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EMBRYONIC AND LARVAL DEVELOPMENT OF AN ORNAMENTAL FIGHTING FISH, *BETTA SPLENDENS* UNDER CAPTIVE CONDITION

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ABSTRACT : The present study was carried out to investigate the embryonic and larval development stages of an air breathing ornamental fish *Betta splendens*. To understand the breeding behaviour of *B. splendens* and to better characterize the embryonic development, since very few research has been done on *Betta* embryology. *B. splendens* embroyonic development was very rapid compared to other species. Essential features of development were early cell division during the first two hours, organ development starting at 9 hours, hatching at 26 hours and the fry becoming free swimming on the 3rd day post hatching. Temperature plays an important role during development of egg. Increased temperature results in faster development and shorter time to hatch. Little research has been done on *Betta* embryology. Therefore, to better understand and characterise the *Betta* embryology additional information and research seemed desirable. Air-breathing starts on the 3rd day post hatching and commenced regular air-breathing on the 8th day post hatching. Apparently, the labyrinthine organ developed between 3rd and 5th day.

Key words : *Betta splendens*, embroyonic development, cell division, hatching and labyrinthine organ.

INTRODUCTION

Aquarium keeping as hobby was started several hundred years ago in Eastern China. The ancient Rome was the first to keep ornamental fishes as pets at home. Keeping ornamental fish in aquarium as hobby became popular in England and Scotland in the eighteen century. It has been realized that aquarium keeping is not only a means of recreation but also a source of earning (Ayyappan, 2006).

Today *Betta splendens* is among the most beautiful and fascinating of all aquarium fish. It attracts many novice aquarium fish holders with their long flowing fins and various unique colors. Males of *B. Splendens* have the most brilliant colors and longest fins (Linke, 1980; Mills, 1986).

Development of the embryo is rapid, with hatching occurring within 48 hours. Rugh (1962) reports that in 10 to 20 minutes after matting, fertile eggs will appear translucent, and unfertilized eggs will appear opaque, although an occasional fertile egg will appear opaque.

Knight (1963) described early embryonic development of the rainbow trout, *Salmo gairdneri*. Excellent photomicrographs and a very thorough explanation were provided. Embryos were described in relationship to time, but they were not staged.

Little research has been done on *Betta* embryology. An early report was that of Duijn (1938). His description began at 24 hours and continued until the young fish were free swimming (no time given). It included only seven photographs taken at various intervals of development. He described the threshing of the tail of the young fish which finally causes hatching by ripping the egg membrane. He pointed out the large size of the eye, which is typical even of mammals and mentioned that 24 hours after hatching, the fry leave the nest.

Groth (1970) reported that development of labyrinthine organ starts after one to two weeks after hatching. In contrast, Balasubramani and Pandian (2008) reported that labrinthine organ developed between 2nd and 5th day post-hatching.

With a view to above reasons, *B. splendens* plays a vital role among Indian fresh water ornamental fish trade. Therefore to understand the breeding behaviour of *B. splendens* and to better characterize the embryonic development, since very few research has been done on *Betta* embryology.

MATERIAL AND METHODS

The study was carried out in the Laboratory of Department of Fishery Biology and Resources Management, Faculty of Fishery Sciences, West Bengal

University of Animal and Fishery Sciences, Kolkata from January to September 2010. From the acclimatized fishes, healthy males and females were selected for breeding at the ratio of 1: 1. Brooders were collected from Gullif Street, near Syambazar, Kolkata, and West Bengal. The breeding experiment was conducted by natural method.

Characteristic of egg

The eggs were usually small and oval in shaped. Egg size depends on the availability and quality of food fed to the spanners. Eggs were translucent when first laid. Infertile eggs turned white and become opaque. The eggs were hatched between 24-36 hours after spawning. Male takes care of them in the nest for next three days until the yolk sac was absorbed and they can swim horizontally. After 3 days of hatching, when larvae started horizontal swimming.

Study of embryonic development

In order to study the embryonic developmental stages, eggs were collected from the spawning tank with a pipette as they dropped from the female. Then they were put on glass slide and observed under Trinocular phase contrast research microscope. Eggs from the same spawn were observed for the entire developmental period. Temperature in the aquarium was maintained at 29°C. Finally, records were made and photo micrographs were obtained of their process. Observations were made frequently during the first two hours, so all of the early cell divisions could be photographed. Then from 2-26 hrs the observations were made hourly.

