Determination of Total Protein Growth of *Dendraster excentricus*

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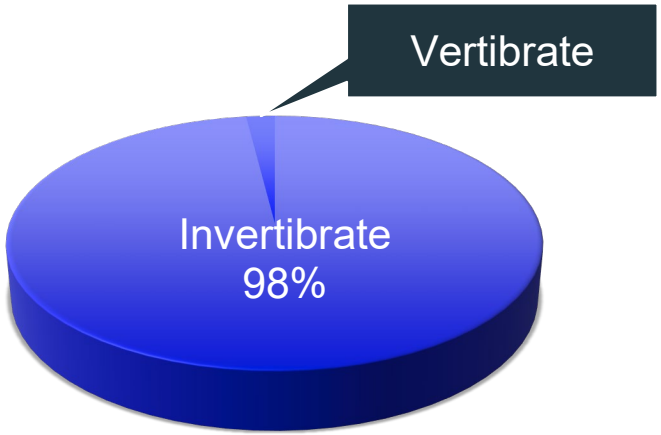
Introduction

- Over 98% of Earth species are invertebrate.
- The development of Echinoidea (i.e., sea urchins) is similar to many invertebrates.
- Development takes place through a planktotrophic larval stage and then metamorphose into a mature adult.



Introduction

- Sea urchins are important model organisms
- Used in research of animal fertilization and the events that characterize early development.

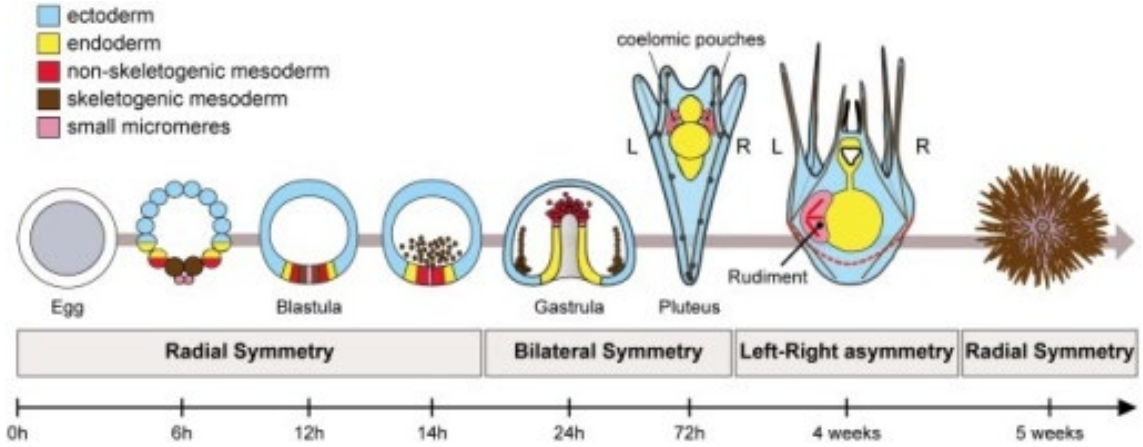


Figure: Bessodes N, Haillot E, Duboc V, Röttinger E, Lahaye F, Lepage T (2012) Reciprocal Signaling between the Ectoderm and a Mesendodermal Left-Right Organizer Directs Left-Right Determination in the Sea Urchin Embryo. PLoS Genet 8(12): e1003121. https://doi.org/10.1371/journal.pgen.1003121

Introduction

- Sea urchins are a commercially valuable source of food for people as well as food source for a variety of marine animals on the food web.

