

A study of the effect of sediment accumulation on the settlement of coral larvae using conditioned tiles

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Abstract. Studies have shown that marine biofilms play an important role in coral larval settlement. Biofilms are formed when substrata are immersed in seawater over a period of time in a process known as conditioning. This study investigated the settlement of *Pocillopora damicornis* larvae on concrete tiles which had been conditioned in running seawater for one, two, four, six and eight weeks, with unconditioned tiles used as controls. Results showed that larval settlement appeared to be higher on unconditioned tiles. Two-way ANOVA indicated no significant differences in larval settlement densities in treatments and controls, but significant differences in the choice of settlement surfaces preferred. Larvae preferred to settle on the sides of the tiles, compared to the top and bottom surfaces. Regression analysis conducted on the weight of sediment accumulated and the density of settled larvae indicated a negative and significant correlation between the two variables. Sediment accumulation on biofilm layers growing on underwater substrata likely affects the suitability of these surfaces for larval settlement. This factor must be taken into account when designing artificial surfaces to enhance coral larval settlement for reef rehabilitation.

Key words: *Pocillopora damicornis*; larval settlement; biofilm; sediment

Introduction

The life history of sessile marine invertebrates is affected by the behaviour of larvae during the planktonic and settlement phases as well as by post-settlement mortality (Keough and Downes 1982). Various environmental, chemical and biological cues influence larval settlement. Crustose coralline algae (CCA), skeleton and coral rubble have been used to provide the necessary cues to induce settlement of corals (Morse et al. 1988; Heyward and Negri 1999; Webster et al. 2004). Biological cues such as marine biofilms also play an important role in coral larval settlement.

Marine biofilm formation occurs on any substrate immersed in natural seawater (Wimpenny 1996). A layer of organic molecules is immediately formed on the clean substrate surface when placed in seawater. During conditioning, marine bacteria, diatoms and algal spores grow on the surface, and sediment also accumulates on the surface to form a complex biofilm layer (Kirchman et al. 1982; Mitchell and Maki 1988; Gilmour 1999). The formation of marine biofilms is a dynamic colonization process that may change over time (Webster et al. 2004; Shikuma and Hadfield 2006). The settlement of larvae is affected by the

length of time a natural marine biofilm has developed on a substrate. The layer of marine biofilm potentially affects the recruitment of larval benthic invertebrates (Characklis and Cooksy 1983; Webster et al. 2004). An increase in the duration of substrate conditioning has been reported to significantly increase the rate of coral larval metamorphosis (Webster et al. 2004).

The absence of sediment on substrate is essential for coral settlement (Harrington et al. 2004). However, coral reefs are increasingly exposed to natural and anthropogenic disturbances in the past few decades (Rogers 1979; Wilkinson 1998). These disturbances include increased nutrient levels, sedimentation, pollution and eutrophication in the marine environment, and have been reported to promote succession changes in reef communities (Birkeland 1987; Belliveau and Paul 2002; Fabricius 2005). A study showed that suspended sediment, and the accumulation of sediment on the upper surface of settlement substrate negatively affected larval settlement of *Acropora digitifera* (Gilmour 1999).

Singapore is a highly urbanised country, and has a steadily expanding economy. The marine environment has contributed to Singapore's success as one of the world's busiest ports and one of the

largest oil-refining centres (Chou and Goh 1998). However, the presence of numerous human activities along the coastline, such as shipyards and coastal development (Chua and Chou 1992), has resulted in degradation of the reefs. High sedimentation ($45 \text{ mg cm}^{-1} \text{ day}^{-1}$) has been recorded in Singapore waters (Low and Chou 1994). Generally, coral reefs do not extend beyond the 6 m depth due to high water turbidity and sediment load (Chou and Tun 2005).

The objective of the present study was to investigate the effect of conditioning artificial tiles in seawater, on the settlement of *Pocillopora damicornis* larvae. The ease of collection and maintenance of *P. damicornis* larvae, in addition to the regular planulating cycle of the adults make this species of coral a suitable test organism for the present study. This study also examined the accumulation of particulate matter on biofilm layers formed on conditioned tiles, and their effects on larval settlement.

