<u>Exploring the Life Cycle of Crepidula Atrasolea</u>

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Abstract

The project focuses on researching a species of slipper snail, *Crepidula Atrasolea.* The *Atrasolea* species serves as a perfect model for furthering research in embryo development. At different stages of development, embryos were harvested and observed under both an epi fluorescent microscope and a confocal microscope. By incubating the embryos at optimal controlled temperatures, then observing them after specifically designated time checkpoints, it was possible to create a timeline of *Atrasolea* embryo development. With fluorescent markers, the embryos were precisely staged, defining sub cellular structures. From this data a schematic timeline as well as a timeline was compiled. Using imaged fluorescent photos detailing the number of cells at each stage as well as its visual appearance, the project contributes to furthering the study of not only slipper snail development but other marine species as well.

Background

From the beginning of time, cells have been recognized as the crucial building blocks of all living matter. As science has developed alongside technology, scientists have begun the exploration of the role that cells contribute to the development of the human body. The human body consists of around 30 trillion cells with over 60 types of tissues (Cooke, LiveScience), a massive and unimaginable number. These cells, forming anything from the brain, bones to the heart, are the most integral part of all living species as we know, all arising from one single fertilized embryo. The intricate process has led to how one-cell develops into trillions, serving unique purposes and functions. Many modern scientists have turned to looking at other species parallel to our own to expand the human knowledge of embryogenesis. One such lab is the Lyon lab that focuses on researching the embryonic development of the black-footed slipper snail, *Crepidula atrasolea,* a species that could potentially open doors in understanding how human embryos develop.

This species serves as a perfect model for biological studies for a number of reasons. *Crepidula atrasolea* is a calyptraeidae gastropod (a family of small cup-sized snails), and of the same taxonomic genus as the Atlantic slipper snail *Crepidula fornicata* (Lyon et. al). Similar to *C. fornicata, C. atrasolea* shares the same lifestyle involving filter-feeding in a scientific environment . However, *C. atrasolea* is a more effective model for embryo development due to its short generation time, year-round reproduction and direct development. These individual snails will typically go from egg to reproductive females in under six months, a considerably short amount of time compared to the 1-2 years for *C. fornicata* (Lyon et. al). As a result, scientists are able to study the life cycle of the embryos in an effective and time-efficient manner. Females will brood encapsulated embryos for an average of three weeks, then the embryos will hatch as juveniles, which can then be raised to continue the breeding cycle. Throughout these three weeks, the embryos go from early cleavage to late organogenesis stages, which is a useful tool for future studies of gene expression and function during developmental stages, as embryos can be collected at different time checkpoints in order to be studied.

Maintaining the Animal Colony

After joining the Lyon lab, I not only focused on my individual project but also learned how to properly care for the complex species of *C. atrasolea.* This started with animal care, which included both feeding and cleaning each sample of adult and breeding snails. This was to be done each day to ensure the most optimal conditions for breeding. In order to clean and feed the snails each day, I used a paintbrush to carefully remove any algae and fecal matter that might have grown around the snails. I would then pour out the old water and replace it with a mix of Phyto-Feast, consisting of a blend of microalgae, and filtered seawater, as a feeding supplement for the snails. Throughout my time at the lab, I helped experiment with different foods to feed the slipper snails, fluctuating from a combination of traditional Phyto-Feast to egg whites in hopes of raising the slipper snails with clearer embryos. Ultimately, referring to the survival rates of the two, the traditional feeding method was more effective in keeping the snails healthy enough to survive and breed. In addition, as needed, I would collect the embryos from the female snails by means of a pair of forceps. While collecting the embryos, it was important to store the collected embryos in phosphate-buffered saline or PBS water to simulate brooding conditions in females. This way, it was possible for the embryos to still continue to develop even after being removed from the female snail.

