Embryonic and larval development of an endangered catfish, *Horabagrus brachysoma*

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ABSTRACT

The fertilised eggs of Asian sun catfish, *Horabagrus brachysoma* (Günther 1864) were demersal and pale yellow in colour with equal perivitelline space. First cleavage appeared at 49 min (± 3 min) and the size of the blastomeres reduced as the development stage proceeded. Morula stage was reached 3 h 37 min (± 15 min) post-fertilization. The embryonic development from morula to hatching took about 16-17 h. The hatching of eggs started at 20 h 46 min (± 28 min) post-fertilization. The newly hatched yolk-sac larvae were 3-4 mm in length. A membranous layer was found covering from behind the head to posterior part of yolk-sac. Eye, mouth, alimentary canal, barbells and pectoral fins were appeared in the 3 days after hatch (dah). The membranous layer started rupturing at fifth day of post-hatch and disappeared during 9-12 dah with the appearance of fins in different parts of body. Pigments on dorsal side of body appeared in 2 dah larvae and the 10-12 dah larva looked pale brown in colour. After 12 dah, the larva morphologically resembled like an adult fish.

Key words: Asian sun catfish, Cat fish egg cell division, Embryonic development, Fertilization, Yellow catfish.

INTRODUCTION

The Asian sun catfish, *Horabagrus brachysoma* is a high priced catfish and is well known as ornamental fish during its early stage as well as food fish during adult stage. The population of this species is declining rapidly due to human interventions and habitat alterations. It is now enlisted as an endangered catfish (Anonymous, 1998). ICAR-Central Institute of Freshwater Aquaculture (CIFA, Bhubaneswar) is involved on its captive production during last few years due to its potentiality as high priced catfish (Sahoo *et al*., 2010a; b). The literature on its embryonic development is very limited to only three stages, while reporting on its induced breeding (Padmakumar *et al*., 2011). However, this study does not cover the entire developmental stages from egg activation to hatching and also the larval development. In literature, only few studies have been reported on the early life history and embryonic development of some bagrid catfishes (Saigal and Motwani, 1961; Arockiaraj *et al*., 2003; Rahman *et al*., 2004; Mollah *et al*., 2011; Adebiyi *et al*., 2013). In view of this, an attempt has been made to study the embryonic development and larval history of the *H. brachysoma* catfish.

MATERIALS AND METHODS

Breeding protocol: The pond raised brood fishes were caught during spawning season (July-August) for breeding operation. The perspective-matured females were selected on the basis of uniform intra-ovarian oocytes collected by catheter and males were selected by seeing free oozing of milt. Ovaprim at the dosage level of 1.0-1.5 mL kg⁻¹ body weight was injected to females for successful ovulation, whereas the males did not receive any hormone injection (Sahoo *et al*., 2014). The males and female were released separately in ferro-cement tanks (1.5 m diameter) with continuous aeration. The readiness of females was judged by seeing the free flow of eggs by applying the gentle pressure on the abdomen. The females were stripped after seeing the free flow of eggs and were thoroughly mixed with the milt stripped from the males for fertilization. The fertilized eggs were properly washed and incubated in flow-through hatchery. The water temperature and dissolved oxygen of the supplied water were 28-30 °C and 5-6 ppm respectively.

Observation of embryonic development: Egg samples were collected in petri dish to observe the embryonic development under a dissecting microscope. The eggs containing round yolk sphere and smooth perivitelline space were considered for embryonic study. The timing of each developmental stage was recorded when more than 50% of eggs achieved the desired stage. A total of three observations were made from the eggs collected from three breeding operations to record the maximum variability of developmental timing. The eggs considered for the study were kept in petri dish under running tap water to avoid shortage of oxygen and abnormal development.

