STUDY ON THE EMBRYONIC AND LARVAL DEVELOPMENT OF AN ENDANGERED SPECIES OF BATA (*Labeo bata)*

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ABSTRACT

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The embryonic and larval development of critically endangered local Bata (*Labeo bata*) was studied at the hatchery of the Field Laboratory Complex, Faculty of Fisheries, Water Quality Laboratory of the Department of Fisheries Management and Field Fertility Clinic Laboratory of the Department of Surgery and Obstetrics, Bangladesh Agricultural University (BAU), Mymensingh during April 2007 to July 2008. This study presents preliminary observations on the embryonic and larval development of *Labeo bata* under laboratory conditions. The brood fish of *L. bata* was collected from the Jamuna river of Sirajgonj district for breeding purpose. The eggs were obtained after reproductive induction. At fertilization; the eggs were 0.7 mm in diameter. Samples were taken every 10 minutes interval till completion of morula and then every 1 hour interval up to hatching. After hatching, newly hatched larvae were observed daily until the attainment of the fingerling stage. The eggs presented brownish-yellow coloration. They were spherical, demersal and adhesive. The stages of embryonic development observed with cleavage, followed by blastula, morula, early gastrula, middle gastrula, late gastrula and until hatching of non-pigmented larvae which displayed total average length of 2.0±0.01 mm, 20 hours after fertilization. First cleavage was recorded within 45 minutes after fertilization and the embryonic rudiments of developing eggs appeared at 10 hours at 27.0°C-31.0°C. The yolk sac was completely absorbed at 66 hours during embryonic development on attainment of 6.2±0.02 mm total length. At the same time the digestive system became fully developed and the larvae searched for feeding.

Key words: *Labeo bata*, embryonic development, first feeding time

INTRODUCTION

Labeo bata, a minor carp, is well known for its excellent taste. It is one of the most popular and favourite food fishes among minor carps and it has high market value for its excellent taste. This species was abundantly available in Bangladesh in the past. However, due to over exploitation and various ecological changes in natural ecosystem, the species is on the verge of extinction. Recent estimate suggests that world wide 20% of all fresh water species are extinct, endangered or vulnerable (Moyle and Leidy, 1992). As a result fish stocks particularly those dwelling in inland open water areas has gradually become endangered (Hussin and Mazid, 2001). Among 266 species, 14 are going to be extinct, condition of 12 has been severely deteriorated and 28 of them is critically endangered or somewhat endangered freshwater fish species, *L. bata* is one of them (IUCN, 2000).

Considering the enormous importance of Bata, information on the early life history of a fish is very important for the optimization of its large-scale seed production, culture and management. Therefore, this study was carried out to highlight some aspects of the early life history of endangered *Labeo bata* (embryonic and larval stages), the developmental biological clock and the first feeding time of *Labeo bata* in relation to various time interval and larval rearing

MATERIALS AND METHODS

The experiment was conducted in the hatchery of the Field Laboratory Complex, Faculty of Fisheries, Water Quality Laboratory of the Department of Fisheries Management and Field Fertility Clinic Laboratory of the Department of surgery and obstetrics, Bangladesh Agricultural University (BAU), Mymensingh during April 2007 to July 2008. Specimens of local bata *(Labeo bata)* were induced to spawn through hypophysation with PG extract at the dose of 5.5 mg PG/kg body weight in the case of female spawners and male fishes were injected with PG extract at the rate of 2.0 mg/kg body weight. Then the eggs were fertilized with normal milt.

The fertilized eggs were washed with fertilization solution (4g NaCl and 3g Urea per liter water) and Tannin solution (0.5 g/l water) for several times to remove stickiness and finally transferred into hatching jars with continuous water circulation. The developing stages of *L. bata* had been observed at every ten minutes interval till the completion of morula and then every one hour interval up to hatching. The eggs were preserved into 70% ethanol solution for further study. Early developmental stages were studied under a stereomicroscope (Olympus, BX41 Research Stereo).

Developing stages had been studied continuously until the embryo started twisting movement, which took from 18 to 20 hours. For clear observation, individuals were temporarily stained with methylene blue. The specimens were measured by placing them over a Petridish having 1.0 mm graph paper at the bottom. Embryonic and larval stages were examined at 27.0°C to 31.0°C temperature. Five to ten specimens were used to describe each stage.

