CHAPTER 4

DEVELOPMENTAL BIOLOGY OF PRISTOLEPIS MARGINATA JERDON, 1849

4.1 INTRODUCTION

Studies on evolution of development have large scale comparisons of diverging taxa in a known phylogenetic context to uncover the developmental pathways of animals. Galis and Sinervo (2002) explained the similarity of early embryos resulting from a variety of developmental constrains (eg., teratogens). Studies on early life stages of fishes are important because the requirement of young fish changes rapidly as a function of age. The embryonic and larval development studies provide useful information for the successful rearing of larvae. Blaxter (1974) suggested that fish eggs and larvae are useful for estimating the fish stock. Most authors accept the division of fish development into five periods; embryo, larva, juvenile, adult and senescence (Kovac and Copp, 1996). The meaning of the term larva, however, has been used rather
vaguely. Definition of larva and larval period involve subtle differences (Rass, 1946; Balon, 1971; Lange et al., 1972; Snyder, 1976; Kendall et al., 1984; Balon, 1990 and Penaz, 2001). Larvae represent temporary intervals inserted in the developmental sequence primarily in order to complete the nutrient provision needed for formation of the definitive phenotype (Balon, 1986 and 1989). In general, two types of definition of larvae emerge: one is characterization by morphological attributes, the other by ecological features (Wake and Hall, 1999). Reports on different developmental stages of fishes are given in Fig. 4.1. The length of development period is highly dependent on temperature and species specificity (Kendall et al., 1984). The hatched out young ones are called yolked larvae. Apart from the adult mode of life, the larvae of most of the fishes share some common characters. Some particular transition stages are evident in most fish larval development. Transition from one stage to another is characterised by a group of characters which represent its genetics. Ditty et al. (2003) have very well described it in gobiid fishes. Kendall et al. (1984) has detailed the early life stages of fish larvae into yolk sac larva, pre-flexion larva, flexion and post-flexion, and the larval phase will be followed by juvenile stage. The larval stages are characterised by feeble swimming movements and rely on reserve food in the form of yolk. Non-functional digestive system, poorly developed musculature and nervous systems are other characters. At the time of complete utilization of yolk, in most cases, the mouth will be functional and the larva starts external feeding. The food it usually intakes, depends upon the mouth size of the larvae, which is a species specific character and has much significance in the development of larval rearing techniques.
Juvenile period is characterised by appearance of characteristic adult-like features as pigmented patterns, and some species specific fine structures. As the growth progressed rays in fins and scales on the skin will become adult-like, and the complete ossification also occurred. The overall body shape also changed.

Different modes of larval development are evident in fishes. It depends upon the fact that to which taxa it belonged to. The development seen in egg scatterers is different from fishes showing parental care. The mouth brooders release very few eggs with big size. The live bearers produce juveniles directly bypassing the larval stage (Wourms, 1981). Here *P. marginata* is found to be a fish showing strong parental care. It showed all aspects of parental care like territory finding and establishment, aggressive defence reactions, nest building, caring egg and young ones. Jones *et al.* (1978), has categorised the sequences, evident in different stages of development of bony fishes as below.

- **Egg**: the embryonic stage after spawning up to hatching inside the egg envelope.
  - **Yolk-sac larva**: stage between hatching and absorption of yolk
  - **Larva**: stage between absorption of yolk and acquisition of minimum adult fin ray complement
  - **Juvenile**: stage between acquisition of minimum adult fin ray complement and sexual maturity with assumption of adult body form (prejuvenile: with assumption of incomplete adult body form)
  - **Adult**: sexually mature
Different Terminologies in connection with Developmental stages

Different scientists in different times have given different terminologies to different phases in the way development bony fishes. It includes scientists like Hubbs (1943), Nikolsky (1963), Balon (1975), Snyder (1981) etc. It has been diagrammatically represented by Kendall *et al.* (1984), as in Fig. 4.1.

