IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 5 Ver. I (May. 2015), PP 26-29
www.iosrjournals.org

Embryogenesis of Heterobranchus Bidorsalis

Takoradi, O. C[2] Yisa, Moses*[1] and Olufeagba, S.O[1]
[1]*Fish Genetics Improvement Laboratory, National Institute for Freshwater Fisheries Research (NIFFR) P.M.B. 6006, New Bussa, Niger State, Nigeria.*
[2] *Federal College of Freshwater Fisheries Techonolgy, P.M.B. 1500, New Bussa, Niger State.*

Abstract: *A detailed embryological study of Heterobranchus bidorsalis was carried out to determine the onset of first mitosis under laboratory condition. Observations on the embryogenesis showed that the formation of animal and vegetal pole occurred 36 minutes after fertilization, while first cleavage which is critical for tetraploidy induction occurred at 37 minutes after fertilization. The 4, 8, 16 and 32 cleavage stages occurred between 38 to 58 minutes after fertilization. Other embryonic stages observed includes the blastula, gastrula, somite, wriggling and hatching stages which occurred at 1 hour 48 minutes, 2 hours 15 minutes, 13 hours 14 minutes, 13 hours 38 minutes and 14 hours 37 minutes after fertilization respectively.*
Key words: *Heterobranchus bidorsalis, Embryo stages, Genetic manipulation.*

I. Introduction

Fish is an important and the cheapest source of animal protein and account for about 37% of Nigeria's total protein requirement (FDF, 2002). Among the cultured food fish in Nigeria, catfish is the most sought after fish species, very popular with fish farmers and consumers and commands a very good commercial value in Nigerian markets (Ezenwaji, 1985; Oladosu et al., 1993; Ayinla et al., 1994). The African catfish *Heterobranchus bidorsalis* is a unique fish belonging to the family Clariidae (catfishes) and distributed throughout Africa (Clay, 1977, Burton 1979). According to Huisman and Richter (1987), two Clariids catfishes *Clarias gariepinus* and *Heterobranchus bidorsalis* are prominent in Africa aquaculture because of their breathing and hardy nature and by virtues of their suitable reproductive strategy, nutritional efficiency and attainment of large size within a short period of time.

Knowledge of embryonic developmental process is important to the fish biologist and aqua culturist because it unmasked life history, mechanics of development, environmental and genetic influences on the ultimate fish structure and form. Recently it usefulness is demonstrated in predicting the precise time of genetic manipulation of fish for efficient aquaculture production (Aluko and Aremu, 2001). According to Olufeagba and Yisa (2003), embryology is the key factor to chromosome manipulation it serves as tool for genome manipulation for improvement in fish culture. The knowledge of the time of initiation of first mitosis is a strategy for chromosome duplication, which helps in producing tetraploids. When tetraploid (4n) are produced they could be used to produce interploid triploid when they are crossed with diploid (2n) , this will help to eliminate the low hatching of triploid production through physical or chemical shock (Olufeagba and Yisa, 2003). 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* (Aluko et al., 2001), interspectic cross between *Clarias anguillaris* and *Heterobranchus bidorsails* (Diyaware et al., 2009). This study was conducted to serve as a tool for genome manipulation for improvement in the culture of this fish.

II. Materials and Methods

Heterobranchus bidorsalis broodstock were obtained from fishermen in kainji Lake Basin, Nigeria. The fish were kept in holding concrete tanks of the Fish Biotechnology Research laboratory of the National Institute of Freshwater Fisheries Research, New Bussa, Niger State to acclimatize, one male and female were collected from the concrete tank with drag net for this experiment

Ovaprim hormone was used to inject the female at a single dosage of 0.5ml per kg of body weight for a latency period of twelve hours. The female was stripped of eggs and male sacrificed to remove the testes, the freshly collected milt were used to fertilize freshly stripped eggs. Physiological saline solution (0.9% Nacl) was added to facilitate fertilization.

Embryological Studies

The embryogenetic chronology of *Heterobranchus bidorsalis* was monitored using Brasser Digital photomicroscope. Forty fertilized eggs were put into a Petri dish with water and viewed under the photomicroscope. Major stages were photographed ranging from unfertilized eggs to hatching and free swimming stage. The time of occurrence of each stage was recorded and pictures uploaded in computer.

