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To cite this article: S Yusuf *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **253** 012023

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Larvae of the Coral *Acropora tenuis* (Dana 1846) Settle Under Controlled Light Intensity

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Abstract. Coral restoration using sexual reproduction could have many advantages over the currently more widely used asexual reproduction methods, in particular for maintaining genetic biodiversity. On-going research on controlled sexual reproduction of corals is seeking ways to achieve higher larval settlement and survival rates. Metamorphic settlement is a critical phase for the survival of coral larvae, due to morphological changes as well as threats of predation and competition. This study aimed to identify the effects of light intensity and substrate positioning on the metamorphosis of competent planula stage larvae. Five day old larvae of the Indo-Pacific coral *Acropora tenuis* (Dana 1846) were obtained from coral spawning under laboratory conditions. The light intensity experiments used 4 treatments: 170, 130, 90 and 45 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$. Substrate positioning experiment treatments were vertical and horizontal orientations of the settlement plates. Coral larval settlement was not significantly correlated with light intensity, despite the higher settlement rates observed under light intensities between 130-170 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$. The highest rate of settlement occurred on the ninth day post fertilisation and was significantly higher ($\alpha < 0.05$, $\text{df}:2$) than the rates on day seven and day eleven. The number of coral larvae settling on horizontal substrate was significantly higher compared to vertical plates, with a ratio of 11:1. This understanding of the factors affecting larval metamorphosis and settlement, in particular the importance of light intensity and substrate orientation, could be applied in the on-going efforts to mass produce juvenile corals for coral reef restoration.

1. Introduction

Reproduction and recruitment are two crucial processes for the development and maintenance of coral populations. During recruitment, a critical phase in the life of aquatic organisms, larvae undergo metamorphosis, a process of morphological change from planktonic larvae to benthic juveniles [1,2]. If this metamorphosis is successful, the survival rate of the settling larvae as juveniles will be higher, and vice-versa. At the time of this body shape change, the internal energy reserves of the larva are depleted, and as they begin a new benthic lifestyle they face many threats including predation and competition. [3]. Coral planula settlement and metamorphosis processes require physical and chemical cues from the environment to induce planulae to initiate settlement and metamorphoses [1]. Experimental research on the metamorphosis and settlement of planula stage larvae has been undertaken using several experimental models [4,5,6,7,8].



At the laboratory scale, planula stage larvae require special handling and conditions during metamorphosis. It is possible to provide appropriate conditions conducive to the early larval and juvenile stages of corals reared for research purposes [9]. Larval development processes can be observed and controlled in the laboratory, including the process of settlement and attachment of planulae to a substrate. Planula stage larvae tend to prefer substrate with a rough surface texture and crevices rather than smooth surfaces, as the former offer a more secure attachment place and some refuge from predation [6,10]. The selection of permanent settlement sites and substrate by planula stage larvae is also influenced by physical factors such as light, salinity, water movement, depth, substrate topography, and sedimentation [11].

The settlement of coral larvae is influenced by light intensity [5]. Planula stage larvae tend to settle when light intensity conditions are appropriate [7]. A study on the influence of ultraviolet radiation (UVR 280-400 nm) on the settlement of *Pocillopora damicornis* larvae [13] found that the radiation in a specific range (PAR, 400-700 nm) was required to maintain energy production from newly settled juvenile to adult life stages, while the influence of light intensity on the settlement and metamorphosis of *Acropora millepora* larvae has also been studied [12]. However there is a lack of information on the metamorphosis response of planula stage larvae to light intensity. How much influence light intensity might have on the settlement and metamorphosis of coral larvae is still largely unknown.

Although corals generally have a very high fecundity, in their natural environment the survival rate of coral larvae is very low. The high levels of environmental stress in the surface layer of the open ocean pose many threats to larval survival [14]. While it is now widely accepted that coral planula settlement and metamorphosis processes require physical and chemical cues from the environment to induce planulae to settle and begin to metamorphose [1], there is still a need for improved knowledge on the settlement and metamorphosis phase of coral planulae. Such information is required not only to better understand natural coral recruitment processes, but also to support the cultivation coral larvae *ex-situ*, whether for research, conservation, or commercial purposes. Furthermore, while it is known that rough substrates are preferable to smooth ones, there is a lack of information on the angle at which settlement substrates should be placed. The aim of this research was to study the effect of light intensity and substrate placement angle on the settlement of planula stage coral larvae and their metamorphosis to the early juvenile stage.

