Reference: *Biol. Bull.* **204:** 241–245. (June 2003)

Settlement Cues in the Locally Dispersing Temperate Cup Coral *Balanophyllia elegans*

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Most studies of settlement cues in sessile marine invertebrates have focused on species with the potential to disperse over great distances. This persistent focus has perpetuated the idea that long-distance dispersing species may rely on cues to settle in specific habitats, whereas short-distance dispersers are delivered directly into a favorable habitat. I tested the effects of water movement and substrate on the settlement of the temperate solitary cup coral Balanophyllia elegans, *a species whose crawl-away larvae disperse just centimeters before settling. Ninety percent of larvae settled within 3 days of release in the presence of both natural rock substrate and moving water, but fewer than 11% settled in the same period when either or both factors were absent. An additional experiment revealed that when natural rock substrate was available, water velocities of less than 25 cm · s^{-1} triggered a 5-fold increase in settlement rate relative to standing water. When settling, the larvae of* B. elegans *respond strongly to the interactive effects of water flow and substrate. Thus, settlement cues may play a significant role in generating the patchy distribution of* B. elegans, *a pattern previously attributed to short-distance dispersal.*

Larval settlement is a critical stage in the life history of sessile marine invertebrates. This transition from a mobile larval stage to a sessile juvenile stage represents a commitment to a particular location, and determines the subsequent conditions that an individual will encounter, including predation pressure (1), severity of physical stress (2), competition for space (2), competition for food (3), and fertilization success (4). Numerous studies have revealed that two factors influence the spatial pattern of settling larvae: a variable supply of larvae, which can lead to a correlated variable settlement pattern (5, 6); and selective larval settlement in response to patchy environmental cues, which can refine settlement patterns set by larval supply (7, 8). Variable larval supply can lead to heterogeneous settlement patterns in both long-distance (scale of kilometers) (6) and short-distance (scale of cm or m) (9, 10) dispersing species. Settlement cues, on the other hand, have been observed primarily in long-distance dispersers (but see 10, 11), and reviews on the topic typically ignore settlement cues in species whose larvae disperse and settle within the area immediately surrounding parent individuals, particularly those with crawl-away larvae (7, 8). The patchy distribution of short-distance dispersers remains largely attributed to patterns of larval supply (12–16); however, few studies have investigated the alternative hypothesis that larval settlement of species with limited dispersal is influenced by environmental cues.

The stony (scleractinian) aclonal cup coral *Balanophyllia elegans* is an ideal species with which to test the widely accepted hypothesis that species with limited dispersal do not show strong settlement response to environmental conditions. Unlike the planktonic larvae characteristic of most marine invertebrate species, which can disperse kilometers, the wormlike planula larvae of *B. elegans* disperse locally by crawling along the substrate (13). Adults of *B. elegans* are patchily distributed at a scale of meters—a pattern largely attributed to an average dispersal distance of just 39 cm, though larvae are capable of traveling more than 1.3 m before settling (13, 17, 18). Thus, larvae generally settle closer to parent individuals than their dispersal potential would predict, indicating that environmental factors may influence the settlement behavior of larvae and contribute to the patchy distribution of adults. Settlement cues have been largely overlooked as a potential cause of the patchy settlement distribution of *B. elegans* and many other species with short-distance dispersal, perhaps because short-distance dis-

Received 30 September 2002; accepted 14 April 2003.

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persal is perceived as delivering larvae directly to habitats that have already proven suitable for reproductive adults (19–22). The alternative hypothesis that the settlement behavior of *B. elegans* is influenced by environmental conditions has not been tested, even though cues often cause species to aggregate where environmental variables are favorable to the fitness of the adults (23, 24), and *B. elegans* belongs to a taxon (scleractinian corals) for which larval settlement cues are common in species with long-distance dispersal (25, 26). The purpose of this study was to establish whether environmental cues influence the settlement behavior of the solitary cup coral *Balanophyllia elegans.*

