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# Induced Spawning Activity of African Catfish (Clarias gariepinus, BURCHELL 1822) Using Different Fish Pituitary Gland

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***Abstract***

*A total of 27 Clarias gariepinus breeders (18 males and 9 females) with the female body weight ranging from 750g to 2100g were used to observe the spawning activity of the fish. The study was conducted to determine the reproductive variables of C. gariepinus, such as gonadosomatic index, fecundity, fertility, and hatchability. The pituitary glands (PG) collected from C. gariepinus, Cyprinus carpio, and Oreochromis niloticus were used for induction. The spawning fecundity of C. gariepinus injected with C. gariepinus pituitary gland extract oscillated at 79, 510 eggs, while the recipient of C. carpio pituitary gland extract yielded 106, 550. However, the fish administered with O. niloticus pituitary gland extract did not respond to the induction since there was no egg yield during the stripping procedure. The gonadosomatic index of C.gariepinus and C.carpio induced fish were 12% and 12.23%, respectively. In terms of fertilization rate, the C. gariepinus (88.4%) is higher than C. carpio (76.6%). Concerning the hatchability rate, it was observed that after 31 hours of incubation, the eggs were hatched with a rate of 58.6% and 67.6% for the C. gariepinus and C. carpio induced fish, respectively. Statistical analysis shows no significant difference both in hatchability rate and fertility rate, which implies that both C. gariepinus and C. carpio PG could be used as effective inducing agents in the spawning activity of African Catfish, C. gariepinus.*

**Keywords:** *Clarias gariepinus, induced spawning, pituitary gland, reproductive variables*

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## 1.0 Introduction

Fish is one of the cheapest sources of animal protein, and it is being used increasingly to correct the deficiency in human diets in the tropics (Dada and Ebhodaghe, 2011). According to Olumuji and Mustapha (2012), the African catfish (*Clarias gariepinus* Burchell 1822) is the most sought-after farmed fish species in West Africa. According to Dekempe and Micha (1994), Hogendoorn (1983), and Richter (1976), as cited by Gadissa and Devi (2013), it has been considered as an ideal species for the development of aquaculture in Africa. Vitule *et al.* (2006) reported that its major habitats are calm lakes, rivers, and swamps in areas that flood on a seasonal basis (De Graaf and Janseen, 1996; Winemiller and Kelso-Winemiller, 1996). *C. gariepinus* has pseudo-lungs, long bodies, and a high capacity to produce mucous as adaptations to living in stagnant environments or out of the water (Doneelly, 1973). Its reproduction is seasonal with gonadal maturation associated with periods of flooding. The maturation process is influenced by changes in water temperature and photoperiod, but the increase in water level is the principal factor influencing their reproduction (Van der Waal, 1974; De Graaf *et al.,* 1995; Yalcin *et al*., 2001).

Artificial propagation of fish is the most promising and reliable way of ensuring the availability of good quality fish seeds all year round and the sustainability of the aquaculture industry (Olumuji and Mustapha, 2012). According to Legendre *et al.* (2000), Adebayo and Fegbenro (2004), as cited by Sahoo *et al.* (2008), the injection of the different inducing agents in fish breeding is adopted for successful ovulation and collection of eggs in diverse cultivable fish species. The reproduction performance (Sahoo *et al.* 2008) is an important parameter to evaluate the proliferation success in captive conditions, which depend on the type of hormone used and its potency, dose of hormone, and maturity status of the fish. Agropedia (2012) reported that pituitary glands are often used to induce the fish to spawn. The hormone selected by the pituitary gland stimulates growth, development, maturity, and ovulation.

Several studies, as cited by Hossain *et al.* (2012), on the induced breeding of *Heteropneustes fossilis* (stinging catfish) are available from the literature and have been reviewed by several authors, including spawning behavior (Kohil and Goswani, 1987), induced spawning (Alok *et al*., 1993; Haniffa and Sridhar, 2002), effects of Carp Pituitary Gland Extract doses on induced breeding (Begum *et al.,* 2001), induced maturation and ovulation (Nayak *et al.*, 2001), induced spawning of catfish (*H. fossilis*) using human chorionic gonadotropin and synthetic hormone (Ovaprim) (Haniffa and Sridhar, 2002). However, induced spawning of *C. gariepinus* using pituitary glands of *Cyprinus carpio, Clarias gariepinus,* and *Oreochromis niloticus* have not been well investigated.

