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COMPARATIVE ASSESSMENT OF EMBRYONIC DEVELOPMENT IN *Clarias gariepinus* FROM BROODERS OF DIFFERENT AGES

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ABSTRACT

The embryonic and early post-embryonic developmental study of African catfish produced from 6, 9, 12 and 15 months old experimental breeders were examined in an indoor incubation water temperature range within 28ºC to 30ºC. The various stages of the eggs development was viewed under a microscope, from unfertilised oocytes to the initial cleavage to hatching stages. Despite the age disparities between the different breeders (6, 9, 12, & 15 months old), there were no significant changes in the embryonic development of the eggs. The precise minute-by-minute timing and thorough activities of every step were examined, the unfertilised oocyte at 0 minutes, fertilised stripped eggs at 2 minutes, blastodisc/animal pole and vegetal pole stage at 30 minutes, and one-daughter cell stage at 34 minutes after fertilisation. The stages of two daughter cells were observed at 38 minutes, four daughter cells at 52 minutes, eight daughter cells at 63 minutes and sixteen daughter cells at 77 minutes. Up to 24 hours after fertilisation, the following stages were observed: thirty-two-daughter cell stage at 80 minutes, morula stage at 100 minutes, blastula stage at 125 minutes, gastrula stage at 320 minutes, somite stage at 583 minutes and hatching commencing stage at 1099 minutes (18 hours: 32 minutes). The study helped to understand the embryonic and early post-embryonic development of African catfish. Fish hatchery operators will benefit from this result, as it provides information on egg and larvae status at different ages of the brooders.

Keywords: Embryonic development, *Clarias gariepinus,* breeders, oocytes, larvae

INTRODUCTION

The African Catfish, *Clarias gariepinus,* is a major cultivated fish of high commercial value in Nigeria and is ideal for captive breeding (Haniffa and Sridhar, 2014). Lack of accurate age of breeders and level of sexual immaturity are among many limitations associated with fry production, and that is why the development of better broodstock management techniques through suitable age of selected breeders are crucial to improvement of egg/fry yield and system efficiency. FAO (2014) revealed that fish seed production demands a thorough understanding of the husbandry and nutritional requirements of broodstock, which greatly affect fecundity, survival, egg size and larval quality. The African catfish, *C. gariepinus,* is distributed throughout Africa. It is of growing economic value in the African aquaculture industry (Kamthorn and Jim, 2016). In Nigeria, *C. gariepinus* is well accepted by fish farmers and consumers and is therefore very indispensable to the sustainability of aquaculture. Research on the development of embryos and early larvae is essential and crucial to the successful rearing of larvae for aquaculture and large-scale seed production (Ochokwu *et al.,* 2015). It is believed that the larval and embryonic phases are extremely sensitive markers of environmental disruptions. In addition, such studies on any cultivable species like *C. gariepinus* might be valuable in directing the husbandry efforts of the fish farmer to be specific state and requirements of each larval stage. A thorough understanding of the fish growth process is crucial for life history research and fish farming. According to Sule and Adiku (1999), embryos reveal a variety of evolutionary relationship traits based on age differences. Even though *C. gariepinus* is a superior animal model for development and embryological research; many aspects of its embryology are still unclear due to the various ages of breeders' offspring (Olufeagba, 1999). The benefits include convenient size, ova transparency, and lack of pigment under a microscope; these make it easier to see changes in organs and tissues (Sule, 2002). In all these, the continuous process of development was to hatching and free swimming stage, yet limited studies have compared the embryonic development of eggs obtained from brooders of varying ages. Thus, this study aimed to observe, document, and compare the embryonic developmental stages of *Clarias gariepinus* eggs obtained from brooders aged six, nine, twelve, and fifteen months, using photomicrographic analysis under controlled indoor incubation conditions. This information is very crucial, in order to maximise larval growth and survival

MATERIALS AND METHODS

Study Area

The experiment was conducted in the indoor and outdoor fish hatchery complex of the Department of Fisheries and Aquaculture, Federal University in Dutsin-Ma, Katsina state. Dutsin-Ma is a Local Government Area in Katsina State, Nigeria, located at 12º 27′ 18" N and 7º 29' 29" E. Local Government Area (Rabe, 2019).

Experimental Design and Set-up

The experiment was designed as a multi-phase, multi-stage study that included target ages of male and female *C. gariepinus* breeders at six, nine, twelve, and fifteen months of age. The animals were raised in identical conditions.

Two tarpaulin tanks were utilised for the management of the grow-out and broodstock outside, with a stocking density of 200 pieces of fish per tank (3 m x 2 m x 2 m area), males and females were separated in two tanks. The breeding was carried out indoors in incubation troughs (40 cm x 30 cm x 30 cm) with each treatment in triplicate.
A light microscope (Olympus Trinocular microscope XSZ-156T) equipped with a digital camera (DCM 130, Res1.3M pixels) and an HP computer mini-5103 was used to meticulously photograph and record each stage of the development.

Experimental procedures
Phase 1
Production and Development of Experimental Parents Breeders
Sourcing, selection of breeders and initial induced breeding exercise
An initial induced breeding exercise was conducted to produce the parent breeders in order to ascertain the ages of the experimental breeders of *C. gariepinus* employed in this experiment, namely 6 months, 9 months, 12 months, and 15 months olds. Four breeders comprising two males and two females were procured from a commercial farm in Dutsin-Ma, Katsina state and the breeding was carried out using hypophysation method with the use of Ovaprim hormone in line with the description of FAO (2015) and Madu (2014).

Development of the Parents Experimental Breeders
When the experimental *C. gariepinus* post-juveniles reached four months of age, they were seaparated based on sex into two distinct, and well-sealed collapsibl tarpaulin fish tanks (3m x 2m x 2m), within the hatchery complex of the Department of Fisheries and Aquaculture at Federal University Dutsin-Ma. Each of the tarpaulin were stocked with 200 post juveniles (200 males and 200 females respectively). The water level was kept at 1m height and the fish were raised to broodstocks using commercial vital feed (pelleted) at graduated sizes of 2mm, 4mm, 6mm, and 8mm (Adebayo, 2006; Owodeinde and Ndimele, 2021). So, the fish were selected for experimentation at 6 months, 9 months, 12 months, and 15 months old in accordance with the experimental design.