Fig. 4.1

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<th>END POINT EVENTS</th>
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<th>Blastopore closure</th>
<th>Tail bud forms</th>
<th>Matching</th>
<th>Yolk sac absorbed</th>
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4.2 MATERIALS AND METHODS

Pristolepis marginata was bred under captive conditions as mentioned in the chapter 3 Part II. The fertilized eggs were collected from the experimental breeding tanks. The eggs were found attached to the pebbles of the nest. Eggs were carefully detached and washed. The fertilized eggs of P. marginata were found to be slightly adhesive. So the collected eggs were attached with dust particles which prevented the internal structures to be observed clearly. The dust particles attached to the eggs were removed by application of potassium chloride (0.02 %) and by using fine drawing filter mesh of 0.25 mm. The method was adapted from Perumalsamy (2006). The studies were conducted in ambient temperature conditions of 28-29 °C. Dissolved oxygen and pH were recorded throughout the experimental period.

Observations were made under trinocular microscope with camera Nikon 90. The fertilized eggs were observed continuously until hatching. The hatched out yolk sac larvae were observed and photographed at an interval of one hour until 10 hours. Further observations were made at an interval of five hours until the larva became 72 hours old. After that, observations made on a daily basis up to the completion of juvenile stage.

Embryonic and larval development

Eggs were sampled immediately after fertilization and thereafter at every one hour intervals. Eggs sampled were fixed in a neutral solution or buffered and different embryonic stages were observed within 4 hours (Fermin, 1991) under compound microscope, measurements of standard length were made using ocular micrometer.
Fertilized and unfertilized eggs

Fertilized and unfertilized eggs were distinguished accordingly to the nature of colour changes, adhesive properties and first polar body extrusion.

4.3 RESULTS

4.3.1 Description of fertilized eggs

The fertilized egg consisted of three regions, the inner oil globule region, the middle yolk portion and the outer cytoplasmic region. The yolk filled the entire space within the egg membrane and it was surrounded by a uniform layer of cytoplasm except at the animal pole where the germ disk was formed. The fertilized eggs of *Pristolepis marginata* were double walled, spherical and demersal, even though it contained a small oil globule of 0.2 mm in diameter. The eggs proper were enclosed inner and outer side with smooth and transparent vitelline membrane. The outer egg membrane is zona radiate, which is heavily laid with fine particles of silt and sand suggesting its adhesive quality. The yolk was brownish yellow in colour. The diameter of the fully swollen fertilized eggs varied from 1.10 mm to 1.50 mm, with an average size of 1.30 mm. The yolk filled the entire space within the egg membrane and it was surrounded by a uniform layer of cytoplasm except at the animal pole where the germ disk was formed. The small oil globule of 0.2 mm in diameter was there, which was incapable of giving the egg enough buoyancy to float. Egg proper is yellowish brown in colour. Details of the development of fertilized eggs and average body measurement of the larval stage up to hatching were described.
The egg stage extended from the spawning up to hatching. Depending upon the stage of embryonic development it showed different phases. The most important feature of the egg stage is the egg envelope. It was found to be slightly adhesive in nature. The fertilized eggs were found attached to the pebbles with which the nest was made of. The eggs were perfectly round in outline. The peri-vitelline space was measured at 0.1 mm in diameter. The yolk mass has a diameter of 0.9 mm to 1.2 mm and was homogenous in nature. The surface of the egg envelope was smooth without any structures. The colour was ‘amber’ or yellowish brown. The larvae were hatched within 30 to 36 hrs after spawning at the ambient temperature prevailed, 28 to 29°C.

4.3.2 Embryonic and Larval development

The first cleavage that divided the blastodisc into two blastomeres, which occurred 30 min after fertilization. The cleavage was meroblastic, ie., the planes of cleavage were vertical. The second cleavage was perpendicular to the axis of the first division at 40 minutes, which was followed by the third cleavage at 45th minute and the 16 celled embryo was formed. Further meridional and vertical divisions of the four blastomeres produced many more but smaller blastomeres between 60th and 80th minutes after fertilization. This divisions resulted in the formation of morula, which looked like a cluster of eggs.

Blastula stage

At this stage segmentation has progressed considerably so that the fissures, incident to cell divisions, are greatly obliterated. It resulted in the dome shaped mass of
cells noticeably elevated over the general outline of the yolk mass to form the blastula. It happened between the 120\textsuperscript{th} and 140\textsuperscript{th} minutes after fertilization.

