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Full Length Research Paper

Embryogenetic studies of intra-specific *clarias anguillaris* hybrid: Implication on genetic manipulation

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The need to carry out a thorough study on the embryogenesis of intra-specific *Clarias anguillaris* to determine the best time and the implication in chromosome engineering research cannot be over-emphasized. In this study, it was observed that the first cleavage into 2-cell stage was 34minutes after fertilization, which gave the right timing for the production of tetraploids. The 4-cell, 8-cell and 16-cell stages occurred at 47 minutes, 52 minutes and 53 minutes respectively. Fifty-four percent (54%) of the embryo attained morula stage at 94 minutes. Other embryonic stages observed include blastula, gastrulation, somite formation and hatching. Hatching started at 18hours after fertilization and lasted for a period of about 6hours. The hatchlings increased in length at 0.2mm/day in the first 5 days after hatching.

Key words: Embryonic, development, intra-specific, *Clarias anguillaris*.

INTRODUCTION

Embryonic development and larval development studies besides providing interesting information in itself are imperative and consequential to the successful rearing of larvae for large scale production of fish seed.(Marimuthu and Haniffa, 2007). According to Olufeagba and Yisa (2003), embryology is the key factor to chromosome manipulation. It serves as a tool for genome manipulation for improvement in the culture of fish.

Studies on the duration of embryonic stages, the determination of the frequencies and the implication in chromosome engineering research were carried out in *Heterobranchus longifilis* (Alukoet *al*,2001),interspecific cross between *Clarias anguillaris* and *Heterobranchus bidorsalis(*Diyawaree*t al*,2009), *Clarias anguillaris* (Onyia *et al,*2009),*Sarotherodonniloticus* (Omotosho 1987), *Tilapia nilotica* (Galman ,1980), *Heterobranchus longifilis* (Olufeagbae*t al*,1999).

The significance of embryogenesis cannot be under-estimated in fish breeding. It is the tool for genetic manipulation and engineering to produce sex-reversal, increased ploidy, development of gynogen and androgen fish (Aluko and Aremu, 2001). It affords hatchery managers the opportunity to determine the time it will take to complete embryo development. The total number of stages of embryogenesis differs from one fish to another. Changadeyae*t al*, 2003, opined that promotion of selective breeding, hybridization and chromosome manipulation will help in improving aquaculture production in Africa. Very few information are available on embryogenesis and ploidy manipulation of indigenous fishes in Nigeria (Olufeagba, 1999).

The objectives of this study are to determine the chronological timing for chromosome manipulation and describe the morphological events of the embryogenesis of intra-specific *Clarias anguillaris.*

MATERIALS AND METHODS

Mature female and male *Clariasanguillaris* breeders harvested from Lake Chad was procured from Gamboru fish market, Maiduguri, Nigeria and was transported to the Department of Fisheries Laboratory, Federal University of

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Table 1: Chronological timing of embryogenesis of intra-specific African mudfish *Clarias anguillaris* under laboratory temperature.

TIMING	STAGES OF DEVELOPMENT
0 minute	Fertilized eggs
06 minutes	Movement of cytoplasm materials to animal pole (blastodisc)
34 minutes	2-cell stage
47 minutes	4-cell stage
52 minutes	8-cell stage
53 minutes	16-cell stage
64 minutes (1hr4mins)	32-cell stage
94 minutes (1hr34mins)	Morula stage
129minutes (2hrs 9mins)	Embryo starts surrounding the yolk
187minutes (3hrs 7mins)	Blastula stage
277minutes (4hrs 37mins)	Somite stage formation
540minutes (9hrs 0min)	Enlargement of somite stage and beginning of intermittent movement.
793minutes (13hrs 22mins)	Heart beat of embryo detectable at 160/minute
1028minutes (17hrs 7mins)	Initiation of hatching of embryo.

Technology Yola, Nigeria, in a jute bag placed into a 50 liter capacity Jerry-can cut horizontally and filled with 10 liters of water. While male and female Clarias *anguillaris breeder*s were acquired from Fish market in Yola. All the breeders were conditioned separately in holding plastic tanks in the Department of Fisheries Laboratory for 2 days.

