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Embryonic and larval development of tara baim (*Macrognathus aculeatus)*

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Abstract: The early developmental stages of local tarabaim, *Macrognathus aculeatus* were studied up to 72 hrs starting from egg fertilization in the hatchery of Field Laboratory Complex and Water Quality Laboratory of the Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. The collection of brood fishes, care, maintenance, and administration of hormone, fertilization of eggs with normal milt, incubation and hatching of eggs were done with a great care. Incubation period of *Macrognathus aculeatus* was 16 - 18 hrs at water temperature 27.0 - 28.0°C and the hatching period was 36-40 hrs. The length of the newly hatched larvae of *Macrognathus aculeatus* was found to be 1.7±0.05 mm which started feeding movement after 66 hrs of fertilization when they reached a length of 5.9mm. This study will help fishery biologists in understanding on the biology and ecology of the fish, which might be of great use to take appropriate measure for sustainable development of culture and management technology of *Macrognathus aculeatus.*
Key words: Tarabaim, larval development and larvae.

Introduction

Macrognathus aculeatus is a common freshwater small omnivorous fish of Bangladesh. It is also found in India and Pakistan. It is commonly found in natural water bodies i.e. haors, baors, beels, river and flood plains of Bangladesh. Unfortunately, the population of this fish has declined in recent years due to various ecological changes. Now its natural population has been declining very fast. Now the fish is considered to be a critically endangered (IUCN, 2000). As such, the species should be protected from being extinct. Though some information exists on the breeding biology of this species (Akhtaruzzaman *et al.,* 1991 and Kohinoor *et al.,* 1997), no information is available on the early development of embryos and larvae of fishes. So it is necessary to study and characterize its various stages of embryonic and larval development to understand the biological clock and culture techniques of the species. This study was carried out to know the early life history of the endangered *Macrognathus aculeatus.*

Materials and Methods

For the experiment twenty brinjal varieties/lines were The experiment was conducted in the hatchery of Field Laboratory Complex and in the Water Quality Laboratory of the Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. Early developmental stages of local tarabaim, *Macrognathus aculeatus* were studied upto 72 hrs starting from egg fertilization.

Collection of egg sample: The eggs were collected randomly from the hatching tray. The developing stages of *Macrognathus aculeatus* were observed at every 5 to 10 minutes interval till completion of morula and then after every one-hour interval up to hatching. The eggs were put into 70% ethanol solution for preservation for further study. At least 10 eggs undergoing embryonic development process were studied to get precise information.

Collection of larval sample: The larval samples were collected from the hatching tray. Initial samples were collected at hourly intervals. At least five larvae were collected and immediately put into 70% ethanol solution for further study. The larvae were examined as soon as they were collected.

Developmental stages: Early developmental stages were studied under a Stereomicroscope (Olympus, SZH10 Research Stereo). Five individuals at each developing stage were examined for confirmation of various stages and the timing of development. The stages of development were observed at every 5 to 10 minutes interval till the completion of morula and then after every one-hour interval till completion of organogenesis. Then their sketches were drawn by using Camera Lucida (Olympus, Japan 306681) fixed on a Stereo microscope (Olympus, SZH10 Research Stereo).

Preparation of embryo or larvae for microscopic study: For the study of development stages, the fish larvae were temporarily stained with ethylene blue for clear observation. Specimens of early stages of *Macrognathus aculeatus* were drawn by hand using a Camera Lucida. (Olympus, Japan 306681) setting on Stereo microscope (Olympus, SZH 10 Research Stereo).

Results

Embryonic development

A short description of embryonic and larval development with relation to the time was presented in Tables 1 and 2.

Unfertilized eggs (Stage 1): The unfertilized eggs of *Macrognathus aculeatus* were spherical in shape. Eggs were greenish in colour. As the specific gravity of eggs was higher than water. So they settled on the bottom i.e. eggs were demersal. The eggs were adhesive. Unfertilized eggs measured 0.6 mm in diameter (Plate A).

