

## Reproductive Performance of African Clariid Catfish *Clarias gariepinus* Broodstocks on Varying Maternal Stress

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**Abstract:** Effects of maternal stress on the reproductive performance of *Clarias gariepinus* broodstocks were investigated using female broodstocks of 450-550 g. The experimental broodstocks were subjected to transportation stress (treatment I), handling stress (treatment II), oxygen stress: one hour out of water before hormone administration (treatment III), two hours out of water before hormone administration (treatment IV), three hours out of water before hormone administration (treatment V), four hours out of water (treatment VI) and control {well conditioned before hormone administration}(treatment VII). The results showed that there was significant difference ( $p < 0.05$ ) among the treatments with the control having the best result of 75, 65, 13.2, 77.5 and 22.5% as %fertilization, %hatching, %deformed larvae, %survival and mortality, respectively. The broodstocks kept out of water for four hours before hormone administration gives the least result with 47.5, 29.5, 28.14, 52.5 and 47.5% as %fertilization, %hatching, %deformed larvae, %survival and %mortality, respectively. Based on the results of this study, proper handling and conditioning of female broodstocks is recommended to ensure successful induced breeding.

**Key words:** Stress, broodstocks, *Clarias gariepinus*, reproductive performance, larvae, hatching, fertilization

### INTRODUCTION

In Nigeria, a major family of catfish that is of commercial interest is the family clariidae. Clariid catfishes constitute a major family of food fish of economic value in sub Saharan Africa. The important genera in this family, *Heterobranchius* and *Clarias* are widely used in Africa aquaculture<sup>[1]</sup>. Among clariid catfish, *C. gariepinus* is the most widely cultivated in Africa, averaging 40MT/ha/year<sup>[2]</sup>. These are prominent in African aquaculture due to their fast growth rate resistance to diseases, tolerance to high density culture, ability to grow on a wide range of natural and low cost artificial foods and ability to withstand low oxygen and pH levels<sup>[3]</sup>. *C. gariepinus* maintain very efficient feed utilization<sup>[4]</sup>.

*Clarias gariepinus* has a wide range of tolerance to climatic conditions and an extensively cultivated species in aquaculture<sup>[5]</sup>. *C. gariepinus* ranked high on the consumer preference table in Nigeria and African countries as it naturally occurs in most stagnant and semi stagnant waters including shallow lakes, ponds, swamps, rice fields and irrigation ditches<sup>[6]</sup>. Availability of fish seed has been recognized as a lucid way of increasing fish production from aquaculture. Brett<sup>[7]</sup> reported two types of stress found in fish as internal stress caused as a result of physiological changes such as breathing and sexual maturation and external stress which include bad handling, poor feeding and sudden changes in water

quality. Internal stressors cannot be controlled, the culturist should focus attention in reducing the external stress factors for soaring yield and productivity<sup>[7]</sup>. The poor response of *C. gariepinus* to artificial propagation and high mortality is due to poor incubation condition and stressing of broodstocks during or after hormone administration. It is therefore imperative to carry out more research on the effects of handling and environmental stress factors as it affects the reproductive performance, growth and survival of the fish especially at their very first stage of development. The obliteration of these factors will go a long way in escalating the quality and quantity of fish seed production which in turn will augment the daily protein intake, thus alleviating the problem of malnutrition since the biological role of fish is largely nutritional.

There are many fish farmers in Nigeria but few have their own broodstocks. This necessitates transporting broodstocks over long distances; hence there is a need to know the effects of transportation stress on the reproductive performance of *C. gariepinus*. Moreover, the harvesting stress, which leads to bruises on broodstocks, has to be investigated to know whether this stress has adverse effects on the reproductive performance of *C. gariepinus*

### MATERIALS AND METHODS

**Experimental fish:** Sexually matured broodfish (450-550g) were collected from Agricultural Development Project

(ADP), Akure, Nigeria for treatment II to VII, while sexually matured broodfish were collected from a renowned fish farm at Okitipupa, Nigeria. The broodstocks were selected based on the external morphological features as described by<sup>[6]</sup>.

The experimental broodstocks were subjected transportation stress (treatment I), handling stress (treatment II), oxygen stress: one hour out of water before hormone administration (treatment III), two hours out of water before hormone administration (treatment IV), three hours out of water before hormone administration (treatment V), four hours out of water (treatment VI) and control {well conditioned before hormone administration}(treatment VII).

**Experimental procedure:** The broodstocks used for treatment II to VII were conditioned for two weeks in holding tanks at Federal University of Technology, Akure, Nigeria and were fed on 40% crude protein pelleted feed at 3% body weight twice daily between 0900-0930hr and 1600-1630hr on split doses.