Phase 2
Stage I: Observation of Ovulatory Responses from the Four Experimental Breeders Produced
The breeding experiment was carried out in the indoor hatchery at different ages of 6, 9, 12 and 15 months using the method previously described for the production of the brooders (FAO, 2015, Madu, 2014). Approximately 10 to 12 hours (latency period) following ovaprim treatment, the females' ovulatory responses were assessed. The start of ovulation was defined as the release of eggs through the genital pore after mild abdominal pressure. Eggs from ovulated females were removed into circular plastic receptacles (fertilisation trays). Following ovulation, the male was slaughtered to expose the testes, which were taken and testes were excised, and the milt was extracted under sterile conditions to release the milt for fertilising the eggs.
In order to perform dry fertilisation, the eggs and milt were combined in a plastic and mixed with bird feathers. Saline solution was later added to extend the milt and the fertilised egg was gently stirred for two minutes before being repeatedly washed with fresh water to get rid of extra milt. Immediately after fertilisation, the eggs were moved to three indoor plastic incubation troughs (50 cm by 30 cm by 30 cm). Ten to fifteen minutes after gamete mixing, the eggs were inspected under a microscope to see if the blastodisc had formed, a sign of successful fertilisation. The fertilised eggs remained transparent for two hours after insemination, however, the unfertilised eggs went white the unfertilised eggs and the spawning mat (kakaban) were carefully removed from the indoor incubation troughs following the standard methods (Puvaneswari *et al.,* 2009).

Phase 3
Microscopy and Photomicrography of the Experimental Eggs
A light microscope (Olympus Trinocular microscope XSZ-156T) with its digital camera (DCM 130, Res 1.3m pixels) and an HP computer were used to observe, track, photograph, and document the embryonic development of the four ages of the experimental *C. gariepinus* eggs produced in real time. A stop watch was used to time the development at both minute and hourly intervals. Ten (10) oocytes from each breeders' eggs experimental were sample into the incubation trough and placed in a petri dish at intervals of a few minutes until they hatched. The oocytes were six, nine, twelve, and fifteen months old.
These samples were kept in an incubating/hatching regulated water temperature range of 28°C to 30°C using heater, and the morphological developments, organogenesis, pigmentation, and temporal sequence of the various developing phases were seen and documented indoors. This lasts for the first four (4) days and till metamorphosis, as well as until hatching (18–24 hours). Egg sizes before and after fertilisation, as well as embryonic development until hatching stage, were further separated into the developmental stages (Olaniyi and Omitogun, 2013). At a magnification of x 40, the developmental stages of eggs from initial cleavage to hatching were viewed under a microscope for a full day.

Documentary
Photomicrograph was used to take important stages of segmentation, blastulations, differentiation of embryo and hatching. The film of the photograph was developed, transferred in to a HP computer for details analysis stages of images viewed through the microscope and snap using digital camera and prints as each stage of eggs embryonic

development produced from the experimental breeders (6 months, 9 months, 12 months and 15 months), while the accurate timing and detailed description of each stage of features appearances was noted and recorded per minute and per hour as necessary.

RESULTS

Unfertilised stripped oocytes

The four distinct unfertilised eggs produced from the experimental breeders at 6, 9, 12, and 15 months of age were oval in shape, opaque, and brownish in colour, according to observations. They possessed a thin perivitelline membrane with protoplasmic layer filling the voids, and they were adhesive (sticky) in nature without oil globules. They had a diameter of 1 ± 0.1 mm. Figure 1a illustrates how spermatozoa enter the eggs through the micropyle. The pictures are based on four distinct eggs produced from the four ages of *C. gariepinus* breeders. the separation of the membrane from the yolk. In the images, the adhesiveness of the eggs has increased to facilitate attachment to the spawning mat or kankaban.

The four experimental *C. gariepinus* breeders eggs, which were 6, 9, 12, and 15 months old, had their unfertilised eggs timed at 0 minutes. The unfertilised eggs from female breeders of *C. gariepinus* produced at ages 6, 9, 12, and 15 months had mean diameters of 1.0 ± 0.1 mm, 1.1 ± 0.2 mm, 1.2 ± 0.2 mm, and 1.3 ± 0.1 mm, respectively. Transparent, round, sticky, and exhibiting brownish and greenish hues, the stripped eggs are free of oil globules. A little perivitelline gap isolated the egg membrane from the remainder of the egg. As seen in figure 1a, the ova started to grow shortly after insemination, increasing the egg's diameter.

Fertilised stripped eggs

The fertilised eggs of the various ages of the *C. gariepinus* female breeders produced at 6 months, 9 months, 12 months, and 15 months old, and their diameters were measured as follows: 1.3 ± 0.3mm, 1.4 ± 0.3mm, 1.5 mm, and 1.6 ± 0.1mm, as illustrated in figure 1b. The time used for fertilisation of the stripped eggs was 3 minutes, 3 minutes, 2 minutes, and 2 minutes respectively. The egg membrane's adhesiveness became more noticeable two (2) minutes after fertilisation, and the eggs stuck to the substratum (the spawning mat in the indoor incubation trough). As seen in figure 1b, the fertilised eggs were easily identifiable to the unaided eye due to a reddish patch (blastodisc) on one pole.

Blastodisc/animal pole and vegetal pole stage after fertilisation

Immediately after fertilisation, the fertilised eggs were identified by a red spot (blastodisc) that was apparent to the unaided eye. One extreme pole had an aggregation of yolk mass (vegetal pole), whereas the other end pole was completely covered in granular cytoplasm (animal pole). Within 30 minutes, 26 minutes, 24 minutes, and 22 minutes following fertilisation, the blastodisc had its first cleavage, dividing it into two blastomeres (Vegetal pole and animal pole) (Figure 2a). Usually, the segmentation was discoidal meroblastic.

