Embryology and larval development of Acteon punctocoelata (Carpenter) (Gastropoda, Opisthobranchiata)

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Brigham Young University - Provo

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EMBRYOLOGY AND LARVAL DEVELOPMENT OF ACTEON PUNCTOCOELATA
(CARPENTER) (GASTROPODA, OPISTHOBRANCHIATA)

A Thesis
Presented to the
Department of Zoology
Brigham Young University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Scott E. Brown
August 1972
This thesis, by Scott E. Brown, is accepted in its present form by the Department of Zoology of Brigham Young University as satisfying the thesis requirement for the degree of Master of Science.
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INTRODUCTION

Acteon (Rictaxis) punctocoelata (Carpenter) is a primitive opisthobranch mollusc found sporadically in central California bays. The primitive nature of this genus is indicated both geologically and morphologically (Fretter and Graham, 1954 and Morton, 1958). This microphagous browser burrows just below the surface of the sandy mudflats, as do its close relatives Haminea, Scaphander, and Philine (Fretter and Graham, 1954). Its strong, sculptured external shell is approximately 1/2 inch long and is encircled with narrow black bands that follow the whorls around the white shell (Fig. 1).

A British species, A. tornatilis, has been the subject of several studies (Pelseneer, 1894; Guiart, 1901; Perrier and Fischer, 1911; Gabe and Prenant, 1952, 1953; Fretter and Graham, 1954; Johansson, 1956; Duncan, 1960).

Acteon punctocoelata, on the other hand, has received little attention. This cephalaspidean was described as a new species by Phillip P. Carpenter (1864, p. 307). As far as the author was able to determine, further work on this animal consists merely of brief descriptive notes by Ricketts and Calvin (1939) and Light (1954).

The objective of this study is to describe the
embryology and larval development of *A. punctocoelata* with primary emphasis being placed on the morphology and ultrastructure of the veliger larvae.
METHODS AND PROCEDURES

On July 22, 1970 forty egg masses and twenty adult specimens of *Acteon punctocoelata* were collected at Lawson's Flat in Tomales Bay, Dillon Beach, California. All adults and egg masses were found on the surface of the mud exposed by a -1.1 foot low tide. In the laboratory adults were maintained in 12 cm culture dishes supplied with running sea water. Egg masses were separated according to their stages of development and were then placed in Stender dishes containing filtered sea water. The sequential development of the larvae was studied at a water temperature of 18° C. Water in these containers was changed daily. Adults and egg masses were also collected on December 26, 1970 and August 10, 1970 and were transported to Brigham Young University for further observation. There the specimens were kept in polyethylene freezer dishes in a medium of "Instant Ocean." Care was taken to wash the samples at regular intervals and to avoid temperature shock. While observing the early developmental stages, small samples were taken from the distal end of the spiral egg mass. These were then placed on depression slides and observed with phase contrast optics. All illustrations were made from living specimens and from photomicrographs taken with a Zeiss Photo II.
Appropriate developmental stages were selected and fixed according to the following procedure:

1. one hour in 5% glutaraldehyde in Millonig's Phosphate Buffer solution plus sufficient NaCl to raise the osmolality to 970 mosm;
2. washed every 10 minutes for one hour in 0.4 phosphate buffer plus sufficient NaCl to reach 980 mosm;
3. post-fixation for two hours in 2% osmium tetroxide (Palade, 1952) in 0.1M phosphate buffer and sufficient NaCl to reach 960 mosm.

After post-fixation, larvae were dehydrated in a graded series of ethanol and acetone, and then embedded in a mixture of Epon 812 and Araldite 506 (Mollenhauer, 1964 Stock II). The larvae were next left in a 25% plastic-acetone solution for one hour and then in a 75% solution overnight. The specimens were then embedded in 100% plastic. Sectioning was done on a Sorvall MT-2 microtome. Thin sections were cut with glass knives at a thickness of 800-1000 Å and picked up with copper grids coated with Formvar. These sections were stained for fifteen to twenty minutes with lead citrate (Reynolds, 1963). They were observed with a Hitachi HS-7 electron microscope. One micron sections were cut with glass knives for observation with the light microscope. Sections were stained with a 1:1 mixture of 1% azure blue in water and 1% methylene blue in 1% borax solution (Richardson, et al., 1960).
RESULTS