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**Microscopic examination:** The developmental stages of the eggs were captured under microscope (CKS41, Olympus) with photographic attachment and time required to reach the stage was noted. The total length and weight of newly hatched larvae were also recorded using ocular micrometre fitted to a compound microscope and with the help of electronic balance (XS 105, Mettler Toledo) respectively. The changes if any in the morphology of larvae were also recorded at three days interval. All the data related to embryonic development were expressed in hour: minute (Mean ± SD).

**RESULTS AND DISCUSSION**

**Egg development:** The time taken for the change of embryonic developmental stage of *H. brachysoma* egg is presented in Table 1. The ovulated and the fertilized eggs were of 0.9-1.2 and 1.1-1.4 mm in diameter respectively. These were non-adhesive and demersal in nature (Fig. 1a). The perivitelline space was unequal around the yolk sphere and was filled with clear fluid.

**Cleavage stages (Single cell to sixty four cell stage):** Single cell stage became clear by the accumulation of yolk free cytoplasm over the animal pole with a protrusion at 00:35±00:02 h, representing early blastodisc or germinal disc stage (Fig. 1b). Cytoplasmic disc became thick and first cleavage furrow developed vertically over the blastodisc. The blastodisc divided into two blastomeres at 00:49±00:03 h (Fig. 1c). Four (Fig. 1d) and eight-cell (Fig. 1e) stages were observed at 01:12±00:03 h and 01:28±00:01 h respectively and remained in two rows. Sixteen (Fig. 1f), thirty two (Fig. 1g) and sixty four (Fig. 1h) cell stages were appeared at 02:01±00:03, 02:22±00:09 and 02:46±00:17 h respectively. The blastomeres at these stages were reduced in size. The cleavage planes were no longer regularly patterned as compared to 2-8 cell stage.

**Morula stage:** During the morula stage (03:37±00:15 h), the blastomeres were further divided into many cells and accumulated around the animal pole, representing a flowery appearance (Fig. 1i). The central blastomeres were very small, compact and darker because of increased cellularity.

**Blastula and gastrula stage:** Blastula stage appeared at 07:03±00:11 h. The marginal blastomeres lost their boundaries and further compressed, where the individual blastomeres were not recognized properly (Fig. 1j). Gastrula stage appeared at 09:30±00:18 h, where sheet of cells migrated from animal pole in both the sides towards the vegetal pole (Fig. 1k). The cell migration continued and covered 40-50% over the yolk sphere called as germ ring, giving a thread like appearance. This germ ring proceeded further with differentiation of slightly broader at one end and narrow at other end during 10:30-11:45 h indicating future cephalic region and tail, respectively. Body axis mostly encircled the vitelline sphere with well differentiated head and tail, which looked like “C” shape (Fig. 1l).

**Twitching and hatching:** Embryo appears fully developed and tail portion was free from yolk sphere leading to twitching movement at 20:06±00:24 h. The embryo began continuous beating inside the egg shell and tried to rupture the perivitelline covering. Twitching rate was 50-60 times per min. In successive time the larvae hatched out at 20:46±00:28 h (Fig. 1m).

**Larval development:** The morphological changes in the larvae of *H. brachysoma* after hatching are presented in Table-2. The newly hatched larvae were transparent, straight and 3-4 mm in length. The mouth and barbels were not distinct. The mouth cleft was visible after 8-10 h of hatching and the jaws were not functional. Complete yolk sac was absorbed at the end of the 3rd day and the larvae started feeding on plankton. At this stage the alimentary canal was distinctly visible. Development of pigments was observed on the head and dorsal side of two days old larvae. The concentration of pigments was increased as the larvae grew. Later on these pigments were gradually expanded and merged with each other during 10-12 days and turn in light brownish colour. This colouration was changed to light yellowish by 20th day of life. Fin folds of hatching were disappeared before 8-9 dah and normal fins in different parts of the body were appeared. The dorsal, caudal, anal, pelvic and pectoral fins were noted with 5-7, 19-21, 23-27, 3-4 and 6-7 fin rays respectively during 12 day of life. At this time, the larvae acquired a definitive phenotype like an adult fish and showed normal swimming behaviour.

