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Embryonic and larval development of *Mystus gulio* (Ham.)

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Abstract
Mystus gulio eggs are strongly adhesive and contain relatively small yolk (0.75~1.0 mm). The egg envelop is thick and transparent. First cleavage (two cells), four cells, eight cells, sixteen cells and multi cells stages were found 20, 25, 35~40, 60 and 70 minutes after fertilization, respectively. The morula stage was visualized within 1.5 h after fertilization. The heart beat visible and the circulatory system commenced after 16 h of fertilization. Embryos hatched 18~20 h after activation of egg. The newly hatched larva measured 2.82±0.03 mm in length and 0.32±0.06 mg in weight. The yolk sac was fully absorbed by the third day though larvae commenced exogenous feeding even before completion of yolk absorption. A 5-day old post larva began wandering in search of food. Ten-day old post larvae endowed with eight branched rays in dorsal fin and seven in caudal fin. Fifteen-day old post larvae had the pectoral spine become stout though the embryonic fin folds had to be disappeared. The length of fingerlings ranged from 25~30 mm after 30 days, and their external features were just like those of an adult except that they were not sexually matured.

Key words: Embryonic development, Larval development, Ontogenic events, *M. gulio*.

Introduction

Nuna Tengra, *Macrones gulio* renamed *Mystus gulio* (Ham.) is a native catfish of family Bagridae and distributed around India to Malay Archipelago especially in estuarine and tidal waters (Jhingran 1997). The decline in total catch of the species is in a state of vulnerable (IUCN, 2000 and Mijkherjee, 2002) in the nature. It is having high market demand and delicious in taste and it has an emerging trend as an aquaculture species in South-west Bangladesh. Despite a paucity of information available about its biology, except some observations on the fecundity, induced spawning, spawning behavior and larvae rearing of *M. gulio* by Sarker *et* al (2002), Alam *et al* (2006a and 2006b) and Islam *et al* (2007), hence quests have been made to study its embryonic and larval development. The present work was undertaken as a part of a study of the catfish, especially to observe early developmental stages.

Materials and methods

Mystus gulio brooders were collected from the reservoir ponds of the brackishwater station (BS) located in the south-west part of Bangladesh using cast nets. After a brief dip in potassium permanganate, the brooders were acclimatized in cisterns (4.5 X 1.5 X 1 m) without food. Spawning was introduced by an intramuscular injection of 1 ml/kg body weight of ovaprim (Alam *et al*, 2006a). Each breeding set consisting of two males and one female was released into a breeding hapa (1.2 x 0.9 x 0.9 m) after injection. The behavior of brooders was observed frequently after injection and the spawning activity appeared to continue first after some 5 hours post injection, though its latency period is 6-8 hours (Alam *et al*, 2006b). Fertilized eggs were collected every hour with the help of a dropper from the breeding hapa and observed its developmental stages. The eggs hatched out first after 18 hours of fertilization. When about 80% of hatchlings were observed with their absorbed yolk sac, feeding was given with boiled and screened hen's egg yolk. Newly hatched larvae and thereafter was also observed its developments. The lengths of the hatchlings were measured by ocular micrometer (1/100 fraction of a millimeter). Descriptions of the developing stages were made on the basis of examining eggs and larvae under microscope (Brand: CETI with JVC closed circuit digital camera and monitor) and digital still photographs of the developmental stages of eggs and larvae were also made.

Results

In the present study, spawning was observed within 6~8 hours after injection of the hormone. Fertilized eggs (Fig. 1) of *M. gulio* were adhesive, demersal and spherical in form. The yolk sphere contained no oil globule. Due to the adhesive nature of the eggs, considerable debris adhered to the capsule of the egg. The grayish-white egg capsule was translucent, where the yolk was brownish. The eggs became opaque as development progressed. The diameter of the egg capsule ranged 0.75~1.0 mm, while the yolk sphere ranged from 0.7~0.9 mm. The developmental stages of *M. gulio* were divided into six stages: embryo, hatchlings, larva, post larva, fry and fingerlings (Jhingran and Pullin, 1985), with each stage having typical anatomical and physiological features. A summary of the timing of the important ontogenic events and structures is presented in Table.1.

