Morphological Embryonic Development Stages of *Barbonymus gonionotus*
(Bleeker, 1850)

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Abstract

Morphological study on embryonic development of *Barbonymus gonionotus* (Bleeker, 1850) was described under laboratory condition. Fertilization occurred at 16 hours after injection. Ten minutes after fertilization, the one cell stage of embryos and unfertilized eggs were observed. The developmental stages were observed and divided into early cleavage stage, morula stage, blastula stage, gastrula stage, somite formation stage, hatching stage and larval stage. The one-cell embryo of fertilized egg develops subsequently through the first, second, third, fourth and fifth cleavage. After thirty-two cell stage, morula stage was observed after 25 minutes postfertilization followed by blastula stage. Gastrula stage appeared at 2 hours 50 minute after fertilization. After gastrula stage, the embryo transformed into bud stage at 5 hours. Further development of a pair of mesodermal somite formation, appearance of notochord and heart, were observed after 9 hours after fertilization. Thirty-three somite formation stage was observed at about 12 hours and 39 minutes. Anal structure was observed and located between 22 and 23 myomeres. After 15 hours, the development of all cells was completed. Hatching occurred about 16 hours after fertilization, the membrane disappeared and the embryo entered the period of free-swimming existence.

Keywords: *Barbonymus gonionotus*, fertilization, cleavage, embryonic development.

1. Introduction
The silver barb, *Barbonymus gonionotus* (Bleeker, 1850) was introduced into Myanmar from Thailand in 1996, and became a well-established culture species. It has been introduced in many Asian countries including Bangladesh not only for its palatability and market ability but also for high yield potential (Hussain *et al.*, 1987).

In vertebrates, as in all sexual animals, the first step in development is the union of male and female gametes, called fertilization. Fertilization is typically external in fish and amphibians, which reproduce in water, and internal in all other vertebrates (Raven and Johnson, 2003). *Barbonymus gonionotus* is one of the most economically important freshwater fish in Thailand. It grows to marketable size within 8-12 months and mature at 4-6 months of age (Saepithakkiat and Leenanond, 1984).

There are several species of barb found in Thai waters, and these include the Beardless, Golden Belly, Hampala, Smith’s Barb and silver barb (*B. gonionotus*). -Silver barb are important food fish in Thailand, and are farmed intensively, as well as frequenting natural waters. The silver barb found in the Mekong River are migratory fish, and can be caught in smaller streams, canals and flooded areas where they move to during the monsoon season (Jaggs, 2007).

The fish eggs are telolecithal, meaning that most of the egg cell is occupied by yolk. Cleavage can take place only in the blastodisc, a thin region of yolk-free cytoplasm at the animal pole of the egg. The cell divisions do not completely divide the egg, so this type of cleavage is called meroblastic. Since only the blastodisc becomes the embryo, this type of meroblastic cleavage is called discoidal (Gilberd, 2003).

The silver barb is a short-cycle species that, like Tilapia, can be farmed with low technology and relatively less effort than other species, being thus popular as a farmed fish in Bangladesh, where it is known as Thai Sharhunti. In fish farms, Silver Barbs
rarely exceed 40 cm in length and 1.50 kg in weight. However, a 2.80 kg specimen was caught in the Teak Tree Lake in Thailand and the rod and reel record with a weight of 13 kg and a length of 90 cm was caught in Malaysia (Rainer and Pauly, 2006).

Objectives of this study work are; to evaluate each of the early developmental stages of silver barb (*Barbonymus gonionotus*) from fertilized egg to larvae, to examine the features useful in the species identification of the larvae, to investigate the early egg and larval development of *B. gonionotus* as a basis for future physiological studies and to suggest the introduction of this valuable species into commercial aquaculture.

2. Materials and Methods

2.1 Study period

The study was carried out from July to September, 2012.

2.2 Study site

Fertilized eggs or embryos of *Barbonymus gonionotus* (Bleeker, 1850) were collected from Tha-yet-kone Fishery Station, Patheingyi Township, Mandalay Division. It is located in 21° 59’ 23.55’ N and 96° 07’ 29.77’ E. (Fig. I)

2.3 Data collection

2.3.1 Selection of breeder

Induced breeding of *B. gonionotus* was done by Tha-yet-kone Fishery Station. The technique of induced breeding for *B. gonionotus* should be used by means of one dose of injection in the period from May to September. Selection of male and female *B. gonionotus* was made. Selected breeders were three years old.

