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Embryology and early ontogenesis of the threatened menoda catfish, *Hemibagrus menoda* (Hamilton, 1822)

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Abstract
Knowledge of the early life stages of fishes portrays the morphological changes they undergo during embryogenesis and early ontogenesis which aids in their culture and preservation. This study investigated the embryonic and larval development of the menoda catfish, *Hemibagrus menoda* from fertilization to post-hatching. The ovulated eggs were placed in convex slides and the embryonic and larval developmental stages studied under the microscope. Unfertilized eggs were sticky, mean egg diameter increased from 0.8 ± 0.00 mm to 1.10 ± 0.01 mm after fertilization. Cleavage stage occurred within 2. 50 hr and hatching occurred at 19.30 hr after fertilization at 24.1 ^{0}C water temperature. Newly hatched larvae were curved, laterally compressed with the body gradually tapering towards the tail and measured 3.0 ± 0.02 mm. The egg yolk became completely absorbed within 68 hours at mean length of 6.5 ± 0.05 mm. Findings from this study might aid in the development of appropriate culture program for this species hence preventing its extinction.

Keywords: *Hemibagrus menoda*; embryonic development; early ontogenesis; conservation

1. Introduction
Increase in human populations has increased the demand for cultured fish [1] and if this demand is to be met in the coming years, then aquaculture will play a major role [2]. *Hemibagrus menoda* is one of the siliruid catfish species that is highly cherished due to its tasty flesh and large size (max. length > 45.0 cm TL) which dwells in rivers, haors and beels of Bangladesh, Nepal and India [3]. However, as a result of unprecedented excavations, chemical pollution, erosion and siltation, the *H. menoda* habitat has become degraded and its populations drastically declined leading to the categorization of the fish as `near threatened' in Bangladesh [4]. To curtail the imminent extinction of this valuable food fish, study of its early life stages and intensive culture has become necessary for its harvest and conservation.
The knowledge of embryonic and larval stages of a species allows for morphological and physiological understanding of the species and helps to control the period of the larvae vulnerability. For example, the high rate of cannibalism observed in cultured fishes is frequently due to inadequate larval food and an improper feeding regime, demonstrating the importance of studying larval development [5]. Early life of fish is characterized by various cellular and morphological changes [6]. Fish life begins with the fusion of male (sperm) and female (ova) gametes to form a zygote and thereafter the embryo undergoes series of developmental changes resulting to hatching. The elucidation of feeding behaviour, prey preference, the ecology of the larvae of cultured fishes, and the marking of developmental time-clocks like yolk sack absorption and mouth opening (which varies among species) will determine the requirements for exogenous food during larval growth [5]. It is pertinent to observe and pinpoint the period of yolk sac absorption by the larvae in order to know the appropriate time for first feeding. With information about appearance of organs, their functionality and their exact time of emergence, it is possible to establish management protocols and specific food recommendations for this critical phase [7]. Some research has been conducted on the early life development of some species in the genus Hemibagrus [6]. Recent studies on *H. menoda* were confined to sex ratio, length-weight relationships and condition factor and its food and feeding habits [8, 9]. In this study, the embryonic development and early ontogenesis of the threatened *H. menoda* was investigated.

This might facilitate its culture and preservation.

2. Materials and Methods

2.1 Broodstock collection and culture

Samples of *H. menoda* were obtained from Kangsha River (Fig. 1) Netrakona, Bangladesh and taken to the hatchery unit of the Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh where the fish were kept in circular tanks for 7 days with continuous water showering for acclimatization. The broodfish were stocked for nine (9) months in rectangular brood rearing earthen ponds (10 x 4.6 m^2) and fed twice daily with trash fish (protein 20.6±0.6%, lipid 6.3±0.7%, ash 1.3±0.1%, moisture 70.5±2.6% and carbohydrates 1.3±0.2%) at 3% body weight. Ready to spawn gravid females and males were selected and injected intramuscularly with PG extract then released into breeding hapa placed in tanks for synchronized spawning at 1♀:2♂ ratio.

