treatments to the control are indicated by: * = < 0.05, ** = < 0.01, *** = < 0.001.

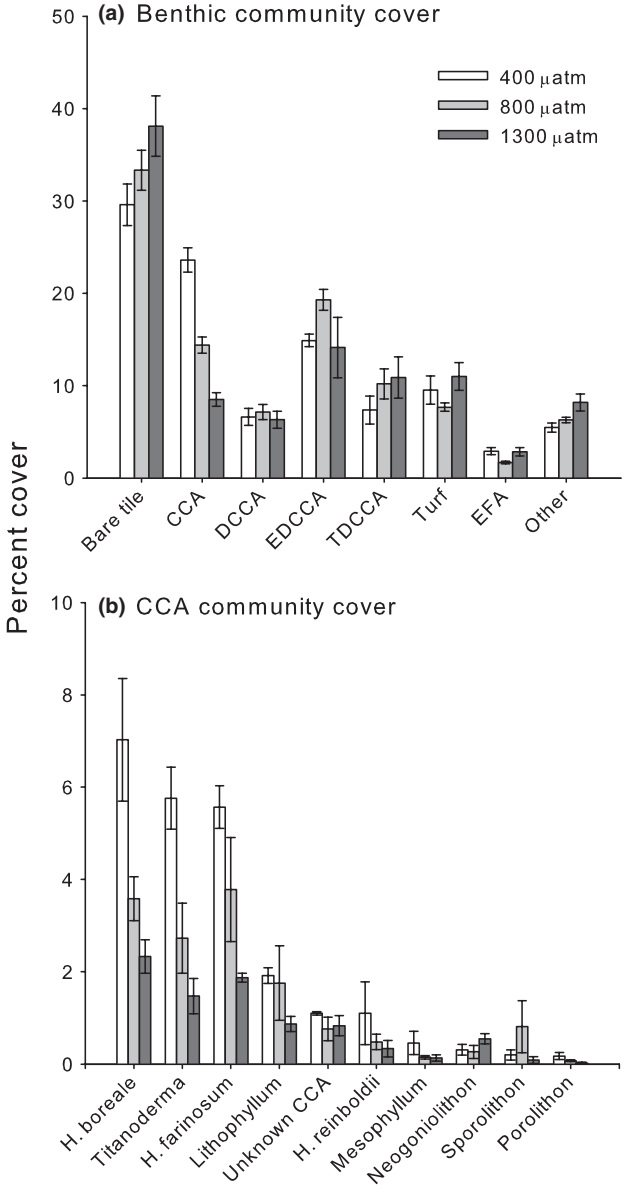

Figure 2 Percent cover of (a) the broad benthic community and (b) the crustose coralline algae community in response to increasing pCO_2. Settlement tiles were exposed to the treatments for 66 days, which involved a 60 day pre-exposure period prior to the 6 day settlement assays (expt. 3). CCA = crustose coralline algae. DCCA = dead crustose coralline algae. EDCCA = endolithic algae in dead crustose coralline algae. TDCCA = turf on dead crustose coralline algae. Turf = filamentous algal turf. EFA = encrusting fleshy algae. Other = biofilm, carbonate, bryozoans, encrusting foraminifera, and unidentified. *H. boreale* = *Hydrolithon boreale*. *H. farinosum* = *Hydrolithon farinosum*. *H. reinboldii* = *Hydrolithon reinboldii*. Data are means ± SEM; n = 3.

experiments. The reduction in settlement was accompanied by a profound decline in the cover of CCA when the settlement substrata were conditioned in elevated CO_2 treatments for 60 days prior to the settlement assays (expt. 1 & 3). While recent studies have also found inverse relationships between elevated pCO_2 and rates of coral settlement (Albright *et al.* 2010; Albright & Langdon 2011; Nakamura *et al.* 2011), and overall CCA cover (Hall-Spencer *et al.* 2008; Kuffner *et al.* 2008; Russell *et al.* 2009; Fabricius *et al.* 2011), our study is the first to directly link benthic community cover with coral settlement and it provides three important novel insights. First, we identified the most susceptible CCA to OA and found that they are the most important taxa for coral settlement, particularly *Titanoderma*. Secondly, we discovered that OA reduced the affinity between the settling larvae and *Titanoderma,* their preferred settlement substrate. Third, we found that similar changes in settlement behaviour occurred under all three

