Induced Breeding and Early Development of Stinging Catfish, *Heteropneustes fossilis* (Bloch) (Siluridae)

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Abstract Induced breeding, embryogenic chronology and larva development of *Heteropneustes fossilis* were studied in this work. One sexually matured female was injected intramuscularly with dry carp pituitary gland suspension at 4 mg/ kg of body weight. After a latency period of eight hours, the female was stripped while the male was sacrificed to remove the testes which were macerated to fertilize the eggs. Incubation was carried out at 27°C and the different stages of embryo were photographed using a Nikon SMZ 800 binocular microscope. Hatching started 23.53 hrs after fertilization and the percentage hatchability was 97.5%. Hatchlings had a mean length of 2.5 mm and mean yolk diameter of 0.1 mm. The yolk was completely absorbed between the third and fourth day of hatching and exogenous feeding started on the fourth day of hatching. The mean percentage survival was 75%. No new works are available. This study on *Heteropneustes fossilis* was carried out to monitor the embryogenic chronology, embryo survival and larva development with the aim of improving their artificial propagation.

Keywords 17 *Heteropneustes fossilis*; Cleavages; Ontogeny; Embryogenesis; Blastomereres; Hatchlings

1 Introduction

Indian catfish (*Heteropneustes fossilis* Bloch, Siluridae) popularly called Singh or stinging fish is fast becoming an important fish species in India. Unfortunately, it has a poor growth rate. At sexual maturity, female weighing about 100 gm can release about 8000 eggs. The eggs become sticky when fertilized and incubation period was reported to be between eighteen to twenty four hours depending on temperature (Singh et al., 1982; as quoted by Thakur, 2003). One of the major problems mitigating against the production of *H. fossilis* is poor survival rate due to mass larvae mortality and cannibalism. There is also lack of technical know how on their artificial production.

The understanding of the embryogenesis is a basic knowledge that is required to achieve success in improving the artificial production of any species and also improve their growth rate. A good knowledge of the embryogenesis will help to improve their culture practice, understand the species adaptations and improve artificial breeding techniques. Apart from these, (Koumoundouros et al., 2001) and (Borcato et al., 2004) also elucidated on the use of the knowledge of ontogeny in understanding the biology of any species and the functional trends and environmental preferences of the different developmental stages. Furthermore, the knowledge is essential for the development of potentials for detailed comparison between species (Verreth et al., 1992). Embryological studies are made easy because of the transparent nature of the embryo and its’ small size (Osman et al., 2008). Published work on the embryogenesis of *H. fossilis* is limited except for works like that of (Haniffa et al., 2002); Thakur (2003); (Thakur et al., 1974); and Kohli and Vidyarthi (1990). However, information on similar catfishes like *Heterobranchus longifilis* (Olufeagba, 1999); *Channa punctatus* (Banerji, 1974); *Clarias batrachus* (Thakur et al. 1974); *Clarias gariepinus* (Osman et al., 2008) and *Channa stratus* (Marimuthu and Haniffa, 2007) are available. To this end therefore, this study on *Heteropneustes fossilis* was carried out to monitor the embryogenic chronology, embryo survival and larva development with the aim of improving their artificial propagation.

2 Materials and Methods

This study was carried out at the Pisciculture Laboratory of the Center for Cellular and Molecular Biology, Hyderabad, India. The parent stocks were collected...
from the rearing tanks in the Pisciculture Laboratory. The female was injected intramuscularly with dry carp pituitary gland suspension at a dose of 4 mg/kg of body weight. After a latency period of eight hours at 27°C, the female was stripped while the male was sacrificed to remove the testes which were macerated in fish ringer (0.75% Nacl, 0.02% KCl and 0.04% CaCl₂). The eggs and spermatozoan were mixed and sufficient water was added to activate the spermatozoan. Mean length of unfertilized and fertilized eggs, embryo and larvae were taken. The eggs were incubated in 200 ml glass bowls inside the laboratory (temperature= 27°C). The water quality was maintained at the optimum condition (dissolved oxygen =5 mg/l, pH= 7.0) by changing water in the incubation bowls every two hours with fresh aerated de-chlorinated water.

Photographs of embryo development from egg stage to when they were 4 days old were taken with binocular microscope (Nikon SMZ 800). The time of occurrence of the different stages of development; egg fertilization, polar cap formation, 2, 4, 8, 16, 32- cell stages, morula, blastula, gastrula, somite formation and hatching were recorded. Two sets of twenty-fertilized eggs were separately monitored in 200 ml glass bowls to determine the fertilization rate, embryo survival rate from time of hatching till when they were four days old. Ontogenic development, endogenous feeding and commencement of exogenous feeding were also monitored. Fry were fed with Artemia and paramecium ad libitum twice in a day starting after yolk absorption.

