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Effect of Lower pH on Settlement and Development of Coral, *Pocillopora damicornis* (Linnaeus, 1758)

Voranop Viyakarn[1], Wipada Lalitpattarakit[1], Narainrit Chinfak[1], Suppakarn Jandang[1], Pataporn Kuanui[1], Somkiat Khokiattiwong[2], and Suchana Chavanich[1]*

[1]*Reef Biology Research Group, Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Phuket Marine Biological Center, Department of Marine and Coastal Resources, Phuket 83000, Thailand*

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Abstract – The effects of pH reduction on the settlement and development of the coral *Pocillopora damicornis* were investigated. Three different pH treatments (pH = 7.6, 7.9, and 8.1) were used. In addition, water quality (temperature, salinity, total alkalinity) around the study site was monitored. The results showed significant differences in the settlement rates of *Pocillopora damicornis* larvae between pH treatments ($p \leq 0.05$). A decrease in pH levels caused a strong decline in larval settlement rate. In addition, at pH 7.6 and 7.9, all larvae were unable to complete metamorphosis, and metamorphosis delay was observed. Field monitoring showed low fluctuation of all seawater parameters within 24 hours, and there was no difference between seasons. From this study, a strong negative effect of pH reduction on *P. damicornis* larvae was observed. Although the function of physiology is still not clearly understood, correlations are likely to exist.

Key words – acidification, recruitment, metamorphosis, coral larvae, carbon dioxide

1. Introduction

Coral reefs are species-rich habitats that have high primary production (Armenteros et al. 2012). However, the recent rapid increase in ocean carbon dioxide (CO_2) concentrations and its effects on corals are of concern (Gattuso and Buddemeier 2000; Hoegh-Guldberg et al. 2007; Edmunds et al. 2013; Venn et al. 2013). More than 25% of anthropogenic CO_2 is absorbed by the oceans (Sabine et al. 2004). The increasing levels of CO_2 in the ocean lead to a reduction in seawater pH and ocean acidification (Hoegh-Guldberg et al. 2007), which have been reported to impair calcification and threaten coral growth (Hoegh-Guldberg et al. 2007; Marubini et al. 2008; Albright and Langdon 2011). Several scleractinian corals such as *Stylophora pistillata* and *Porites astreoides* have shown a decrease in calcification when partial pressure of CO_2 is increased and saturation state of aragonite is decreased (Erez et al. 2011; Crook et al. 2013; Venn et al. 2013). However, Comeau et al. (2013) have shown inconsistencies in the calcification responses of other scleractinian corals when subjected to increasing partial pressure of CO_2, suggesting that such calcification responses of corals and reef organisms are not yet well understood.

Recruitment is an important step for ecosystem sustainability. Coral recruitment is a process that includes settlement, metamorphosis, and growth of youngest juvenile corals (Harrison and Wallace 1990). Several physical factors such as temperature, salinity, ultraviolet radiation, and pollution have an influence on coral recruitment (Harrison and Wallace 1990; Mundy and Babcock 1998; Markey et al. 2007). Recent studies have shown that corals are affected by global climate change and that there is a resulting reduction in recruitment and fertilization rates (Randall and Szmant 2009; Albright et al. 2010). When pH decreased, the rates of recruitment and fertilization were approximately reduced by 29% and 45%, respectively (Randall and Szmant 2009; Albright et al. 2010). However, some studies have reported that corals can adapt to adverse pH conditions (Hendriks et al. 2010; Nakamura et al. 2011; Gabay et al. 2014).

So far, corals of the Indo-Pacific region, particular in Southeast Asia have been poorly studied with respect to the effects of rising oceanic CO_2 concentrations. Most studies

*Corresponding author. E-mail: suchana.c@chula.ac.th

have been carried out in Australia and Japan (Hoegh-Guldberg et al. 2007; Nakamura et al. 2011; Chua et al. 2012). Little is yet known about the response of corals in Thailand, and no study so far has investigated the impact of ocean acidification on coral larvae in Thailand. Understanding how pH affects calcifying organisms, such as corals and their larval stages, is a critical step in predicting their survival under future ocean acidification conditions. In this study, we investigated the effect of pH reduction on larval settlement and development of *Pocillopora damicornis.*

2. Materials and Methods

Coral larvae samples

Five colonies of *Pocillopora damicornis* (Linnaeus, 1758) were collected 1 week before the new moon at 5-7 m depth from Khao Ma Choa Reef (MCHO) (10°35′54.6″N, 100°56′52.6″E), in Sattahip Bay, Chon Buri Province, Thailand. In Chon Buri, *Pocillopora damicornis* releases planulae during the daytime and nighttime between first and fourteenth day after the new moon (Kuanui et al. 2008). The coral colony samples were then placed in aerated tanks at the coral hatchery and nursery facility at Samae San Island until larvae were released. The larvae were then collected fresh, and pooled all together before they were randomly transferred to experimental glass containers.

