Changes in Protein Growth Rate During Larval Development Of *Dendraster excentricus* (Sand Dollar)

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Abstract:

Researching larval development is an important process in understanding how differing environmental stimuli affect their growth and ultimately the recruitment of the species. The experiment aimed to determine the total protein growth of *Dendraster excentricus* and questioned whether protein growth rates remained constant throughout the pluteus larval stage. In the experiment, *D. excentricus* larvae were cultured in a 20-liter vessel at a temperature of 16°C and fed 10,000 cells/ml of *Rhodomonas sp*. (red algae) Samples of larvae were taken every two days. Protein was measured using a BCA protein Assay kit (Pierce), a BSA standard, and a Synergy H1 BioTek Plate Reader. Stages of larval development were quantified according to number of arms grown and noting the dates when development of the rudiment was observable by microscope. It was discovered that protein growth was not constant. The protein growth rate in early development (day 0-3) was 5.51 ng day^{-1} , and in later development (day 20-21) was 150.53 ng day^{-1}. Rates increased with the age of *D. excentricus.* This was evident by the rate of biomass accumulation after day 7 and especially after day 14. Defined increases in protein growth rates were stratified corresponding with pivotal stages of development, such as the growth of arms and the formation of the rudiment. Our findings provide a quantitative understanding of the of the importance of food supply during the larval development stages of *Dendraster excentricus.*

Introduction:

The development of Echinoidea (i.e., sea urchins) are like many invertebrates where fertilization is external during a broadcast spawning event. Development takes place through a planktonic larval stage followed by metamorphose into a mature adult. Previous research has shown sea urchins to be an important indicator species of ecosystem health. Echinoids have also served as a model organisms for understanding fertilization, cell division (cleavage) and other events that characterize early stages of development during embryogenesis. Sea urchin larvae are a major food source for a variety of marine animals. Therefore, the health of sea urchin populations has a direct influence on the health of the marine ecosystems.

Given that early development of sea urchin larvae are typified by large amounts of mortality, it is important to understand how changes in marine environments will affect larval development and metamorphic success to the juvenile stage. An important way to quantify growth is through measuring changes in protein content since protein composes more than half of the larvae biomass and it is required for all metabolic transformations.

The purpose of this experiment is to investigate total protein growth (Biomass) of *Dendraster excentricus* (Sand Dollar). Larvae will be grown at 10,000 cells/ml *Rhodomonas sp.* (red algae) and their rate of total protein biomass accumulated will be measured throughout their larval development. The data will be instrumental in understanding if different larval stages have similar protein growth rates. Such data is important for assessing energy efficiencies of different organisms and if they will have similar responses to changing environmental conditions (ie .Food Concentrations).

Methods:

Spawning

- *Dendraster excentricus* was collected off the coast of San Pedro, CA.
- Spawning was induced by injection of 0.5M KCl.
- Fertilized eggs were cultured at 16˚C for three days.

Feeding and Larval Husbandry

- Larva were reared in a 20L bucket containing filtered sea water held at 16˚C .
- Initial feeding of 10,000 cell/mL *Rhodomonas sp.* (Red algae) took place on the 3rd day post fertilization.
- Water change and feeding took place every two days following the first feeding.
- Larval Cultures were restocked to target algal concentrations using the BD ACCURI C6 PLUS Flow Cytometer to count algal cultures.

Sampling

- During water changes samples of larvae were taken at 500 and 1000 ind/ul and placed into 2ml microcentrifuge tubes.
- The samples were then centrifuged for 10 minutes, sea water was decanted, and protein samples were held in a -80˚C freezer.
- Samples were run trough BCA protein assay after completion of spawn.

Protein Biomass

- Measurement of protein biomass of the larvae samples were made using a (BCA) Protein Assay Kit (Pierce), a Fisher flat bottom 96 well plate, and the Synergy H1 BioTek Plate Reader
- Samples were suspended in Nano pure water in a 2ml dolphin tube in a ratio of 1um/1 larvae. The samples were then centrifuged, sonicated, and homogenized using a vortex shaker before they were added to a 96 well plate (Fisher) with Nano pure water in proportions of Np+sample= 150ul.
- BCA reagents were added to the 96 well plat. BSA (Bovine Serum Albumin) was used as a known standard for referencing protein content from spectrophotometric absorption values given after the plate of protein assays was run.
- The reaction produces a purple-colored product due to the chelation of two molecules of BCA with one cuprous ion. Spectrophotometric absorption values were then measured using the Synergy H1 BioTech Plate reader.

Results:

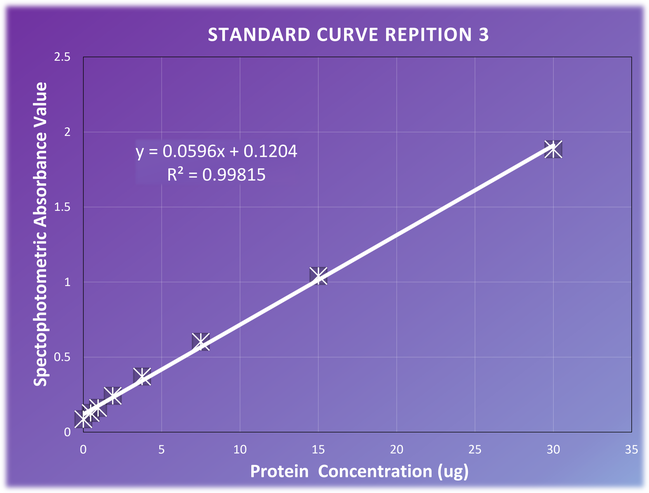

Figure 1. Standard curve of Absorbance vs Protein concentration (microgram) was given by using serial dilution of BSA standard in the first two columns of the 96-well plate.

