EMBRYONIC DEVELOPMENTAL STAGES OF AFRICAN GIANT CATFISH *Heterobranchus longifilis* (Valenciennes, 1840) (TELEOSTEI, CLARIIDAE)

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ABSTRACT
The embryonic developmental stages in Heterobranchus longifilis were assessed. Matured specimens of male (1.9kg) and gravid female (2.3kg) broodstock of H. longifilis were selected. The female broodstock was injected with 1.1ml ovaprim and stripped after the latency period of 11 hours. The ovulated eggs collected were fertilized with the male milt. Fifty five fertilized eggs were distributed randomly into each of labeled 13 transparent plastic bowls, filled with 50 ml of freshwater. Hatchability rate and egg developmental stages were examine simultaneously every two hours with microscope. The first cleavage cell division occurred 30 minutes after the fertilization of eggs while the morula stage was observed within 2 hours. The blastula stage occurred between 2 and 8 hours, while the gastrula stage was 12 and 18 hours. Thereafter, neurulation period and embryonic body formation appeared. The optical vesicle and auditory vesicle formed. Finally, muscular contraction, tail formation, heartbeat, and hatching occurred. The young larva emerged from the embryonic membrane at 24.46 hours with vigorous lashing of the caudal region against the chorion membrane. The average weight and length of the yolk larvae were 0.005g and 0.43 cm respectively. The percentage of fertilization and hatchability rates was 82.50 and 65.10% respectively.

Key Words*:* Fish, embryogenesis, fertilization, Reproduction

INTRODUCTION
Heterobranchus species (Plate 1) belong to the family Clariidae and are mainly characterized by the presence of dorsal and adipose fins which differentiate them from *Clarias* species. However, the length of the adipose fin further differentiates, especially between *H. bidosalis* from *H. longifilis* in which the equidistance of the two fins identifies the latter with a black spot at the adipose fin compared to the former with longer dorsal fins, and a black spot at the adipose fin (Reed *et al.,* 1967). Several researchers have reported on genetic improvement and induced breeding, growth rate, and adaptability of *H. longifilis* to different artificial diets (Wilfred-Ekprikpo, 2014; Ofor, 2001; Ayinla *et al.*, 1994, Nwadukwe; 1993, Dada and Olarewaju, 1996, Oladosu *et al*. 1993, Ovie *et al.,* 1997). Several authors have also studied the embryological development of culturable species including *Clarias anguilaris* (Aluko, 1994), *Tilapia zilli* (Omotosho, 1989), *Heterobranchus bidorsalis* (Olaniyi and Omitogun 2014), and *H. longifilis* (Olufeagba *et al*., 1999). The genesis of progeny development starts with the union of male and female gametes during the fertilization of eggs. A zygote is formed and embryonic development starts and ends at hatching.

The embryonic studies typically support phylogenetic development by revealing very important developments insitu from the time the yolk starts concentrating at the centre thereby increasing the perivitellin space resulting in the animal and vegetal poles formation for further segregation of serial cell divisions. The morula, blastula, and gastrular stages are the advanced developmental stages after which sensitive organs are noticed before hatching. The embryological studies in fish life are of great significance in aquaculture production as well as taxonomical studies (Rahman *et al.,* 2009). This research work aimed to study the embryonic development of *H. longifilis* providing scientific information for successful breeding programmes.

MATERIALS AND METHODS
The experiment was carried out at Badore experimental fish farm of the Nigerian Institute for Oceanography and Marine Research, Lagos, Nigeria located between Longitude 06030' 25" E and 06032'28" E and Latitude 03036'19" N and 03039'17" N.

The length and weight of the matured male and female were measured using a meter rule and Camry EK4150 sensitive scale respectively. The matured specimens of male (1.9kg) and gravid female (2.3kg) broodstock of *H. longifilis* were selected and used to study the developmental stages of fertilized eggs (Olaniyi and Omitogun 2013). The female broodstock was injected with 1.1ml ovaprim and stripped after the latency period of 11 hours. The female fish was stripped and the ovulated eggs collected were fertilized with the male milt. Thirteen small transparent plastic bowls were set up in the Fish Health Laboratory and labeled 2–26 hours, each filled with 50 ml of freshwater and 55 fertilized eggs of an average weight of 0.053g were randomly sampled and placed in each bowl to physically

examine the hatchability rate and egg developmental stages simultaneously with two hourly microscopic examinations of the egg cells divisions. The development stages of the egg to hatching was monitored at a magnification of x100 for 27hours under a trinocular microscope with scope photo digital camera DCM 350, 369k pixels (USB 2.0), and computer operating system Microsoft window XP, professional version 2002. The water temperature was measured with a mercury thermometer while pH and dissolved oxygen were measured with a digital meter respectively.

