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The embryonic development of *Clarias gariepinus* fertilized eggs subjected to different water temperature interval in an indoor hatchery in Jos

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Abstract
Embryonic development of *Clarias gariepinus* fertilized eggs subjected to different water temperature in an indoor hatchery in Jos was investigated**.** Gravid female and matured male *C. gariepinus* (800g each) were selected for this experiment. The female was hormonally induced with ovaprim at 0.5mlkg^{-1} and kept for 14 hours latency period at room temperature of 28.1 ^{0}C. The eggs were stripped and carefully mixed with the milt from sacrificed male for fertilization and incubated in aerated five different tanks (2 x 1 x 0.5m^{3} at 10cm water depth) with different water temperatures (24 ^{0}C, 26 ^{0}C, 28 ^{0}C, 30 ^{0}C and 32 ^{0}C) in three replicates. The processes of hatching were restricted to a particular temperature according to each treatment with the aid of adjustable electric thermostat heater. Description of the egg developing stages at every 30 minutes interval were made by examining live specimens under a microscope and the process was repeated at the various stages until the eggs hatched into larvae while microphotographs of were taken by digital camera. Water quality parameters apart from the water temperature of source of water used for this study were not significantly different from each other. Embryonic development of *C. gariepinus* fertilized eggs in treatments 28 ^{0}C – 30 ^{0}C were not significantly different (p>0.05) from each other but they were significantly successful (p<0.05) than other treatments (24 ^{0}C, 26 ^{0}C and 32 ^{0}C). Water temperature at 28 ^{0}C – 30 ^{0}C is highly recommended for successful embryonic development of *C. gariepinus* fertilized eggs into hatchlings.

Keywords: *Clarias gariepinus*, Embryonic development

1. Introduction
Aquaculture, the fastest growing food production sector [4], has the potential to help address the world's growing food supply demand. *C. gariepinus* is one of the most suitable species for aquaculture in Africa [3] but there is no sufficient information on the early development of the *C. gariepinus* fish. So it is necessary to undertake proper study to characterize its various stages of embryonic and larval development to understand the biology of this species as life starts with the union of male and female gametes. As soon as the egg is fertilized by a sperm, the zygote is formed and embryonic development starts and ends up at hatching. The hatchlings further undergo organogenesis and appear as like as their parents, thus end the larval stages. Egg development in the ovary is maternally derived and is predetermined in the ovary but its genetics complex is determined at the very instant of fertilization [11]. The embryonic and larval stages of fish are very sensitive to environmental disturbances. Temperature is an important environmental factor affecting fish embryo development and the survival and growth of fish larvae [1], several studies have been carried out on the effect of temperature on the early development and survival of cultured and wild fish species [10, 6].
An increased in temperature within an optimal range leads to faster development and shorter hatching time [1] and incubation temperature outside a species optimal range have been shown to have severe detrimental effects on hatchability and survival [2]. Moreover studies on larval development of any cultivable species are useful in directing the hatchery efforts of fish farmers to succeed in their efforts on fish seed production by promoting larval growth and survival.
The objective of this study is to provide a complete description of the effects of temperature on embryonic developmental stages of *C. gariepinus* before hatching. This investigation will help in the improvement of fish breeding in aquaculture in Plateau State.

2. Materials and methods

2.1 Study area

The research was carried out in the automated thermo-controlled fish hatchery of Global Aquaculture and Allied Ventures (GAAV) in Jos-South. The farm has all the required hatchery facilities (underground ponds, concrete tanks, plastic tanks, working space, standard hatchery, constant water and power supply) for induced breeding of this fish species.