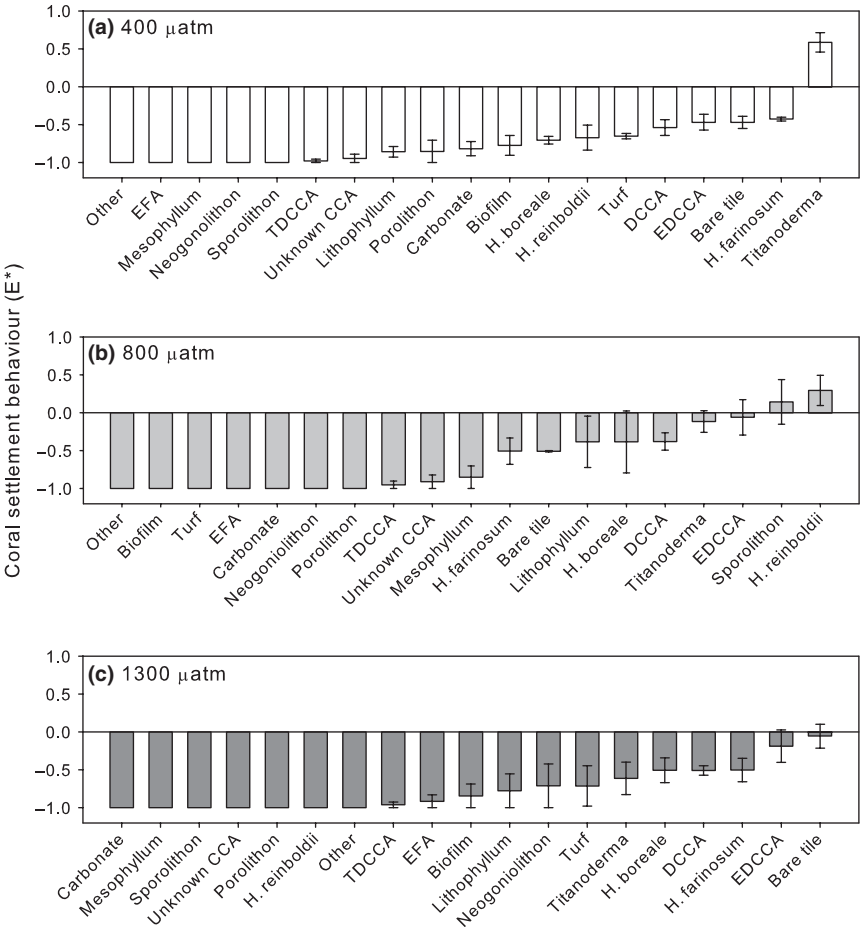

Figure 3 Coral (*Acropora millepora*) settlement behaviour of the substrata that the coral larvae preferred (> 0), avoided (< 0), or randomly (∼ 0) settled on in response to (a) 400, (b) 800, and (c) 1300 μatm pCO_2 using Vanderploeg and Scavia's electivity index (E^*). Settlement assays occurred with settlement tiles and larvae exposed to the treatments for 60 and 6 days, respectively (expt. 3). See Fig. 2 for abbreviated substrate definitions. Data are means ± SEM; $n = 3$.

experimental conditions. As we explain below, this surprising result implies that coral settlement behaviour is mediated by cues associated with coralline algae that appear to be highly sensitive to elevated pCO_2.

We designed our experiments to distinguish the effects of OA on the settling organisms (corals) from the settlement surfaces (the benthic community on the tiles). In experiment 1, we subjected tiles to a 60 day exposure to elevated pCO_2 that resulted in profound changes to the coralline algal assemblage. When these tiles were then placed in control (ambient) conditions with coral planulae, the settlement behaviour of the larvae was disrupted. Because the larvae never experienced OA conditions in this experiment, the result implies that prolonged exposure of substrates to OA may alter the cues associated with CCA that are used by larvae to settle preferentially on *Titanoderma*. To examine the influence of OA on the settling larvae themselves (expt. 2), we exposed them to OA treatments during the 6 day settlement assays. In this case, all benthic substrata were preconditioned in control seawater prior to the experiment and were exposed to the treatment seawater for the 6 day period during the assays. Again, we found the same qualitative disruption to larval settlement behaviour, suggesting that the 6 day exposure of the benthic community to OA disrupted the signalling from the CCA, and potentially that the larvae may also be directly affected by elevated pCO_2. In the third experiment, we found a similar qualitative result when larvae were subjected to OA and offered settlement substrates that had also been exposed to the treatments for a 60 day period.