3 Results

The female freely released eggs on gentle striping after eight hours of latency period. The eggs were green, translucent, round and moderately sticky with a mean diameter of 1mm. The eggs were surrounded by jelly and oily chorion wall (Figure 1a). Immediately after fertilization, the micropile closes up and oil globules were produced and soon dissolved again. The chorion wall swelled up and was lifted up from the newly fertilized egg (Figure 1b). There is accumulation of cytoplasm at the anterior part to form the animal pole (blastodisc) and yolk at the posterior part to form the vegetal pole (Figure 1c). All subsequent cell divisions occurred in the blastodisc, while the posterior part developed to form the nutritive yolk. The mean fertilization rate was 97.5% (Table 1).

| Stages of development | First set | Second set | Mean % survival |
|------------------------|-----------|------------|-----------------|
| Unfertilized eggs      | *20(100)  | *20(100)   | 100             |
| Fertilized eggs        | 19 (95)   | 20 (100)   | 97.5            |
| 2- cell stage          | 19 (95)   | 19 (95)    | 95.0            |
| 4- cell stage          | 19 (95)   | 19 (95)    | 95.0            |
| 8- cell stage          | 17 (85)   | 18 (90)    | 87.5            |
| 16- cell stage         | 17 (85)   | 18 (90)    | 87.5            |
| 32-cell stage          | 17 (85)   | 16 (80)    | 82.5            |
| 64-cell stage          | 17 (85)   | 16 (80)    | 82.5            |
| Gastrula               | 16 (80)   | 15 (75)    | 77.5            |
| Advanced gastrula      | 16 (80)   | 15 (75)    | 77.5            |
| Pre hatching           | 16 (80)   | 15 (75)    | 77.5            |
| Hatching               | 16 (80)   | 15 (75)    | 77.5            |
| Day 1                  | 16 (80)   | 14 (70)    | 75.0            |
| Day 2                  | 16 (80)   | 14 (70)    | 75.0            |
| Day 3                  | 16 (80)   | 14 (70)    | 75.0            |
| Day 4                  | 15 (75)   | 14 (70)    | 73.5            |

Note: *Number of eggs sampled per sets=20. Figures in parentheses represent % survival in relation to number of egg fertilized

Because the eggs were mildly adhesive, it was easy to observe the rate of development of each egg individually. Details on the time of cleavage and their description are presented in Table 2. The first cleavage which was meroblastic in nature producing two blastomeres from the blastodisc occurred 14 – 18 minutes after fertilization (Figure 1d). The second line of division which was perpendicular to the first line of division occurred within 31-34 mins after fertilization producing four blastomeres (Figure 1e). Within 41-45 mins of fertilization, the third cleavage took place producing 8 cells arranged in two rows of four cells (Figure 1f). The 16- cell stage occurred between 55-58 mins after fertilization (Figure 1g). As from the fourth cleavage which produced sixteen cells, the blastomeres were difficult to count as all consecutive divisions led to reduction in cell size. Not long after the last cleavage occurred, the 32-cell stage started at exactly the 60th mins after fertilization till the 63rd mins after fertilization (Figure 1h). The 6th round of cleavage produced 64-cells, and this took place 80minutes after fertilization (Figure 1i). Some blastomeres cover other ones at this stage making counting of individual cells impossible. Between this period and the 187th minutes after fertilization, four rounds of cleavages occurred leading to the formation of the Morula stage with
elevated rhomboid multicellular blastodisc (Figure 1j). The blastodisc soon flattens into lozenge shaped blastula (Figure 1k) at the 240th minutes after fertilization. The Gastrula was formed at the 426th mins after fertilization (Figure 1l). The thinning out of the blastodisc continues leading to formation of the advanced gastrula at 673rd minutes after fertilization and then the development of the embryonic shield at the 843rd mins after fertilization (Figure 1m). The margin of the blastoderm advances around the yolk downward. Figure 1n represents epiboly of 70%, while Figure 1o represents 75% epiboly. Figure 1p represents 80% epiboly followed by 90% epiboly represented in Figure 1q. Somite formation started from the 587th

