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Observation on the Embryonic and Larval Development of Silurid Catfish, Gulsha (*Mystus cavasius* Ham.)

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Abstract: In order to study the early development stages, eggs and milts were obtained from the ripe specimens of *M. cavasius*. For females stripping was done after 6 h of 10 mg kg⁻¹ body weight carp PG extract injection. Then the eggs were fertilized in the laboratory and subsequent development stages were studied. The average diameter of fertilized eggs were observed to be 0.5 mm. First cleavage (two cell), four cell, eight cell and multi cell stages were observed after 00:40, 00:55, 01:10, 02:50 h of fertilization, respectively. Morula, gastrula and yolk plug stages were found 4, 6 and 7 h after fertilization. Notochord was slightly observed after 8 h of fertilization. Head and tail were differentiated after 09:30 h of fertilization. Hatching occurred within 19±2 h after fertilization and larvae were measured 1.28 mm, when the water temperature was 27 to 29.5°C. The newly hatched larva was devoid of mouth, dorsal, ventral, pectoral fin and pigments. Barbel appeared to be slightly visible in the larva just after 6 h of hatching and clearly visible after 12 h of hatching. The yolk sac was completely absorbed when the larva was 48 h old. All the morphological and other organal development occurred within 12 days of post larval period.

Key words: *M. cavasius*, embryo, larvae, development

INTRODUCTION

Mystus cavasius locally known as Gulsha is one of the indigenous small catfishes once available in flood plains, swamps and canals of Bangladesh^[1]. It is a favourite fish to the consumers and has a great demand fetching high price in the market. In recent years, due to natural and man induced hazards in aquatic ecosystem such as physical reduction of water areas, siltation and erosion of river basins, application of pesticides in rice cultivation and release of chemical effluents from industrial plants, together with hydrological changes taking places due to numerous flood control measures, natural breeding ground of this fish species and their habitats have been severely degraded^[2]. This has created a serious problem to genetic resources of this important silurid catfish and has become gradually threatened in Bangladesh. Indeed this fish is in the brink of extinction^[2]. Keeping this in mind, in order to increase its production and as well as to preserve the gene pool, seed production technology through artificial propagation was developed by the Bangladesh Fisheries Research Institute^[3]. Expect some observation on the morphology, ecology, food and feeding habits, fecundity, induced breeding and culture technique of *M. cavasius*^[3-6] no published information about the early development of the fish is available.

Early life-history information is an essential requirement for optimization of mass seed production, culture and management^[7]. So, it is necessary to study and characterize the various stages of embryonic and larval development of *M. cavasius*. Therefore, an attempt was made to study gamete sizes, fecundity, every successive changes of zygote and larvae, their developmental timetable and sizes in a control rearing condition.

MATERIALS AND METHODS

Collection and maintenance of broods: The experiment was conducted during the natural breeding season of *M. cavasius* from June to August 2002 in the laboratory of the Freshwater Station, BFRI, Mymensingh. The mature female and male fishes were collected from the brood ponds and were transferred to the cistern of the BFRI hatchery and acclimatized for at least 6 h before used for the experiment. The male and the female brood fishes were identified by the external characteristics according to Mollah^[8].

Fecundity and gamete size: Mature female of various size were used for study of fecundity. A total of 12 female brood fishes weighing from 32.5 to 60.0 g were injected

with acetone dried pituitary hormone (PG) at a dose of 10 mg kg^{-1} body weight^[9]. The injected females were then kept in a spawning hapa (made of glass nylon). Ovulated fishes were then stripped to collect eggs. The testes of males were taken out by excising the abdomen with the help of scissors and forceps. Diameter of eggs and length of sperm head were measured (egg at 10×10 in a well slide, sperms at 40×10 on a plain slide) by a compound microscope, using the methods of Amy^[10].

Fertilization, embryonic and larval development:

Fertilization was done by mixing a few drops of diluted sperm into one petridish containing 20 ml stripped egg suspension. Sperms were then mixed homogeneously by bind feather and the excess sperm and blood were removed by 3-4 consecutive washes with water. The fertilized egg were then transferred into a small incubation jar and maintained ambient water temperature and dissolved oxygen through continuous aeration. Six replicate trails were performed through out the breeding season. 19 h after fertilization, the hatchling (larvae) was come out from the egg envelope and was transferred into shallow cisterns/tray 48 h after fertilization, when the yolk sacs of eggs were fully absorbed, the larvae were fed With boiled egg-yolks for three days and after that hatched artemia cysts were supplied until 12 days for their normal growth and development. All the developmental stages of embryos and larvae were observed at different time intervals upto that stage. At each stage, specimens were fixed in 10% formalin for more detailed specimens, provided information on the times required for embryos to attain specific development stages. In each experiment, the times after fertilization for 50% of the embryos to develop to 2-cell, 4-cell, 8-cell, multi-cell, blastula, gastrula, yolk plug and hatching stages were estimated, following fujisawa^[11].