III. Results and Discussion

Fertilization initiates the first steps in chain of embryonic development (Haylor, 1993). Plate 1 show Stages of embryonic development in *Heterobranchus bidorsalis* under laboratory temperature of 26-28^{0}c. Eggs were fertilized after activation with spermatocytes, and 36 minutes after fertilization the fertilized eggs divided into vegetal and animal (blastodisc) pole. The yolk concentrated at the vegetal pole and the cytoplasm at the animal pole. Diyaware et al. (2009) made similar observation in *Claris anguilleris* x *Heterocbranchus bidosalis* crosses where the formation of an animal and vegetal poles occurred within 42 minutes after fertilization. The egg started cleavage process as the blastodisc divides producing two cells of equal sizes at 37 minutes after fertilization. This result is similar to that of Onyia et al. (2009) who reported 34 minutes of cleavage in *Clarias gariepinus*. The knowledge of the first cleavage is critical in chromosome manipulation to produce tetraploids. In a similar work done by Aluko et al. (2001) using *Clarias gariepinus* observed that the first mitosis took place 34 minutes after fertilization which was in agreement with the present study. On the other hand Olufeagba et al. (2004) reported 40 minutes of cleavage stage in *Heterobranchus longifilis*. The first cleavage furrow that was clearly visible divided the blastodisc into two distinct blastomeres. It should be noted that the two blastomere represent the two telophase poles of mitosis and the formation of cleavage furrow. Other work reported similar furrow in L.punctatus and P.carisicans. (Cardoso et al., 1995; Makeeva and Emelyanova, 1993) chromosome engineering approaches is possible if the two cell stage is known which will open opportunity for the precise time of the first mitotic metaphase (Aluko et al., 2001).

The second division perpendicular to first division was noticed one minute later and four equal cells were formed at 43 minutes after fertilization, third cleavage was effected i.e. five minute after the second cleavage and eight visible cells arrange in row were formed. At 48 minutes the cells divided into sixteen and were becoming difficult to count, further division of the cell into 32 cells stage at 58 minutes after fertilization was observed. The cells were smaller and tend to lie on themselves which were difficult to count individually. They could be referred to as morula stage. At 1 hour 48 minutes the blastula stage was observed, where further division occur. The cells arranged in a dome shape formed over the yolk mass. This is after the cleavage has produced over 100 cells; the embryo is called a blastula (http://education.yahoo/references/gray/subjects/subject/2009). The blastula is usually a spherical layer of the cells (the blastoderm) surrounding a fluid filled or yolk filled cavity (blastocoels). At 2 hours 15 minutes the gastrula was notice; the blastoderm cell started spreading over the yolk mass and become thickened with a small portion called blastodisc becoming weakened to form the embryonic shield. During gastulation cells consequently forming two (in dipioblastic animals) and three (triploiblastic) germ layers. The embryo at this process is called gastrula(http://education.yahoo/references/gray/subjects/subject/2006). At 13 hours 14 minutes the somite stage of the cell division was observed. Somitogenesis is the process by which somite (primitive segments) are produced. This segmented tissue could be differentiated into skeletal muscles vertebra and dermis of all vertebrate. Wriggling stage was noticed at 13 hours 38 minutes and it was observed that the tail separated from the yolk and due to swift movement of the tail, the chorion breaks and thus hatching occurred at 14 hours 37 minutes, the hatchling has observed attached itself to the chorion with weak movement and later detached and started swimming.

In conclusion, the timing for the first Cleavage in this study is 37 minutes and can be used for chromosome manipulation to produce tetraploid *Heterobranchus bidorsalis*. It also highlighted the morphological changes that occurred during embryogenesis of this species.

References

[1]. Aluko P.O; A.A. Nlewadim and A. Aremu (2001). Observation of fry cannibalism in Clarias gariepinus (Burchell, 1822). J. Aqua. Sci. 16:1- 6.

[2]. Ayinla OA, Kayode O, Idoniboye-Obu OIE, Oresegun A, Adidu VE (1994). Use of tadpole meal as substitute for fish meal in the diet of H. bidorsalis (Geoffrey St Hillarie 1809). J. Aqua. Trop. 9(I): 25-33.