RESULTS AND DISCUSSION

Embryonic Development: A short description of embryonic development is presented in Table 1. The fertilized eggs were found adhesive, sticky, demersal and brownish-yellow in color. The average diameter of the egg was 0.8±0.01 mm. Slight swelling was observed immediately after the fertilized eggs. The fertilized eggs had a spot (blastodisc) on one pole and were readily recognizable through naked eye. The blastodisc was divided into two distinct cells by vertical cleavage within 45 minutes after fertilization (Figure 1D) and after 55 minutes a second cleavage was observed forming four cells (Figure 1E). Third cleavage forming eight cells was recorded after 80 minutes of second cleavage at 28.5°C (Figure 1F). The 16 cell stage developed within 90-110 minutes (Figure 1G). Subsequent cleavage increased cell number (Figure 1H) and reached morula stage (Fig 1I) within four hours and 30 minutes of fertilized. A cap like structure was seen over the animal pole, which gradually increased in size (Figure 1J).

The blastoderm started invading the yolk by spreading over it in the form of a thin layer (Figure 1K). The formation of germinal ring around yolk was clearly visible and that about half of the yolk occupied by blastoderm in (Figure 1L). Blastoderm coverd ¾ th of the yolk and embryonic shield was clearly visible and appearance of optic rudiment in gastrulation stage (Figure 1M). The total completion time of this stage was form 8.30 hours to 12.00 hours.

The yolk plug was identified by the completed invasion of the yolk within 14.00 hours by gradual spreading over the germ layer. At this stage the head and tail end of the embryo was clear and distinguishable (Figure 1N). The embryo was elongated and encircled the yolk materials. Both tail head ends were clearly differentiated (Fig 1O). From this time embryo started occasional twisting movement. The tail gradually detached from the yolk mass. This stage was obtained in 18-20 hours (Figure 1P). The twisting movements, which gradually became vigorous and egg-capsules were weakened and ruptured. The embryos ruptured the egg shell by the continuous movement. Hatching took place at 31°C. Larvae emerged with its tail portion first in 18-20 hours after fertilization. Hatching was continued for two hours because the entire embryo did not hatch out at a time. The newly hatched spawn measured 2.0±0.01 mm just after hatching (Figure 1P).

Hatching: Newly hatched larvae (2.5±0.05 mm) (Figure 2A) were slender, straight and transparent, gradually tapering towards the tail. Larvae silver in color and the yolk sac attached to the body. The hearts of the larvae were functional in between head and the anterior margins of the yolk.

Two hour post hatching (Figure 2B): The length of the larvae was 2.6±0.05 mm. Larvae turns into silver in color, Yolk sac still remained attached to the body, larvae slender, transparent showing internal organs.

Four hour post hatching (Figure 2C): The total length was measured 2.7±0.05 mm. Body of the larvae becomes more transparent. Head and body laterally compressed and yolk sac partially decreased.

Six hour post hatching (Figure 2D): Melanosphores appeared on the head, around or on the yolk sac. The anterior part began to thicken and stronger. The length of larvae was 2.8±0.02 mm.

Eight hour post hatching (Figure 2E): The yolk sac partially reduced. The tail becomes thickened. The length of larvae was 2.9±0.01 mm.

Ten hour post hatching (Figure 2F): A tubular pulsating heart appeared. Yolk sac reduced. Eye and anus slightly visible. Intestine also appeared. The length of larvae was 2.9±0.09 mm.

Twelve hour post hatching (Figure 2G): Chromatophores seen in the eye only. Ventral embryonic fin fold more prominent. Pectoral fin bud appeared. The larvae increased 3.0±0.05 mm in size.

Sixteen hour post hatching (Figure 2H): More melanosphores appeared on the head and body. Brain was not differentiate from body. Interior part of the yolk globular in shape. The length of larvae was 3.2±0.02 mm.

Twenty hour post hatching (Figure 2I): The total length of the larvae measured 3.3±0.02 mm. Newly chromatophores appeared above eyes. The Yolk sac became thinner.

Twenty four hour post hatching (Figure 2J): Operculum becomes visible and dark eyes pigmented. Myomeres were partially visible. Prominent pectoral and pelvic fins fold. At this stage, the length of the larvae was 4.4±0.01 mm.

