

**Rise with the Incandescent Sun: A look at Diel Cycling and growth of *Dendraster
excentricus* in Response to Incandescent Light**

Morgan Thompson

FHL 470: Research in Marine Biology

Metamorphosis in the Ocean and across Kingdoms

Spring 2019

Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250

Contact information:

Morgan Thompson

Department of Biology

University of Washington

Seattle, WA 98195

thompmor@uw.edu

Keywords: Diel cycling, Incandescent light, Larvae, Entrainment, Metamorphosis

Rise with the Incandescent Sun: A look at Diel Cycling and growth of *Dendraster excentricus* in
Response to Incandescent Light

Abstract

The pluteus larvae of the sand dollar *Dendraster excentricus* and their diel vertical distribution have been well studied in the field and in lab. However, there has not been much focus on how the larvae and their vertical distribution change as a response to entrainment by an incandescent light source. In order to test this, two groups of the *D. excentricus* plutei were set to different day/night cycles, one receiving 12 hours of light, the other receiving only 5 hours. The swimming patterns and growth of these two treatments were observed and compared. The results indicated that the larvae could be entrained to incandescent light as vertical distribution and growth varied between the two treatments.

Introduction

Sand dollars in the genus *Dendraster* are common along the west coast of the United States. In particular, *D. excentricus* can be found in large numbers spanning from southern Alaska to southern Baja California in suitable habitats of shallow water and in a sandy substrate (Mooi, 1997). The spawning season of the echinoids is summer to early autumn, and the echinopluteus of the sand dollar is a planktonic feeding larva (Merrill, 1970). These larvae, as with many other zooplankton, undergo diel vertical migration (DVM) within the water column, rising slowly to the surface of the water as night approaches and descending in the water column as light levels increase (Pennington & Emlet, 1986; Longhurst, 1976).

While *Dendraster* larvae have responded strongly to UV light, they do not seem to react to incandescent light by altering their vertical position in the water column (Pennington & Emlet,

1986). As such, the question arises as to whether larvae can be entrained to a day/night cycle by an incandescent light. By examining the relative locations of larvae functioning on different but overlapping light/dark schedules, it is possible to determine whether the larvae may have been entrained by the incandescent light as they should behave differently when examined at the same time. Light quality and intensity have been shown to influence the survival and metamorphosis of other larvae, so it stands to reason that incandescent light could have a regulating effect on the metamorphosis of sand dollar plutei (Gao *et al.* 2016).

The relative locations of the long vs short-day larvae were examined and compared soon after the first light exposure for the short-day larvae and right before the end of that exposure. If entrainment had been successful, the expectation was that the two treatment groups would exhibit differences between their swimming patterns. Larvae were also staged at the end of the experiment to determine whether day length had had an impact on growth.

Materials & Methods

Fertilization and Set Up

Eight sand dollars were collected from curated sand dollar tanks at Friday Harbor Labs. Each individual was injected with 0.75 ml of 10.5°C 0.55 M KCL into the mouth with approximately 0.25 ml of the solution into three of the available openings. The needle was reused across specimens but rinsed with RO water between injections to avoid contamination of sperm.

Sperm was collected by Pasteur pipette, deposited into an Eppendorf tube, and stored in the fridge. A small sample of sperm was placed under the microscope to check for sperm motility. Only very active sperm was considered for fertilization. Eggs were collected by placing

spawning female sand dollars upside down on a completely full beaker of filtered sea water (FSW) and kept at sea water temperatures. Similar to collected sperm, eggs were examined under the microscope to check for quality prior to fertilization (i.e. had no visible germinal vesicles, and had a fully expanded jelly coat, the extent of which can be seen in this species via the scattered red pigment granules in the jelly).

