EMBRYONIC AND LARVAL DEVELOPMENT OF THE SNAIL *CALLIOSTOMA LIGATUM* UNDER LABORATORY CONDITIONS

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ABSTRACT

Larval development of animals is important to document as this information helps determine evolutionary relationships, and assists in the identification of larvae at the species level. I observed the development of the snail *Calliostoma ligatum*. Embryo was recognizable as a veliger in three days. Zygotes hatched on day four as non-feeding veliger with a small velum and a transparent shell. Day five veligers had more pronounced shell, though still largely transparent. Larvae are bad swimmers and rarely were found off the bottom of the culture dish. All larvae died after five days. More cultures would be needed for a more complete observation of development from larva into the juvenile.

INTRODUCTION

Larval development in gastropod species is highly variable, and it is therefore important to document the development of each individual species. Documentation of this process will help in identifying veliger larvae when found in the wild, spawned and away from the adult. This identification can be used to assess diversity of plankton at the species level in scientific studies. From my own experience in conducting plankton tows twice a week during the spring I found gastropod veliger larvae in very low numbers. Being able to identify what species of gastropod veliger I found may have given me insight as to the reproductive biology and life history of the species that is not available through lab work.

Developmental biology is important because it helps us to study the connections and determine the relatedness of different species. This is useful in classification because we can use the developmental characteristics to systematically organize species found in nature. There are thousands of gastropod species living, and thousands more in the fossil record. Ongoing study is needed to determine how they relate. DNA analysis has changed much of the known organization of gastropod species. Much of the known relations were reinforced with DNA analysis, but many have been changed accordingly. DNA analysis plus the study of morphology and development can give us insight into developmental constraints that end in converging characteristics. Previously such characters might have signaled a relatively recent speciation, but this new tool will help determine which characteristics were shared before, and which came after divergence.

I will not be looking into the answers of these questions, as they are far beyond the scope of my research. I will however describe in detail the development of the gastropod *Calliostoma ligatum.*

MATERIALS AND METHODS

Calliostoma ligatum specimens were collected by hand at Portside mudflats. Induced spawning by warming and aggravation in order to retrieve gametes. Small total numbers, consisting of one culture of approximately 100 eggs was kept and observed. The culture was held between 12 and 13 degrees Celsius. The culture was kept in filtered sea water (FSW) that was changed daily. FSW was changed by pipetting water out of culture through a 50 μm filter, then placing remaining FSW and embryos in a fresh dish and replenishing the supply of FSW. Embryos/larvae were observed twice daily for the first two days and once daily for the remainder of development. Observations were made using a compound light microscope.

RESULTS

At fertilization (Fig 1, A): Egg dark green to brown. Egg approximately 220-230 μm in diameter, outer jelly coat approximately 650 μm in diameter. Fertilization envelope irregular, touching egg at one side with a relatively large gap on the other. Pronucleus seen as lighter green area in egg. Approximately 100 eggs. Sperm long and thin, very small, tail very small and nearly invisible on compound microscope (Fig 1, B). Many sperm in jelly coat (Fig 3, D).

After fertilization (Fig 1, C; Fig 3 A): Egg mono-colored green brown. No other visible change.

2-Cell stage (Fig 1, D): 2 hrs 30 min after fertilization (AF). Two cells, equal holoblastic cleavage (Fig 3, B). Approximately 30 zygotes developing.

8-Cell stage (Fig 1, E; Fig 3, C): 7 hrs AF. Eight cells, spirally cleaving. Outer jelly coat disintegrating.

Gastrula stage (Fig 1, F; Fig 3 D): 24 hrs AF. Green brown gastrula, opaque, cell outlines barely distinguishable around perimeter. Gastrulation by epiboly, blastopore to be mouth. 1 day 4hrs AF, trochoblasts apparent (Fig 2, A). 15 individuals still living.

Gastrula with prototroch (Fig 2, B; Fig 3, E): 2 days 4 hrs AF. Prototroch encompasses entire vegetal pole. Prototroch cells large, about 30-40 make prototroch. Dark region in center of prototroch visible. Some have lobe forming off of one side, mantle secreting cells. Rotating inside fertilization envelope. 15 individuals still living.