**Embryonic development:** The ovulated eggs of *H. brachysoma* were noticed 0.9-1.2 mm in diameter, whereas the fertilised eggs incubated in hatchery were increased to 1.1-1.4 mm in diameter, which may be due to imbibition of water. The egg size of fishes *viz.* *Mystus montanus*, *M. cavasius* and *Rita rita* belongs to same family was reported

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Time (h:min)</th>
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<tbody>
<tr>
<td>Fertilization</td>
<td>00:00</td>
</tr>
<tr>
<td>Blastodisc</td>
<td>00:35±00:02</td>
</tr>
<tr>
<td>Two cell</td>
<td>00:49±00:03</td>
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<tr>
<td>Four cell</td>
<td>01:12±00:03</td>
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<tr>
<td>Eight cell</td>
<td>01:28±00:01</td>
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<tr>
<td>Sixteen cell</td>
<td>02:01±00:03</td>
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<tr>
<td>Thirty two cell</td>
<td>02:22±00:09</td>
</tr>
<tr>
<td>Sixty four cell</td>
<td>02:46±00:17</td>
</tr>
<tr>
<td>Morula</td>
<td>03:37±00:15</td>
</tr>
<tr>
<td>Blastula</td>
<td>07:03±00:11</td>
</tr>
<tr>
<td>Gastrula</td>
<td>09:30±00:18</td>
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<tr>
<td>“C” shape embryo</td>
<td>10:58±00:19</td>
</tr>
<tr>
<td>Twitching</td>
<td>20:06±00:24</td>
</tr>
<tr>
<td>Hatching</td>
<td>20:46±00:28</td>
</tr>
</tbody>
</table>

*Time shown in the table for each developmental stage is the average value of three observations (Mean ± SD).*
Fig 1: Embryonic development of *Horabagrus brachysoma* (a) fertilized egg (b) blastodisc (c) two cell (d) four cell (e) eight cell (f) sixteen cell (g) thirty two cell (h) sixty four cell (i) morula (j) blastula (k) gastrula (l) “C” shape embryo (m) hatchling
The fertilized eggs of *H. brachysoma* took 03:37±00:15 h to reach morula stage in the present study. Different catfishes had different timing to reach morula stage. Whereas, it was appeared within 3.3 h in *Clarias gariepinus* (Bruton, 1979) and in *Heteropneustes fossilis* it was achieved within 1.5 h (Thakur, 1974). The time taken to reach morula stage in *M. cavasius* was similar with the present catfish (Rahman et al., 2004), but different as compared to 2.75 h in *R. rita* and 1.5 h in *M. montanus* (Arockiaraj et al., 2003; Mollah et al., 2011).

Gastrula stage appeared at 09:30±00:18 h which was approximately double as compared to *M. cavasius* (Rahman et al., 2004). This time variation during embryonic development might be due to species specific.

The growing embryo of *H. brachysoma* was occupied the entire perivitelline space by 19 h of embryonic development and the twitching movement was observed at 20:06±00:24 h. Arockiaraj et al. (2003) reported similar twitching period (1.5-2 h) and pause of twitching (1/30sec) in *M. montanus*. However, the hatching time was bit faster (20-21 h) in the *H. brachysoma* catfish as compared to 24-26 h at 23-25 °C in *M. seenghala* (Saigal and Motwani, 1961). This may be due to the impact of slightly high temperature of the incubated water i.e. 28-30 °C. Islam (2005) also reported the hatching time of Thai Pangas within