Once two viable males and two viable females had been procured, water was decanted from the egg beakers such that there was only 100 ml of water and eggs left at the bottom. Eggs were then transferred to two additional 150 ml beakers filled with 100 ml of FSW. A clean glass Pasteur pipette transferred a small amount of sperm into a 15 ml centrifuge tube filled with 10 ml of FSW, pipetting the mixture up and down until a cloudy suspension had been achieved. Ten drops of the suspension were added to the beakers of stirred up eggs. After ten minutes, $\geq 80\%$ fertilization was determined by observing the presence of halos under a microscope. After hatching, density was determined by placing 500 μl of larvae in well plates, counting them in replicates of three and averaging those counts. Then, using the known density, larvae were transferred from each beaker into 1500 ml jars and fed *Rhodomonas* and *Dunaliella*, the cell counts of which had been determined with a hemocytometer (Hodin *et al.* 2018).

Experiment

The four jars of larvae were stored in a windowless, temperature-controlled room at 22°C on a shaker table completing 72 rpm. The room, lit by incandescent bulbs 1 m above the larval cultures, provided $16.09 \mu\text{mol m}^{-2} \text{s}^{-1}$ of light (a HydroFarm quantum par meter was used to measure the light). Two jars (LA and LB) were set on a 12:12h light/dark cycle, the lights on between 10:30AM and 10:30PM. The other two jars (DA and DB) were on a 5:19h light/dark cycle, only receiving light between 1:30PM and 6:30PM. A five-hour day length was chosen as

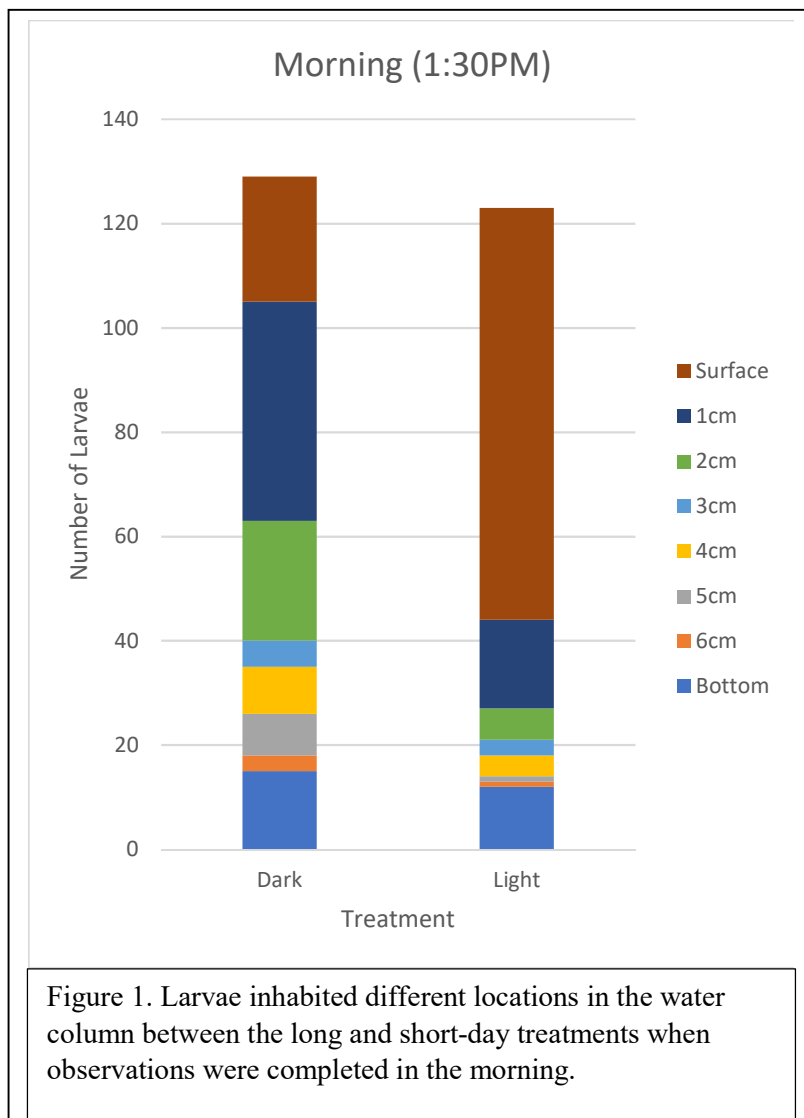
it was the relative day length (not including civil twilight hours) on the shortest day of the year in southern Alaska: the northern limits of *D. excentricus*'s range (Alaska Channel, 2019). At all other times, the short-day jars were under a box covered in thick black plastic and duct tape which prevented any light from reaching the larvae. Every other day beginning three days post fertilization, a water change was carried out on each culture and the larvae were fed. Each jar was reverse filtered with a 40-micron filter, larvae set aside, the jars cleaned, and any stragglers recovered.