Fertilized eggs (Stage 2): Fertilization of eggs took place as soon as the sperm enters into the eggs. Almost immediately a cortical reaction closed the microphyle which denied the entry of more sperm. The fertilized eggs were found adhesive, sticky, demersal, and green brownish in color. Immediately after fertilization the diameter of the egg was found to

be 0.8 mm. Slight swelling and a spot (blastodisc) on one pole were observed in eggs and were readily recognizable through naked eye within 15 mins of fertilization (Plates B and C).

Two celled stage (Stage 3): The first cleavage of eggs occurred within 30 mins at 27^0C. It was partial or meroblastic, forming a transitory blastula stage. The blastodisc was divided into 2 distinct cells by vertical cleavage within 35 mins post fertilization (Plate D).

Table 1: Summary of embryonic development process of *Macrognathus aculeatus* in the wet laboratory

Phase	Plate No.	Stage	TAF (hrs)	Mean temperature (°C)	Mean total diameter (mm)	Characteristics
Unfertilized eggs	A	1	**0.00**	**27.0± 0.15**	**0.7±0.11**	Eggs spherical greenish demersal and adhesive.
Fertilized eggs	B	2	**0.00**	**27.5±0.22**	**0.8±0.11**	Eggs spherical green, demersal and adhesive.
Blastolation (Segmentation)	C	3	**0.10**	**27.5±0.22**	**1.6±0.11**	After 45 mins appearance of 1st cleavage, this restricted to small disc of cytoplasm at animal pole, dividing blastodisc into two blastomeres.
	(D-E)	4	**1.10-2.30**	**28.0±0.22**	**1.6±0.11**	The 2nd division of the two blastomers resulted in four blastomeres.
	F	5	**3.20**	**28.5±0.22**	**1.6±0.11**	Formation of 8 blastomeres
	G	6	**4.10**	**29.5±0.22**	**1.6±0.11**	Attainment of 16 cell stage
	H	7	**5.15**	**30.5±0.22**	**1.6±0.11**	Appearance of multiple cells
Morula	I	8	**7.20**	**32.0±0.15**	**1.6±0.11**	Blastomere visible at the animal pole, which gradually increased in size over time.
Gastrulation	J	9	**15.10**	**32.0±0.15**	**1.8±0.11**	Blastomeres started invading the yolk in the form of a thin layer
Gastrulation	L	11	**22.10**	**32. 5 ± 0.22**	**1.9 ± 0.11**	Blastoderm covered 3/4 of the yolk. Embryo shell visible. Appearance of optic rudiment.
Yolk plug Stage	M	12	**30.15**	**32.5 ± 0.22**	**2. 0 ± 0.11**	Completion of yolk invasion. Appearance of Rudimentary head and tail
Organogenesis	N-O	13	**32.10-33.15**	**32.5 ± 0.22**	**2.2 ± 0.11**	Notochord becomes visible, auditory and optic vessels developed
Just before hatching	P	14	**36.0-40.0**	**32.5 ± 0..22**	**2.6 ± 0.11**	Completion of hatching. Newly hatched eggs started slow forward movement.

Four celled stage (Stage 4): A second cleavage was observed forming four cells within 45 mins (Plate E). It was at right angle to the first.Eggs measured 1.4±0.02 mm in diameter.

Eight celled stage (Stage 5): Third cleavage forming eight cells was recorded after 70 mins of second cleavage at 27.5^0C (Plate F). The diameter of the eggs was 1.4±0.02 mn.

Sixteen celled stage (Stage 6): The sixteen cells stage was developed within 80 to 135 mins (Plate G). The diameter of the eggs was 1.4±0.02 mm.

Multi celled stage (Stage7): The more the cleavage occurred and the more the blastomeres were formed. These cleavages enhanced the next cleavages. The sixteen celled stage in quick succession transformed into 32, 64, 128 cells stage and so on dividing geometrically. This occurred so quickly that it was very difficult to observe or count all the cells. So, it was generally referred to as multi celled stage. Eggs were measured 1.4±0.02 mm (Plate H).

Morula stage (Stage 8): The blastomeres after repeated cleavage resulted into morula stage Within 4.20 hrs after fertilization. A cap like structure was seen over the animal pole, which was gradually increased in size (Plate I).

