The effect of age on the adult-produced larval settlement cue in *Dendraster excentricus*

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Mary Gates Research Endowment
Spring 2019
FHL 470 Research in Marine Biology
Spring 2019

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Keywords: Dendraster excentricus, sand dollar, settlement, larva

Abstract

A settlement cue produced by adult Pacific sand dollars, *Dendraster excentricus*, maintains dense aggregations of the species by inducing the metamorphosis of their larvae. This process is well-studied, but the unknown details are many. In May 2019, while studying at Friday Harbor Laboratories on San Juan Island, I attempted to add to the knowledge of this process by hand-rearing larvae and introducing them to sand which had been conditioned by adults of different sizes. The settlement data collected, while limited and nonsignificant, suggests that the 12-day timeline was sufficient for the adults to condition the surrounding sand, and that it is produced by adults, both big and small. Any nuance in the response to the cue remains to be discovered and could shed light on the adaptations to communal living in Echinoderms.

Introduction

A common life history in the ocean is the transition of planktonic larvae to bottom-dwelling adult (Hodin et al 2015). It is a story full of risk, trade-off, and if the individual is lucky, reward. The site selection process is, arguably, the most important chapter. Settling and metamorphosing in the right spot can bring abundant food, ample protection from predation, and sufficient opportunities for reproduction in the organism’s next life stage. On the other hand, an area unsuitable to any of those same processes can drastically lower fitness and quickly eliminate genomes from the population. It is a big risk, especially for sessile and slow-moving organisms, and many species have evolved methods to make the site selection process more successful by reading the chemical and/or physical factors of a site (Burke 1984). One of these methods involves taking a cue from one’s elders and settling near them.

The Pacific sand dollar, *Dendraster excentricus*, forms dense aggregations in shallow waters in the northern Pacific, interspersed with large stretches devoid of the species (Highsmith 1982). This pattern has led many to study the site selection process of its larvae. Adult *Dendraster* are thought to produce a small peptide pheromone into the surrounding sand that induces settlement in conspecific youngsters (Burke 1984). This could be evolutionarily advantageous for multiple reasons. The presence of adults suggests a suitable habitat for the growth and survival of the species. It also means that there will be plenty of adults of the same species around when it comes time to reproduce (Tamburri et al 2007). Finally, adults can provide predation protection. Specifically, the bioturbation activities of adults control populations of *Leptochelia dubia*, a tube-building crustacean that is a major predator on juvenile *Dendraster* (Highsmith 1982).

Settling and growing up with your elders may have its disadvantages though. Juvenile survival rates in *Scaphechinus mirabilis*, a sand dollar of the Northwestern Pacific, were shown to plummet with an increase in adult density, due to increased bioturbation, leading to juvenile burial and lack of sufficient feeding opportunities (Takeda 2008). Even before settlement, suspension feeding adults may consume their own larvae (Woodin 1976). Despite these risks, sand which has been home to adult sand dollars consistently leads to more rapid settlement and metamorphosis of pluteus larvae in laboratory studies of multiple echinoid species (Pearce and Scheibling 1990, Highsmith 1982).

Many questions concerning this settlement cue remain. While there is some evidence for the chemical being related to the digestive tract or the gonads, it is not entirely clear where in the body it is produced, nor at what age adults begin to produce it (Burke 1984, Highsmith 1982). If it is related to the gonads, it may follow that the cue is not produced until sexual maturity. If, on the other hand, this chemical is produced by all adults regardless of size, there may be a difference in the response of larvae to the cues of adults of varying age. I attempted to gain insight into this process by introducing competent larvae to sand which had been occupied, and therefore conditioned, by adults of different sizes as a proxy for age. On the one hand, I expected the cue to become stronger and more effective in causing settlement in larger adults but wondered if the danger of metamorphosing within dense beds of large adults has been influential in the attractiveness of the cue emitted by smaller individuals. If this is the case, larvae would be expected to respond more readily to a young adult cue and exhibit higher settlement.

Materials and Methods

This experiment was conducted at Friday Harbor Laboratories, San Juan Island, WA in May 2019.

Sand Conditioning

Dendraster individuals of varying sizes were collected from the established, flow-through tanks on the side of Fernald Laboratory at Friday Harbor Laboratories. The larger animals in these aquaria were collected by hand at low tide on Crescent Beach, Orcas Island, WA (48.6947, -122.8963) in July 2018, and the smaller individuals were the product of culturing by J. Hodin in 2017 and 2018. A group of 5 large dollars (7.3-8.3cm across) was haphazardly chosen and weighed. Another group of 5 large individuals was selected to match this weight closely and two additional groups of smaller dollars (0.9-3.3cm) were separated into groups weighing ¼ this weight. The animals were then submerged into a common outdoor flow-through sea table in 14x14x20 cm plastic containers containing a ratio of 0.42 g/mL False Bay sand. Sand for conditioning treatments and control was collected from the low intertidal at False Bay (48.4821, - 123.0708), a location known to once contain a population of *Dendraster* (J. Hodin, personal communication). This sand, therefore, despite being absent of an adult cue, should be otherwise suitable for sand dollar survival. The dollars were left to condition this sand for 12 days before sand extracts were made for the settlement experiment.