One daughter cell stage after fertilisation

The image depicts the extrusion and development of a cell stage that was distinguished by the buildup of protoplasmic layer at the submicropylar region or animal pole (Figure 2b). The one-daughter cell phases were observed at 34, 30, 25, and 20 minutes respectively.

Two daughter cell stage after fertilisation

The initial meridional and meroblastic mitotic division, which forms two blastomeres as a characteristic of telolecithal eggs, is depicted in this stage as seen in the photograph (Figures 3a and 3b), two-daughter-cell phases were observed at 38, 34, 30 and 25 minutes respectively.

Four daughter cell stage after fertilisation

All of the eggs had four blastomeres because the second mitotic meroblastic division formed here at a straight angle to the first division (Figures 4a and 4b). Four-daughter-cell phases were observed at 52, 48, 44, and 40 minutes respectively.

Eight – cell stages after fertilisation

Similar to the first division, the third mitotic division produced two parallel rows, giving each egg four paired cells (Figures 5a and 5b) Eight-daughter-cell phases were visible at 63, 60, 55, and 50 minutes respectively.

Sixteen – cell stages after fertilisation

The fourth mitotic divisions produced 16 blastomeres and appeared vertically to the first cell cleavage. The cells were observed, arranged in layers of four by four blocks (Figures 6a and 6b). sixteen daughter-cell phases were observed at 77, 71, 66, and 60 minutes, respectively, based on the age differences of the eggs.

Thirty – two cell – sixty – four cell stages after fertilisation

Major divisions in these early morula stages were latitudinal and resulted in the production of a second layer of cells. Constant cleavages and divisions led to the heterogeneous formation of numerous cells until they were extremely difficult to count (Figures 7a and 7b). The thirty-two daughter cell stages were visible at 80, 75, 70, and 65 minutes respectively.

Morula stages after fertilisation

This happens when multiple cell divisions result in a large number of blastomeres. The image depicts the mulberry, a multicellular stage (Figures 8a and 8b). The morula stages were apparent at 100, 95, 90, and 85 minutes respectively.

Blastula stages after fertilisation

These stages shown in the photograph viewed indicate proliferation of the blastocoele to form a dome-shaped

structure that enveloped major part of the yolk (vegetal pole). Blastula stage was viewed at 125 min, 120 min, 115 min and 110 min respectively (Figure 9a and 9b).

Gastrula stages after fertilisation

The expansion of the blastoderm signified the change from blastula to gastrula. With the random moving transitional waves, the gastrulation began with the onset of epiboly. One important characteristic of a living embryo at this stage is the uniform and random movement of quasi-peristaltic waves. In reality, any embryos that do not participate in this movement are dead and will begin to darken in colour. The embryo can travel within the perivitelline spaces thanks to this movement on the yolk sphere surface, which ultimately causes the perivitelline fluid mixture to mix. It causes hypoblasts and epiblasts to develop.

This movement came to an end when the blastopore closed, and morphogenesis—the formation of the embryonic axis—began. The pollster (cephalic bud) and tail bud were then visible at these stages as illustrated in figures 10a, 10b, 10c, 10c, 10d, 10e, and 10f, the gastrula stages were observed at 320, 315, 310, and 305 minutes respectively.

Somite stages after fertilisation

At this point, the paired blocks of cells that formed the vertebral column at the back of the embryo show early somite development. After that, somite development emerged cephalocaudally. Additionally, a strong disagreeable stink and a choking scent or smell were present from this point until the next (hatching) (Figures 11a and 11b). The somite stage was visible at 583, 579, 574, and 570 minutes respectively.

Hatching stages after fertilisation

The embryo breaks off from the chorion or egg capsule through the tail in this stage's image. Additionally, there were sporadic embryonic peristaltic movements observed just prior to hatching (at least an average of nine twitching movements per minute at the beginning because they were more frequent). Following the initial heartbeat, which ranged from 115 to 160 beats per minute, it was observed that the tail bud around the yolk sac was somewhat detached from the centre portion.

Although the anterior portion of the head is growing longer and bulbous in shape, the encephalic portion, or head, is firmly fixed to be the yolk sac. Eventually, it broke off due to repeated, regular twitching; that is, the tail bud beat and separated the chorion or egg capsule/membranous covering upon hatching. The unpleasant and repulsive smell remains the hallmark of the hatching period. Then, other mental development phases were observed, including the onset of the circulatory system and pigmentation (Figures 12a, 12b, 12c, and 12d). Hatching starts at 1099 minutes (18 hours:32 minutes), 1094 minutes (18 hours:23 minutes), 1090 minutes (18 hours:17 minutes), and 1085 minutes (18 hours:08 minutes) respectively.

Therefore, the observation made post fertilisation, revealed that the antero-posterior axis was distinguishable, cephalic portion being broader and embryonic rudiment became distinct with two somites. The anterior particle elongated to form the tail fold. The maximum size of the coiled embryo at this time were 0.76 mm and the eye vesicle were demarcated. About 6-8 somites hatchling emerged from the eggs capsules at 18 hrs.

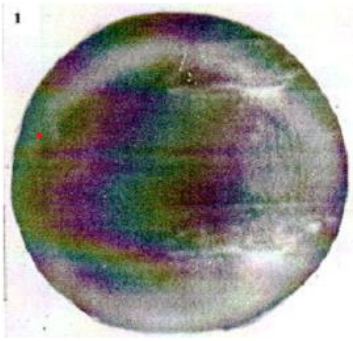


Figure 1a: The embryonic stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders unfertilised egg.