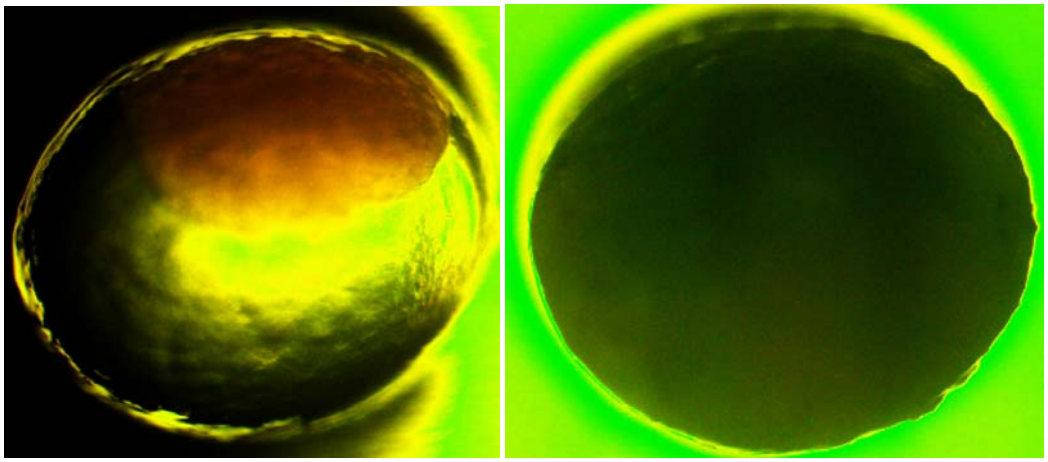
2.2 Experimental design

Gravid and healthy *Clarias gariepinus* brood fish and matured male (800g each), were selected according to sexual dimorphism for the breeding experiment. Seven days before being subjected to hypophysation, the breeders were separated by sex, in a three recirculating concrete tanks. The female Brooders were weighed and was hormonally induced with ovaprim at 0.5mlkg^{-1} of fish and allowed to spawn after a 14 hour latency period at room temperature of 28.1 ^{0}C The eggs were artificially stripped (dry method) without anaesthesing the female, inseminated and incubated in an aerated concrete tank (2 x 1 x 0.5m^3) at 10cm water depth and managed as described by [12]. During incubation, five water temperatures of 24 ^{0}C, 26 ^{0}C, 28 ^{0}C, 30 ^{0}C and 32 ^{0}C were introduced to each of the concrete tanks in three replicates. The processes of hatching were restricted to a particular temperature according to treatment with the aid of an adjustable electric thermostat heater. The description of the egg developing stages at every 30 minutes interval were made by examining live specimens under a microscope and taking microphotographs of the developmental stages of the fertilized eggs to larvae.

3. Results

The results of embryonic development of *Clarias gariepinus* fertilized eggs subjected to different water temperature in an indoor hatchery in Jos is shown in plate 1 to 5. Embryonic development of *C. gariepinus* fertilized eggs in treatments 28 ^{0}C – 30 ^{0}C were not significantly different (p>0.05) from each other but they were significantly successful (p<0.05) than other treatments (24 ^{0}C, 26 ^{0}C and 32 ^{0}C). After fertilization, the eggs swell and presented a spot (blastodisc) on one pole in all the treatments. After 2hrs of incubation the embryonic development of the eggs with temperature of 24 ^{0}C turned white, opaque and had turbid contents (Plate 1). These follow the same for temperatures of 26 ^{0}C, 30 ^{0}C and 32 ^{0}C which turned white, opaque and have turbid contents within the time intervals of 3:30mins, 5:30mins, and 2hrs (Plates 2, 4 and 5) respectively. The water temperature of 28 ^{0}C favours significantly the embryonic development of the eggs more than the other treatments and the eggs are hatched into larval within the period of 24hrs. The embryonic development of the catfish was divided into seven periods; zygote, cleavage, blastula, gastrula, segmentation, pharyngula and the hatching period. The cleavage was typically meroblastic and the first division (2 celled stages) occurred 30minutes after fertilization. Followed by the second cleavage completing 40 minutes after fertilization The 16 celled stage was reached an hour after fertilization. Yolk invasion started 5 hours after fertilization and completed 7 hours after fertilization. The head and the tail of the embryo became distinguishable at the end of the gastrula stage and the notochord could be clearly seen 13 hours after fertilization, the caudal region detaches from the yolk mass and become free.