Six hrs to the time of the hormone administration, ripe females used for treatment II to VII were harvested from the holding tank and brought to the Laboratory while the broodstocks for treatment I were transported to the Laboratory the same day from Okitipupa using 25l. The temperature, pH and the dissolved oxygen were 27°C, 6.45 and 3mg l<sup>-1</sup>, respectively. The female broodstocks used for treatment II were with bruises due to poor harvesting while broodstocks used for treatment III, IV, V and VI were conditioned for two weeks but were kept out of water for one, two, three and four hours, respectively before hormone administration. The female broodstocks for treatment VI (control) were conditioned for two weeks. They were kept inside holding tanks until they were administered with hormone.

The broodstocks were induced by injecting Ovaprim, a synthetic hormone (Aqualife Syndel International Inc. Vancouver, B.C. Canada) at the rate of 0.5mL 1000g<sup>-1</sup> body weight. Ovulation occurred 14 -18h after injection and gentle pressure was applied to the antero-posterior direction on the abdomen of two female brood fish to strip them of eggs. Male brood fish were anaesthetized, sacrificed and their testes removed. Milt was collected after dissection of the testes and immediately preserved in 0.9% NaCl solution. Stripped eggs were later fertilized with milt after sperm activation was initiated by the addition of 5 mL fresh water and checked for motility by microscopic examination. After 1 min of gentle stirring, fertilized eggs were rinsed in fresh water to remove excess milt and treated with talcum powder for 15-30 min to inhibit adhesiveness of the egg jelly coat and to prevent clumping and suffocation of the eggs during incubation. Eggs were incubated in glass tanks (60 X 40 X 40cm<sup>3</sup>). A blower and air stones provided continual aeration and

temperature maintained at 27± 1°C. Hatching occurred 24-26h later. The larvae were left for three days to absorb their yolk.

**Reproductive performance parameters:** The number of eggs released was determined by subtracting the weight of the broodstock after spawning (Wii) from the weight before spawning (Wi) in grams and multiplying the difference by 700 (1gm = 700eggs) Viveen *et al.*<sup>[6]</sup>.

Fertilisation rate was determined when the eggs generally reached the 4-8 celled stage of embryonic development. For calculating percent fertilisation, a sample of about 30eggs from each replicate of each treatment were carefully taken on petri dish containing water and the number of fertilised and unfertilised eggs were counted under a microscope (40 times magnification). The fertilisation rate was then calculated by the following equation.

$$\text{Fertilisation rate} = \frac{\text{Number of fertilised eggs}}{\text{Total number of eggs counted}} \times 100$$

The eggs were then transferred to their original lot for hatching. After hatching, the numbers of hatchlings within each batch were carefully counted and the hatching rate was calculated using the following equation.

$$\text{Hatching rate} = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs in a batch}} \times 100$$

$$\text{Percentage deformed larvae} = \frac{\text{Number of deformed larvae}}{\text{Total number of larvae}} \times 100$$

$$\text{Survival rate} = \frac{\text{Number of hatchlings alive up to larva stage}}{\text{Total number of hatchlings}} \times 100$$

**Statistical analysis:** All data collected were subjected to one-way analysis of variance (ANOVA) test using the SPSS Statistical package (SPSS Inc. V3.0. Chicago, Illinois) and where significant differences were indicated, means were tested using Least Significant Difference (LSD) test at p = 0.05 significance level. All percentage data were transformed to arc sin values prior to analysis<sup>[8]</sup>.

## RESULTS AND DISCUSSION

Artificial breeding of *Clarias gariepinus* was successfully carried out through the use of synthetic hormone (ovaprim) single knockout dose of 0.3 mL per

**Table 1: Weight loss and number of eggs released by *Clarias gariepinus* broodstocks on varying stress**

Treatment	Weight before spawning	Weight after spawning	Weight loss	No of eggs released
I	490.14±1.14	449.87±1.33	40.26±0.19 <sup>b,c</sup>	28185.00±133.64 <sup>b,c</sup>
II	500.05±0.07	465.55±0.64	34.50±0.57 <sup>c</sup>	24150.00±395.98 <sup>c</sup>
III	525.00±0.36	478.75±1.60	46.25±2.76 <sup>b</sup>	32375.00±1930.40 <sup>b</sup>
IV	500.06±0.08	466.36±1.18	33.70±2.26 <sup>c,d</sup>	32590.00±1583.92
V	500.15±0.07	471.65±1.48	28.50±1.56 <sup>d</sup>	19950.00±1088.94
VI	465.25±1.57	442.85±0.01	22.40±1.56 <sup>c</sup>	15680.00±1088.94
VII	475.06±1.28	367.12±1.08	107.94±73.64 <sup>a</sup>	75558.00±51546.67

1 gm = 700 eggs, Viveen *et al.*<sup>[6]</sup> Values are means ± SD from three replicates. Means in each row with different superscripts are significantly different  $p < 0.05$