I tested the effect of water movement and substrate on the settlement rate of *B. elegans* larvae, since both factors influence the settlement behavior of other scleractinian corals (25, 27). In the first of two experiments, single larvae were placed in polycarbonate cups (75 × 75 mm diameter × height) within 24 h of release, and exposed to one of three substrate treatments and one of two water-movement treatments. The substrate treatments were live rock (rock collected from *B. elegans* habitat), sterilized rock (live rock that had been boiled for 2 h in de-ionized water), and polycarbonate (no additional substrate added to cup). The water-movement treatments were moving water (stream of seawater entering cup at 17 $\mathrm{ml \cdot s^{-1}}$) or standing water (no stream of water). Larvae were monitored daily for settlement, indicated by irreversible metamorphosis from worm-like crawling larvae (3–5 mm long) to radially symmetrical sessile polyps (~1.2 mm diameter) (28). Larvae in all treatments were observed to crawl until settlement or end of the experiment at a rate consistent with Gerrodette's observation of 0.03–0.04 $\mathrm{cm \cdot min^{-1}}$ (13). In laboratory conditions, Gerrodette (13) found that larvae of *B. elegans* settled an average of 3 days after release, and remained competent to settle for more than 14 days. To determine if the experimental treatments of my study shifted the timing of settlement relative to that average, larvae were recorded as either settling or not settling in the first 2 days of the experiment (due to a 1-day lag between larval release and placement in experimental treatments). Differences between treatments in the proportions of larvae settled during the first 2 days were evaluated by chi-square analysis. Results showed that the proportion of larvae settling during this period was 9 times higher in treatments with both natural substrate (live or sterilized rock) and moving water than in treatments with either or both conditions absent (Fig. 1A). This dramatic difference ($\chi^2 = 90.30$, $P < 0.001$, df = 1) demonstrates that the settlement rate of *B. elegans* larvae is strongly affected by both environmental factors.

A second experiment was conducted to estimate the water velocity necessary to trigger settlement of *B. elegans*, and to verify that the high settlement rate exhibited by larvae in the first experiment was due to water movement rather than to some artifact such as varying concentration of solutes in the inflowing seawater or depletion of oxygen in the standing water treatment. Single larvae were placed in polystyrene petri dishes within 24 h of release, and exposed to one of two substrate treatments—sterilized rock (live rock that had been boiled for 2 h in deionized water) or polystyrene (no additional substrate added to petri dish)—and to one of two water-movement treatments—moving water (shaker table at 80 rpm) or standing water (stationary table). Water movement was generated by an orbital shaker table, rather than a stream of seawater, to eliminate potential variability of inflowing seawater. Shallow polystyrene petri dishes (60 × 15 mm) were used instead of polycarbonate cups to ensure ample surface area for diffusion of oxygen into the water. Settlement was scored and analyzed similarly to the first experiment. As observed in the first experiment, settlement rates were significantly higher in the presence of both moving water and rock substrate than when either or both conditions were absent ($\chi^2 = 19.7$, $P < 0.001$, df = 1). Based on the petri dish dimensions, it was determined that water velocities of less than 25 $\mathrm{cm \cdot s^{-1}}$ were sufficient to trigger a 5-fold higher settlement rate relative to standing water (Fig. 1B), a range of water flow commonly encountered by *B. elegans* within its geographic range (29).

To validate the *a priori* 2-day settlement criterion used for analysis, and to examine settlement patterns of *B. elegans* on a finer temporal scale, the shaker table experiment (Fig. 1B) was allowed to run for 8 days, and the cumulative proportion of larvae settled was recorded daily. The plot of larval settlement over time illustrates that the sterilized rock-moving water treatment diverged sharply from the other three treatments within the first day, a difference that persisted for the remainder of the 8-day experiment (Fig. 2). Settlement did not occur in the polystyrene-standing water treatment until the 8th day, suggesting that in poor conditions settlement can be delayed, taking up to 4 times longer than in favorable conditions. In many marine species with selective larvae, settlement is similarly delayed to lengthen the search time for suitable habitat (7).

The significantly higher settlement rate of *B. elegans* larvae exposed to water movement and rocky substrate is probably due to both the reliability of the cues as indicators of suitable settlement areas (30) and the potential costs of not using those cues (31). Water movement is critical for delivery of food, nutrients, and dissolved gasses to scleractinian corals, and has been correlated with their distribution on the scale of meters (32). The results of this study suggest that correlation could be due in part to selective larval settlement. It is generally presumed that the mechanical characteristics of water movement influence the settlement behavior of larvae (33–35), and this study did not test the alternative hypothesis that the flux of solutes correlated with water movement could have induced settlement. Nevertheless, water movement proved to be an excellent predictor of settlement behavior in *B. elegans.* Settlement rates were also strongly affected by substrate. Though larvae were capable of settling on the artificial (polycarbonate and polystyrene) surfaces tested, settlement rates were dramatically lower than on natural substrates (live and sterilized rock). The lack of biogenic structures may indicate an inhospitable habitat. Conversely, the relatively smooth surface of polycarbonate may mimic the smooth surfaces of encrusting organisms, such as ascidians and algae, that can overgrow *B. elegans.* Potential interactions with spatial competitors are known to affect the settlement choices of marine larvae (36), and competition for space is a significant factor in establishing the post-settlement distribution of *B. elegans* in the rocky subtidal (18, 37, 38). The comparison between substrates of natural rock and polycarbonate limits our ability to predict substrate preference under natural conditions; however, the results are valuable in demonstrating one extreme in the timing of settlement (Fig. 2).