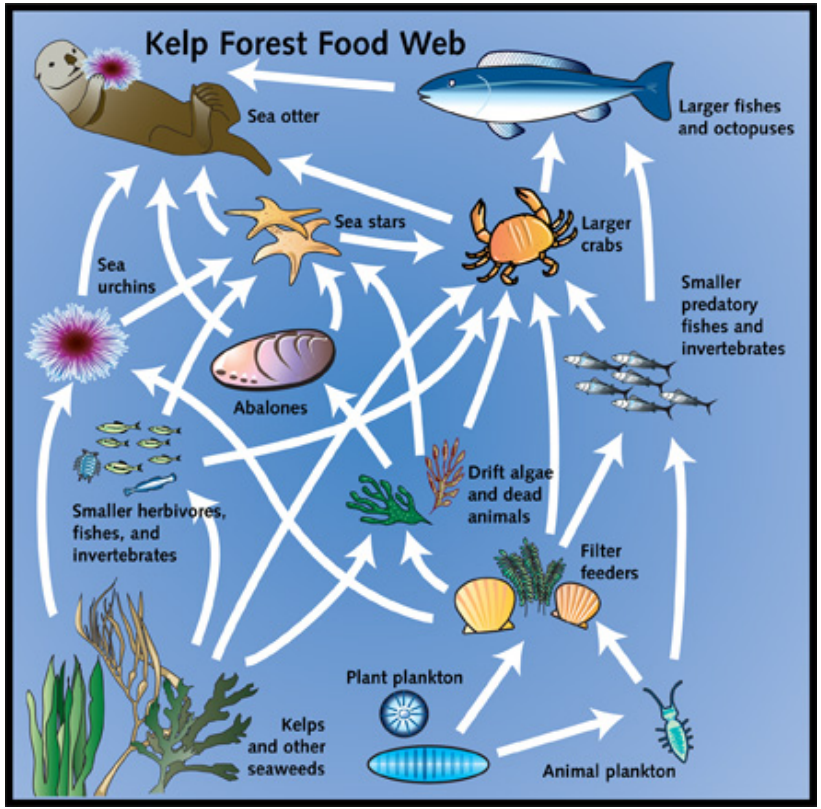


Figure: http://science.jrank.org/kids/article_images/chains_p33.jpg

The Research

Sea urchin larvae are typified by large amounts of mortality.

- We need to understand how changes in environments will affect larval development and metamorphic success to the juvenile stage.
- A broadcast spawning event can be see in the figure to the right.

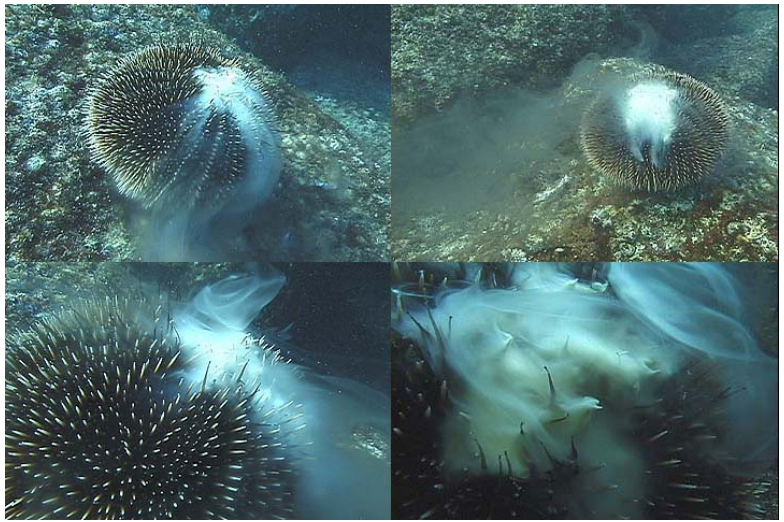


Image: http://ykcomparativeanatomybio2.weebly.com/uploads/5/4/0/4/54043635/3073115_orig.jpg

The Research

Protein composes more than half of the larvae biomass and it is required for all metabolic transformations.

- Measuring changes in protein content can be used to quantify growth.
- The developmental stages of Sea Urchins can be seen in the figure to the right.

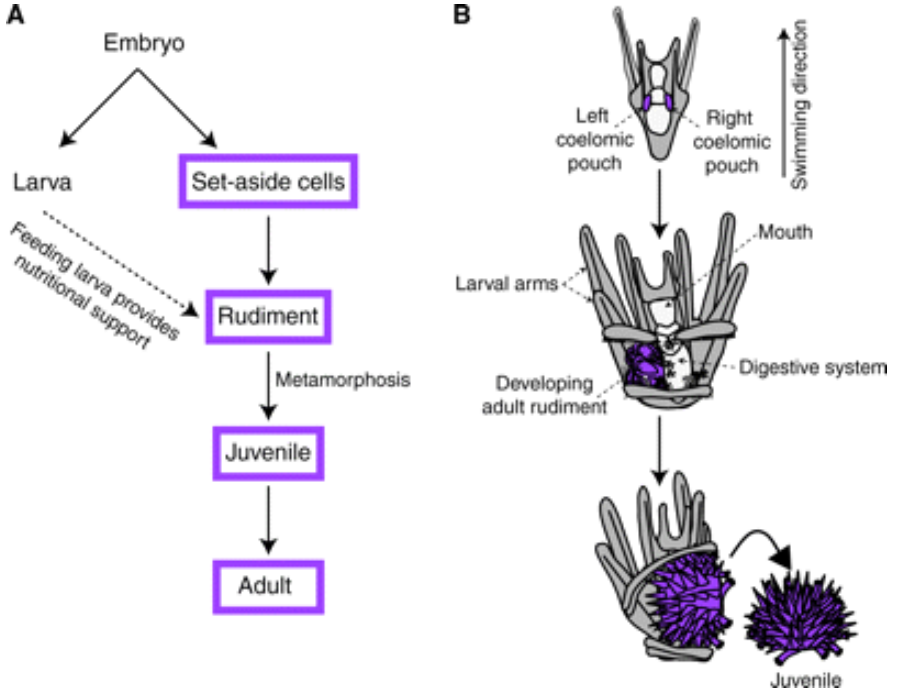


Figure: A conserved germline multipotency program
Celina E. Juliano, S. Zachary Swartz, Gary M. Wessel
Development 2010 137: 4113-4126; doi: 10.1242/dev.047969

The Research

By measuring protein we want to understand….

- How differing environmental conditions might influence larval development.
- If similar larval forms pursue different strategies to maximize nutrient acquisition and fuel their required growth and development?