The development of fish seed production has been identified as a rational way of augmenting the dwindling fish supply from the capture fisheries (FAO, 1990). The *C. gariepinus* grows rapidly; it is disease and stress-resistant and is highly productive in polyculture with many species (Ayinla, 2003). Thus, the study was conducted to induce the spawning activity of *C. gariepinus* to help increase the production of the said commodity. In this relation, increasing the seed production of *C. gariepinus* can help boost the aquaculture industry in the Philippines since it is highly accepted in both local and foreign markets. The study aimed to determine the fecundity, gonadosomatic index, fertility rate, and hatchability rate of *C. gariepinus* induced with *C. gariepinus, C. carpio and O. niloticus* pituitary gland extracts.

## 2.0 Materials and Methods

### *2.1. Description of the Study Site*

The entire research work was carried out at RG Aqua in Monkayo, Compostella Valley from December 20, 2014 to January 26, 2015. Monkayo lies between 7.82$^{o}$ North latitude and 126.08$^{o}$ East longitude and 58 meters above sea level. The climate of Monkayo is tropical and has a significant amount of rainfall even during the driest month. It has an average annual temperature of 26.5$^{o}$C., and the average rainfall is 2,957 mm.

### *2.2. Broodstock Source and Management*

Twenty-seven (27) healthy broodstocks of African Catfish (*C. gariepinus*) (9 females and 18males) were purchased from RG Aqua in Monkayo, Compostella Valley, and were conditioned in an earthen pond (15m x 12m with a depth of 20 inches) for one week and were fed with commercial feeds twice daily at 7:00 A.M and 5:00 P.M at 5% of the total fish biomass.

### *2.3. Experimental Design and Set-up*

Complete Randomized Design (CRD) was used for this study. The experiment consisted of three (3) treatments (T1:C. gariepinus PG, T2:C. carpio PG, T3:O. noliticus PG; (1 female: 2 males breeders for all treatments) with three (3) replicates per treatment. The female broodstocks were administered with pituitary gland extracts of C. gariepinus, and C. carpio and O. niloticus with 1ml/kg concentration of female catfish body weight. Flow-through of water was carried throughout the experiment.

### 2.4. Collection of Pituitary Gland

The pituitary glands were collected from C*. gariepinus*, *C. carpio,* and *O. niloticus*, which were used for induction. The pituitary gland is situated on the ventral side of the brain, just below the hypothalamus. The dorsal side of the head was first chopped with the knife to gain access to the gland. The gland was removed intact without causing any damage to it because this would result in loss of potency (Agropedia, 2012).

### 2.5. Preparation and Preservation of Pituitary Gland Extracts

The collected glands were immediately preserved because the glycol- or murco- protein contained in them can be degraded by the enzymatic action. The glands were kept in fresh 60% acetone inside a vial until used. Then, the glands were taken out of acetone, put on a filter paper, and were allowed to dry at room temperature for one hour. Before administration, the glands were weighed before maceration. Weighing is essential for accurately determining the dose to be given by replicates of each treatment. The gland was macerated in a small bowl by adding a measured distilled water to homogenize. After homogenization, it was drawn into a syringe for injection. This process is adopted from the methods of Agropedia (2012).

### 2.6. Pituitary Gland Injection

The female *C. gariepinus* breeder was injected intramuscularlyat an angle of 30-45º at the dorsal fin with a 1ml/kg body weight of female catfish dose of hormone in every treatment. Each injected breeder was secured in different holding basins to prevent them from inflicting injury on one another. This process is adopted from the methods of Olumuji and Mustapha (2012).

### 2.7. Stripping, Fertilization, and Incubation

Injected female breeders were removed from their respective holding basins after 12 hours and were stripped into a clean dry bowl. Testes of male breeders were removed by laceration of the abdomen and were stored in a refrigerator until they were used. Upon stripping, the testes were squeezed into the eggs to fertilize. The fertilized eggs were spread on a hatching basin that served as an improvised hatching tank with a flow-through of clean freshwater until the eggs were hatched. The pH and temperature were monitored every hour from the incubation of the eggs until the hatching period (adopted from the methods of Olumuji and Mustapha, 2012).

### 2.8. Data Analysis

The fertilization rate and hatchability rate were calculated by the following equation according to Adebayo (2006): To determine the Fertilization Rate (FR), the equation is obtained in (No. of Fertilized Eggs) / (Total No. of Eggs) x 100. For the Hatchability Rate (HR), it was obtained in (No. of eggs hatched) / (Total No. of Fertilized Eggs) x 100. Gonadosomatic index (GSI) was determined by using the equation of Howaida *et al*. (1998), (Weight of Gonad) / (Body Weight) x 100. For the fecundity, the ovaries were carefully weighed after removing excess water on filter paper, and then the number of eggs per gram counted. Finally, it was calculated to show the total number of eggs (Dada and Ebhodaghe, 2011). The PAST software was used to analyze the data using One-Way Analysis of Variance (ANOVA) at $p < 0.05$ probability levels. Regression analysis was used to correlate the physicochemical parameters and the reproductive variables.

