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Embryonic and larval development stages of *Oreochromis niloticus* eggs during its artificial incubation

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Abstract
Embryonic and larval development stages of *Oreochromis niloticus* eggs during its artificial incubation were conducted in transparent water filter incubation jar at 29±0.11°C. Embryo developments were monitored and captured for defining characteristics from just fertilized egg to post yolk sac fry under digital microscope at 45× magnification. A bright yellow ellipsoidal egg describes a just fertilized egg was observed on the 1st day. Development of a rod-like structure was noticed at the dorsal part of developing embryo on the 3rd day. Hatching of the embryo occur on the 5th day while on the 12th day a well-developed swim-up fry with functional mouth, eye, and fins emerged. This research would be valuable to tilapia stakeholders interested in genetic manipulation techniques for improve *Oreochromis niloticus* production.

Keywords: *Oreochromis niloticus*, nile tilapia, breeding, embryonic stages, larval development and incubation

1. Introduction
The global production of tilapia in 2020 was 4, 407,200 tonnes which represented nine percent of the total (49, 120, 500 tonnes) finfish in inland aquaculture produced in the world [1]. Tilapia is the second species after carp that is most cultured in the world [2]. Culture of monosex progeny, preferably males grow faster and to a larger size than females during the culture period [3]. However, Fuentes-Silva [4] reported that manual separation of sexes, environmental manipulation, hybridization, sex reversal and genetic manipulation methods like androgenesis, gynogenesis, polyploidy and transgenesis are techniques involve in monosex tilapia fingerlings production. Artificial incubation during breeding allows close monitoring of the developing embryo and developmental stages crucial for sex reversal and chromosome set manipulations. Besides, it has also been reported that the manipulation of incubation temperature at the embryonic, larval, and juvenile stages stimulates growth-related gene expression which results in better growth performance of *Anabas testudineus* [5]. However, it was reported that important internal and external characteristics of the embryonic and larval development of *O. niloticus* occur under different stages named zygote, cleavage, blastula, gastrula, segmentation, and pharyngula [6, 7]. Agbebi [8], Olaniyi and Omitogun, [9] accentuated that embryological studies of fish species are essential for improvement on its breeding, aquaculture potentials and biodiversity. Moreso, a key understanding on embryonic and larval development stages of *O. niloticus* is the basis for its genetic improvement techniques for better production output. Therefore, this study was aimed at investigate and describe the embryonic and larval development stages of *O. niloticus* egg during its artificial incubation.

Materials and Methods
Eighty-one (1M:2F) *Oreochromis niloticus* broodstock from the Ejide Farms were selected and manually sexed following the description of Worldfish [10] by looking at the genital papillae of the fish. Prior to pairing, both sexes were fattened with 45% crude protein fish feed separately for 15days before pairing them in three breeding hapa nets of 3 × 3 m at a stocking density of 3 fish/m2 [11].

Three days after paring, the mouth of the female brood stocks were checked [12] to obtain fresh fertilized eggs used for the study. Eggs collected were cleaned and incubated in transparent water filter incubation jar fabricated using water filter containers of 2 litres capacity at a density of 150 eggs per liter. Embryo were monitored and captured for defining characteristics from just fertilized egg to post yolk sac fry under digital microscope at 45× magnification. The characteristic changes of Zygote (Z), Cleavage (C), Blastula (B), Gastrula (G), Segmentation (S), Pharyngula (P) and Hatching (H), Early Larval (EL) and Late Larval (LL) were studied under the embryonic development progress [8, 6, 13 9]. Time of distinguishable stages of embryo development with reference to time of freshly fertilized egg was recorded in hours post fertilization (HPF) and day post fertilization (DPF), counting the time of freshly fertilized egg collected as 0h and first day of fertilization.