The Egg Mass

The newly deposited egg mass is a milky white spiral composed of three or four loops of similar diameter. The coil is spawned out of the genital aperture which is located antero-laterally on the right side of the adult (Fig. 1) and consists of some 40,000 capsules surrounded by a jelly matrix. Each capsule measures 150 microns in diameter and generally contains only one egg, although some house two. The entire mass, when uncoiled, measures from one to one and a half inches in length.

Early Cleavages

By the time the entire coiled egg mass is deposited, cleavage has already begun at the distal end. Growth and differentiation of the embryos at the proximal end of the spiral lag slightly behind those at the distal end throughout development. The first two divisions are holoblastic and equal, whereas the third is unequal and forms the first quartet of micromeres characteristic of spiral cleavage. Asynchronous cleavage is indicated by the appearance of 3- and 6-cell stages. These stages are then followed by 8- and 16-cell stages (Table 1). Approximately 54 hours
Fig. 1. Adult *Acteon punctocoelata* laying an egg mass.
# TABLE 1

**DEVELOPMENTAL TIMES OF ACTEON PUNCTOCELELATA CULTURED AT 18°C**

<table>
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<th>Stage</th>
<th>Hrs. Between Stages</th>
<th>Cumulative Hrs.</th>
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<tbody>
<tr>
<td>Two cell</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Three cell</td>
<td>0.75</td>
<td>2.25</td>
</tr>
<tr>
<td>Four cell</td>
<td>0.25</td>
<td>2.50</td>
</tr>
<tr>
<td>Six cell</td>
<td>2.50</td>
<td>5.00</td>
</tr>
<tr>
<td>Eight cell</td>
<td>1.50</td>
<td>6.50</td>
</tr>
<tr>
<td>Sixteen cell</td>
<td>4.50</td>
<td>11.00</td>
</tr>
<tr>
<td>Blastula</td>
<td>43.00</td>
<td>54.00</td>
</tr>
<tr>
<td>Early gastrula</td>
<td>22.00</td>
<td>76.00</td>
</tr>
<tr>
<td>Blastopore elongates</td>
<td>7.00</td>
<td>83.00</td>
</tr>
<tr>
<td>Appearance of foot rudiment</td>
<td>2.00</td>
<td>85.00</td>
</tr>
<tr>
<td>Cilia first observed with light microscope</td>
<td>1.00</td>
<td>86.00</td>
</tr>
<tr>
<td>on velar lobe rudiments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Separation of velar lobes</td>
<td>6.00</td>
<td>92.00</td>
</tr>
<tr>
<td>Cilia first observed with light microscope</td>
<td>12.00</td>
<td>104.00</td>
</tr>
<tr>
<td>on foot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Velar retractor muscles functional</td>
<td>17.00</td>
<td>122.00</td>
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<tr>
<td>Hatching</td>
<td>42.00</td>
<td>164.00</td>
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after spawning, large yolky macromeres are surrounded by smaller, clear micromeres. The embryo then elongates at one end and becomes triangular in shape. Gastrulation begins 76 hours after the first cleavage. After 83 hours the blastopore lengthens out forming a narrow slit. At this stage slight rocking movements of the embryo are evident presumably due to the short, developing cilia. As is common in opisthobranch development, there is no definite trochophore stage. Development proceeds directly to the formation of the veliger larvae.

Early Veliger

Although it is not possible to observe the short, developing velar cilia with a light microscope at this early stage, their presence is obvious as the embryo begins to rotate within the capsule. As development proceeds, a single tuft of longer cilia appears on one side of the region that will become the velar lobes. Shortly thereafter another tuft of several long cilia appears on the opposite side of the velar lobe rudiment (Figs. 3, 4). The cilia which appear between these two tufts beat arhythmically and intermittently.