Thirty four hour post hatching (Figure 2K): The total length of the larvae measured 5.3±0.01 mm. Myomeres were visible. Color of the larvae changed to silver- yellowish and mouth cleft formed.

Thirty six hour post hatching (Figure 2L): The eyes increased in size and pigmented. Pectoral fin more prominent. Brain lobe visible mouth cleft formed. The larvae reached to 5.5±0.05 mm in size.

Thirty eight hour post hatching (Figure 2M): Pectoral fin fold well developed. Mouth cleft more prominent. The eyes increased in size. At this stage, the length of the larvae was 5.6±0.02 mm.

Forty four hour post hatching (Figure 2N): Opercula fold appeared. Brain lobe clearly and mouth cleft easily distinguished. The heart started functioning. The larvae reached to 5.8±0.01 mm in size.

Forty eight hour post hatching (Figure 2O): The total length of the larvae measured 5.9±0.02 mm. Yolk sac convex interiorly and air bladder distinct. Large black chromatophores observed on head. Gills prominent and air bladder elliptical in size.

Fifty eight hour post hatching (Figure 2P): Eyes fully pigmented. Pectoral fin bud more pronounced. The jaws more pigmented. At this stage, the length of the larvae was 6.0±0.01 mm.

Sixty six hour post hatching (Figure 2Q): The total length of the larvae measured 6.2±0.02 mm. Brain lobe fully visible. Yolk sac completely disappeared and larvae started feeding.

Seventy two hour post hatching (Figure 2R): Myomere clearly visible. The larvae were silver-blackish and transparent in color. Larvae swim actively. The larvae reached to 6.5±0.02 mm in size.