Research Project

My research project while at the Lyon lab surrounded creating a concrete and referenceable timeline of the developmental stages of the *C. atrasolea* embryos. As a lab that focuses on collecting snails at varying timelines during embryonic development, it is crucial to understand visible landmarks between each stage as well as the time elapsed. After moving through animal care, I discovered that there was no established timeline to fulfill these needs that are important whilst studying the species. Referencing timelines made for other marine species, I put forth the task of creating a timeline of my own for *C. atrasolea.* Through 17 separate trials, I collected an estimated 450 embryos from the mother snail all at different stages. I then incubated them at 4 degrees celsius, to mimic temperatures of the species in the wild in the ocean. After checking back on the collected embryos at specific time indicators, I was able to document the split or cleavages of the embryo either through observations under a light microscope or by staining the nuclei and organs of the embryos. While more distinct stages, such as the difference between 2-cell and 4-cell embryos were observable under a light microscope, other stages required the imaging of the embryos. To image embryos, it was necessary to first fix the embryos with a chemical, called paraformaldehyde 4% in PBS, then dehydrate them with methanol. After rehydration, I was then able to stain them with primary and secondary antibodies that would make these cells fluoresce. I used phalloidin to stain the cytoskeleton of the embryos in order to distinguish one cell from the other, making it easier to count the amount of cells in each embryo. I also used Hoechst 3232 to stain the nucleus of the cell in order to count how many cells were present at each time point. Finally I also used Poly-U to stain the messenger RNAs in order to create clearer images of the targeted cells. I then merged these images together to create a corresponding image in order to correctly stage the embryos (Figure 1). After collecting enough information to develop a solidified timeline between each cleavage ranging from the beginning of 2-cell until late epiboly (2-cell, 4-cell, 8-cell, 16-cell, 20-cell, 24-cell, 25-cell, crown, gooseberry, round, epiboly), I compiled this information to create a timeline, accompanied by diagrams or pictures of each stage (Figure 2). This diagram is extremely useful to help future researchers or undergraduates in the lab to stage the embryos, which is a necessary step when collecting them, as well as knowing how long one must wait until the collected embryo is at the desired cleavage stage.

Reflection and Conclusion

From my time in the Lyon lab, I learned not only about the species and scientific processes itself but applicable life skills that included project management and responsibility.

Whether it was collecting and caring for the snails themselves, it was important to contribute my own efforts for the betterment of the lab. I also gained valuable experience within the lab, learning about the processes of fixing embryos and staining them to reveal the different organelles within a cell. As I continue my time in the Lyon lab, I will take the time to reaffirm my confirmed data with more trials. Due to the short increments of 2 to 4 hours between checking the cleavages, the time taken out of the incubator to check the stage may have potentially slowed down the development of the cleavages. By using longer increments in the future, I could adjust the timeline to create it more accurate to the species itself. Overall, the experience shaped my skills not only as a researcher but as a person, learning how to take charge of my own projects when given the scientific freedom.

IMAGE 1: The image of a round embryo fixed 20 hours after entering the 4-cell stage.