Planula experiment

To investigate the effects of lower pH on the settlement and development of *P. damicornis* larvae, three different pH treatments (pH = 7.6, 7.9, and 8.1) were conducted. The treatment with pH 8.1 (an average ambient pH measured in the field) served as the control. To adjust the pH levels of the seawater in individual experimental chambers, seawater was simultaneously bubbled with pure CO_2 with a pH regulator connected to a pH electrode. The stability of the pH in all containers was confirmed every 2 hours by using a handheld pH meter. The ambient seawater temperature was also controlled at 28°C. In the experiment, ten planulae were randomly added to each 2-litre container. A total of three replicate containers were set up for each treatment. During the experiments, settlement rates were counted and calculated to percentages. Settlement in this experiment was referred to when larvae attached to the substrate and started metamorphosis. After the larvae settled, their developmental stages were also observed under a stereo-light microscope. The data were collected at a different chamber at each time point. The experiments were run for 31 hours, and 50% of water in each container was changed daily. A one-way ANOVA test followed by Tukey's pairwise mean comparison was used in Systat Program to test the differences in settlement rates of coral larvae in different pH treatments.

Monitoring seawater characteristics

Water quality parameters (temperature, salinity, and total alkalinity [TA]) were monitored around Sattahip Bay every 3 months from December 2013 to July 2014. Water samples were collected every 8 hours for 24 hours (10.00 am, 18.00 pm, 02.00 am, and 10.00 am of next day). Two replicate samples were collected each time. For TA analyses, the water samples were first filtered using filter paper (GF/F 25 mm), and TA was measured using both classical and automated Gran titration at the same time for comparison. Dissolved organic carbon, pCO_2, HCO_3^-, CO_3^{2-}, and CO_2 were then calculated using the CO2SYS program (Lewis and Wallace 1998).

3. Results

Significant differences were found in the settlement rates of *Pocillopora damicornis* larvae between pH treatments ($p \leq 0.05$) (Fig. 1). A decrease in pH levels caused a strong decline in larval settlement rates. The control (pH = 8.1) had the highest average settlement rate (26.67 ± 8.82%), while the lowest settlement rate (3.33±3.33%) was at pH 7.6. At pH 7.9 and 7.6, the results showed that the majority of larvae did not settle and were still at the swimming stage (Fig. 1).

After touching the bottom of the chambers within 24 hours, the planulae started to settle. In normal control pH, the metamorphosis of *P. damicornis* was divided into eight stages following Kuanui et al. (2008): 1) search for suitable substrate,

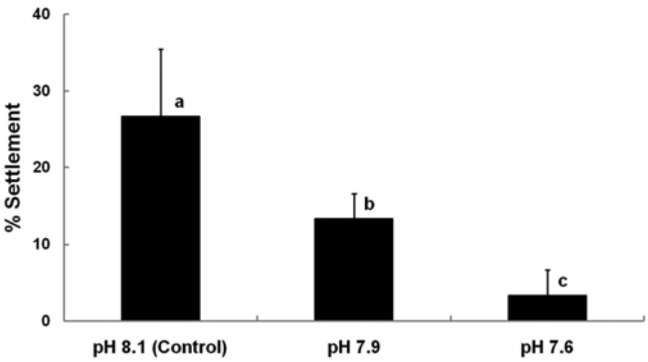

Fig. 1. Mean percentage of settlement rates of *Pocillopora damicornis* plus 1 SE in three different pH conditions