Six days post fertilization, larvae were transferred from their large jar cultures to small 250 ml (about 10cm x 5cm x 10cm) tissue culture flasks. A turkey baster was used to mix the cultures and transfer 150 ml of larvae and water (10% of the total culture) to a 300 ml beaker (in order to measure amount of culture transferred). Those 150 ml were gently poured into the flask and the beaker was rinsed between uses. A flask was made for each of the jars and the containers were left on the shaker table until each of the flasks were prepared. In 3 min increments, cultures were removed from the shaker table and left to sit undisturbed for 20 minutes to let the larvae situate themselves in the water column (Pennington & Emlet, 1986). After the rest period, the flask was analyzed to determine the relative location of the larvae within the water column. They were scored as being at the surface, on the bottom, or at -1 to -6 cm below the surface of the water then returned to the shaker table after observation and left in their small cultures until the process was repeated at 1:30PM the next day after which the larvae were placed back in their jars. This process was repeated two days consecutively, one day with a water change, one day without. Analysis was done in excel and R using a two-way ANOVA with replication and a subsequent Tukey test.

Growth Examination

To determine whether there was a difference in the growth of the two larval conditions, 7 larvae from each culture were examined. On the eighth day after fertilization, the measured larvae were randomly gathered from their large cultures with a Pasteur pipette and observed. The larvae were then staged by counting the number of arms and the maximum number of crosshatches on any developing adult spines the larva may possess (Heyland & Hodin, 2014).

To determine whether any differences in growth could be due to feeding, the cultures were stirred well and reverse filtered, 50 ml of fluid collected from each culture and spun down. The