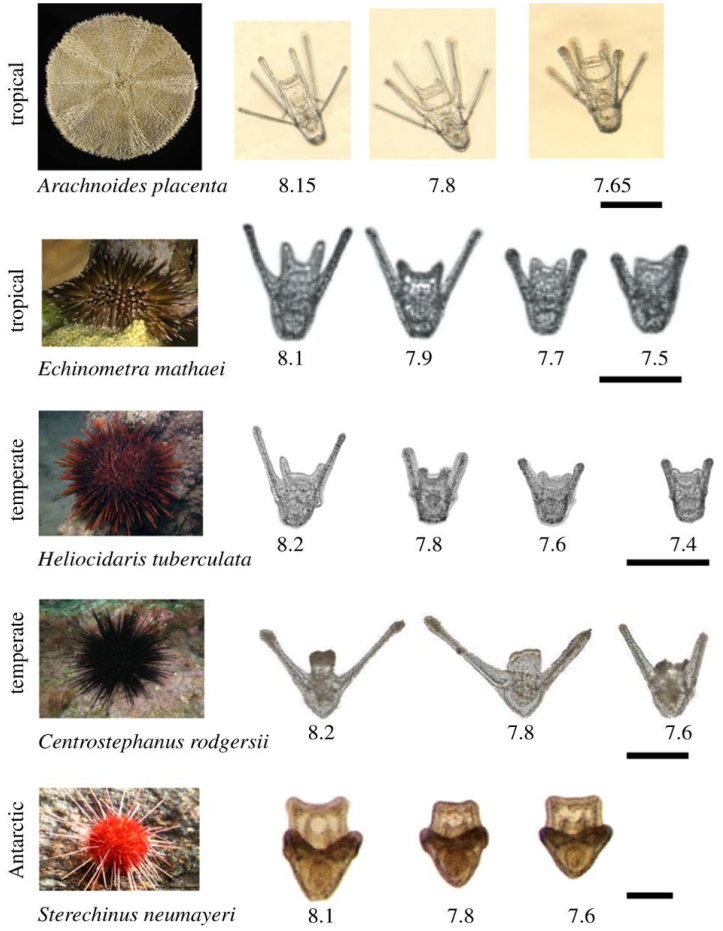
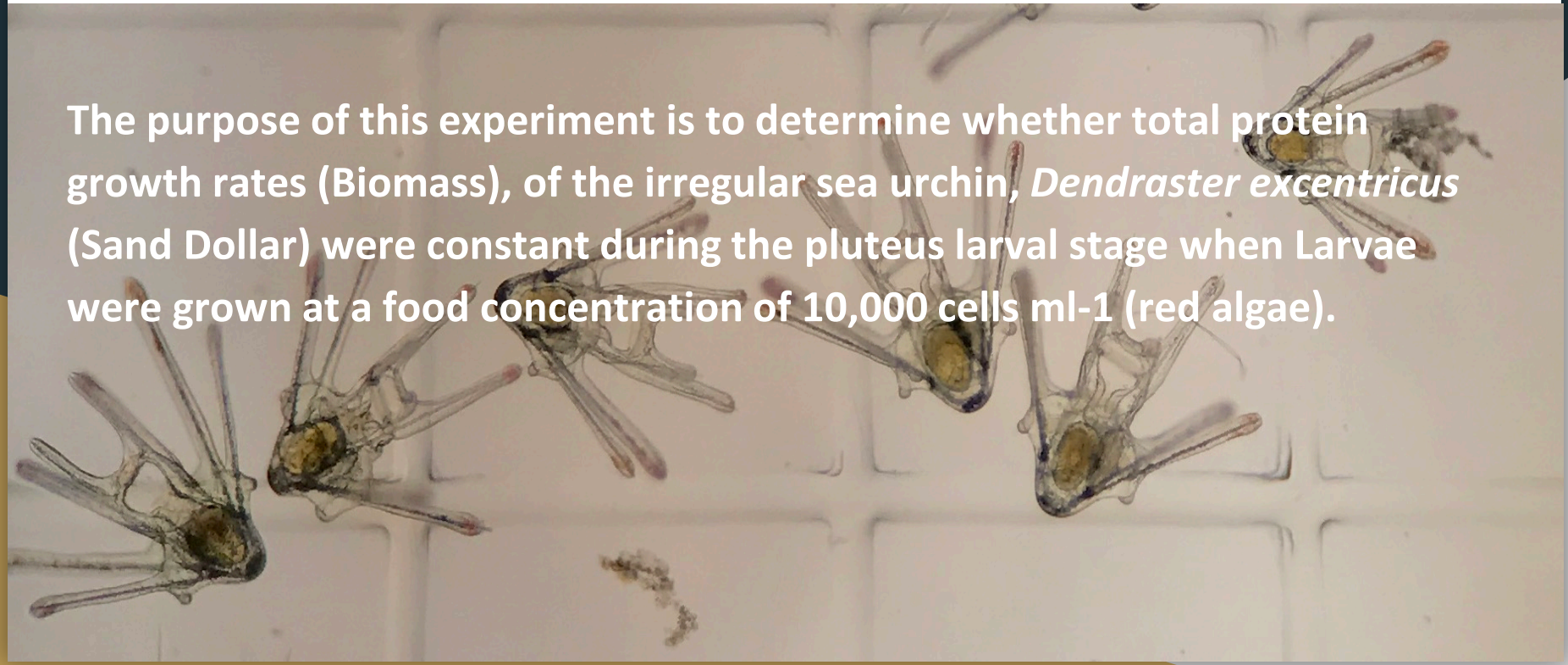