The percentage fertility was calculated with respect to the number of eggs set aside in a separate bowl using the formula by Honji *et al.*, (2011, 2012) and Olaniyi and Omitogun (2012, 2013) while the percentage hatchability was deduced using Don and Avtalion (1986) and Omitogun *et al*., (2012) methods.

$$\% \text{ fertility} = \frac{Numbersoffertilizedegg X\ 100}{Total\ Number\ of\ egg\ released}$$

$$\% \text{ hatchability} = \frac{Numberofhatchedlarvae X\ 100}{Numberoffertilizedeggs}$$

PLATE 1 – Broodstock of *Heterobranchus longifilis*

RESULTS

The embryonic development of *H. longifilis* characterized into 19 periodic developmental stages started immediately the egg was fertilized (Plate 2). The average weight and length of the yolk larvae were 0.005g and 0.43 cm respectively; while the percentage fertilization and hatchability rates were 82.50% and 65.10% respectively. The experiment was carried out within a water temperature range of 26.5oC -27.0oC. The pH and dissolved oxygen at spawning were 6.8 and 5.5ppm respectively. The yolk content was observed under the microscope, converging to the centre of the egg immediately after fertilization. The oocyte became denser thereby creating more perivitelline space with two distinct thin membrane layers (Plate 2, D). The fertilized eggs are divided into vegetal and animal (blastodisc) pole in less than 30 minutes after fertilization. The animal pole (germ pole) consisted of pigment granules (nucleus and cytoplasm) whereas the vegetal pole (vitelline part) was mostly yolky. The protoplasmic bulge at the animal pole (one-cell stage) to the third mitotic cell division (8 cell stage) occurred in less than 45 minutes after fertilization. The cell divisions from the fourth mitotic stage to the sixth form of cleavage were rapid and occurred within 30 minutes (Plate 2, F, and G).

Morula stage was observed at 1½ - 2 hours after fertilization, then further development ensued ausing the cleaved blastomeres to become highly compacted and heavily consolidated forming deeply coherent cells with the blastoderm, on top of large yolk cells. At this stage, two blastula levels were noticed thus: high and low (Plate 1K and 1L). Epiboly continues during the gastrulation period and the percent epiboly described the fraction of the yolk cell that the blastoderm covers. Hence at 50% epiboly (Plate 1L), the blastoderm margin is at 50% of the entire distance between the animal and vegetal poles.

Gastrulation started at 8 hours after fertilization with internal processes of epiboly, the formation of the germ ring along the entire circumference of the blastoderm and the embryonic shield was apparent at the animal polar end. Neurulation followed gastrulation by transforming the gastrula to neurula and this was noticed about 14 hours after fertilization (Plate 10). In fish (teleosts), only secondary neurulation occurred unlike in birds, amphibians, and mammals with primary and secondary neurulations. The advanced form of somite formation was noticed between 14 – 22 hours after fertilization with clear cell differentiation between the head and the caudal region revealing matured somite blocks - body pigmentation, otic

placode, optic primordium, notochord, and spinal cord.

