

Effects of ocean acidification on metamorphosis: brooding and spawning larvae

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Abstract. The effects of ocean acidification on metamorphosis were tested using the larvae of a spawning coral, *Goniastrea retiformis* and a brooding coral *Leptastrea cf transversa* in Guam. Four treatment levels of $p\text{CO}_2$ were used, corresponding to current levels of atmospheric CO_2 (approximately 380) and three values projected to occur later this century 600, 750 and 1000 ppm). Metamorphosis was not consistently affected by $p\text{CO}_2$ in either species. These results suggest that the mode of reproduction does not affect the larval response to $p\text{CO}_2$ and furthermore, there will be no direct effects of ocean acidification on settlement rates of reef corals, at least in the near future

Key words: acidification, climate change, coral reefs, dispersal

Introduction

Climate change has the potential to detrimentally affect coral reefs. In particular, increasing ocean acidity, as a result of increased atmospheric concentrations of CO_2 , is expected present a severe threat to marine ecosystems before the end of this century (Kleypas et al. 1999; Orr et al. 2005). Ocean acidification is caused by alteration of sea water chemistry through CO_2 absorption from the atmosphere leading to a decrease in seawater pH and carbonate ion concentrations (CO_3^{2-}). Recent changes in ocean chemistry, including a decrease in pH of 0.1 units since the 1950s, have reduced the rates of calcification in many marine organisms (Langdon 2000; Cooper et al. 2008; Pörtner 2009). By 2050, ocean pH is expected to be lower than at any point in the last 20 million years (Caldeira and Wickett 2003; Guinotte et al. 2006; Turley et al. 2007).

To date, much of the research on the effects of ocean acidification on corals has focused on adults, in particular, the process of calcification. However, the early life history stages of marine organisms are also likely to be sensitive to changes in ocean chemistry (Portner et al. 2004; Byrne 2011). Hypercapnia, defined as an increase in the partial pressure of CO_2 in the respiratory fluids, affects acid-base regulation, oxygen transport and metabolic function (see review by Portner et al. 2004) and seawater artificially enhanced with CO_2 reduces sperm motility (CO_2 narcosis, e. g. Havenhand et al. 2008, Morita et al. 2010)

Many ecological processes are affected by differences in the life history traits of organism. For example, the mode of larval development in marine invertebrates (often called the reproductive mode), affects patterns of dispersal (Baird et al. 2009b), rates of recruitment (Hughes et al 2002), and post-settlement mortality (Marshall et al. 2010). Corals have two contrasting modes of reproduction: brooding and broadcast spawning. These modes of reproduction are distinctive in many ways (Baird et al. 2009b). However, potentially the most important difference in terms of the larva's response to stress, such as increase acidity, is whether or not they contain photosynthetic symbionts, *Symbiodinium*. Interestingly, all brooding larvae (except those of Isoporans.) contain *Symbiodinium* on release from the mother, where only four out of 85 genera of broadcast spawning species have *Symbiodinium* in the eggs (Baird et al. 2009b). Symbiotic organisms are generally more susceptible to stress: for example, few other organisms' responses to the small change in sea surface temperature association with mass bleaching events (Baird et al. 2009a; McClanahan et al 2009). Similarly, *Acropora* larva experimental infected with *Symbiodinium* have higher rates of mortality when exposed to temperatures 2°C higher than ambient (Yakovleva et al 2009), and naturally occurring levels of ultra-violet radiation (Baird et al unpublished data) when compared to larvae of the same species that lack *Symbiodinium*.

Here, we test for an effect of $p\text{CO}_2$ on metamorphosis in the larvae of a brooding coral that

contains *Symbiodinium*, *Leptastrea cf. transversa* and a spawning coral that does not, *Goniastrea retiformis*

Material and Methods

Collection of gravid colonies

Goniastrea retiformis is a hermaphroditic broadcast spawner. The eggs of *G. retiformis* lack *Symbiodinium* which are typically not taken up by larvae until settlement (Fig. 1a, Babcock and Heyward 1986). *Leptastrea cf. transversa* is a gonochoric brooder. Larvae are released containing *Symbiodinium* that have been transmitted from the mother to the developing oocytes (Fig. 1b)