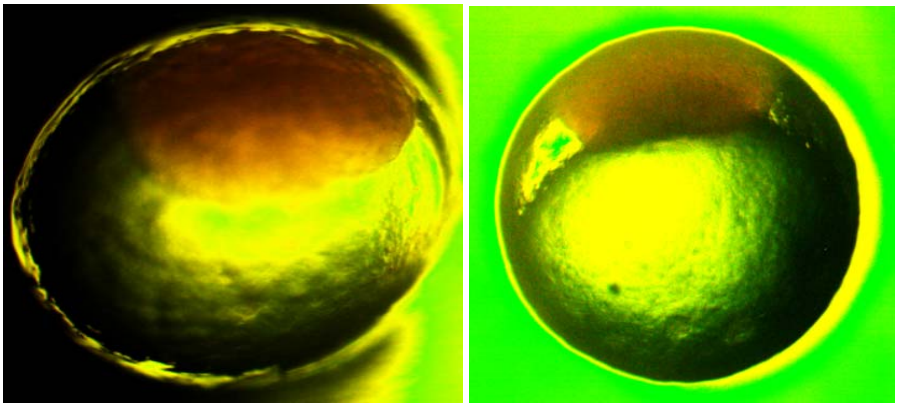
In the final stage of the embryonic development, the growing embryo occupied the entire previtelline space exhibiting a frequent twitching movement by lashing the tail against the egg capsule. Suddenly after a few seconds the larvae free itself through violent whipping movement of the tail which eventually ruptured the egg capsule. The newly hatched larvae were slender, straight, and transparent and were gradually tapering towards the tail. Hatching occurred 23 – 24 hours after spawning. Also the new hatchlings were characterized by the presence of an almost round yolk sac and ranged between 2.8 – 3.0 mm in length and tried to hide in any refuge they could find while some gathered on the edges of the aquarium. At this developmental stage the new hatchlings had no vent or mouth, no swim bladder as the breathe by absorbing oxygen through the fine blood capillaries that surround the yolk sac while still attached to the gut. Two days after hatching the larvae swam freely and by the third day, the larvae had almost completed its morphogenesis by absorbing its yolk sac.



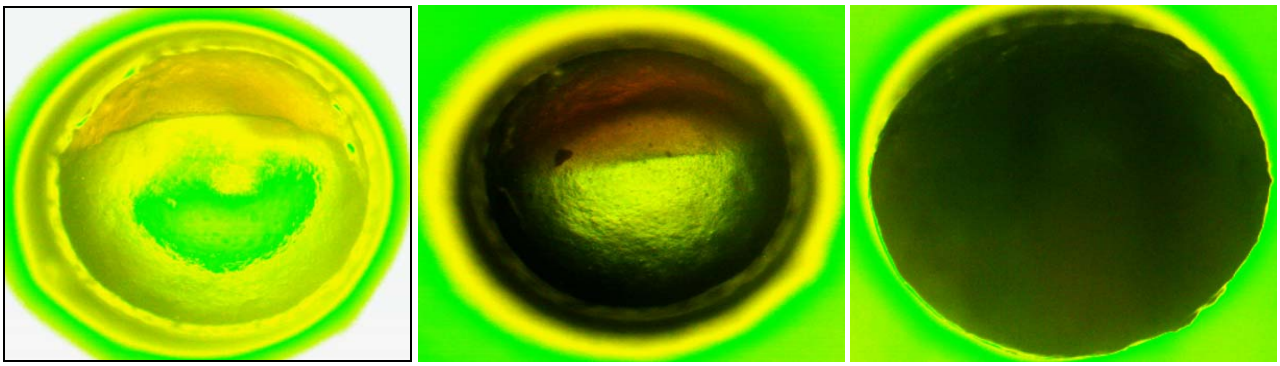
A
A – Blastula period,

B
B – Dead egg

Plate 1: Embryonic and larval development of *Clarias gariepinus* under 24 ^{0}C water temperature