Monitoring of air-breathing frequency and development of labyrinth organ

In this experiment we were monitoring air-breathing frequency of the fighting fish and observations were made on 1, 2, 3, 5 and 8 days post-hatching (dph). This included 3 beakers, each containing 100 ml of water with column height of 5 cm. The fry were observed individually for 1 hr and mean of three individuals were taken. The number of times the fish visited the surface was counted; these values were calculated for the number of visits/h.

Statistical analysis

The Mean, Standard error, statistical methods were used in the present study.

RESULTS AND DISCUSSION

Embryonic development

In the present study with reference to the age of the embryo rather than by stages. Following fertilization the egg absorb some water, become swollen and there is a separation of the chorion from the vitelline membrane leading to the formation of the perivitelline space. The chorion then hardens and, thus, serves to protect the embryo during the earliest stages of development. Before cell division started, the egg was about 1 millimeter in diameter. Cleavage involved only the animal hemisphere, so it was meroblastic and discoidal. The animal pole was distinguished only after cleavage started.

Embryos development during first hour

The first visible cell appeared about 10 minutes after fertilization (Plate 1; a). After about one-half hour (Plate 1; c) this cell has flattened. By three-fourths hour (Plate 1; d) the first cleavage occurred. The second cleavage occurred by 1 hour (Plate 1; e).

Embryos development during 1st hour through 8th hours

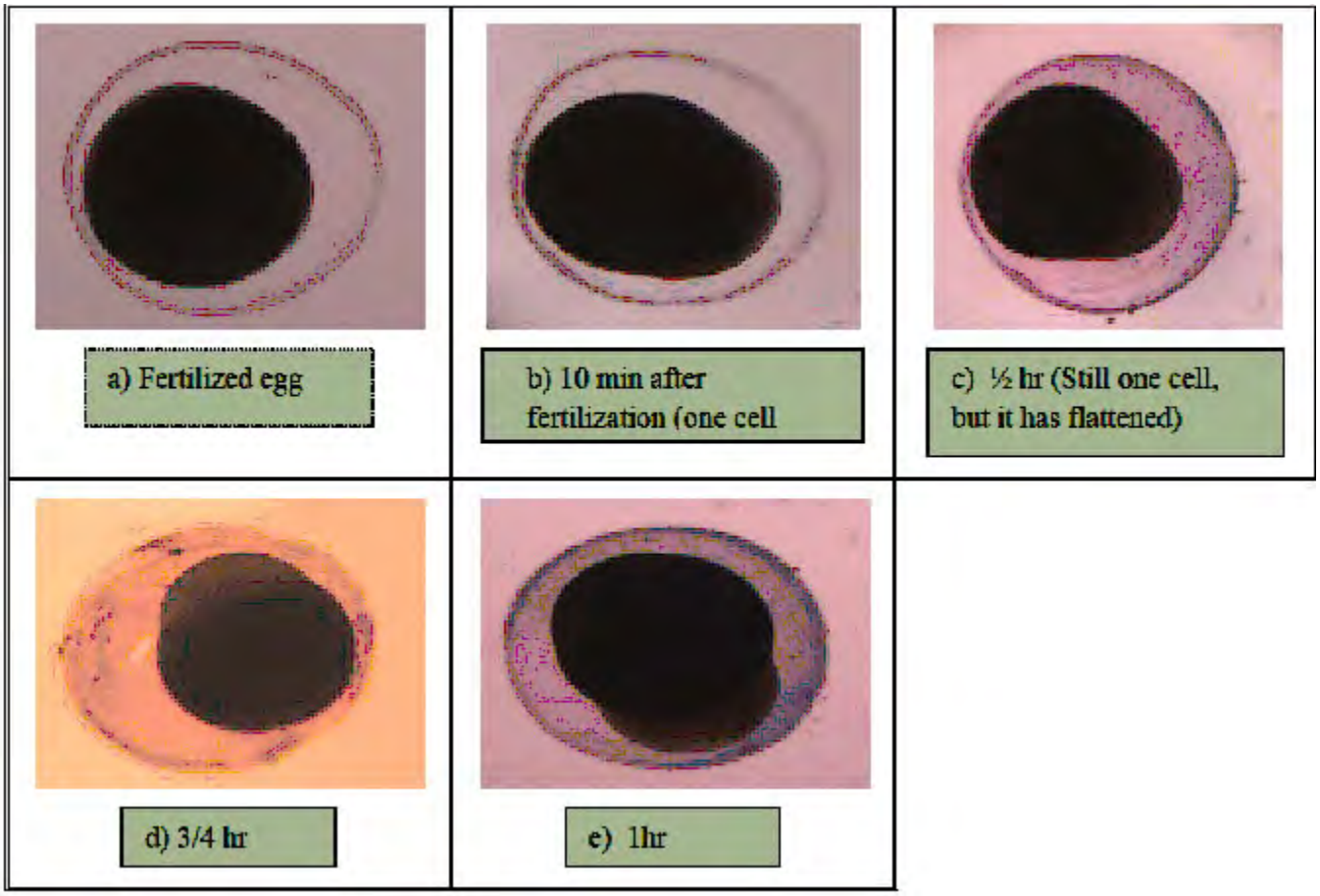
By one and one-half hours, the third cleavage (Plate 2; a) had occurred and followed by fourth cleavage on the second hours (Plate 2; b). From 3 hours to 8 hours (Plate 2; c to Plate 3; d) the germinal disc flattened and grew around the yolk. The cells extending around the yolk tended to squeeze it out of its spherical shape, and a distinctive yolk plug appeared at about 7 hours (Plate 3; c).By 8 hours (Plate 3; d) the germinal disc had fully encircled the yolk.

