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High CO_2 inhibits substratum exploration and settlement of coral larvae

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ABSTRACT: Biological and physical factors affecting coral recruitment are critical in influencing the recovery of coral communities after disturbance. While ocean acidification (OA) can reduce coral settlement and the early growth of coral recruits, the impact of OA on coral larval swimming behavior is unknown. Here, we investigated the effects of elevated CO_2 on the swimming behavior and settlement of coral larvae of 2 common *Acropora* species. Larvae were exposed to 4 CO_2 partial pressure (pCO_2) conditions consistent with the current Intergovernmental Panel for Climate Change predictions for the next few centuries (pCO_2: 393, 853, 1485 and 3022 µatm; pH: 8.1, 7.8, 7.6 and 7.3) in 2 laboratory experiments. We found that bottom exploration, expressed as the proportion of *A. cytherea* and *A. pulchra* larvae present in the bottom part of experimental cylinders, decreased by 92 and 98%, respectively, from the ambient to highest CO_2 treatment. When offered the choice to settle on the crustose coralline algae *Titanoderma prototypum*, a well-known positive settlement cue, the percentage of larvae that settled on the fragments declined rapidly as pCO_2 increased, with no larvae settling in the highest CO_2 treatment. These results suggest that OA may negatively affect coral recruitment via direct effects on larval swimming behavior, with larvae avoiding benthic probing in response to high CO_2.

KEY WORDS: Ocean acidification · Settlement · Recruitment · Coral larvae · Larvae behavior · *Acropora*

1. INTRODUCTION

Over the past 250 yr, anthropogenic emissions of carbon dioxide (CO_2) have caused an increase in atmospheric CO_2 from a concentration of approximately 280 ppm prior to 1750, to over 400 ppm at present (Keeling et al. 2005, IPCC 2014). Furthermore, it is predicted that atmospheric CO_2 will have increased by 2100, depending on model type and assumptions, to between 730 and 1088 ppm (IPCC 2014). Ocean uptake of atmospheric CO_2 alters the carbonate chemistry of seawater and has already reduced the global saturation state of the oceans by 20% since 1800 (Orr et al. 2005, Raven et al. 2005). Organisms that accrete calcium carbonate, such as reef-building corals and calcifying algae, are directly affected, as calcification is reduced in seawater with lower pH due to depleted carbonate saturation (Schneider & Erez 2006, Anthony et al. 2008, Jokiel et al. 2008, Kuffner et al. 2008, Diaz-Pulido et al. 2012). These studies support the hypothesis that ocean acidification (OA) threatens the long-term viability of coral reefs globally (Hoegh-Guldberg et al. 2007).

However, calcification is not the only process affected. Many ecological processes, such as coral recruitment, herbivory, trophic integrity and connec-

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tivity, which are essential for coral reef resilience, are also impacted by OA (Knowlton 2001, Mumby et al. 2007). Coral recruitment is crucial for coral reef recovery following disturbance. This process is highly dependent on coral larval settlement and microhabitat selectivity, the latter of which drives subsequent post-settlement survival rates (Harrington et al. 2004, Ritson-Williams et al. 2009, 2016, Price 2010). Coral settlement can be reduced by OA (Albright et al. 2010, Nakamura et al. 2011). Typically, crustose coralline algae (CCA) stimulate coral settlement and metamorphosis and act as preferred settlement substratum for larvae of numerous coral species (Morse & Morse 1996, Harrington et al. 2004, Ritson-Williams et al. 2009, Price 2010, Sneed et al. 2014, Tebben et al. 2015, Doropoulos et al. 2016, Gómez-Lemos et al. 2018). Interactions between settlement cues and coral larvae can be altered by environmental factors, such as OA, temperature, oxygen concentration and boat noise (Doropoulos et al. 2012, Webster et al. 2013, Lecchini et al. 2018). Notably, OA can reduce coral settlement by disrupting the chemical and microbial cues produced by CCA, which act as signalling mechanisms used by coral larvae to settle (Albright & Langdon 2011, Doropoulos et al. 2012, Doropoulos & Diaz-Pulido 2013, Webster et al. 2013).