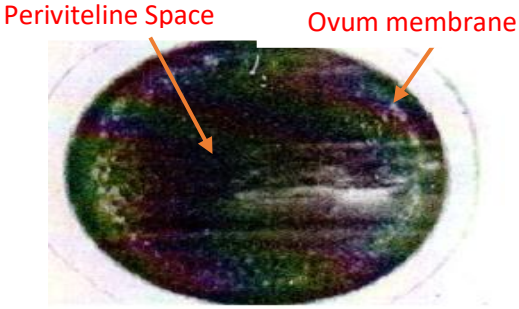
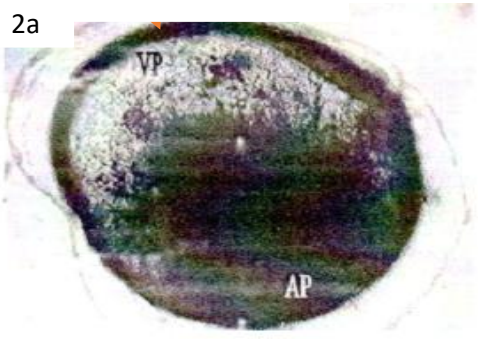


Figure 1b: The embryonic developmental stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders fertilised egg.

magn

Vegetal Pole feature developed at 30min

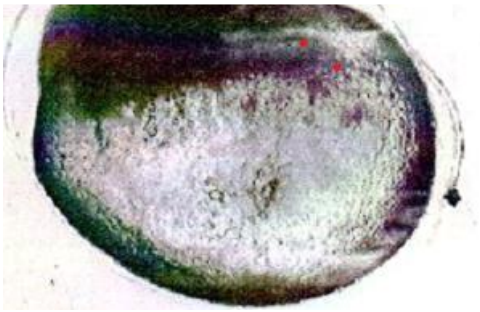
2a



magn

2b

One-cell stage feature developed at 34min



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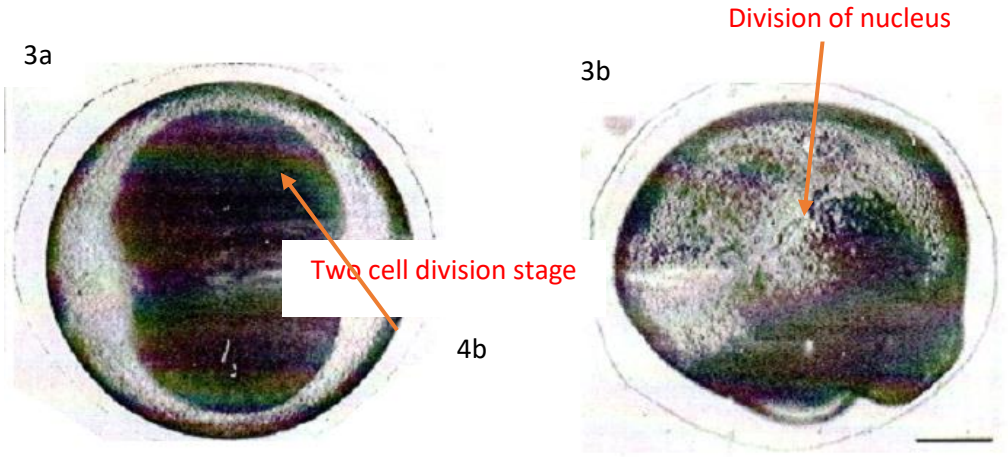


Figure 3a & 3b: The embryonic developmental stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders egg in Two-cell stage: (a) Top view: (b) lateral view. magnification X 40.

Figure 4a & 4b: The embryonic developmental stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders egg in Four daughter-cell stage, (a top view (b) lateral view magnification X40.

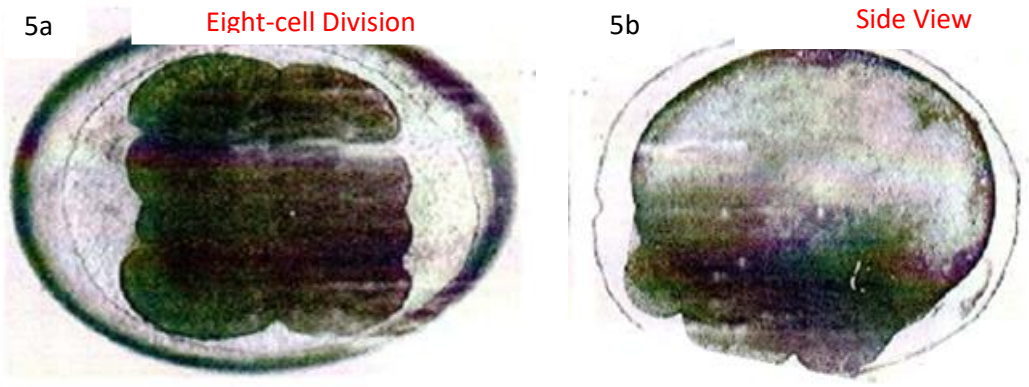


Figure 5a & 5b: The embryonic developmental stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders egg in Eight daughter-cell stage, (a) Top view (b) lateral view magnification
X 40 respectively.

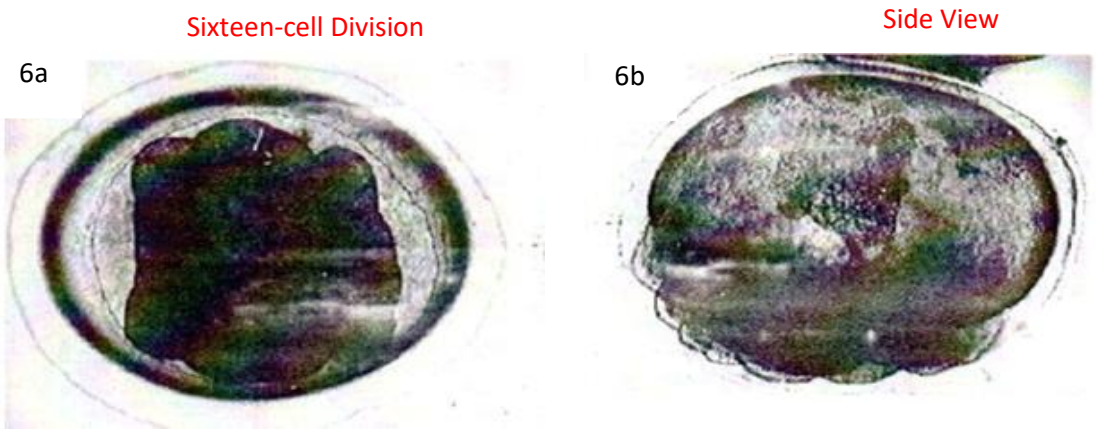


Figure 6a & 6b: The embryonic developmental stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders egg in Sixteen daughter-cell stage, (a) top view: (b) lateral view, magnification X 40.