Hormone injection and egg fertilization

Ovaprim hormone was injected at a dosage of 0.5ml per kilogram body weight for the female *C.angullaris* while 0.25ml per kilogram body weight dose was given to the male and was kept separately in plastic basins covered with net to prevent them from jumping out. Males were sacrificed and the testes removed, cut from the lobe into a Petri dish containing physiological solution (9g sodium chloride in 1L of water). The female was stripped of the eggs after ten (10) hours latency period into a dry plastic bowl and the milt was used to fertilize the eggs. Physiological saline solution was added to aid fertilization.

Embryogenesis

About 100 fertilized eggs were collected from the time of fertilization into a petri dish containing water placed under a photomicroscope (ZIESS, Stemi 2000) for viewing and photographing of each embryological stage. The fertilized eggs were monitored from time of fertilization to hatching and exogenous feeding. The unfertilized eggs were monitored up to the time the eggs died. The observations of the stages were done at 5 minutes intervals in the first one-hour and thereafter, observation were carried out at hourly intervals. Photograph of each embryological stage was taken and printed.

RESULTS AND DISCUSSION

The fertilized eggs were adhesive and the formation of animal and vegetal poles started four minutes after fertilization. Fifty percent of the fertilized eggs started and reached this stage after 20 minutes (Tables1 and 2). Similar observation was reported in *Heterobranchuslongifilis* where the formation of animal and vegetal pole occurred within 22 minutes after fertilization (Olufeagba and Yisa, 2003), which was 2 minutes later than *C. anguillaris.* The animal pole further divided vertically into two leaving the vegetal pole to develop as yolk. This result agreed with Onyia *et al,* (2009), who observed the formation of vegetal and animal poles using purebred *Clarias anguillaris* from Jos.

The first cleavage (2-cell stage) occurred 34 minutes after fertilization. The knowledge of the first cleavage is critical in chromosome manipulation to produce triploids and tetraploids. In a similar work done by Aluko *et al*. (2001) using *C. anguillaris*, observed that the first mitosis took place 34 minutes after fertilization, which was in agreement with the present work. Onyia *et al* (2009) using *C. anguillaris* reported the first cleavage at 39 minutes. On the other hand, Olufeagba *et al*. (2004), reported 40 minutes after fertilization in *H. longifilis.*

The first cleavage furrow that was clearly visible, divided the blastodisc into two distinct blastomeres. It should be noted that the two blastomeres represent the two telophase poles of mitosis and the formation of cleavage furrow. Other workers reported similar furrow

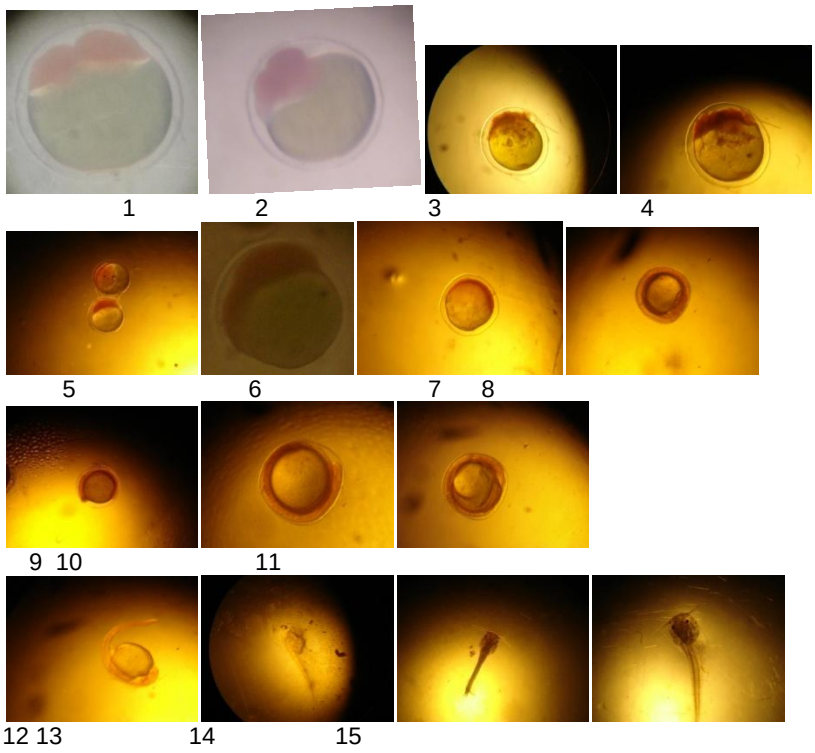
Table 2: Embryonic development of intraspecific hybrid of *Clarias anguillaris*

