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Effect of pituitary gland from spent and unspent female brood fish on the spawning performance of African catfish (*Clarias gariepinus*)

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ABSTRACT

Pitutary gland produces, accumulates and stores gonadotropic hormone which plays active role in ovulation as intermediary between central nervous system and gonads. However, information on the efficacy of pituitary gland from newly spent *Clarias gariepinus* for inducing ovulation is yet established. The effect of pituitary gland from spent and unspent female fish on the induced breeding performance of *Clarias gariepinus* was tested. The experiment was set up in a completely randomized design (CRD). Nine (9) brood stock distributed into three treatments were used for the experiment (Treatment 1 was injected with 0.5ml synthetic hormone (Ovulin), Treatment 2 was injected with pituitary from already spent female fish and Treatment 3 was injected with pituitary from unspent fish) and each treatment was replicated three times. The result showed that Treatment 2 had the highest mean values for fecundity rate (122.92%) and was not significantly different from the other treatments. It also has higher value for fertilization rate (60.67%) and high stripping percentage but lower ($P < 0.05$) hatching rate (13.619) when compared with Treatment 1 which had the highest value for hatching rate (22.79%) followed by Treatment 2 (19.739%) with statistical similarity ($P > 0.05$). The present research demonstrated that *C. gariepinus* can successfully be induced to ovulate with pituitary from spent female fish, ensuring maximum use of brood fish and production of high-quality eggs and normal hatchlings.

Keywords: *Clarias gariepinus*; induced breeding; gonadotropic hormone; pituitary gland; spent fish; unspent fish

INTRODUCTION

The success of any fish farming operation depends on the availability of a ready supply of fish larvae for on- growing to market size (Rottmann *et al*., 2003). The rearing of the larvae to the fry stage is most critical in the cycle of fish seed production in hatcheries, therefore, the rearing of the larvae under controlled hatchery conditions requires the development of specific culture techniques. Reproduction technique is one of the factors that affect the performance of any fish farm as it can either be natural or artificial (Adebayo and Popoola, 2008; Owodiende and Ndimele, 2011).

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The output of natural propagation in fish is very low and cannot meet the protein requirement of its consumers (FAO 2004, FAO,1996). In view of this an artificial propagation technique under more controlled conditions has been discovered to produce reliable sources of fish fries and fingerlings distribution centre (FAO,1996). Artificial breeding otherwise known as Hypophysation (Naeem *et al*., 2005) is practiced with the involvement of reproductive hormones. Induced breeding through hormone treatment and artificial incubation of fertilized eggs has advantages of better rate of fertilization and hatching, better conditions for growth and survival of larvae to fingerling and better protection of larvae against unfavourable environmental condition and predators (Woynarovich and Horvath, 1980). However, most of the hormones that are generally used for induced breeding are deficient in various ways. For example, Deoxycorticosteroid Acetate (DOCA) causes severe ulcer on the injected female; Human Chronic Gonadotropin (HCG) is very expensive; Common carp (*Cyprinus carpio*) pituitary gland material are not easily accessible to small scale fish farmers, although Ovulin and Ovaprim (Salmon Gonadotropin Releasing Hormone) had recorded numbers of success but the price is very high (Owodeinde and Ndimele, 2011). The report by Hill *et al*. (2009) revealed average success rates of 50% ovulation, 54% spermiation and 1.3% mortality were recorded after injection of different species with ovaprim. Also, ovaprim has been used successfully for induced breeding in different families of fish like *cyprinidae* (Hill *et al*. 2005), *Characidae* and *Cobitiidae* (Yanong *et al*., 2009). However, the price of ovaprim increased indiscriminately due to import duties, therefore, to reduce the cost of production arising from cost of ovaprim, Ovulin or ovatide, there is need to find an alternative cheaper spawning aid. African Catfish pituitary hormone is said to be readily available and cheaper than any other hormone (Adebayo and Popoola, 2008) and can be prepared in a suspension (Fagbenro *et al*., 1998). This study was therefore designed to determine the effect of pituitary gland from spent and unspent female fish (*Clarias gariepinus*) on the reproductive performance and survival of fry (*C. gariepinus*). Pitutary gland produces, accumulates and stores the gonadotropic hormone which plays active role in ovulation as an intermediary between the central nervous system and the gonads. However, information on the efficacy of the pituitary gland from spent *Clarias gariepin*us for inducing ovulation immediately it is stripped is yet established.