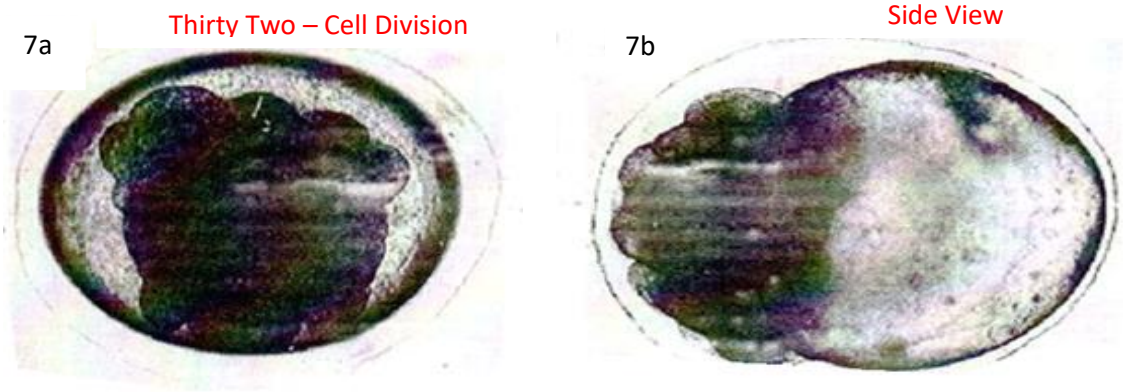


Figure 7a & 7b: The embryonic developmental stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders egg in Thirty-two daughter-cell stage, (a) Top view: (b) lateral view magnification X 40.

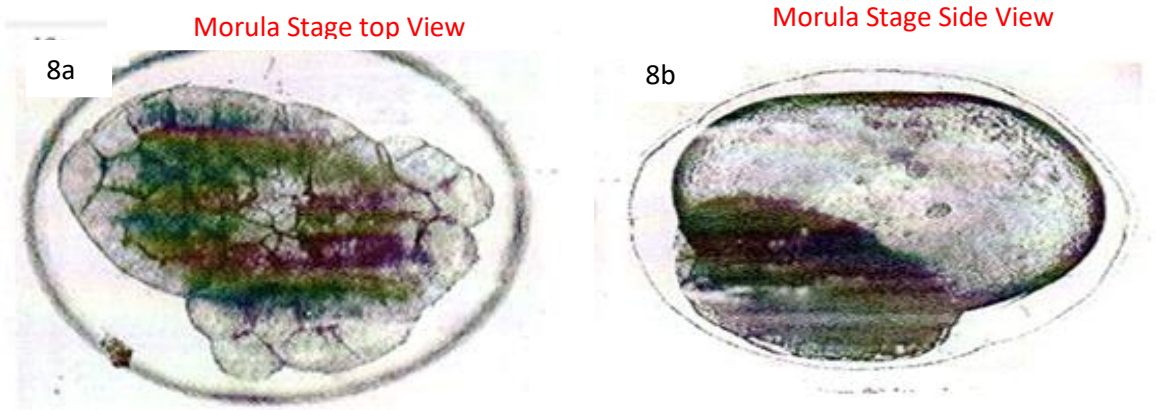


Figure 8a & 8b: The embryonic developmental stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders egg in Morula stage (a) Top view: (b) lateral view magnification X 40.