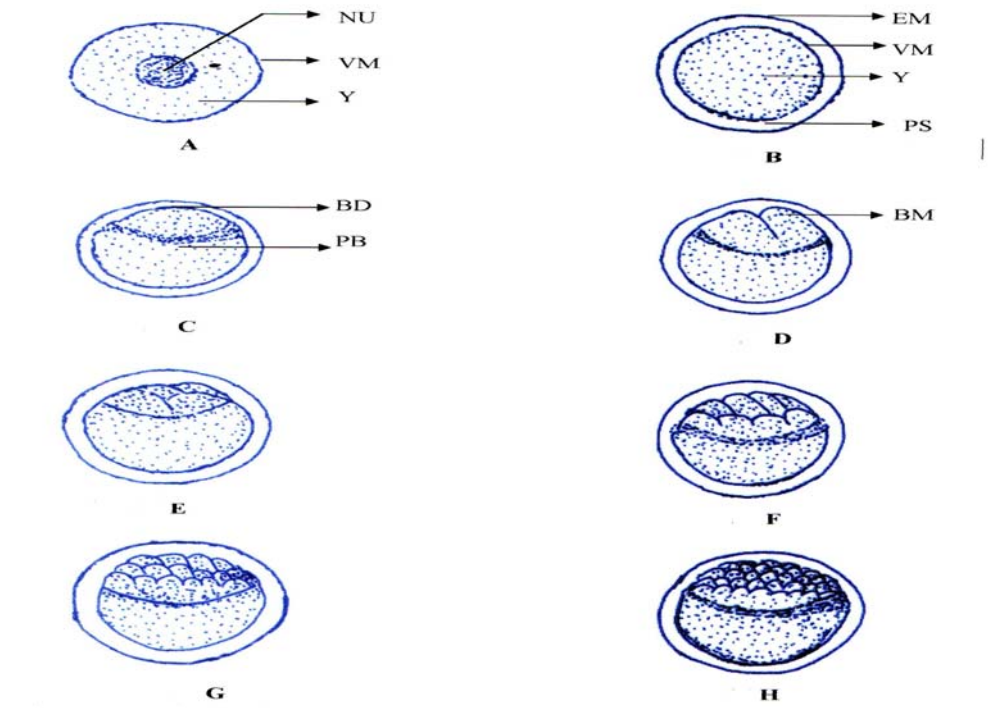


Figure 1. Unfertilized and fertilized eggs with different developmental stages

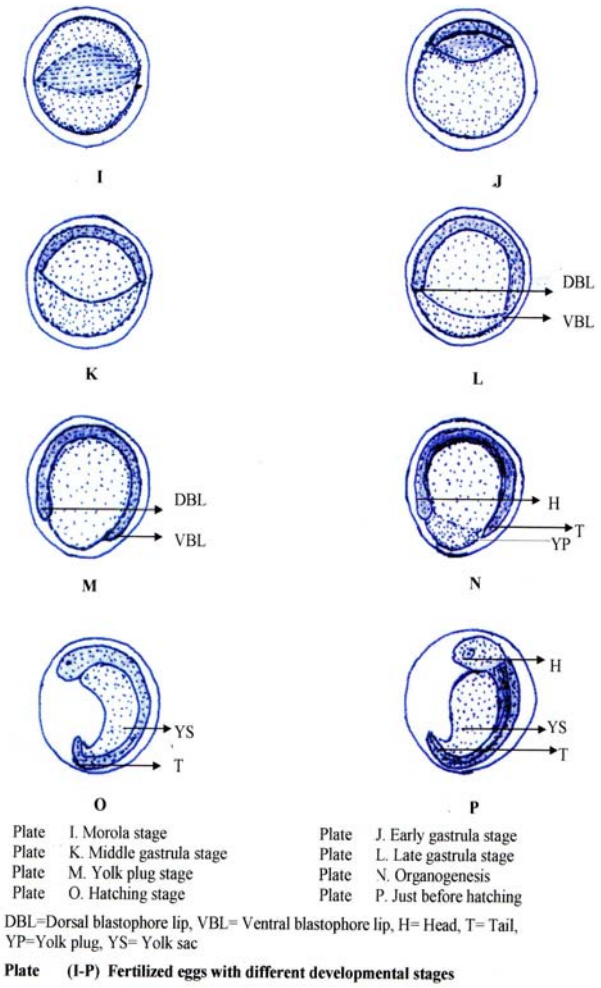
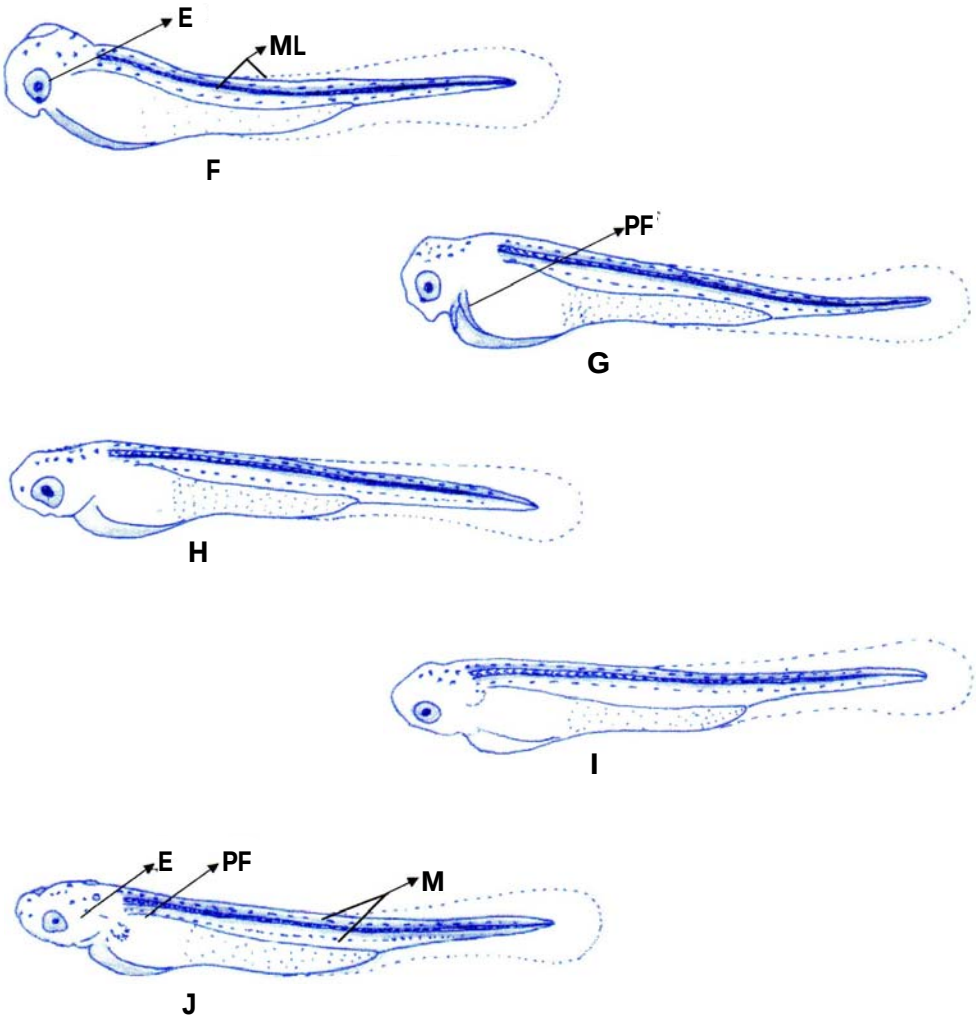