Measurement of embryos and larvae: All morphometric measurements of embryos and larvae were made on freshly prepared specimens, following the method of Rehman *et al.*^[12] and McEdward^[13] with slight modification. Briefly the stages of development were observed and measured at every 5 to 10 min interval till the completion of morula and then after every 1 h interval till hatching. When hatching was completed, the observations were continued at every 6 h for the first day and thereafter at every two days interval to study the each developmental stage, samples ere collected randomly from the spanning hapa and rearing cistern. Larvae were killed in 10% formalin-water and were concentrated by settling to the bottom of a vial. A few drops of formalin-water containing about 5-10 larvae were put

under an elevated cover slip on a microscope slide and finally measured by a compound microscope using a presetting objective micrometer and the free hand sketch of the specimens were made.

RESULTS

Embryonic development: The average sperm size was $3.72 \mu\text{m}$ (range $3.10\text{-}4.96 \mu\text{m}$). The fertilized eggs of *M. cavasius* were spherical in shape, slightly adhesive, light pinkish in colours and demersal type. The average diameter of fertilized egg was observed 0.50 mm (range: $0.49\text{-}0.51 \text{ mm}$). Fertilized eggs had a reddish spot (Blastodisc) on one pole and readily recognizable through naked eye (Fig. 1A). The blastodisc was divided into two cells blastomeres resulted into four equal blastomeres 00:45 h after fertilization (Fig. 1B). The blastomeres 00:55 h after fertilization (Fig. 1C). Third division formed 8 blastomeres was observed increased cell numbers and passed through 16 cells, 32 cells, multicell (Fig. 1E) and reached morula stage (Fig. 1F) within 03:40 h of fertilization. Blastomeres started invading the yolk by spreading over the yolk in the form of a thin layer then gastrulation ring was observed after 5 h of fertilization (Fig. 1G). Gastrulation spreads to the whole yolk and the yolk plug stage was formed (Fig. 1H). After 8 h of fertilization, the earliest indication of the embryo was discernible and embryo reveal rudiments of optic cups (Fig. 1I). In about 09:30 h from fertilization, the head and tail ends become differentiated (Fig. 1J). At that time, the terminal portion of head and tail was slightly round in shape. Following this stage, the number of somites increased gradually. At 19 h from fertilization, both tail and head end head end were clearly visible. The embryo became elongated encircling the yolk sphere. The heart was beating actively. Tubular rod like notochord was appeared and some of the embryos show occasional faint twisting movement (Fig. 1K). As the embryo advances in development, the movement becomes more and more vigorous. With further twisting movements, the embryo was able to release out from the surrounding membranes (Fig. 1L). The stage was observed just before hatching and time required $19 \pm 2 \text{ h}$ from fertilization. In a 19 h old embryos, the embryonic fin fold was distinct both on the dorsal and ventral sides. All the eggs were kept in a same hatching jar but hatching period was varied from 17 to 21 h.

Larval and post larval development

Newly hatched larvae: Newly hatched larvae were slender, transparent and devoid of pigmentation in mouth and pectoral fins. The anal fin-fold extended up to the yolk