Veliger larvae, just hatched (Fig 2, C; Fig 3, F): 4 days AF. Region inside of prototroch being pulled in. Prototroch starting to enlarge, obvious ring around mouth end. Most veligers have a lobe coming off side, transparent shell starting to form. Bad swimmers, most laying on side in dish in remains of jelly coat. Effective stroke of prototroch goes anterior to posterior. Velum cone shaped from prototroch to center. 10 individuals still beating cilia (living).

Veliger larvae with shell (Fig 2, D; Fig 3, G): 5 days AF. Prototroch continues to enlarge, gap in ciliary band formed on ventral side. Cilia longer and more effective. All larvae found on their sides at bottom of dish. Foot forming on posterior end, has orange color seen in the

adult. Foot has folds indicating enlargement as well as muscles forming. Shell much more pronounced, spiral shape becoming clear. Pre-torsion. 3 individuals still living.

After day five all larvae were dead.

DISCUSSION

With the obvious lack of effective swimming velum these larvae do not disperse very far. Their short time spent in the zygote and larval stages indicates that the juveniles of this species prefer to end up near where they were spawned. At the collection site, in the rocks at the edge of the Portside mudflat, there was a large population ranging in size from very small to a shell length of about 2 cm. Their need for a specific rocky substrate may make dispersal rather low on the list of priorities, the first being to find a suitable habitat. This is probably why they don't waste energy on producing a large velum, as their velum hardly got any wider than the body itself. The ciliary beating was also rather asynchronous compared to other veligers, like those from *Janolus fuscus* whose cilia beat in a synchronized fashion to move. *C. ligatum* also put significant early energy into producing a large foot. The foot comprised one entire end of the larvae at 5 days, whereas other species produce a foot much later in development, choosing to invest more energy on other body parts. All of these characteristics are part of a biology that works toward dispersing very little and moving almost directly into the juvenile phase.

The observations that I made would have been more complete had I been able to keep my culture alive for a longer period of time. I do not know what could have been done differently as these are non-feeding veligers that are known to be bad swimmers. According to Megum F. Strathmann's observations in his book Reproduction and Development of Marine Invertebrates of the Northern Oregon Coast the larvae go through torsion around 9 days after fertilization. At

12 days Strathmann observed pediveligers which settled and metamorphosed at 14 days. After this the juveniles developed a large shell, and digitate cephalic tentacles (Strathmann, 1987).

Much of the difficulty in observing this species is the fact that they are opaque, making observation of internal organs and gastrulation impossible. During gastrulation I was able to see a darker region that moved up from the velum posteriorly, but could not determine what it was as the mouth formed at a spot more ventral and therefore must not have been the archenteron. With a confocal microscope and much dedication one would be able to map out the movement of internal organs by staining nuclei and muscle development at different stages. Not only were the internal organs beyond observation, but the shell had very fine detail that was hard to see using a compound microscope. Looking at shell development through a scanning electron microscope would have been very informative.