Very few studies have investigated the effects of OA on the pre-settlement phase of corals. OA can reduce gamete fertilisation (Albright et al. 2010), but its effects on larval metabolism and survival are negligible (Suwa et al. 2010, Nakamura et al. 2011). Although coral larvae are weak swimmers and depend mostly on ocean currents for dispersal over long distances, they have the ability to use their swimming behavior to control their position within the water column and to increase the probability that they will be transported to suitable settlement substrata (Szmant & Meadows 2006, Hata et al. 2017). Depending on chemical or physical cues, they can swim down to explore the substrate and eventually settle, or they can swim up to avoid their present environment by being transported away by currents (Raimondi & Morse 2000, Gleason et al. 2006, 2009, Szmant & Meadows 2006, Ritson-Williams et al. 2009, Lagos et al. 2015, Da-Anoy et al. 2017). In sub-optimal habitats, they can even delay or avoid settlement (Graham et al. 2008, Tran & Hadfield 2013). Several factors, including oxygen concentration, hydrostatic pressure and water-soluble chemicals, have been shown to influence vertical swimming behavior in coral larvae (Stake & Sammarco 2003, Gleason et al. 2009, Jorissen & Nugues 2021). However, the effect of OA on coral larval swimming behavior has never been studied. If OA acts as a negative cue for swimming larvae, it could reduce the number of larvae reaching the reef and exploring the substratum, with detrimental downstream effects on coral recruitment.

In the present study, we aimed to explore the behavioral response and settlement preferences of 2 species of broadcast spawning corals (*Acropora cytherea* and *A. pulchra*) to a range of CO_2 concentrations. We conducted 2 separate laboratory experiments over the same range of CO_2 concentrations: one assessing the swimming behavior of coral larvae inside vertical cylinders (Expt 1) and one testing their settlement rates on the CCA *Titanoderma prototypum* in a settlement assay (Expt 2). We hypothesized that coral larvae would respond by avoiding downward substratum exploration and settlement in elevated partial pressure of CO_2 (pCO_2) conditions.

2. MATERIALS AND METHODS

2.1. Coral colony collection and larval rearing

Colonies of *Acropora cytherea* (n = 12) and *A. pulchra* (n = 8) were collected 1 d after the new moon in September and October 2018, respectively, from the back reef at ca. 2 m depth on the west coast of the island of Moorea, French Polynesia (17° 33′ 14.4″ S, 149° 53′ 7.5″ W) and transported to the CRIOBE research station. Colonies were kept in aquaria with flow-through sand-filtered water and constant aeration and checked each evening at 19:00 h. When signs of imminent sperm–egg bundle release were observed, colonies were isolated in 10 l buckets. Bundles from several colonies (n = 10 and 7 for *A. cytherea* and *A. pulchra*, respectively) were collected and mixed. The mixture was distributed over several small plastic containers (500 ml) containing filtered seawater (FSW; 0.45 µm) and left to fertilize for 2 h. After rinsing the sperm, embryos were kept on an agitator at slow speed in a 12 h light:12 h dark regime within a temperature range of 26–28°C. This temperature range falls within the typical temperature range (26–30°C) reported year-round at ca. 2 m depth in the back reef of Moorea (Edmunds et al. 2010). FSW was changed every 12 h by partially draining the old containers and then pipetting the larvae into new containers with fresh FSW. Dead larvae were removed simultaneously with the water changes. After 5 d, larvae were actively swimming in all directions and were assumed to have reached competency (Randall et al. 2020). They were used in experiments within the following 3 d, with Expt 1

occurring on Days 5–7 and Expt 2 on Day 5. Experiments were conducted in the same temperature-controlled room, and thus water temperature in all experimental treatments stayed between 26 and 28°C. Due to differences in spawning time, both experiments were run separately for each species.