Plate I. Morola stage
Plate J. Early gastrula stage
Plate K. Middle gastrula stage
Plate L. Late gastrula stage
Plate M. Yolk plug stage
Plate N. Organogenesis
Plate O. Hatching stage
Plate P. Just before hatching

DBL=Dorsal blastophore lip, VBL= Ventral blastophore lip, H= Head, T= Tail, YP=Yolk plug, YS= Yolk sac

Plate (I-P) Fertilized eggs with different developmental stages

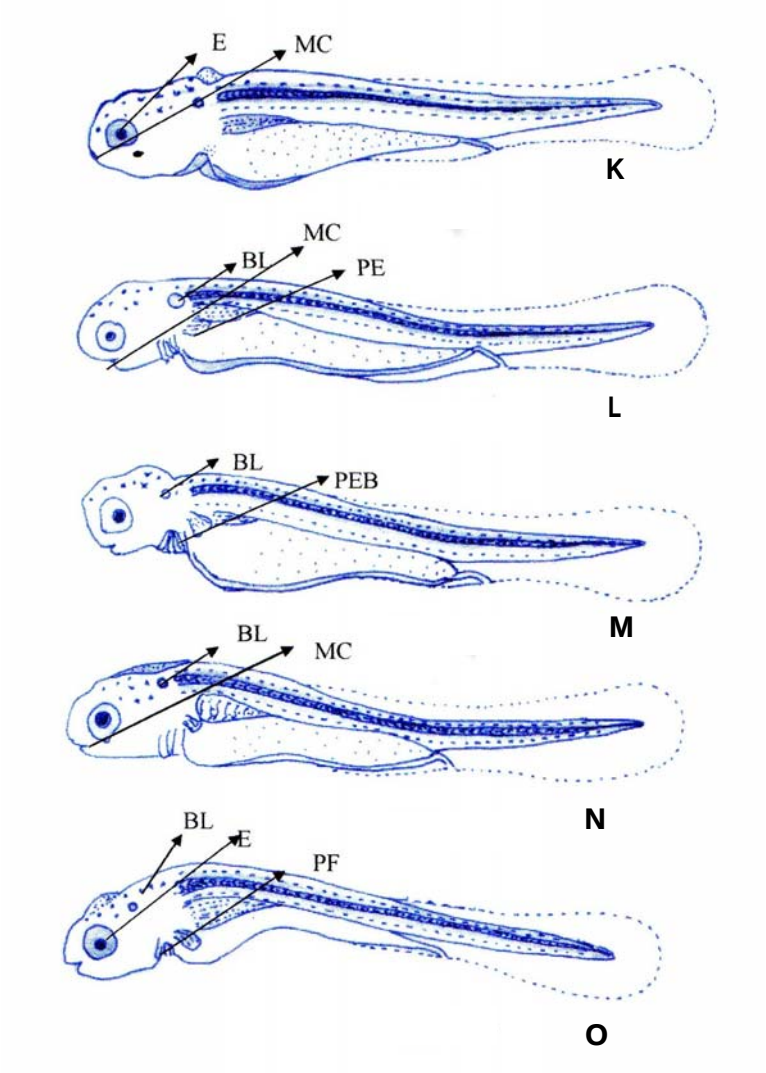
Figure 1. Fertilized eggs with different developmental stages (Contd.)