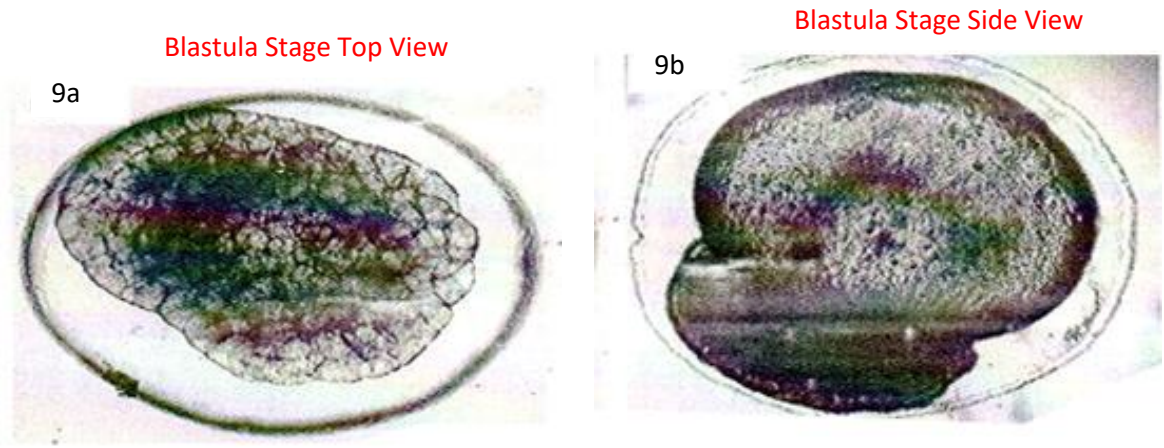
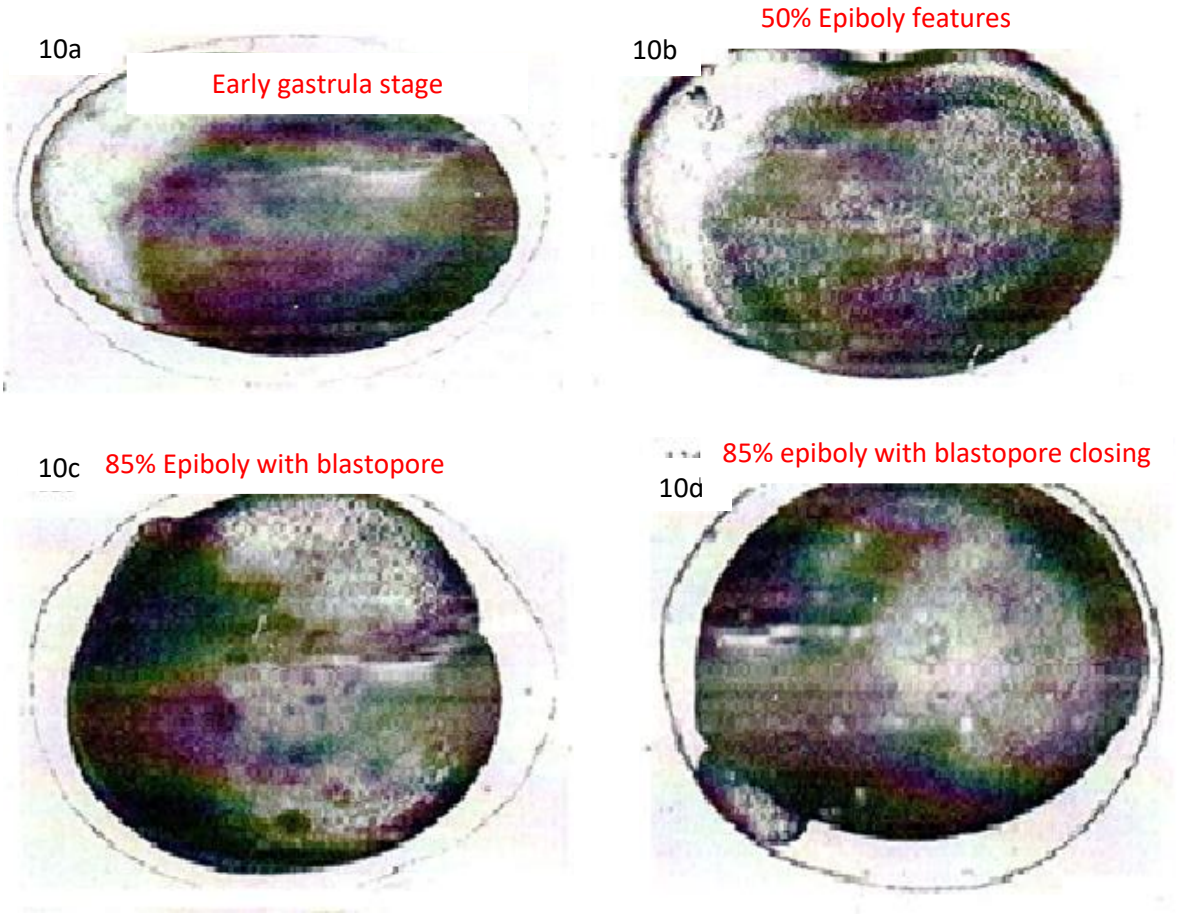
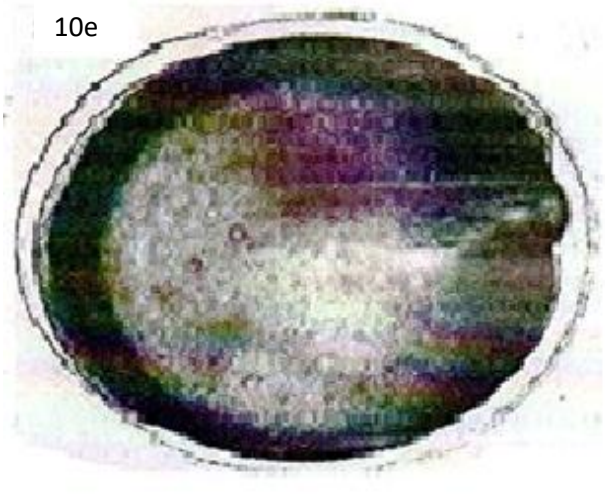


Figure 9a & 9b: The embryonic developmental stages of 6, 9, 12 & 15 months old *Clarias gariepinus* breeders egg in Blastula stage (a) Top view: (b) lateral view
magnification X 40.



Epiboly completed

10e



Gastrola ends and early somite block

10f

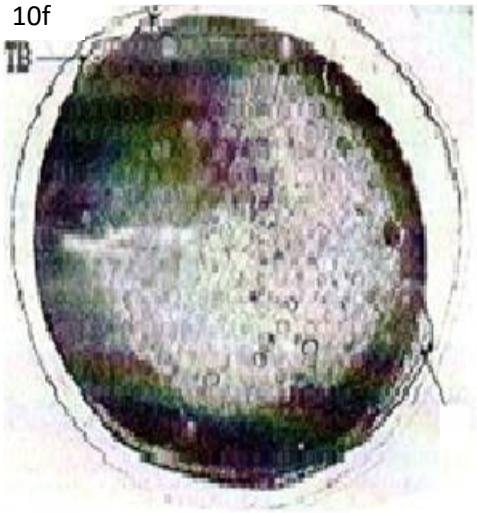
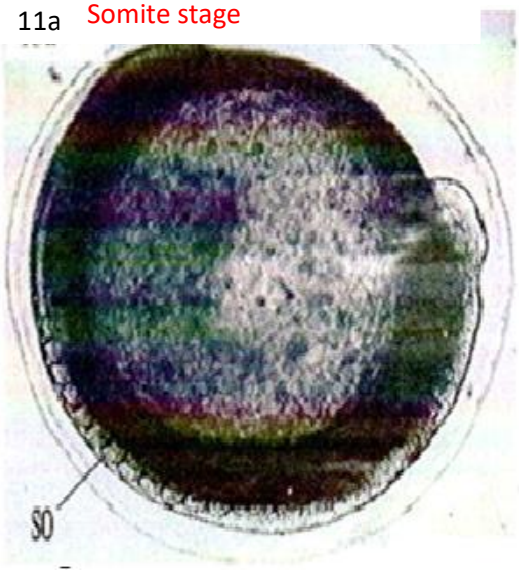


Figure 10a, 10b, 10c, 10d, 10e & 10f: Clarias gariepinus breeders' eggs at 6, 9, 12, and 15 months of gestational age in the Gastrula stage (a) early gastrula; (b) 50% epiboly, with transitional wave movement indicated by the arrow;

(c) 85% epiboly with blastopore wide open; (d) 95% epiboly. Magnification X 40.

