EMBRYONIC DEVELOPMENT IN *Clarias gariepinus* (BUCHELL, 1822) UNDER LABORATORY CONDITIONS

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ABSTRACT

The embryonic development in Clarias gariepinus was studied under laboratory conditions. The development stages of eggs starting from first cleavage to hatching were examined microscopically. The accurate timing and detailed description of each stage were recorded. Photomicrograph of important stages, segmentation, blastulation, differentiation of embryo and hatching, was taken. The result shows that the blastodisc (polar cap) appeared 35±1 minutes after fertilization. The first cleavage dividing the blastodisc into two blastomeres occurred 15±0.5 minutes after the polar cap formation. The larva emerged from the egg case 22 hours after fertilization at a water temperature of 25.1±1.5 ^{O}C. This result will assist in better management of C. gariepinus, enhance their survival to fry and increase the supply of fingerlings in Nigeria.

Keywords: Embryonic development, *Clarias gariepinus*

INTRODUCTION

Knowledge of fish development process is important because of its role in life history studies and in fish culture practice. Sule and Adikwu (1999) reported that embryology unfold many features of evolutionary relationship. Much of the details of *C. gariepinus* embryology remain yet to be fully understood in spite of the fact that *C. gariepinus* is a better animal model for developmental and embryological studies. The merits include lack of pigment, ova transparency and convenient size. These facilitate observation of change in organs and tissues under the microscope (Sule, 2001).

A generalized account of embryonic development in fish are based largely on the works of (Battle, 1944; Carr, 1942) who indicated that in *Clarias anguilaris* the first cleavage of the fertilized eggs occurs 22 minutes after fertilization.

Despite the growing interest in the culture of African catfishes, the early life cycle of the group has not been thorough investigated for *Clarias gariepinus*.

Detailed embryological development of *Heterobranchus longifilis* has been reported (Olufeagba, 1999) and in *Clarias anguilaris* by Aluko, (1994). Detailed information on ontogeny of *Lucania parva* has been reported by Crawford and Balon (1994) and in *Tilapia zilli* by Omotosho (1989). In all these, continuous process of development was reported from the fertilized egg to hatching and free swimming stage.

The aim of this study was to investigate the embryonic development in *Clarias gariepinus* under laboratory conditions.

MATERIALS AND METHODS

Breeders of *Clarias gariepinus* were obtained locally from Lake Alau, Maiduguri. The males have an average weight of 450.0±0.3g with females having an average weight of 350.0±0.8g. The females were injected with ovaprim (Gonadotropic hormone) at 0.5 ml per kilogram of fish body weight after 10 hours latency period; the females were stripped by applying slight pressure on the abdomen. This led to running out of the eggs which were collected in a plastic container. The males were sacrificed to expose the testes which were removed and squeezed to let out the milt for fertilizing the eggs. Dry fertilization was carried out by mixing the milt and the eggs in a plastic bowl using feather. Measurements of diameter

of the egg were made to the nearest 0.01 mm using a microscope with micrometer.

Fertilized eggs were removed from the container and put in well aerated water in 60 x 30 x30 cm^3 glass aquaria at a temperature of 25.1±1^oC. Monitoring of the development stage of the fertilized eggs immediately after fertilization until the free – swimming stage was carried out.

Fifty fertilized eggs were removed randomly from incubating tank into a Petri dish in 5 batches of 10 eggs per batch. Selected eggs were viewed under the photomicroscope immediately after fertilization and at 5 minutes intervals for the first 3 hours, later at an hourly and two hourly intervals until hatching. The development stages of eggs starting from first cleavage to hatching were examined microscopically at a magnification of x 1000 for 22 hours.

Photomicrograph was used to take important stages of segmentation, blastulation, differentiation of embryo and hatching. The film of the photograph was developed and prints of each stage produced. The accurate timing and detailed description of each was done.

The newly hatched larvae were reared for 21 days in indoor aquaria. The fish larvae were fed with zooplankton mostly monia harvested from fertilized ponds. They were later transferred to the outdoor tanks (2 x 2x 1.m^3) and reared until they attained mean weight of 5.0 g.

RESULTS

Figure 1 shows the visible embryogenetic chronology of *Clarias gariepinus* immediately after fertilization up to the commencement of free-swimming of larva. The stages of embryonic development and time after fertilization are explained in table 1.

Fertilized Eggs: The fertilized eggs were brownish in colour spherical and adhesive. The mean egg size before fertilization was 0.65±0.02 mm and 1.01±0.19 mm after fertilization (Figure 1a)

Embryonic Development (Cleavage): The blastodisc (polar cap) appeared about 35 minutes after fertilization (Figure 1b) and the first cleavage dividing the blastodisc into two blastomeres occurred 15 minutes after polar cap formation (Figure 1c), The second cleavage perpendicular to the first, followed within 55 minutes after fertilization (Figure 1d). The eight cell stage was reached later in 10 minutes (Figure 1c). The fourth cleavage, which is parallel to the second one occurred 2 hours 5 minutes after fertilization and the 16 – cell stages was obtained (Figure 1f). The 32 cell stage followed by the sixth cleavage in another 4 minutes later. The Morula followed in 2 hours 9 minutes after fertilization (Figure 1g) Blastula was reached in 1 hour 3 minutes later (3 hours 17 minutes after fertilization) It was observed that as successive cleavage occurred the blastomerer decreased in size.