Fig 1: Photograph of the Kangsha River at Jaria Zanzhail Netrakona, Bangladesh

2.2 Collection of egg and larval samples

About 50 fertilized eggs were randomly collected using an enamel plate from the breeding hapa and placed in a rectangular hatching tray (85 x 40 x 12 cm) with continuous water flow. Using an ocular micrometer attached to the eyepiece of the microscope and a stage micrometer on the slide panel, the development of the embryo was checked at intervals of 0, 5, and 10 minutes from fertilization to last stage of cleavage, 30 minutes from morula to late blastula and after every one hour from early gastrula to hatching. A minimum of 10 eggs undergoing embryonic development process were examined by placing in a convex slide containing distilled water (Plate 1). Larval samples were collected from the hatching tray first at 1 hr interval and subsequently the time interval increased. Minimum of 6 larvae were occasionally immobilized with 10% formalin before being examined under the microscope.

Plate 1: Convex slide containing egg samples used for the observation of embryonic and larval development of *H. menoda.*

2.3 Observation of developmental stages

The larval development time and total length (TL) from hatching were recorded to the nearest minute and mm, respectively. The TL of the larvae was taken by placing the larvae on a Petri dish with a graph paper attached beneath. The images of morphological changes in the larvae in each day were captured through a camera attached to a compound microscope (Olympus Lab EC 20X ~1500X) and specimens labeled accordingly.

3. Results

3.1 Embryonic development stages of *H. menoda*

3.1.1 Unfertilized to fertilized eggs

Mature eggs of *H. menoda* were greenish, spherical, sticky and demersal and measured 0.8±0.00mm in diameter (Fig. 2a). The fertilized eggs appeared transparent, spherical, sticky and demersal (Fig. 2b), showed double thin membranes and its diameter increased to 1.10±0.01mm.

3.1.2 Cleavage stages

This starts with the blastodisc, also referred to as the one – cell stage which results to a protoplasmic bulge at the animal pole (Fig. 2c). Egg diameter was 1.1 ± 0.01 mm at 15 min after fertilization (af). The first cleavage (2-cell stage) occurred 30 min af at 25.5 ^{0}C. The blastodisc divided vertically into two discoidal parts through the blastodisc but not the yolk region (Fig. 2d). Mode of cleavage was meroblastic. The second cleavage occurred 40 min af which resulted in four blastomeres of equal sizes (Fig. 2e). Third cleavage forming eight cells was observed 1.20 hr af and the egg diameter was 1.1 ± 0.01 mm (Fig. 2f). Fourth cleavage resulted to the formation of 16 cells while 32 blastomeres were formed and arranged in 4 x 8 pattern (Fig. 2h) at 2.20 hr af. Successive cleavages increased with time from 32 to 64, 128, etc, though the blastomeres decreased in size. Due to the numerous cells that accrued, it was cumbersome to count the eggs thus referred to as multi – cell stage (Fig. 2i). This occurred between 2.30 – 2.50 hr af and the egg diameter was measured as 1.4 ± 0.01 mm.

3.1.3 Morula stage

Morula stage was attained within 4.20 hr (Fig. 2j) at 25.7 ^{0}C characterized by continuous but irregular cell divisions which appeared compacted over the animal pole.

3.1.4 Blastula and gastrula stages

Early blastula was observed 4.50 hr af at 26.1 ^{0}C as the cleaved and compacted cells formed blastoderm over the yolk sphere with 1.4 ± 0.01 mm egg diameter (Fig. 2k). Late blastula which marked the commencement of epiboly occurred 5.20 hr af with 1.4 ± 0.01 mm egg diameter, whereby the mound of blastoderm spread over the yolk region with a clear differentiation of the posterior and anterior ends of the embryo (Fig. 2l). At early gastrula stage, the germ ring forms a dome shaped structure at 8.00 hr af (Fig. 2m) and the blastoderm started to increase by about ¼ of the yolk sphere leading to 30% epiboly. At late gastrula stage, blastoderm covered ¾ of the yolk (75% epiboly) and the embryonic shield was visible from the animal pole at 9.30 hr post fertilization (Fig. 2n).