Embryo

The time required to develop from the first cleavage to formation of an embryo was about 1 h. The embryonic development of *M. gulio* was usually completed within 18~20 h after fertilization. The first cleavage commenced 20 min after fertilization when the blastodisc divided into two blastomeres (Fig. 2). Within another 5 min, the four cell stage was obvious. The eight cell stage was reached after 10~15 min. Sixteen blastomeres (Fig. 3) were noticeable within the next 20 min, and the number of cells doubled (64-cell stage) in the following 10 minutes. The morula stage (Fig. 4) was visualized within 1.5 h after fertilization. By about the seventh hour, the head and tail

ends of the embryo were distinguishable (Fig. 5). Myomers differentiated between 9 and 11 h of development (Fig. 6). In the 15-somite stage, the optic vesicles appeared, and in the 14th to 16th hours (Fig. 7).

Table 1. Ontogenic events in the early developmental stages of *M. gulio*.
(Each value is the average of five observations)

Age (h)	Ontogenic events
	Cleavage
0.2~1.0	2~16 cells stages
1.0~1.5	Morulla stage
	Formation of embryo
1.5~2.0	Blastulla stage
2.0~3.5	Germinal ring formed; embryonic shield formed; and more than half of yolk invaded
3.5~5.0	Yolk invasion two-thirds complete
5.0~7.5	Yolk invasion complete
	Differentiation of embryo
9.0	Embryonic rudiment distinct
11.0	2~3 myomers; eye vesicle demarcated
12.0	7~8 myomers; heart rudiment visible; demarcation of brain
14.0	12~17 myomers; heart and tail differentiated
16.0	Entire space inside egg occupied by embryo; heart beat visible; tail begins to separate from the yolk; blood circulation commenced; embryo making frequent movements
18.0	Hatching begins
20.0	Larva hatched (almost 80%)
	Larva (post hatching)
Newly hatched	2 mm long; unpigmented eyes; no fin buds; mouth not yet formed Head and body faintly yellow
3~8	3 mm long; displaying unpaired dorsoventral fin; heart and brain distinct; yolk sac elongated
12~20	Caudal fin begins to separate; pigmentation of eyes; alimentary canal distinct; pectoral fin buds appear
25~35	Mouth beginning to differentiate; pigmentation of body
40~48	Mouth opens; jaw movements begin; barbles formed
	Post larva
Third day	4 mm long; head prominent; yolk sac absorbed
Fifth day	5 mm long; body grayish black; pectoral fin clearly recognizable

