Macro and Microscopic Changes During Regeneration and Aestivation in Echinoderms

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Introduction

- Echinoderms, which are closely related to chordates within the deuterostome group, are recognized by their distinctive capacities in physiological processes, including regeneration of lost body parts and organs, and aestivation when submitted to stressful conditions.
- Sea cucumbers can regenerate their entire digestive system after evisceration (García-Arrarás & Greenberg, 2001). The regeneration of the intestine takes about a month in the aquarium. During this time, the sea cucumbers do not eat and rely on their body tissues to provide the energy for the regeneration process.
- Sea cucumbers also undergo aestivation, where they lower their energetic needs during stressful periods. Specimens of the sea cucumber *A. japonicus*, experience a loss of overall body mass as a means of conserving energy and resources during this inactive phase. (Zhao et al., 2022). Similarly, other organisms, such as the planarian flatworm (Felix et al., 2019) have also been observed to withstand prolonged starvation by reducing their body size.
- By studying *H. glaberrima* as a model, we aim to unravel how holothurians can endure extended periods without nutrients, while simultaneously engaging in the complex process of regeneration.
- In this project we aim to study the macroscopic and microscopic changes in the sea cucumber during regenerative processes and during prolonged starvation.
- Cellular studies will be done focusing on the coelomocytes, cells found in the coelomic cavity that are also found among the tissues of various body parts (Fu-Shiang Chia et al, 1996). These cells act as the first line of defense for these animals, after an injury or an infection (Li et al., 2018).

Objectives and Hypotheses

Aim:

- To understand the ongoing processes that mediate aestivation and regeneration in the sea cucumber *H. glaberrima.*

Hypotheses:

- There will be a reduction in overall body size in all groups as they are recycling their own cellular components to survive.
- We anticipate this reduction to be most pronounced in the regenerating group due to their heightened energy demands during tissue regrowth.
- There will be a change in the cellular populations of the coelomic fluid as part of the adaptation process.

Methods

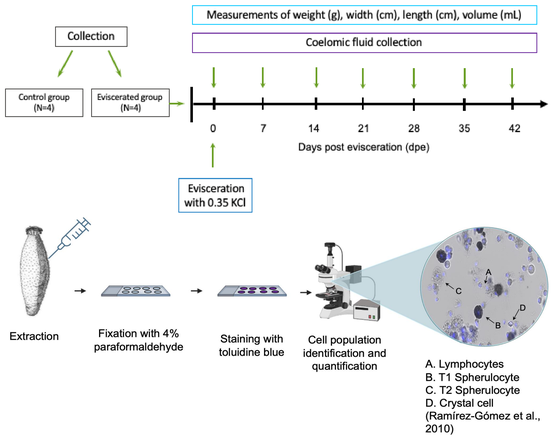

Fig. 1 This diagram illustrates the experimental design, indicating what was done each day. The same methods were applied to the control group, exempting evisceration.

Fig. 2 This figure demonstrates the technique used for coelomic fluid collection.

A. Lymphocytes
B. T1 Spherulocyte
C. T2 Spherulocyte
D. Crystal cell
(Ramírez-Gómez et al., 2010)

Results

Macroscopic

Non – eviscerated group

Eviscerated group

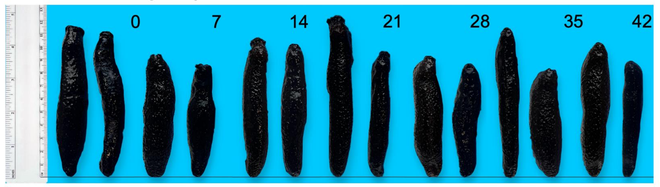

Fig. 3 –Comparison in body size of the non – eviscerated group with the eviscerated group. Body size shrinkage is more evident in the eviscerated group.

Length of *H. glaberrima*

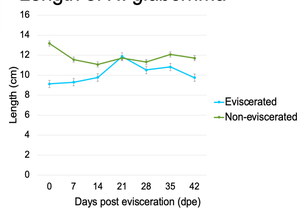

Fig. 4 – Average length of eviscerated and non- eviscerated animals throughout the 42-day experimentation period.

Downward trend in weight of *H. glaberrima*

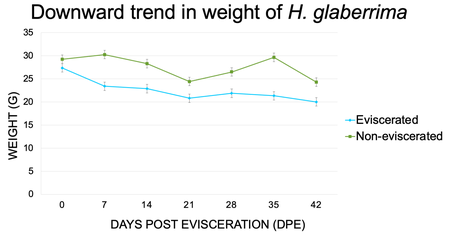

Fig. 5 – Average weight of eviscerated and non- eviscerated animals throughout the 42-day experimentation period. A downward trend is observed in both groups.