## 3. Results and Discussion

A total of 27 African catfish with a ratio of 1 female : 2 males (*C. gariepinus*) breeders with the female body weight ranging from 750g to 2100g were used to observe the spawning activity of the fish.

### 3.1. Fecundity of C. gariepinus

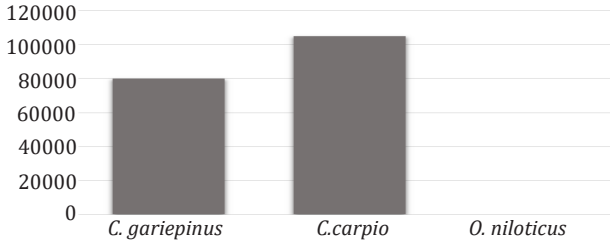


**Figure 1.** The fecundity of C. gariepinus induced with *C. gariepinus, C. carpio,* and *O. niloticus* pituitary gland extracts

Figure 1 shows the fecundity of *C. gariepinus* using different treatments. The spawning fecundity of *C. gariepinus* injected with catfish pituitary gland extract oscillated from 56430 to 92700 with a mean of 79510. In contrast, the recipient of *C. carpio* pituitary extract yielded from 56700 to 187200 with a mean of 106550. However, the fish injected with *O. niloticus* pituitary gland extract did not respond to the induction since there was no egg yield during the stripping procedure.

Fecundity varies from one species to another, depending on the environmental conditions, length, age, etc. (Musa and Bhuiyan, 2007). The experimental fish have a high fecundity during the study, and this is in agreement with the findings of Sydenham (1980), which pointed out that the high fecundity of the African catfish seemed to protect the species as they do not show parental care to their young. It is also an indication of the season as high fecundity is usually recorded during the peak of rains; this period favors the survival of catfish since flooding exposes the more foraging area to fish, making food and hiding places available to both young and adult fish as cited by Yusuf *et al.* (2013).

The morphological characteristics of *C. gariepinus* eggs and a hatchling of the fertilized egg have a thick membrane in which the gamete of a male fish penetrates a membrane. It was observed in this study that unfertilized eggs of 50.38µm were slightly bigger than that of fertilized eggs of 45.02 µm. Fish eggs are translucent with clearly defined yolk or oil globules.

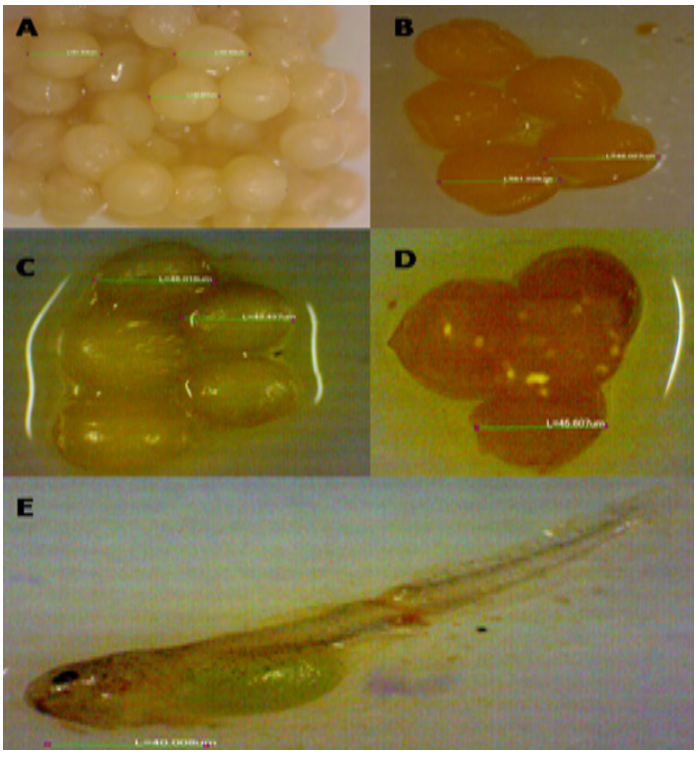


**Figure 2.** Morphological Characteristics of Egg (A-B, unfertilized egg, 50.38µm; C-D, fertilized egg 45.02µm; E, hatchling of scale bar of 40µm)

The size of the eggs in this study contradicts the result of Macfarlane *et al.* (2009) in sockeye salmon under sperm-limiting conditions; they found out that eggs that were successfully fertilized had significantly larger surface areas than eggs that failed to achieve fertilization. Fertilized eggs had a surface area approximately 7 percent greater than unfertilized eggs. This difference could not have been an artifact of our correction for the swelling of unfertilized eggs because the surface area of fertilized eggs was also more significant than the uncorrected surface area of unfertilized eggs, although the magnitude of the difference was reduced.