Formation of Embryo: The blastoderm cells started spreading over the yolk mass 6 hours 10 minutes after fertilization. Yolk mass invasion progressed considerably 6 hours 30 minutes after fertilization. The eggs reached the late gastrula stage and yolk mass invasion was completed. Gastrulation was completed (Figure 1f) after another 40 minutes.

Differentiation of Embryo: Early stages of somite formation started 7 hours 10 minutes after fertilization. Advanced form of somite formation was observed 13 hours later. Head and tail were clearly differentiated. 15 somites were counted while the embryo became C – shaped. In another 1 hour, myotomes appeared, the embryo developed further and looked like a girdle over the yolk mass (Figure 1i). The embryo elongated and the tail became separate from the yolk mass. In another 1 hour 30 minutes, the head separated completely from the yolk mass and the heart beat was noticed. The tail further elongated, while the embryo developed further and movements of the body could be observed approximately 20 hours after fertilization. In another 2 hours later, the yolk mass further reduced and the tip of the tail extended nearer to the head. 21 hours after fertilization, the embryo was fully differentiated and was about to hatch.

Hatching: The elongated tip of the tail struck against the head end of the egg shell causing the later to break, the head came out first. The larva shook off the shell and emerged completely from the egg case at about 22 hours after fertilization at a water temperature of 25.1^oC (Figure 1j).

Larval Development: The newly emerged larva had unpigmented eyes and no fin buds, the mouth is not formed and the anus situated posterior to yolk mass. The yolk sac projected anteriorly near end of the larva.

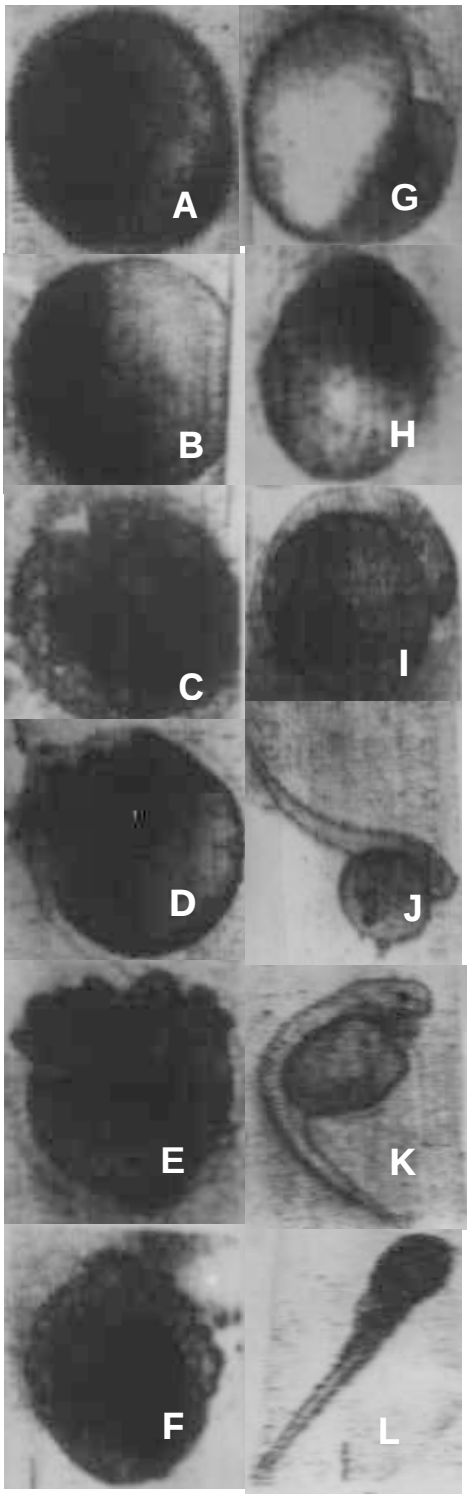

Figure 1: Stages in embryonic development in *Clarias gariepinus* under laboratory condition (27±1.5°C). ***A= Fertilized egg, B= Animal and vegetal pole, C= Two cells at first cleavage, D = Four blastomeres, E = Eight blastomeres, F = 16-32 cell stage, G = Morula/Blastula, H = Gastrulation, I = Advanced stage of somite formation, J = Hatchling (Day1), K = Hatchling (Day 2) and L = Hatchling (Day 3).***

Black pigmented cell were noticed in the fin fold except the tip of the caudal region. Pigments were also scattered in the yolk mass and on the head and body of the larva. The larva swims slowly up to the surface of the water and then gradually drops down and remains suspended in the column of water in an oblique position with head down in the Petri dish in an inclined posture (Figure 1k).