2.2. Expt 1: larval vertical swimming behavior assay

The effect of elevated pCO_2 on larval downward substratum exploration was tested by examining larval distribution within 500 ml graduated cylinders (height: 360 mm; diameter: 53 mm) filled to a water column depth of 27 cm. Treatments comprised FSW with 4 different pCO_2 concentrations: ambient (pH 8.1, 393 µatm) and 3 elevated (pH 7.8, 853 µatm; pH 7.6, 1485 µatm; pH 7.3, 3022 µatm) levels of CO_2 (Table 1). The first 3 treatments were chosen to represent present-day (~400 µatm) and projected CO_2 levels for ~2100 under the RCP 8.5 model of the Intergovernmental Panel for Climate Change (IPCC 2014) and for ~2200 according to simulations by Caldeira & Wickett (2003). The last treatment (pH 7.3, 3022 µatm) was added to simulate particularly low pH concentrations that can occur at the surface of benthic organisms in darkness due to respiration and calcification (e.g. pH 7.0 for the calcifying algae *Halimeda discoidea*, de Beer & Larkum 2001; pH 7.4 for hermatypic corals, Kühl et al. 1995). For example, pH at the surface of coralline algae can decline by ~0.35 units under darkness compared to ambient seawater (Hurd et al. 2011). Elevated CO_2 seawater was obtained by slowly diffusing industrial-grade CO_2 gas into 240 l sumps while continuously stirring the water and measuring the pH with a 914 pH/Conductometer (Metrohm) until it reached the desired levels. Enriched seawater was then distributed from the sumps into the cylinders. The calibration of the pH meter was checked before the start of each experiment and recalibrated with Mettler-Toledo calibration buffers to 0.01 pH units when necessary. Alkalinity was measured on water samples (50 ml) from each sump at the start of the experiments. Alkalinity replicates within a sample were analyzed until a maximum 2% error was met, using a Metrohm AGCH-9100 automatic titrator (Metrohm). The carbonate chemistry of the control and experimental seawater was calculated with CO2SYS (Lewis & Wallace 2006) using pH, total alkalinity, salinity (mean ± SE: 36.5 ± 0.3 ppt; n = 6) and temperature as inputs, with the constants from Mehrbach et al. (1973) refitted by Dickson & Millero (1987).

On each of the 3 consecutive days during which Expt 1 was run, 2 replicate cylinders were randomly assigned to each of 4 pCO_2 treatments. Increasing the level of replication was logistically not feasible for the observer (H.J.), hence our choice to run the experiment over several days. At the start of the experiment at 21:00 h, 15 actively swimming larvae were introduced into the middle of each cylinder. Larvae were haphazardly removed from various larval rearing containers for use in the cylinders. The cylinders were then covered with individual cloths to ensure full darkness. Every 30 min for a period of 2 h, the number of larvae that were swimming in the bottom 3 cm (equivalent to 50 ml), middle and top 3 cm of the cylinder were counted by briefly illuminating (~20 s) each cylinder with a UV light. The pH of the cylinders was measured at the start and end of each experimental run with a pH probe submerged 5 cm from the surface. There was no significant difference in pH between the pCO_2 values at the start and end of each experiment in the graduated cylinders (Table S1 in the Supplement at www.int-res.com/articles/suppl/m689p047_supp.pdf). Preliminary measurements in the bottom 3 cm, the middle and the top 3 cm of 4 randomly selected cylinders over a 4 h duration at 2 pCO_2 concentrations (pH 7.8 and 7.3) showed no differences in pCO_2 concentrations within the water column of the cylinders (repeated

Table 1. Physical and chemical seawater values for CO_2 treatment levels. Temperature, pH and total alkalinity (TA) are means (±SEM) of 6 replicates. CO_2 partial pressure (pCO_2), HCO_3^-, CO_3^{2-} and aragonite saturation (Ω) were calculated using CO2SYS (Lewis & Wallace 2006)

Treatment	Temp (°C)	pH	TA (µmol kg^{-1})	pCO_2 (µatm)	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	$\Omega_{aragonite}$
pH 8.1	26.6 (±0.04)	8.06 (±0.01)	2368 (±12)	393 (±10)	1794 (±24)	235 (±6)	3.8 (±0.05)
pH 7.8	26.6 (±0.04)	7.77 (±0.01)	2365 (±19)	853 (±14)	2030 (±21)	139 (±3)	2.2 (±0.05)
pH 7.6	26.6 (±0.04)	7.56 (±0.01)	2371 (±20)	1485 (±13)	2153 (±16)	90 (±4)	1.4 (±0.04)
pH 7.3	26.6 (±0.04)	7.27 (±0.01)	2374 (±14)	3022 (±22)	2256 (±19)	48 (±4)	0.8 (±0.07)

measures ANOVA; pH 7.8: $F_{2,18}$ = 1.10, p = 0.908; pH 7.3: $F_{2,18}$ = 0.11, p = 0.896).

