

## Development of Egg Masses in Fresh Water Pulmonate Snail *Indoplanorbis exustus*

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### **Abstract**

*Egg capsules were generally laid at night or early in the morning by I. exustus. The freshly laid eggs varied from 4.0 to 5.0mm in their diameter. Each egg mass of I. exustus was observed as flattened and like a coiled whorl. The proper egg capsules of I. exustus were flattened, oval and rectangular and had a diameter from 0.5 to 0.7mm. The egg capsules lie side by side in 2-3 rows. The shape of egg capsule was more or less rectangular, the average length being 0.7mm and width 0.5mm. The number of capsules varied in each egg mass. In I. exustus, they were generally 7-60. Normally each capsule contained only one egg.*

### **Introduction**

Molluscs have played important roles in strengthening our knowledge of fundamental processes in developmental biology. In freshwater Pulmonates, the embryonic development is direct and takes place in eggs. Eggs which comprise a zygote surrounded by perivitelline fluid and membrane are embedded in jelly and enclosed in a common egg capsule. Bhramachary (1992) stated that embryonic development is a ceaseless process till eclosion, which involves several aspects such as zygote metabolism, cleavage, blastulation, gastrulation and organogenesis. The morphological and behavioral aspects of the development of *Lymnaea* have been well documented (Morril 1982, Voronezskaya et al 1999). Dewitt (1954) studied the reproduction, embryonic development and growth in the pond snail *Physa gyrina* Say. He mentioned the mechanism of copulation, oviposition, breeding parameters like eggs, egg capsule, cleavage and larval development and growth of *P. gyrina*. Cumming (1993) worked on the reproduction and variable larval development of an ectoparasitic snail, *Turbonilla* sp. He described the chronology of larval development and mentioned only one larval stage, veliger in the snail species. Okusu

(2002) made an observation on the embryogenesis and development of *Epimения babai*. Herman et al. (1994) and Sarker (2002) described the reproduction and embryology of the different species of *Lymnaea*. But still now, detailed work on embryology and development of *Indoplanorbis exustus* is completely lacking. The juveniles leave the egg capsule using their radula to gnaw the surrounding membranes (Costil 1997). Termination of egg-laying is very likely determined by a synergetic action of environmental factors, it is suggested that these factors control the activities of the CDCH- producing neuroendocrine caudo dorsal cells in the brain.

Early larval development is the most sensitive during molluscan development (Rumrill 1990), particularly in the intertidal zone where environmental stresses can be frequent and harsh (Gosselin & Ghia 1995). Many molluscs enclose their eggs within benthic egg masses that may reduce early larval mortality. Among gastropods, these egg masses can be grouped into two main types, capsular and gelatinous. Capsular egg masses include leathery egg capsules of neogastropods in which the embryos are surrounded by a tough capsule wall. Gelatinous egg masses are deposited by heterobranchs and some other caenogastropods, with the embryos embedded in a gelatinous matrix and surrounded by a microscopic vitelline membrane. The egg masses of some species appear to provide protection against predation, extremes of temperature and salinity, desiccation, microbial infection and ultraviolet radiation (reviewed by Rawlings 1999, Przeslawski 2004). Despite such protection, embryos within egg masses are still vulnerable to environmental stresses, including surface fouling (Biermann et al 1992). The morphological, morphometric and behavioral aspects of the development of *Lymnaea stagnalis* have been well documented by Cumin (1972), Marois and Croll (1991), Mescheryakov (1990), Morrill (1982), Voronezhskaya et al (1999). Studies on breeding and development of gastropods have been done by many workers. Dewitt (1954) reported the reproduction, embryonic development and growth in the pond snail, *Physa gyrina*. Hurling (1928) for the first time described the hatching of eggs of aquatic gastropods. Bondesen (1953) showed the rupturing from the egg membrane by the radula in *Lymnaea stagnalis* and three pulmonate families, Physidae, Lymnaeidae and Planorbidae.

## Materials and Method

The snails *Indoplanorbis exustus* were collected from local pond near Aurangabad, Maharashtra, India. The snails were maintained in tap water in laboratory for acclimatization. The snail population ranged between 8-12 mm shell lengths was maintained in large plastic troughs with continuous water refreshment and aeration. Snails were provided with fresh vegetative food like algae, mulberry leaves etc., twice a day.

To study the embryology and development of eggs, egg masses were collected in troughs. Daily observations were made to study development of embryo.

### **Result:**

Egg capsules were generally laid at night or early in the morning by *I. exustus*. The freshly laid eggs varied from 4.0 to 5.0mm in their diameter. The egg capsules lie side by side in 2-3 rows. The shape of egg capsule was more or less rectangular, the average length being 0.7mm and width 0.5mm. The number of capsules varied in each egg mass. In *I. exustus*, they were generally 7-60. Each egg capsule contained single ovum which was suspended in the usual viscous nutritive medium.