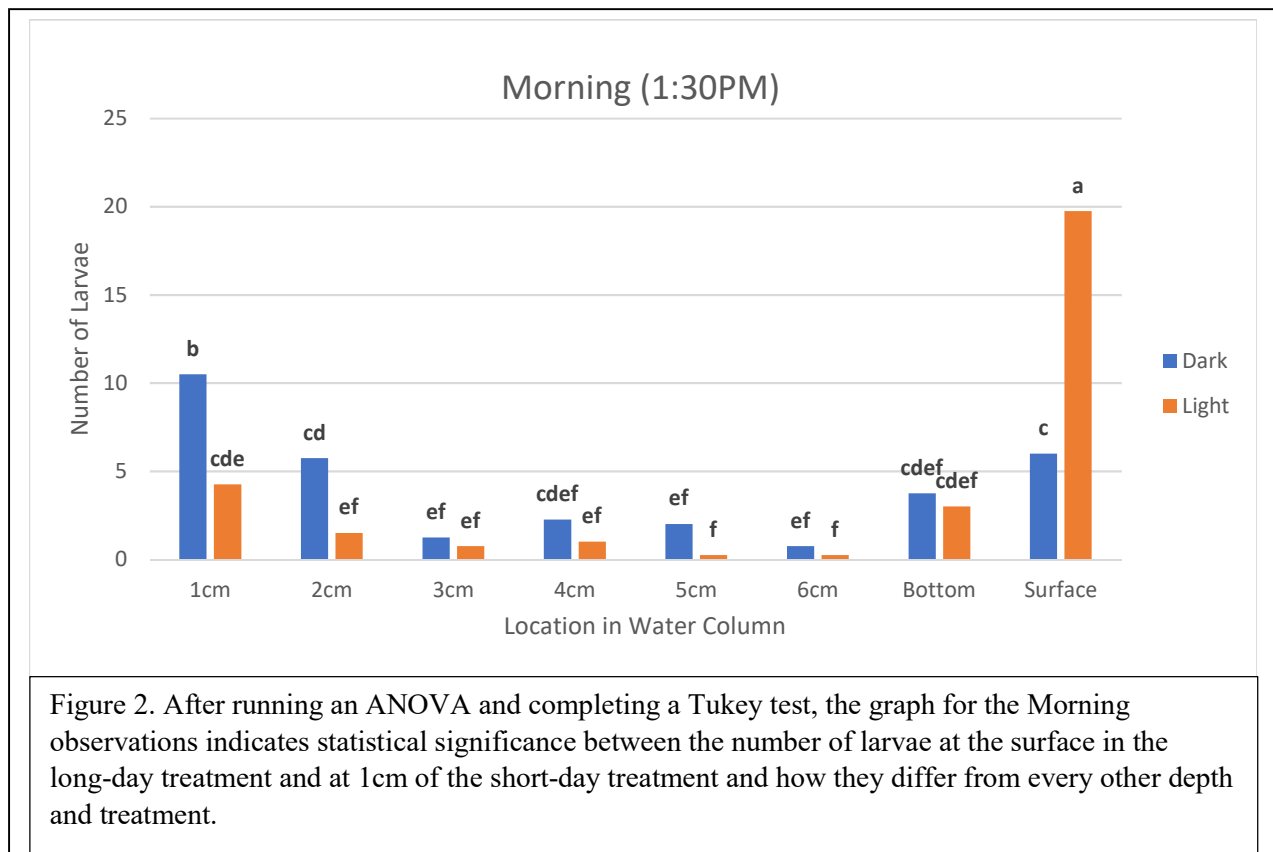
pellet was resuspended in 5 ml of FSW. Three replicates of each sample were examined with the hemocytometer, 32 squares counted in each replicate. These measurements were then averaged and treated with a t-test.

Results

Swimming Behavior. When observations were done in the ‘morning’ (at 1:30PM: the beginning of the short-day treatment’s exposure time), it was found that the interaction between treatment and location in the water

column was significant with a p value of $7.13e-16$. The long day treatment had larvae found most