Mouth formation immediately precedes the development of the foot rudiment. The foot becomes ciliated over its entire surface, except for the posterior region where the operculum will be secreted. Within the proximal
Figs. 2-4. 2, Early veliger showing operculum (Op), digestive gland (Dg), stomach rudiment (St) and the first muscle to appear (Mus). 3, Early veliger showing foot rudiment (Ft), developing locomotor cilia (Ci) and central yolk mass (Yk). 4, Early veliger showing further development of locomotor cilia.
portion of the foot two prominent statoliths are formed that rotate continually within the statocyst.

Larvae at this stage are still very opaque due to high yolk concentration in the cells. The greatest concentration of yolk is restricted to the center of the embryo while the foot rudiment and the velum are clear and essentially free of yolk granules. As this supply of yolk is utilized the embryo becomes less opaque, and differentiation of the internal organs can be observed.

The first organs to appear are the stomach rudiment and the digestive gland, neither of which has a lumen at this stage (Fig. 2). The first larval muscles connect the stomach rudiment to the mantle. Shortly thereafter, other muscles appear that extend from the shell membrane to structures referred to by Pelseneer (1910) as anal glands. These have subsequently been referred to as simply "clear vesicles" (Thompson, 1958). The function of these vesicles is unknown. There are several muscles in this region that contract periodically, pulling the mantle away from the shell to form a shell cavity.

The large larval retractor muscles are also seen to differentiate at this time. These are concentrated at first in the visceral region, and then they are spread out into the foot and velum, forming a complex network of muscle fibers. With the elaboration of these muscles, independent movements of the foot and velum are now able to take place and larvae are able to withdraw within the
veliger shell either partially or entirely.

Located between the first branches of the columnar muscles that extend into the velum is the larval kidney. It is approximately the same size as the statocyst and is the most deeply pigmented organ of the veliger.

Mature Veliger

Behavior Before Hatching

As the muscles extend the velum, velar locomotor cilia begin to beat, effecting a spinning motion of the veligers at short irregular intervals. This movement is generally backwards, but forward movement also occurs. When the velar muscles contract, the large velar cilia cease to move, and the larvae lie motionless within the capsule. During such quiescent periods shorter cilia of the subvelar ridge, foot, and gut continue their constant metachronal motion.

Hatching

The muscular, ciliated velum plays a vital role in the hatching of mature veligers. Just prior to hatching the capsule membrane becomes extremely pliable. This weakened membrane is then ruptured by the action of the velar cilia and the violent rocking motions of the veligers. Larvae rotate within the capsule as they rock back and forth, thus weakening the entire membrane in preparation for escape.
Fig. 5. Mature veliger showing internal structures at the time of hatching.
Behavior After Hatching

Once outside the egg capsule and freed from the surrounding jelly matrix, *A. punctocoelata* swims in a variety of patterns. Larvae frequently traverse the dish swimming in a straight line with the velum fully extended in front of the animal. Circular motions are observed that sometimes take the form of a backward somersault and at other times are right or left handed circles parallel to the bottom of the container housing them. Locomotor cilia can be stopped abruptly and withdrawn to a position above the velar lobes. This causes the veligers to sink to the bottom of the container. A few cilia begin to beat slowly, and shortly the remainder of the cilia resume normal, coordinated beating. While on the bottom they are observed to spend a great deal of time moving with the velum directly in contact with the substrate. At other times they merely lie on the bottom for a short time with the shell in contact with the sand and the velum extending upwards. While in this inverted position the long velar cilia beat slowly and intermittently.

The Foot

The foot is a very prominent structure with both dorso-ventral and longitudinal muscles which are not arranged in any definite pattern within the pedal sinus (Fig. 5). Short cilia are arranged in definite bands on
the foot. Especially long, stiff cilia are present on both sides of the foot.

The Digestive System

Medial to the foot is the mouth into which the food is swept by cilia. Veligers were seen to feed on the one-celled algae Monochrysis lutheri Droop, 1953. The food is moved through the gut by cilia that beat metachronally. Once food enters the stomach it is mixed by the circular motion set up by the cilia. Although the stomach itself never contracts, muscles attached to it move it around considerably within the body cavity thus aiding in mixing the food. The surface of the stomach and hindgut are covered with irregular shaped bodies of unknown function.