The phenotypic characteristics of the eggs are greenish in color, demersal, spherical, and non-adhesive. Mollah *et al.* (2011) reported in their study in Rita rita that fertilized eggs were transparent, demersal, spherical, non-adhesive, and brownish with a diameter ranging between 1.3 to 1.6 mm. The first cleavage occurred within 25-30 min post-fertilization at the temperature of 28±1°C. Hatching started 22 h post-fertilization and was completed within 24 h at the same temperature range. Newly hatched larvae were 2.0 mm in length, devoid of mouth and pigmentation, and started feeding within 48-60 h post-hatching.

### *3.2. Reproductive Variables of C. gariepinus*

The gonadosomatic index (GSI) of C. gariepinus PG and *C. carpio* PG induced fish were 12% and 12.23%, respectively (Table 1.). However, for *O. niloticus* PG induced fish, no GSI was observed. Statistical analysis revealed no significant difference (*p*-value of 0.9986) among the treatments of PG in *C. gariepinusin* terms of GSI. It implies that all treatments did not vary in their effectivity in the GSI.

**Table 1.** The reproductive variables of *C. gariepinus* induced with *C. carpio, O. niloticus,* and *C. gariepinus* PG extract.

| | Reproductive Variables | | |
|---|---|---|---|
| | GSI (%) | Fertility Rate (%) | Hatchability Rate (%) |
| *C. gariepinus* | 12 | 88.4 | 58.8 |
| *C.carpio* | 12.23 | 76.6 | 67.6 |
| *O. niloticus* | - | - | - |

In terms of fertilization rate, the *C. gariepinus* PG (88.4%) induced fish is higher than the *C. carpio* PG (76.6%). There was no fertilization in *O. niloticus* PG-induced fish. Statistical analysis revealed no significant difference (*p*-value of 0.8976) in the treatments of PG in *C. gariepinusin* terms of fertilization rate. It is inferred that the two (2) treatments (*C. gariepinus and C. carpio*) did not vary in the rate of their fertility.

On the hatchability rate, it was observed that after 31 hours of incubation, the eggs were hatched with a rate of 58.8% and 67.6% for the *C. gariepinus* PG and *C. carpio* PG-induced fish, respectively. For *O. niloticus* PG-induced fish, no hatchability of eggs was recorded because there was no occurrence of fertilization. There is no significant difference (*p*-value of 0.6743) among treatments regarding the hatchability rate.

This result is supported by the study of Gadissa and Devi (2013) who reported the fertility rates of catfish and carp-induced fish were 76.93% and 80.53%, respectively. In the study of Agbebi (2013) on the *C. gariepinus*, fertilization occurred between 17 h and 21 h after hormonal injection. Milts that were delayed for 2, 4, and 6 h at 19°C were used to fertilize freshly stripped eggs. The highest percentage was obtained when the sperm was delayed for 2 h, which achieved 69.6% fertilization. In this category, newly collected milt, eggs stripped at 10 h yields had 45% fertilization, 40% fertilization was recorded for stripped eggs at 12 h, at 14 h of latency which had a percentage fertilization of 70%.

However, in the study of Gadissa and Devi (2013), the fishes injected with catfish and carp pituitary extract resulted in 45.30% and 42.93%, respectively. The result contradicted when 45.30% fishes were injected with catfish and carp pituitary extract. The present study contradicts the results of Gadissa and Devi (2013, which gained higher rates in fertility and hatchability while the hatching occurred within 33 hrs which is longer than the present study. Observations on other studies showed that with catfish pituitary extracts (80%) fertility rate for *C. gariepinus* (Hecht *et.al,* 1982) was obtained by induced spawning. However, using carp pituitary extract (at a dose of 3mg/ kg body weight) for induced breeding of *C. batrachus* reported an 85% fertility rate (Thakur and Das, 1986). Through homoplastic hypophysation of *C. gariepinus* 80% hatchability rate was recorded (Britz and Hecht, 1988). The hatching rate was less (Hecht *et al*., 1988; Hogendoorn and Vismans, 1980) (65%) for *C. lazera* with carp pituitary extract as inducing material. There is a considerable variation in the hatchability rates of the two donor species, as observed in the present study. It is inferred that the hatchability of the incubated eggs not only depends on the potency of the hormones injected but also on the incubation facilities, temperature as well as other water quality parameters.