To assess the effects of species and pCO_2 treatment on larval downward substratum exploration, the percent of larvae found in the bottom 3 cm of the cylinders was analyzed using a linear mixed effects model to approximate a repeated-measures ANOVA ('lmer' function, 'lme4' package; Bates et al. 2015), with species (2 levels), pCO_2 treatment (4 levels) and time point (4 levels) as fixed factors and day as a random factor. Model selection was performed by generating a set of models with all possible combinations of the terms in the global model, using the 'dredge' function ('MuMIn' package; Bartoń 2020). The best fit model was selected using corrected Akaike's information criterion (AIC) scores. A type III ANOVA with Satterthwaite's method on the main effects was produced ('survey' package; Lumley 2021). Tukey's honestly significant difference (HSD) post hoc tests were conducted to test for significant differences within pCO_2 treatments for each species ('emmeans' package; Lenth 2021). Prior to analysis, data were tested for normality (QQ plots) and homogeneity of variance (Levene's test). All statistics were performed using R (v. 3.3.5; R Core Team 2021).

2.3. Expt 2: larval settlement assay

To test for the effect of elevated pCO_2 on coral larval settlement, larvae were placed in 200 ml sealed glass jars filled with FSW from the same pCO_2 concentration treatments described in Expt 1 (Table 1). A small piece of *Titanoderma prototypum* or aragonite plug was placed in each jar. In the Pacific, the genus *Titanoderma* has a remarkable capacity to induce the settlement of several coral genera, including *Acropora* (Harrington et al. 2004, Price 2010, Doropoulos et al. 2012) and is easy to identify to species level *in situ*. Fragments of *T. prototypum* (approximately 4 cm^2 of live algal surface) were collected on the day of the experiment from the back reef at ca. 2 m depth on the north coast of Moorea (17° 28′ 51.5″ S, 149° 50′ 52.6″ W) using a hammer and chisel. After cleaning off any epiphytes, each fragment was cut using pliers to a 1 cm^2 (top surface area) size. The remainder of the specimen was examined using a dissecting microscope and verified to species level using anatomically based taxonomic schemes (Gordon et al. 1976, Adey et al. 1982; see representative photographs in Fig. S1). One single piece was cut out from one CCA fragment. Therefore, each piece represents one CCA individual. Aragonite coral frag plugs (Ocean Wonders) were cut to a similar size to serve as a control for the CCA settlement cue. At the start of the experiment at 19:00 h, 15 actively swimming larvae were introduced into each jar together with either a *T. prototypum* piece or an aragonite fragment. Larvae were haphazardly removed from various larval rearing containers for use in the jars, and CCA and aragonite fragments were randomly assigned to individual jars. Six replicated jars were used per pCO_2 concentration and settlement substratum (CCA vs. aragonite) treatment combination (total: 48 jars). At 09:00 h the next morning, the number of coral larvae that had successfully settled on the fragments or were still swimming were counted under a binocular microscope (NB: no settlement was observed on the jar itself). We did not distinguish between larvae settling on the live algal surface on top of the CCA fragments and larvae settling on the bare rock on which the encrusting alga was growing.

The pH was measured at the start and end of the experiment, as in Expt 1. There were no significant differences in pH values at the beginning and end of the experiments (paired *t*-test: *A. cytherea* t_{23} = 1.25, p = 0.224; *A. pulchra* t_{23} = −1.357, p = 0.188). Almost no settlement occurred on the aragonite fragments (see Section 3.2 below). Therefore, only settlement data on *T. prototypum* were analyzed. To assess the effects of species and pCO_2 treatment on larval settlement, the percent of larvae that settled in different pCO_2 concentration treatments was analyzed using a 2-way ANOVA, with species (2 levels) and pCO_2 treatment (4 levels) as fixed factors. Tukey's HSD post hoc tests were conducted to test for significant differences between pCO_2 treatments and between species ('emmeans' package). Prior to analysis, data were tested for normality (QQ plots) and homogeneity of variance (Levene's test). All statistics were performed using R (v. 3.3.5).

3. RESULTS

3.1. Expt 1: larval vertical swimming behavior assay

There were significant effects of coral species and elevated pCO_2 on the percentage of larvae in the bottom 3 cm of the graduated cylinders as well as a significant interaction between these factors (Fig. 1, Table 2). There was no significant difference in the percentage of larvae in the bottom 3 cm of the graduated cylinders across time points. The percentage of larvae in the bottom 3 cm declined from an average (±SEM) of 25.83 ± 6.90% in the ambient CO_2 treat-