Gastrula stage (Stage 9): The blastoderm started invading the yolk by spreading over the Yolk in the form of a thin layer. Gastrulation resulted within 8.00 hrs of fertilization. Eggs were measured 1.4±0.01 mm in diameter (Plate J).

Middle gastrula stage (Stage 10): This stage appeared at 9.30 hrs after fertilization. The formation of germinal ring around yolk was clearly visible and that about half of yolk was occupied by blastoderm.The diameter of the eggs was 1.4±0.01 mm (Plate K).

Late gastrula stage (Stage 11): Blastoderm covered 3/4th of the yolk and embryonic shield was clearly visible. Optic rudiment was appeared. The diameter of the eggs was 1.5±0.01 mm (Plate L).

Yolk plug stage (Stage 12): The yolk invasion was completed by gradual spreading over the germ layer. Rudimentary head and tail appeared and became differentiated. It was seen 12.00 hrs after fertilization. It measured 1.6±0.01 mm in diameter (Plate M).

Table 2: Summary of embryonic development process of *Macrognathus aculeatus* 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**	**2.1±0.15**	Larvae black in color, yolk sac attached to the body, Larvae slender, transparent showing internal organs.
2	2.00	**B**	**2.3±0.15**	Larvae turns into yellow color, yolk sac still remained attached to the body, larvae slender, transparent showing internal organs.
3	4.00	**C**	**2.4±0.15**	Body of the larvae becomes more transparent and takes cylindrical shape.
4	6.00	**D**	**2.6±0.22**	The anterior part becomes more prominent and stronger
5	8.00	**E**	**2.7±0.11**	The yolk sac partially reduced. The tail becomes thickened.
6	10.00	**F**	**2.9±0.19**	A tubular pulsating heart appeared. Yolk sac reduced. Eye and anus slightly visible. Intestine also appeared.
7	12.00	**G**	**3.0±0.15**	Chromatophores seen in the eye only. Ventral embryonic fin fold more prominent. Pectoral fin bud appeared.
8	16.00	**H**	**3.2±0.22**	Interior part of the yolk globular in shape.
9	20.00	**I**	**3.3±0.22**	Newly chromatophores appeared above eyes. Yolk sac became thin .
10	24.00	**J**	**4.4±0.11**	Operculaum becomes visible. Dark eyes pigmented. Myomeres partially visible. Prominent pectoral and pelvic fins fold.
11	34.00	**K**	**5.3±0.11**	Myomeres visible. Colour of the larvae changed to ash colour
12	36.00	**L**	**5.5±0.14**	The eyes increased in size and pigmented. Pectoral fin more prominent. Brain lobe visible mouth cleft formed
13	38.00	**M**	**5.6±0.22**	Pectoral fin fold well developed. Mouth cleft more prominent. The eyes increased in size.
14	44.00	**N**	**5.8±0.11**	Opercula fold appeared. Brain lobe clearly and mouth cleft easily distinguished. The heart started functioning
15	48.00	**O**	**5.9±0.22**	Large black chromatophores observed on head. Gills prominent and air bladder is slightly visible.
16	58.00	**P**	**6.0±0.11**	Pectoral fin bud more pronounced. The jaws more pigmented.
17	66.00	**Q**	**6.2±0.22**	Brain lobe fully visible. Yolk sac completedly disappeared and larvae started feeding.
18	72.00	**R**	**6.5±0.22**	Myomere clearly visible. The larvae were silver blackish and transparent in colour. Larvae swim actively.

Organogenesis (Stage 13): The head and tail end of the embryo was differentiated (Plate N). The embryo was elongated and encircled the yolk materials. Both tail and head ends were clearly differentiated and the beating heart was visible (Plate O). Heart rudiment, pectoral fin buds, otocysts and gill rudiment appeared one by one. The pectoral fin appeared first of all the fins as a bud and then the fin rays were formed. At this stage eye lack of pigment, the notochord in cellular structure became visible within 14.00 to 15.30 hrs. when auditory and optic vessels developed.