MATERIALS AND METHODS

Experimental site

The study was carried out in the fish hatchery facilities of the Department of Fisheries and Aquaculture, Faculty of Agriculture Usmanu Danfodiyo University, Sokoto, Nigeria situated on latitude 13˚ 7′ 78″ N and longitude 05º 12′ 25″E at 275m above sea level. The site is situated in the extreme end of Sudan savannah agro-ecological vegetation zone of Nigeria. It's characterized with an annual average rainfall of 565mm from (May to October) with average temperature of 27ºC with peak at 40ºC (April) and 15ºC (December to January).

Broodstock Selection

The broodstock of the *C. gariepinus* was obtained from the department of fisheries and aquaculture Usmanu Danfodiyo University; and was acclimatized in the concrete tank of the Fisheries Department Farm/hatchery for 24 hours. The mature gravid females were

selected based on extraction of few eggs by depressing of the fish abdomen using the finger. Mature males were also selected based on their reddish pointed genital papillae, as reported by (da- graaf and Janssen, 1996).

Experimental Setup

The experiment was subjected to a completely randomized design (CRD) with 3 experimental sets in replicates 3 x 3 giving a total of 9 experimental units. The treatments were designated as T1, T2 and T3. Treatment T1 was injected 0.5ml synthetic hormone (Ovulin) administered to both Male and Female brood stock base on the size of the fish as control, treatment T2 100% Pituitary extract from spent female and T3 100% pituitary from unspent Female base on the size of the brood fish.

Hormone Injection

The male and female broodstocks were weighed separately using a 10kg Camry Premium Table weighing balance (0.01g). The females were injected based on their weight with a pituitary gland extract collected from the mature *C. gariepinus* females (pituitary from these spent females were from those initially injected with ovulin to induce ovulation before use for this experiment). The pituitary gland extract was administered intramuscularly at the recommended rate of 1ml per kg body weight of female fish and half dosage for male (Legendre *et al*,.1986). The Ovulin (synthetic hormone) was administered intramuscularly (above the lateral line, towards the tail) at the recommended rate of 0.5ml per kg body weight of female fish and half dosage for male. After the injection, the brood stock was kept back into the concrete tank, with the males being separated from the females for a latency period of 9 hours.

Collection of Milt and Eggs

The milt was collected by sacrificing the male. The two lobes of the male's testes were removed, cleaned with tissue paper and kept in a cleaned Petri dish. The abdomen of the female was well cleaned with tissue paper in order to avoid contact between the eggs and water. Then the females were stripped of their eggs by a gentle application of pressure on the abdomen to release the eggs were collected in a dry, well cleaned plastic bowl.

Artificial fertilization

The testes of the male were cut open using a razor blade and the milt was squeezed out, and then 0.9% saline solution (NaCl) was added to the milt in order to facilitate fertilization after which the milt was used in fertilizing the already stripped eggs, hence the percentage fertilization was calculated (Ovie and Ovie 2010).

Incubation and hatching

Incubation and hatching of eggs were carried out in 9 aerated hatchery plastic bowls with each measuring at least 70 l. Each hatchery bowl was filled with water containing

incubation nets of 1mm mesh size for substrate attachment of eggs. The fertilized eggs were evenly spread on the incubation nets in the tanks at temperatures between 27-32 °C.

Data Collection

Reproductive Performance Indices

Total number of eggs spawned: The total number of eggs spawned (Spawning fecundity) were estimated by counting the number of eggs in 1g of egg mass, multiplied by the weight of stripped eggs (Sahoo *et al.*, 2005).