Eight mature colonies of *G. retiformis* were collected from the fringing reef in Pago Bay, Guam (13° 25'N, 144° 48'E) and maintained in outdoor aquaria until they spawned on the night of 15th June 2009. Following spawning, larvae were cultured as outlined in Babcock et al. (2003) and maintained in 0.2µm filtered sea water (FSW) until competent to settle (Connolly and Baird 2010). Approximately 20 colonies of *L. cf. transversa* were collected from the lagoon behind the fringing reef fronting the Guam Port Authority (13°27'54.98"N, 144°40'16.10"E) on the 15th June and maintained in flow through aquaria at the University of Guam Marine Laboratory. At night, the water flow was stopped, and in the morning larvae were collected from the aquaria with pipettes. A total of 75 larvae were collected on the 17th June 2009 and the larvae were maintained in 0.2µm filtered sea water for three days prior to the settlement experiment that commenced on 20th June 2009

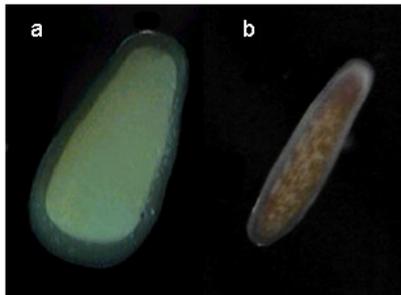


Figure 1(a) A broadcast spawned larva (*Goniastrea retiformis*) with no symbionts (b) a brooded larvae (*Leptastrea cf. transversa*) with symbionts evident as brown splotches in the gastroderm

Experimental manipulation of ocean acidification

The CO₂ tanks were prepared in a temperature control room (25°C-26°C). A CO₂ mixing system developed by Munday et al. (2009) was used to manipulate pCO₂ in seawater. Four levels of pCO₂ (ambient 380 ppm, 600 ppm, 750 ppm and 1000 ppm) were used, as recommended by the EPOCA protocol (Barry et al. 2010). CO₂ concentration was measured in ppm units using a CO₂ gas probe (Vaisala ®). The mixed gases were bubbled through 70 l sump tanks to

enrich the water. The enriched water was flowed through replicates via a one-way flow-through system. pH conditions and corresponding total alkalinity (TA) were measured for each treatment by using auto Gran titration (APHA 2320). Experimental calcite (Ω_{calc}) and aragonite saturation (Ω_{arag}), carbonate (CO_3^{2-}) and bicarbonate ion concentration (HCO_3^-) were calculated from TA, pH, salinity and CO₂ concentration (pCO₂) using CO2SYS (Pierrot et al. 2006; Table 1).

The effect of acidification on metamorphosis

Ten *G. retiformis* larvae from each of the 3.5 l jars were placed into each well of a 6-well Iwaki cell culture plate with a modified meshed lid. Similarly, one *L. cf. transversa* larva was placed in each well with a total of 18 larvae per pCO₂ treatment. A 5 mm x 5 mm crustose coralline algae (CCA), *Hydrolithion sp.* was placed in each well to induce metamorphosis. The plates were then immersed in the four pCO₂ treatments and the number of larvae completing metamorphosis was assessed after 24 h. Larvae were defined as metamorphosed if a basal disc had been deposited (Baird and Babcock 2000).

Data analysis

Differences in the mean number of *G. retiformis* larvae completing metamorphosis among the four pCO₂ treatments were tested with 1-way ANOVA. Tukey's HSD multiple comparison test was used to determine which pCO₂ levels differed. Any bias in these data was explored by residual analysis and if detected the data were transformed as noted in the ANOVA tables. A contingency table was used to test whether the number of *L. cf. transversa* larvae settling was independent of the level of pCO₂.

Results

Ocean acidification did not affect metamorphosis as predicted in either species. The mean number of *G. retiformis* larvae completing metamorphosis did not differ among the different pCO₂ levels ($F_{1,46} = 2.386$, $p = 0.1292$; Fig. 2). While the number of larvae completing metamorphosis in *L. cf. transversa* was dependent on the level of pCO₂ ($\chi^2_{1,3} = 9.0625$, $p = 0.02847$), the pattern was not that expected if the response was does dependent. The highest number of *L. cf. transversa* larvae metamorphosed in the 1000 ppm the lowest number in the 750 ppm treatment and there was no difference between ambient and 500 ppm (Table 2).

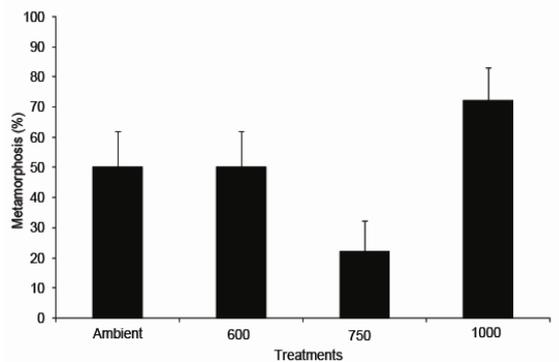


Figure 2: Metamorphosis success in *G. retiformis* propagules under acidity treatments (SE).