Healthy, mature and ripe females and males were identified visually. Females with soft, elastic, bulging bellies and swollen, reddish vents were taken as ripe ones. Oozing males was considered as ripe ones (Chaudhuri, 1962). An average
body weight of 500 kg and average body length 8-10 inches of both males and females of

*B. gonionotus* were used in this research (Figure-1 A and B).

![Image](https://example.com/image.png)

**Figure 1.** Map of Tha-yet-kone Fishery Station

### 2.4 Study area

Injection time was recorded and eggs were examined to observe. The collected eggs were brought to the laboratory, Department of Zoology, Mandalay University. Unfertilized eggs and fertilized were differentiated.

### 2.5 Data analysis

Fertilized eggs were poured into the petridish containing water. Water temperature was 28° C to 32° C and studied under compound microscope in fresh condition. Then unfertilized eggs, embryo, and larva were measured to the nearest micrometer and an average was taken.

The developing embryo was divided into 8 parts according to Freeman and Bracegirdle (1978) and Jones *et al.*, (1978). Early cleavage stage (1 to 32 cells) (Figure-2 A to G), morula stage (Figure-2 H-I), blastula stage (Figure-2 J to L), gastrula stage (Figure-2 M-N), bud stage (Figure-2 Q), somite formation stage (Figure-2 P to S), hatching stage (Figure-2 T) and larval stage (Figure-3) were studied and recorded. The
photomicrographs were taken with the use of Olympus DP12 Microscope Digital Camera at all developmental stages from fertilized eggs to larvae of *B. gonionotus*. The chronological data were also recorded (Table.1).

3. Results

3.1 Development of embryo

In the present study, the developmental stages were divided into 8 parts. Early cleavage stage (1 to 32 cells), (Figure 2 A to G), morula stage (Figure 2 H to I), blastula stage (Figure 2 J to L), gastrula stage (Figure 2 M to N), bud stage (Figure 2 O), somite formation stage (Figure 2 P to S), hatching stage (Try to hatch) (plate 2 P) and larval stage (plate 3).

At 6 hours after injection, the eggs were examined to observe the fertilization. The fertilized eggs were easily separated from the unfertilized eggs because fertilized eggs were transparent and the unfertilized eggs were opaque. Cleavage occurs in a cytoplasmic cap, the blastodisk, on the large mass of yolk. The first two cleavage furrows appeared vertical, at right angles to each other. The third followed, forming eight cells.

3.2 Unfertilized egg

Unfertilized eggs are a spherical shape, enclosed in the transparent Chorion. The color of the egg is dark gray. Animal pole and vegetal pole cannot be differentiated. They are observed to be sticky (Figure 2 A).

3.3 Early cleavage stage (1-32 cell)

3.3.1 The one-cell egg stage

One-cell embryos or fertilized eggs of *Barbonymus gonionotus* were observed at about 10 minutes after fertilization. The fertilized eggs were yellowish-white egg capsule, non-sticky, transparent while the yolk was pale yellow or green and
granular. The eggs of *B. gonionotus* became translucent as development progressed. The average diameter of the fertilized egg and yolk were 81.3±3.24μm and 23.81±1.68μm. Development is initiated without cleavage. The germinal disc or blastodisc begins to form over the yolk (Figure 2 B).

### 3.3.2 The 2-cell stage

The embryo continued to develop and the first cleavage. This stage was first observed after 15 minutes of fertilization, meroblastic (partial) cleavage, restricted to animal pole and the blastodisc divided into two equal halves of blastomeres. Measurement of the embryo and each blastomere were 28μm and 14μm (Figure 2 C).

### 3.3.3 The 4-cell stage

The second cleavage plane emerged at right angles to the first, producing an embryo of the 4-cells egg stage at 20 minutes after fertilization. The average diameter of embryo and each blastomere were 28μm and 7μm.

### 3.3.4 The 8-cell stage

At 4:50 hours the second cleavage was followed by the third cleavage of 8-cell stage 25 minutes after fertilization. Which was noticed as two rows of four cells over the yolk. The measurement of each blastomere was half in size but the embryo was not much different from that of 4-cell stage.

### 3.3.5 The 16-cell stage

At 30 minutes after fertilization, the fourth cleavage furrowed and resulted in the 16-cell embryo stage, in four rows of four cells over the yolk. Blastomere getting smaller than those of the previous stage.

### 3.3.6 The 32-cell stage

At 35 minutes after fertilization, the fifth cleavage furrows
and resulted in 32-cell stage embryo stage. In this stage, elevated blastoderm formed into a globule-shaped blastomeres. The split is unequal, since the size of animal pore blastomere is much smaller than the vegetal pore. The number of blastomeres were difficult to count as they were increasing and changing in size and shape. The shape of the egg was rounded.