Embryos development during 9 hours through 12 hours

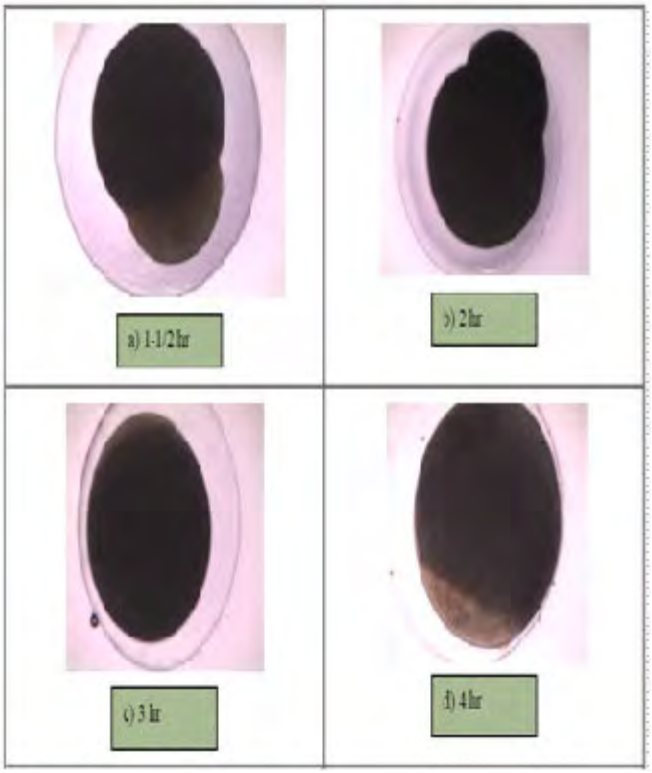
By 9 hours the cephalic end, neural keel and optic lobes was apparent (Plate 4; a). The tail region then starts to differentiate on 10 hours and starts extending around the yolk (Plate 4; b). During this hour the caudal knob (tail region) was still attached to the germinal layer.