<table>
<thead>
<tr>
<th>Age of larvae in dah (days after hatching)</th>
<th>Yolk sac Length/height (mm)</th>
<th>Length (mm)/Weight of larvae (mg)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 dah</td>
<td>1.2-1.5/1.1-1.2</td>
<td>3.1-4.4/1.1-1.6</td>
<td>Head looks bent due to attachment with the yolk sac membrane. Heart is visible at the anterior part of yolk sac. Body is straight with fin fold. Yolk sac is oval and uniform. Mouth, eyes, alimentary canal and gills are not clearly differentiated. Barbels are also not clear, but bud like protrusion is marked at the head tip. Larvae show tail lashing movement at this stage. Finfoold persists and rays are visible in the membrane. Pectoral fin is prominent with 3-4 fin rays. 3-4 pairs of barbells are clearly visible in almost all the larvae. Yolk sac lost its uniformity and substantially absorbed from the anterior part. Heart beat is faster (120-137/min) as compared to newly hatched larvae. Eyes, mouth and alimentary canal are prominent. Opercular movement is visible. Pigments are also visible on the dorsal side of body. Larvae are more active with discontinuous swimming.</td>
</tr>
<tr>
<td>3 dah</td>
<td>0.83-1.23/0.36-0.95</td>
<td>5.4-6.7/2.2-3.1</td>
<td>Finfold on the body as seen at 3rd day, starts rupturing irregularly. Bifurcation of caudal fin is visible. Barbels are prominent. Eyes are prominent, round and black in colour. Pigments are increased in number and are more prominent. Alimentary canal is detectable. The larvae showed normal swimming. Dorsal fin (3-4 rays), pectoral fin (5-6 rays) and pelvic (2-3 rays) are detectable. Anal fin demarcation is visible with 20-24 fin rays. Body pigments on dorsal side are more prominent. The larvae look like an adult fish. The pigments lost their boundaries and spread over the body turn brownish. Dorsal and pectoral spines are prominent with serration. Bifurcation of caudal fin is more prominent and heterocercal in structure having 19-21 fin rays. Dorsal fin (5-7 rays), pelvic (3-4 rays), pectoral (6-7 rays) and anal fins (23-27 rays) are prominent.</td>
</tr>
<tr>
<td>6 dah</td>
<td>-</td>
<td>7.5-8.2/4.1-6.3</td>
<td>1.2-1.3, 0.49-0.51 and 1.3-1.6 mm diameter, respectively (Arockiaraj et al., 2003; Rahman et al., 2004; Mollah et al., 2011). In the present study unequal perivitelline space around the yolk sphere was filled with clear fluid, which may be acting as a cushion to protect the embryo from injury. The yolk sphere of the egg in the present study was recorded yellow in colour, whereas it was reported green in <em>Clarias batrachus</em> (Thakur, 1980).</td>
</tr>
<tr>
<td>9 dah</td>
<td>-</td>
<td>8.3-9.7/11.1-14.5</td>
<td>Different catfishes had different timing to reach morula stage. Whereas, it was appeared within 3.3 h in <em>Clarias gariepinus</em> (Bruton, 1979) and in <em>Heteropneustes fossilis</em> it was achieved within 1.5 h (Thakur, 1974). The time taken to reach morula stage in <em>M. cavasius</em> was similar with the present catfish (Rahman et al., 2004), but different as compared to 2.75 h in <em>R. rita</em> and 1.5 h in <em>M. montanus</em> (Arockiaraj et al., 2003; Mollah et al., 2011).</td>
</tr>
<tr>
<td>12 dah</td>
<td>-</td>
<td>9.3-14.3/15-25</td>
<td>Gastrula stage appeared at 09:30±00:18 h which was approximately double as compared to <em>M. cavasius</em> (Rahman et al., 2004). This time variation during embryonic development might be due to species specific.</td>
</tr>
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| 1.2-1.3, 0.49-0.51 and 1.3-1.6 mm diameter, respectively (Arockiaraj et al., 2003; Rahman et al., 2004; Mollah et al., 2011). In the present study unequal perivitelline space around the yolk sphere was filled with clear fluid, which may be acting as a cushion to protect the embryo from injury. The yolk sphere of the egg in the present study was recorded yellow in colour, whereas it was reported green in *Clarias batrachus* (Thakur, 1980). |