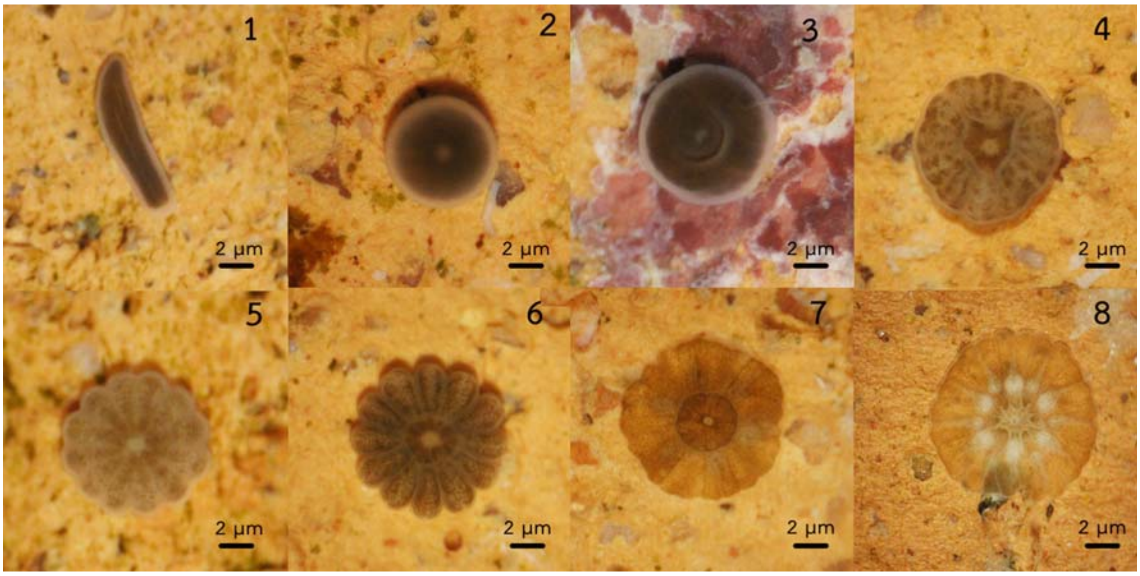

Fig. 2. Eight stages of larval development of *Pocillopora damicornis* in a control pH condition

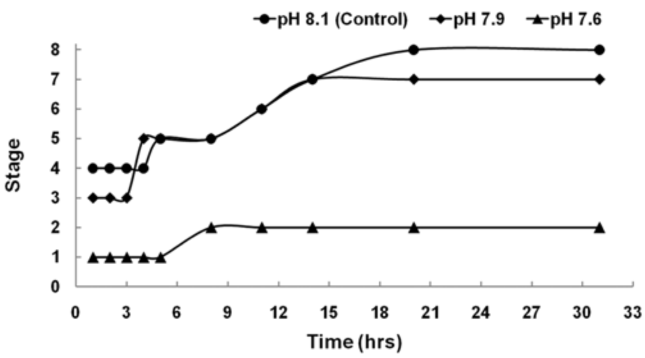

Fig. 3. Stages of larval development of *Pocillopora damicornis* and time durations in three different pH conditions

2) start of attachment, 3) depression of shape, 4) start of polyp formation, 5) polyp formation, 6) tentacle formation, 7) tentacle and exoskeleton formation, 8) tentacle and exoskeleton observation (Figs. 2 and 3). Polyp formation with tentacles and exoskeleton was observed within 24 hours after it started to settle. However, at pH 7.6 and 7.9, all larvae were unable to complete metamorphosis, and metamorphosis delay was observed (Fig. 3). Meanwhile, at pH 7.6, it was clearly shown that the pH reduction impeded overall metamorphosis of *P. damicornis* larvae. It is also interesting to note that all larvae in the experiments remained alive and were actively swimming through to the end of the experiment in all pH treatments.

For the water quality monitoring, in situ seawater characteristics around Sattahip Bay are presented in Table 1. Our observation revealed that, overall, the fluctuation of all seawater parameters within 24 hours was low, and there was no difference between seasons.

4. Discussion

The response of corals to the increase of CO_2 levels or the decrease in pH can be species-dependent (Edmunds et al. 2012). Some corals exhibit pH resistance (McCulloch et al. 2012). The resistance to pH decrease may be attributed to the capacity to buffer pH and protection mechanisms against adverse pH conditions (McCulloch et al. 2012; Gabay et al. 2014). Gabay et al. (2014) demonstrated that soft corals maintained the integrity of their hospice sclerites when subjected to changing pH conditions. Bignami et al. (2013) also showed that larvae of cobia fish displayed some resistance to ocean acidification. This resistance may be caused by the naturally variable environmental conditions that this fish species usually encounters (Bignami et al. 2013). Marine organisms found in highly variable environments or within a large geographic range are likely to resist and adapt to ocean acidification conditions better than those found in less variable environments or within a narrow geographic range (Dixson et al. 2010; Bignami et al. 2013).