Embryos development during 13 hours through 16 hours

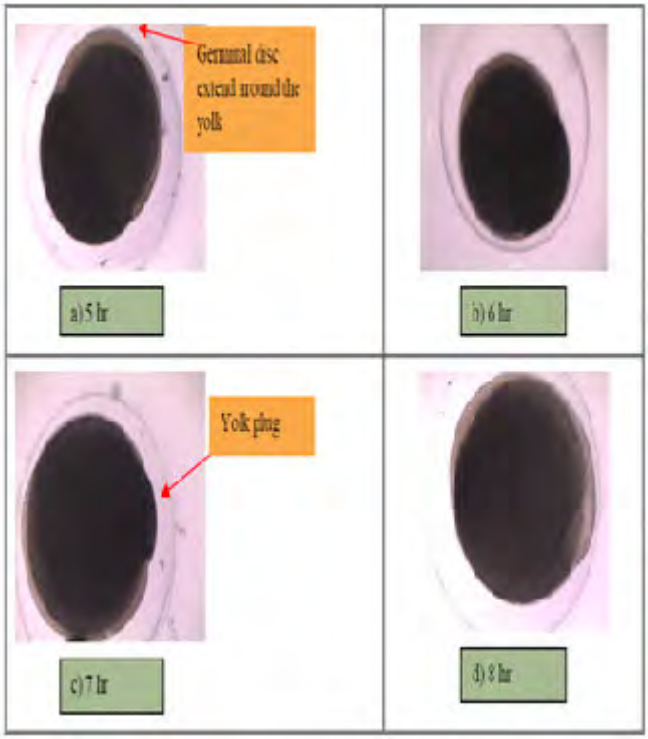
When the development reached 13 hours (Plate 5; a) the heart beat was first observed and the caudal knob had already started to grow free of the germinal layer. It was beating only 25 beats per minute. In one hour's time (Plate 5; b) the heart rate made a noticeable increase to 95 beats per minute. Somites were noticeable and eye lenses were distinct in this hour of development. The blood flow was also observed in the caudal region as it passed from the dorsal artery through capillaries between the somites into the ventral vein and back into the heart. From 15 hours (Plate 5; c) the embryo starts growing and occupying the previtelline space. By 16 hours (Plate; d), the first chromatophores were appearing as dark spots on the dorsal surface. The heart rate had increased to 130 beats per minute.



(Plate 1)



(Plate 2)

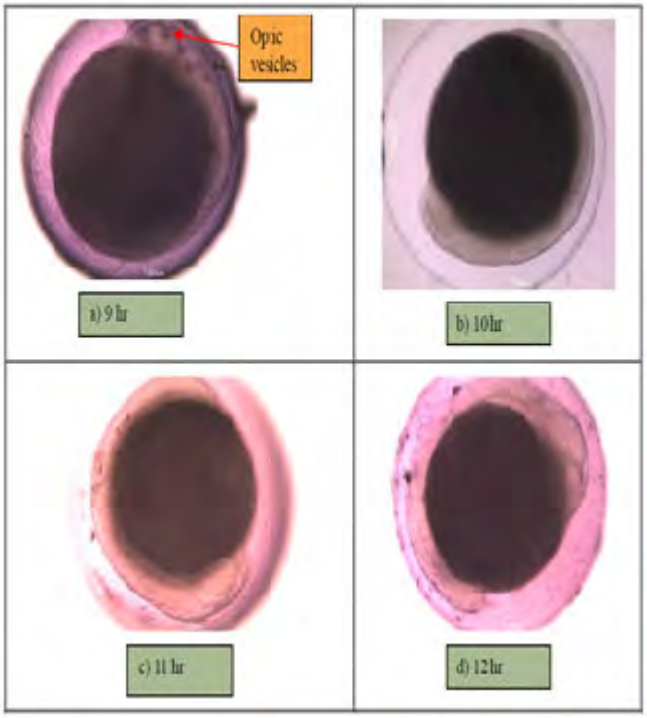


(Plate 3)

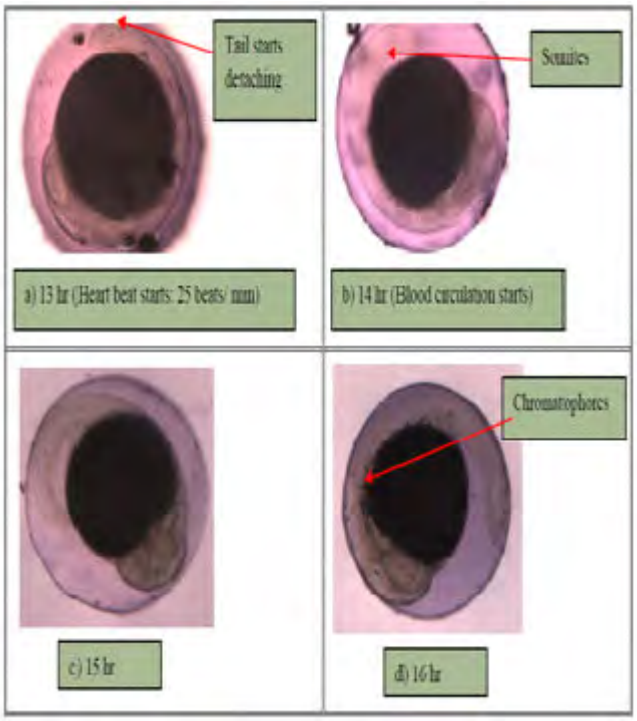
Plate 1 : Embryos development during first hour.