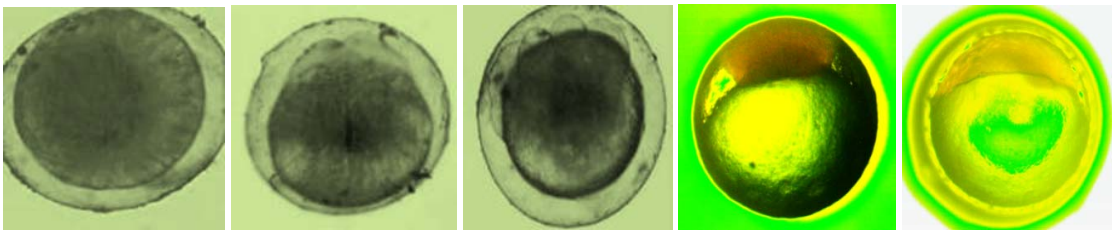
A B



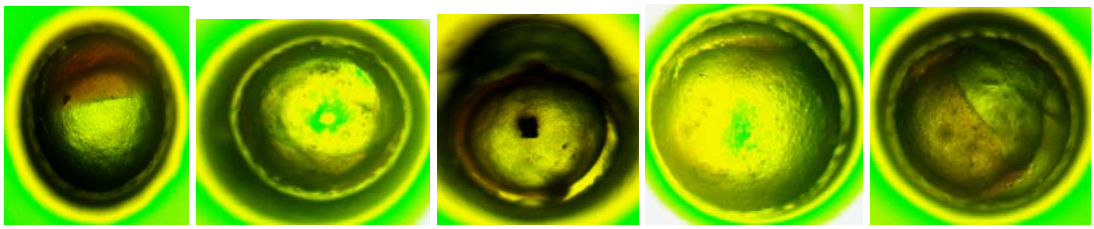
C D E

A – Blastula period, B – C cleavage period, D – Blastula period, E – Dead egg.

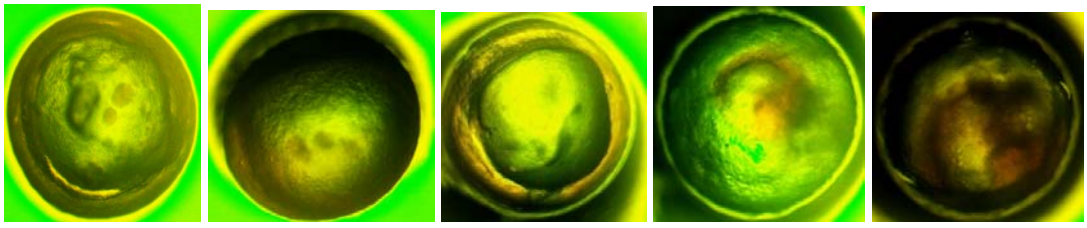
Plate 2: Embryonic and larval development of *Clarias gariepinus* under 26 ^{0}C water temperature