**Gastrula stage**

Due to rapid and repeated division, the blastoderm flattened and expanded slowly down towards the vegetal pole over the yolk mass to form the gastrula. Gastrulation occurred 3.5 to 4 hours after fertilization. It occurred by a flattening of the blastoderm followed by thickening and widening of a small portion of the blastodisc to form the embryonic shield. The blastodermal cells have completely invaginated the yolk and the germ ring was seen as a marginal vegetal poles. The median embryonic ridge condenses to form the keel. The embryonic ridge later becomes more distinct and got slightly raised up from the adjoining cell mass. A slight thickening of one end of the embryonic ridge, differentiated into cephalic region became visible by 4\textsuperscript{th} hour after fertilization.

**5.00 hrs old embryo**

The embryo appeared as a narrow thickening band encircling the yolk mass. Rudimentary optic vesicles were formed in greatly pronounced cephalic region of the embryo. The paired optic capsules made their appearance but otoliths have not yet been formed. Embryo started to develop myotomes, and portion of the head and tail of the embryo could also be identified. By seventh hour after fertilization three main parts of brain were distinctive and there was a lateral expansion of the prosencephalon to form
the optic bud. The pericardial sac was ill-defined. Pigment spots appeared over the yolk sac.

**Myotomes stage (8.00 hrs old) (Plate 4.1 Fig. 1)**

At 8.00 hours the heart rudiment had become more elongated and it exhibited twitches. It started pulsating very feebly which increased later and was erratic at times. No blood pigment was observed. The embryo had encircled about three quarters of the yolk and possessed 8 to 10 pairs of somites; the optic cup and optic vesicles were enlarged. An olfactory vesicle appeared for the first time at this stage as a small depression anterior to the optic vesicle. The auditory capsules appeared as very conspicuous oval depression with a thickened rim, enclosing two distinct circular concretions, the rudiments of the otoliths. Brain vesicle was formed at this stage.

At 13\(^{th}\) hour of fertilization there was elongation of pericardial cavity and the circulation of blood was visible. Blood flow was observed at the posterio-ventral direction. The brain and heart were further enlarged and melanophores on the yolk surface had increased. The somites were more closely packed. Heart beats became visible. From this stage onwards, further development entailed general increase in embryo size, gradual decrease in yolk size and differentiation of various organs.

**Newly hatched larva (Plate 4.1 Fig. 4)**

The newly hatched larva of *P. marginata* showed similarities towards characteristic cichlid larvae (El Zarka, 1956). It measured 2.1±0.3 mm in average total length. The heavy yolk sac of 1 mm diameter, was another noticeable feature. It lacked
functional mouth, eye pigment, and differentiated fins. Hubbs (1943) termed this stage of development of yolk sac larvae as ‘alevin’, which could be applied in this study also. A continuous median fin fold was the sole organ of locomotion and orientation. It had pale yellow coloured body; eyes were unpigmented. Mouth was not formed. Lens formation had advanced and otoliths were distinct. The larva is generally quiescent and remains at the bottom resting horizontally on its yolk.

**One day old larva (Plate 4.1 Fig. 5)**

At this stage the length of the larvae measured at 2.5±0.4 mm. size of the yolk sac was considerably decreased. Caudal fin began to separate. Alimentary tract was visible and extended towards 20th somite; but there was no trace of food pectoral fin appeared. A few faintly developed melanophores were seen in cephalic region. Lens also became pigmented at the same time. Melanophores started to appear on the neural crest region of the embryo. Blood flow and blood cells became clearly visible.

<table>
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<tr>
<th>Table 4.1 Embryogenesis and larval development of Common Catopra, <em>Pristolepis marginata</em></th>
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<tr>
<td><strong>Embryogenesis</strong></td>
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<td>16 blastomeres</td>
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<td>Morula stage</td>
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Blastula stage (dome shaped blastoderm) 02.00

Gastrula stage (germinal ring moves towards the vegital pole. Slightly thickening of one end of the embryonic ridge, differentialted cephalic region 03.30

Embryo appears as a narrow band encircling the yolk mass. Rudimentary optic vesicles found, paired optic capsules formed; otoliths have not yet formed. 04.00

The head and tail ends of the embryo could be identified; 14 myotomes were seen. 04.30