Water movement and substrate appear to be of such importance to the coral *B. elegans* that the larvae have developed a sensitivity to those factors when settling. For the species to have evolved such settlement preferences, water movement and substrate must vary at a spatial scale smaller than the potential larval dispersal distance of only 1.3 m. Gradients of topography and water movement similar to those tested in this study exist over a range of scales from millimeters to kilometers in the rocky subtidal where *B. elegans* occurs (29), and the lower end of that range is well within the larval dispersal distance.

The selective behavior of *B. elegans* larvae probably translates into an effect on dispersal patterns and adult spatial distributions. Gerrodette (13) found that the larvae of *B. elegans* moved randomly away from parent individuals

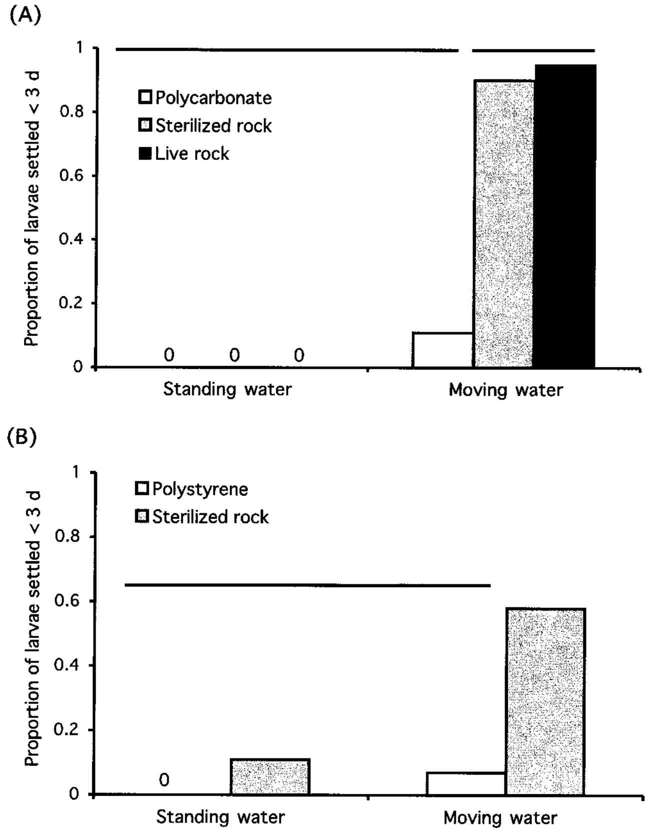

Figure 1. (A) A significantly higher proportion of *Balanophyllia elegans* larvae settled during the first 2 days of the experiments in treatments with both natural substrate (live or sterilized rock) and moving water than in treatments with either or both conditions absent ($\chi^2 = 90.30$, $P < 0.001$, df = 1). The two treatments of sterilized rock and live rock in moving water treatments were similar ($\chi^2 = 0.367$, $P > 0.54$, df = 1), and all other treatments were similar to one another ($\chi^2 = 5.66$, $P > 0.1$, df = 3). All experiments were conducted in January–March 1998 at the Long Marine Laboratory of the University of California at Santa Cruz in water temperatures of 14.0–16.5°C. Larvae used in the experiments were spontaneously released in the laboratory by a group of individuals collected from a depth of 4–8 m below MLLW at the Breakwater in Monterey, California (36°37′N, 121°53′W) in December 1997. Live rocks (15 × 15 × 10 to 25 × 20 × 15 mm) were collected from the same area, and were encrusted primarily by coralline algae. Sterilized rocks were collected as live rocks and prepared by boiling for 2 h in deionized water, a preparation which largely inactivates the polysaccharide and peptide in the crustose coralline algaes that respectively induce settlement of other corals and molluscs (39, 40). Thus the similarly high rate of larval settlement in both the live and sterilized rock treatments (in presence of moving water) was probably due to the texture of the substrate, and not to inductive compounds in the coralline algae.