**Table 2: Effects of maternal stress on fertilization and hatching rates of eggs and percentage deformity and survival rate of hatchlings**

Treatment	% Fertilization	% Hatchability	% Deformity	% Survival
I	52.50±3.54 <sup>b</sup>	34.50±2.12 <sup>b,c</sup>	25.32±3.49 <sup>a</sup>	67.50±3.54 <sup>b</sup>
II	49.00±1.41 <sup>b,c</sup>	34.50±3.54 <sup>b,c</sup>	24.86±4.21 <sup>a</sup>	62.50±3.54 <sup>c</sup>
III	58.50±2.12 <sup>b</sup>	42.50±3.54 <sup>b</sup>	17.78±3.14 <sup>b</sup>	62.50±3.54 <sup>c</sup>
IV	52.50±3.54 <sup>b</sup>	32.00±2.83 <sup>c</sup>	27.52±0.69 <sup>a</sup>	60.00±0.00 <sup>b,c,d</sup>
V	49.00±1.41 <sup>b,c</sup>	31.00±1.41 <sup>c</sup>	29.22±1.57 <sup>a</sup>	57.50±3.54 <sup>d</sup>
VI	47.50±3.54 <sup>c</sup>	29.50±0.71 <sup>c</sup>	28.14±2.69 <sup>a</sup>	52.50±3.54 <sup>d</sup>
VII (control)	75.00±7.07 <sup>a</sup>	65.00±7.07 <sup>a</sup>	13.21±1.53 <sup>b</sup>	77.50±3.54 <sup>a</sup>

Values are means±SD from three replicates. Means in each row with different superscripts are significantly different  $p < 0.05$

average weight of 475.11g females and 0.2mL per average weight of 475.01gm male were successfully used to induce spawning in *C. gariepinus*. Latency time recorded in this study was found to be between 11-18 hours at temperature range of 23.50-23.77°C. This finding is in agreement with that of<sup>[4]</sup> who reported 8-12 hrs at temperature ranging from 22.5-31.0°C.

Broodstocks subjected to transportation stress for 2-3 hrs without using oxygen when compared with control shown a significant reduction on the average number of eggs released, % fertilization, % hatching, % survival of the larvae and a significant increased in the number of deformed larvae and mortality. This finding is in agreement with<sup>[9]</sup> findings that improper handling of spawners during transportation affects successful artificial inducing breeding of *C. gariepinus*. The effect could be as a result of hormonal imbalance caused by transporting the spawner with low dissolved oxygen. Charkroff<sup>[10]</sup> reported that fish begin to be stressed when the dissolved oxygen level falls below 4mg/litre.

Broodstocks with bruise used for artificial fish propagation when compared with control shown a reduction on the number of eggs released, % hatchability, high-deformed larvae and mortality. This findings is in agreement with Alatis<sup>[11]</sup> report that spawners with bruise and poor health when used for induced breeding have adverse effect on the reproductive performance of the fish as a result of injuries which exposed the spawners to easy infection by bacteria and other micro organisms.

There were also inverse relationships in the reproductive performance parameter (number of eggs released, fertilization, hatching, deformed larvae and mortality) between the well-conditioned fish (control) and the spawners placed on concrete floor for 1-4 hrs (Table 1 and 2). This finding is an agreement with

Ayinla<sup>[12]</sup> that placing *Clarias* and *Chrysiichthys species* on concrete floor makes them to gradually lose their maturity stages. This study shows that lifting broodstocks out of water for 1-4 hrs has adverse effect on the reproductive performance and survival of the larvae of *Clarias gariepinus*.

There were significant difference ( $p < 0.05$ ), in the percentage fertilization, hatching, deformed larvae and survival between the treatments under different stress conditions. This finding confirmed Brett<sup>[7]</sup> report that poor response of fish to artificial propagation is due mainly to wrong latency period and stressing of brooders before or after hormone injection.

The result of the study revealed that handling stress, transportation stress has significant effect on the performance of hormone which resulted in reduced number of eggs released by the female spawners. It also had adverse effect on the percentage fertilization, hatchability, deformity, mortality and survival of the larvae. Hence stress factors resulting from handling should be eliminated or reduced to ensure that quality and quantity fish seeds are available for fish culturist at affordable prices.

Broodstock with bruise and bad health should not be used for seed propagation rather a healthy fish free from injuries and disease should be used to ensure good results.

Water is a medium where fish performs all its biological characteristics, that is water to fish is life and as such broodstock should always be kept inside water both before and after hormone administration to enhance the performance of hormone. If the above suggestions are stringently adhered to and water parameters well monitored and high-quality feed available, there will be good quality and quantity of fish seed available, to the

culturist at affordable price and this will lessen the problem of shortage of fish fingerlings which is one of the main constraints to aquaculture expansion in Nigeria.

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