Figure: The stunting effect of a high CO_2 ocean on calcification and development in sea urchin larvae, a synthesis from the tropics to the poles
Maria Byrne, Miles Lamare, David Winter, Symon A. Dworjanyn, Sven Uthicke
Published 26 August 2013.DOI: 10.1098/rstb.2012.0439

The Experiment:

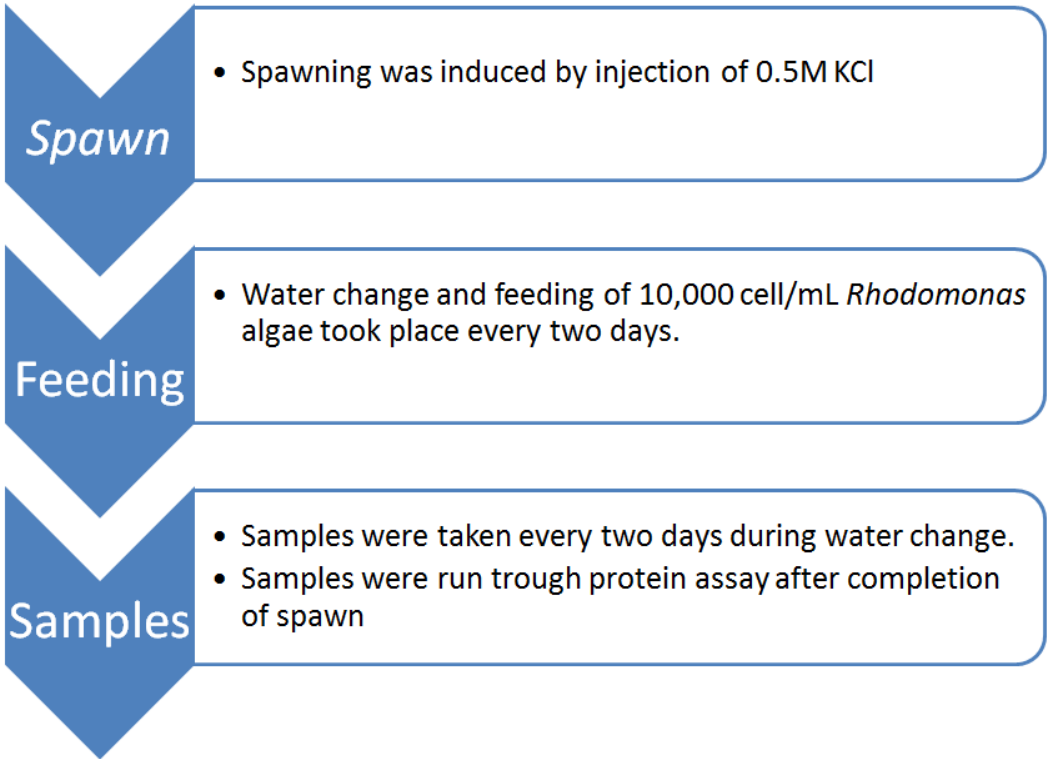


The purpose of this experiment is to determine whether total protein growth rates (Biomass), of the irregular sea urchin, *Dendraster excentricus* (Sand Dollar) were constant during the pluteus larval stage when Larvae were grown at a food concentration of 10,000 cells ml-1 (red algae).

The Experiment

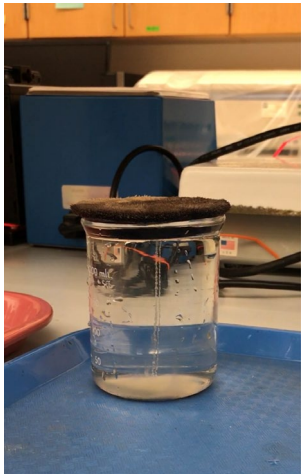
- The data will be instrumental in understanding if different larval forms of echinoids have similar protein growth rates.
- Such data is important for assessing energy efficiencies of different organisms and if they will have similar responses to changing environmental conditions.