Downward trend in length by width

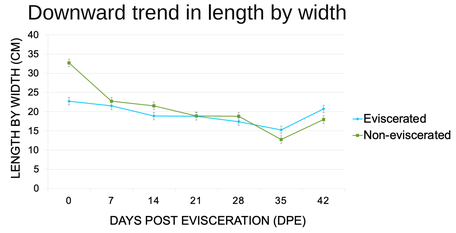

Fig. 6 – Average length by width of eviscerated and non- eviscerated animals throughout the 42-day experimentation period.

Microscopic

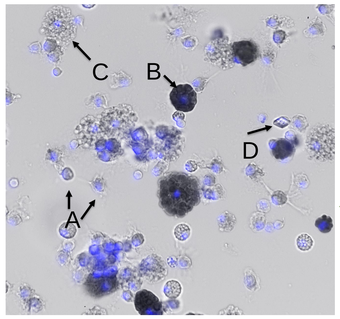

Fig. 7 – Cell population in coelomic fluid. Different types of coelomocyte populations of *H. glaberrima* have been characterized according to their morphological and physiological properties. In our laboratory, we identified:
A) Lymphocytes
B) Type 1 Spherulocytes
C) Type 2 Spherulocytes
D) Crystal cells
These cells may play important roles in processes like regeneration and dealing with challenging conditions like aestivation. (Ramírez-Gómez et al., 2010)

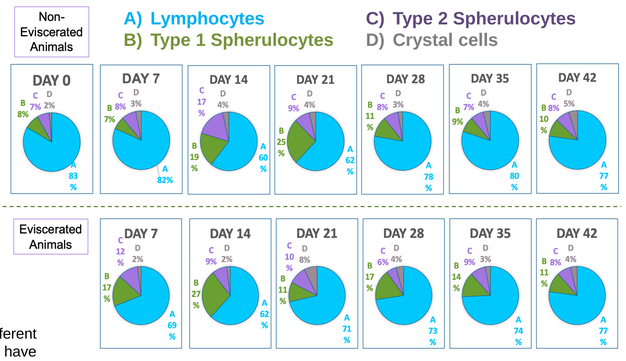

Fig. 8 – Comparison of the percentage of each coelomocyte population in non-eviscerated animals and eviscerated animals. A significant difference can be observed in cells A and B in eviscerated animals, compared to non eviscerated animals, especially in the first stages of regeneration ($p > 0.01$).

Starvation effects in sea urchin, *P. lividus*

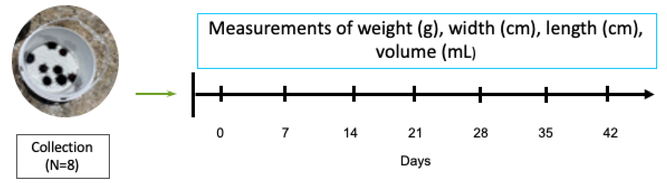

Fig. 9 – This is the method we used to observe physical changes in P. lividus for 42 days. None of the animals were fed.

Fig. 10 – This figure represents the body size of the largest and smallest sea urchins in the experimental group in days 0, 7, 21, 28, and 42. No significant change in size is observed.

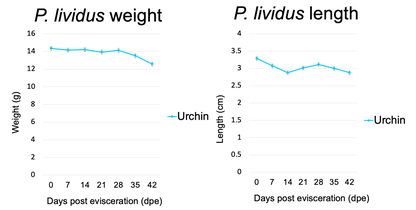

Fig. 11 – These graphs show the average weight and length of *P. lividus* during the 42-day experimentation period. No significant changes are observed. The sudden drop in weight could be due to loss of spines.

Conclusions

- Body size in all animal groups showed a slight downward trend, indicating possible adjustments in response to the experimental conditions.
- Eviscerated sea cucumbers demonstrated an ability to maintain their body size similar to their non-eviscerated counterparts.
- The fact that both eviscerated and non-eviscerated groups showed only slight downward trends in body size without significant differences suggests that sea cucumbers have mechanisms to mitigate the potential impact of organ loss on their overall growth and energy allocation.
- Differences in cell populations between the eviscerated and non-eviscerated groups were found. In eviscerated animals, during the first stages of regeneration there is an increase in type 1 and type 2 spherulocytes at an expense of a decrease in lymphocytes.
- After 42 days of starvation, eviscerated and non-eviscerated animals show similar percentages of coelomocytes as day 0 animals.

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