3.3.7 Early morula stage

At 40 minutes after fertilization, the fifth cleavage followed by irregular cleavage, resulted in an embryo with several blastomeres, which were extremely difficult to count. Thus resulting in a rounded ball of cells.

3.3.8 Late morula stage

This stage was occurred at 55 minutes after fertilization. The blastoderm consist of an outer and inner layer of smaller cells row. The cells were relatively smaller than the previous stage due to rapid cell division. There was no major change.

3.3.9 Early high blastula

At 1 hour and 5 minutes after fertilization. The bulging blastoderm consist of a small bubble-shaped cytoplasm.

3.3.10 Middle high blastula

Beginning at the tenth cell division, the mid-blastula transition was detected at 1 hour and 35 minutes after fertilization. Cell division was slow, and cell movement was evident. The blastoderm started to extend slightly over the yolk. The cells of the blastoderm gradually became smaller than in the previous stage, however with no major change in the shape of blastula.
3.3.11 Flat blastula

At 2 hours and 5 minutes after fertilization, the blastoderm flattened. The cells at the vegetal edge of the blastoderm merged with the underlying yolk cell, this fusion formed a ring of nuclei. A cap of cells arched over the blastoderm.

3.3.12 Early gastrula stage

At 2 hours and 50 minutes after fertilization, early gastrula stage appeared with no significant change in the egg diameter. The cell at the vegetal pole inverginated into the interior. The peripheral edge of the blastoderm extended over the surface of the yolk and encircled one-third of the yolk.

3.3.13 Late gastrula

At 4 hours and 10 minutes after fertilization, half of yolk was covered by blastoderm as the epiblast. During migration, one side of the blastoderm became noticeably thicker than the other, which later becomes the dorsal side of the embryo.

3.3.14 Bud Stage

At 5 hours after fertilization, the head and tail region began to appear. The embryo was slightly elongating along the long axis and roughly bean-shaped. The gastrula was still only two-layered (ectoderm and endoderm) and the third layer (mesoderm) appeared at this time.

3.3.15 Somite formation

At 7 hours and 25 minutes after fertilization the eye vesicle could be observed. The embryo increased in size and 6-somites were visible at the middle portion of it. At 5 hours 57 minutes, 11-somite and notochord were observed. At 9 hours after fertilization, 25-somite and optic vesicle were clearly visible. The embryo was distinctly elongated (9.6 μm). Head and tail ends were distinct. The anterior part of the
neural tube could be soon distinguished. The heart beating started to circulate colourless plasma. Caudal section of the embryo detached from the yolk sac.

3.3.16 33-Somite formation

At 12 hours and 39 minutes after fertilization, 33-somite was appeared. The embryo increased in size (11.3μm). Digestive organs were developed posterior to the yolk sac. Anal structure was observed and located between 22 and 23 myomeres.

3.3.17 Hatching Stage (Trying to hatch)

At 15 hours and 14 minutes after fertilization, the larva appeared transparent with large eyes and more active, in an attempt to hatch. The yolk sac disappeared completely and the swim bladder was filled with gas.

3.3.18 Hatching Stage or Larva

Hatching began 16 hours after fertilization. The average length of the larvae was 1.96±0.03mm. One day old larva was observed to be still transparent, increasing pigmentation.
Table 1. Chronological data of the development of *Barbonymus gonionotus* at 28 °- 32 °C

| No | Stages of Development  | Time taken   |
|----|------------------------|--------------|
| 1  | Unfertilized egg       | 0 hour       |
| 2  | One celled stage       | 10 minute    |
| 3  | Two celled stage       | 15 minutes   |
| 4  | Four celled stage      | 20 minutes   |
| 5  | Eight celled stage     | 25 minutes   |
| 6  | Sixteen celled stage   | 30 minutes   |
| 7  | Thirty-two celled stage| 35 minutes   |
| 8  | Early morula           | 40 minutes   |
| 9  | Late morula            | 55 minutes   |
| 10 | Early blastula         | 1hr 15 minutes |
| 11 | Middle high blastula   | 1hrs 35 minutes |
| 12 | Flat blastula          | 2 hrs 05 minutes |
| 13 | Early gastrula         | 2 hrs 50 minutes |
| 14 | Late gastrula          | 4 hrs 10 minutes |
| 15 | Bud stage              | 5 hours      |
| 16 | 6- somite formation    | 7 hrs 25 minutes |
| 17 | 11-somite formation    | 8 hrs 57 minutes |
| 18 | 25-somite formation    | 9 hours      |
| 19 | 33-somite formation    | 12 hrs 39 minutes |
| 20 | Try to hatch stage     | 15 hrs 14 minutes |
| 21 | Hatching stage         | 16 hours     |
A. Unfertilized egg  
B. One-cell stage  
C. Two-cell stage  
D. Four-cell stage