3.1.5 Neurula/yolk plug stage

After 11.30 hr post fertilization, the yolk invasion was completed by gradual spreading over the germ layer (Fig. 2

o). Notochord was formed at this stage and the rudimentary head and tail became distinct at the anterior and posterior end, respectively (Fig. 2 p).

3.1.6 Organogenesis stage and just before hatching

Organogenesis/somite stage is the segmentation stage of embryonic development and occurred within 15.00hr af. It was characterized by formation of somites and an optic or `C′ shaped embryo (Fig. 2 q). Start of heart beating, appearance of rudiments of gill buds was observed and embryo encircled the whole yolk sphere at 26.1 ^{0}C, 1.4 ± 0.01mm egg diameter. Continuous and vigorous twisting movement was observed from 19.30hr af (Fig. 2 r). As a result, the egg capsule weakened and ruptured and the larvae emerged with the tail region while the head remained attached to the egg yolk. The diameter of the egg just before hatching was 1.9 ± 0.03 mm while the temperature was 24.6 ^{0}C. The hatching time was within 1.15 hr from when the first larvae hatched to the time when 50% hatched.

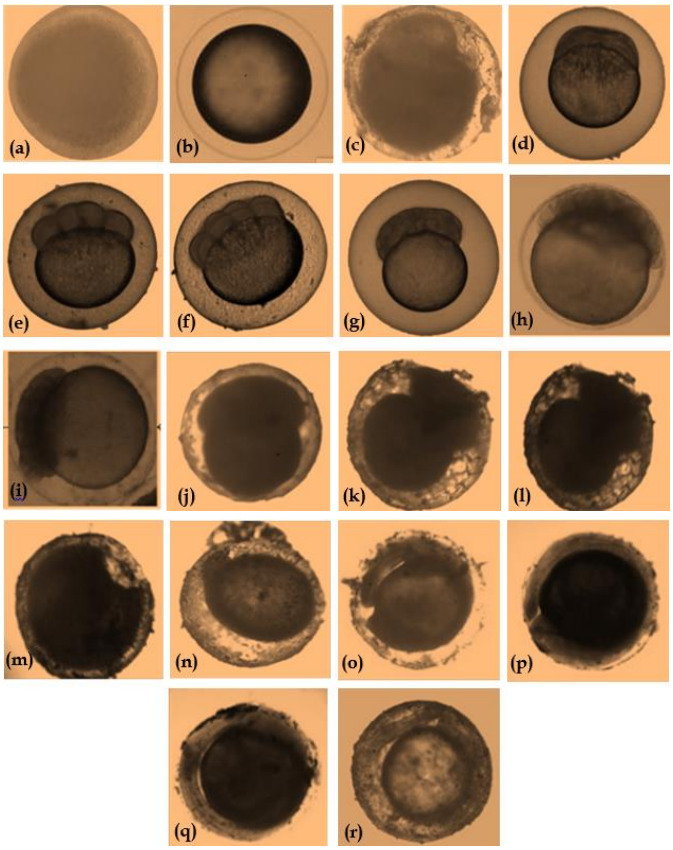

Fig 2(a – r): Embryonic development stages of *H. menoda*

a) Unfertilized egg, b) fertilized egg, c) Blatodisc stage d) 2-cell stage, e) 4-cell stage, f) 8-cell stage, g) 16-cell stage, h) 32-cell stage, i) multi-cell stage, j) morula stage, k) early blastula stage, l) late blastula stage, m) early gastrula stage, n) late gastrula stage, o) early neurula stage, p) late neurula stage, q) organogenesis stage, and r) just before hatching.