The time taken for the usual cleavage to reach eight cell stage was 01:28±00:01h in this catfish whereas the time taken from first cleavage to eight cell stage was reported 0:35-0:40 h in *M. montanus* (Arockiaraj et al., 2003) and 1.5 h in *R. rita* (Mollah et al., 2011). This might be due to species variation even though they are belongs to the same family. The reduced sizes of blastomeres at sixteen cell stage onwards were not followed regular patterned like 2-8 cell stage. This reduction in size of blastomeres may be due to further division of blastomeres, which is a common feature reported in many teleost during embryonic development (Thakur, 1980; Rahman et al., 2004; Gonzalez-Doncel et al., 2005).
24 h at 26 °C, which also lingered to 32 h at 20 °C. Embryonic development and hatching for C. gariepinus requires 21-26 h at 20-30 °C (Bruton, 1979).

**Larval development:** At the time of hatching the larvae of *H. brachysoma* were noticed transparent, straight (3-4 mm) and head looks bent due to the attachment over the yolk sac. This type of morphometry at hatching is found in other catfishes also such as *R. rita* (Mollah et al., 2011) and *H. fossilis* (Puvaneswari et al., 2009). The total length of hatching was reported 4-5 mm for *Heterobranchus longifilis* (Ogunji and Rahe, 1999), 3.6 mm for *C. gariepinus* (Bruton, 1979) and 2 mm for *R. rita* (Mollah et al., 2011). These variations of length among the larvae may be related to the egg size of the species. Bagarinao and Chua (1986) have also suggested positive correlation of egg size with the hatchlings. In the present study, mouth and barbels were not distinct just after hatching. Whereas, Adebiyi et al. (2013) reported that the mouth formation and appearance of barbels were also ambiguous at first day of hatching in *Hemibagrus nemurus*. The mouth cleft was visible after 8-10 h of hatching in the present study. However, different species showed variation for this period such as 36 h for *Channa striatus* (Marimuthu and Hanifa, 2007) and 3-4 h for *H. longifilis* (Ogunji and Rahe, 1999). In the present study complete yolk sac was absorbed at the end of the 3rd day of life, when the larvae started feeding on plankton during its rearing. The functional mouth was also observed when the larvae of *H. longifilis* started feeding after 48 h of hatching (Ogunji and Rahe, 1999). At this stage the alimentary canal was distinctly visible in *H. longifilis* (Ogunji and Rahe, 1999) which was also observed at the end of 3rd day in *H. brachysoma* larvae in the present study. Verreth et al. (1992) was also in opinion that the morphological and functional alimentary canal was only completed in the larvae at the onset of feeding. Development of pigments on the head and dorsal side of two days old larvae was observed during its larval development. As the larvae grew, the concentration of pigments was increased. These pigments gradually expanded and merged each other during 10-12 days of life turn light brownish colour. This colouration was changed to light yellowish by 20th day of life. Similar appearance of pigments on the body of catfish larvae was reported for *R. rita*, *H. fossilis* and *Rhamdia quelen* (Pereira et al., 2006; Puvaneswari et al., 2009; Mollah et al., 2011). The changes of colour were also reported in post larvae of *M. mantanus* to orange (Arockiaraj et al., 2003) and *C. striatus* to purple red (Yackov and Ali, 1992). The fin fold of newly hatched larvae were disappeared in 8-9 days in old larvae and normal fins in different parts of the body appeared. Similar appearance of finfold was also documented in *H. fossilis* (Puvaneswari et al., 2009) and *Pangasius sutchi* (I slam, 2005).

**CONCLUSION**

The present findings will be helpful in better understanding of the embryonic development of other bagrid catfishes. The pattern of larval development may also provide a basis related to ontogeny and phylogenetic studies.

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