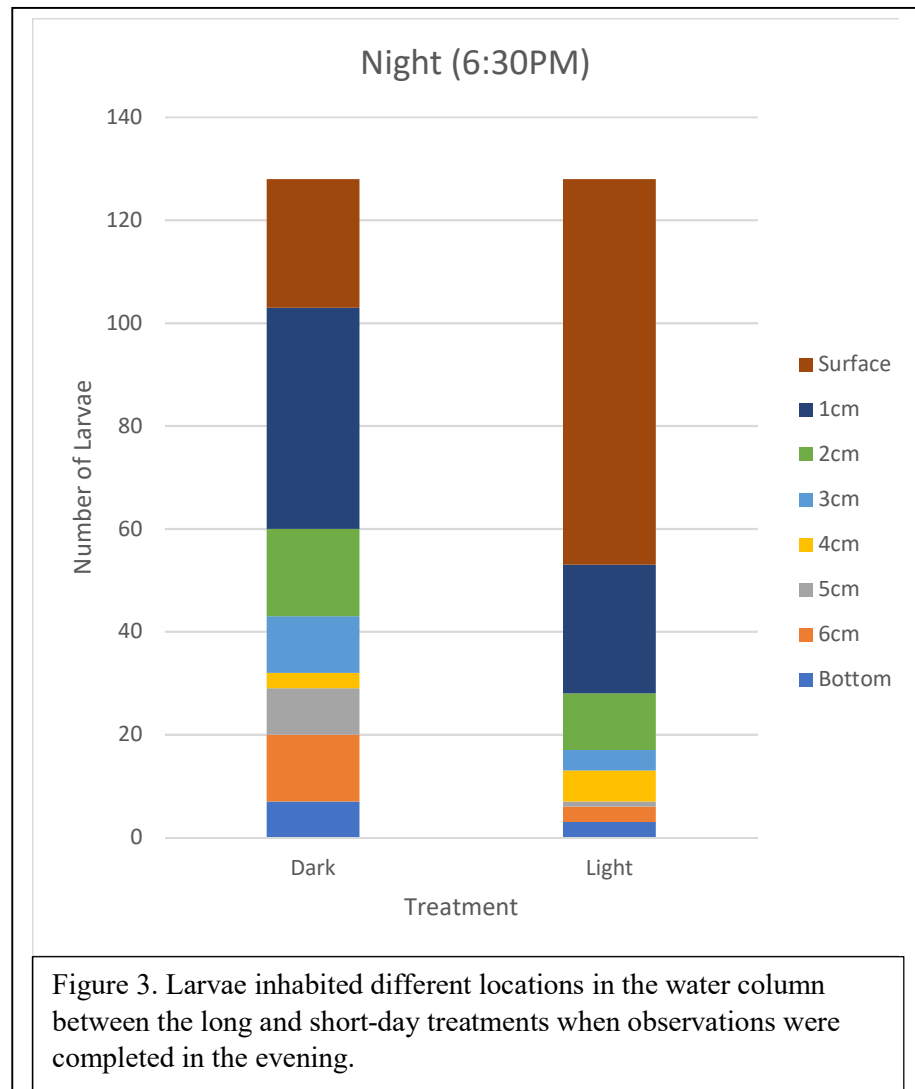
often at the surface of the water which was significantly greater than the average number of larvae found there in the short-day treatment. The short-day treatment had a greater occurrence of larvae at lower points in the water column for the morning observations whereas the long day treatment has next to no larvae between 5 and 6 cm below the water's surface (Figure 1). See figures for all other crosswise comparisons (Figure 1 & 2).



When observations were done in the evening (at 6:30PM), it was found that the interaction between treatment and location in the water column was significant with a p value of 4.19E-12. The long day treatment had larvae found most often at the surface of the water which was once again significantly greater than the average number of larvae found there in the short-day treatment. The short-day treatment had a greater occurrence of larvae at lower points in the water column for the morning observations whereas the long day treatment has next to no larvae

between 5 and 6 cm below the water's surface (Figure 4). See figure for all other crosswise comparisons (Figure 4).

Staging. All larvae examined were at the same 8 arm stage so examining the difference between the treatments came down to the crosshatches of forming spines. When the total number of larvae of either of the two treatments (regardless of replicate) had their number of crosshatches averaged, it was seen that the long-



day treatment larvae averaged 4.28 crosshatches and the short-day larvae averaged 2.57 crosshatches. The results of the food analysis indicated that there was no significant difference between the amount of food each treatment had left after two days.

Additionally, it should be noted that on the final day of the experiment, 9 days post fertilization, 19 larvae were found to have spontaneously settled in jar LA, 22 in LB, 1 in DA, and 0 settled in jar DB.

Discussion

The results suggest that larvae can be entrained with an incandescent light as there appears to be a significant difference between the locations of the larvae of the two treatments when tested at the same time. The difference between vertical migration patterns would be expected so long as the larvae could be entrained by incandescent light as the length of exposure to the light was the only manipulated variable between the two treatments.