The lumen of the large digestive gland communicates with that of the stomach. Fluid is seen to circulate between these organs, and the contents of the two are mixed. Occasionally the digestive gland is seen to contract, emptying its contents into the stomach.

Ultrastructure of Epithelial Cells

Epithelial cells of both the foot and the velum have a similar ultrastructure. Cilia have the typical 9 + 2 arrangement of microfibrils and are surrounded by a membrane continuous with the plasma membrane (Fig. 6). Basal bodies (Fig. 11) are associated with each of the cilia.
Fig. 6. Ultrastructure of mature veliger foot.
Numerous clavate microvilli with expanded tips and narrow bases are located between the cilia. Cross sections reveal that the microvilli lack the internal filaments that characterize cilia (Fig. 12). These microvilli have prominent hair-like filaments radiating from the surface (Figs. 6, 7, 8).

Numerous granules of varying size and density are present in these epithelial cells (Fig. 6). Glycogen granules (Fig. 9) are the smallest and most numerous. These aggregate in rosettes or "alpha particles" (Fawcett, 1966). Zymogen (Fig. 10) appears in the form of densely staining membrane bound secretion droplets. Large mucous droplets (Fig. 6) are also present in both the foot and velum. Lipid granules (Fig. 6) are less dense than the zymogen. These appear to be surrounded by a limiting membrane.
Fig. 7. Section through velum showing glycocalyx (Glx), microvilli (Mv), and cilia (Ci). 55,800X

Fig. 8. Glycocalyx (Glx) showing its delicate, filamentous nature. 59,400X
Fig. 9. Epithelial cell of foot showing glycogen aggregates or "alpha particles" (arrows). 11,550X

Fig. 10. Epithelial cell of foot showing zymogen granules (Zy), mitochondria (M), chromatin material (Chr), and pore in nuclear envelop (arrow). 28,800X
Fig. 11. Epithelial cell of the velum showing basal bodies (arrow), Golgi apparatus (Go), and mitochondria (M). 22,800X

Fig. 12. Epithelial cell of the velum showing microvillus in cross section (Mv), and septate desmosome (arrow). 90,000X
DISCUSSION

The egg mass of *Acteon punctocoelata* is considered "type B" according to Hurst's (1967) classification. When first spawned it is milky white, but it gradually changes to a yellowish hue as the time of hatching approaches. The entire jelly matrix becomes flaccid at this time, and simultaneously the egg capsule membrane becomes very pliable presumably due to the action of enzymes produced by the veligers (Gohar and Abul-Ela, 1957a and 1957b and D'Asaro, 1965). The rocking motions of the veliger weaken the entire membrane; therefore, the point of rupture does not appear to be in any specific region nor through a softened plug in the aperture of the egg capsule as reported by D'Asaro (1965) for the queen conch, *Strombus gigas*.

The same types of cilia on the mature, hatched veliger of *Acteon punctocoelata* (Fig. 5) are present in numerous other opisthobranchs and have been the subject of many studies. The long, immovable cilia found at the sides of the foot are postulated to be sensory in function by Thompson (1958) and Williams (1970). At least a third of the opisthobranch veligers examined by Hurst (1967) had such special, probably sensory, cilia characteristically placed.
The velum bears two types of cilia: the long, locomotor cilia and the short, subvelar cilia. The fact that the former do not beat continually but periodically stop abruptly is indicative of the type of nervous coordination reported by Carter (1926). Further evidence of nervous control is that cells bearing five to ten cilia that are separated from the velum beat continually for several hours. This was also observed by Carter (1926) and led him to the conclusion that the nerve endings found among these cells are regulatory and cause the simultaneous stopping of the cilia.

When the veligers lie in an inverted position on the substrate, only a few of the locomotor cilia beat at a time, creating currents that bring food to the veligers. As food is brought near the larvae, it enters definite feeding currents set up by the short, subvelar cilia and the bands of cilia on the foot. The arrangement of these bands is identical to that described by Thompson (1959). These cilia beat metachronally and never demonstrate the intermittent beating that is indicative of nervous control.