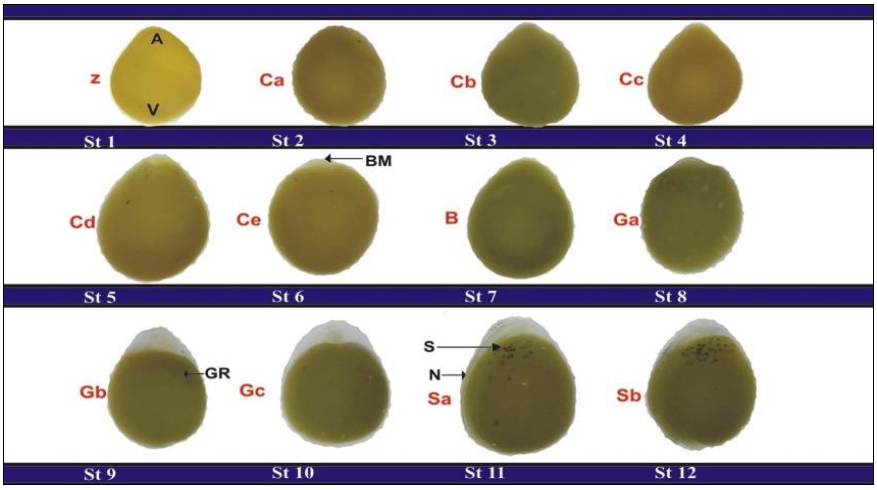
Photomicrography of defining characteristics in different stages were processed using CorelDraw X3 software. Water quality parameters such as pH, alkalinity, ammonia, nitrite, nitrate and general hardness during egg incubation were monitored using Pondlab 200 water test kit while water temperature was measured three times daily using a digital thermometer model number GMK-910T.

Results

The result of water quality parameters during egg incubation is presented in Table 1. Illustrations of the embryological stages of *Oreochromis niloticus* from fertilized eggs to post yolk sac fry incubated at 29±0.11 °C is presented in Plate 1. Table 2 presents detailed information on the timing and description of morphological events at different stages. The developmental stages were grouped into embryonic development and larval development. These two developmental groups were further divided into nine periods; Zygote (Z), Cleavage (C), Blastula (B), Gastrula (G), Segmentation (S), Pharyngula (P) and Hatching (H), Early Larval (EL) and Late Larval (LL). These nine periods were further divided into twenty-six stages which are detailed presented in Table 2 and Plate 1. Stage 1 in the zygote period (0-2 HPF) is characterized by a bright yellow ellipsoidal egg (Plate 1z). The egg is made up of large volume of yolk that serves as nourishment and energy to the developing embryo. Stage 2-6 (Plate 1Ca-Ce) in the cleavage period (2-6 HPF) consisted of five stages which are described by a greenish brown egg and mitotic divisions that resulted in the formation of blastomeres at the blastodisc. Stage 7 (Plate 1B) in the blastula period (6-22 HPF) described an epithelial layer called the blastoderm which is the germination point in an ovum where the embryo develops. The blastoderm became more visible within Stage 8-10 (Plate 1Ga-Gc) in the gastrula period (22-55 HPF) on the 2DPF. An anterior outward-growth was noticed on the developing embryo at 40-55 HPF in Stage 10 (Plate 1Gc). Stage 11-12 in the segmentation period (55-64 HPF and 3 DPF) is divided into two stages (Plate 1Sa-Sb). The formation of black pigment called the somite and a rod-like structure was noticed on the 3DPF at 55-60 HPF. Also, at 60-64 HPF the number of black pigments increases and the rod-like structure became prominent. Stage 13-15 in the pharyngula period at 64-84 HPF comprised of three stages (Plate 1Pa-Pc) and are characterized by the appearance of the developing structure of the head and neck on the 3 DPF at 68 HPF. The structure of the head became visible, the beating of the heart is observed as blood circulate within the developing embryo which is still enclosed in the chorion at 70-84 HPF on 4 DPF (Plate 1Pc). Stage 16-18 in the hatching period (84-110 HPF and 4-5 DPF) is divided into three stages (Plate 1Ha-Hc). The break-off of embryo out of the chorion was observed on the 4 DPF, head, eyes and hair-like tail structure were seen on the larvae at 94 HPF (Plate 1Ha). The larvae eyes became black in color and more visible at 102 HPF (Plate 1Hb). At 110 HPF (Plate 1Hc) the tail region became straightened, yolk sac reduction with better developed structure of head and eye were observed. Stage 19-24 in the early larval period on 6-9DPF were divided into six stages (Plate 1Ela-Elf). Stage 19-23 of the early larvae period at 142-190 HPF were characterized by yolk absorption, well developed eye, and movement of the jaws (Plate 1Ele). A great reduction in the size of the yolk sac with a better developed mouth, operculum, caudal fin and eye were noticed at 206 HPF on the 9 DPF (Plate 1Elf). Stage 26 in the late larval period on the 12 DPF was characterized by complete absorption of yolk sac as fry were seen swimming free on the surface of water (Plate 1Llb). At this stage, the swim up fry had developed to a level that supplementary food can be ingested through the mouth and each fry weighing 0.015g.