### *3.3. Physico-chemical parameters*

Table 2 revealed that treatment 1 (*C. gariepinus* PG) reached a higher pH and temperature of 7.5 and 25.8ºC, while treatment 3 (*C. carpio* PG) obtained 7.73 and 24.96ºC, respectively. Ndimele and Owodeinde (2012) cited that these values fall within the range of (24 - 32°C for temperature) and (6.5-9 for pH), as reported by Boyd (1979), while Chapman (2012) testified that the optimum spawning and embryo development of 25-27°C and pH 6.5-9.0 as the best for tropical fishes.

The incubation time, hatching rate, and larval survival of *C. gariepinus* were compared at pH levels between 4.0 and 10.0. The incubation time extended from 17 hours at pH 6.5. – 8.5 to 20 hours at pH 4.5 and 9.5. No hatch occurred at pH 4.0 and 10.0. The mean hatching rate increased from 31.18% at pH 4.5 to 69.84% at pH 8.0 and then declined to 34.21% at pH 9.5. Larval activity was depressed at low and high pH, whereas larvae were very active at pH 7.5-8.5. The results indicate that the optimum pH range for normal hatching and larval survival of *C. Gariepinus* is pH 7.5-8.5 (Nchedo and Chijioke, 2012).

The result of the linear regression shows the associated *p*-values of 0.35, 0.29 and 0.07 for the fertilization rate, hatchability rate, and GSI, respectively, in relation to the temperature. Concerning the pH, associated p-values are 0.51, 0.75, and 0.48 for the fertilization rate, hatchability rate and GSI, respectively. Since the *p*-values are greater than 0.05, the study revealed that there is no significant difference between the pH and temperature and the reproductive variables such as fertility rate, hatchability rate, and GSI. It was observed during the conduct of the study that the pH and temperature fell on the suitable values for the good fertility and hatchability rate of the eggs.

**Table 2.** The relationship of pH and temperature to the reproductive variables of induced *C. gariepinus.*

| Parameters | R | P Value |
|---|---|---|
| pH vs Hatchability | -0.16 | 0.75 |
| pH vs. Fertility Rate | 0.31 | 0.51 |
| pH vs. GSI | 0.35 | 0.48 |
| Temperature vs. Hatchability | 0.52 | 0.29 |
| Temperature vs. Fertility Rate | 0.46 | 0.35 |
| Temperature vs. GSI | -0.72 | 0.07 |

Oleyese (2006) reported that the spawning temperature ranged between 24.30-27.66ºC for *C. gariepius,* while the highest hatchability of 77.84% was recorded at 27.66% for fish injected at 8 pm. Since ovulation response to hormonal treatment is temperature influenced (Phelps *et al.,* 2007), degree-hours at spawning give a realistic indication of the time for stripping females.

**4.0 Conclusions**

The spawning fecundity of *C. gariepinus* injected with catfish pituitary gland extract oscillated with 79510 eggs, while the recipient of *C. carpio* pituitary extract yielded 106550 eggs. However, the fish injected with *O. niloticus* pituitary gland extract did not respond to the induction since there was no egg yield during the stripping procedure. The gonadosomatic index of *C. gariepinus* PG and *C. carpio* PG induced fish were 12% and 12.23%, respectively. Fertilization rate of *C. gariepinus* PG (88.4%) is higher than *C. carpio* PG (76.6%). It was observed that after 31 hours of incubation, the eggs were hatched with a rate of 58.8% and 67.6% for the *C. gariepinus* PG and *C. carpio* PG induced fish, respectively. It is also observed that the fertilization rate is inversely proportional to the hatchability rate.

The pituitary gland extracts from *C. carpio* has the highest hatchability rate, followed by *C. gariepinus* pituitary gland.

The result revealed that the treatment with *C. gariepinus* PG reached a higher pH and temperature of 7.5 and 25.8$^{o}$C, while the treatment with *C. carpio* PG obtained 7.73 and 24.96$^{o}$C, respectively. Generally, results show that both *C. gariepinus* and *C. carpio* PG could be used as inducing agents.

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