Materials and Methods

Preparation of settlement substrates and larval collection

Cement tiles (of dimensions: 10x10x1cm) containing 10% of coral rubble (10% CR) were used as settlement substrates. Tiles (placed horizontally) were conditioned in flow-through seawater systems in indoor aquaria. Tiles were conditioned for 1, 2, 4, 6 and 8 weeks, making up five treatments. All experiments were conducted at the Tropical Marine Science Institute under aquarium conditions. Adult colonies of *P. damicornis* were collected from Singapore reefs and maintained in aquaria for planulation. Planulation occurred after the new moon, and *P. damicornis* larvae were collected in overflow collectors.

Effect of duration of tile conditioning on coral larval settlement

The larval settlement experiments were carried out in March 2007. A single experimental tile was placed in a 2 L polyethylene tank containing UV-treated seawater and 20 larvae were introduced into each treatment tank. Four replicate tanks were prepared for each treatment and Controls, which comprised clean, unconditioned tiles. The numbers of coral larvae which had settled on each tile were enumerated after a 10-day exposure experimental period.

Determination of particulate matter

In a larval settlement experiment repeated in February 2008, a determination was also made of the amount of particulate matter that had settled on the experimental tiles.

After the end of the exposure experiment and enumeration of settled larvae, particulate matter that may have collected from the top surfaces of settlement tiles were washed off with gentle agitation in clean filtered UV-treated seawater. Particulate matter removed from individual tiles was filtered in the laboratory onto Whatman GF/C filter paper. The filter paper was then dried in an oven at 65°C over 24h, and the dry weight of the filtered particulate matter obtained thereafter.

Statistical analysis

A log (x+1) transformation was applied to the data (density of settled larvae) before statistical treatment. Two-way Analysis of Variance (ANOVA) with Tukey's post-hoc test were conducted on the density of settled coral larvae to determine if significant differences in settlement existed in the different treatments (different tile conditioning durations), and if coral larvae had a preference for a tile surface (top, sides, or bottom). Linear regression analysis was performed on the dry weights of particulate matter and numbers of settled larvae to determine possible relationships.

Results

The 10% CR tiles conditioned in aquaria for periods from 1 to 8 weeks showed different degrees of biofilm formation (Fig. 1).

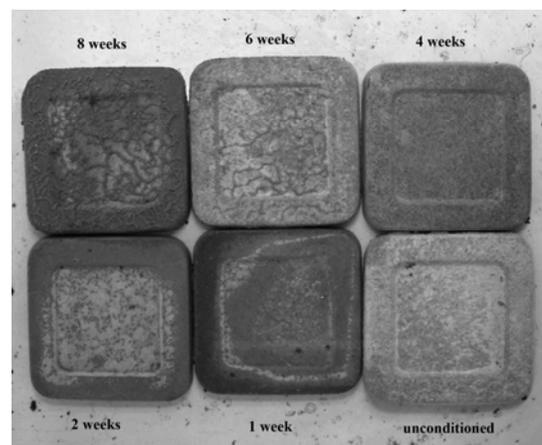


Figure 1: Surfaces of tiles conditioned for different periods of time in a running seawater system in indoor aquaria, showing different degrees of biofilm formation.

Two-way ANOVA on density of larval settlement data showed no significant interaction between the duration of tile conditioning and preferred settlement surfaces (top, sides, or bottom) on experimental tiles ($p > 0.05$; Table 1). Tukey's test revealed no significant differences in the mean settlement densities on tiles conditioned for different periods of

time ($p > 0.05$), but significant differences in settlement on different surfaces of the tiles ($p < 0.05$, Table 1 and Fig. 2).