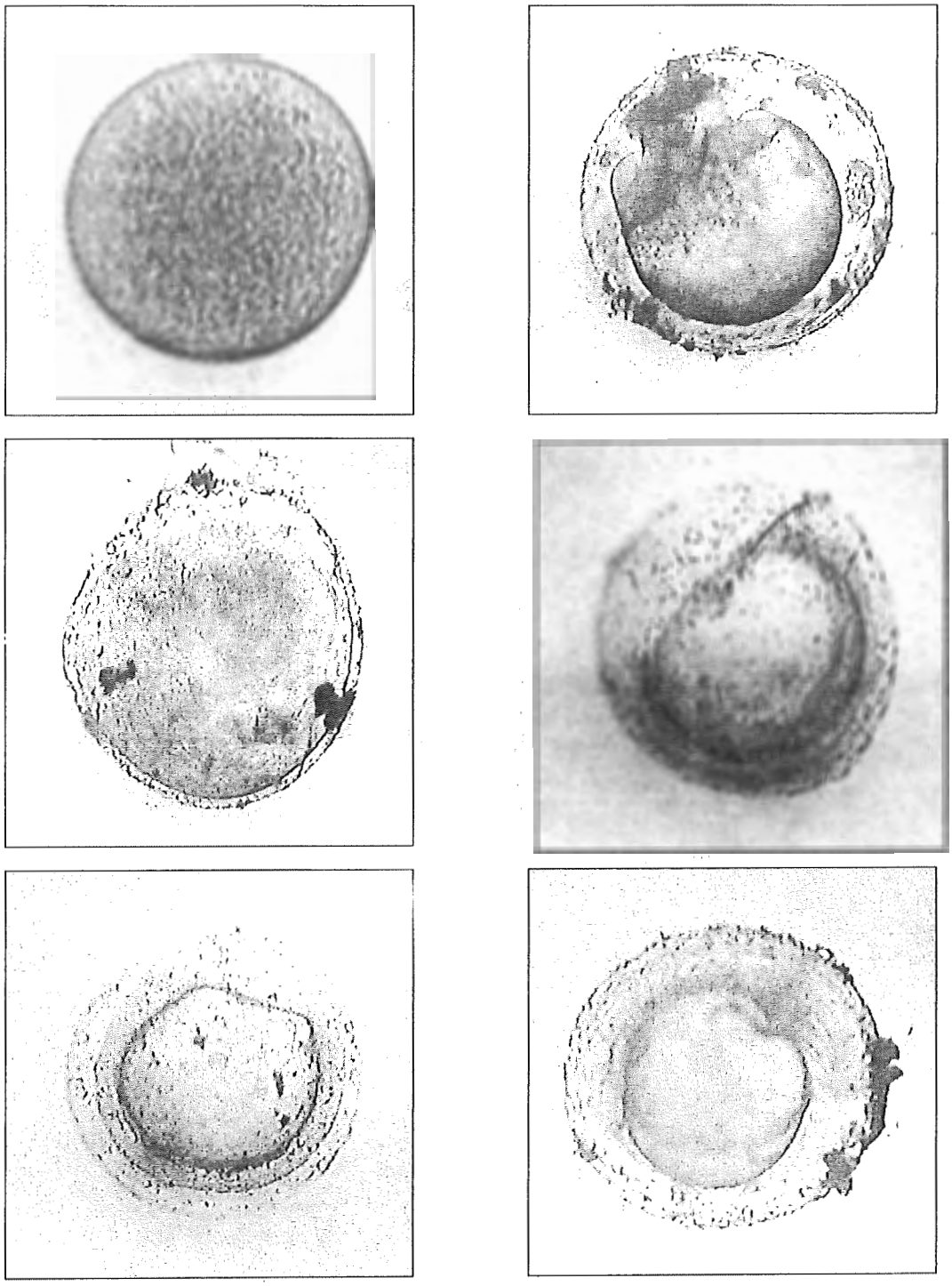

Plate 1. Developmental stages of *M. gulio*. Fig.1: Fertilized egg; Fig.2: Formation of two blastomeres; Fig.3: Sixteen cells stage; Fig.4: Morula stage; Fig.5: Seven-hour old embryo; Fig.6: Ten-hour old embryo.