Relative fecundity (RF)

$$Relative\ Fecundity = \frac{Total\ number\ of\ eggs}{Body\ weight\ (g)}$$ De Kimpe and Micha (1974)

Stripping percentage

This was calculated according to Brizuska (2003) as follows:

$$Stripping\ percentage = \frac{Weight\ of\ stripped\ eggs}{Body\ weight} \times 100$$

Percent fertilization

The percent fertilization was estimated from the number of unfertilized eggs by the equation:

$$Percent\ fertilization = \frac{N-n}{N} \times 100$$ Hogendoorn (1979).

Where, N = total number of eggs spawned
n = number of unfertilized eggs

The number of unfertilized eggs were determined when the eggs have developed to the middle gastrula stage (6–8h after fertilization) by random collection of 50 eggs sample with a sieve from each experimental unit and placed on a petri dish containing water. The samples were then observed under Kyowa electronic microscope (Model XSZ-21) at 40 magnifications. The number of opaque eggs were regarded as unfertilized while the translucent eggs containing embryonic eyes were regarded as fertilized. The eggs were then returned back to the corresponding unit for hatching.

Percent hatchability

Hatchability was determined from the direct data count of numbers of hatchlings at one day old as follows (Ayinla and Nwadukwe, 1990):

$$Percent\ hatchability = \frac{Number\ of\ hatchlings\ (one\ day\ old)}{Total\ number\ of\ fertilized\ eggs} \times 100$$

During the incubation period, a sample of 7g fertilized eggs were incubated in each experimental unit.

Data Analysis

The data obtained was subject to one way analysis of variance (ANOVA). To determine significant differences ($P<0.05$), the treatments means were separated using Duncan's multiple range tests. SPSS statistical package for windows software version 20. was used to run the data and statistical.

RESULTS

The result of the latency period is as shown in Table 1. The average latency period to complete ovulation is 9 hours for all the three treatments under the average mean temperature of 29.50±0.211, 30.70±0.84, 31.07±0.89 and 29.78±0.25 for morning, afternoon, evening and night respectively. There were no significant ($P>0.05$) differences between the treatments. However, the stripping percentage shown in Table 1, indicates that brood fish injected with pituitary from spent fish i.e. treatment 2 has the highest stripping percentage (17.4845±0.60) when compared with treatment 3 induced with pituitary from unspent fish (15.6949±0.49) and treatment 1 induce with Ovulin (6.5796±0.43). There was no significant($P>0.05$) difference among the treatments. The result of relative fecundity of eggs for both the three treatments showed that Treatment 2 with brood fish induce with pituitary from spent fish has a better relative fecundity (122.92±19) when compared to treatment 3 and treatment 1, (110.33±3.42 and 46.2545±1.03) respectively. There were no significant ($P>0.05$) differences between the treatments. The result on percentage fertilization rate indicates no significant difference among treatments ($P>0.05$). Percentage hatchability of eggs as shown in Table 1, revealed that treatment 1 had the highest percentage hatchability (22.79±2.65) when compared with treatments 2 (13.619±0.89) and 3, (19.739±0.25). There were no significant ($P>0.05$) differences between treatments in the hatchability of eggs.

Table1: Induce spawning performance of (*clarias gariepinus)* on Ovulin, pituitary from already spent and unspent female fish

Parameters	T1	T2	T3
Latency period (hours)	9 hours	9 hours	9 hours
Average weight of brood fish	$749.33±3.48^{b}$	$606.67±12.02^{c}$	$1200.00±11.55^{a}$
Stripping percentage	$6.5796±0.43^{c}$	$17.4845±0.60^{a}$	$15.6949±0.49^{b}$
Relative fecundity	$46.2545±1.03^{c}$	$122.92±4.19^{a}$	$110.3355±3.42^{b}$
Percentage fertilization	$56.00±14.19^{a}$	$60.67±1.76^{a}$	$62.67±4.67^{a}$
Percentage hatchability	$22.79±2.65^{a}$	$13.619±0.89^{b}$	$19.739±0.25^{b}$