2. Methods

Planula stage larvae were obtained from the captive spawning of *Acropora tenuis* colonies at the Orpheus Island Research Station (OIRS), James Cook University, Palm Island Park, Central Great Barrier Reef, Australia. Prior to this research the larvae were reared for five days under intense supervision. Each experimental unit comprised a 10 L plastic bin (35 x 18 x 20 cm³) placed in a large fibreglass tank filled with sea water to maintain a stable temperature regime. The density of coral larvae in each bin was 1 planula per ml, giving a total of around 10,000 planulae/unit.

The experimental design for testing the effect of light on coral settlement was based on the theory that coral larvae avoid settling on substrate where the light intensity is too high. Light intensity on the reef crest and slope tends to vary between 100–200 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ [15]. The average intensity of direct sunlight at the research site was 295 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$, while under the transparent fibreglass roofing the average light intensity was 228 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$.

In order to reduce the light intensity reaching the larval rearing units, several layers of black netting were used. Light intensity reaching the units varied with the number of layers used, with four treatments (S1 to S4) as follows: S1, 1 layer (170 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$); S2, 2 layers (130 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$); S3, three layers (90 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$); and S4, four layers (50 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$). The percentages of ambient sunlight (average light intensity) reaching the experimental units under each treatment were: S1: 60%; S2: 45%; S3: 30%; and S4: 15%.

This research used a fully randomised design, with four levels of light intensity (independent factor) and 5 repeats. The dependent variables were: (a) larval settlement time, and (b) number of settled coral juveniles/polyps. Within each unit, substrate orientation (vertical or horizontal) was also a

two-level independent factor. Artificial substrate (10 x 10 cm² settlement plates) was provided in each unit. The preference for vertical or horizontal settlement surfaces was tested by placing the plates vertically or horizontally.

During this research, the settlement of planula stage larvae was divided into two phases: the swimming planula stage and the settled stage. Swimming planulae were sampled using a 5 ml pipette; 10 replicate samples (1 ml each) were taken from the water column of each unit at each sampling time. Settling planulae were sampled by scrapping the artificial substrate (settlement plates) using a 5 ml pipette, again collecting 1 ml with 10 replicates per experimental unit [16]. Sampling was carried out at two day intervals (day 7, 9 and 11).

Each sample was placed in an Iwaki glass, and the number of larvae was counted under a microscope. The newly settled juveniles or polyps were observed on day 16. The number of settled juveniles on each substrate plate was counted directly under a microscope.

The data were tabulated and were analysed descriptively (graphically) as well as quantitatively. Analysis of Variance (ANOVA) was applied to evaluate statistical significance.

3. Results

3.1. Settlement of Coral Planulae

The coral planula stage larvae exhibited settlement behaviour from day 5 (experimental set up) until day 11 under all light intensity treatments, however with different settlement rates (Figure 1a). The mean (\pm standard deviation) proportion of settled larvae was higher under the higher light intensity treatments, with 52.22% (\pm 22.93 %) under treatment S1 and 51.67% (\pm 29.26 %) under treatment S2. Lower settlement levels were observed under treatment S3 (42.11% \pm 34.48%) and S4 (36.99 \pm 37.11%). Although the final settlement percentages were not significantly different at the 95% confidence level, at higher light intensity the larvae tended to settle earlier.

The planula stage larvae exhibited a settlement peak on day 9 (Figure 1b). The ANOVA results (Table 1) show that the mean proportion of settled planulae (73.62% \pm 10.69 %) on day 9 was significantly different (at the 95% confidence level) from the proportions on day 7 (29.87 \pm 15.88%) and day 11 (33.75 \pm 13.22%).