Our results showed that lower pH had considerable effects on the settlement and development of *Pocillopora damicornis* larvae (Figs. 1 and 3). Reduction in pH resulted in delays of settlement and metamorphosis. Coral larvae were unable to complete metamorphosis at pH 7.6 and 7.9. Under normal

Table 1. Average seawater characteristics at different times around Sattahip Bay, Chon Buri Province

	Time			
	10:00	18:00	02:00	10:00
	Average ± SE	Average ± SE	Average ± SE	Average ± SE
Salinity (psu)	30.67 ± 0.59	30.38 ± 0.38	29.89 ± 1.00	30.60 ± 0.78
Temperature (C)	28.68 ± 0.42	28.96 ± 0.52	28.27 ± 0.15	29.19 ± 0.71
pH	8.12 ± 0.03	8.11 ± 0.02	8.12 ± 0.06	8.10 ± 0.07
TA (µmol/kg)	2,148.37 ± 45.44	2,144.27 ± 49.45	2,178.70 ± 4.12	2,156.75 ± 46.25
DIC (µmol/kg)	1,880.12 ± 39.10	1,859.98 ± 60.71	1,895.84 ± 23.24	1,896.44 ± 40.67
pCO_2 (µmol/kg)	480.19 ± 4.00	437.76 ± 52.25	492.65 ± 45.56	468.29 ± 61.59
HCO_3^- (µmol/kg)	1,673.07 ± 32.97	1,642.89 ± 65.75	1,677.68 ± 34.92	1,694.78 ± 35.23
CO_3^{2-} (µmol/kg)	195.51 ± 6.14	206.51 ± 8.39	207.12 ± 12.96	189.15 ± 5.27
CO_2 (µmol/kg)	11.58 ± 0.09	10.58 ± 1.26	11.04 ± 1.23	12.51 ± 0.17

pH conditions (pH 8.1), the larvae would have settled within 0.5 hours, and within 20 hours, their exoskeletons would fuse with substrates (Kuanui et al. 2008; Lee et al. 2009). The settlement rate of coral larvae in this study under normal conditions was similar to that of Lee et al. (2009). After 40 hours, polyps, tentacles, and exoskeletons would be observed (Kuanui et al. 2008). Kurihara (2008) showed that high pCO_2 had negative impacts on the fertilization, cleavage, larval settlement and reproductive stages of several marine calcifiers, including echinoderm, bivalve, coral and crustacean species. Kurihara (2008) also pointed out that corals were unaffected by high pCO_2 until the larval stage, and the impact was observed after coral settled.

In this study, we also found that pH did not have an effect on the swimming ability of coral larvae. Similar to our findings, some species of coral and fish larvae did not show abnormal swimming behaviors under acidified conditions (Kurihara 2008; Munday et al. 2009; Esbaugh et al. 2012). However, the acidified conditions could disrupt the olfactory mechanisms of fish larvae (Munday et al. 2009). In contrast, some studies have shown that acidification affected the swimming activity and some behaviors of reef fish by reducing the auditory response of fishes (Simpson et al. 2011; Domenici et al. 2012).

Settlement of coral larvae typically requires chemical cues such as crustose coralline algae, which play an important role in reef formation (Adey 1998), and there is a correlation between the settlement rates and crustose coralline algae (Harrington et al. 2004). However, crustose coralline algae are vulnerable to acidification because their skeleton is made of magnesium calcite, and the recruitment and growth rates of crustose coralline algae could be inhibited and reduced in elevated carbon dioxide conditions (Kuffner et al. 2008). Thus, acidification can affect coral larval settlement through reduced crustose coralline algae (Kuffner et al. 2008).

The monitoring of water quality in the study area showed that the fluctuation of all seawater parameters within 24 hours was low and within normal ranges (Table 1). Therefore, scleractinian corals in the area seem unlikely to be experiencing the effect of acidification yet. Comeau et al. (2013) pointed out that the reefs in the central South Pacific may not be affected by intensified ocean acidification because the seawater saturation states for $CaCO_3$ in the region are high and are thought to be supersaturated. However, continuous monitoring is important to determine the dominant and resistant coral species in future ocean acidification conditions.

In conclusion, we observed that pH reduction has a strong negative effect on *P. damicornis* larvae. Although the function of physiology is still not clearly understood, correlations are likely to exist. Further studies on the physiology of larvae and the effect of ocean acidification are necessary.

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