There are two possible explanations of our results. The most parsimonious explanation is that even a daily exposure of the benthos to OA disrupts the signalling mechanisms used by coral planulae to preferentially settle upon *Titanoderma*. That is, the outcome for settlement was the same whether the tiles were pre-conditioned for 60 days, causing profound changes in coralline cover, or 6 days during the experiment. This explanation is consistent with all three experiments and accounts for the lack of an additive impact of OA on settlement when both larvae and substrates were exposed to OA. The most likely mechanism is that larval settlement behaviour is mediated by bacteria and/or chemical cues associated with CCA because settlement was disrupted even when the cover of coralline algae was unchanged (exp. 2). These results imply that the cues associated with algal morphogens and/or the bacterial communities associated with the CCA thalli are highly sensitive to changes in water chemistry. It was recently demonstrated that microbial communities associated with biofilms grown on glass slides were altered after 11 days in elevated pCO_2 (Witt *et al.* 2011). There is a precedent for the role of bacteria in facilitating settlement (Johnson & Sutton 1994; Negri *et al.* 2001; Webster *et al.* 2004), but neither the taxon specificity (to *Titanoderma*) nor the sensitivity to OA have been shown, and future work should isolate whether it is changes in bacterial communities and/or the morphogens associated with CCA that alters the preference of larval settlement under elevated CO_2.

An alternative, albeit not mutually exclusive, explanation is that coral settlement on CCA is disrupted by exposure of either partner to

OA conditions (i.e. exposure of either the larvae or the algae). This explanation is consistent with recent reports of the impacts of elevated pCO_2 on coral larvae metabolic rate (Albright & Langdon 2011; Nakamura *et al.* 2011) and metamorphosis (Albright *et al.* 2010; Albright & Langdon 2011; Nakamura *et al.* 2011), and on fish larvae olfactory ability (Dixson *et al.* 2010; Munday *et al.* 2010). It has also been shown that invertebrate larvae become less discriminating in the selection of their preferred substrate for settlement when they are under stress (Marshall & Keough 2003). Thus, the coral larvae may have lost their selectivity for *Titanoderma* due to the stress related to OA. Yet, this explanation is not entirely satisfactory for the following reasons. Firstly, we have to accept that the similarity in outcome from manipulating the settlement substrata versus the planulae is coincidental (i.e. the disruption to either partner has the same overall outcome). Secondly, we cannot easily account for the absence of a clear additive effect when both partners were perturbed simultaneously.

Previous studies of coral recruitment in both spawning and brooding corals, including those from the families Acroporidae, Agariciidae, Pocilloporidae, and Poritidae, and stemming from both the Atlantic and Indo-Pacific, have found that coral larvae have an innate ability to settle preferentially on a single CCA genus, *Titanoderma*, and that ensuing survival is greatest on this substrate compared to any other (Harrington *et al.* 2004; Arnold *et al.* 2010; Price 2010; Ritson-Williams *et al.* 2010). Here, we found that OA presents two problems for settling corals. Not only is *Titanoderma* exceptionally sensitive to OA, such that its availability is compromised, but larval behaviour switches from a high preference to settle on *Titanoderma* to avoidance. Corals have previously been shown to settle on other substrata, including *Hydrolithon* spp. and bare tile that the larvae preferentially settled upon at the elevated CO_2 treatments in this study, but this occurs at lower rates of settlement and survival (Harrington *et al.* 2004; Arnold *et al.* 2010; Price 2010; Ritson-Williams *et al.* 2010). *Titanoderma* has been proposed to be a good facilitator of coral settlement because it does not slough off tissue and therefore provides a persistent substratum for recruits, while some species of *Hydrolithon* and other CCA slough their tissue to remove fouling organisms (Harrington *et al.* 2004; Ritson-Williams *et al.* 2010). Thus, while the settlement of corals onto previously avoided substrata at elevated pCO_2 in our study implies that their post-settlement survival may be reduced, empirical investigations of the long-term survival of recruits on different substrates at elevated pCO_2 are needed to directly test this hypothesis as it may be an adaptive trait.