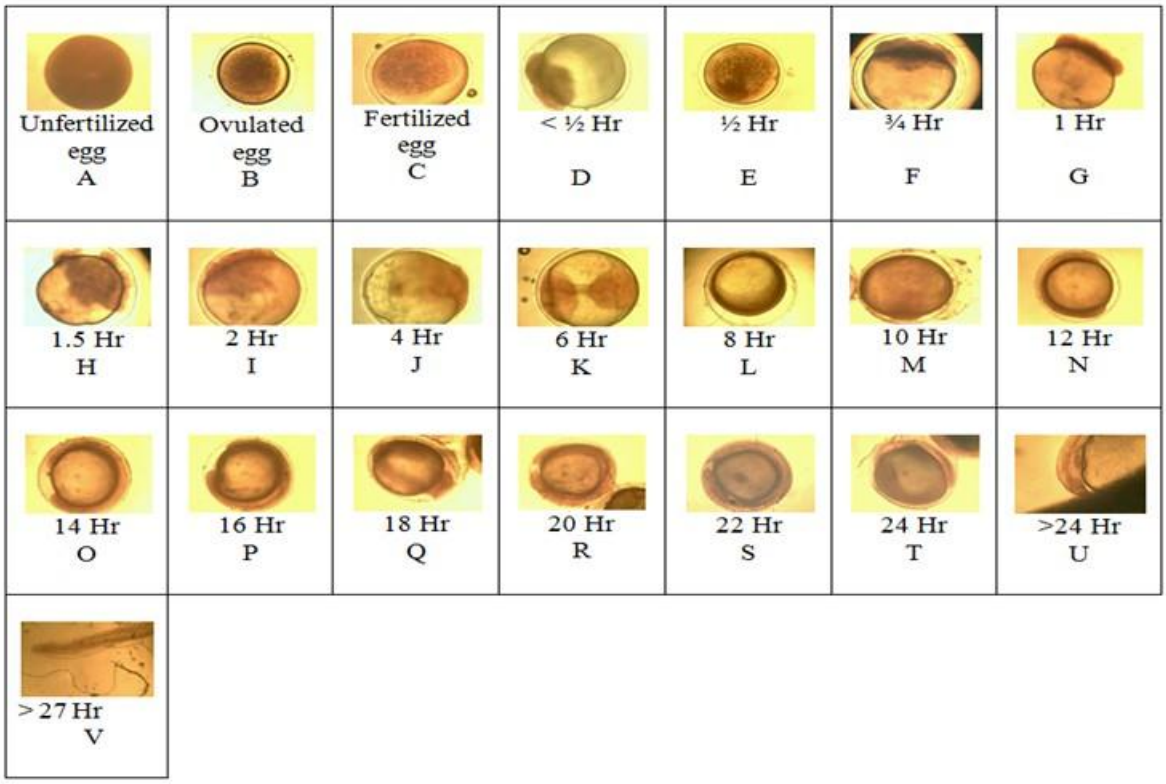

PLATE 2: Embryonic developmental stages of *Heterobranchus longifilis*
Key to Plate 2
A: Egg before injecting the female broodstock, B: Ovulated egg (Before stripping), C: Fertilized egg, D: Separation of the animal pole and vegetal pole, E: 1 – 8 cell stage, F: 16 – cell stage, G: 32 – 64 cell stage, H- I: Morula, J: Blastula (High), K: Blastula (Low), L: 50% Epiboly stage, M: 100% Epiboly, N: Gastrulation and formation of somite blocks - somite block, notochord, and early emergence of optic primordia, muscular movement noticed, O: Neurulation – formation of otic placode, distinct optic primordium P – Q: Primary organogenesis and pharygula period, muscular contraction of the somite block increased, R – S: Distinct vertebrae column and tail membrane, T: Heartbeat movement and caudal muscular contraction, U: Hatched larva showing the head region and heartbeat, V: Newly hatched larva showing the tail region, the position of the anus and the flow of body fluid along the body cavity, kupffer's vesicle distinct.

DISCUSSION

The water temperatures after ovaprim administration and before stripping were 250C and 270C respectively while pH and dissolved oxygen at spawning were recorded as 6.8 and 5.3ppm respectively. The temperature range during the experiment was 26.50C – 270C which agreed with the temperature requirement for egg development to hatching in *H. longifilis* (Freund *et al.*, 1995 and Olufeagba,1999), *C.gariepinus* (Kamler *et al.*, 1994) and river catfish, H*emibagrus nemurus* (Adebiyi *et al.*, 2013). The pH corresponded with the International standard for freshwater fish culture. An increase in dissolved oxygen above 5ppm influences the active metabolic rate and reproduction processes of fish as reported by Brain (2006) and Ita *et al*., (1995).

The latency period was 11 hours at 26oC contrary to 14 hours in *Heterobranchus bidorsalis* (Olaniyi and Omitogun, 2014). The percentage fertilization and hatchability rates were 82.50% and 65.10% respectively which was similar to *Heterobranchus bidorsalis* (85.67%, 68.33% as reported by Olaniyi and Omitogun (2014) probably due to the size of broodstock used with matured eggs and milts. The formation of animal and vegetal pole occurred in less than 30minutes compared to 45minutes after fertilization in *H. bidorsalis* which was similar to observations by Olufeagba *et al.*, (2004). The rapid cell divisions from 4 – 64 blastomeres agreed with Takoradi *et al.*, (2015). The early observations of morula, blastula, gastrula, and neurular in *H. bidorsalis* could be the effect of temperature (28.5 ± 0.5°C) under which the embryonic developmental stages were subjected to. The secondary neurulation caused the formation of a solid neural keel and a neural rod which advanced to form the neural tube (Papan and Campos-Ortega 1994).

The blastoderm thickness varies in many embryones during the blastula stage in the sense that the blastula rotates on the animal – vegetal axis thereby revealing one side to be thinner and flatter than the other side which later (the former) form the dorsal region of the embryo in advance development (Schmitz and Campos-Ortega 1994).

Before hatching, the muscular contraction of the larvae was initially gentle at an average interval of 8 – 12 seconds and later became vigorous with series of radial and rotational movements especially a few millimeters posteriorly from the point of yolk attachment towards the caudal parts. The contraction was propelled by the well-aligned consolidated muscular blocks of myotomes and supported by sclerotomes (Plates:2, R, S, and T). The heartbeat shortly before hatching was at the average of 92 beats/minute, which gradually reduced to 76 beats/minute. This was similar to 72beats/min. reported by Olaniyi and Omitogun (2014), for *H. bidorsalis.* The larvae finally hatched at 24.46 hours.

CONCLUSION

This study has provided scientific information on fertilization rates, hatching rates and incubation period (time to hatching), thus is very vital to the successful breeding of *H. longifilis* for all year round fingerling production.

ACKNOWLEDGEMENT

The authors wish to acknowledge the West Africa Agricultural Productivity Programme (WAAPP - Nigeria) for funding this research study. We are also grateful to the Executive Director of NIOMR for his support and supervision of the project.

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