Embryogenetic Studies of *Clarobranchus*

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ABSTRACT

Detailed embryological studies of *Clarobranchus* (*Clarias gariepinus X Heterobranchus bidorsalis*) was carried out to determine the onset of first mitosis, under laboratory temperature condition. Observation on embryogenesis showed that the formation of animal and vegetal poles were first observed at 19 minutes after fertilization. 2-cell attained at 38 minutes, 4-cell stage at 43 minutes and 8-cell stage was reached at 60 minutes. Other embryonic stages observed include morula, gastrulation, somite, first wriggling movement and hatching embryos emerged at 21 hours after fertilization. The average length of the hatchling was 3 mm. Daily growth was observed by the measurement of average length of 1, 2 and 5 days old hatchlings.

KEYWORDS: Fish, Fertilization, Embryonic Development and *Clarobranchus*

Introduction

It is pertinent to have knowledge of embryonic and larval development in different fish species to better understand the biology of different fish species in the areas of organ development, nutritional needs and environmental preferences (Borcato *et al*., 2004; Koumoundouros *et al.*, 2001). Egg and larval development studies of fish are also useful in understanding the systematic or genetic relationship useful for identification of spawning sites (Meijide and Guerrero, 2000). Disruption or abnormalities in developmental stages of embryos and larvae are often considered as indicators of disturbances in the environment. These stages are also useful in ontogenic and phylogenetic studies (Verreth *et al.*, 1992; Legendre and Teugels, 1991). Embryonic and larval development in cultured fish is important and evident in agricultural practices and fish production. Specifically, the needs of each larval stage and use of such information are key steps for increasing larval growth and maximizing survival (Puvaneswari *et al.*, 2009).

Heterobranchus bidorsalis and *Clarias gariepinus* are the river catfish found in most African countries. It is economically important for commercial fisheries and aquaculture and it has high food value because it is rich in omega 3-polyunsaturatedfatty acid (Rainboth, 1996; Mesomya *et al.*, 2002).The study was aimed at providing information on the embryogenesis of *Clarobranchus*, determining the developmental stages and by producing a photomicrographical description of embryogenesis of *Clarobranchus.*

Materials and Methods

The study was carried out in the Fisheries Laboratory, Modibbo Adama University of Technology Yola, Adamawa State. The female *Clarias gariepinus* (1 kg) was collected from the Department of Fisheries farm and male *Heterobranchus bidorsalis* was obtained from the commercial farm with body weight 5.6 kg. The female was induced with Ovaprim hormone. The dry method of artificial fertilization was used. Milt from the male was pooled and used to fertilize the eggs. Fertilized eggs were collected in a petridish and allowed to hatch inside the petridish for easy developmental stages determination. Timing of development, photomicrograph and morphological features were used to identify developmental stages according to Kimmel *et al*. (1995). Embryonic and larval development was observed by taking digital images using Leica DM 750 light microscope attached with a Leica digital camera ICC 50. Measurement of larvae was done by using a ruler. The time of appearance of each developmental stage was recorded.

Results

The formation of the animal and vegetal pole that divides the blastodisc into 2 blastomeres occurred within 19 minutes' post fertilization with the movement of eggs into the animal and vegetal pole. The animal pole further divided vertically into two, leaving the vegetal pole to develop as yolk. The cell division was clearly visible. Dividing the blastodisc into 2-cell stage occurred at 38 minutes' post fertilization. The second cleavage (4-cell) occurred at 43 minutes after fertilization, 8-cell stage was noticed within 60 minutes' post fertilization respectively.

The 16-cell stage occurred at 73 minutes' post fertilization. The 32-cell stage and 64-cell stage was difficult to observe as the line of division became difficult to recognize, and they occurred at 102 minutes and 124 minutes after fertilization. The other developmental stages and timing are shown in Table 1 and the photomicrographs in Plate 1. The newly hatched larvae measured 4 mm, while 2 and 5 day hatchlings were 6 mm and 7 mm respectively.

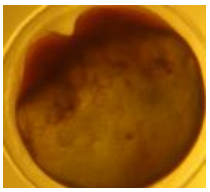
Discussion

The embryonic chronology of *Clarobranchus* reported in this study is similar to other freshwater Siluriformes as *H. bidorsalis, H. longifilis, C. gariepinus, C. anguillaris*. (Onyia *et al*., 2010, Aluko, 1994, Kamler *et al*., 1994 and Mollah *et al*., 1983).The fertilized eggs of *Clarobranchus* became adhesive, which is similar to those of other catfish such as *H. bidorsalis, H. longifilis, C. gariepinus, C. anguillaris.* (Onyia *et al.*, 2010, Aluko, 1994, Kamler *et al*., 1994 and Mollah *et al.*, 1983). The mode of cleavage recorded in the present observation is also similar to other catfish species; *Pangasius sutchi* (Islam, 2005).