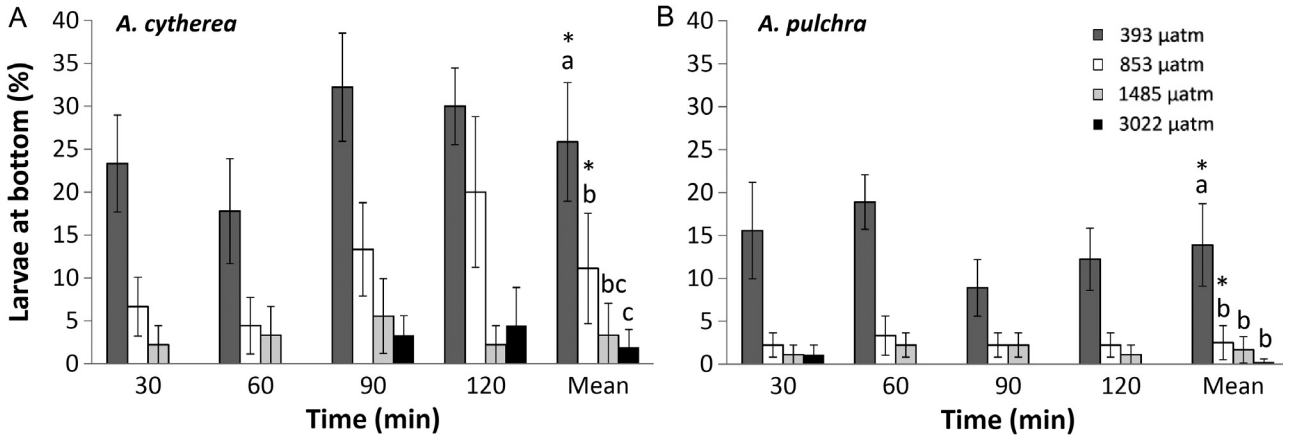

Fig. 1. Percent of coral larvae (mean ± SE, n = 6) of (A) *Acropora cytherea* and (B) *A. pulchra* swimming in the bottom 3 cm of water column depth of 27 cm in the graduated cylinders for the 4 pCO_2 treatments. Letters above bars indicate significant differences ($p < 0.05$) among pCO_2 treatments on data averaged across time points for each species; asterisks indicate differences between coral species for each treatment (Tukey's HSD)

Table 2. ANOVA (Type III) summary with Satterthwaite's method of linear mixed effects model on the effects of coral species (*Acropora pulchra* and *A. cytherea*), pCO_2 treatment (393, 853, 1485 and 3022 µatm) and time point (30, 60, 90 and 120 min) on the percent of larvae in the bottom 3 cm of the cylinders. Significant p-values ($p < 0.05$) are in **bold**

Fixed factors	*F*	df	p
Species	24.68	1	**<0.001**
pCO_2 treatment	50.53	3	**<0.001**
Time point	3.27	3	0.072
Species × pCO_2 treatment	4.60	3	**0.004**
Random factors	Variance		SD
Day	11.50		3.39

ment to 1.94 ± 2.06% in the highest CO_2 treatment for *Acropora cytherea* and from 13.89 ± 4.81 to 0.28 ± 0.34% for *A. pulchra*, representing reductions of 92 and 98% in bottom exploration between the ambient and highest CO_2 treatments for each species, respectively. Post hoc tests showed that there were significant decreases in bottom exploration by *A. cytherea* from the ambient to 853 µatm treatment, and from the 853 to 3022 µatm treatment. For *A. pulchra*, bottom exploration rapidly declined as pCO_2 increased, and almost no larvae were found exploring the bottom in the elevated CO_2 treatments. In this species, there was a significant reduction in bottom exploration from the ambient to 853 µatm treatment, while bottom exploration in all 3 elevated CO_2 treatments did not statistically differ from one another. Fewer *A. pulchra* larvae explored the bottom compared to *A. cytherea* larvae.