11a Somite stage



11b Somite completed stage

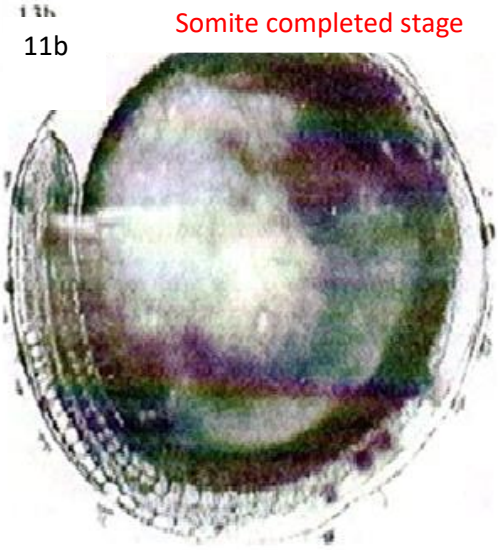


Figure 11a & 11b: The Clarias gariepinus breeders' eggs at 6, 9, 12, and 15 months of embryonic development, magnified by 40 times in the Somite stage. (a) stage of the somite (b) stage of the finished somite
(movement before hatching), somite block.

Hatching lavae

Hatched Larvae

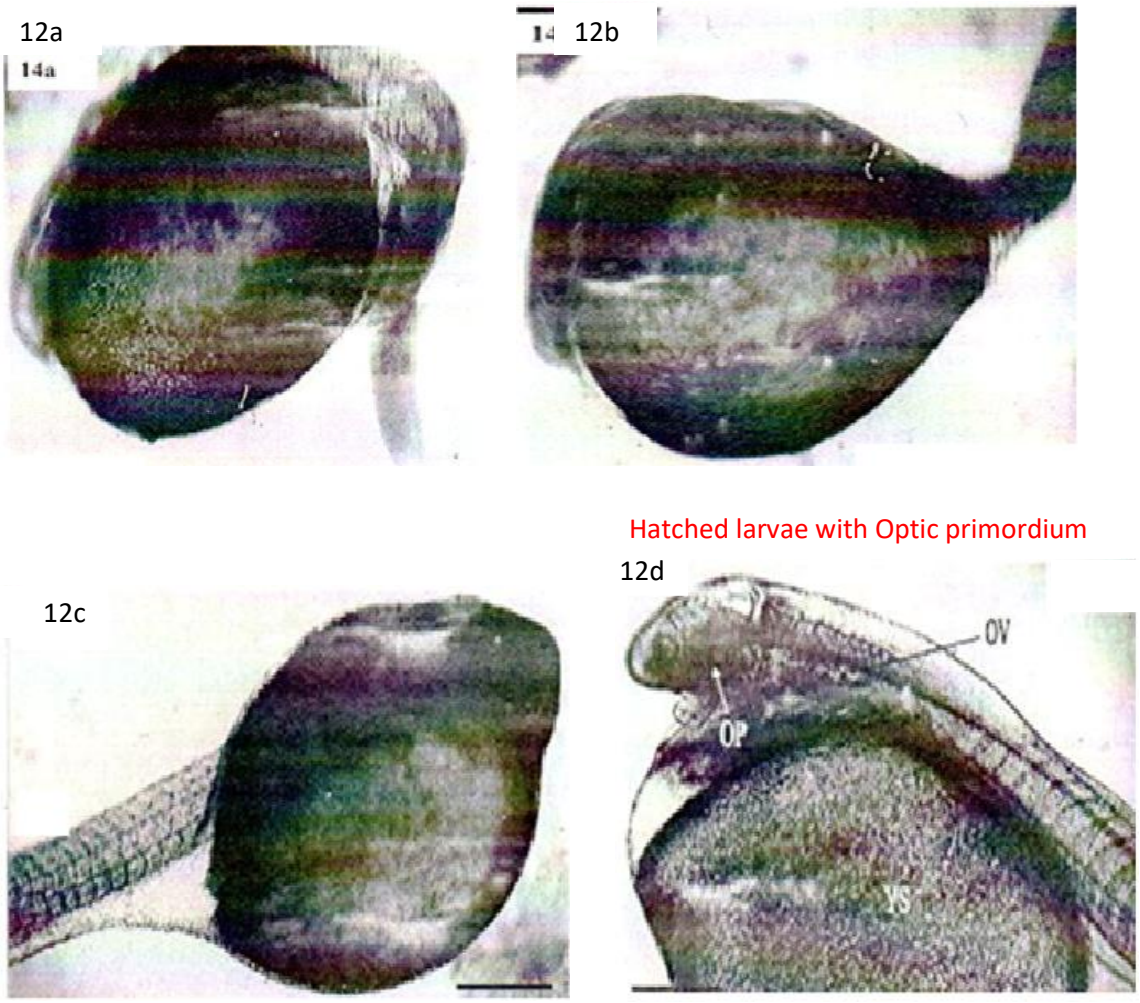


Figure 12a, 12b, 12c & 12d: The early stages of hatching Clarias gariepinus breeders' eggs at 6, 9, 12, and 15 months of embryonic development. Magnification X 40.

DISCUSSION

Early maturation in fish is being achieved through genetic selection or improved nutrition, indicating a relationship between maturation and growth. In this study full maturation of *Clarias gariepinus* was established to be attained at 6months of age. It revealed that maturity is related to age in the experimented fish, the study also hinted that maturity was more related to age than size. This indicate that age cannot be totally excluded in the determination of maturity, the age of maturity in fish appears to increase with size. This study noted that maturity depends more on age than size since in all the experimental fish (6, 9, 12 and 15months old) are all matured, but in varying degrees of maturity. So also, in the classification observed in the different ages of the ovary, development owing to the different appearances it can take ovarian development.

