EMBRYOGENESIS OF *Hetrobranchus longifilis* (CURVIER AND VALENCIENNES, 1840)

S.O. OLUFEAGBA[1], P.O ALUKO[2], J.S. OMOTOSHO[1], S.O. OYEWOLE[1], A. RAJI[1] and O. OGUNDELE[1]

[1]*Federal College of Freshwater Fisheries Technology (FCFFT) PMB 1500, New Bussa, Nigeria*

[2]*National Institute for Freshwater Fisheries Research (NIFFR) PMB 6006, New Bussa, Nigeria*

ABSTRACT

Studies on development of *H.longifilis* (Curvier and Valenciennes, 1840) were conducted at a temperature of 25EC (1Ec) in aquaria tanks continuous development were monitored with the use of wild Heerbrugy photomacroscope and length of yolk and larva were monitored using Stereo olympus microscope with ocular micrometer.

The division into animal and vegetal poles was observed 22 minutes after activation. The first cleavage occurred 65 minutes after activation while the second division which was perpendicular to the first line of division occurred 74 minutes after activation.This was quickly followed by the third and fourth cleavage at 80th and 82nd minutes after activation respectively.Morular stage was reached at 4 hours 20 minutes with formation of optic bud at 14 hours 35 minutes.

Developing embryo hatched after 27 hours of activation at a mean length of 6.63 and mean yolk length of 2.17. Yolk size decrease at an average rate of 38.5% till the 5th day of total absorption. Growth of larvae proceeded faster in tail-anus region than in anus-snout portion of the body

The rate of yolk absorption and larva development(survival) as monitored in this work gives important information in Research and development programme for *H. longifilis* larva - an important aspect of Research development and implementation of appropriate technologies in small scale fisheries

INTRODUCTION

Heterobranchus longifilis (Curvier and Valenciennes 1840 is one of the major clarid catfishes in Africa and they form a major family that are of high economic value. They are commonly cultured in ponds, tanks and cages. They live in swamps and rivers where they are caught all year round in most freshwater and swamps with a variety of gears.

According to Teugels *et al* (1990), Genus *Hetrobranchus* consists of four main species which are *Heterobranchus bidorsalis* (Geoffery St. Hilaire 1809) *Heterobranchus longifilis* (Curvier and Valenciennes 1840). *Heterobranchus isopteris* (Bleeker, 1863) and *Heterobranchus boulenge* (Pellegram, 1922). Despite their economic value and wide distribution there exist a dearth of information on the general biology of this species. Though some studies on embryology have been conducted on Clarias (Madu, 1989) Aluko (1994) and on non catfishes Cardoso *et al* (1995) no effort has been made to study the survival in the embryonic stages of the fish.

This study on the embryology is therefore:

1. To study the survival rate (with time) of eggs development before and after hatching up to the free swimming stage.

2. To study the rate of yolk absorption until the beginning of external (exogenous) feeding.

MATERIALS AND METHOD

H. longifilis broodstock were collected from the wild and kept in concrete tank fed with 40% crude protein NIFFR feed. The specimen were collected from the concrete tank when they had ripe eggs and transferred to the Fish Genetic Improvement Laboratory (NIFFR).

The breeders were injected with ovaprim C at 0.5ml/kg and the breeders were hand stripped, after a latency period of 12 hours. Fertilization was performed by the "dry method" the fertilized eggs were placed in a 60x30x30cm aquaria, one third filled with water with aerators and Kakabans which serve as egg collector. The water in the aquaria had the following (mean parameters) DO=5mg/6 pH=6.5and temperature 26CE temperature.

A batch of 5 embryos was monitored in fresh eggs from fertilization to hatching under Wild Heerbrugy Photomacroscope, and measurements of the developing embryo (Yolk length and width, Total length, Heartbeat rate per minutes) were taken under the Stereo binocular olympus microscope with the aid of calibrated micrometer (x4, 50 divisions =1.25mm =25um/division).

A high level of precaution were taken to avoid unnecessary agitation or excitement through touch, light, noise etc which could affect some parameters especially, the heartbeat rate.

RESULTS AND DISCUSSION

The morphological characteristics and embryonic development of *H.longifilis* was divided into 13 convenient stages which extended from mature oocyte to hatching. Detail of the morphological description and time of occurrence of stages are shown in Table 1. The percentage fertilization success of egg was 98% and the percentage hatching rate was 49.6%.

Table 1: Stages of the embryonic development in *Heterobranchus longifilis*

NO	STAGE	TIME(MIN/HRS)	DESCRIPTION
1	Mature oocyte	Omins	The unfertilized egg of *H.longifilis* is greenish and oval in shape with a mean diameter of 1.22mm. Freshly extended eggs re adhesive,surrounded by a uniform layer of cytoplasm which is transparent.
2	Fertilized egg	22 mins.	The fertilized egg shrinked few movement after fertilization with a mean diameter of 12m.
3	Animal and vegetal pole	22 mins	Shrinkage of the yolk away from the membrane, and accumulation of cytoplasm at the animal pole to form animal pole (blastodisc) and vegetal pole.
4	2 cell stage	1.5hr	This was observed as a vertical division of the animal pole producing two cells of equal sizes.
5	4-cell stage	1.14hr	Second line of division was perpendicular to the first line of division producing 4 cells which are still of equal size.
6	8-cell stage	1.20hr	Cell are seen as heaps on top of the "round lower yolk", cell size are becoming irregular
7	16-cell stage	1.22hr	Cell are clearly seen as irregular in size and could be difficult to count.
8	32-cell stage	2.45hr	Further division of the cells. Some cell tend to lie on another cell. Many further division producing many cells, irregular in size.

Table 1: (Contd.)