3.2. Expt 2: larval settlement assay

No *A. cytherea* larvae and only one *A. pulchra* larva settled in the containers with the aragonite fragments, demonstrating that settlement and metamorphosis do not occur as a result of increasing pCO_2 alone. Subsequently, only the settlement data in response to *Titanoderma prototypum* fragments were analyzed. Despite the relatively low settlement rates in the ambient treatment (ca. 10–15%), there were significant effects of coral species and elevated pCO_2 on the percentage of larvae that settled on *T. prototypum* fragments (Fig. 2, Table 3). The percentage of larvae that settled on *T. prototypum* fragments declined rapidly as pCO_2 increased, from an average of 14.40 ± 3.18% in the control pCO_2 treatment to 0% in the highest pCO_2 treatment for *A. cytherea* and from 11.10 ± 2.22 to 0% for *A. pulchra*, representing reductions of 100% in settlement rates between the ambient and highest CO_2 treatments for each species. Post hoc tests showed that there were significant decreases in settlement rates in *A. cytherea* from the ambient to the 853 µatm treatment and from the 853 to 1485 µatm treatment. For *A. pulchra*, post hoc test results matched those found in Expt 1. Settlement rates rapidly declined as pCO_2 increased, and almost no larvae settled in the elevated CO_2 treatments. There was a significant reduction in settlement rates from the ambient to 853 µatm treatment, while settlement rates in all 3 elevated CO_2 treatments did not statistically differ from one another. Fewer *A. pulchra* larvae settled on *T. prototypum* compared to *A. cytherea* larvae.

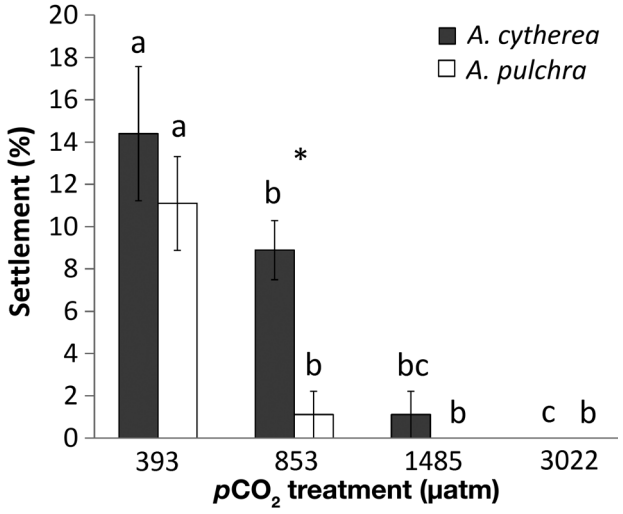

Fig. 2. Percent (mean ± SE, n = 6) of *Acropora cytherea* and *A. pulchra* larvae that settled on *Titanoderma prototypum* fragments in response to increased pCO_2. Letters above bars indicate significant differences ($p < 0.05$) among pCO_2 treatments for each species; asterisk indicates differences between coral species for each pCO_2 treatment (Tukey's HSD)

Table 3. Two-way ANOVA summary on the effects of coral species (*Acropora pulchra* and *A. cytherea*) and pCO_2 treatment (393, 853, 1485 and 3022 µatm) on the percent of larvae that settled on *Titanoderma prototypum* fragments. Significant p-values ($p < 0.05$) are in **bold**

	F	df	p
Species	7.66	1	**0.009**
pCO_2 treatment	28.60	3	**<0.001**
Species × pCO_2 treatment	2.43	3	0.080

4. DISCUSSION

CCA are often the most preferred settlement substrata of spawning corals due to the presence of algal morphogens (Morse et al. 1988, Heyward & Negri 1999, Harrington et al. 2004) and associated epiphytic bacterial communities (Negri et al. 2001, Webster et al. 2004, Gómez-Lemos et al. 2018). Reduction in coral settlement with increasing pCO_2 has been associated with declines in CCA cover and recruitment rate (Hall-Spencer et al. 2008, Kuffner et al. 2008, Fabricius et al. 2011, Doropoulos et al. 2012) and with altered CCA composition of the settlement substrata (Albright & Langdon 2011, Doropoulos et al. 2012). However, short-term studies using single species of CCA (i.e. without changing CCA abundance) found similar reductions in coral settlement, suggesting that OA reduces coral settlement by rapidly altering the chemical cues associated with the CCA thalli and/or their associated microbial community (Doropoulos et al. 2012, Doropoulos & Diaz-Pulido 2013, Webster et al. 2013). The negative settlement response to OA in our second experiment is consistent with these studies, providing support for this hypothesis. However, our first experiment demonstrated that OA reduces bottom exploration in coral larvae within minutes of exposure. This finding is important, as it suggests that the reduction in settlement observed in the second experiment, and potentially earlier studies, could be due to direct effects of OA on coral larval swimming behavior, with larvae avoiding benthic probing in response to elevated pCO_2.