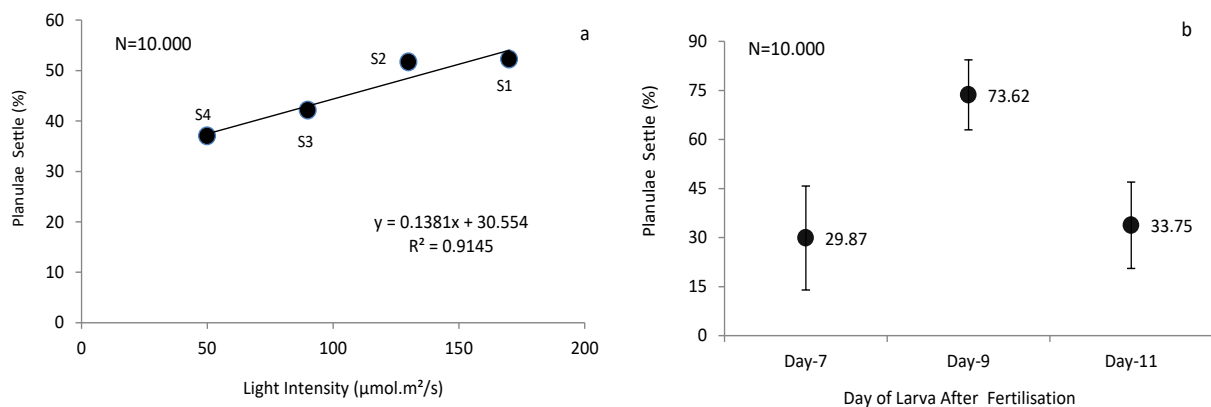


Figure 1. Percentage of larval settlement on artificial substrates (a) by light intensity treatment (on day 11); and (b) by days after fertilisation

Table 1. Analysis of Variance (ANOVA) of *A. tenuis* planula stage larvae settlement

| SK | JK | DF | KT | F | Sig (0,05). |
|-----------------|----------|----|----------|----------|-------------|
| Corrected Model | 19803,89 | 11 | 1800,354 | 3,437065 | 0,005 |
| Intercept | 80466,78 | 1 | 80466,78 | 153,6196 | 0,000 |
| Light intensity | 789,8889 | 3 | 263,2963 | 0,500401 | 0,684 |
| Day * Light I | 19803,89 | 11 | 1800,354 | 3,437065 | 0,005 |
| Error | 12571,33 | 24 | 523,8056 | | |
| Total | 112842 | 36 | | | |
| Corrected Total | 32375,22 | 35 | | | |

3.2. Metamorphosis of Coral Planulae

From day 12 onwards, the planula stage larvae were not only settling but beginning to attach to the substrate and metamorphosing to form juvenile colonies consisting of a single polyp. A visual record was made of the juvenile coral recruitment process, from planula stage larvae to new polyps attached to the substrate (Figure 2). By day 16, the total count of settled juveniles (Figure 3a) was not significantly different between light intensity treatments (at a confidence level of 95%). However, despite the high within treatment variability (indicated by the high standard deviations), the number of settled juveniles was substantially lower (80.67 ± 68.04) under the lowest light intensity treatment (S4, $50 \mu\text{mol. m}^{-2}.\text{s}^{-1}$) compared to the other three (higher) light intensity treatments (S1: 138.33 ± 148.80 ; S2: 158 ± 150.44 ; S3: 137 ± 14.8). Furthermore, the second lowest light intensity (S3, $90 \mu\text{mol. m}^{-2}.\text{s}^{-1}$) had a lower maximum, around half of the maximum juvenile counts under the two highest light intensity treatments (S1, $170 \mu\text{mol. m}^{-2}.\text{s}^{-1}$ and S2, $130 \mu\text{mol. m}^{-2}.\text{s}^{-1}$). These results indicate that settlement rates may decline at lower light intensities (below around $100 \mu\text{mol. m}^{-2}.\text{s}^{-1}$).