ACKNOWLEDGEMENTS

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REFERENCES

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Fig 1

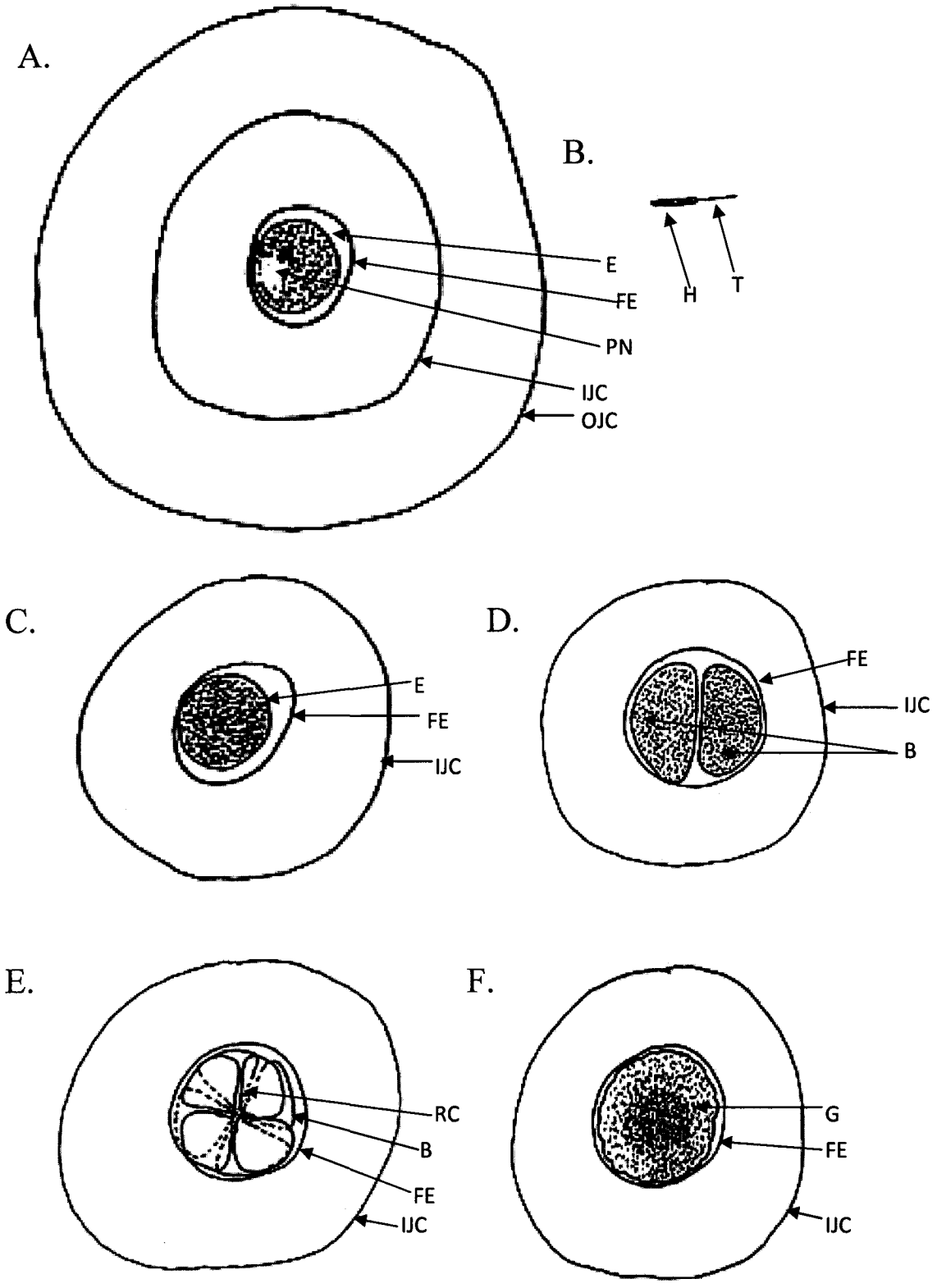

Fig 2

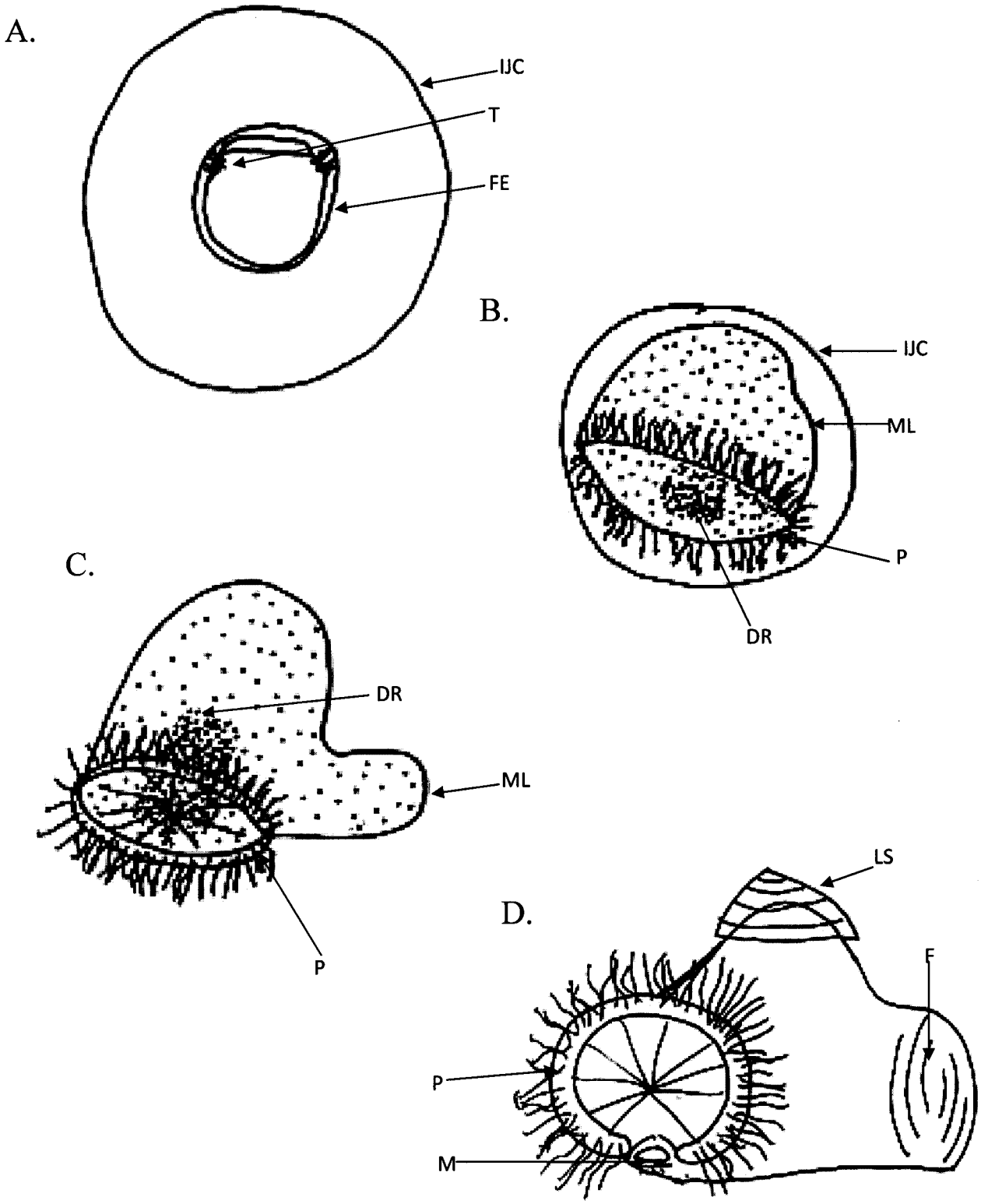

Fig 3

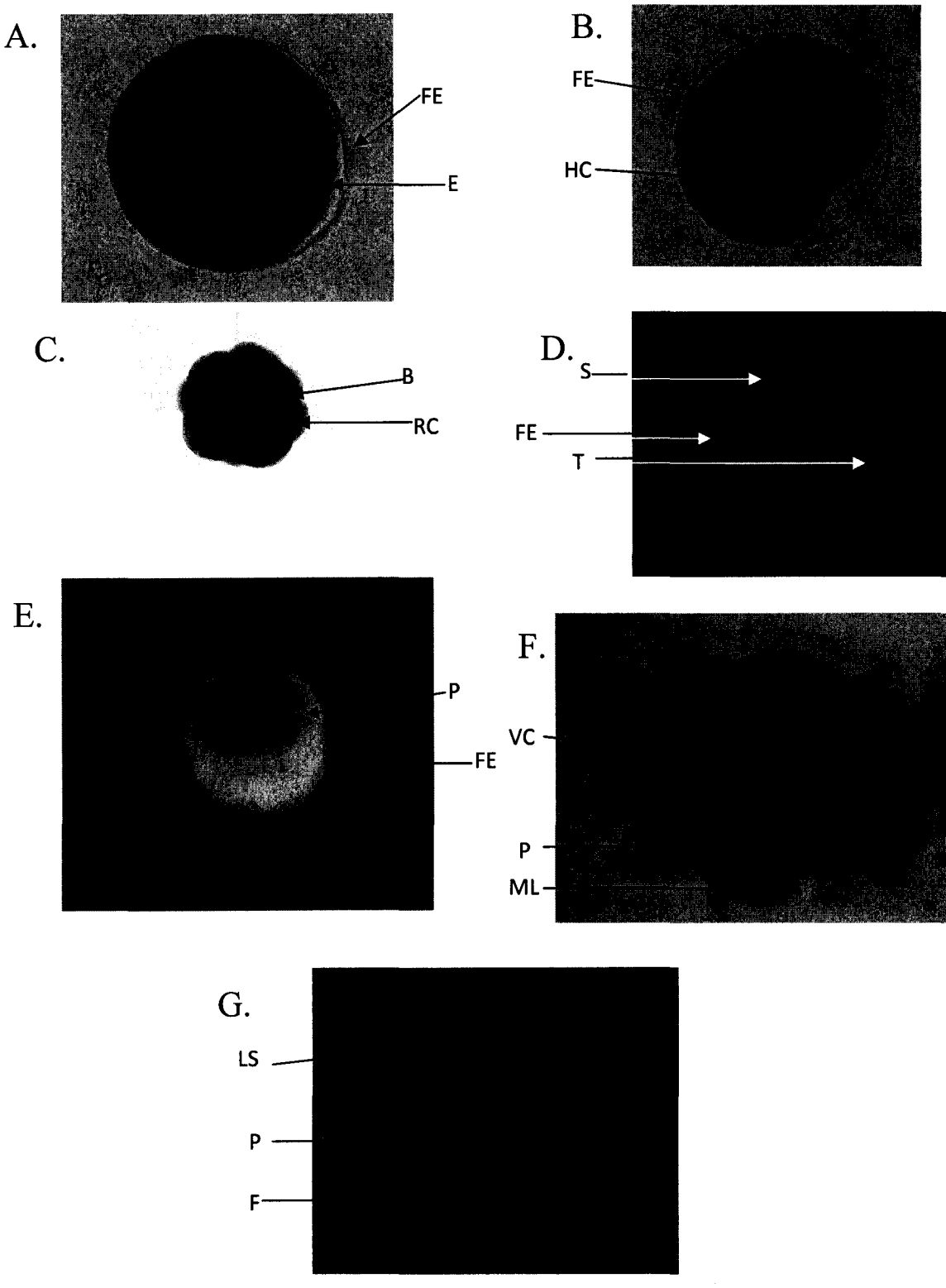

Fig 1: (A) Unfertilized egg with pronucleus. (B) Sperm. (C) Fertilized egg, (embryo). (D) 2-cell stage embryo. (E) 8-cell stage embryo. (F) Gastrula.

Fig 2: (A) Gastrula with trochoblast cells. (B) Gastrula with prototroch and early mantle lobe, darker region in center. (C) Recently hatched veliger with darker region extending posteriorly and enlarging mantle lobe. (D) Veliger with foot forming, early larval shell visible and a gap in the prototroch where mouth will be.

Fig 3: (A) Egg. (B) Embryo undergoing first equal holoblastic cleavage. (C) 8-cell stage embryo. (D) Gastrula with trochoblasts. (E) Gastrula with prototroch. (F) Recently hatched veliger, small velum and small mantle lob forming. (G) Veliger with foot and visible larval shell.

Abbreviations: Inner jelly coat (IJC), outer jelly coat (OJC), egg (E), fertilization envelope (FE), pronucleus (PN), head (H), tail (T), blastomere (B), radial cleavage (RC), gastrula (G), trochoblast (T), mantle lobe (ML), prototroch (P), dark region (DR), larval shell (LS), foot (F), mouth (M), holoblastic cleavage (HC).