[3]. Bruton, M.N.(1979): Systematic and biology of Clarias catfish in the culture of sharp toothier Catfish (Clarias gariepinus) in Southern Africa.Pp. 2358

[4]. Clay, d. (1977) Biology of tropical catfish family (Clariidae) with special emphasis on it suitability for culture of forehead marine ser, man rep. 11-58.

[5]. Cardoso E.L., Alves, M.S.D.Ferreirs, R.M.A.and Godinho H.P. (1995): Embryogenesis of the neotropical freshwaterSiluriformePseudoplaiystoma Corus can 3. Aquati Living Resour, 1995 8, 343 - 346.

[6]. Diyaware, M.Y., Haruna,A.B., Abubakar, K.A. and Olufeagba, S.O.(2009).Embryogensis of hybrid African catfish. Proceedings of 33rd Annual Conference of Genetics Society of Nigeria (Eds. J.A. Morakinyo, G. Olaoyo, M.A. Belewu, and T.R. Fayeye). Pp 192-196.

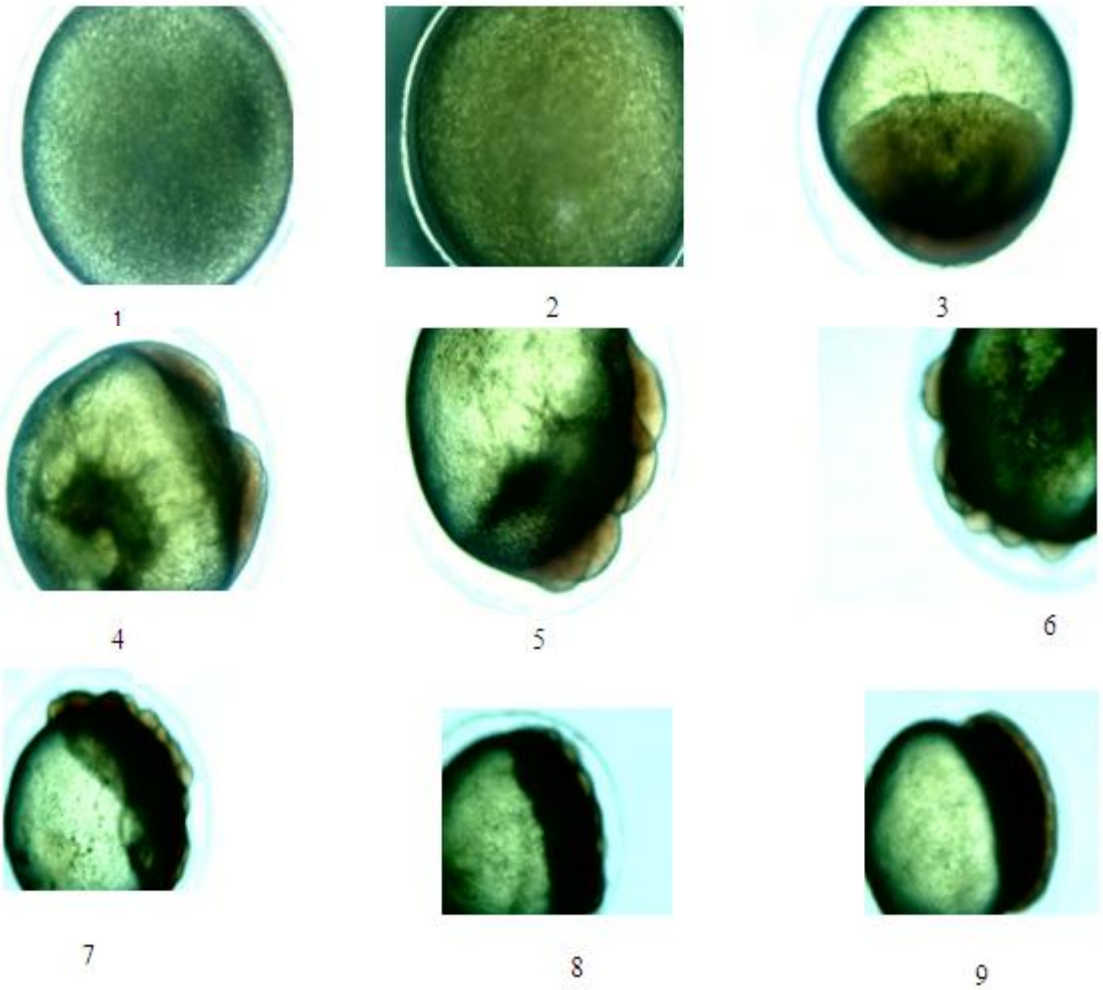
[7]. Ezenwaji HMG (1985). African Clarias taxonomy implication for field work. Proceeding of the 4th Annual Conference of the Fisheries Society of Nigeria (FISON) Held at Port-Harcourt 26th -29th November 1985, pp. 191-196.