The results of our ~15 h settlement assays disagree with those of Webster et al. (2013), who found no reduction in larval settlement of *A. millepora* on the CCA *Hydrolithon onkodes* using 18 h assays or after a 24 h pre-exposure of larvae to acidified conditions followed by settlement assays with CCA extracts. Short-term exposure to low pH also did not affect the settlement rates of several brooding coral species (Albright et al. 2010, Albright & Langdon 2011, Anlauf et al. 2011). Webster et al. (2013) only found reduced settlement when the CCA was exposed to acidified conditions for a period of 6 wk, overall suggesting that OA does not affect the physiology and searching behavior of coral larvae. However, our results are consistent with those of Nakamura et al. (2011), who reported reductions in larval metamorphosis of *A. digitifera* after only 2 h pre-exposure to seawater at pH 7.6 followed by settlement assays using CCA extracts. Nakamura et al. (2011) also found a significant reduction in larval metabolic rates, as expressed by oxygen consumption, after 3 d exposure, indicative of an effect of OA on larval physiology. Taken together, these results suggest that OA could negatively affect coral recruitment through multiple mechanisms, including the disruption of chemical cues used by coral larvae to select a settlement site, the reduction of the abundance of appropriate settlement substrates and the alteration of larval swimming behavior as suggested by this study. Unfortunately, this study did not allow us to estimate the relative importance of each of these mechanisms. It does not preclude that chemical and/or microbial changes occurred to the CCA during the settlement assays and that those played a role in the reduction in coral settlement.

Coral larvae can change their vertical position in the water column according to chemical and physical cues (Gleason et al. 2009, Hata et al. 2017). A multi-

tude of larval behaviors including photo-, geo-, baro- and oxytaxis are potentially used during coral dispersal and settlement (Mundy & Babcock 1998, Raimondi & Morse 2000, Stake & Sammarco 2003, Gleason et al. 2006, Jorissen & Nugues 2021). Using the same experimental setup as our study, Gleason et al. (2009) showed an increase in bottom exploration in larvae of 2 brooding species in response to reef water relative to oceanic water, suggesting that substances carried in reef water could be used by larvae to select a settlement site. Similarly, Dixson et al. (2014) demonstrated that coral larvae responded positively to water-borne cues from coral-dominated reefs but negatively to cues from algal-dominated areas in 2-channel Atema flumes. At a smaller spatial scale, the latter authors also found that coral larvae can respond to water-borne cues from specific benthic organisms (i.e. CCA, conspecifics, macroalgae; Dixson et al. 2014). pH regimes vary enormously across small spatial and temporal scales on the reef and according to benthic organisms (Suzuki et al. 1995, Anthony et al. 2008, Hauri et al. 2010, Cornwall et al. 2013). Our study demonstrates that larvae will avoid exploring benthic substrates under acidified conditions. Since a slight water movement may be enough to transport larvae away to a new location, this behavior might help larvae to reach a more favorable environment. However, this will come at the cost of delaying settlement. Short-term exposure to low pH can cause decreased metabolic rates in coral larvae, which is thought to act as an energy-saving strategy for stressful conditions over short periods (Guppy & Withers 1999, Albright et al. 2008, Suwa et al. 2010, Albright & Langdon 2011, Nakamura et al. 2011). Assuming that the cost of delayed settlement is balanced by other advantages, such as increased post-settlement survival by settling in a more favorable environment, delaying settlement under low pH conditions combined with decreasing metabolism may be a short-term coping strategy for enhanced survival. However, this strategy might become detrimental under chronic CO_2 elevation and result in larvae having to settle with low energy reserves, or not settling at all. To better predict the effect of OA on coral recruitment, further research should investigate the swimming response of larvae that have been previously exposed to acidified conditions and test whether their avoidance behavior declines over time or varies in relation with energy reserves.