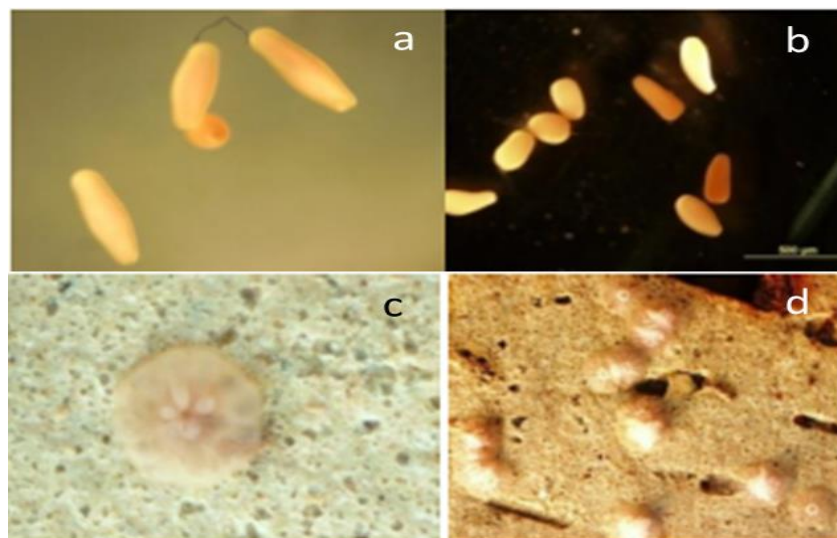


Figure 2. a.b. Planula stage larvae prior to settlement; c.d. juvenile polyps newly settled on the artificial substrate

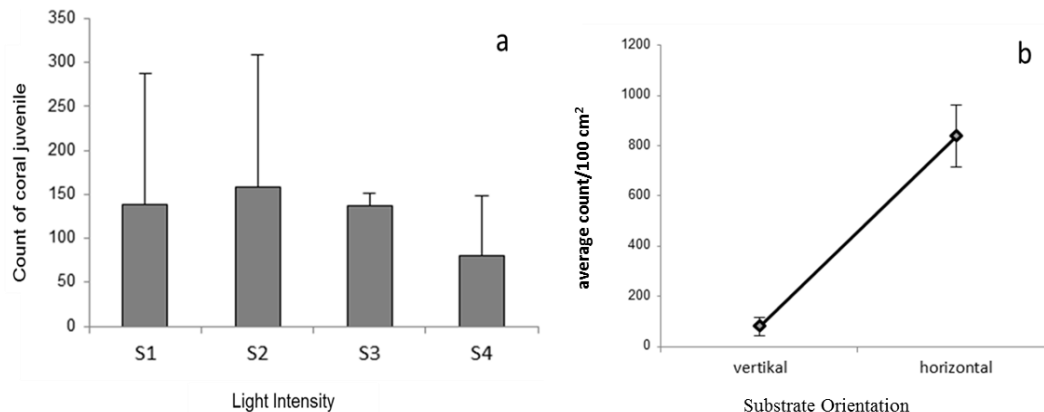


Figure 3. Larval recruitment: (a) count of coral juvenile recruits by light intensity; (b) recruit density by substrate orientation.

Observations of settlement outside the artificial substrate plates showed an increase in settlement with reducing light intensity from treatments S1 to S3, with a sharp drop in recruitment under the lowest light intensity, S4. The Univariate ANOVA ($df=3$; $\alpha=0,05$) model (Table 2) did not show a significant effect of light intensity on juvenile coral count. However the ANOVA does indicate a significant effect from substrate orientation ($df=1$; $\alpha 0,05$) as well as from the combined influence of light intensity and substrate orientation ($df=7$; $\alpha 0,05$).

Table 2. ANOVA for light intensity and substrate orientation effects on *A. tenuis* juvenile settlement

| SK | JK | DF | RK | F | Sig. |
|-----------------------|------------------------|----|-----------|-------|-------|
| Corrected Model | 24704,167 ^a | 1 | 24704,167 | 4,897 | 0,038 |
| Substrate orientation | 24704,167 | 1 | 24704,167 | 4,897 | 0,038 |
| Light intensity | 22304,833 | 3 | 7434,944 | 1,312 | 0,298 |
| Light * Orientation | 77183,167 | 7 | 11026,167 | 3,016 | 0,032 |
| Error | 110977,667 | 22 | 5044,439 | | |
| Total | 171410,000 | 24 | | | |
| Corrected Total | 135681,833 | 23 | | | |

The results of the substrate orientation experiment show that *A. tenuis* larval settlement was much higher on horizontal than vertical plates, with a ratio of 11:1 (Figure 3b). Furthermore, settlement on vertical plates was still higher than settlement on the bin surface outside the artificial substrate provided. The number of juveniles counted on vertical plates (mean \pm standard deviation) was 78 ± 36.34 , while on horizontal plates the mean count was 838 ± 125.24 .