### **Development of eggs in *I. exustus***

Embryos of pulmonate gastropods undergo direct development from fertilized egg to juvenile snail inside egg capsules filled with aluminous perivitelline fluid, also known as capsular fluid (CF). The CF provides the nutrition required for normal development of embryos, which is a secretory product of the albumen gland (Raven, 1958; Morrill et al., 1964; Heras et al., 1998). Development of *I. exustus* takes place in within the eggs, from which the young hatch as crawling juvenile snails. Eggs hatch after 8-10 days.

Unsegmented eggs of *I. exustus* are spherical and two polar bodies are seen at the animal pole, one on top of the other. Eggs are heavily yolked.

**1<sup>st</sup> day:** - The first cleavage division occurred about 2 hrs after the egg-laying. The first cleavage is vertical; it passed through animal pole to vegetal pole dividing the egg into two halves. The two celled embryo underwent second equal division of cleavage within next hour. The second division is at right angles to the first and results in the formation of four blastomeres of similar size. After each division, daughter cells become almost spherical in form, but later flatten against each other to form a single sphere. The third division is horizontal which is unequal. At this

division four smaller cells (micromeres) appear at the animal pole and four larger cells (macromeres) at the vegetal pole. The cleavage cavity is seen clearly during the above divisions. It under go 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> division. About 9 hours after commencement of the first division, the blastula is formed, which is a spherical mass with an outer layer of larger cells and a core of smaller inner cells. In next 9 hours time the blastula becomes the gastrula. At this stage, the egg flattens at the vegetal pole where the elliptical opening of the gastrula is situated and the whole embryo begins to show slight movements.

**2<sup>nd</sup> day:** - During 26-28 hrs of gastrulation a very slow jerking movement of the developing embryo was noticed. 28 hours after the beginning of the first cleavage the gastrula reaches the early trochophore stage. On either side of the vegetal pole lateral lobes bearing cilia have developed. At the animal pole a few vacuolated cells get raised from the surface of the embryo which indicates the position of the future shell (Lankester 1874).

**3<sup>rd</sup> day:** - The next 24 hrs of incubation period was passed for the attainment of adequate development as the late trochophore and shows further increase in size. The endodermal region which forms a large part of the embryo is seen through the ectoderm as a bunch of cells. The ventral region just below the mouth grows relatively faster and becomes the foot. Towards the latter part of the day the heart is seen pulsating and the embryo has now attained the early veliger stage. The mantle flap is seen at the posterior margin. By the end of the day, tentacles can be recognized as small projections at the anterolateral margin and the larva thus develops into a fully formed veliger.

**4<sup>th</sup> day:** - The embryo on; the fourth day of development is characterized by the presence of well developed eyes, tentacles and a creeping foot. The occurrence of these characters is generally recognized as showing the definite molluscan phase (Lankester, 1874). At the beginning of the 4<sup>th</sup> day of incubation embryo became slightly elongated.

**5<sup>th</sup> day:** - In external appearance, there is very little change except that the embryo has growth larger. The movement is less vigorous compared to the trochophore and veliger stages. After five days foot region sharply became demarcated from visceral mass.

**6<sup>th</sup> day:** - By this time the larva is found to have used up most of the nutritive material within the capsule. In *I. exustus* the hatching begins to take place either by the end of 6<sup>th</sup> day or early 7<sup>th</sup> day of development.

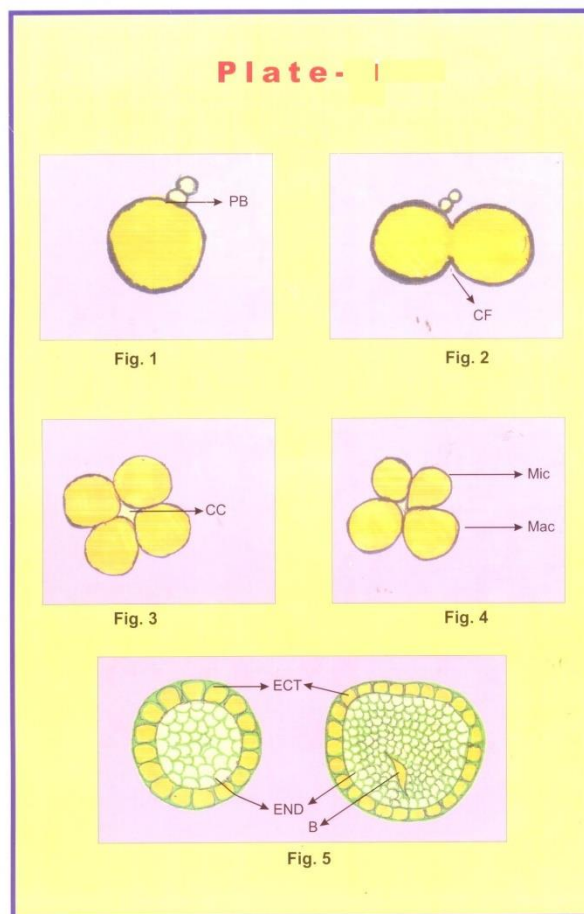
The egg takes usually 8-10 days to come out from the egg capsule. Naturally the egg capsule becomes tough after drying. Just before hatching the developing embryo uses its radula to procure its escape from the tough membrane. At first the radular action is random and without apparent function. At the time of commencement of hatching, the embryo of *I. exustus* rasps the inner wall of the capsule with the radula, which by this time has developed fully. The rasping movements are at random to begin with, but subsequently occur on the outer aspect of the wall only, where a small rupture appears. Following this, the embryo exhibits rating movements all around the capsule and enlarges the initial opening by further action of the radula. The head lobe and foot are pushed out gradually through this aperture. A large opening has now been formed and ultimately the floor of the radula. The head lobe and foot are pushed out gradually through this aperture. A large opening has now been formed and ultimately the floor of the capsule is ripped open. The embryo then pushes itself into the surrounding jelly with the foot and head lobe extended. The movement within the jelly is affected by alternate contraction and expansion of foot and head lobe and eventually the embryo pushes itself out of the mass.