This study overlooked metabolic processes occurring on the reef surface, which have the potential to modulate the effects of OA in reef communities (Hurd et al. 2011, Cornwall et al. 2013). Corals and other benthic reef organisms are able to alter their immediate chemical microenvironment near their surfaces (Kühl et al. 1995, de Beer & Larkum 2001, Anthony et al. 2011, Hurd et al. 2011, Cornwall et al. 2013, Jorissen et al. 2016). These changes in surface chemistry are the result of metabolic processes occurring within the diffusive boundary layer at a scale of µm to mm (Kühl et al. 1995, de Beer & Larkum 2001, Hurd et al. 2011, Jorissen et al. 2016). However, they may also occur on a larger scale in regions of low flow, such as within macroalgal canopies (Barott & Rohwer 2012, Cornwall et al. 2013). Typically, seawater within these layers/regions has higher pH than ambient seawater during daytime due to photosynthesis, whereas pH is lower during nighttime due to respiration (Kühl et al. 1995, de Beer & Larkum 2001, Anthony et al. 2011, Cornwall et al. 2013). On macroalgal surfaces, pH values between 7.0 and 8.1 have been observed in darkness due to respiration and calcification, which is far lower than the predicted values of pH under OA scenarios for the coming century (de Beer & Larkum 2001, Caldeira & Wickett 2003, Larkum et al. 2003, Riebesell et al. 2009, Hurd et al. 2011). Our experimental results support the fact that coral larvae will avoid low pH surface layers during the night. This behavior could help them avoid settling in unfavorable environments. However, if low pH areas are widespread (e.g. in algal-dominated reefs), it could result in decreased coral settlement even when mainstream pH has no effect on larval swimming behavior. In contrast, high pH surface layers during daytime could potentially alleviate the negative effects of OA on coral larvae. Whether the negative effects of OA could be ameliorated in the light or exacerbated in the dark needs to be further tested using experimental observations of coral larval behavior and settlement in the presence of different benthic organisms under conditions simulating present-day and OA scenarios and varying irradiance and flow.

Settlement rates in the ambient treatment averaged 14% for *A. cytherea* and 11% for *A. pulchra*. While these rates were unexpectedly low, they are similar to those (~15%) of 5 d old *A. palmata* larvae on *Titanoderma prototypum* fragments reported by Ritson-Williams et al. (2010). In a similar study conducted 1 yr earlier in Moorea, 54% of *A. cytherea* larvae settled on *T. prototypum* fragments in plastic wells filled with 12 ml FSW (Jorissen et al. 2021). These differences in settlement rates could be due to variations in the health or size of coral larvae and/or the health of *T. prototypum*. For example, small-sized larvae can experience lower settlement compared to

large-sized larvae (Hartmann et al. 2013). Alternatively, the environmental conditions in the jars may have been less suitable for coral settlement compared to those in the wells. Because our jars were sealed and did not allow for gas exchange, larval and bacterial respiration could have reduced oxygen concentrations in the jars during our ~15 h assays and created an additional source of larval stress, which reduced settlement (Jorissen & Nugues 2021). However, reductions in oxygen concentrations would likely have been coupled with decreased pH in the jars, which is not supported by pH measurements at the beginning and end of the assays. Although the reasons behind the low settlement rates in the ambient treatment found in this study remain unclear, we can reasonably assume that all treatments were equally affected and thus that differences among treatments can be attributed to differences in pCO_2 concentrations.

This study did not follow any acclimation procedure and used coral larvae as soon as they were competent. We cannot rule out the fact that the acute changes in pCO_2 concentration may have induced a physiological shock in the larvae, especially when they were moved from a pH of 8.1 immediately to a pH of 7.3. However, large pH concentration gradients may develop between the reef surface and the bulk seawater (described above). Thus, it is possible that larvae are exposed to large pH fluctuations upon reaching the benthos in the field. Future experiments could consider acclimating coral larvae to the desired pH levels or exposing them to pH concentration gradients.

Titanoderma sp. is known to be a highly inductive CCA for coral metamorphosis (Harrington et al. 2004, Ritson-Williams et al. 2010). The abundance of *Titanoderma* sp. and the preferential settlement of coral larvae on this taxa were both demonstrated to decrease under elevated pCO_2 (Doropoulos et al. 2012). Although much emphasis has been placed on the impact of OA on the ability of CCA to induce coral metamorphosis in coral larvae, there are still only a few studies on the effects of OA on coral larval settlement behavior. To our knowledge, this study is the first to examine the impacts of OA on substratum exploration in coral larvae and adds to the growing body of literature demonstrating the negative effects of anthropogenically induced OA on the recruitment of marine invertebrates. Our findings indicate that even small increases in pCO_2 concentrations can significantly decrease explorative larval swimming behavior and settlement. The compounding nature of increasing pCO_2 on successive early coral life history stages suggests that the consequences of OA on coral populations might be more severe than originally perceived. Although further studies are needed to fully understand the impacts of OA on coral recruitment, the reduction in larval benthic probing in response to elevated pCO_2 raises serious concerns for the recovery of reefs under future OA conditions predicted to occur into the next century.

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