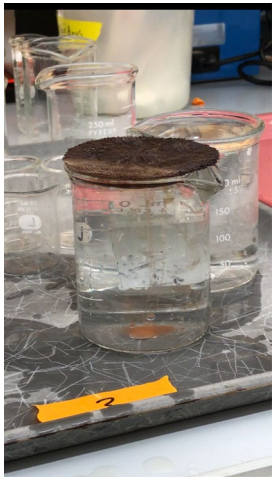
Materials & Methods

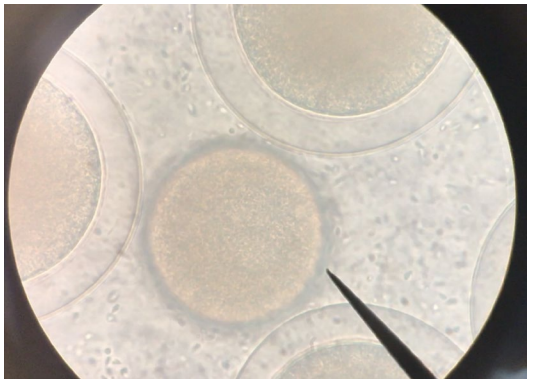


Spawning:

- D. excentricus were collected off the coast of San Pedro, Ca
- Spawning was induced by injection of 0.5M KCl
- Fertilized eggs were incubated at 16˚C For 3 days

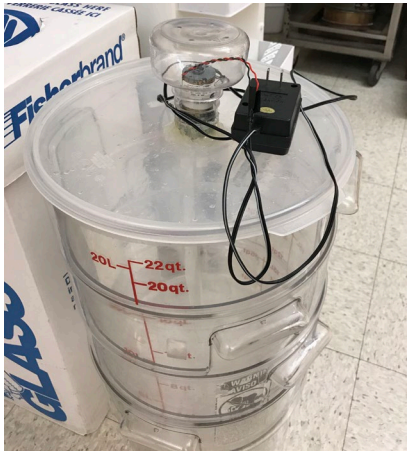






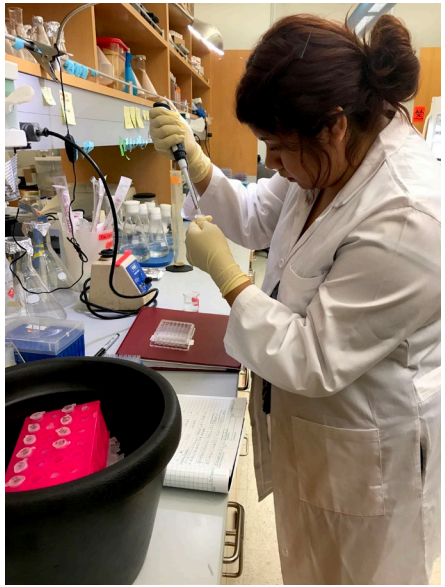
Water change & Feeding:

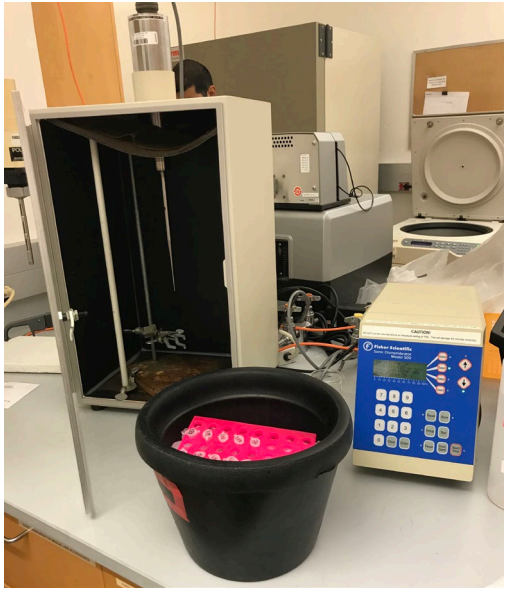
- Larvae were reared in a 20 L bucket @ 16°C
- Water was circulated via a motorized paddle .
- Salt water was changed every other day
- Samples of larva were counted, and algae concentration counted using flow cytometry.
- Larvae were fed a concentration10 cell/ul Rodomonus (Red Algae

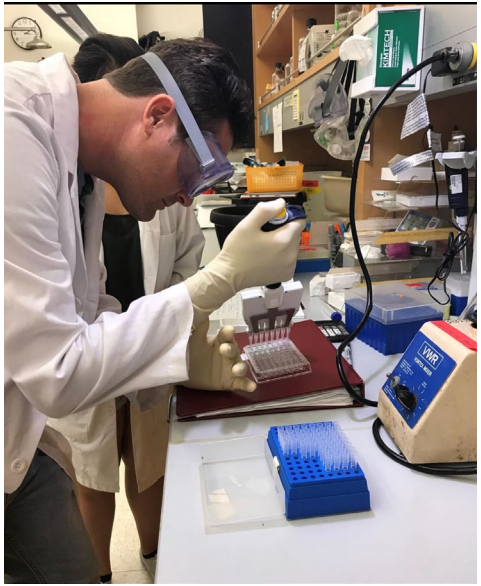


BCA Protein Assay

- Samples were diluted with NPW so that there was 1 ind/ul.
- Samples were then sonicated to lyse the cells and homogenize the sample.