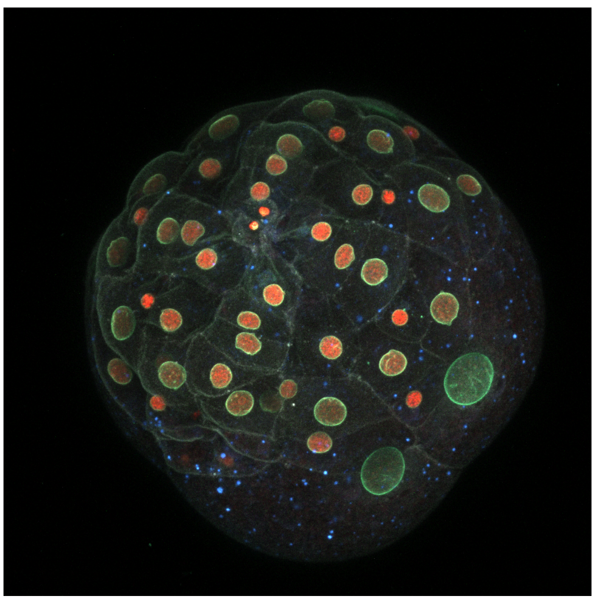


IMAGE 2: The compiled timeline and diagram

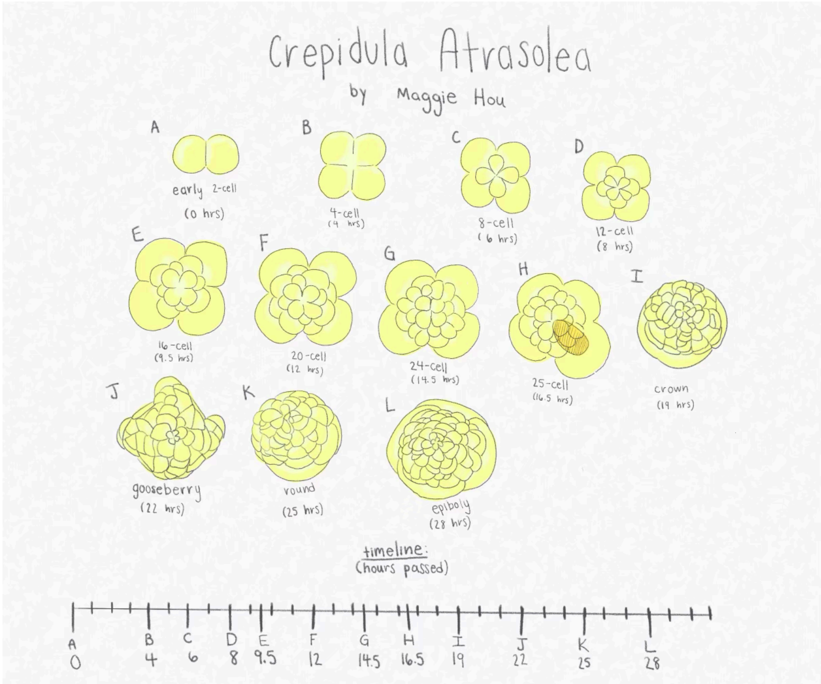
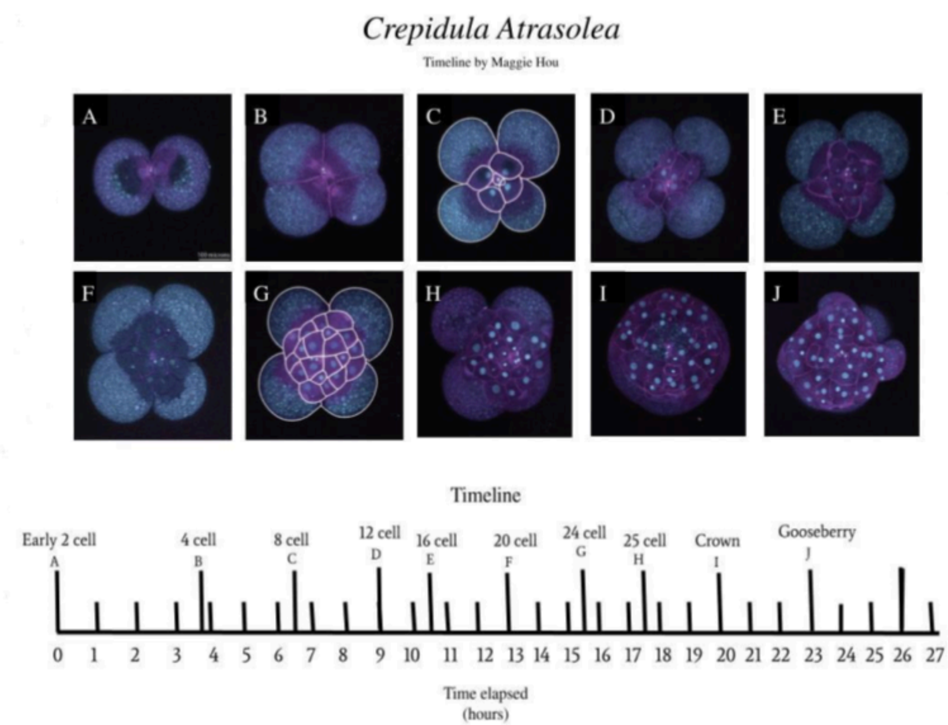


IMAGE 3: A complete timeline using imaged embryos of the *Atrasolea.* (Courtesy of Stephanie Neal)



Works Cited

Jonathan Q Henry, Maryna P Lesoway, Kimberly J Perry, C Cornelia Osborne, Marty Shankland, Deirdre C Lyons. *Beyond the sea: Crepidula atrasolea as a spiralian model system.* The International Journal of Developmental Biology. https://pubmed.ncbi.nlm.nih.gov/29139534/.