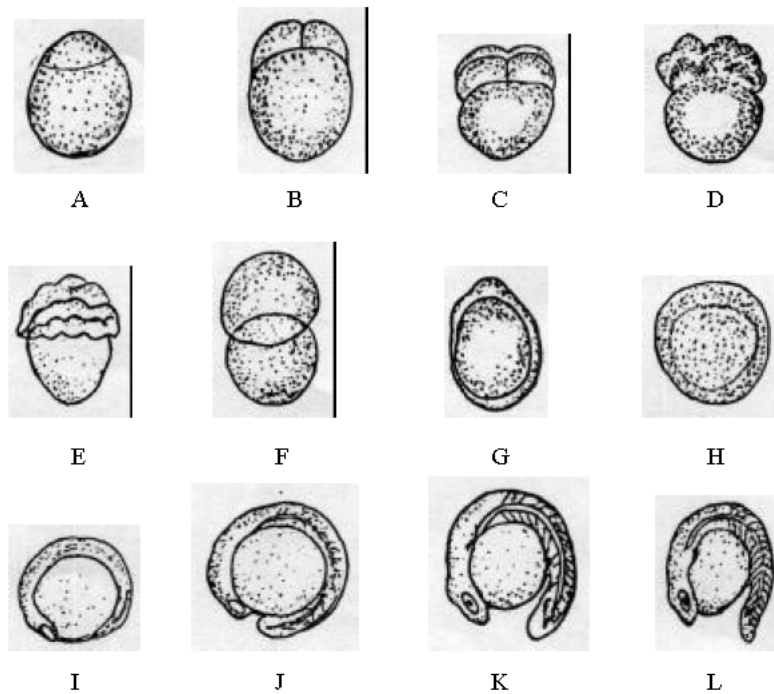


Fig. 1: Embryonic development of *M. cavasius*. Fertilized egg- A) blastodisc just formed; B) 2-celled stage; C) 4-celled stage; D) 8-celled stage; E) multi-celled stage; F) morula stage; G) gastrulation; H) yolk plug stage; I) 8-h old; J) 9.30 h old; K) twisting movement; L) just before hatching

Table 1: The development stage of gulsha (*Mystus cavasius*) eggs and embryos at ambient water temperature of 27-29.5°C

Hours after fertilization	Development stage	Diam (mm)	Morphology/Character
00:00	Fertilized eggs	0.50	Spherical, demersal, adhesive to substratum, pinkish in colour.
00:15	Blastula stage	0.61	Blastodisc formed at pole.
00:45	Two cell stage	0.61	
00:55	Four cell stage	0.61	
01:10	Eight cell stage	0.61	
02:00	Multi cell stage	0.61	
03:40	Morula stage	0.71	Blastoderm flattens
05:00	Gastrulation stage	0.71	Blastomeres started Invadins the Yolk and form a thin layer
06:30	Yolk plug stage	0.82	Rudiment of notochord
08:00	Eight hours old Embryos	0.90	Optic disc slightly visible
09:30	Nine and half hours Old embryos	1.02	Head and tail slightly appear. Notochord developed.
15:00	Twisting movement	1.02	Both head and tail clearly visible
19:00	Just before hatching	1.28	Eggs capsule wakens, twisting more vigorously and larvae hatched out

sac. The yolk sac was oval in shape and pale greenish in colour. The diameter of yolk sac was 0.75 mm. The anas was situated almost at mid ventral part of the body. Rudiment of maillary was slightly appeared. In total 30-35 of myotomes was disenrmed. Among them 10-16 myotoes were pre-anal. The mean length of hatching was 2.61 mm (Fig. 2A).

Six h old larvae: The length of larvae measured averagely 3.12 mm at this stage. The brain was slightly visible. Prominent notochord was found and barbell partially appeared in 6 h old larvae. Two chromatophores were present on the front side of the body. Optic lobe was formed. Melanophores were prominent on head and gradually decreased through lateral sight of the body (Fig. 2B).

Twenty four h old larvae: The length of larvae measured averagely 3.98 mm with reduced yolk sac. Two pairs of barbel appeared and among then the maxillary pair was the longest. The yolk sac was reduced which was 0.7 mm in length. Mouth was clearly visible.

Twelve h old larvae: The average length of larvae was measured to be 3.34 mm with reduced yolk sac. Two pairs of mandibular barbells had appeared. Eyes and anas visible. The pectoral fin fuds and mouth cleft had formed. The pigmentation in the eye had become pronounced. Some melanophone observed on the head region. Brain becomes distinct (Fig. 2C).