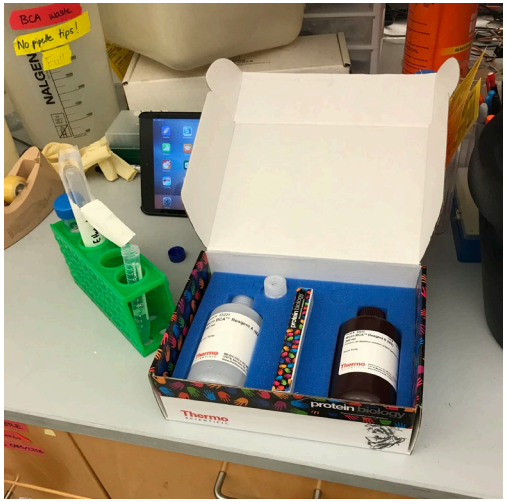






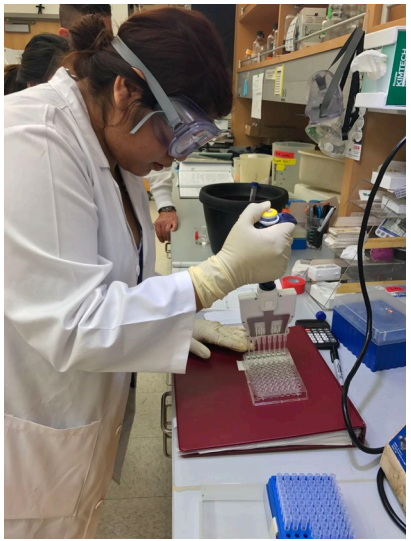
BCA Protein Assay

- Dilute Bovine Serum Albumin (BSA) was used as a standard of known protein concentration.
- BCA was the working reagent. Which binds copper to protein, changing its color!



BCA Protein Assay

- The Standard, Samples, NPW, and BCA were added to a 96well plate
- A Biotek Plate Reader was used to determine spectrophotometric absorption values.



Results

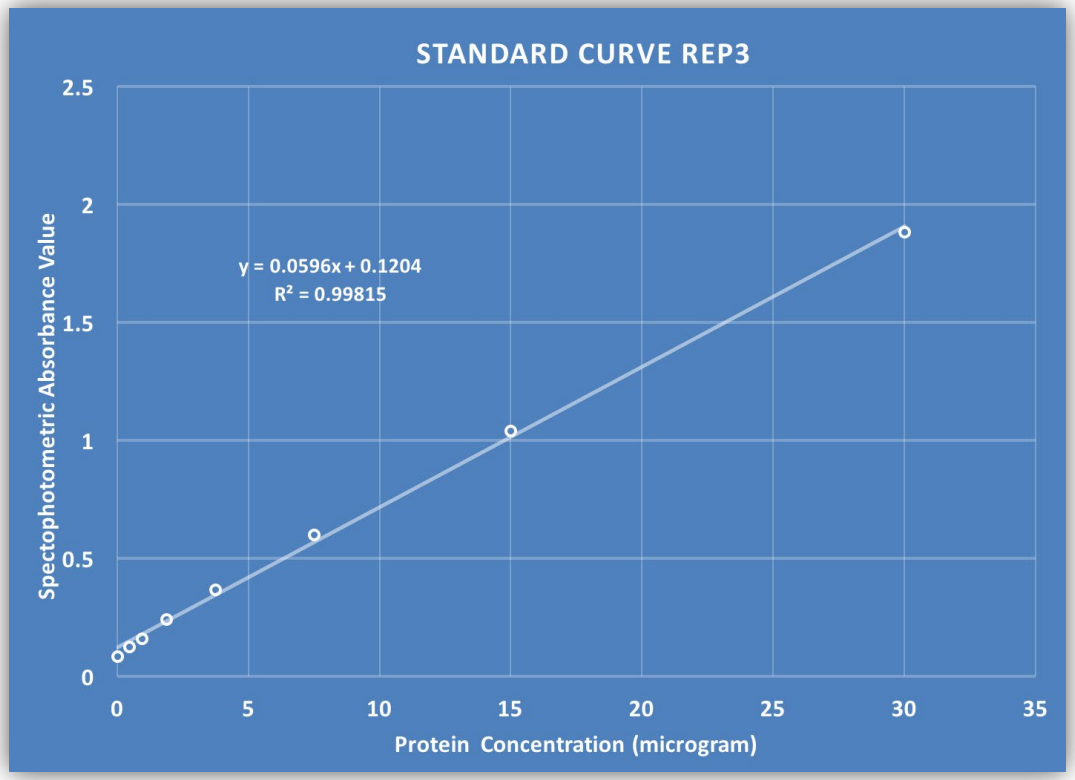


Figure 1:
Standard curve of Absorbance vs Protein (µg) was given by serial dilution of BSA Standard in the first two columns of the 96 well plate.