Table 1: Mean ± Standard error of water quality parameters

Water quality parameters	Mean ± Standard error
Water temperature (°C)	29 ± 0.11
pH	7 ± 0.11
Alkalinity (ppm)	267±0.05
NH_3 (mg/l)	0.05±0.03
Nitrite (mg/l)	0.06±0.03
Nitrate (mg/l)	0.63±0.03
General Hardness (ppm)	249±0.05



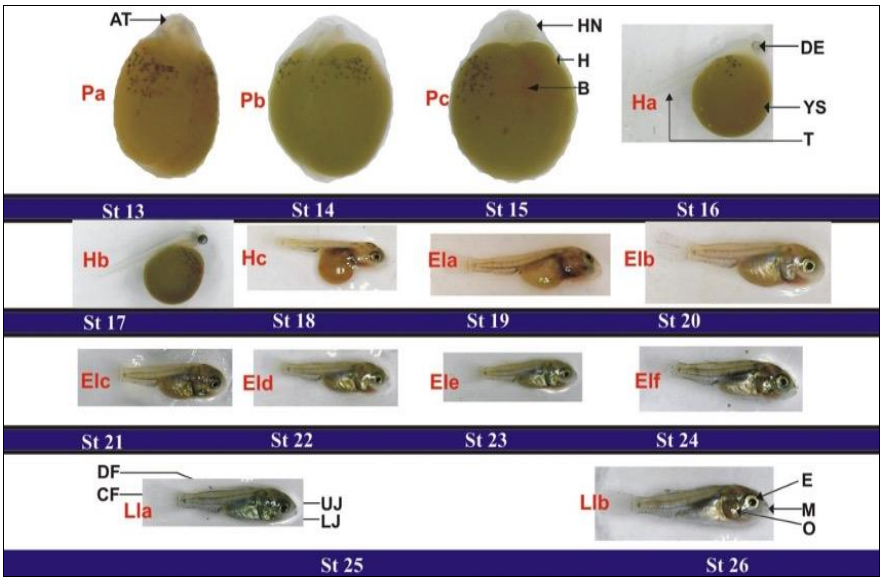


Plate 1: Photomicrography of the embryonic stages of *Oreochromis niloticus* from fertilized eggs to post yolk sac fry

Table 2: Timing and description of morphological events of *Oreochromis niloticus* eggs during its artificial incubation

Groups	Period	Stages of development	Time from fertilization		Description of morphological events
			Day(s) post fertilization (dpf)	hour(s) post fertilization (hpf)	
Embryonic Development	Zygote (Z)	1	1	0-2	A banana yellow ellipsoidal egg weighting 0.008g
	Cleavage (C)	2	1	2-3.5	2 cells, a greenish brown egg
		3	1	3.5-4	4 cells
		4	1	4-4.5	8 cells
		5	1	4.5-5.5	16 cells
		6	1	5.5-6	32 cells
	Blastula (B)	7	1	6-22	This is characterized by an epithelial layer called the blastoderm which the germination points in an ovum where the embryo develops.
	Gastrula (G)	8	2	26-36	The blastoderm became more visible and formation of germ ring.
		9	3	36-40	
		10	3	40-55	
	Segmentation (S)	11	3	55-60	Somite formation which are located on the mesoderm along the neural tube. Development of rod-like structure known as notochord seen at dorsal part of developing embryo
		12	3	60-64	The number of somites increased.
	Pharyngula (P)	13	3	64-68	A series of externally visible anterior tissue band lying below the early brain give rise to the structures of the head and neck
		14	3	68-70	Appearance of the developing structure of the head and neck
		15	4	70-84	The structure of the head became visible, the heart beat as blood circulates within the developing embryo which is still enclosed in the chorion.
	Hatching (H)	16	4	84-94	Break-off of the embryo out of the chorion, eyes and hair-like tail appeared, head appeared. Distinct heart was visible and functioned actively.
		17	5	94-102	
		18	5	102-110	
larval Development	Early larva (EL)	19	6	110-142	Yolk absorption, the eye is well developed, and the larvae gradually began to move its jaw.
		20	7	142-149	Reduction in the size of the yolk sac
		21	7	149-153	
		22	8	153-175	
		23	8	175-190	A great reduction in the size of the yolk sac.
		24	9	190-206	Mouth is well developed. Fins become prominent and the fry are seen swimming free.
	Late larva (LL)	25	10	206-248	Yolk sac is fully absorbed and the swim up fry had developed to a level that supplementary food can be ingested through
		26	12	248-288	