4. Discussion

The settlement of planula stage larvae on day 9 probably represents an exploratory phase where the planulae were still exploring the substrate present and its suitability. The drop in settled larvae on day 11 indicates that a proportion of these larvae were not yet fully ready to settle and metamorphose, so that they returned to a swimming mode after having checked out the available substrate. Such behaviour has been reported in other species. For example, initial larval settlement rates of *A. solitaryensis* peaked at 3–4 days after spawning, and decreased gradually. In contrast, some larvae of *A. solitaryensis* began to settle permanently 3–4 days after spawning with a peak occurring at 6–7 days [2].

The observed preference of *A. tenuis* larvae for horizontal substrate is similar to the results reported by [17], with 2.8 times more juveniles on horizontal substrate compared to vertically oriented

substrates. However the opposite has also been recorded from studies in the wild; for example, [6] found significantly more recruits on vertical substrates, although horizontal substrate was more common. It was suggested that this might be due to the deposition of sediment particles on the horizontal substrate, together with higher levels of competition and predation, as well as higher settlement rates of algae, all of which could impede the settlement of planula stage larvae.

Of the many factors affecting settlement, one factor which can attract larvae is the presence of a biofilm layer on the substrate surface [7]. According to [18], this biofilm is formed by bacteria which can stimulate the settlement and metamorphosis of planula stage larvae. Biofilm composition can vary between sites as well as with depth and over time [19].

During this research, planula stage larvae of the coral *Acropora tenuis* showed increased settlement activity under higher light intensity levels, and vice-versa. Although the final settlement percentages were not significantly different at the 95% confidence level, at higher light intensity the larvae tended to settle earlier. This indicates that *A. tenuis* juveniles tend to prefer a relatively high level of light intensity. This is in line with research by [5] reporting over 50% larval settlement under a light intensity of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This result is also similar to findings reported by [15], and is likely related to the habitat of *Acropora tenuis*, a species which is mainly found in shallow waters, so that the larvae would be likely to have become adapted to relatively bright conditions through natural evolutionary processes. In some tropical regions, *A. tenuis* is found in reef flat zones where light intensity can be as high as $500\text{-}1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

According to [15], excessively high light intensity will adversely affect the settlement of coral larvae. Conversely, as proposed by [5], in coral species which have become adapted to habitats with lower light intensity, larval settlement and metamorphosis would likely be impeded by strong light. It has also been reported that in very bright conditions, coral larvae will tend to settle and subsequently metamorphose on the underside or hidden (shaded) faces of available substrate [20].

Other studies have reported a highly significant but non-linear relationship between relative light intensity on the settlement plates and the positions of coral spat [21]. On the reef slope, a range of $100\text{ - }200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ has become a widely accepted reference value as an ideal light intensity range for the settlement of almost all coral species. However this research found results similar to [15], where juvenile *Goniastrea aspera* and *A. tenuis* were mostly found in areas with ambient light penetration of 70% or higher. Thus, the spatial distribution of settled larvae of these two species was within the habitat of existing adult colonies (broodstock), i.e. in brighter and/or shallower areas of the reef.

Under controlled (laboratory) conditions, some potential problems encountered in the wild can be minimised. For example, substrate with coarse surfaces and crevices can be provided to cater to the known preference of *A. tenuis* in terms of settlement substrate. Light and substrate orientation can also be adjusted to the optimum conditions found in this study, and similar studies can be conducted for other species, especially those with different habitat distributions in the wild.

However, it is worthy of notice that there is a lack of studies on the settlement of coral larvae on smooth surfaces. The presence of biofilm on smooth surfaced substrate can act as a biomagnification factor to attract larvae and promote juvenile settlement, while another factor is the tendency of planula stage larvae to settle in groups [1,22]. Biofilm is produced by layers of bacteria and can stimulate the settlement and metamorphosis of planula stage larvae, which become pear shaped and sink to the substrate [1]. Coral larval metamorphosis can also be triggered by increasing the level of basic requirements such as zooxanthellae as a source of energy, and calcium for skeleton formation (Heyward and Negri 1999). These aspects are suggested as avenues for further research on larval settlement in corals.

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