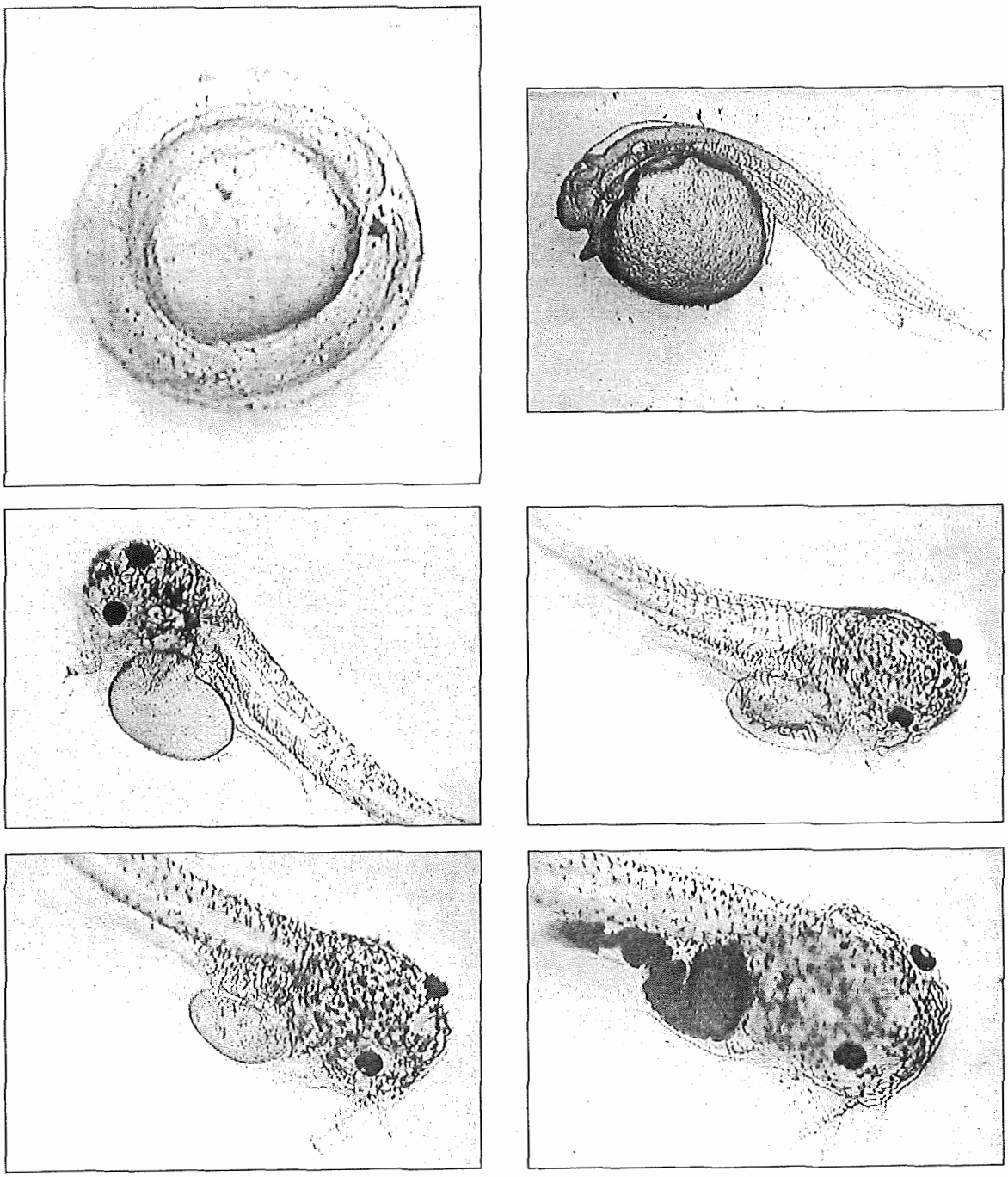

Plate 2. Developmental stages of *M. gulio*. Fig.7: Optic vesicle appeared (14^{th}~16^{th} h); Fig.8: Just hatched out larva; Fig.9: Six-hour old larva; Fig.10: One-day old larva with tiny protuberance of barbles; Fig.11: Two-day old larva with urinogenital opening distinct; Fig.12: Three-day old larva commenced exogenous feeding.

In the final stage of embryonic development, the growing embryo occupied the entire previtteline space and by about 1.5~2.0 h before hatching, it exhibits frequently twitching movement. After a pause of about 30 sec, this frequent movement suddenly culminated in a violent jerk breaking the previtelline membrane and the hatching emerged tail first (Fig. 8).

Hatchling

Length and weight measurements of newly hatched larva are given in Table 2. Hatchlings of *M. gulio* showed a laterally compressed transparent body, characterized by the presence of an almost round yolk sac, occupying about one-third of the total length. Chromatophores were completely absent. The mouth, alimentary canal and gills were not yet differentiated. The primitive streak of the notochord were quite prominent; about 25~27 myomers were distinct and another 7-8 were apparent in the tail region. The tip of the tail was rounded and the fin fold was differentiated, but not very distinct. Newly hatched larvae were not that active and generally they moves with water flow and few of them remain resting on the sides of the hapa where water current is much lower.

Larva

A relatively broad space appeared between the head and anterior margin of the yolk in 2~3 h old larvae (Fig. 9). This space facilitated the accommodation of the developing heart. Buccal invagination was appeared in 6~8 h old larvae and the alimentary canal formed as a straight tube emerging from the posterodorsal aspect of the yolk sac. The anal opening was closed and was situated between 10^{th} and 12^{th} myomers, which was about half of the length of the larva at this stage. The heart commenced to bit at a rate of 105~110 beats/min. Barbles appeared in the form of tiny protuberance in 1-day old larvae (Fig .10). The upper and lower jaws were formed, and the lower jaw showed occasional movements. The urinogenital opening was distinct and situated just posterior to the anal opening in 2-day old larvae (Fig.11). The heart beats at a rate of 120~130 beats/min. The pectoral fin buds appear as a moderate elevation. Intestinal coiling of the alimentary canal was noticeable. The yolk was exhausted by the end of the third day of development, and larvae commenced exogenous feeding even before completion of yolk absorption (Fig .12).