Just before Hatching (Stage 14): The embryo further elongated and was gradually differentiated. The tail was gradually become detached from the yolk mass. This stage was obtained in 17.00 to 18.00 hrs (Plate P). Embryo started occasional twisting movement. The twisting movements, which gradually became vigorous, eggs-capsule weakened and got ruptured. Hatching took place at 27.0±0.02°C. The embryos ruptured the egg shell by the continuous movement. Larvae emerged with its tail portion first in 18.00 to 20.00 hrs after fertilization. Hatching was continued for 2 hrs because the entire embryo did not hatch out at a time. Newly hatched spawn measured 1.7±0.01 mm just after hatching in Plate P. The rate of development of the embryo had varied, accordingly to the variation in the temperature of Hatching

Zero hour post hatching (Stage 1): Newly hatched larvae (1.7±0.05 mm) were slender, straight and transparent, gradually tapering towards the tail. Hearts of the larvae were functional in between head and the anterior margins of the yolk (Plate A1).

Two hour post hatching (Stage 2): The length of the larvae was 2.0±0.05 mm. The color of larvae was green black. Yolk sac remained attached to the body. Melanophore bands appeared on posterior end of the body (P1ate B1).

Four hour post hatching (Stage 3): The body was transparent. The yolk sac became partially decreased. Two vertical bands of melanophores appeared at the posterior end. The total length was measured 2.2±0.05 mm (Plate C1).

Six hour post hatching (Stage 4): Melanophores appeared on the head, around the yolk sac or on the yolk sac. The anterior part began to thicken. The colour of the yolk sac was brown-yellowish. The total length was measured 2.5±0.02 mm (Plate D1).

Eight hour post hatching (Stage 5): The yolk sac was partially reduced. Melanophores appeared more prominent. The length of the larvae was 2.6±0.01 mm (Plate E1)

Ten hour post hatching (Stage 6): A tubular pulsating heart appeared. Eye and anus become slightly visible. Intestine was visible. Notochord appeared. The length of the larvae was 2.7±0.09 mm (Plate F1)

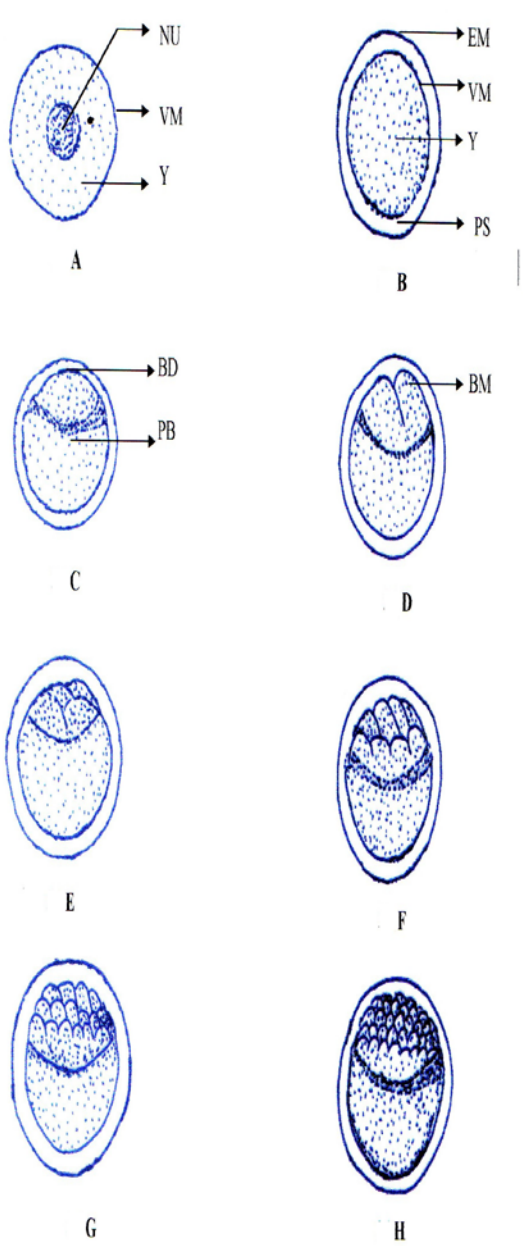

Plate A. Unfertilized egg
Plate B. Fertilized egg
Plate C. Blastodisc just formed
Plate D. Two celled stage
Plate E. Four celled stage
Plate F. Eight celled stage
Plate G. Sixteen celled stage
Plate H. Multi celled stage