T1 = Ovulin; T2 = Pituitary from spent fish; T3 = Pituitary from unspent fish

Table2: Mean water quality parameter during the experiment

Parameters	Morning	Afternoon	Evening	Night
Water temperature (°C)				
Mean	29.50±0.211	30.70±0.84	31.07±0.89	29.78±0.25
Minimum	21°c	29°c	30°c	29°c
Maximum	31°c	33°c	34°c	32°c
pH				
Mean	7.47±0.038	7.40±0.062	7.34±0.051	7.37±0.049
Minimum	7	6	7	7
Maximum	8	8	8	8
Electrical Conductivity (µm/cm)				
Mean	423.85±17.93	402.15±18.88	395.12±20.24	421.62±19.69
Minimum	223	195	193	195
Maximum	567	487	484	499

DISCUSSION

The average time taken to complete ovulation in this research work was 9 hours under mean temperature of 30.26°c. Maradun *et al*. (2018) reported the latency period of 9.67 hours under the mean temperature 30°c. Shinkafi, 2014, recorded 8 hours latency period for catfish *C. gariepinus* at water temperature of 30°c when Ovatide was used. These shows that the temperature influences induced spawning and eggs quality index. The time taken to complete any stage is related to water temperature and often decreases with an increase in temperature as reported by Legendre *et al*., (1986).

The stripping percentages of ovulated eggs were 6.5796±0.43 for treatment 1, 17.4845±0.60 and 15.6949±0.49 treatments 2 and 3 respectively of the broodstock body weight which compares with those of Abubakar *et al*., (2013) that recorded stripped percentage as 11.77% on cross of exotic Dutch Clarias, *H bidorsalis* and *H. longifilis* using Ovatide hormone.

From this research work, it was observed that fecundity value in treatment 1 (Ovulin) had 46.2545±1.032 for treatment 2 (spent) had 122.92±4.19 which is the highest percentage and for treatment 3 (unspent) had 110.33±3.42. this is in consonance with the findings of Adebayo and Popoola., (2008), who mentioned that the relative fecundity of *C. gariepinus* and *H. longifilis* is between 70 and 106 eggs/g body weight.

The fertilized eggs usually developed normally if the incubation conditions (Temperature, pH, oxygen, EC, cleanliness etc) are adequate (FAO, 2011). These factors were taken care of during the experimental period and resulted percentage fertilization up to 56% for treatment 1 (Ovulin) and 60% recorded for treatment 2 (spent) and 62% for (unspent). These are lower when compared to Legendre *et al*., (1986), who obtained higher percentage of fertilization in *Heterobranchus longifilis* when treated with HCG 76%. However, Khan *et al.*, (2014) reported a lower percentage fertilization of 56% on Giant River Catfish (*Sperata seengala*) with ovatide.

Percentage hatchability of eggs as shown in this research work revealed that treatment 1 (Ovulin) gave a better performance with 22.79% when compared to treatment 2 (spent) which had 3.6196% and treatment 3 (unspent) that has 1.9739. Mosses *et al*. (2005), reported for Kainji strain of Clarias 58.58% and *C. gariepinus* 52.44% when pituitary from female fish was used. Artificial spawning was induced in female African giant catfish *H. bidorsalis*

by single intramuscular hormone injection of carp pituitary suspension (CPS), homoplastic pituitary suspension (HPS) and human chorionic gonadotropin (HCG) in Nigeria with percentage normal larvae of 81 to 86% recorded (Adebayo and Fagbenro 2004).

CONCLUSION

Induced breeding of *C. gariepinus* using pituitary gland from spent and unspent female fish, induced final maturation and ovulation as well as high survival rate of larval of the study fish. In addition, the study revealed that high fertilization, hatching, stripping percentage and relative fecundity were achieved in all treatments. It is recommended that fish seed production can be encouraged through the use of natural hormone which is more readily available unlike synthetic analogue whose supply varies with changes in import duties.

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