Table 1 Results of 2-way ANOVA on coral settlement density crossed with factors: duration of tile conditioning (0, 1, 2, 4, 6, and 8 weeks) and tile surfaces (top, sides or bottom), and results of Tukey's test. (* indicates significant treatment effects)

Factors	df	F	p
Duration of conditioning	5	1.923	>0.05
Tile surface	2	15.800	<0.05*
Duration × Surface	10	1.977	>0.05

The mean number of settled larvae showed a tendency to decrease with increasing periods of tile conditioning (Fig. 3). The highest settlement rate occurred on the clean, unconditioned tiles (Controls), and there was a gradual decrease in larval settlement on tiles conditioned for longer periods of time (Fig. 3). These results indicated that the larvae preferred to settle on clean, unconditioned 10% CR tiles.

The mean density of larvae that settled on the sides of the tiles were significantly greater than at the top surfaces of the tiles (Fig. 2).

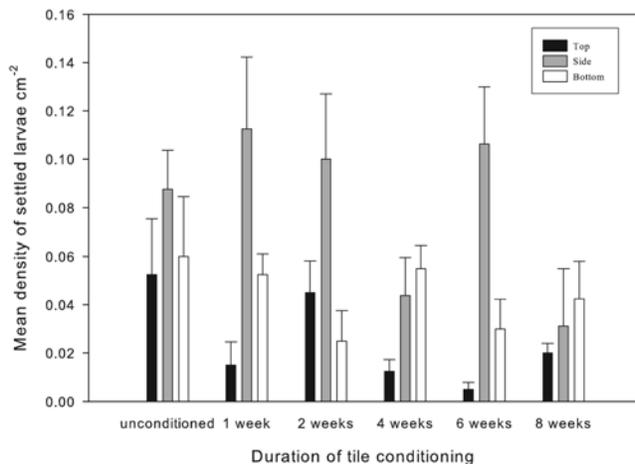


Figure 2: Mean density of settled *P. damicornis* larvae observed on different surfaces of tiles conditioned for different periods of time. (Means of 4 replicate aquaria; Error bars show standard error)

Regression analysis indicated a significant, negative relationship between the mean number of settled larvae and weight of particulate matter on experimental tiles ($r^2 = 0.512$, $p < 0.05$, $n = 24$; Fig. 4).

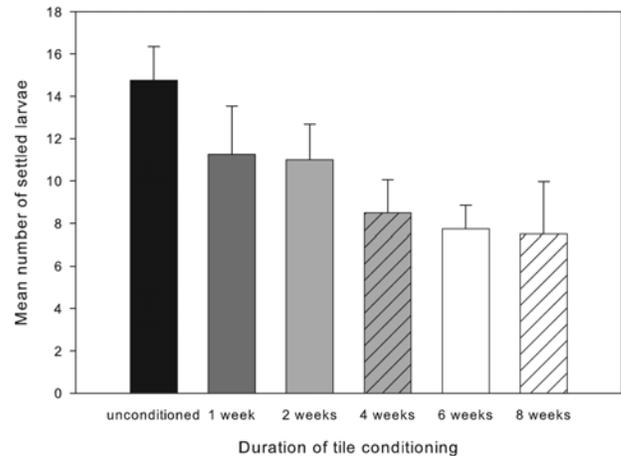


Figure 3: Mean number of settled *P. damicornis* larvae observed on 10% CR tiles conditioned for different lengths time over a 10-day experimental period in aquaria. (Means of 4 replicate aquaria; Error bars show standard error)

Discussion

P. damicornis larvae showed decreasing rates of settlement in relation to the duration of tile conditioning. In the experiment, larvae had a clear propensity to settle on clean tiles, suggesting that conditioning of 10% CR tiles was not necessarily a requirement for promoting larval settlement. A possible explanation of the results obtained may be that the longer duration of tile conditioning in a natural flow-through seawater tank also resulted in the accumulation of higher amounts of fine sediment on the top surfaces of the tiles.

In the present study, larvae seemed to have a marked preference for the sides (vertical surfaces) of the tiles for settlement. In a settlement orientation study of the corals, *Platygyra sinensis* and *Oxypora lacera*, larvae were significantly affected by physical and biological factors under natural conditions (Babcock and Mundy 1996). Settlement on vertical surfaces was also observed in an experiment which reported that tiles positioned between angles of 60° and 90° induced higher larval settlement (Carleton and Sammarco 1987).