NU= Nucleus, VM = Vitelline membrane, Y = Yolk, EM = Egg membrane, PS = Perivitelline space, BD = Blastodisc, PB = Periblast, BM = Blastomere

Plate (A-H) Unfertilized eggs and fertilized eggs with different developmental stages

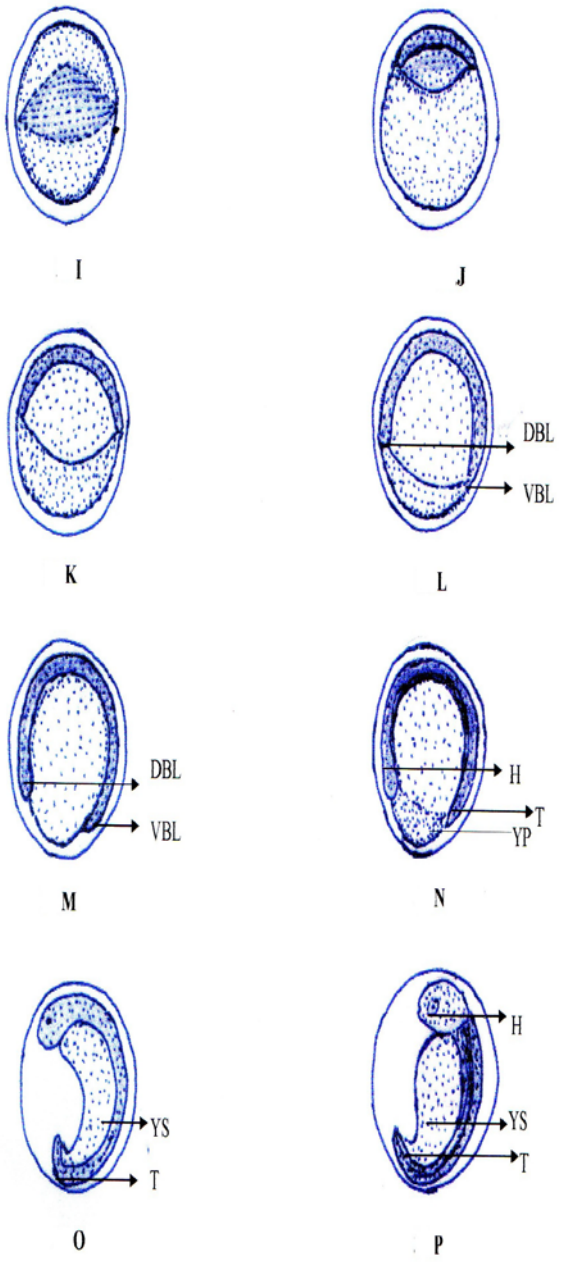

Plate I. Morola stage
Plate J. Early gastrula stage
Plate K. Middle gastrula stage
Plate L. Late gastrula stage
Plate M. Yolk plug stage
Plate N. Organogenesis
Plate O. Hatching stage
Plate P. Just before hatching

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

Twelve hour post larvae (Stage 7): Pectoral fin bud appeared. Melanophore bands were very much prominent at the posterior end *of* the body. A large number *of* melanophores also appeared above the eye and around the yolk sac. The larvae were increased to 2.8±0.05 mm in size (Plate G1).

Sixteen hour post larvae (Stage 8): More melanophores appeared on the head and body. Brain did not differentiate from the body. Yolk sac slightly decreased. Myomere were still partially visible. The length of the larvae was 2.9±0.02 mm (Plate H1).

Twenty hour post hatching (Stage 9): The total length of the larvae measured 3.0±0.02 mm. The eyes were slightly pigmented. External melanophores appeared dorsally on head. Myomeres were partially visible. The yolk sac became thin (Plate I1).

Twenty four hour post hatching (Stage 10): Chromatophores were visible in the head and above the eyes. Eyes became pigmented and dark in colour. Pectoral and pelvic fin bud appeared. Air bladder was visible. Anal became distinct. Myomere was partially visible. The larvae were increased to 4.2±0.01 mm in size (Plate J1).