3.2 Larval development stages of *H. menoda*

Table 1: Description of larval development stages of *H. menoda*

Age of larvae	Stage	Figure no. 3	Mean total length (mm)	Characteristics
Newly hatched larvae	1	a	3.0 ± 0.02	Newly hatched larvae were curved with laterally compressed body. Yolk sac roundish, dark and attached to the gut.
2 hrs	2	b1&b2	3.2 ± 0.03	Notochord and melanophores become slightly visible.
4 hrs	3	c	3.2 ± 0.03	The eyes become slightly visible, melanophores appear at the posterior end.
6 hrs	4	d	3.2 ± 0.03	Barbels slightly appear in form of knobs, melanophores visible on head, yolk sac slightly decreased.
8 hrs	5	e	3.2 ± 0.02	Melanophores more visible at the posterior end.
10 hrs	6	f	3.3 ± 0.02	A tubular pulsating heart appeared, eye and anus become more visible.
12 hrs	7	g	3.4 ± 0.03	Chromatophores appeared in the eyes only, pectoral fin buds become visible.
18 hrs	8	h	4.0 ± 0.03	More distinct pigmentation with melanophores, mouth cleft and pairs of barbels visible.
24 hrs	9	i	4.30 ± 0.03	Eyes become darker, pectoral fin buds appeared and the hatchlings swim freely.
27 hrs	10	j1&j2	5.0 ± 0.02	Myomeres become more visible, yolk sac diminished.
38 hrs	11	k	5.5 ± 0.02	Eyes darker in colour, rudiments of gills opening appeared, yolk sac reduced.
48 hrs	12	l	5.8 ± 0.02	Yolk sac drastically reduced, chromatophores visible at caudal region, head distinct from the rest of the body, myomeres clearly visible.
56 hrs	13	m	6.0 ± 0.02	Yolk sac slightly visible, barbells, pectoral and dorsal fins prominent.

68 hrs	14	n1&n2	6.5 ± 0.05	Yolk sac completely absent, mouth well defined, barbells broad, fins very prominent, larvae swim actively and first feeding was observed.

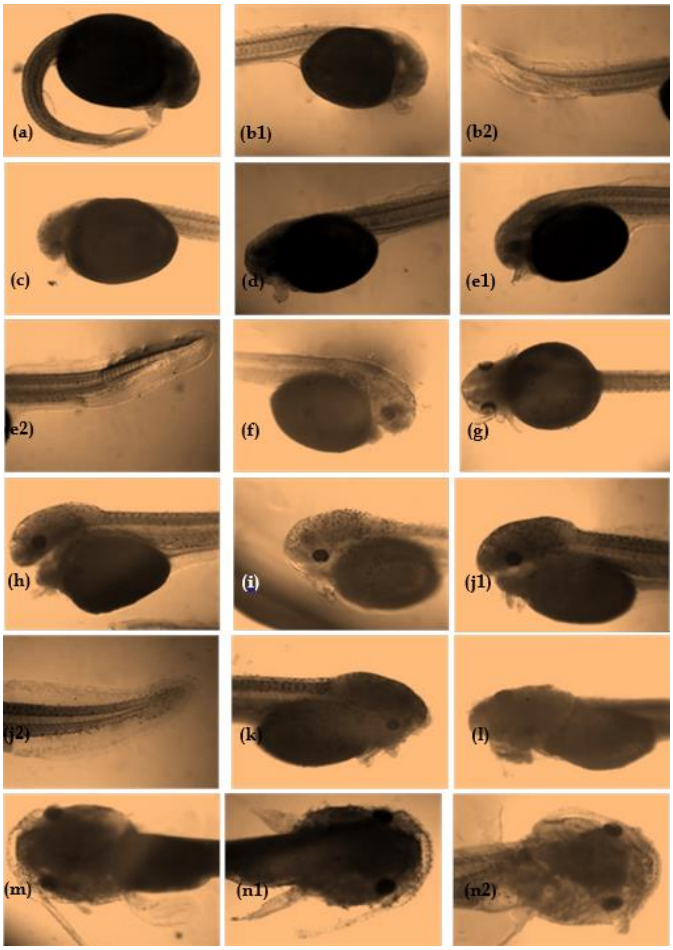

Fig 3(a – n): Larval development stages of *H. menoda*

a) Newly hatched larva, b) 2hr old larva, c) 4 hr old larva, d) 6 hr old larva, e) 8 hr old larva, f) 10 hr old larva, g) 12 hr old larva h) 18 hr old larva, i) 24 hr old larva j) 27 hr old larva k) 38 hr old larva, l) 48 hr old larva, m) 56 hr old larva, and n) 68 hr old larva.