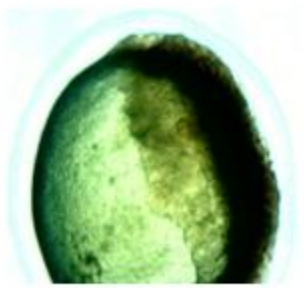
[8]. Federal Department of Fisheries (FDF), (2002): Fisheries Statistics of Nigeria.

[9]. http://education.yahoo/references/gray/subjects/subject/2009.

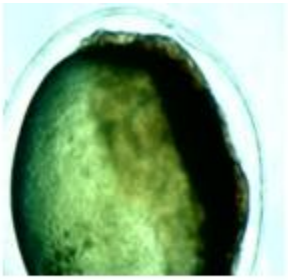
[10]. http://education.yahoo/references/gray/subjects/subject/2006.

[11]. Haylor, G.S. (1993): Aspects of the Biology and culture of the African Catfish Clarias gariepinus (Burchell, 1822) with particular reference to Developing African Countries. In: recent Advances in Aquaculture, Vol. IV. Ed. J.F. Muir and R.J. Roberts 1993. Blackwell scientific publication 235-293.pp.
[12]. Huisman, E.A and Richter, C.J (1987) Reproduction, growth, health, control and aquaculture potential Africa catfish (burchell 1822) aquaculture, 63, 1-14.
[13]. Makeeva,A.P. and N.G.Emel' yanova(1993). Early development of channel catfish Ictalurus punctatus. Journal of Icthyology 33:87-103.
[14]. Marimuthu,K. and M.A. Haniffa(2007). Embryology and Development of the Striped Snakehead Channa striatus. Taiwania 52 (1): 84-92.
[15]. Oladosu GA, Ayinla OA, Adeyemo AA, Yakubu AF, Ajani AA (1993). A Comparative study of the reproductive capacity of the African catfish species Heterobranchus bidorsalis (Geoffrey) Clarias gariepinus Burchell and their hybrid "Heteroclarias" ARAC Tech. Pap. 92: 1 – 5.
[16]. Olufeagba, S. O., Agbebi, O.T. and Otumbusi, S.O. (2004). Determination of first mitosis for induction of ploidy manipulation in Heterobranchus bidiorsalis (Geoffroy St. Hilarire, 1809). Proceedings of the 29th conference of genetic society of Nig., October 11th – 14th, 2004. 63-66.
[17]. Olufeagba, S.O., and Yisa, M. (2003). Studies on embryogenesis for induction of polyploidy and diploid mitogynogenesis in Heterobranchus longifilis (Pisces Clariidae).Nigerian J.Genet 18, 73-80.
[18]. Onyia,L.U., Ladu,B.M.B. and Olufeagba,S.O.(2009). Embryological Studies of African Mudfish (Clarias anguillaris) Proceedings of 33rd Annual Conference of Genetics Society of Nigeria,27-30 september,2009.

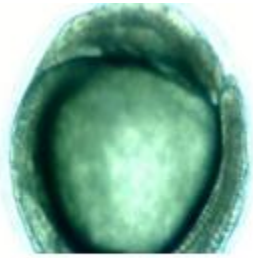




10



11



12

Plate1: The stages of embryo developmen‡ in Heterobranchus bidorsalis

1	Unfertilized Egg.	7	16-cell stage	13	Wringling stage
2	Fertilized Egg.	8	32-cell stage	14	Hatching stage
3	Animal and vegetal pole.	9	Morula stage	15	Embryo stage
4	2-cell stage	10	Blastula stage		
5	4-cell stage.	11	Gastula stage		
6	8-cell stage.	12	Somite stage		