Post larva

In 5-day old post larvae, streaks denoted rudimentary rays, which appear in the caudal fin. The pectoral fin was differentiating and was in the form of a flap just behind the operculum; at this time, sidewise movement of the larvae commenced. The yolk was completely absorbed and began wandering in search of food. The phenomenon of aerial respiration began on the seventh day of development. Ten-day old post larvae endowed with eight branched rays in the dorsal fin and seven eight in the caudal fin, and at this stage, the outline of the brain in the cranial cavity could clearly be seen under a microscope. Fifteen-day old post larva showed seven-eight anal fin rays, and the pectoral spine had become stout. The embryonic fin folds had yet to disappear. Vertebral

segmentation of the notochord took place with distinct neural and hemal spines especially in the caudal region. Pigmentation was more pronounced throughout the head and body.

Table 2. Average measurement of hatchlings and post larva of *M. gulio*

Aging	Length (mm)	Weight (mg)
At hatching	2.82±0.03	0.32±0.06
10~12 h old hatchlings	3.16±0.06	0.47±0.04
1-day old hatchlings	3.36±0.09	0.65±0.08
2-day old hatchlings	4.05±0.25	0.87±0.08
3-day old hatchlings	4.63±0.08	0.98±0.08
4-day old hatchlings	5.65±0.07	1.15±0.07
5-day old hatchlings	5.87±0.08	1.25±0.07
10- day old hatchlings	14.87±1.45	55.68±3.21
15- day old hatchlings	17.64±2.11	89.63±4.52

Fry

Twenty-day-old fry ranged 19.0-21.0 mm in length. Fry swam actively and were observed to voraciously feed on plankton. Fry displayed a dorsal fin with branched rays. The body became dark due to the accumulation of pigments.

Fingerling

This stage began on the 30th day and lasted for the next 15 days. Thirty days after hatching, the pectoral, pelvic, dorsal, caudal and anal fins showed seven-eight, six-seven, eight, nineteen and seven-eight rays, respectively, representing the full complement of rays. On day 30, fingerlings were 25.0–30.0 mm in total length and externally resembled the adult suggesting the end of the fingerling stage.

Discussion

Changes in the pattern of the entire structure of an organ or of a specific organ in relation to the environment are decisive for evaluating the developmental pattern of a species (Balon 1999). Since the egg envelop is thick, translucent and sticky, observations on the development of *M. gulio* are difficult (Kovac 2000). Ontogenic events during the ovular phase (cleavage stage) did not markedly differ from those in *Heteropneustes fossilis* or *Channa marulius* (Khan 1926, Mookherjee 1945). Changes in structure emphasized the thresholds between embryonic, larval and post-larval development from the onset of cleavage, or at the time of organogenesis, respectively (Kovac 2000, Carlos *et al.*, 2002).

The first cleavage was found 30 min after fertilization in *Nandu nandus* and 20 min in *Ompok pabda* (Das *et al.* 2002 and Kohinoor *et al.* 1997). The yolk sac of *M. gulio* was fully absorbed by the third day, when the larvae measured 4.63±0.08 mm and weighing 0.98±0.08 mg (Alam *et al.* 2006). Whereas, yolk sac absorption was completed in 56 hrs and 48 hrs for *N. nandus* and *O. pabda*, repectively (Das *et al*, 2002 and Kohinoor *et al*,

1997). Disappearance of yolk sac for other similar catfishes like *Mystus montanus* (Raj *et* al, 2003) and *Mystus macropterus* (Wang *et al* 1992) was found also in the third day.

Fish farmers are much less familiar with the culture of catfish species because of the lack of breeding and feeding techniques and non availability of seeds from the wild (Meehan 2002). Despite this small scale operation have been attempted for *M. gulio* and the culture of other catfishes e.g. *Heteropneustes fossilis* and *Clarias batrachus* have been achieved in the past (Marguiles 1997). The high fecundity, short embryonic period, fast development of sense organs of *M. gulio* suggest that it may be a suitable species for commercial seed production.

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