Thirty four hour post hatching (Stage 11): Myomeres were visible. Colour of larvae changed to silver-yellowish. Mouth cleft formed. At this stage; the length of the larva was 5.0±0.01 mm in size (Plate K1).

Thirty six hour post hatching (Stage 12): At this stage, the length of the larva was 5.2±0.04 mm. The pectoral and pelvic fin buds were found. The colour of the larvae was whitish-black. Eyes became whitish black (Plate L1).

Thirty eight hour post hatching (Stage 13): Eyes were increased in size and became densely pigmented. Brain lobe was visible. Pectoral and pelvic fin fold well developed. Myomeres were partially visible. At this stage, the length of the larva was 5.3±0.09 mm (Plate M1).

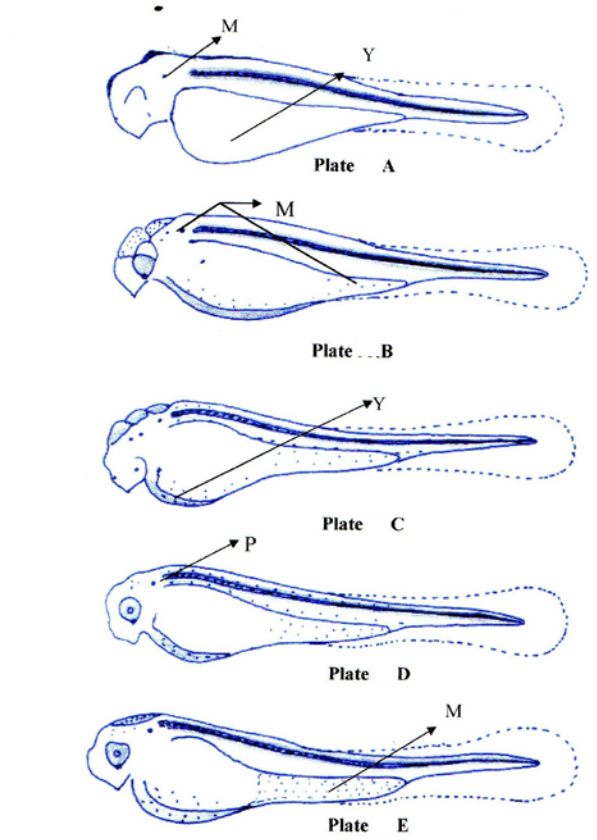

Plate A. Zero hour old larvae
Plate B. Two hour old larvae
Plate C. Four hour old larvae
Plate D. Six hour old larvae
Plate E. Eight hour old larvae

M = Melanophore, Y = Yolk, PM = Prominent melanophore

Plate (A-E) Different developmental stages of newly hatched larvae

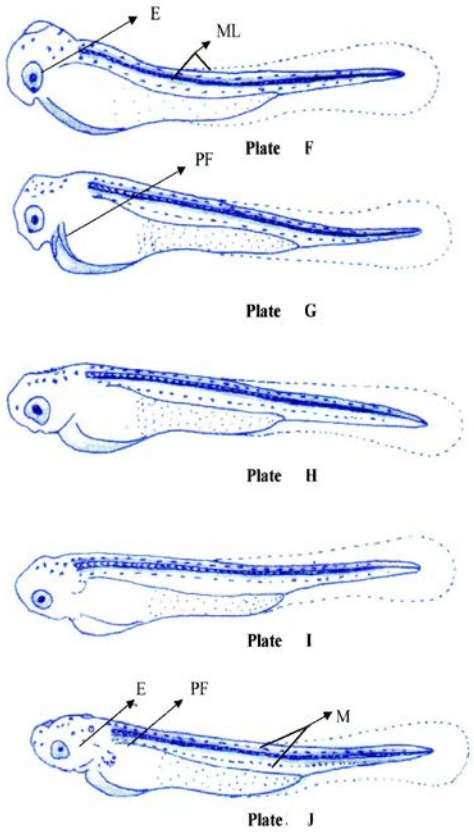

Plate F Ten hour old larvae
Plate G Twelve hour old larvae
Plate H Sixteen hour old larvae
Plate I Twenty hour old larvae
Plate J Twenty four hour old larvae

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

Plate (F-J) Different developmental stages of newly hatched larvae

Forty four hour post hatching (Stage 14): Opercula fold appeared. Brain lobe clearly visible and mouth cleft easily distinguished. The heart functioned actively. Upper and lower jaws were fully formed. The larvae were increased to 5.6±0.01 mm in size (Plate N1).