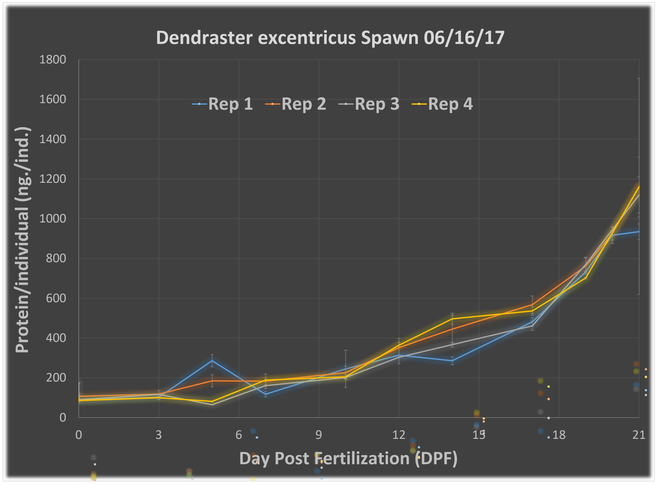

Figure 2. Raw data from rep 1, 2, 3 and 4 of protein content from *Dendraster excentricus* larvae samples.

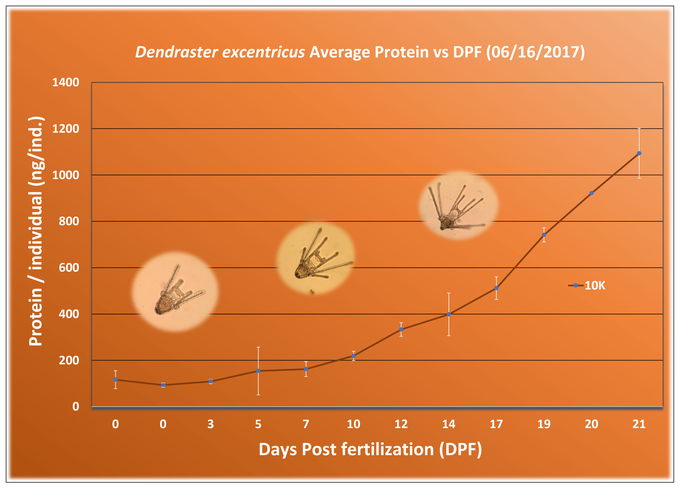

Figure 3. D. excentricus average protein content from rep 1, 2, 3 and 4 from day zero (eggs) through day 21 of post fertilization. The error bars represent the standard deviation. (N=1)

Results:

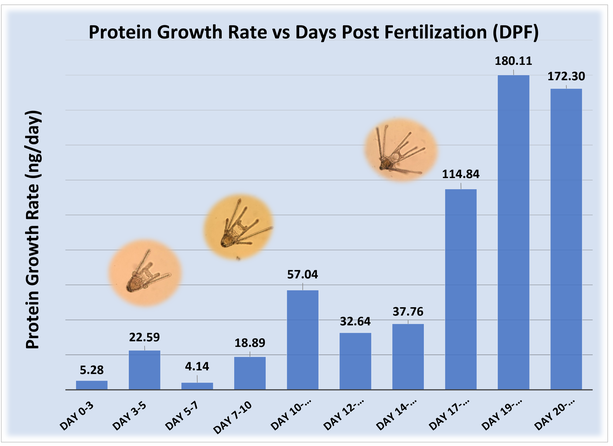

Figure 4. Protein growth rate calculated from average protein content (fig.3) over the intervals of day post fertilization.

- Unfertilized eggs had an average protein biomass of 123.33 ng egg-1 and is smaller compared to the larvae (day 21) with an average of 1071.92 ng individual-1.
- Early in development (days 0-7), protein biomass was slow then growth rate began to markedly increase after day 7 when larvae reached the six-arm stage.
- From day 14 to 21, protein biomass substantially increase and coincided with the formation of the juvenile rudiment and the eight-arm stage.
- Protein biomass and growth rate increased with larvae age.

Discussion:

Research conducted by Hart and Strathmann (1994) discovered phenotypic plasticity of feeding structures from larvae of *D. excentricus* played a significant role in phenotype-environment matching.

- Cultured larva and fed them a different food concentrations.
- Low-fed larvae had longer ciliated arms than high-fed larvae, in order to capture more food particles. It delayed their growth to metamorphosis into adults.
- The high-fed larvae had shorter arms and developed much faster, thereby shortening larval duration.
- These responses are considered adaptive, however current study is necessary for understanding how these responses influence the larva's ability to grow.

Protein growth is not constant, it varies during the pluteus stage of larval development. This raises several questions.

- When are larvae are most susceptible to stunted growth or mortality if deprived of food?
- At what food concentration and developmental stage would the effects of food availability be the most consequential in terms of metamorphic success to the juvenile stage?
- Would physiological adaptations to food shortages maintain recruitment?

Future research:

Focus on the protein growth response different algal concentrations at different times during larval development.

- Understanding of the quantitative impact of food type and availability on larval development and species recruitment.
- Further research, from a biochemical perspective, on population biology of *D. excentricus* as well as developmental strategies employed in different environments.

References:

M. W. Hart and R. R. Strathmann, "Functional Consequences of Phenotypic Plasticity in Echinoid Larvae," *The Biological Bulletin* 186, no. 3 (June 1994): 291-299.

Acknowledgements:

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