(B) A second experiment was conducted to estimate the rate of water movement necessary to trigger settlement of *Balanophyllia elegans,* and to ensure that the strong settlement response exhibited by the larvae in the first experiment was due to water movement and not to some characteristic of inflowing water such as changes in concentration of solutes. Results were qualitatively similar to the first experiment: natural substrate and water movement acted together to induce a significantly higher rate of settlement relative to the other treatments in which either or both factors were absent ($\chi^2 = 19.7$, $P < 0.001$, df = 1). Results of the latter set of treatments were statistically similar ($\chi^2 = 4.12$, $P > 0.1$, df = 2). For the moving water treatments, petri dishes were placed on a shaker table with circular motion after larvae had been allowed to adhere to tested substrate for 1 h. Rocks remained relatively motionless in moving water treatments, and larvae remained adhered to substrate in all treatments for the duration of the experiment (when collecting larvae from brood stock, several vigorous blasts from a transfer pipette were commonly required to dislodge larvae from substrate, due in part to a mucous trail). By multiplying the circumference of the petri dish (18.84 cm) by the orbital rate of the shaker table (80 rpm), the wave generated by the shaker table was estimated to reach a velocity of 25 cm · s^{-1} at the edge of the dish and to diminish toward the opposite side. Since the water itself would have traveled more slowly than the wave due to encounter with the sides and bottom of the dish, 25 cm · s^{-1} is a conservative estimate of the velocity required to trigger preferential settlement.

For both experiments, differences between treatments in proportions of larvae settled during the first 2 days were assessed by chi-square analysis. Contingency tables with expected values of less than 5 in greater than 20% of the cells were analyzed by log-likelihood ratio goodness of fit test to avoid bias in the chi-squared statistic. When contingency table analysis showed significant results, the tables were partitioned to determine the treatment or combination of treatments responsible for the experiment-wide statistically significant result. Sample size was 14–20 for all treatments. Histogram bars under shared horizontal lines do not differ significantly at $P < 0.05$.

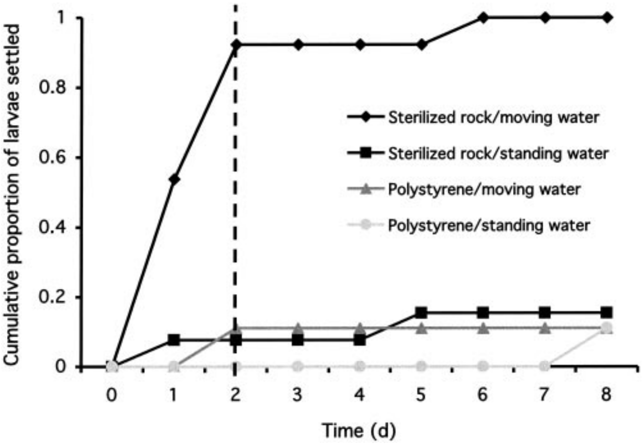

Figure 2 As detected by the chi-square analysis, cumulative larval settlement in the sterilized rock-moving water treatment shows a marked departure from the other three treatments by day two of the experiment. This relationship persisted for the remainder of the 8-day experiment, though larvae continued to settle through the end of the experiment. Values at the 2-day mark used for statistical analysis (indicated by the vertical dashed line) were thus an accurate descriptor of longer-term behavioral trends.

in a pattern analogous to diffusion at a rate of $\sim 10\ \mathrm{cm \cdot d^{-1}}$. The results of this study indicate that as larvae move randomly about an area that is heterogeneous with respect to substrate and water movement, they are more likely to settle and accumulate in areas where specific conditions are present. Thus, the patchy distribution of *B. elegans* that has been quantified in several studies may be due to preferential settlement in areas of higher water movement and rugose substrate. Such selectivity for water movement and topography leads to patchy distribution in other sessile marine invertebrates, including barnacles (33), ascidians (34), bryozoans, hydroids, and polychaetes (35).

The crawl-away larvae of *Balanophyllia elegans* are extremely sensitive to environmental conditions, and they base their settlement behavior on factors including water movement and substrate. This suggests that the environment is variable enough at the scale of dispersal (centimeters) to have generated an adaptive advantage for selective larvae, just as in more widely dispersing species. Though the relative importance of water movement and substrate as settlement cues remains to be tested in the field for *B. elegans*, these two factors are likely to be significant in establishing the settlement patterns of this species with short-distance dispersal.

Acknowledgments

I thank A. Boxshall, M. Carr, D. Lohse, and P. Raimondi for insightful discussion. They, along with M. J. Greenberg, B. Silliman, R. Wahle, J. Witman, and two anonymous reviewers, improved earlier versions of this manuscript with their comments. J. Pearse, P. Raimondi, and B. Steele made lab space and expertise available for this study. Divers A. Boullard, A. Boxshall, D. Huang, S. Lonhart, and M. Readdie assisted in field collections, and D. Canestro supported field operations. D. Huang provided a place for me to stay during portions of this study. Funding was provided by a University of California President's Undergraduate Fellowship, a Friends of Long Marine Lab Student Research Award, and a UCSC Department of Biology Independent Study Research Support Award.

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