Plate 2 : Embryos development during 1st hour through 4th hours.

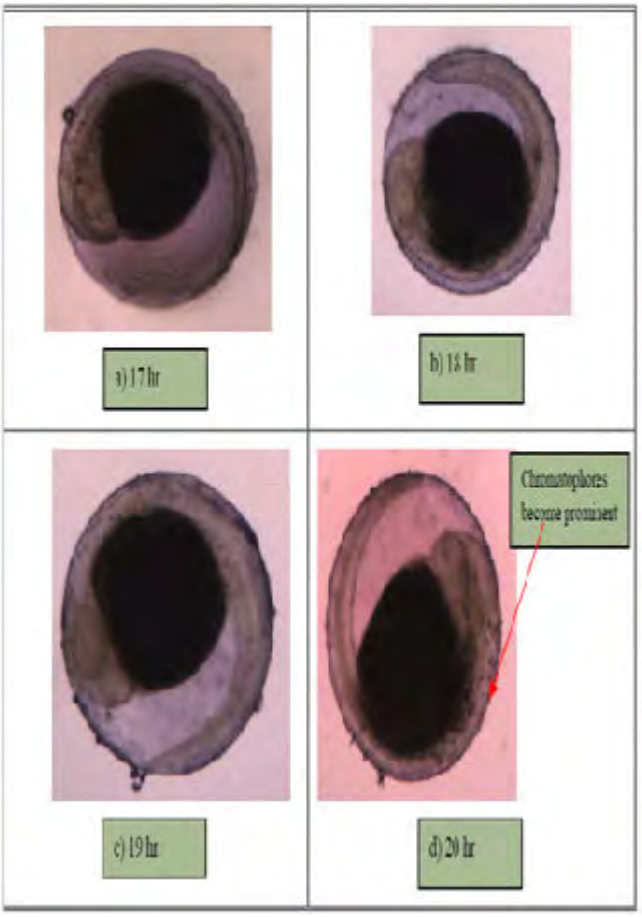
Plate 3 : Embryos development during 5th hours through 8th hours.



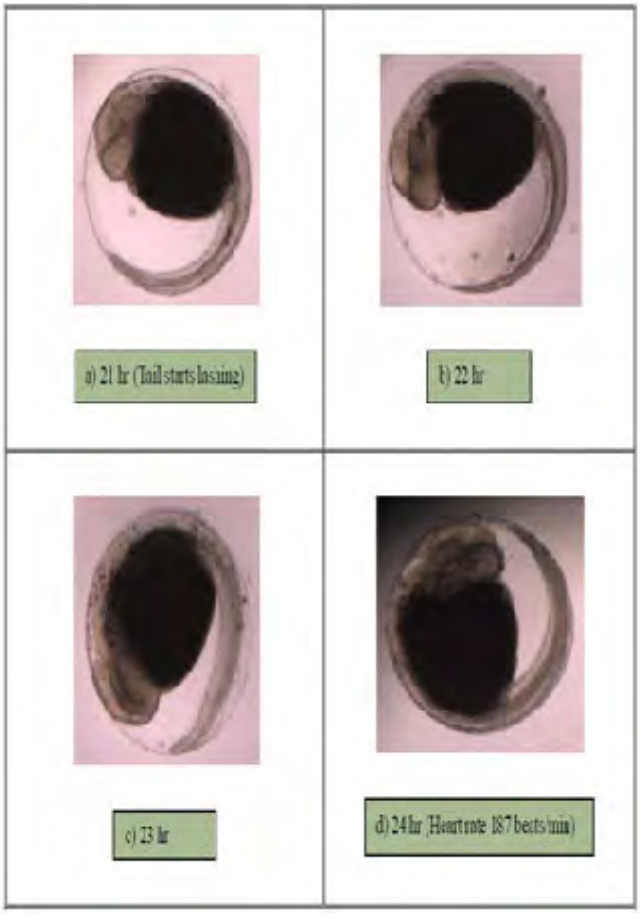
(Plate 4)



(Plate 5)