Results:

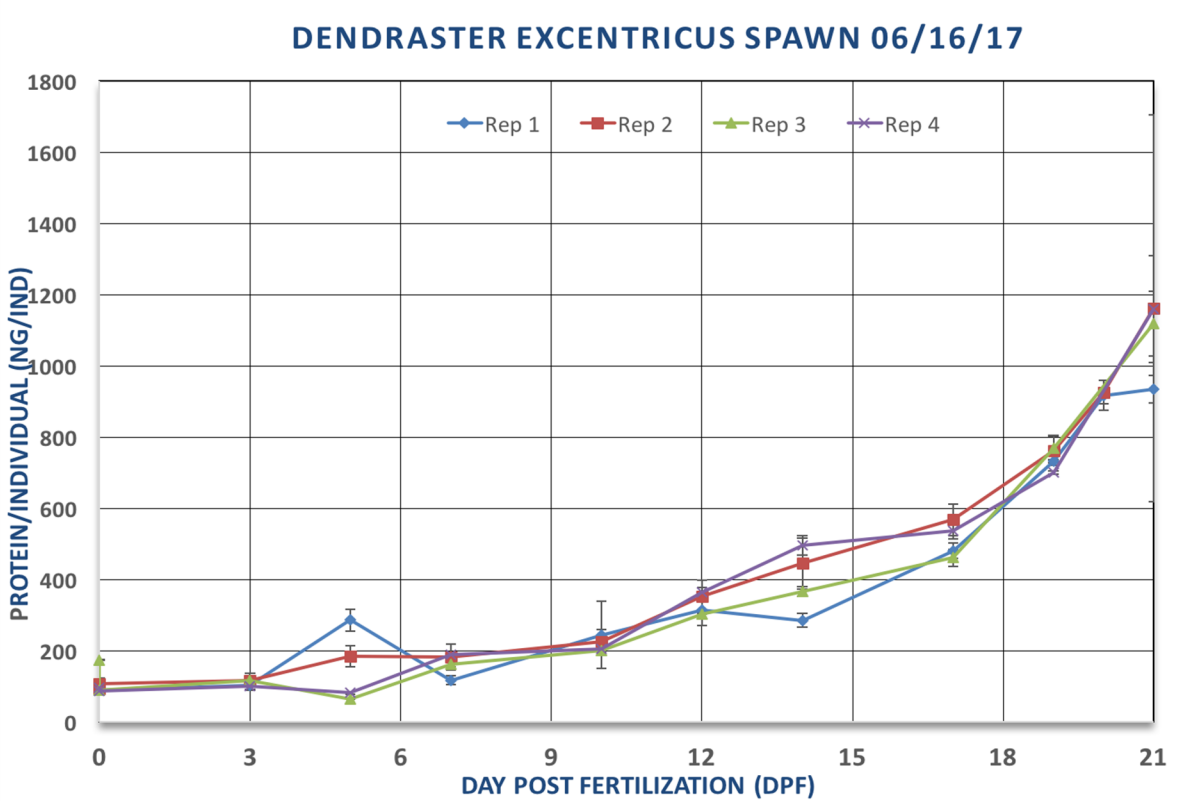


Figure 2:
Raw data of from Rep 1,2, 3, and 4 protein measurements of *D. excentricus* with standard deviation noted.

Results

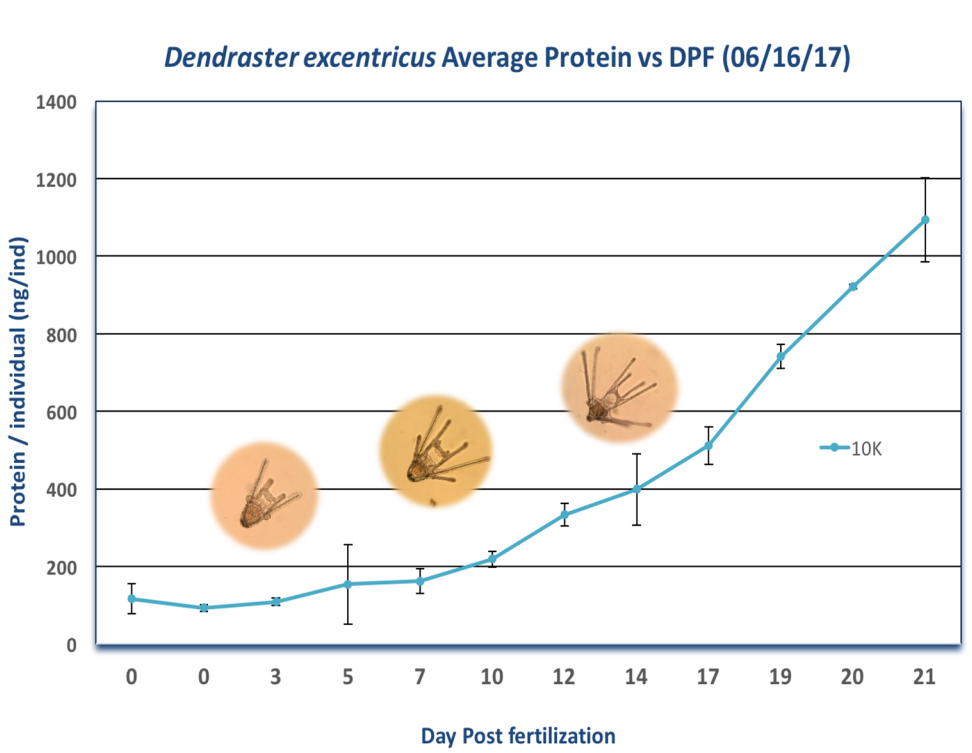


Figure 3:
Average total protein *D. excentricus* from day zero (eggs) through day 21 (pluteus larva) post fertilization with standard deviation noted.