After the initial tear the animal probes a small opening and the young snail glides away on its foot. The process of hatching takes place when the stored embryonic food supply is exhausted. It was found that hatching in *I. exustus* does not occur by means of enzymes or in the osmotic manner as in other pulmonates, but purely by mechanical means. The newly hatched larvae which are yellowish in color and show fine striations on their surfaces measuring 0.55mm across the shell. The pseudo branch is well developed which is a characteristic feature of the members of the family-planorbidae (Pennak 1953).

## Discussion

Breeding season of *I. exustus* was found in June-October. July is found to be the high breeding month of *I. exustus*. The climate in the area of Maharashtra is characterized by seasonal rainfall from June to October and peak in the month of July and less from November. The rainfall during the period from July to October 2013 was maximum and temperature is constant. The number of capsules varied in each egg mass. In *I. exustus*, they were generally 7-60. Normally each capsule contained only one egg. The eggs were suspended within a nutritive fluid which provided nourishment for the growth of the embryo. In *I. exustus* the egg was found to be composed of

capsular membrane, jelly and capsular fluid. Embryos of pulmonate gastropods undergo direct development from fertilized egg to juvenile snail inside egg capsules filled with albuminous perivitelline fluid, also known as capsular fluid (CF). The CF provides the nutrition required for normal development of embryos, which is a secretory product of the albumen gland (Raven 1958, Morrill et al 1964, Heras et al 1998).



**Plate I Diagrammatic representation of developing embryo of *I. exustus***

B – Blastopore

CC – Cleavage cavity

CF – Cleavage furrow



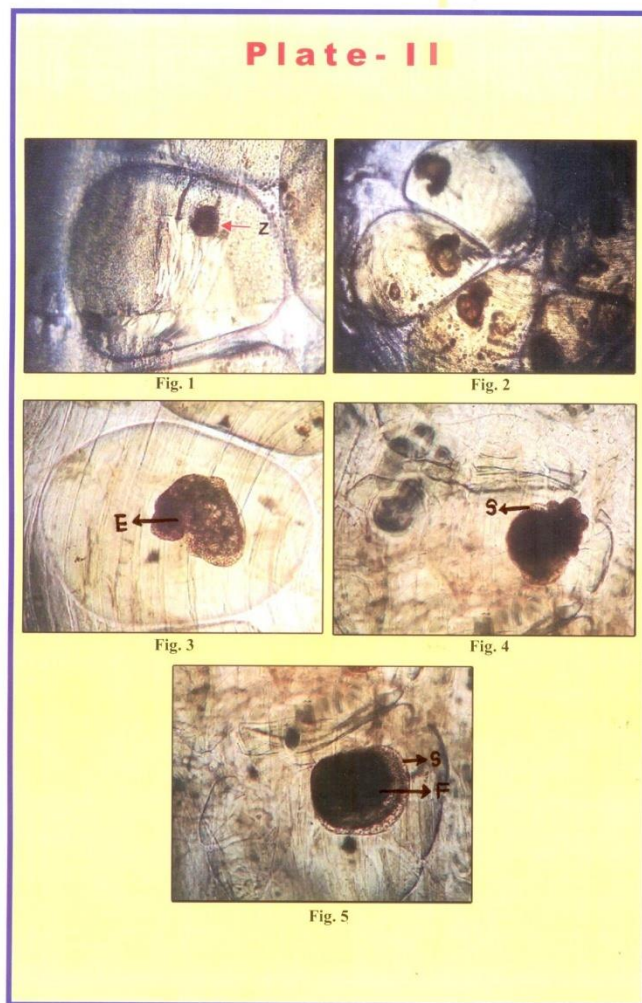
ECT – Ectoderm

END – Endoderm

Mac – Macromere

Mic – Micromere

PB – Polar bodies



**Plate II Micrograph of the embryological development of *I. exustus***

S – Shell

F – Foot

E – Eye

Z - Zygote

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