24 somites were found; the optic cup and optic vesicles were evident; Otoliths clearly visible; brain vesicle formed 05.00

30-36 somites found; notochord was in the process of formation; the gut as a straight tube is further developed as an extension up to 14 somites 06.00

Eye lens in the process of formation; otoliths fully developed; 36-42 somites found 8.00

42-46 somites found; fore, mid and hind brain lobes are differentiated; fin folds formed 10.00

Lens formed in the rudimentary eye; olfactory vesicles, heart formed; blood circulation started 14.00

Embryo hatched out into yolk sac larva, rupturing the egg envelope near the head region 24.00

**Larval and postlarval development**

Pale yellowish in colour; pigmented eyes; fin buds formed; mouth not yet formed 24.00 (After hatching)

Caudal fin begins to separate; faint pigmentation of eye; alimentary tract not formed; pectoral fin bud appeared 36.00
Caudal fin rays distinguished; mouth started to function 96.00

Head prominent; yolk sac remains as a rudiment; larvae commenced external feeding 120.00

Pectoral fin the form of flap; gill arches noticeable 120.00

Feed actively on zooplankton; dwells at the bottom zone of the rearing tank 120.00

Voracious feeding noticed; pectoral fins become stout 144.00

Resembles adult in all features except sexual maturity 30 days

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**Two days old larvae (Plate 4.1 Fig. 6)**

The larva increased in size with a mean total length of 3.1±0.3 mm, and a minimum and maximum of 2.7 and 3.3 respectively. The heart chambers were well differentiated and the rate of beat increased to 140 to 150 per minute.

**Three days old larvae (Plate 4.1 Fig. 7)**

On the third day of development the average total length of the larva became 3.5 mm. The larva showed swimming movements. The opercula and caudal fin rays were distinct at this stage. The lower jaw was vibrating intermittently.
Four days old larva

At this stage the larva measured between 3.4 mm and 3.8 mm in length. The yolk sac had reduced to 1 mm in diameter. Well developed pectoral fins were visible. Swimming movements became more co-ordinate. The head had straightened out and the dorsal and anal fins had taken shape.

Five days old larva

On the fifth day the mouth became functional and a very scanty yolk became remained in the yolk sac. The larvae grew to an average total length of 4.1 mm.

Six days old larva

By sixth day the total length reached up to 4.3 mm. The ventral fin began to differentiate. Caudal fin rays were distinguishable; melanophores started to move towards ventrally and laterally.

Post larval development (Plate 4.1 Fig. 8)

The transformation into post larval stage occurred at the end of the sixth day after hatching. The most important features at this point were the full absorption of yolk sac and the formation of a small oval shaped stomach. Larvae commenced exogenous feeding. Vent opening was found at about 23rd myotome; pigmentation of head was considerably pronounced. Pectoral fin is differentiated; gill and gill rakers with well developed gill lamellae were seen clearly. Gill flaps were well developed over the gills and flap movement were noticed. Larvae actively feed on zooplankton.
Twenty days old larva

At this stage larva becomes a juvenile. The colour is light greenish and body is fully covered with minute scales.

Thirty days old larva

At this stage, the fish resembled an adult. Mouth has well developed teeth. Juveniles were omnivorous in feeding habit. The developmental stage at hatching was influenced mainly by temperature and oxygen (Heming, 1982; Penaz et al., 1983 and Eckmann, 1987). And apart from this physical factors and predatory invertebrates are also responsible for high mortality. In the present study after hatching, hatchlings showed movements especially for escapism and hiding.

Yolk sac also played a major role after hatching; some fishes have remnants of yolk sac after post larval stages. Balon (1989) stated that it is of little consequence to young ones after hatching, but before transition to exogenous feeding, a free embryo, an eleuthero embryo or yolk sac larvae; what is imperative is recognition of a difference between direct ontogeny without a larvae and indirect ontogeny with larvae. Yolk absorption is a very slow process which depends on temperature (Snyder, 1976 and Kamler, 1992). According to Balon (1971) onset of exogenous feeding occurs over a wide range of developmental features and enormous physiological, ecological and behavioural significance (Kovac and Copp, 1996). The pattern of exogenous feeding occurrence depends upon the size of yolk sac and condition of animal body size (Burton, 1979 and Heyer et al., 2001). In the present study yolk sac was completely absorbed on
the 7th day of larval development (4.5 to 4.7 mm total length) and mouth opened on 1st day of post larval stage and feeding commenced in 5 hrs after the mouth was opened. The same pattern of larval feeding was also noticed in *Cichlasoma dimerus* (Baerends and Baerends-Van Roon, 1950). Disappearance of yolk sac has been observed during post larval stages and more mortality was recorded in this stage due to various factors.