Results

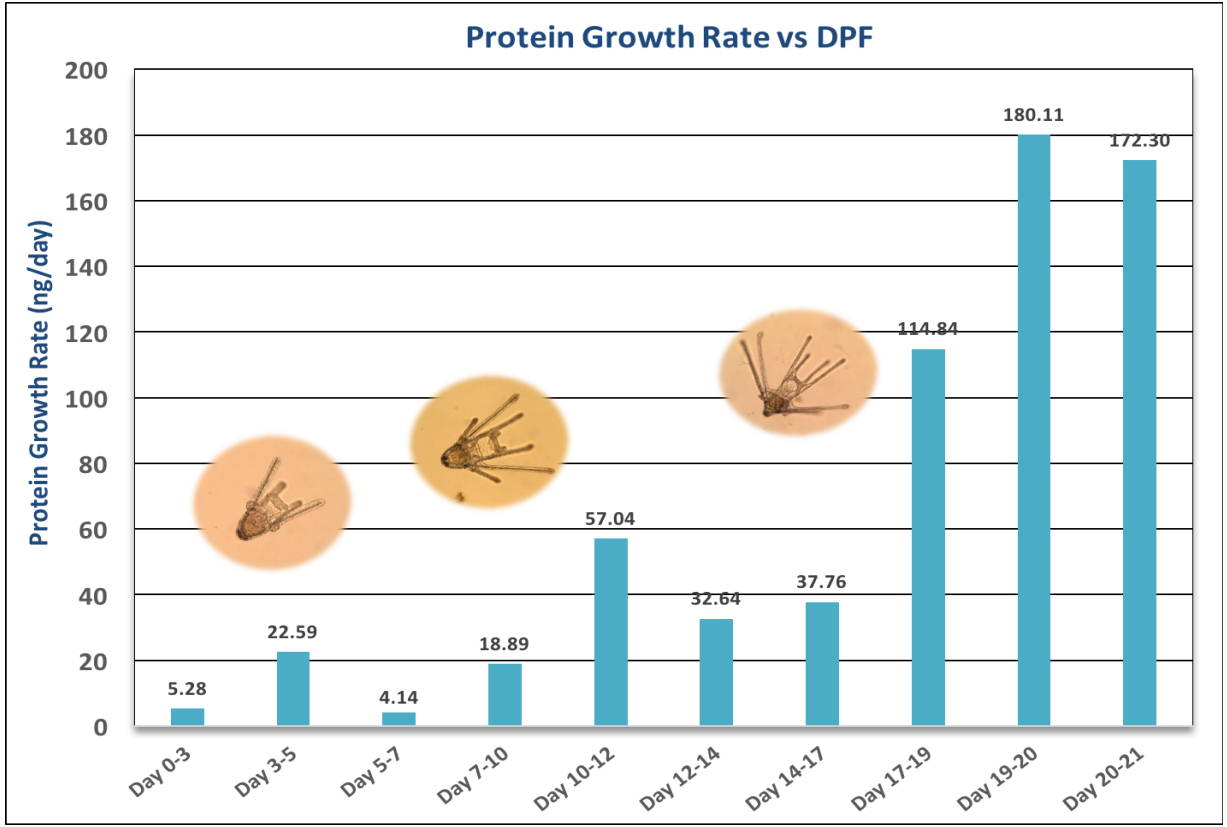


Figure 4:
Protein Growth rates of *D. excentricus* over of intervals of DPF

Discussion

Protein growth rate was not constant which was evident by the rate of biomass accumulation after day 7 and especially after day 14.

- Changes in protein growth rates correlated with the addition of appendages such as arms and the formation of the rudiment.

The finding that protein growth was not constant but varies during the pluteus stage of development raises many questions.

- When during development are the larvae are most susceptible to stunted growth or mortality if deprived of food?
- In early larval stages or in later stages when more protein is produced, and the rudiment is forming?

Discussion

What food concentration would affect development and at what stage? Does Algal type influence development.

Would physiological adaptations to food shortages mitigate mortality rates and maintain successful recruitment of the species?

Michael W. Hart and Richard R. Strathmann (1994) investigated phenotypic plasticity of feeding structures from larvae of *Dendraster excentricus*. The larvae with scarce food invested their energy into longer arms to adapt their environment which delayed metamorphosis into adults.

What are the limits the species can adapt to food shortages?

Discussion

Future research needs to be made measuring total protein biomass of *D. excentricus* involving a sample of greater biological variance.

Further research which includes more than one species should be taken on to compare the developmental strategies of different echinoids (sea urchins) and their physiological limitations to changing environmental stressors (Food supply).

Given the data obtained, it is important to investigate if there are times during development when food limitation would be more harmful to the survival and recruitment of the species.