Treatment	Settled	Not settled
Control	9	9
500	9	9
750	4	14
1000	13	5

Table 2: The metamorphosis success of *Leptastrea sp.*

Discussion

Metamorphosis was not consistently affected by $p\text{CO}_2$ in either species. These results suggest that the mode of reproduction does not affect the larval response to $p\text{CO}_2$ and furthermore, there will be no direct effects of ocean acidification on settlement rates of reef corals, at least in the near future.

Our results are consistent with other recent research on the effect of ocean acidification on coral larval metamorphosis. A reduction in metamorphosis is typically only apparent at very high levels of $p\text{CO}_2$ or when mediated by ocean acidification induced changes in the quality of the substratum (see review by Albright 2012; Chua 2012). There are a number of reasons why corals may be robust to ocean acidification. Adult corals thrive in an environment with high natural fluctuations in pH (Gagliano et al. 2010) which may pre-adapt the offspring to withstand high level of OA. For example, sea urchins that live in tidal pools with high natural fluctuations in pH produce offspring that are robust to OA (Byrne 2011). Similarly, larvae of the Sydney Rock Oyster (*Saccostrea glomerata*) produced by adults subjected to elevated $p\text{CO}_2$ during gametogenesis had faster development, greater shell length and metabolic activity in elevated CO_2 conditions, compared to

juveniles from wild caught adults (Parker et al. 2012). Similarly, brooded larvae produced by adult byzoans (*Bugula neritina*) grown in elevated copper concentration were larger, dispersed farther and were tolerant of copper toxicity (Marshall 2008). Such maternal effects are likely to be greater in species with larvae that rarely leave the maternal habitat, such as those species that brood larvae. However, we found no difference between these two coral species with different modes of larval development. Alternatively, the process of calcification may pre-adapt corals to fluctuation in pH. During calcification, corals elevate the extracellular pH under the calciblastic epithelium by 0.2-0.5 units (Venn et al. 2011) and therefore, corals may have evolved mechanism for the tissue to adjust to high pH.

In conclusion, projected ocean acidification levels in the near future appear unlikely to have major ecological effects on coral settlement. The lack of major effects on larval ecology does not mean that ocean acidification is not a threat to other stages in a coral's life history. There are strong theoretic and empirical reasons for expecting an effect on physiology (Portner et al. 2004) and patterns of gene expression vary dramatically in coral larvae as levels of $p\text{CO}_2$ increase (Moya et al. 2012). The consequences of energy expenditure on cellular acid-base regulation and lowered metabolism are perhaps unlikely to be apparent in short term ecological experiments. In particular, growth, reproduction and competitive ability are all likely to be affected by increases in ocean acidity over a longer time frame (Anthony et al. 2008; Fabricius et al. 2011). In addition, increased concentrations of atmospheric CO_2 are also resulting in the warming of the ocean via the green house effect (Hendriks et al. 2010). Consequently, marine organisms must deal with both high $p\text{CO}_2$ and high temperatures and future work should explore the possible synergistic effects of these stressors on coral larval ecology

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Table 1: Water chemistry parameters (\pm SE) value throughout the experiment with saturation values of Aragonite and Calcite and actual saturated values of $p\text{CO}_2$ in treatments tanks calculated using CO2SYS using parameters: K1, K2 from Mehrbach et al 1973 refit by Dickson & Millero 1987; KHSO_4 from Dickson 1990; pH: NBS scale/kg- H_2O (Pierrot et al 2006)

Treatment	pH	TA ($\mu\text{mol/kg}$ as CaCO_3)	Temperature ($^\circ\text{C}$)	Ω calc	Ω arag	$p\text{CO}_2$ calculated
Ambient	8.08 (0.02)	2076 (40)	25.6(0.1)	3.5 (0.1)	2.3 (0.1)	443 (21)
600	8.02 (0.02)	2094 (43)	25.3 (0.1)	3.1 (0.1)	2.0 (0.1)	523 (24)
750	8.01 (0.02)	2015 (37)	25.2 (0.1)	3.0 (0.1)	2.0 (0.1)	531 (29)
1000	7.87 (0.02)	2036 (47)	25.3 (0.1)	2.3 (0.1)	1.5 (0.1)	775 (32)

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