F. Ten hour old larvae G. Twelve hour old larvae
H. Sixteen hour old larvae I. Twenty hour old larvae
J. Twenty four hour old larvae

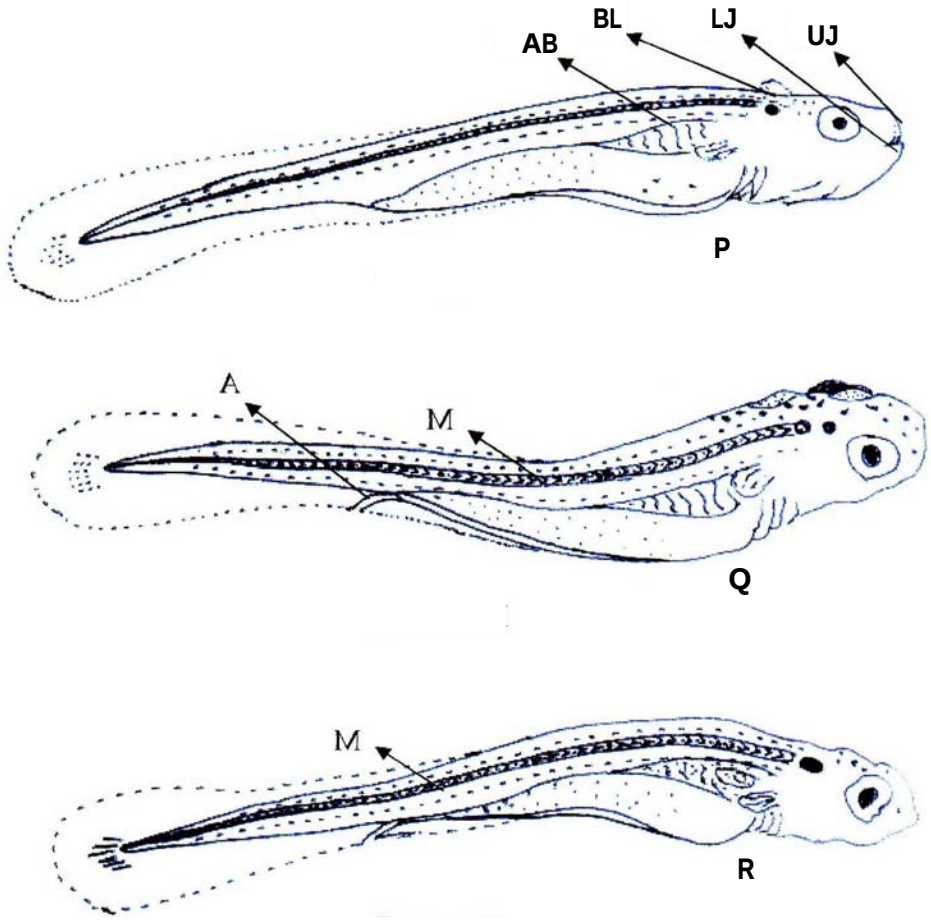
E = Eye PFB = Pectoral fin bud
M = Myomere ML = Melanophore line

Figure 2. (F-J) Different developmental stages of newly hatched larvae



K: Thirty four hour old larvae L: Thirty six hour old larvae
M: Thirty eight hour old larvae N: Forty four hour old larvae
O: Forty eight hour old larvae
MC = Mouth cleft, E = Eye, PFB = Pectoral fin bud BL = Brain lobe

Figure 2. (K-O) Different developmental stages of newly hatched larvae



P: Fifty eight hour old larvae Q: Sixty six hour old larvae
R: Seventy two hour old larvae
BL = Brain lobe AB = Air bladder, A = Anus, UJ = Upper jaw, LJ = Lower jaw,
M = Myomeres

Figure 2. (P-R) Different developmental stages of newly hatched larvae

Table 1. Summary of embryonic development process of *Labeo bata* in the laboratory