Titanoderma is a cryptic, early successional species with relatively rapid growth, creeping morphology and delicate, thin thalli (< 500 μm) (Steneck 1986; Ringeltaube & Harvey 2000; Littler & Littler 2003). Its morphology and cryptic, opportunistic nature make it indicative of fresh substratum with relatively benign levels of stress such as parrotfish grazing or sediment scour. Such environments are likely to be ideal for coral settlement because new substratum is likely to possess fewer competitors (Vermeij & Sandin 2008) and parrotfish predation can be problematic for coral recruits (Penin *et al.* 2010). However, we hypothesize that some of the traits that make *Titanoderma* such an important settlement inducer might predispose a particular sensitivity to OA. It has previously been demonstrated that elevated pCO_2 decreases the abundance and recruitment of coralline algae, in both field (Hall-Spencer *et al.* 2008; Fabricius *et al.* 2011) and laboratory (Kuffner *et al.* 2008; Russell *et al.* 2009) settings. While these reports (Hall-Spencer *et al.* 2008; Kuffner *et al.* 2008) found an inverse competitive relationship between CCA and turf cover as pCO_2 increased, we found that reduced CCA was accompanied by an increase in the amount of bare tile rather than turf. This suggests that the reduction of CCA cover was not a consequence of space competition with turfs, but a direct effect of OA on CCA. In our study, the three most sensitive taxa of CCA to elevated pCO_2 (*H. boreale*, *H. farinosum*, and *Titanoderma* spp.), are all early successional species with rapid growth and thin thalli (< 500 μm) (Steneck 1986; Ringeltaube & Harvey 2000; Littler & Littler 2003). In contrast, later successional CCA taxa that have thicker crusts (> 500 μm) (e.g. *Sporolithon*, *Neogoniolithon*, *Porolithon*) (Steneck 1986; Ringeltaube & Harvey 2000; Littler & Littler 2003) may be more resistant to OA. While further studies are needed to test this hypothesis, our results show that increasing levels of dissolved CO_2 are likely to have profound consequences for the functional diversity of coralline algal communities.

Our research has demonstrated the ecological mechanics of how ocean acidification may interfere with a critical process important to the resilience of a diverse marine ecosystem. This occurred by a reduction to the abundance of the preferred substrate for larval settlement, and a disruption to an intimate ecological interaction between the coral larvae and its preferred substrate during settlement. The altered interaction between coral settlement and the CCA community suggests that future recruitment of individuals may be impaired by CO_2 concentrations predicted to be reached this century. These type of impacts of increased CO_2 on non-trophic ecological interactions between species are just starting to be experimentally demonstrated (e.g. Connell & Russell 2010; Dixson *et al.* 2010; Diaz-Pulido *et al.* 2011), but suggest profound consequences on the recovery potential of shallow marine ecosystems (e.g. coral reefs) following local and global disturbances.

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AUTHOR CONTRIBUTIONS

CD, SW, GDP and PJM designed the study, CD and SW conducted the study, CD and GDP collected the data, and CD and PJM analysed the data. CD wrote the first draft of the manuscript, and all the authors contributed substantially to the interpretation and final version of the paper.

REFERENCES

Albright, R. & Langdon, C. (2011). Ocean acidification impacts multiple early life history processes of the Caribbean coral *Porites astreoides*. *Glob. Change Biol.*, 17, 2478–2487.

Albright, R., Mason, B., Miller, M. & Langdon, C. (2010). Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. *Proc. Natl Acad. Sci. USA*, 107, 20400–20404.

Anderson, M.J. (2005). *PERMANOVA: A FORTRAN Computer Program for Permutational Analysis of Variance*. Department of Statistics, University of Auckland, New Zealand.

Andersson, A.J., Kuffner, I.B., Mackenzie, F.T., Jokiel, P.L., Rodgers, K.S. & Tan, A. (2009). Net loss of $CaCO_3$ from a subtropical calcifying community due to seawater acidification: mesocosm-scale experimental evidence. *Biogeosciences*, 6, 1811–1823.

Anthony, K.R.N., Kline, D.I., Diaz-Pulido, G., Dove, S. & Hoegh-Guldberg, O. (2008). Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proc. Natl Acad. Sci. USA*, 105, 17442–17446.

Arnold, S.N., Steneck, R.S. & Mumby, P.J. (2010). Running the gauntlet: inhibitory effects of algal turfs on the processes of coral recruitment. *Mar. Ecol. Prog. Ser.*, 414, 91–105.