Twenty four h old larvae: The length of larvae measured averagely 3.98 mm at this stage. Three pairs of barbel appeared and among them the maxillary pair was clearly

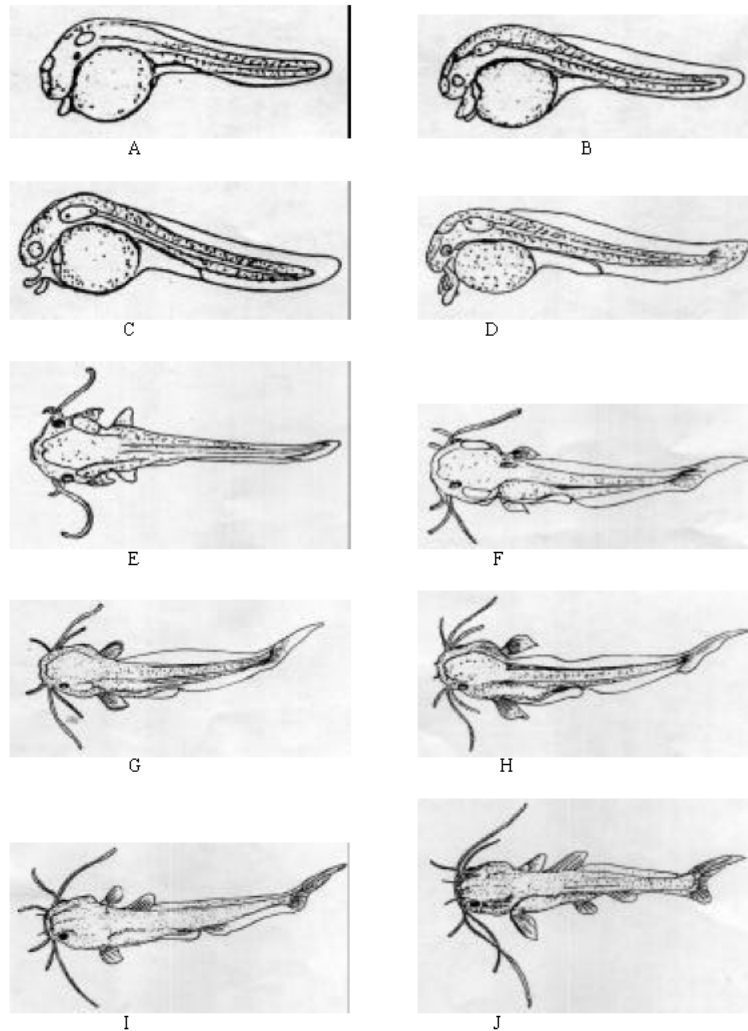


Fig. 2: Larval development of *M. cavasius*. Larvae- A) newly hatched; B) 6 h after hatching; C) 12 h after hatching; D) 24 h after hatching; E) 48 h after hatching; F) 96 h after hatching; G) 6 days after hatching; H) 8 days after hatching; I) 10 days after hatching; J) 12 days after hatching

visible. The total myotomes were counted to be 35-40 and among them 9-11 myotomes were pre anal and 29-31 myotomes were post anal. Upper and lower jaws that were found were pre anal and 29-31 myotomes were post anal. Upper and lower jaws that were found were totally formed (Fig. 2D).

Two days old larvae: Mouth cleft was distinguished clearly. Opercula fold was appeared at this stage. Myomeres became more developed. The yolk sac was completely absorbed and the larvae started wandering here and there in order to search foods. A pouch like stomach was formed. The elementary canal became short and straight. More melanophore had appeared on body. Eyes were fully pigmented. Pelvic fin was clearly visible. Pectoral fin visible with 2 spines. The

barbells had increased in length and the maxillary pair became longest than the others. In the stage was 4.13 mm in length (Fig. 2E).

Four days old larvae: More melanophore was appeared on the head and body. The head had broadened than body and became round in shape. Formed on the caudal fin and 8-10 rays could be discerned. The dorsal fin fold had broadened to the caudal fin. The rays of pectoral fin had developed. Radials was started to appear in the ventral fin fold posterior to the anus, were indicating the formation of anal fin (Fig. 2F).

Six days old larvae: The six days old post larvae were measured to be 5.66 mm in length and 1.08 mg in weight. Anal fin was separated from anus. Head had become

more pigmented than ventral part of the body. Adipose fin was not free from caudal fin. Spine of pectoral fin was well developed. About 10-15 rays could be discerned in the caudal fin (Fig. 2G).

Eight days old larvae: The eight days old larvae were 7.40 mm in length and 3.88 mg in weight. Four pairs of barbell were clearly observed. Dorsal fin was also clear with 6-7 fin rays at this stage. Large number of chromatophores was present on the body and head (Fig. 2I).

Ten days old larvae: The ten days old post larvae were measured 8.62 mm in length and 6.0 mg in weight. Caudal fin had developed with 7-8 fin rays. The adipose fin was free from caudal fin. The dorsal fin had developed with 5-6 rays (Fig. 2J).