Forty eight hour post hatching (Stage 15): Air bladder was distinct. A few black chromatophores were found in a row from the posterior to the auditory concentrations up to the base of the caudal fin. Large black chromatophores were observed on head. The larvae were increased to 5.7±0.02 mm in size (Plate E1).

Fifty eight hour post hatching (Stage 16): Eyes were fully pigmented. Pectoral fin bud was more pronounced. The length of the larva was 5.8±0.01 mm (Plate P).

Sixty six hour post hatching (Stage 17): The larvae were 5.9±0.09 mm in length. The brain lobe was fully visible. Yolk sac completely disappeared and larvae had started feeding. Gills were prominent. Air bladder was elliptical. Prominent large black chromatophores were seen on the head. Notochord was in an upward position only at the very terminal past. Pectoral fin folds became distinct and rudimentary rays developed in caudal fins (Plate Q1).

Seventy two hour post hatching (Stage 18): Eyes were fully pigmented. Distinct black chromatophores were seen behind the eye. Dorsal and ventral fin folds were persistent. Myomere was clearly visible. The larvae were silver-blackish and transparent in colour. Larva swims actively. The larvae were 6.0±0.09 mm in total average length (Plate R).

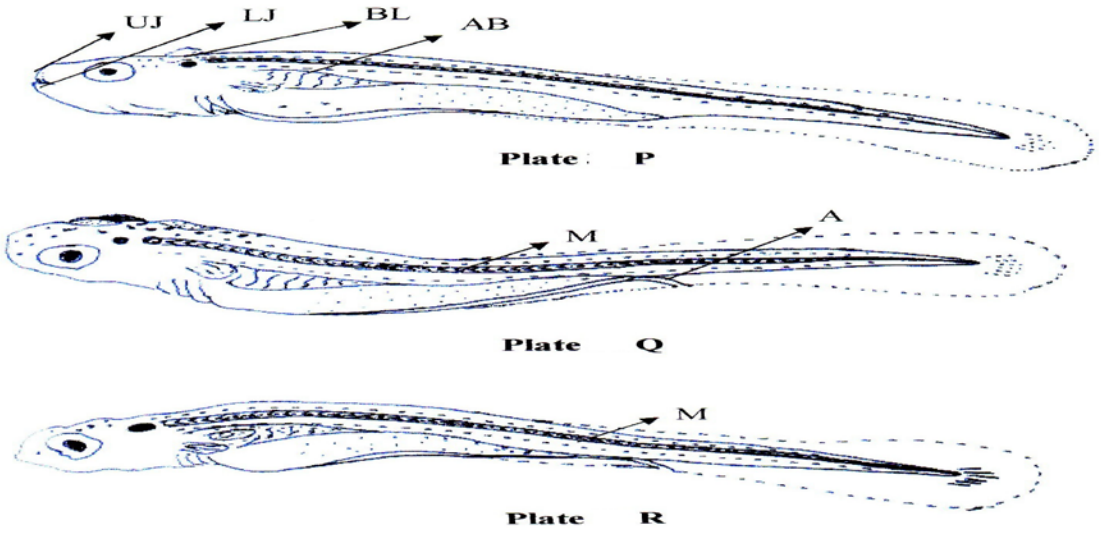

Plate P Fifty eight hour old larvae Plate Q Sixty six hour old larvae
Plate R Seventy two hour old larvae

BL = Brain lobe, AB = Air bladder, A = Anus, UJ = Upper jaw, LJ = Lower jaw, M = Myomeres