Connell, S.D. & Russell, B.D. (2010). The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *P. R. Soc. B-Biol. Sci.*, 277, 1409–1415.

Diaz-Pulido, G., Harii, S., McCook, L.J. & Hoegh-Guldberg, O. (2010). The impact of benthic algae on the settlement of a reef-building coral. *Coral Reefs*, 29, 203–208.

Diaz-Pulido, G., Gouezo, M., Tilbrook, B., Dove, S. & Anthony, K.R.N. (2011). High CO_2 enhances the competitive strength of seaweeds over corals. *Ecol. Lett.*, 14, 156–162.

Dickson, A.G. & Millero, F.J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Res.*, 34, 1733–1743.

Dixson, D.L., Munday, P.L. & Jones, G.P. (2010). Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecol. Lett.*, 13, 68–75.

Doney, S.C., Fabry, V.J., Feely, R.A. & Kleypas, J.A. (2009). Ocean acidification: the other CO_2 problem. *Annu. Rev. Mar. Sci.*, 1, 169–192.

Fabricius, K.E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G. *et al.* (2011). Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Clim. Change*, 1, 165–169.

Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M. *et al.* (2008). Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, 454, 96–99.

Harrington, L., Fabricius, K., De'Ath, G. & Negri, A. (2004). Recognition and selection of settlement substrata determine post-settlement survival in corals. *Ecology*, 85, 3428–3437.

Heyward, A.J. & Negri, A.P. (1999). Natural inducers for coral larval metamorphosis. *Coral Reefs*, 18, 273–279.

Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Greenfield, P., Gomez, E. *et al.* (2007). Coral reefs under rapid climate change and ocean acidification. *Science*, 318, 1737–1742.

Jansen, E., Overpeck, J., Briffa, K.R., Duplessy, J.-C., Joos, F., Masson-Delmotte, V. *et al.* (2007). Palaeoclimate. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M. & Averyt, K.B., *et al.*). Cambridge University Press, Cambridge, pp. 433–498.

Johnson, C.R. & Sutton, D.C. (1994). Bacteria on the surface of crustose coralline algae induce metamorphosis of the crown-of-thorns starfish *Acanthaster planci*. *Mar. Biol.*, 120, 305–310.

Knowlton, N. (2001). The future of coral reefs. *Proc. Natl Acad. Sci. USA*, 98, 5419–5425.

Kroeker, K.J., Kordas, R.L., Crim, R.N. & Singh, G.G. (2010). Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol. Lett.*, 13, 1419–1434.

Kuffner, I.B., Walters, L.J., Becerro, M.A., Paul, V.J., Ritson-Williams, R. & Beach, K.S. (2006). Inhibition of coral recruitment by macroalgae and cyanobacteria. *Mar. Ecol. Prog. Ser.*, 323, 107–117.

Kuffner, I.B., Andersson, A.J., Jokiel, P.L., Rodgers, K.S. & Mackenzie, F.T. (2008). Decreased abundance of crustose coralline algae due to ocean acidification. *Nature Geosci.*, 1, 114–117.

Langdon, C., Takahashi, T., Sweeney, C., Chipman, D., Goddard, J., Marubini, F. *et al.* (2000). Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Global Biogeochem. Cy.*, 14, 639–654.

Le Quere, C., Raupach, M.R., Canadell, J.G., Marland, G., Bopp, L., Ciais, P. *et al.* (2009). Trends in the sources and sinks of carbon dioxide. *Nature Geosci.*, 2, 831–836.

Lechowicz, M.J. (1982). The sampling characteristics of electivity indexes. *Oecologia*, 52, 22–30.

Lewis, P.D.E. & Wallace, D.W.R. (2006). MS excel program developed for CO_2 system calculations. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge.

Littler, D.S. & Littler, M.M. (2003). *South Pacific Reef Plants: A Diver*'s Guide to the Plant Life of South Pacific Coral Reefs. Offshore Graphics, Washington, D.C.

Marshall, D.J. & Keough, M.J. (2003). Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. *Mar. Ecol. Prog. Ser.*, 255, 145–153.

Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M. *et al.* (2007). Global climate projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M. & Averyt, K.B., *et al.*). Cambridge University Press, Cambridge, pp. 747–845.