Figure 1 Stages of embryonic development in *Heteropneustes fossilis*. a=egg. b=zygote. c=fertilized egg. d=2-cells stage. e=4-cells stage. f=8-cells stage. g=16-cells stage. h=32-cells stage. i=64-cell stage. j=Morula stage. k= blastula. l= gastrula stage m=Embryonic shield. n=70% epiboly. o=75% epiboly. p=80% epiboly q=90% epiboly r=somite formation. s. 12-somites. t = Heart formation (arrow). U=13-somites v= 15-somites w=17-somites x=20-somites y= 25-somites
Table 2 Embryonic development of *Heteropeustes fossilis*

| Stages            | Description of Morphological events                                                                 |
|-------------------|-----------------------------------------------------------------------------------------------------|
| Unfertilized egg  | Round, greenish and slightly adhesive with a mean diameter of 1 mm.                                 |
| Zygote            | The zygote swelled up. It was translucent with jelly chorion wall.                                 |
| Fertilized eggs   | Expansion of yolk away from the chorion wall. Accumulation of cytoplasm at the anterior part to form animal pole (blastodisc) and yolk at the posterior part to form. Vegetal pole. All cell divisions occurred in the blastodisc. |
| 2-cell stage      | First cleavage at the animal pole. Meroblastic type of division producing 2 cells.                 |
| 4-cell stage      | Second cleavage producing four cells at the animal pole. Line of division perpendicular to the first line of division (meroblastic). |
| 8-cell stage      | Each of the four cells divided into two producing eight cells arranged in two rows of four cells.   |
| 16-cell stage     | Fourth cleavage producing sixteen cells that now becomes difficult to count as consecutive divisions led to reduction in cell size. |
| 32-cell stage     | Fifth cleavage producing 32 cells                                                                  |
| 64-cell stage     | Sixth cleavage producing 64 cells                                                                   |
| Morula            | Further divisions leading to the formation of multicellular blastodisc                              |
| Blastula          | Cell flattened out forming epiboly, as embryonic shield on the animal pole                          |
| Gastrula          | Formation of germinal ring with two somites                                                       |
| Advanced gastrula | Formation of cephalic and caudal edges, with 22 somites and rudimentary eyes Embryonic shield Initiation of gastrula wriggling movement, olfactory pit, and otolith, cardiac beats to aid rudimentary fluid movement. |
| 25 myotome stage  | Body segmentation completed. First fluid movement initiated as heart contraction started beating initially once per 60 mins. This gradually increases with increase in time. |
| Pre hatching stage| Wriggling movement increased as chorion wall still enclosed the embryo, heart beat increased to 68 times per minute. |
| Hatching          | Rupture of the chorion wall as embryo contracts and tail first emerged, followed by the trunk and head region. |

Figure 2 Embryogenetic chronology of *Heteropeustes fossilis*
3 eggs representing 13.5% of total fertilized eggs died and these soon turned whitish making it easy to identify and were removed. At the gastrula stage, few eggs also died. These were seen in form of attenuated embryos (Figure 4c). One hatching was seen dead with loosed body texture at about twenty hours after hatching (Figure 4d)

4 Discussion
Artificial propagation was successfully carried out in *H. fossilis* and the photographs of the embryological chronology were taken revealing the stages in the development of the organs and systems in the body. The latency period of eight hours reported in this work was quite short compared with the 18 to 24 hours and 21 to 24 hrs reported for the same species by Haniffa and Sivasubbu (2002) and Roy and Pal (2006) respectively. The mean fertilization rate recorded (97.5%) was higher than 70 to 75% reported by Haniffa and Sivasubbu (2002) and 93% reported by Roy and Pal (2006) for the same species. These variations could be due to differences in the ripeness of eggs used or methods of activation that were however not reported by the authors. The shortness in the incubation period (22 hrs 8 mins) is an advantage for quick and mass artificial production of this species.

The embryogenetic chronology was similar to other freshwater siluriforms like *C. gariepinus* (Kamler et al., 1994), *C. gariepinus* (Osman et al., 2008) and *H. longifilis* (Olufeagba, 1999). According to the report of (Thakur et al., 1974) on *H. fossilis*, the first cleavage occurred in about 30mins after fertilization and the 16 cell-stage occurred in about 100 mins after fertilization under 26°C room temperature, this is slightly different from the observation in this work carried out at 27°C. The completion of all cleavage stages and hatching within 24hrs at 27°C in this work was similar to the reports of (Freund et al., 1995) and (Kamler et al., 1994) in *H. longifilis* and *C. gariepinus* respectively. It has been reported that hatching occurred between 23 to 24 hrs after fertilization at 29°C in *Channa striatus* (Marimuthu and Haniffa, 2007), 16 to 18 hrs in *H. fossilis* at a temperature of 26°C (Kohli and Vidyarthi (1990), 23.1hrs in *H. longifilis* at a temperature of 26°C (Olufeagba, 1999) and 24 hrs in
C. punctatus at a temperature of 28°C (Banerji, 1974). From all indications, the rate of embryogenetic development is dependent on temperature during incubation.