Figure 2 Barbonymus gonionotus female (A) and male (B)
E. Eight-celled stage

F. Sixteen-celled stage

G. Thirty-two cell stage

H. Early morula stage

I. Late morula stage

J. Early blastula stage
K. Late blastula stage

L. Flat blastula stage

M. Early gastrula stage

N. Late gastrula stage

O. Bud stage

P. 6-somite formation stage
Figure 3 Developmental stages of egg (A-T)
Figure 4. Larval stage of *Barbonymus gonionotus*

4. Discussion

Bhuiyan *et al.* (2006) indicated that the breeding season of *Barbonymus gonionotus* (Bleeker, 1850) lasted from April to September, but April to May and July to August were the best periods for spawning.

According to this, the present experiment of the study on the embryonic development of *B. gonionotus* had been done in the best period of July to September 2012. The reproductive performance with no loss in fertilization capacity was obtained.

Akhter *et al.*, (2003) described the mean fertilized egg of *Barbonymus gonionotus* diametre was significantly (P<0.05) lower (95.77) than that of the other strains. The highest value was observed in Bangladesh and crossbred, 113.35 mm and 111.40 mm, respectively. In this study, the mean diameter of the fertilized egg was 81.3±3.24μm.

Gall (1974) and Islam *et al.* (1973) conducted egg size which might influence the hatchability and growth rate of fry. Several investigators suggest that egg size depended on the size of the female parent. Selection of larger egg size might be an important index because a larger egg could produce a larger fry (Gall 1974; Fowler 1972; Dunham *et al.* 1983). The present experiment, larger size range between 8-10 inches of both sexes was used. The data obtained from experiment clearly reflected to assume of these authors. Fertility also variable concerned with female parent body size.
The fish naturally spawned within 4.59 to 5.34 hours after administration of carp pituitary homogenate. During this period, air temperature ranged from 28.9° to 29.8°C. Hussain et al. (1987) reported that the natural spawning of *Puntius gonionotus* was accomplished in the spawning hapas within 4 to 6 hours after decisive injection at the ambient temperature of 27°-29°C.

Cleavage is characterized by cell division and gastrulation is characterized by major rearrangements of cells. Cells of the blastula undergo major rearrangements within the embryo to reach the gastrula and neurula stages. Gastrulation is the process by which the embryo forms a distinct endodermal tube that constitutes the early gut. The space enclosed within the gut is the gastrocoel, or archenteron. Neurulation is the process of forming an ectodermal tube, the neural tube. This tube is a forerunner of the central nervous system and encloses the neurocoel (Kardong, 2010). The result of the present study was similar to those previous research described by Kardong (2010).

Datta (2005) described embryonic development of *Pantius sarana* that 30 minutes after fertilization, the blastodisc began to form over the yolk, following first, second and third cleavage. Sixty-four cell stages were observed after 70 minutes post fertilization followed by morula stage in two hours. Yolk plug stage appeared after five hours. The cephalic and caudal end of the embryo had differentiated after 10-15 hours. After 16-21 hours, the gut appeared faintly posterior to the yolk sac, leading to the anus, and movement of the embryo could be seen within the egg. After 22 hours, movement of the embryo was observed. The eggs began to hatch after 23 hours.

Chaudhary et al., (2008) mentioned that *Barbonymus gonionotus* is an important tropical fish species on account of its fast growth rate, palatability to a wide range of culture conditions.
In Thailand, for induced breeding of *B. gonionotus*, a 2-injection procedure was a normal practice (Srisuwantach, 1981). It took 12 hours from the first injection to ovulation. Hussain *et al*. (1987) mentioned that natural spawning was accomplished in the spawning hapas within 4-6 hours after decisive injection at the ambient temperature and that a significant increase in the mean period from injection to spawning between the experiments was the result of lower temperature and not the effect of dosage. Chaudhuri (1962) reported that *Puntius sarana* (Ham.) hatched out within 13-17 hours.

In this research, same species of fish was conducted, fertilization time was similar to the previous research and however, this study was different on time of morula stage, at about 70 minutes after fertilization. The time of differentiation into cephalic and caudal end was also significantly different, so also the hatching time in *B. gonionotus* was approximately 16 hours.

Although *B. gonionotus* is an introduced species, the results of the present study indicated that *B. gonionotus* can easily be cultured and reared as an important source of protein supplement for local populace.

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