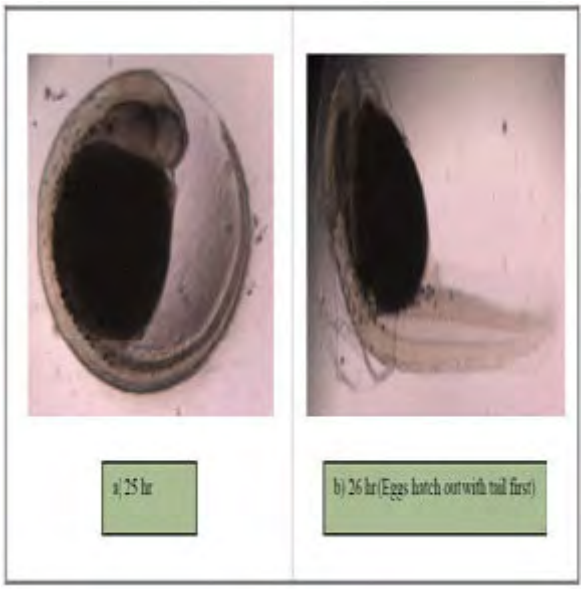


(Plate 6)

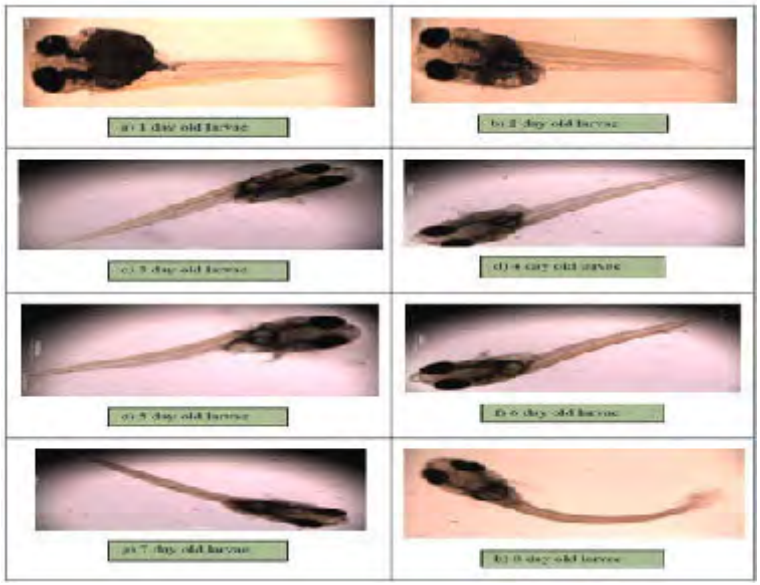


(Plate 7)