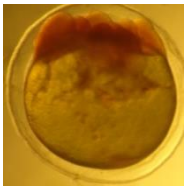
Table 1: Chronological Embryonic Development in *Clarobranchus* under Laboratory Temperature

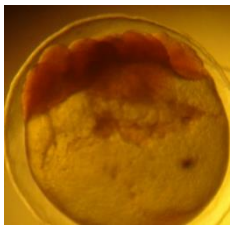
No	Stages	Time(Mins)	Description
1	Matured egg	0 mins	The unfertilized egg of *C. gariepinus* was golden and oval in shape. Fresh eggs were adhesive and surrounded by a layer of mucus which was transparent.
2	Fertilized egg	0 mins	Fertilized egg expands a few second after fertilization and become hardened.
3	Animal and vegetal pole	19 mins	Expansion of the yolk away from the membrane, and accumulation of cytoplasm at the anterior pole to form animal pole blastodisc and vegetal pole.
4	2-cell	38 mins	This was observed as a vertical division of animal pole producing two cells of equal size.
5	4-cell	43 mins	Second line of division producing4-cells of equal size.
6	8-cell	60 mins	Cells were seen as heap on top of the round lower yolk, cells were irregular in shapes.
7	16-cell	73 mins	Cells were clearly seen as irregular in size
8	32-cell	102 mins	Further division of cells. Some cells tend to lie on another cell, irregular in size.
9	64-cell	124 mins	Further division of cell and are difficult to count.
10	Morula	142 mins	Further division of cells producing many more cells, but small blastomeres, the morula decreases
11	Blastula	150 mins	Further division, cell producing mass of cells elevated over the general outline of the yolk mass. (Like dome shaped head)
12	Gastrulating	1020 mins	The embryo develops germ rings. Cephalic and caudal edges which formed at, advanced stage of the blastula.
13	First wriggling movement	1260 mins	Long somites start to move both sides within the chorion wall. It starts with the movement in 25 seconds, but this rate gradually increased with time.
14	Hatching	1320 mins	Violent movement of tail to either side against the current wall, followed by contraction of the embryo. As a result of contraction, the chorion wall breaks, and hatching occurs. Hatching not yet strong to swim vigorously in the petri dish.

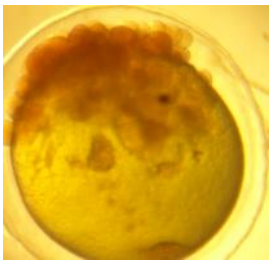

Fertilized egg

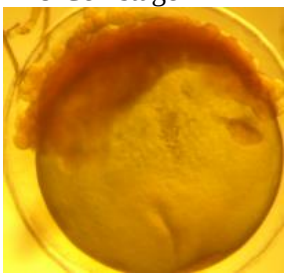

2-Cell stage

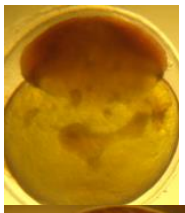

4-Cell stage

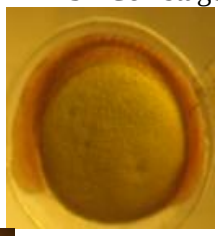

8-Cell stage

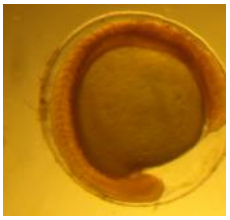

16-Cell stage


32-Cell stage


64-Cell stage

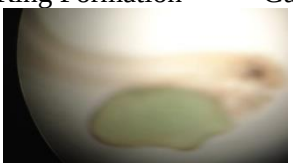







Morula stage Blastula stage Ring Formation Gastrula stage


Newly hatched


24-hour old


48-hour old


5-day old

Plate 1: Photomicrograph of some Developmental Features of *Clarobranchus*

In the present study the first cleavage occurred 38 minutes after fertilization and 16-cell stage was reached in 73 minutes after fertilization. Olufeagba (1999) observed similar result in *H. longifilis* and 16-cell stage at 82 minutes. Onyia *et al*. (2010) observed similar cell division with *H. bidorsalis* and 16-cell stage at 69 minutes. Aluko (1994) observed the cell division at 35 minutes after fertilization and 16-cell stage at 125 minutes in *H. longifilis.* Thakur *et al.* (1974) observed that first cleavage, 16-cell stage, and morula stage in *H. fossilis* were attained within 30, 70-80, and 100 minutes respectively post fertilization. Gastrulating stage was reached at 1020 minutes (17 hrs) post fertilization. In this study, wriggling movement and hatching were observed at 20 and 21 hrs respectively, which is similar to the result or observation reported by Onyia *et al. (*2010).

The observations on one day old larva of some catfish species were similar to *Clarobranchus* (Plate 1). A day old larvae of *M. cavasius* were smaller than those of *Clarobranchus*. The yolk sac was reduced and two pairs of barbels were observed. De Amorim *et al*. (2009) reported that one-day-old *R. quelen* had optical cups, notochord, embryonic fin, myomeres and developed circulatory system similar to *Clarobranchus*. Also, barbels were noticed in *H. fossilis*; but this was contrary to observation on one-day-old larvae of *Clarobranchus,* no barbels were observed at this stage. Ogunji and Rahe (1999) reported that mouth opening in *H. longifilis* occurred at 3-4 h post hatching, this was evident in *Clarobranchus*. Onyia *et al*. (2010) reported the length of 9 mm in 5-day old *H. bidorsalis* which is contrary to the present report of 7 mm of 5-day old *Clarobranchus.*

Conclusion

Clarobranchus has a short embryonic development period and sense organs developed rapidly. The study provides essential information on the embryonic and larval development of *Clarobranchus* and such information could also be beneficial for comparative studies and as a basis for further studies on the ontogeny of *Clarobranchus.*

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