Spawning and Fertilization

Dendraster adults from the established tanks (2 males and 2 females) were each injected with 0.75 mL of 0.55 M KCl in reverse osmosis water on the oral side, shaken lightly, and monitored for signs of spawning. When eggs were seen, females were placed aboral side down on top of beakers of filtered sea water to catch falling eggs.

Males were placed under dissecting scope and a pipette was used to collect and transfer concentrated sperm into tubes that were immediately refrigerated.

After the viability of all gametes was confirmed, a 2x2 genetic cross was performed to increase genetic diversity. Eggs from each female were split in half and fertilized with 10 drops of diluted sperm solution, 1 by each male. The presence of fertilization halos was used to calculate a 90% fertilization success rate after 15 minutes. Beakers of embryos were left covered overnight at 19°C. After 18 hours, hatched and swimming larvae were decanted off the top, larval concentration was estimated, and approximately 750 of each cross was combined into a 3L glass jar. Larvae were fed and placed in indirect sunlight on a Gyrotory shaker-model G2 at 75 rpm at room temperature (RT), approximately 19°C, for the remainder of the experiment. The concentration of larvae was lessened by half, 2- and 4-days post fertilization (dpf), to accelerate maturation. The concentration from 4 dpf until settlement was 1 larvae/ 4 mL.

Water Changing and Feeding

A nearly complete water change was completed every 2 days after hatching. All but ~100 mL was removed from each jar via reverse filtration with a 70 µm filter and replaced with fresh RT filtered sea water. Larvae were then fed 2500 cells/mL *Rhodomonas spp.* and 3000 cells/mL *Dunaliella tertiolecta*, split into 4 jars, immediately following water changes. Algae was cultured in flasks and cells were counted by hemocytometer to standardize. Healthy feeding was determined by monitoring the guts of the larvae, which exhibited a consistent golden-yellow color throughout rearing, suggesting that they were consuming both species.

Settlement Experiment

Larvae 11 dpf were tested for competency in 40 mM excess K. Haphazardly chosen larvae showed 2% settlement after 1 hour of exposure to the K inducer and 2 hours recovery in RT filtered sea water.

On May 29th, larvae 13 dpf were introduced to 1 of 3 treatments: large *Dendraster*-conditioned sand extract, small *Dendraster*-conditioned sand extract, or *Dendraster*-free sand extract. An additional set of larvae was introduced to 3 replicates of established *Dendraster* tank sand extract as a positive control. A common extract strength was prepared by comparing the opacity to 50% extract from the established *Dendraster* tank, which was shown to successfully induce settlement at 30% concentration in previous runs. Each well plate well was filled with 8 mL sand extract and 15 larvae and checked after 4 hours for settlement. Larvae were considered settled when they had settled onto the bottom and metamorphosed into their juvenile form (see Fig.2C in Hodin et al 2015). Each larva was transferred out of wells when counted, for accurate record keeping.

Data Analysis

The average number of larvae settled per well was calculated for 6 replicates in each of 3 treatments. A single factor ANOVA was performed with the Analysis Toolpak in Excel 2016 (v16.0).

Results

Metamorphosis of larvae was not found to be significantly correlated to the presence or absence of adults, nor to the size of adults that had conditioned the sand (single factor ANOVA, $p>0.05$). The trend, however, suggests that the sand was in fact conditioned by both sizes of adults in the given time (12 days), as the adult-free treatment was the only to produce no settlement (Figure 1).

These results were most likely limited due to a lack of larval competency, and not a lack of settlement cue, as the positive control of established *Dendraster* tank sand extract showed the same average settlement of 0.3333 larvae per well.

Discussion

The lack of competent larvae was a deterrent to a more informative experiment. Hodin et al reported *Dendraster* larvae reaching competency at 12 dpf when reared between 20-22℃ (2015). Considering this timeline, our expectation of competency by 13 dpf when reared at 19℃ was potentially unreasonable and the larvae appear to have needed at least a couple additional days to mature before settlement experiments were performed. Genetically identical larvae which were reared slightly warmer showed signs of competency as early as 9 dpf (M. Thompson, personal communication). This illustrates the large influence of temperature in larval development speed.