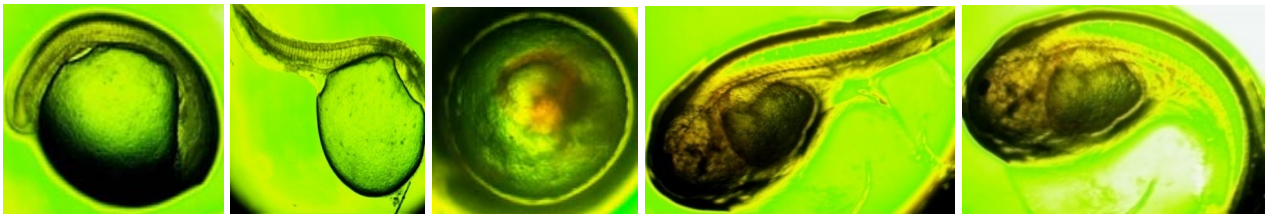
A B C D E



F G H I J



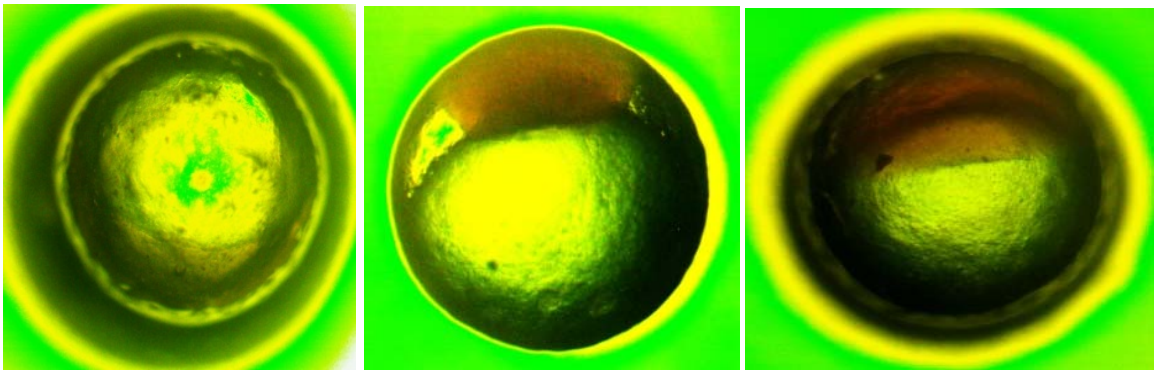
K L M N O



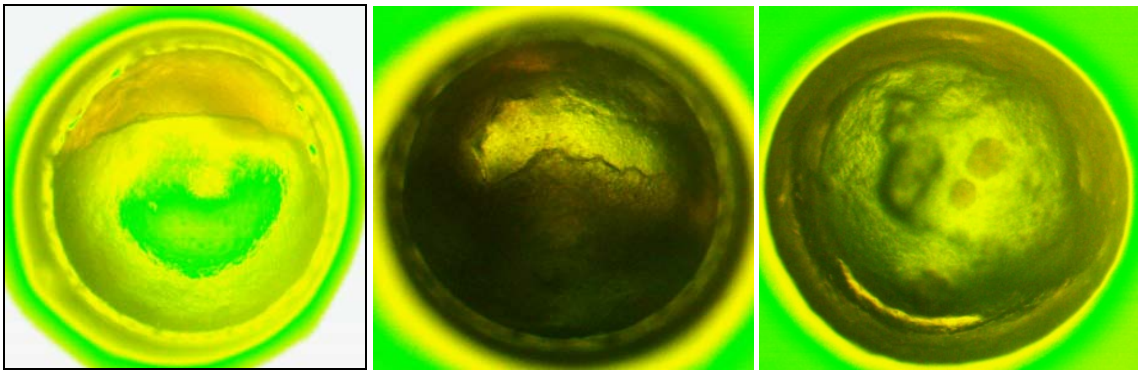
P Q R S T

A – Fertilized egg, B – C cleavage period, B - 2 blastomeres, C – 8 blastomeres, D – G Blastula period, H – M Glastula period, N – O segmentation period, P – Q pharyngula period, R – hatching, S – T three days old embryo

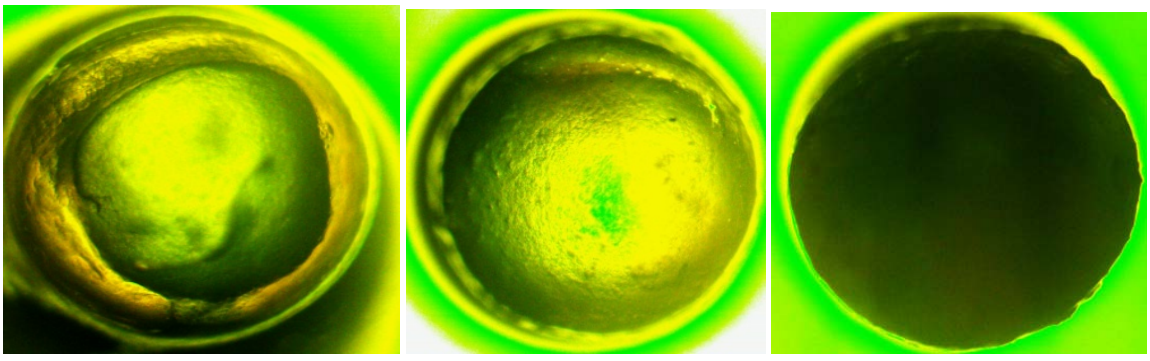
Plate 3: Embryonic and larval development of *Clarias gariepinus* under 28 ^{0}C water temperature



