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Nudibranch Embryological Development

During the ten-week course in Comparative Embryology and Larval Biology we cultured multiple invertebrate species and raised them to their larval stages. Of all the larval forms we raised during the term my favorite are the veliger protoconch larvae of the nudibranchs, which are in the class gastropoda, Phyla Mollusca. Nudibranch development does not seem to be widely studied. There is much literature on factors that affect the timing of metamorphosis and nutritional affects on growth rates in the laboratory environment but the variety of ways that Nudibranch young develop is not nearly such an easily investigated subject. Many of the articles I found are decades old, however I believe that the information is still accurate. I will be focusing mainly on the species *Hermissenda crassicornis* while using data from other aolid type species.

Hermissenda crassicornis is a eurytrophic carnivorous opisthobranch that lays its eggs in a pink or white gelatinous matrix, counter-clockwise in a tight spiral. The diameter of the egg mass has been found to increase with increased body size as do the number of eggs in a mass and the number of eggs per capsule. Diameters of the egg masses were found to be from 0.24 cm to 3.62 cm. Estimated numbers of eggs in the mass were 6.9×10^3 to 1.0×10^6 and the number per capsule ranged from one to nine. Individual eggs are approximately 160 micrometers. Oxygen levels within the egg mass vary as embryos develop and their consumption goes up. A dramatic gradient is mostly avoided by the small size of the mass or as in some species which lay a larger mass, the ribbon like form which exposes the most surface area as possible.

After fertilization spiral cleavage produces a sterioblastula. The vegetal side then begins to flatten. Gastrulation results in a cup shaped gastrula with the ventral blastopore becoming

asymmetrical before it closes. Polar bodies are typically visible stuck to the animal pole all through gastrulation. A shell cap forms on the posterior end of the embryo and increases in size as the shell gland spreads interiorly. Two anal cells appear to the right and in front of the mantle fold on the ventro-lateral surface, while at the same time the velar lobes and foot are growing. The cilia used for locomotion elongate and cause the embryo to rock inside the egg capsule. The shell forms faster than the mantle fold migrates and so a perivisceral cavity forms at the posterior end of the shell. The visceral mass is compact and opaque. The retractor muscle is visible but does not seem to function during formation of the shell (makes sense since it is used to retract the velum). A miscellaneous group of cells surrounds the muscles origin, just dorsal and to the left of the apex of the shell. The anal cells disappear before completion of the shell. Apparently torsion does not involve a 180° twist of the cephalo-pedal elements and the movement of the anal cells may be the only real ontogenetic evidence of torsion.

The foot elongates as it grows ventrally and becomes ciliated very heavily only mid-ventrally and at the tip but not laterally. An operculum forms on the dorsal surface and several long stiff compound flagella protrude. A ciliated subvelar ridge begins to develop and the visceral organs start to fill the perivisceral cavity and eventually leave only the dorsal mantle cavity empty. The yolk content is still high and so individual organs are difficult to see (let alone photograph).

When the larval shell has been completely secreted the mantle fold along the shell aperture becomes thinner and begins to withdraw along the inner surface of the shell. At first the vellum can't be accommodated by the mantle cavity and so velar lobes can not be fully withdrawn until te mantle has regressed three quarters of the way to the shells apex.

Two red eye spots appear and then the propodium begins formation just on the ventral side of the mouth. By this time the mantle is two thirds of the way back from the apex and the sub velar ridge is well developed and completely ciliated. Once the mantle has reached the apex it fuses with the epithelial layer that covers the visceral mass. The veliger is then probably only attached at the retractor muscles origin. Once the propodium is fully developed the action fo the foot is visible. They move around very little inside the shell despite the constant beating of their velar cilia. The organs are still packed with yolk but the foot and velum are less opaque. The larva is then ready to hatch.