DISCUSSION

The breeding season of *M. cavasius* runs from May to August^[3,14]. During the breeding season, mature males and females were identified by Mollah^[8] briefly: abdomen of females was soft and male, respectively. The mature fish were injected with carp pituitary hormone at the dose of 10 g kg⁻¹ body weight in female and 4 g kg⁻¹ body weight in male fish. The egg diameter of *M. cavasius* was varied from 0.49 to 0.51 mm with an average diameter 0.5 mm while the sperm head length from 1.25 to 2.0 µm. Kohinoor *et al.*^[15] found that the fertilized eggs of *O. pabda* were light pinkish in colour. In the present study the fertilized eggs of *M. cavasius* was found light pink to yellowish in colour. This variation might be occurred due to complex result of the species specificity, type and amount of food and the surrounding ecosystem. Similar phenomena were also observed in the eggs of other fishes^[16-19]. This might be happened due to variation of environmental condition of the surrounding ecosystem. In the present study, the average diameter of egg immediately after fertilization was observed to be 0.50 mm. Kohinoor *et al.*^[15] observed the average diameter of eggs to be of 1.18 mm in *pabda* (*Ompok pabda*). Thaker and morula stage attained in 00:30 h and 01:30 h, respectively after fertilization of egg. Kohinoor *et al.*^[15] found that the morula stage of *pabda* was attained in 01:20 h after fertilization. In the present study the morula stage of *M. cavasius* was attained in 01:20 h after fertilization. In the present study the morula stage of *M. cavasius* was attained within 02:20 h after fertilization that differ from that of *pabda*. The average hatching period was observed in *M. cavasius* to be of 19 h after fertilization of egg at a water temperature range of

27.5 to 29.5°C. Whereas, Thakur and Das^[20] observed 18 h in shinghi (*H. Fossilis*) and 21 to 24 h in magur (*Clarias batrachus*) at 25 to 29°C. On the other hand, Kohinoor *et al.*^[15] found that the average hatching period in *pabda* (*O. pabda*) was 20:30 h after fertilization of egg at a water temperature range of 25.3 to 28.7°C. Time requirement of egg hatching was inversely related to their incubation temperature^[21]. The barbells appeared after one day of hatching on *H. Fossilis*^[20] and Kohinoor *et al.*^[15] observed that the barbells of *O. pabda* appeared 12 h after fertilization of egg. In case of *M. cavasius*, barbell partially appeared in 06 h old larvae took 12 h to clearly appear the barbells, which was similar to *O. pabda*. The development of embryo and the variability of hatching time in fertilized egg of most of the fish generally influenced by the temperature of water^[22,23]. The length of newly hatched larvae of *M. cavasius* found to be 2.59 to 2.62 mm. Rahman^[23] found the length of the fresh hatching in case of *A. testudineus* was 1.9 to 2 mm which was more or less similar to the present study. From this study it was found that the pectoral fin buds and mouth cleft appeared in 12 h old larvae. In case of *O. pabda* fin buds and mouth cleft were found in 12 h after hatching^[15] which was similar to *M. cavasius*. In the present study, myotomes of newly hatched larvae were clearly visible and counted 30 to 35, among them 10 to 13 pre-anal. Whereas, Kohinoor *et al.*^[15] found that the greater number of 36 to 40, myotomes at newly hatched larvae in case of *O. pabda* of which 10 were pre-anal. The larvae started feeding at 48 h after hatching. Barua^[24] found *C. batrachus* larvae started feeding keeping a part of internal food in the yolk sac was reported by Das^[25] for *Carassius auratus* larvae. Conservation of evolutionary importance for the perpetuation of the species.

In the present study it was observed that the yolk sac fully absorbed in 48 h old larvae. Kohinoor *et al.*^[15] reported the same in *O. pabda* at room temperature. But Rahman^[23] reported complete yolk absorption in 144 h old larvae of *A. testudineus* room temperature that was not similar to the present study, perhaps due to the species variation.

During the present investigation the embryonic and larval development of *M. cavasius* were studied in a narrow range of ambient temperature. Further studies should be conducted with brood fish drawn from different regions so the reliable and precise information could be obtained. This knowledge will help sustainable development of culture as well as management technology of *M. cavasius* to protect the species from being extinct.

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