A B C



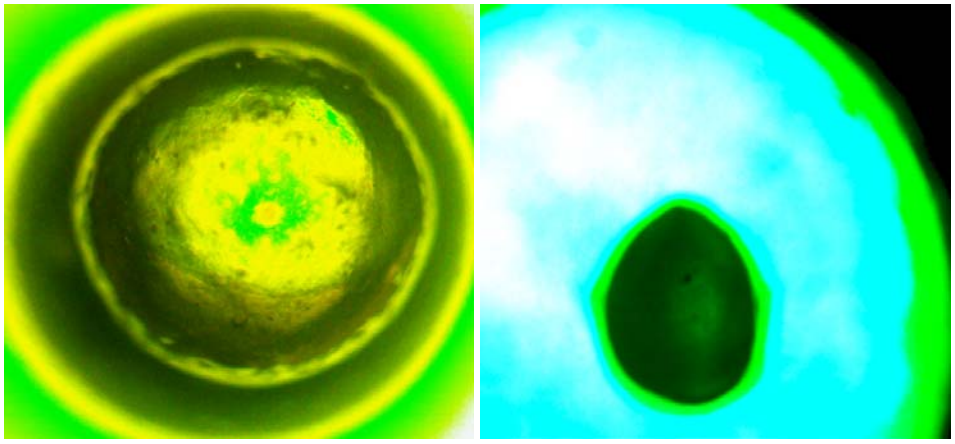
D E F



G H I

A – Fertilized egg, B – C cleavage period, D – Blastula period, H – Glastula period, I – Dead egg.

Plate 4: Embryonic and larval development of *Clarias gariepinus* under 30 ^{0}C water temperature



A B

A – Blastula period, B – Dead egg

Plate 5: Embryonic and larval development of *Clarias gariepinus* under 32 ^{0}C water temperature

4. Discussion

Temperature plays a vital role in the development of eggs. At 28 ^{0}C, fertilized eggs were adhesive, transparent, and spherical with diameters ranging between 1.13 and 1.16 mm and yellowish-white in colour. According to EI-gamal, 2009 [3], fertilized eggs develop properly if water quality parameters such as oxygen and temperature are within tolerable limit and faecal matter and other wastes are removed appropriately. However, it often happens that some eggs die after a brief period of development, either during the morula stage or before the closing of the blastopore. At the beginning, all the eggs appear to be healthy and well developed in all the different temperature treatments. Later, some of the eggs become white or opaque which may be due to injuries sustained during stripping. The unfertilized eggs are not distinguishable from the fertilized ones at the beginning as they swell in the same way and polarization also proceeds likewise. But they lag behind with the first cleavage and the normally hillock-shaped animal pole takes an unusual form, it becomes elongated and pointed as seen in the temperature treatments of 24 ^{0}C and 32 ^{0}C. After the 16 or 32 cell stage, some cells starts to separate off from the cell mass. Each cell is clearly visible in the perivitelline space. The healthy developing eggs are transparent or shinning and their contents are clear, at this stage they can be clearly distinguished from the bad eggs, which are white, opaque and have turbid contents. As the healthy egg reaches the blastopore closing stage, the good and the bad eggs are clearly distinguishable. This is the stage when the fertilization and hatchability rate can be determined as observed in the temperature treatment of 28 ^{0}C. The result disagrees with Sule, 2004 [13] who reported that *C. gariepinus* hatches at 25.1 ^{0}C. But however, agrees with Sule, 2004., Olaniyi & Omitogun, 2014 and Olaniyi& Ofelia., 2014 [13, 9, 8] who reported that *C. gariepinus* hatches at 28 ^{0}C. The study also agrees with Okunsebor, 2015 [7] who reported that the fertilization rate at the temperatures of 28 ^{0}C and 30 ^{0}C gave a higher value of above 80% for the hatching of *Heterobranchus bidorsalis* eggs. Optimum temperature range of 28 ^{0}C – 30 ^{0}C plays an important role for improving fertilization. This study provides a complete description of the embryonic and larval developmental stages of *C. gariepinus*. The effects of temperature on the embryonic development of its eggs will help in the improvement of the breeding, aquaculture potentials and biodiversity of the fish species in Plateau State.

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