Increasing sediment accumulation was observed on the top surfaces of tiles conditioned for longer periods of time in the study repeated in February 2008. The results also demonstrated that the presence of fine sediment decreased the effectiveness of potential suitable substrates for larval settlement of *P. damicornis* (Fig. 4). Sediment has been reported to inhibit the settlement of coral larvae (Hodgson 1990; Babcock and Davies 1991; Babcock and Mundy 1996). Larvae respond to negative chemical or physical cues due to the accumulation of sediment layers on the substrates (Hodgson 1990). Another

study demonstrated that suspended sediments and the accumulation of sediment on the upper surfaces significantly inhibited larval settlement of *Acropora digitifera* on CCA-covered substrates (Gilmour 1999).

It is likely that differences in microbial density and microbial community composition results from conditioning tiles for different durations of time. Few studies have focused on the role of the microbial biofilms on coral settlement. In tropical waters, the composition of marine biofilms fluctuate over time (Webster et al. 2004). Surfaces with different compositions, densities, ages and origins of biofilm attract different marine invertebrate larvae to settle (Parsons et al. 1993; Wiczorek and Todd 1998; Patel et al. 2003), and many larvae prefer to settle and metamorphose on multi-species biofilms (Kirchman et al. 1982; Patel et al. 2003). The community composition of the biofilm depends on the bacterial species which colonise the substrates. For example, *Pseudoalteromonas* bacteria isolated from CCA was able to induce larval settlement of *Acropora willisae* (Negri et al. 2001). Although the conditioned tiles may have developed a complex layer of microbial communities on their surfaces, this may have enhanced the accumulation of silt and sediment, rendering the surfaces unfavourable to the coral larvae. More studies are needed to investigate and to understand the role that microbial communities play in coral larval settlement.

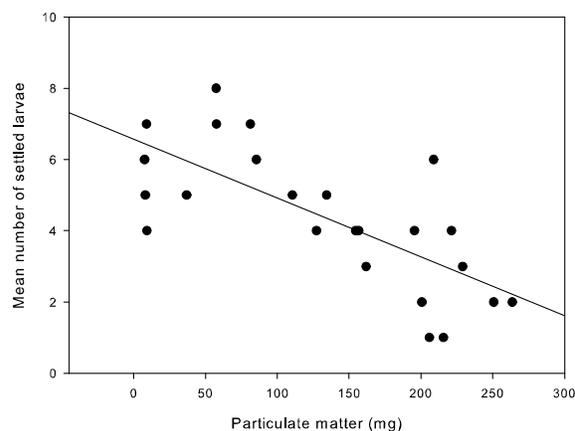


Figure 4: A negative relationship between mean number of settled larvae and the weight of particulate matter on conditioned tiles. The solid line through the data represents a least-squares regression.

Pocillopora damicornis is a brooder with a monthly planulation cycle. The larvae of coral species that are brooders tend to demonstrate more general responses to cues for metamorphosis and settlement (Baird and Morse 2004). It would be interesting to investigate the effects of substrate conditioning with sediment-stress on larvae from other species of corals which are

broadcast spawners, to determine if similar responses are elicited.

In conclusion, increasing durations of substrate conditioning did not enhance larval settlement, possibly due to increasing accumulation of fine sediment on the substrates. Therefore, the characteristics and properties of substrates should be carefully considered in the selection of substrate surfaces to ensure high larval settlement. More research is necessary to investigate the physical and chemical properties of artificial substrates, the composition of biofilm/sediment layer found on biologically conditioned tiles, and species-specific preferences for coral larval settlement.

Acknowledgements

Thanks are due to Juan Walford, Sin Tsai Min, Michelle Lee and staff from the Tropical Marine Science Institute and Reef Ecology Laboratory, National University of Singapore for assistance in research and field work. This project was partially funded by the NIE/NTU AcRF Grant, RP5/02 GPL held by the first author, National Institute of Education, Nanyang Technological University.

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