There are definite currents in the lumen of the digestive gland which indicate that it is lined with the same type of metachronally beating cilia as the entire digestive tract. Thompson (1962) reported cilia in the digestive gland of Tritonia hombergi.

The foot contains a pedal sinus (Fig. 5) that at times appears to be a prominent structure and at other times
is completely absent. This is due to the fact that the dorso-ventral and longitudinal muscles are able to contract the foot and obliterate the cavity. Histological research by D'Asaro (1965, 1966, 1969) describes an identical sinus in the prosobranchs Strombus gigas, Thais haemastoma floridana, Bursa corrugata and Distorsio clathrata.

Analysis of the ultrastructure reveals elements not previously reported in veliger larvae. The external coating of the epithelial cells is composed of microvilli and delicate filaments radiating from them. These filaments were first observed by Yamada (1955) in the fine structure of mouse gall bladder epithelium. Choi (1963) identified this coating, by histochemical methods, as a substance high in mucopolysaccharides. Structures similar to those in Acteon larvae have been found in the cuticle of oligochaetes (Krall, 1968), pogonophorans (Gupt, et al., 1970), human colonic mucosa (Rifaat, et al., 1965) and the intestine of parasitic nematodes (Wright, 1963). Bennett (1963) has suggested that a polysaccharide-rich component is of widespread and possibly of universal occurrence on all cell surfaces. He has proposed the general term "glycocalyx" for all such cell coats.
SUMMARY

1. *Acteon punctocoelata* spawns a spiral egg mass.

2. Early cleavages are asynchronous, spiral and begin as soon as the egg mass is deposited.

3. Velar lobes, foot, operculum, cilia, and statocysts appear while the interior of the larvae is still obscured by yolk.

4. Hatching occurs by vigorous side-to-side movements of the veliger that weaken the egg capsule membrane. At 18° C this occurs 164 hours after the first cleavage.

5. Structures observed in the mature veliger at the time of hatching are the following: velum, subvelar ridge, foot, operculum, statocyst, esophagus, stomach, hindgut, digestive gland, kidney, anal glands, muscles, and sensory cilia.

6. Eyes are absent.

7. Mature veligers exhibit a complex musculature and a high degree of nervous coordination.

8. Once freed from the egg capsule membrane and the surrounding jelly matrix, veligers swim using similar patterns described for a variety of other opistho-branches.
9. The veligers are planktotrophic.

10. Ultrastructure examination of the epithelial cells of the mature veliger revealed glycogen, zymogen, lipid, mucous, microvilli, glycocalyx, and septate desmosomes none of which have been reported in veliger larvae.
LITERATURE CITED


Carpenter, P. P. 1864. Supplementary Report, British Association for the Advancement of Science, 1:307.


EMBRYOLOGY AND LARVAL DEVELOPMENT OF *Acteon punctocoelata* (CARPENTER) (GASTROPODA, OPISTHOBRANCHIATA)

Scott E. Brown
Department of Zoology
M.S. Degree, August 1972

ABSTRACT

The embryology and larval development of *Acteon (Rictaxis) punctocoelata* (Carpenter), a cephalaspidean opisthobranch collected at Lawson's Flat, in Tomales Bay, Dillon Beach, California was studied. This primitive gastropod has not previously been investigated. Live observations were made from egg masses spawned in July and December. Sequential stages were timed from the first cleavage to hatching. Hatching occurred 164 hours after the first cleavage. At the time of hatching the mature veliger is fully formed with a velum, subvelar ridge, foot, operculum, statocyst, esophagus, stomach, hindgut, digestive gland, kidney, anal glands, muscles, and sensory cilia. Behavior inside and outside the egg capsule and morphology closely paralleled that of other opisthobranchs. Ultrastructure examination of the epithelial cells revealed glycogen, zymogen, lipid, mucous, microvilli, glycocalyx, and septate desmosomes none of which have been reported in veliger larvae.

COMMITTEE APPROVAL:
VITA

Scott Ellsworth Brown