Even though the experiment had to be conducted prematurely, the trends that were beginning to develop are interesting. Firstly, my 12-day timeline seems to have been sufficient for adult *Dendraster* to condition the surrounding sand. While a single adult has been reported to condition a small bowl of sand in as little as 20 hours (Highsmith 1982), this is the first time that a group of sand dollars has been allowed to condition a larger volume of sand in a relatively short timeline, in a manner more closely resembling wild populations.

Settlement in both the large and small adult treatments, and a lack of settlement in the False Bay control, suggests adults begin to produce the cue at an early age. When considering wild populations, this trend fits nicely. In some populations, the dense adult presence is thought to limit juvenile survival, the population being maintained by the immigration of young adults moving in from the edges when they are large enough to hold their own (Takeda 2008). If *Dendraster* larvae can detect the cue produced by these juveniles on the edge, settling on the outskirts of the bed with the generation before could offer some of the benefits of communal living, including predation protection and reproductive success, without quite so many risks (Tamburri et al. 2007). Additional runs need to be performed to determine if there is any nuance in the attractiveness between the cues of the younger and older adults, but the potential evolutionary implications are intriguing.

I would be amiss not to mention a potential source of error in this experimental setup. The presence of sand extract in the experimental wells made it difficult to locate larvae and not all larvae were recovered in each replicate. One study used filtered sea water conditioned with only sand dollar faeces to produce the same settlement effect as sand, pointing not only to the location of the chemical cue but also to an easier way of observing settlement (Pearce and Scheibling 1990). My collaborator Shannon Cefalu was looking at ways to make settlement observation easier. While her results were not significant, such work could make this sort of study much more accurate (Cefalu 2019).

Having a deeper understanding of gregarious larval settlement could be helpful in future conservation efforts across phyla. This knowledge could prevent the wasting of valuable time and resources and allow for more focused and successful initiatives. If young adults can indeed condition sand in less than two weeks, this leads to the possibility of rapid bed formation if larvae are present. Of course, this cue is only one of many factors influencing the settlement of *Dendraster* larvae and there is much still to learn about the others and the interactions between them. Every study which unravels one more piece of this puzzle helps us gain understanding into the evolutionary sophistication of many marine benthic species and their planktonic larvae.

Figures

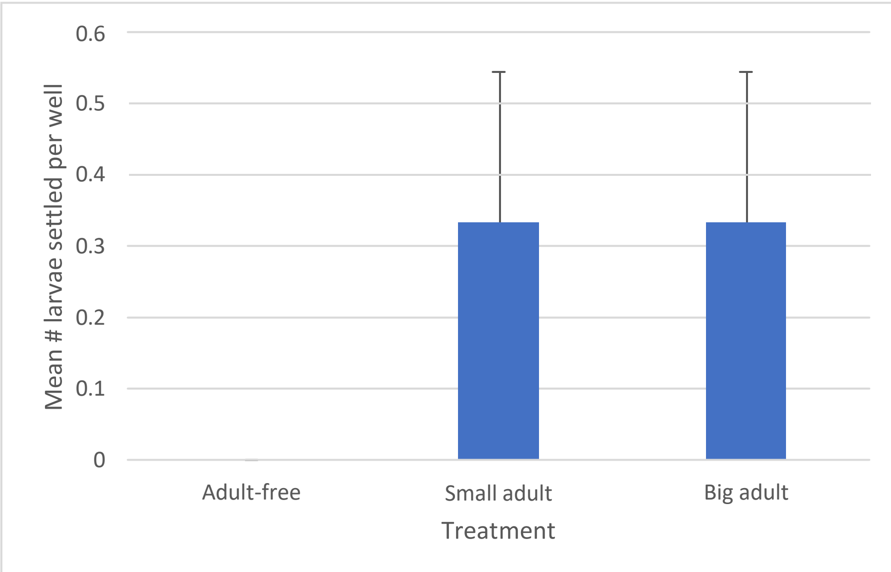

Figure 1: Bar graph showing the mean # of larvae settled per well in treatments of adult-free, small adult-, and big adult-conditioned sand extracts. Values are means ±Standard error. A single factor ANOVA showed no significant effect of treatment on settlement (ANOVA, df=2, $p>0.05$).

Acknowledgments

I would like to thank Shannon Cefalu for helping to rear our shared larval culture and Alexi Lund, Morgan Thompson, and Katie Gonzalez for help with spawning and fertilization. I would also like to thank Dr. Jason Hodin for his guidance and expertise during this foray into marine embryology. For help with data analysis and general morale I want to thank Karly Cohen. To the rest of the Zoobot 2019 class, I could not have done this without your laughter and friendship, so thank you. Lastly, thank you to Friday Harbor Laboratories for the use of their facilities.

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