STAGES	DESCRIPTION OF MORPHOLOGICAL EVENTS
Unfertilized egg	Round, golden brown and slightly adhesive with a mean diameter of 1mm.
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 division 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 division leading to the formation of multicellular blastodisc.
Gastrula	Formation of germinal ring with two somites.
Advance gastrula	Formation of cephalic and caudal edges, with 22 somites and rudimentary eyes.
Embryonic shield	Initiation of wriggling movement, olfactory pit, and cardiac beats to aidrudimentary fluid movement.
25 myotome stage	Body segmentation completed. First fluid movement initiated as heart contraction started beating initially once per 60minutes. 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 emerge, followed by the trunk and head region.

in*Ictaluruspunctatus and Pseudoplatystomacorsicans* (Cardoso *et al.*, 1995; Makeeva and Emel'yanova, 1993). Chromosome engineering approach is possible if the duration of the 2-cell stage is known, which will open opportunities for the precise timing of the first mitotic metaphase (Aluko *et al*, 2001). A foundational information has been provided at the 2-cell stage of cleavage in this present study for the takeoff of genetically improved *C. anguillaris* using tetraploidy and mitogynogenesis technologies. Tetraploidy induction has been carried out targeting the first mitosis (Karyokinesis).The method of producing polyploidy through diploid zygote often leads to aberrations (Myers, 1986, Newport and Kirschner,1982). Olufeagba *et al*, 2009 reported that when 4N (female) are used in crossing with diploid (male), the interploid is expected to be normal. This is possible since the imbalance in nuclear cytoplasm ratio during early stages of embryogenesis in direct triploid induction does not occur. The 4-cell, 8-cell, 16-cell and 32-cell stages occurred at 47minutes, 52minutes 53minutes and 64minutes respectively (Table 1). It was not possible to accurately count the 64-cell stage because the cells were numerous.

The morula stage occurred 94 minutes after fertilization. Fifty percent of the embryo reached this stage in one of the fields. Other morphology of the fertilized eggs occurred before the somites formation (Table 2). At 7hours 7minutes, somites began to form and enlarged until intermittent movement. At 11hours 44minutes, the embryo completely encircled the yolk (blastophore) with increased wriggling within the chorion. The first hatchling emerged after the rupture of the

PLATE 1: Embryological development under normal laboratory temperature in intra-specific
C. anguillaris.



KEY: 1.2-cell stage 2. 4-cell stage 3. 8-cell stage 4. 16-cell stage 5. Morula stage 6. Blastula stage7. Embryo began encircling the yolk 8.Embryo completely encircled yolk 9. Gastrula stage 10. Head fully differentiated 11. Newly hatched embryo12. Closing of the blastophore 13. 24-hour old hatchling 14. 4-day old hatchling15. 9-day old fry.

Table 3: Daily growth in length of intra-specific *C. anguillaris*

DAY	LENGTH(mm)
1	**2.6**
2	**2.8**
3	**3.0**
4	**3.2**
5	**3.4**

chorion wall at 18 hours after fertilization. Fifty-nine percent hatchlings emerged at 22 hours after fertilization and in the next two hours, all the fertilized eggs were hatched. All the stages are shown in Plate I as photomicrographs.

The growth of the hatchlings from the first twenty-four hours to the fifth day after hatching, showed increase in length (mm). It showed the fry increased by 0.2mm daily (Table 3). Marimuthu and Haniffa (2006) observed that growth in length of *Channastriatus* larvae increased at about 2mm per day. From the authors observation, the rate of increase in length of *C. striatus* larvae was greater than *C. anguillaris* in the first five days after hatching though they belong to different families.

CONCLUSION

A foundational information has been produced at the 2-cell stage of cleavage in this present study for genetic improvement of C. anguillaris using tetraploidy and mitogynogenesis.The timing for the first cleavage had been determined and chromosome manipulation to produce tetraploids of intra-specific C*. anguillaris* is now possible. Tetraploid females can also be crossed with diploid males to produce triploids of the species with better growth. The study also highlighted the morphological changes that occurred during embryogenesis of the fish.

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