Mehrbach, C., Culberso, C.H., Hawley, J.E. & Pytkowic, R.M. (1973). Measurement of apparent dissociation-constants of carbonic-acid in seawater at atmospheric pressure. *Limnol. Oceanogr.*, 18, 897–907.

Morse, D.E., Hooker, N., Morse, A.N.C. & Jensen, R.A. (1988). Control of larval metamorphosis and recruitment in sympatric Agaricid corals. *J. Exp. Mar. Biol. Ecol.*, 116, 193–217.

Mumby, P.J., Harborne, A.R., Williams, J., Kappel, C.V., Brumbaugh, D.R., Micheli, F. *et al.* (2007). Trophic cascade facilitates coral recruitment in a marine reserve. *Proc. Natl Acad. Sci. USA*, 104, 8362–8367.

Munday, P.L., Dixson, D.L., McCormick, M.I., Meekan, M., Ferrari, M.C.O. & Chivers, D.P. (2010). Replenishment of fish populations is threatened by ocean acidification. *Proc. Natl Acad. Sci. USA*, 107, 12930–12934.

Nakamura, M., Ohki, S., Suzuki, A. & Sakai, K. (2011). Coral larvae under ocean acidification: survival, metabolism, and metamorphosis. *PLoS ONE*, 6, e14521.

Negri, A.P., Webster, N.S., Hill, R.T. & Heyward, A.J. (2001). Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae. *Mar. Ecol. Prog. Ser.*, 223, 121–131.

Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A. *et al.* (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 437, 681–686.

Penin, L., Michonneau, F., Baird, A.H., Connolly, S.R., Pratchett, M.S., Kayal, M. *et al.* (2010). Early post-settlement mortality and the structure of coral assemblages. *Mar. Ecol. Prog. Ser.*, 408, 55–64.

Price, N. (2010). Habitat selection, facilitation, and biotic settlement cues affect distribution and performance of coral recruits in French Polynesia. *Oecologia*, 163, 747–758.

Raimondi, P.T. & Keough, M.J. (1990). Behavioural variability in marine larvae. *Aust. J. Ecol.*, 15, 427–437.

Ringeltaube, P. & Harvey, A. (2000). Non-geniculate coralline algae (Corallinales, Rhodophyta) on Heron Reef, Great Barrier Reef (Australia). *Bot. Mar.*, 43, 431–454.

Ritson-Williams, R., Paul, V.J., Arnold, S.N. & Steneck, R.S. (2010). Larval settlement preferences and post-settlement survival of the threatened Caribbean corals *Acropora palmata* and *A. cervicornis*. *Coral Reefs*, 29, 71–81.

Rodriguez, S.R., Ojeda, F.P. & Inestrosa, N.C. (1993). Settlement of benthic marine invertebrates. *Mar. Ecol. Prog. Ser.*, 97, 193–207.

Russell, B.D., Thompson, J.A.I., Falkenberg, L.J. & Connell, S.D. (2009). Synergistic effects of climate change and local stressors: CO_2 and nutrient-driven change in subtidal rocky habitats. *Glob. Change Biol.*, 15, 2153–2162.

Schneider, K. & Erez, J. (2006). The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*. *Limnol. Oceanogr.*, 51, 1284–1293.

Steneck, R.S. (1986). The ecology of coralline algal crusts: convergent patterns and adaptive strategies. *Annu. Rev. Ecol. Syst.*, 17, 273–303.

Vermeij, M.J.A. & Sandin, S.A. (2008). Density-dependent settlement and mortality structure the earliest life phases of a coral population. *Ecology*, 89, 1994–2004.

Wallace, C.C. (1985). Seasonal peaks and annual fluctuations in recruitment of juvenile scleractinian corals. *Mar. Ecol. Prog. Ser.*, 21, 289–298.

Webster, N.S., Smith, L.D., Heyward, A.J., Watts, J.E.M., Webb, R.I., Blackall, L.L. *et al.* (2004). Metamorphosis of a scleractinian coral in response to microbial biofilms. *Appl. Environ. Microbiol.*, 70, 1213–1221.

Witt, V., Wild, C., Anthony, K.R.N., Diaz-Pulido, G. & Uthicke, S. (2011). Effects of ocean acidification on microbial community composition of, and oxygen fluxes through, biofilms from the Great Barrier Reef. *Environ. Microbiol.*, 13, 2976–2989.

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