Phase	Plate No.	Stage	Time after fertilization (hours)	Mean temperature (^{O}C)	Mean total diameter (mm)	Characteristics
Unfertilized eggs	A	1	0.00	27.0± 0.05	0.7±0.01	Eggs spherical, brownish-yellow, demersal and adhesive.
Fertilized eggs	B	2	0.00	27.5±0.02	0.8±0.01	Eggs spherical, brownish-yellow, demersal and adhesive.
Blastolation (Segmentation)	C	3	0.10	27.5±0.02	1.5±0.01	After 45 mins appearance of first cleavage, this restricted to small disc of cytoplasm at animal pole, dividing blastodisc into two blastomeres.
	(D-E)	4	0.45-0.55	28.0±0.02	1.5±0.01	The second division of the two blastomers resulted in four blastomeres.
	F	5	1.20	28.5±0.02	1.5±0.01	Formation of eight blastomeres.
	G	6	1.50	29.5±0.01	1.5±0.02	Attainment of 16 cell stage.
	H	7	2.30	30.5±0.05	1.6±0.02	Appearance of multiple cells.
Morula	I	8	4.30	30.5±0.02	1.6±0.02	Blastomere visible at the animal pole, which gradually increased in size over time.
Gastrulation	J	9	8.30	30.5±0.02	1.7±0.01	Blastomeres started invading the yolk in the form of a thin layer.
	K	10	10.00	30.5±0.02	1.7±0.02	Germinal ring become distinct occupying a large part of the yolk.
	L	11	12.00	30.5±0.01	1.8±0.01	Blastoderm covered ¾ th of the yolk. Embryo shell visible. Appearance of optic rudiment.
Yolk plug Stage	M	12	16.00	30.5± 0.02	1.8±0.02	Completion of yolk invasion. Appearance of Rudimentary head and tail.
Organogenesis	(N-O)	13	16.00-18.00	31.0±0.01	1.9±0.02	Appearance of heart rudiment pectoral fin buds and gill rudiment. Notochord becomes visible, auditory and optic vessels developed.
Just before hatching	P	14	18.00-20.00	31.0±0.02	2.0±0.01	Started of hatching and detached from the yolk mass. Newly hatched individuals started slow forward movement and embryo ruptured the egg capsule.

Table 2. Summary of larval development process of *Labeo bata* in the laboratory (Different developmental stages of newly hatched larvae)

Stage No.	Age (hrs)	Plate No.	Mean total length (mm)	Characteristics
1	0.00	A	2.5±0.05	Larvae silver in color, yolk sac attached to the body, Larvae slender, transparent showing internal organs.
2	2.00	B	2.6±0.05	Larvae turns into silver color, yolk sac still remained attached to the body, larvae slender, transparent showing internal organs.
3	4.00	C	2.7±0.05	Body of the larvae becomes more transparent. Head and body laterally compressed. Yolk sac partially decreased.
4	6.00	D	2.8±0.02	The anterior part becomes more prominent, thicken and stronger.
5	8.00	E	2.9±0.01	The yolk sac partially reduced. The tail becomes thickened.
6	10.00	F	2.9±0.09	A tubular pulsating heart appeared. Yolk sac reduced. Eye and anus slightly visible. Intestine also appeared.
7	12.00	G	3.0±0.05	Chromatophores seen in the eye only. Ventral embryonic fin fold more prominent. Pectoral fin bud appeared.
8	16.00	H	3.2±0.02	Interior part of the yolk globular in shape. Pectoral fin rudiment, faintly visible.
9	20.00	I	3.3±0.02	Newly chromatophores appeared above eyes. Yolk sac became thin.
10	24.00	J	4.4±0.01	Operculum becomes visible and dark eyes pigmented. Myomeres partially visible. Prominent pectoral and pelvic fins fold.
11	34.00	K	5.3±0.01	Myomeres visible. Color of the larvae changed to silver- yellowish. Mouth cleft formed.
12	36.00	L	5.5±0.05	The eyes increased in size and pigmented. Pectoral fin more prominent. Brain lobe visible mouth cleft formed
13	38.00	M	5.6±0.02	Pectoral fin fold well developed. Mouth cleft more prominent. The eyes increased in size.
14	44.00	N	5.8±0.01	Opercula fold appeared. Brain lobe clearly and mouth cleft easily distinguished. The heart started functioning actively.
15	48.00	O	5.9±0.02	Yolk sac convex interiorly and air bladder distinct. Large black chromatophores observed on head. Gills prominent and air bladder elliptical in size.
16	58.00	P	6.0±0.01	Eyes fully pigmented. Pectoral fin bud more pronounced. The jaws more pigmented.
17	66.00	Q	6.2±0.02	Brain lobe fully visible. Yolk sac completely disappeared and larvae started feeding.
18	72.00	R	6.5±0.02	Myomere clearly visible. The larvae were silver-blackish and transparent in color. Larvae swim actively.