4. Discussion

The study of the embryonic and larval development stages of *H. menoda* has enabled us to identify its characteristic early life history stages and period of yolk sac absorption. The fertilized eggs were transparent, spherical, sticky and dermersal. Rahman *et al*. [10] found that fertilized eggs of *Mystus cavasius* were spherical in shape, slightly adhesive, light pinkish and demersal. Similar results were also found in *Puntius sarana*, *Cyprinus carpio*, *Hemibagrus nemurus* and *Pangasius pangasius* [11-14]. The adhesiveness of the eggs of *H. menoda* could be due to its preference for muddy and fast flowing waters. It also indicates a behavioural strategy in its natural habitat whereby the eggs are confined to a single location until hatched. This observation corroborates with Husysentuyt and Adriaens [15] who mentioned that the eggs of armoured catfish *Corydoras aeneus* (Gill, 1858; Callichthyidae) were found to be very adhesive and opined that it could be because the species is known to inhabit turbid and turbulent waters. Unlike the eggs of *H. menoda*, non adhesive eggs were reported for *Rita rita* [16] and *Trichogaster fasciata* [17]. After fertilization, the mean egg diameter of *H. menoda* increased from 0.8 ± 0.00 mm to 1.10 ± 0.01 mm. The mean diameter of fertilized eggs found in this study is within the range reported for some other catfishes such as 1.15±0.15mm in *Colia nasus* [18], 1.00-1.30 mm in *Rita rita* [16], 1.18 mm in *Ompok pabda* [19], 1.50 mm in *Hemibagrus nemurus* [6] and 1.2 – 1.4mm found in fertilized eggs of *Channa striatus* [20]. However mean egg diameter of 0.50 mm was reported for *Mystus cavasius* and *Trichogaster fasciata*, which is smaller than that found in the present study. The variability in the egg diameter of fishes is attributable to differences in the age of spawner, water temperature and salinity. Lee [21] found that eggs produced under low salinity conditions were significantly larger than those produced at higher salinity levels. The blastodic formed 15 mins af. Similar observation was made in *Puntius sarana* [11]. In the present study, first cleavage (2-celled) was typically meroblastic and occurred within 30 mins af at 25.5 ^{0}C water temperature, followed by the second cleavage which was completed 40 mins af. Similar time was observed in *Heteropneustes fossilis* [22] and *Rita rita* [16] where the first cleavage began in about 30 mins af at 27-28 ^{0}C water temperature. *Mystus cavasius* was within 40-50 mins af [23]. However, longer time of 1.05 hr was observed before first cleavage in *Mastacembelus pancalus* [24]. These differences could be due to cell cleavage being highly conserved among distinct phyla [25]. The 8-celled, 16-celled, 32-celled and multi-celled stages were completed within 1.20, 1.40, 2.20, 2.50 hr, respectively af. In *Hemibagrus nemurus*, same sequence of stages occurred within 1.00, 1.15, 1.30 and 1.45 hr af [6], while longer time frame was observed in *Mastacembelus pancalus* which occurred within 1.20, 2.20, 3.10 and 5.20, af. The morula stage was completed 4.20 minutes af. Morula stage was completed within 1.20, 3.30, 4.00 and 6.00 hrs af in *Mystus cavasius* [10], *Puntius sarana* [11], *Carassius carassius* [26], respectively. This could be due to differences in species and temperature. In *Hemibagrus nemurus* [6] and Rita *rita* [16] blastula stage was completed within 4.30 hr and 3.00 hr af, respectively. In the present study, blastulation was attained within 5.20 hr at 26.4 ^{0}C water temperature. Gastrulation in *H. menoda* with 75% epiboly was reached at 9.30 hr post fertilization and the embryonic shield became visible from the animal pole. Marimuthu and Haniffa [20] reported that gastrula stage in *Channa striatus* was reached 9.00 hrs af. The embryo at this early stage is more susceptible to mechanical factors such as pressure and shock especially during early gastrulation [27]. The yolk invasion was completed 11.30 hr post fertilization, the rudimentary head and tail became visible at the anterior and posterior end during the yolk plug stage. In *Channa punctatus*, the yolk invasion was completed at 9 hrs af [28, 29]. Heart beat in the embryo of *H. menoda* was observed within 15.00hr af accompanied with appearance of rudiments of gill buds. This stage (somite) was characterized by formation of an optic embryo. The somite stage in *Rita rita* occurred within 13.30 hr post fertilization whereby some of the mesodermal cells divided the body of the embryo in a number of somites and visibly showed optic cups [16]. Just before hatching, continuous and vigorous twitching movements against the chorion facilitated successful hatching within 19.30 hr (incubation time) af at 24.1 ^{0}C water temperature and hatching period lasted 1.15 hr. The hatching time of *H. menoda* in this study obtained at 19.30 hr at 24.1 ^{0}C was