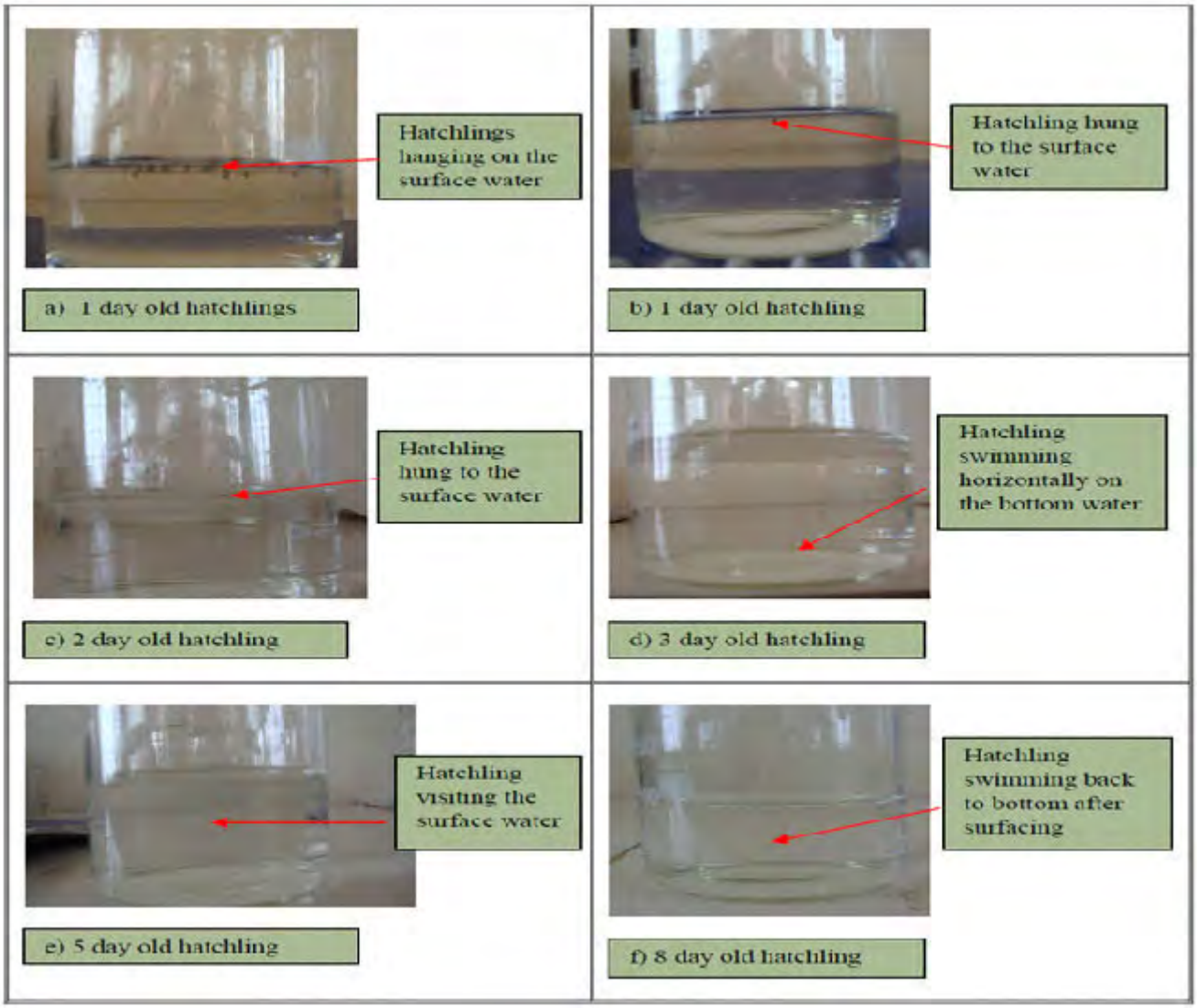
Plate 4 : Embryos development during 9th hours through 12th hours. **Plate 5:** Embryos development during 13th hours through 16th hours.
Plate 6: Embryos development during 17th hours through 20th hours. **Plate 7:** Embryos development during 21st hours through 24th hours.



(Plate 8)



(Plate 9)



(Plate 10)

Plate 8 : Embryos development during 25th hours through 26th hours. **Plate 9:** Larval development during 1st to 8th day old of larvae. **Plate 10:** Air- breathing frequency of 1st, 2nd, 3rd, 5th and 8th day post-hatching (dph)**.**

Table 1 : Air breathing frequency of 1st, 2nd, 3rd, 5th and 8th day post-hatching (dph). Each value represents the mean of three individuals and each individual were observed for 1 hour.

Air breathing frequency (times/h) on the 1st, 2nd, 3rd, 5th and 8th day post-hatching(dph)				
First day	**Second day**	**Third day**	**Fifth day**	**Eight day**
Hung to the surface	Hung to the surface	2±0.8	7±0.9	10±0.9

All values are the means with standard error of the mean (± SE).

Embryos development during 17th hours through 20th hours

During 17 hours to 20 hours (Plate 6; a to 6; d) the embryo had already occupied two-thirds to three-fourths over the previtelline space. The heart rate remained stable at 130 beats per minute during 17 hrs to 18 hrs. At 19 hours the embryos were very active and the heart rate increased to 155 beats per minute.

Embryos development during 21st hours through 26th hours

By 21 hours (Plate 7; a) lashing of tail were first observed and the embryo becomes very distinct. In the final hours of embryonic development i.e. at 23 to 25 hours, the growing embryo occupied the entire previtelline space (Plate 7; c to Plate 8; a). The heart rate again increased to 187 beats per minute. Embryo exhibited frequent twitching movement

Larval development during 1st to 8th day old of larvae

The hatchlings were transparent, reddish in colour and characterised by the presence of dark eye and dark oval yolk sac. The hatchlings ranged between 3.5 and 3.7 mm in length. Observation of the fry immediately after hatching in the aquarium disclosed that they were hanging tail down from the bubble nest. At times they fell from the nest. Then they were sucked up by the male and blown back into the nest. Sometimes by furious wiggling they made it back themselves.