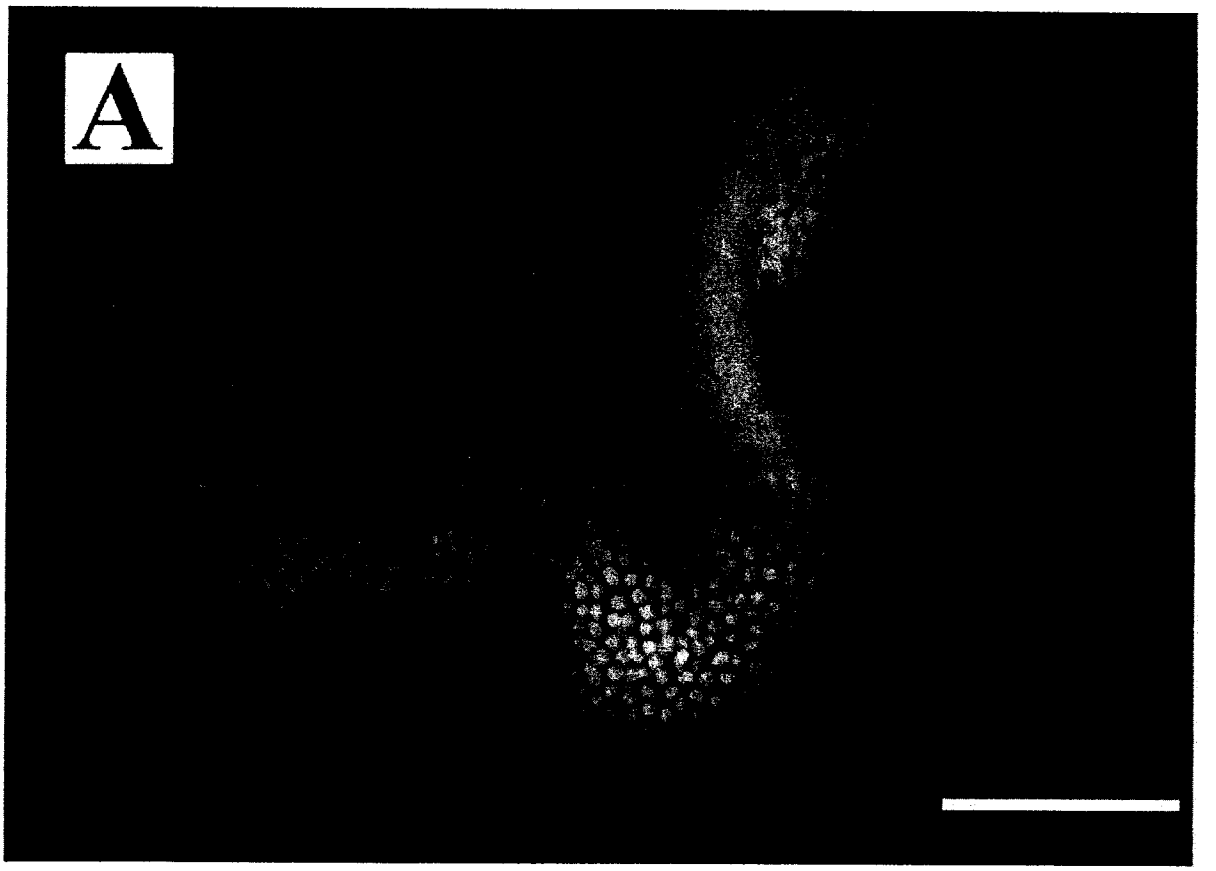
Once hatched larva spend anywhere from a day to 140 days happily swimming about while they try to become competent. *Hermissenda* must spend at least 34 days in the veliger stage however some species, such as *Cuthona nana* are ready to metamorphose in just one to two days. At this exciting point in a young nudibranchs life they must cease swimming or crawling with their head withdrawn into the shell aperture. The action of the locomotory cilia becomes more erratic and then they are thrown away or ingested and the entire velum is absorbed. They grow one pair of tentacle buds and two pairs of cerata buds on their dorsal surface. Their bodies become completely separated from the shell by a constant contraction of the retractor muscle and they are free to crawl away, a whole new life awaiting them.

I obtained a culture of the *Hermissenda crassicornis* eggs from the flow table in the embryology lab. They had almost certainly been laid within 24 hours of collection. I then put them into a medium sized finger bowl with filtered seawater of a standard 35.The mean temperature of the surrounding water was 13° C. I changed the water by filtering with a 50 micrometer mesh and replacing it with the water obtained from Craig Young's lab. This was done approximately every two days. Once the veligers hatched I fed them every several days

with half and half *Tertiolecta and Rhodomonas lens* algae. Every second water change I also changed the finger bowl by pipeting the larvae up individually and putting them into the clean environment. I also told them how wonderful and cute they were.