NO	STAGE	TIME(MIN/HRS)	DESCRIPTION
9	Morula stage	4.20hr	Further division of the cells produced many more but smaller blastomere, but the morula size further decreased.
10	Blastula state	9.20hr	Further division producing mass of cells elevated over the general outline of the yolk mass (like a domeshaped head)
11	Gastrulation stage	11.1hr	Embryo develop germ rings. Cephalic and caudal edges which were formed at advance stage of the blastula.
12	First wriggling movement	21.10hr	The long somite (18-21) start to move to both sides within the chorion wall. It started with 1 movement in 25 seconds, but this rate gradually increase with time. Presence of the olfactory pit, Otolith, cardiac beats to aid rudimentary fluid movement.
13	Hatching	23.19hr	Violent movement of tail to either sides against the wall-chorion, this is followed by contraction of embryo and hatching. Because of contraction, the chorion wall break and hatching occurs (interesting stage to watch.

Table 2: Showing the percentage survival of the various stages in embryonic development

Stage of development	No of survival	Percentage Survival
Egg number	222	
Fertilization rate	218	98
Animal and vegetal pole	218	98
1 cell stage	216	97.3
4 cell stage	216	97.3
16 cell stage	215	96.5
Morula stage	210	95
Gastrulation	194	88
Embryonic shield	165	74.3
Optic bud formation stage	148	67
Hatching	110	49.6

The percentage survival of the different stages of embryogenic stages as monitored in the experiment is shown in Table 2 and described on Fig 1. The fertilization rate was high (98%)
and (survival during) developmental stages until the morula stage when mortality starts to set in till hatching period. Rate of development here was similar to what Aluko (1994) early reported for *Clarias anguillaris*. There is the need for more study to step up the survival rate of longifilis survival at the embryo stage which will invariably increase the overall % hatchability.

The observation in this experiment were similar to those of other studied sample of the family Claridae (Madu 1989, Aluko 1994). The stages of development recorded in this work was faster than those quoted, this could be as result of the temperature under which this work has carried out.

The functional importance of the heart is evident in its early formation,though at rudimentary form. Cardiac contraction also was very few but continue to increase with time. Omotosho (1987) observed this in O.niloticus. An initial Heartbeat rate of 137/minutes was observed in this work, which later decreased till the 5th day. This observation was similar to what Shellon and Stephens (1980) observed in Dorosoma potenense as quoted by Omotosho (1987).The high tension generated was necessary for hatching to be initiated through the breaking of the chorion wall which demand more energy hence more heart pumps to circulate "energy nutrients" round the body. There is need to further research this area.

Yolk absorption rate is shown in figure 3a & b. There is a continuous decrease in yolk size from day 1 (hatching) to day 5 where we had total absorption for is not the same day after day,the highest absorption was on the 3rd day and 5th day. More nutrients are necessary to support the increase in body size. For aquaculture, there is the need to commence exogenous feeding at d time when high % yolk absorption commence (the mixed zooplankton must be filtered very well with double layer seram cloth to avoid big size zooplankton and water beetle from getting access to the fragile fry and harm them (Madu, 1996 per comm). Summary of the relationships of mean total length, yolk length and Heart beat rate are shown in table 3 and represented in Fig 4.

The manuer of egg division in all vertebrate is directly linked with the yolk proportion with the cytoplasm (blastodisc). The cleavage in eggs of *H.longifilis* (telocithic egg is) meroblastic with the initial blastomere remaining in continuity with the yolk globules as indicated by Kinmel and Law (1985). The characteristics of yolk development in *H longifilis*
H. longifilis i.e the flattening of the blastula) and the epiboly of the "animal pole" of the developing blastodisc cell during gastrulation is similar to what Cardoso *et al* (1995) observed in their work.

Table 3: Table showing men body length, yolk length and hear beat rate (YL=Yolk, length, YW-Yolk width, TL=Total length, HB/M =Heart beat/ minute).

DAY	YL	YW	TL	HB/M
1	67	55	193	137
2	64	45	224	103
3	53	37	271	103
4	25	28	303	110
5	2	1	338	104

There is clear delimitation between the embryos body and the yolk sac (food). The kupffer"s vesicle is a transient structure in *H. longifilis* and this could be a characteristic marker in its embryo identification (Laale, 1985) as quoted by Cardoso *et al.* 1995.

REFERENCE

Aluko P.O. (1994) Preliminary experiment in Triploidization of *Clarias anguillaris* by Cold and Warm shock. *NIFFR 1994 Annual Report.* pp 74 - 84 NIFFR, New Bussa

Cardoso E.L., Alves, M.S.D. Ferreirs, R.M.A. and Godinho H.P. (1995): Embryogenesis of the neotropical freshwaterSiluriforme*Pseudoplatystoma Coruscans. Aquati Living Resour,* 1995 8, 343 - 346.

Kimmel C.B; R.D. Low (1985): Cell lineage of Zebrafish blastomere:Cleavagepattern and Cytoplasmic bridges between cells *Der.Biol.* 108: 78 - 85.

Madu C.T.(1989): Hatchery management of the mudfish *Clarias anguilaris* (L). Ph.D thesis, University of Jos,pp 140-143.

Omotosho J.O. (1989) Studies on eggs and larval development in *Sarotherodon niloticus* (LIN) Treiravas. *Nigeria Journal of Applied Fisheries and Hydrobilogy 2:* 45 -53.

Teugels G.G; Denayer T. and Legendre U. (1990): A systematic rension of the African catfish genus *Heterobranchus* Geoffroy-Saint-Hailaire, 1809 (Pisces: Clariidae). Zoological *Journal of the Linnean Society 98:* 237-257.