When the hatchlings were one day old they hung to the surface. The size of the hatchlings ranged between 4.33 and 4.36 mm in length. The pectoral fin bud was moving periodically with no particular rhythm. Again it was observed that two day old larva still hung to the surface. But on the third day after hatching, the fry were no longer hanging from the bubble nest but were swimming in normal horizontal position and the yolk material was almost completely absorbed. By fourth day, the larvae started exogenous feeding and were fed with freshly prepared infusorians.

After 1 hour, the rate of embryonic development was quite slow which was completed within 0-26 hours (Plate 2 to 8). After 26 hours the larvae were hatched out with the yolk sac (Plate 8; b). Embryo with yolk sac was fully absorbed after the end of 3rd day of hatching (Plate 9; c). After yolk sac absorption the spawns were reared upto 8 days and fed with different kinds of natural or formulated feeds (Plate 9; c to 9 h). From 9th day onwards the larvae were considered as young ones of *Betta splendens*.

To study the embryonic development, the fertilized eggs were observed under Trinocular Phase contrast microscope repeatedly. From the study, it was found that after water hardening only the cleavage started at the animal pole. It was also observed that the cleavage (embryonic development) was rapid upto 1 hour (Plate 1: a, b, c, d, and e).

Groth (1970) also reported that development of *Betta* embryo is very rapid during the first hour and slows down after 1 hour, with hatching occurring within 48 hours when temperature was 26°C. According to Groth (1970), the development of many eggs was timed and stages appeared at the same hours consistently. The relatively greater consistency of *Betta* may be only apparent. Development in 2 days rather than 23 makes an hour of trout development correspond to about 5 minutes in *Bettas*. Knight (1963) noted that trout eggs varied markedly during the first few days of development. Temperature is one of the most important factors influencing the rate of development of eggs and fry (Herzig and Winkler 1986). It has been described for many species that increased temperature within an optimal range results in faster development and shorter time to hatch. In the present study, temperature was maintained at 29°C and eggs hatch out at 26 hrs. Groth (1970) reported that embryos of *B. splendens* start hatching by 44 hrs when temperature was 26°C. He also stated that metabolic rates of fish were dependent on environmental temperature and embryonic development might be varied by altering the water temperature.

Little research has been done on embryology of *Betta*. An early report was that of Van Duijn (1938). His description began at 24 hours and continued until the young fish were free swimming (no time given). It included only seven photographs taken at various intervals of development. He described the threshing of the tail of the young fish which finally causes hatching by ripping the egg membrane. He pointed out the large size of the eye, which is typical even of mammals and mentioned that 24 hours after hatching, the fry leave the nest. A photographic essay on *Betta* embryology by Daugs (1967) appeared in a hobby publication. No information was

supplied concerning technical aspects of his work. Cohen (1962) observed developing *Betta* embryos from the time of fertilization through 96 hours. She made a comparison with the Zebra fish, *Brachydanio reric* and found that the development of these two species closely paralleled each other for the first 24 hours. Her report was not illustrated. Therefore, to better understand the *Betta* embryology additional information and research seemed desirable.

Air-breathing frequency and development of labyrinthine organ

During the present study, it was observed that on the first and second day post hatching (dph), the hatchlings hung to the surface (Plate 10; a, b & c). But on the 3rd day, the hatchling no longer hung to the surface and was swimming in normal horizontal position (Plate 10; d). The number of times 3 day old hatchling visited the surface water to breathe air was 2 to 3 times/h (Table 10; e). On the 5th and 8th day, this air-breathing frequency has increased to 7 to 8 times/h and 10 to 11times/h respectively (Table 10; f). Apparently, we can assume that the air-breathing labyrinth organ starts developing between the third and fifth day post-hatching (dph).

Vivekanandan (1977) traced the ontogenetic development of surfacing behaviour in the obligatory air breathing murrel, *Channa striatus*, and showed that the fish did notcome to the surface on hatching, but began to regularly breathe air when it attained a body weight of 750 mg. Groth (1970) and Ayyappan (2006) reported that development of labyrinthine organ starts after one to two weeks after hatching. In contrast, Balasubramani and Pandian (2008) reported that labyrinth organ starts developing between 2nd and 5th day post-hatching which was similar and supports this study. However, a detail study is required to clarify the controversary of Groth (1970) and Ayyappan (2006).

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