The detailed developmental biological studies of *Clarias gariepinus* egg embryonic and early post-embryonic stages has been documented in this study, which will be useful in enhancing the survival of *Clarias gariepinus* larvae. The results obtained in this study corroborate earlier embryological studies on *Clarias gariepinus* (Legendre *et al*., 2021). Moreover, sequential and chronological photomicrographs of the embryonic and larval developmental stages shown in this study from the eggs of different ages (6 months, 9 months, 12 months and 15 months old).

Experimental female *Clarias gariepinus* broodstock produced for this experiment showed various detailed features of the developing *Clarias gariepinus* larvae in their different timing periods and under the same controlled water temperature during egg incubation (28°C – 30°C), respectively.

Immediately after in vitro fertilisation, *Clarias gariepinus* eggs became more adhesive as previously observed in other species from the order siluriformes such as *Rhinelepis aspera* (de Graaf *et al*., 2016), *Heteropneustes fossilis* (Puvaneswari *et al*., 2019: Korzelecka-Orkisz *et al*., 2022) and *Heterobrnachos longifilis.* The observation is however, different from *Rhamdia quelen*, *Rhamdia hilarii*, and *Pseudoplatystoma coruscans* eggs (Moreire *et al*., 2018) that are non-adhesive (Puvaneswari *et al*., 2019). The eggs were translucent and a greenish brown colour that actually depends on the individual parent stock. Some of the female breeder eggs were deeply greenish but faintly brownish – coloured eggs, while some have faintly greenish and deeply brownish oocytes, such relative differences are not uncommon with the species.

The arrangement of egg cleavage occurs as the established discoidal meroblastic mitotic division which was commonly occurs in tololecithal eggs (Richer *et al*., 2017), having large quantity of yolk in fish eggs and concentrated at one end/side. The meroblastic cleavage, narrowed to the animal pole, is common in many teleost fish (Richer *et al*., 2017). While the first mitotic cleavage viewed at 38min after fertilisation occurs at a cell-staged protoplasmic bulge that emerged first, the cleavage furrow was maridional and undercuts the blastodisc, leaving the underlying yolky parts and resulting in two equal blastomeres. This is in line with the previous observation of Iswanto *et al.* (2015). The second cleavage appeared at 55 min, was also meroblastic, however, at right angles to the first furrow and gave four equal blastomeres. The third mitotic division observed at 67 min, was similar to the first plane by having two parallel furros that resulted in four paired blastomeres. Hence, the cleavage in operation were in relation to the furrow that firstly occurred and followed the embryonic developmental processes passing through morula (Many called stage) to blastula and gastrulation, where involution of cells took place. When gastrulation is completed, the pollster and the tail bud were exposed, which serves as good identification for the cranial and the caudal parts of the developing embryo.

Also, the body segmentation continued whereby blocks of somites were developed cephalocaudally and finally, matching occurs. Hatching was noticed mainly by facilitated muscular actions/contraction, wriggling and twitching of the embryo inside the eggs membrane and causing eventual lasting of the caudal portion against the chorion, which assisted the larvae to break out from the eggs capsule. The developmental process from fertilised egg to hatching, like other biological processes, is dependent upon the controlled water temperature; that is the higher the water temperature, the faster the eggs hatch with respect to the maturity of the eggs due to the age of the breeders. This, in turn, enhances the better survival of the larvae (de Graaf & Janssen, 2019). It was noted that a continuous supply of freshwater through a flow-through system also enhances hatching and survival due to cleansing of the developing embryo from the dirt of the hatching egg shell. Before hatching, breathing was noticed with the heartbeat range of 115 – 160 minutes which is different from 20 – 80 minutes in *Heteropneustes fossilis* (Korzelecka-Orkisz, *et al.,* 2022). Also, during somite formation and shortly before hatching started, the characteristic foul and offensive smell increased and reflected active embryogenesis activities.

In the present study, hatching of *C. gariepinus* egg started at 18 hrs with egg produced by 6 month old breeder, at 20 hrs with egg produced by 9 month old breeder, while 22 hrs with egg product by 12 months old breeder and at 25h with egg produced by 15 month old breeder. These results were latter than that obtained by Hogendeoom and Vismans (1980) who reported hatching 21h at about the same temperature, 29^{o}C. Legindre *et al*. (2021) reported that hatching normally occurred at 14 – 18h after fertilization at temperature of 25^{o}C and 33^{o}C. The hatching time recorded in this study supported previous studies that high temperature led to high hatching rates (Legendre *et al*., 2021; Hogendoorn and Vismans, 1980).

After the hatching, body pigmentation and onset of blood circulation were noticed. The mouth was closed and presence of large yolk sac were recorded. Optic primordial and the optic vesicle with its two otoliths are easily visible and develop to provide the needed visual and auditory functions after developing larvae. In the early hours after hatching, emergence of tiny bulbs or knots of growing barbells was observed, while the mouth was still closed. Also, body pigmentation started becoming evident as the development progressed. The mouth opened by the second day, and there was an increase in length of the barbells as the larvae grew in size. The barbells are necessary appendages and need to be well developed prior to active feeding (Korzelecka-Orkisz *et al*., 2022).
They are useful in searching for food as taste buds develop (Korzelecka-Orkisz *et al*., 2022). Tiny melanophores were noticed on the anterior part of the head and spread caudally, then became more pronounced and condensed on the body parts as development continued. On the third day, significant body parts were well developed and observed. The operculum, the fins, among others; the eyes were fully uncovered to be utilised, including the barbells in searching for food as the yolk had been reduced significantly. Feeding prior to this period influences survival and growth performances (Legendre *et al*., 2021); at the fourth day, the alimentary canal was well developed, and the larvae were seen even to pass excreta. At a week old the pectoral fin is readily visible to the naked eye.

CONCLUSION

This study bridges a significant knowledge gap in the embryonic and early larval development of *Clarias gariepinus*, providing sequential morphological details that will aid hatchery management and fish developmental biology. By establishing that viable eggs can be obtained from brooders as young as six months without compromising embryonic quality, the findings contribute to optimizing seed production strategies. Furthermore, the developmental timeline described under controlled thermal conditions can serve as a reference for enhancing survival, synchronizing hatchery operations, and improving the availability of catfish fingerlings in Nigeria.

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