The day old larva had yolk sac that was considerably reduced. Faint pigmentation of the eyes was observed to have commenced. The hind gut was clearly visible. Melanophores were scattered on the head and trunk and also on the dorsal fin fold.

The yolk sacs of the two day old larva were very much reduced. The eyes were fully pigmented and pectoral fins elongated. The mouth was fully formed and the oesophagus was distinctly visible. There was pigmentation on the dorsal part of the body which was denser on the caudal peduncle region. There were a few melanophores over the posterior portion of the gut. The remnant of the yolk sac was observed as a small streak. The mouth was opened and the intestine was fully formed. The optic lens was shiny. The fry made slow directed movements with occasional jerks and moved on the surface as well as at the bottom of the water column (Figure 1l).

DISCUSSION

The embryology of *Clarias gariepinus* is similar to that of fishes like *Clarias anguilaris* (Kamler *et al.,* 1994) *Clarias macrocephalus* (Mollah and Tan, 1983). The completion of cleavage and hatching within 22 hours at 25.1 °C agrees with the reports of Freund *et al.* (1995), for *Heterobranchus longfilis* and Kamler *et al.* (1994) for *Clarias gariepinus*.

The early development of the optic vesicles is an indication that the eye was functional before the larva started active swimming. This will also help the larva to detect and identify their food well in advance of mouth formation and start of exogenous feeding. Similar observation was made in *Sarotherodon niloticus* (Omotosho, 1989). Head and blood vessel formation at twenty hours after fertilization suggest their functional significance as systems were laid ahead of time as embryo increase in size to allow for proper nutrient circulation in the embryo. The rate of heart beat continue to increase as the embryo matures, there is also an increase in somatic contraction and swimming activities noticed is in agreement with

Table 1: Embryonic development and time after fertilization in C*larias gariepinus* under laboratory condition

No	Stages of development	Time after fertilization	Description
	Unfertilized egg	0 min.	The unfertilized egg of *C. gariepinus* with a mean diameter of 0.65 ± 0.02 mm
A.	**Fertilized egg**	0 min.	The fertilized eggs expand few seconds after fertilization with a mean diameter of 1.01 ± 0.19 s.
B.	**Animal and Vegetal pole (Blastodisc)**	35 min.	Expansion of the yolk away from the membrane and accumulation of cytoplasm at the anterior to form animal and vegetal pole.
C.	**2 – cell stage**	40 min.	This was observed as a vertical division of the animal pole producing two cells of equal sizes
D.	**4 – cell stage**	55 min.	Second line of division was perpendicular to the first line producing 4 cells which were still of equal sizes.
E.	**8 – cell stage**	1 hr. 5 mins.	Cells were seen as heaps on top of the "round yolk".
F.	**16 – cells stage** **32 – cell stage**	2 hrs. 5 mins.	Cells were seen as irregular in size and could be difficult to count. Further division of cells. Some cells tend to lie on another cell. Many further division producing many cells, irregular in size.
G.	**Morula stage** **Blastula stage**	2 hrs 9 mins. 3 hrs. 17 mins	Further division of cells produce many more but the morula size decreased. Further division producing mass of cell elevated over the general outline of the yolk mass (like a dome-shaped)
H.	**Gastrulation stage**	7 hrs 10 mins	Embryo develops germ rings. Cephalic and caudal edges which were formed at advanced stages of the blastula.
I.	**First wriggling movement**	21 hrs	The long somite started from both sides within the chorion wall. It started with 1 movement in 25 seconds, but this rate gradually increased with time.
J.	**Hatchling**	22 hrs.	Violent movement of tail to either side against the chorion wall, followed by contraction. As a result of which the chorion wall breaks and hatching occurred

the report of Freund *et al.* (1995) for *H. longfilis.* The structured appendages for swimming like fin rays and the skeletal structure were developed in preparation of the larva entry into the swimming stage.

Apart from sex organs all the organs and system of the fish were already formed in the fry by the time of final yolk absorption. Time of yolk absorption is very significant as exogenous feeding must commence forty eight hours after hatching that is, before final yolk absorption to enable the fry to get used to natural food. This practice has been found to reduce high 4th day mortality noticed in routine hatchery management due to complete yolk absorption (Madu, 1989).

Kamler *et al.* (1994) reported observed temperature induced changes of early development and yolk utilization in the African catfish, *Clarias gariepinus* and *C. macrocephalus*. Generally, low temperature slows down the rate of embryo development. Egg to fry survival in the sea trout has been reported by Rubin and Glimsater (1996). They identified four critical phases, which are spawning phase, incubation phase, and emergence of the alevin phase and growing of the fry.

This study on embryonic development of *C. gariepinus* has bridged a gap in the knowledge of developing eggs and larvae morphology. Furthermore, useful information is provided for routine fish hatchery operators. This will allow for better management of fertilized egg to fry stages for higher survival and increased *C. gariepinus* fingerlings supply in Nigeria.

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