**4.4 DISCUSSION**

*Pristolepis marginata*, is a nandid fish showing perfect parental care. It showed all aspects of parental care like territory finding and establishment, aggressive defence reactions, nest building, caring egg and young ones. It is also characterised by low fecundity, high survival rate, large egg size, which is characterised by members of the family cichlidae (Pandolfi *et al.*, 2009). It is shown that the family nandidae has high degree of genetic correlation with family cichlidae. (Baerends and Baerends-Van Roon, 1950).

The fertilized eggs of *P. marginata* possessed an oil globule of 0.2 mm in diameter. Studies also showed that the eggs are demersal and seen attached to the stones in the pebble nest. The size of the oil globule was not enough to give the eggs a buoyant nature. The oil globules would have provided energy for the developing embryo or served as a additional source of nutrients. The presence of oil globule is a feature in marine fishes as labrids, most carangids, mullids, lethrinids, clupeids, serranids (Kendall *et al.*, 1984). The first signs of pigmentation was found in the neural crest region at the end of two days’ growth.
Larval stage was marked by the period in between the time of hatching and attainment of complete fin ray counts and beginning of squamation. One of the most important events during the larval stage is flexion of the notochord that accompanied the hypochordal development of the homocercal caudal fin. Based on this Kendall *et al.* (1984), divided this period into preflexion, flexion and postflexion stages. These stages were very well evident in the course of development of *P. marginata*.

The pigmentation of the retina before the larvae hatched indicated that the eyes were functional before the larvae were capable of active swimming. The advantage is that, the larvae were able to detect and identify their food well in advance of the development of the mouth and alimentary canal. Same condition was observed by Omotosho (1987) in a cichlid fish, *Saratherodon niloticus*. Pigmentation was another important characteristic during larval development. Most species had a distinct pigmentation pattern during larval stages (Kendall *et al.*, 1984). This pattern was not stable but developed during ontogeny and disappeared shortly after squamation. In the present study stellate melanophores were recorded. During larval stage and after that they disappeared in the juvenile and later adult stages. Similar melanophore formation has been reported in the larvae of the perch *Perca fluviatilis* (Kendall *et al.*, 1984), which gradually disappeared in the later stages. Similar observations were reported in the larval stages of grayling *Thymallus thymallus* (Urho, 1996). The intensity of larval pigmentation varied depending upon the type of water body in which the larvae live (Urho, 1996). The size at hatching of *P. marginata* varied between 1.9 mm and 2.3 mm. According to de Ceichomski (1966) and Blaxter (1969), larval size at hatching period in many fish species is associated with egg size, incubation period and the time during
hatching period at which the larva hatched. Differences in larval size were probably due to variations in the egg size. At hatching, development of the digestive system was not completed, and the stomodeum was not perforated. This suggested that the larvae were living absolutely on the yolk until the yolk was completely absorbed. A particular period is needed for the complete development of the digestive tract and the opening of the stomodeum.

Hodges and Behre (1953) explained the considerable lapse of time between fertilization and the first cleavage as observed in the present study as the time required by the cytoplasm to concentrate at the surface of the yolk and move to the pole to form the blastodisc. When compared to the first cleavage the successive cleavages occur at rapid rates.
Plate 4.1 Developmental Stages of Pristolepis marginata Jerdon

Fig 1  Fertilized egg after 10 hrs
Fig 2  Fertilized egg after 15 hrs
Fig 3  Fertilized egg after 20 hrs
Fig 4  Newly hatched larva
Fig 5  Larva after 24 hrs
Fig 6  Larva after 48 hrs
Fig 7  Larva after 3 days
Fig 8  Larva after one week

EY: Eye, FF: Fin fold, FR: Fin rays, MY: Myomeres, OG: Oil globule, YO: Yolk,