The essential features of all the above larvae are summarized in Table 2

In the present study, we succeeded in obtaining the larvae of *L. bata* by artificial fertilization. The fertilized eggs were round, transparent, demersal and adhesive. The color of the fertilized eggs was brownish-yellow. Mookerjee (1945) found more or less similar in case of *Labeo rohita*. The diameter of *L. bata* eggs after fertilized was increased from 0.7 to 0.8 mm, while according to Chakraborty and Murty (1972) in *L. rohita* the diameter ranged between 4.1 and 4.8 mm with an average 4.4 mm. The difference of the egg diameter was due to the species and brood size of major carp. The size of the swollen fertilized eggs may vary widely even within species. Therefore, it could not be used as a diagnostic character for distinguishing one from another (Chakraborty and Murty, 1972). The two cells, four cells, eight cells, sixteen cells and multiple cell stage of *L. bata* were found within 45, 55, 80, 110 and 150 minutes after fertilized, respectively. In *L. rohita*, the same series occurred at 35, 45, 70, 95 and 135 minutes after fertilization (Mookerjee, 1945). According to Rahman (1975) the same developmental stages appeared in case of *Anabus testidineus*, after 15, 20, 45 and 97 minutes of fertilization, respectively.

Morula stage was found in four hours and thirty minutes after fertilization where Mookerjee (1945) observed the same stage in *L. rohita* five hours and 45 minutes after fertilization. This variation might be due to species difference and temperature. The gastrula stage was observed in *L. bata* at 8.30 to 12.00 hours after fertilization of egg at 30.5°C. Galman (1980) observed *Tilapia nilotica* that gastrulation initiated in five hours at 26°C-27°C.

The heart rudiment, gill rudiment and pectoral fin buds of *L. bata* appeared after 17 hours and 30 minutes, 18 hours and 16 hours of fertilization in the present study. Whereas, Mookerjee (1945) observed the heart rudiment, gill rudiment and pectoral fin buds in 15 hours and 50 minutes, 14 hours and 50 minutes and 13 hours and 30 minutes, respectively in *L. rohita*. The incubation period of *L. bata* was 18-20 hours at water temperature 30°C-31°C, which was almost similar in case of *Cirrhinus mrigala* (Chakraborty and Murty, 1972) at 25°C -30°C.

The length of the newly hatched larvae of *L. bata* was 2.5±0.05 mm. However, Mookerjee(1945) found that the newly hatched larvae of Catla (*Catla catla*) were from 4.2 to 4.7 mm in length. The apparent deviation in the size of hatchlings of *Labeo bata* from that of *Catla catla* might be related with the size of these species which is much larger than the *Labeo bata*. In the larval stage, the development of pectoral fin bud 12 hrs after hatching in *L. bata* was similar to *C. mrigala* (Khan, 1943). According to Chakraborty and Murty (1972), the development of the ventral embryonic fin fold of *C. mrigala* was more prominent which was similar to this study. In the larval stage 24 hours after hatching, operculum appeared but not extended over gills, pectoral and pelvic fins were prominent and air bladed was visible, which were similar to *L. rohita and C. mrigala* (Khan, 1943; Chakraborty and Murty, 1972). In *L. bata*, the yolk sac was convex anteriorly, air bladder distinct, chromatophores were found on the head behind the eyes and in the auditory region, gills were prominent and air bladed was elliptical. These studies were similar to *L. rohita* and *C. mrigala* (Khan, 1943; Chakraborty and Murty, 1972). The yolk sac of the local bata was completely disappeared 66 hours after hatching and brain lobe was fully visible, but Chakraborty and Murty (1972) found these developments in *C. mrigala* within 72 hours. The larvae of *L. bata* started feeding after 66 hours of fertilization when they reached a length of 6.2±0.02 mm.

The embryonic and larval development of *L. bata* were studied at an ambient temperature of 27°C-31°C. The rate of larval development of the larvae varied in other species. This variation is thought to be temperature dependent, the higher the temperature the quicker was the development.

The present work generated some information on the early life history, developmental stages and commencement of first feeding time for larval rearing. This study will help the fishery biologist in understanding the biology and ecology of the fish, which might be of great use to take appropriate steps for the sustainable development of the culture and management technology of *L. bata.*

In view of the above findings and discussion, it may be concluded that the embryonic and larval development of *L. bata* is essential to know from the study of its history, culture of fry and fingerlings for nursery and protect this species from endanger.

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