Should the experiment be repeated, there are several things that could be controlled for and different tests that should be conducted. When water changes were completed, the larval cultures had to be removed from their controlled environment. So, every other day, the larvae were exposed to ambient sunlight as well as fluctuating temperatures. This could have skewed the results as the larvae weren't solely raised on incandescent light. That the larvae could have a day/night cycle set by incandescent light means that future experiments can be done on the circadian rhythm with exacting controls over light cycles. Also, the difference in growth is an interesting thing to note as it may indicate that *Dendraster excentricus* larvae require a certain amount of light in order to function and grow at an optimal rate. A study on larval teleosts found that a lack of light led to deformed and deceased larvae, and that the same might be possible in echinoderm larvae (Villamizar *et al.* 2011). Perhaps further experiments on settlement in response to day length could provide interesting results as larvae are usually spawned during a long day period and the long-day larvae were the ones with the greatest proportion of spontaneous settlement.

In conclusion, the larvae of *Dendraster excentricus* can be entrained by way of an incandescent light. This can be shown by differences in swimming patterns and vertical distribution. Differences in light also had an unforeseen effect on the growth rate and perhaps the

settlement of the larvae. Therefore, beyond entrainment, light or the lack thereof can lead to differences in the metamorphosis of sand dollar larvae.

Acknowledgements

I would like to thank Jason Hodin for reviewing my paper and for helping me set up my experiment. Thank you, Karly Cohen, for helping me review and for your assistance with statistics. I would also like to thank Mo Turner for her immense help with statistics. Thanks to Haleh Mawson for her editing help.

Alaska Channel. 2019. Alaska Daylight Hours Calculator. Retrieved May 20, 2019, from <http://www.alaska.org/weather/daylight-hours>

Gao, X., M. Zhang, X. Li, C. Song, Y. Liu. 2016. Effects of light quality and intensity on the growth, survival and metamorphosis of *Haliotis discus hannai* Ino larvae. *Aquaculture Research*. 48(7): 3369-3382.

Heyland A. and J. Hodin. 2014. A detailed staging scheme for late larval development in *Strongylocentrotus purpuratus* focused on readily-visible juvenile structures within the rudiment. *BMC Dev. Biol.* 14: 22.

Hodin J., A. Heyland, A. Mercier, B. Pernet, D.L. Cohen, J.-F. Hamel, J.D. Allen, J.S.

McAlister, M. Byrne, P. Cisternas and S.B. George. 2019. Culturing echinoderm larvae through metamorphosis. In: KR Foltz and A Hamdoun (eds), *Methods in Cell Biology*. Echinoderms – Part A. 150. Chapter 6. Pages 125-169. Elsevier: New York.

Longhurst, A. R., 1976. Vertical migration. *The Ecology of the Seas*, edited by D. H. Cushing & J. J. Walsh, W. B. Saunders Co., Philadelphia, pp. 116-137.

Merrill, R., Hobson, E. 1970. Field Observations of *Dendraster excentricus*, a Sand Dollar of Western North America. *American Midland Naturalist*, 83(2): 595–624.

Mooi, R. 1997. Sand Dollars of the Genus *Dendraster* (Echinoidea: Clypeasteroidea): Phylogenetic Systematics, Heterochrony, and Distribution of Extant Species. *Bulletin of Marine Science*, 61/2: 343-375. Accessed May 26, 2019 at <http://www.ingentaconnect.com/content/umrsmas/bullmar/1997/00000061/00000002/art00009?crawler=true>.

Morris, R., D. Abbott, E. Haderlie. 1980. *Intertidal Invertebrates of California*. Stanford California: Stanford University Press.

Pennington, J., R. Emlet. 1986. Ontogenetic and diel vertical migration of a planktonic echinoid larva, *Dendraster excentricus* (Eschscholtz): Occurrence, causes, and probable consequences. *Journal of Experimental Marine Biology and Ecology*. 104(1-3): 69-95.

Villamizar, N., B. Blanco-Vives, H. Migaud, A. Davie, S. Carboni, F. J. Sánchez-Vázquez. 2011. Effects of light during early larval development of some aquacultured teleosts: A review. *Aquaculture*. 315(1–2): 86-94.