As observed in this work, the pattern of division in the formation of blastomeres in many catfishes is comparable; discoidal meroblastic cleavage is the rule (Kamlar et al., 1994; Osman et al., 2008; Olufeagba, 1999; Thakur et al., 1974b; Freund et al., 1995; Marimuthu and Haniffa, 2007; Kohli and Vidyarthi, 1990; Banerji, 1974). The timing in most catfishes are also relatively similar as observed by (Osman et al., 2008) in Clarias gariepinus; Marimuthu and Haniffa (2007) in Channa striatus and Olufeagba (1999) in H. longifilis. The mean percentage hatchability obtained was 97.5 % (Table 1) which was slightly below the 100% hatchability that was obtained in this same species before (Majumda, personal comm. 2008), and higher than 60 to 60.5 reported for same species by Haniffa and Sivasubbu (2002). The reason why we did not achieve 100% fertilization rate in this work probably could be due to hypoxial, unripe or over ripe conditions among the eggs and stress during stripping (Figure 4a), and fertilization. Stevens as quoted by (Kamlar et al., 1994) described non-viable eggs in Roccus saxatilis to be due to hypoxial within the ovary after ovulation through changes in the ovarian fluid, and this along with other factors like stage of ripeness of eggs affects the final rate of fertilization.

The mean length of the recently hatched fry (Figure 3a) obtained in this work (2.5 mm) and the yolk diameter (0.1 mm) were different from that reported for the same species by Thakur (2003) who gave 2.72 mm as the length of the fry. In similar species, Marimuthu and Haniffa (2007) reported 3.4 mm as the length of newly hatched Channa striatus fry. It is a known fact that the age and size of broodstock affect the size of eggs and subsequently the size of fry. This opinion was established in teleosts by Bagarinao and Chua (1986). This may be the factor majorly responsible for the variation in the mean values of the different authors, more so non gave hints on the size of the female used which may give indication on the sizes of the eggs.

The early development of the optic vesicles was an indication that the eye were functional before the hatchlings started active swimming and this will help to detect food well in advance of mouth formation, development of stomodium, and start of exogenous feeding. The early formation of the heart (Figure 1t) and rudimentary blood circulation at the embryonic shield stage were significant. This implies its functional significance as the network was laid before the embryo increased in size to allow for proper nutrient circulation in the embryo. Though heart beat rate was low initially, the rate continued to increase as the embryo was maturing. Similar observation has been reported in Clarias gariepinus by (Osman et al., 2008) The somatic contraction observed in the embryonic stage has been reported in Channa striatus (Marimuthu and Haniffa, 2007) and H. longifilis (Olufeagba, 1999) and this usually serves as precursor to hatching.

In hatchery management, knowledge of the time of yolk absorption and appropriate starter food are very significant. This is because exogenous feeding must commence few hours to the time of final yolk absorption so that the fry are familiar with external foods. This practice has been found to reduce mortality associated with “4th day mortality” syndrome in most Clariidae. The mouth gap of H. fossilis was observed to be smaller than the usual Artemia, and few that dare eat them had their thin walled stomach spliced by the claws of the Artemia due to improper digestion (Figure 4e). Feeding fry with smaller plankton like Moina micrura and Rotifers will aid higher survival. Similar observation was reported by Villegas and Lumasaga (1991) in similar small catfish like milkfish. Generally, catfish fry are known to have high preference for live zooplankton. Apart from the use of live zooplankton, early survival of fry also depends on water quality and other management practices that will ensure removal of pathogenic microbes. After hatching, the egg shell (chorion wall) has the potential of serving as primary initiator of fungus infection that can cause fry mortality and must be removed, to avoid water pollution. Complete yolk absorption is pointers that fry is commencing exogenous feeding (Figures 3e). The observation on the survival pattern in this work is a pointer to the need for good management at egg incubation stage as high mortality could occur unnoticed. The short duration and easy handling of the embryo stage along with their nutritive value (Thakur, 2003) suggests this species as a very good candidate for aquaculture.
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