Plate (P-R) Different developmental stages of newly hatched larvae

Discussion

The larvae of *Macrognathus aculeatus* were obtained by artificial fertilization. Fertilized eggs were rounded, transparent, demersal and adhesive. The colour of the fertilized eggs was greenish brown. Mookerjee (1945) found more or less similar colour in case of *Labeo rohita*. The diameter of *Macrognathus aculeatus* eggs after fertilization was increased from 0.6±0.00 to 0.8±0.01 mm, while according to Chakraborty and Murty (1972), the diameter of the fertilized eggs of L. *rohita* ranged between 4.1 to 4.8 mm with an average 4.4 mm. The two cell stage, four cell stages, eight cell stages, sixteen cells and multiple cell stage of *Macrognathus aculeatus* were found within 35, 45, 70, 95 and 135 minutes after fertilization, respectively. In case of L. *rohita* same series of stages were occurred at 35, 45, 70, 95 and 130 minutes after fertilization (Mookerjee, 1945). Morula stage was found four hours after fertilization where as Mookerjee (1945)) observed the same stage in the case of L. *rohita* 5 hrs and 45 minutes after fertilization. This variation was due to the species difference and temperature. The gastrula stage was observed in *Macrognathus aculeatus* at 8.0 to10.40 hrs after fertilization of egg at a temperature of 27.0±0.02°C. Galman (1980) observed initiation of gastrulation within five hours in case of *Tilapia nilotica* at 26.0 to 27.0°C. The heart rudiment, gill rudiment and pectoral fin buds of *Macrognathus aculeatus* appeared after 15.0 hrs, 15 hrs and 30 minutes and 14 hrs and 10 minutes of fertilization, whereas, Mookerjee (1945) observed the same characteristics in 15 hrs and 5 minutes, 14 hrs and 50 minutes and 13 hrs and 30 minutes in the case of L. *rohita.* Incubation period of *Macrognathus aculeatus* was 16 - 18 hrs at water temperature 27.0 - 28.0°C, which was almost similar in case of *Cirrhinus mrigala* (Chakraborty and Murty, 1972). The length of the newly hatched larvae of *Macrognathus aculeatus* was found to be 1.7±0.05 mm. But Chakraborty (2005) found the newly hatched larvae of olive berb, *Puntius sarana* to be 4.2 to 4.7 mm. The apparent deviation in the size of hatchlings of *Macrognathus aculeatus* from that of *Puntius sarana* might be related with the size of the olive barb which is much larger than the *Macrognathus aculeatus*. In larval stage at 12 hrs after hatching, the development of pectoral fin bud of *Macrognathus aculeatus* appeared which was similar to *Cirrhinus mrigala* (Khan, 1943) and according to Chakraborty and Murty (1972), the development of ventral embryonic fin fold of *C. mrigala* was *more* prominent which was very similar to this study. Twenty four hours, after hatching operculum appeared but did not extend over gills; pectoral and pelvic fins were prominent and air bladder was visible, which were similar to *L. rohita and C. mrigala* (Khan, 1943 and Chakraborty and Murty, 1972). In *Macrognathus aculeatus,* the yolk sac was convex anteriorly, air bladder distinct, chromatophores was found on the head behind the

eyes and in the auditory region, gills were prominent and air bladder was elliptical. These aspects of organogenesis were similar to *L. rohita and C. mrigala* (Khan, 1943 and Chakraborty and Murty, 1972). Larval stage 66 hours after hatching, the yolk sac of the Tarabaim was completely disappeared and brain lobe was fully visible but Chakraborty and Murty (1972) found these developments in C. *mrigala* within 72 hrs. Larvae of *Macrognathus aculeatus* started feeding movement after 66 hrs of fertilization when they reached a length of 5.9 mm which strongly supported by the findings of Bruton (1979). During the present investigation the embryonic and larval development of *Macrognathus aculeatus* were studied at an ambient temperature of 26.5 to 27.5°C. The rate of development of the larvae varied from other species. This variation seems to be temperature dependent. The higher the temperature the quicker was the development (Hoar and Randal, 1969).

The findings of the experiment will help fishery biologists in understanding on the biology and ecology of the fish, which might be of great use to take appropriate measure for sustainable development of culture and management technology of *Macrognathus aculeatus*.

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