earlier than that obtained by Mollah and Tan [30] at 22.00 hr for *Clarias macrocephalus* at 30 ^{0}C, and Adebayo and Fagbenro [31] at 21.0 hr at 28.5 ^{0}C for *Heterobranchus bidorsalis*. The difference could be due to variation between species and oxygen supply rather than temperature.

The newly hatched larvae were 3.0 ± 0.02 mm in length, which is similar to the 3.0±0.2 mm reported for the species of the same genus *Hemibagrus nemurus* [6] but higher than the 2.0 mm reported for *Rita rita* [16], 2.62 mm for *Mystus cavasius* [10] and 2.7±0.2mm for *Coilia nasus* [32]. The difference was obviously due to inherent species size. The presence of yolk sac attached to the gut confirms the autonomous feeding character of the larva post hatching. Pectoral fin buds became visible on the 12 hr old larvae which is consistent with the observations of Rahman [33] for *Anabas testudineus* and Kohinoor *et al*. [19] for *Ompok pabda*. After 24 hours, dark eyes and barbells were distinct and the larvae swim freely, unlike the case of *Trichogaster fasciata* larvae that swam freely on day 3 [17]. The yolk sac was slightly visible and mouth cleft open in the 56 hour old larvae. The yolk sac of *H. menoda* larvae was completely absorbed within 68 hours at mean length of 6.5 ± 0.05 mm, swam actively and fed on the egg yolk emulsion provided as first feeding. The yolk sac of *Mystus montanus* was fully absorbed only after third day at 5-5.5 mm [34]. *Carassius carassius* also switched to exogenous feed at 3 days when yolk sac was absorbed [26].

In the present study, it has been observed that hatching takes place in *H. menoda* within shorter period than some other catfishes. This is an added advantage for its culture potential in terms of cost to the farmer. The identification of the period to exogenous feeding due to yolk sac absorption is a very critical period in fish development which if neglected could lead to cannibalism and predation. Finding from this study might aid in the development of appropriate culture program for this species hence preventing its extinction.

5. Conclusions

Study of embryonic and larval development of *H. menoda* revealed that hatching occurred within 19.30 hrs af at 24.1 ^{0}C, and the yolk sac of the hatched larvae was completely absorbed within 68 hours af at mean length of 6.5 ± 0.05 mm, which is a shorter time than in other catfishes like *Mystus montanus* and *Carassius carassius*. Identification of time for first feeding is very critical and prevents loss of fry to the farmer due to cannibalism and death.

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