My results were comparable to the literature for *Hermissenda* raised at 11-13° C and the data for *Phestilla melanobranchia* raised at 22°C. Eggs collected on 5/15/07 began hatching in between 5/19 and 5/22/07 with all individuals hatched by 5/25/07. Individuals began dying off by 5/31/07 with the hail and hearty still alive and swimming when I released them from the boat dock on 6/8/07.

The development of the larvae would have been better observed if I had been able to observe them more often prior to hatching. I would have liked to rear them to the metamorphosis stage however that will have to wait for another time. When I am able to repeat the experiment I will know exactly what needs to be done. And not only will I allow sufficient time but understand that by providing certain chemical cues I can induce them into metamorphosising after 34 days if they do not do so on their own.



Egg mass of a doris nudibranch

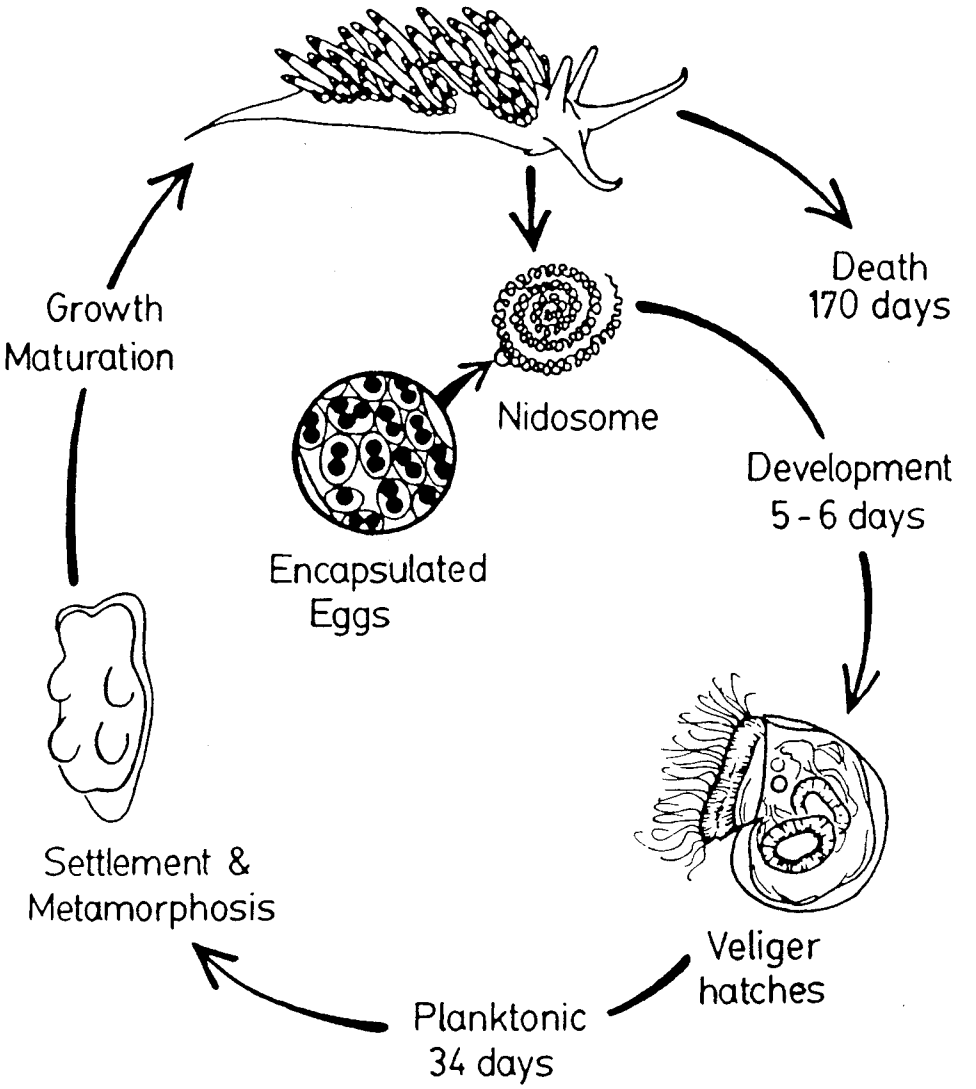


Figure 1. Life cycle of Hermissenda.