(St) Stage, (Z) Stage 1 in Zygote period, (Ca-Ce) Stage 2-6 in cleavage period, (B) stage 7 in blastula period, (Ga-Ge) stage 8-10 in gastrula period, (Sa-Sb) stage 11-12 in segmentation period, (Pa-Pc) stage 13-15 in pharyngula period, (Ha-He) stage 16-18 in hatching period, (Ela-Elf) stage 19-24 in early larval period, (Lla-Llb) stage 25-26 in late larval period, (A) Animal pole, (V) Vegetal pole, (BM) Blastomeres, (GR) Germ ring, (S) Somite, (N) Notochord, (AT) Anterior Tissue, (HN) developing Head and Neck, (H) functional heart, (B) Blood circulation, (DE) Developing Eye, (YS) Yolk Sac, (T)

Hair-like Tail, (DF) Dorsal fin (CF) Caudal Fin, (UJ) Upper Jaw, (LJ) Lower Jaw, (E) Eye, (M) Mouth, (O) Operculum.

Discussions

The color changes in the zygote in this study was similar to the findings of Malik [13] who reported yellow fertilized eggs at the first day and later observed a yellow brown yolky egg at second day. Siddique [7] also affirmed the colour changes of Nile tilapia eggs from Initial yellowish eggs to eggs gradually became greenish color. The present findings on the eggs color during the embryonic development of tilapia are almost positively consistent with the findings of several studies [14, 6, 12, 15]. The cell divisions in Stage 2-6 (Ca-Ce) were less visible when compare to several embryonic study on catfish [8, 16, 17, 18, 9] because tilapia zygotes are heavily yolk [12]. This is in agreement to what had earlier been reported by Olaniyi and Omitogun [9] that cleavage pattern during cell division is dependent on the amount of yolk, its distribution and proportion it occupies with respect to the cytoplasm that constitute the blastodisc. Meyer and Meyer [19], Valeta [15] laid emphasis on the relationship between incubation period and water temperature, it was stated that temperature has a strong indirect influence on the length of time required for the development of the incubating embryo, hatching of the fish-larvae and further development into fry with the recommended range 23-32ºC. However, Hussain [14] reported that hatching of *O. niloticus* eggs take place after 70-90 hours in the mouth of female *O. niloticus* at 28±1ºC coupled with parental care for another 6-10 days until the swim up stage which was in agreement with the finding of this study. A study on the effect of temperature on egg development in an attempt to improve hatching success and fry production in *Oreochromis karongae* was conducted by Valeta [15] where the shortest hatching period was 7.3 days, which was observed at the highest incubation temperature (29°C) and the longest hatching period was 14.7 days at the lowest temperature (25°C). Furthermore, the outcome of this study is similar to the findings of Malik [13] who divided the embryonic development of Nile tilapia at 28.02±0.12°C water temperature into six phases: Zygote (0-1 h to 4 HPF), cleavage (2-4 HPF), blastula (4-20 HPF), gastrula (20-40 HPF and 2 DPF), pharyngula (40-88 HPF and 2-4 DPF), hatching (88-116 HPF and 4-5 DPF), larval (116-274 HPF and 5-12 DPF) and juvenile (306-672 HPF and 13-28 DPF). The result of this study was in agreement with Hussain [14] who reported that fertilized eggs of *O. niloticus* needs an average about 10-12 days to complete the cycle of development (both embryonic and larval stages). The findings of Ahmed [12] who achieved 15 days post-yolk sac stage at 25°C whereby yolk sac was fully absorbed and fry started to feed on supplementary food at the rate of 10% while average body weight reached 0.0168 g was not in agreement with result recorded from this study due to differences in temperature.

Conclusion

In this study, it can be concluded that *Oreochromis niloticus* undergoes several morphological changes from fertilized egg to post yolk-sac fry. However, the morphological changes described contribute immensely to the understanding of its biology, artificial incubation and also have implications for genetic manipulation, ecological and conservational studies of *Oreochromis niloticus*. Moreso, it was revealed that hatching of the embryo occur at day 4th after fertilization while yolk-sac was fully absorbed at day 12th after fertilization.

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