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## Spawning response of African catfish (*Clarias gariepinus* (Burchell 1822), Claridae: Teleost) exposed to different piscine pituitary and synthetic hormone

**Gadisa Natea, Mulugeta Wakjira, Tokuma Negisho and Megerssa Endebu**

### Abstract

Spawning response of African catfish (*Clarias gariepinus*) exposed to different piscine pituitary extracts and synthetic hormone was evaluated. Nine African catfish gravid females were divided in to three treatments and injected with hormones intramuscularly. Data on spawning fecundity, fertility rate, and hatchability rate and induction hours were generated. The highest mean in spawning fecundity (9731.6eggs/g) was observed in groups injected with pituitary extract of African catfish followed by the group injected with pituitary extract of Common carp (*Cyprinus carpio* Linnaeus, 1758) (5,813.8eggs/g). However, the spawning fecundity/g body weight/female did not show significant difference ( $p = 0.073$ ) among the groups. The mean hatchability rate was high (73.3%) in groups injected with pituitary extract of African catfish followed by the group injected with pituitary extract of Common carp (63.5%). The use of pituitary extract from African catfish is more effective for induction as compared to Common carp pituitary and synthetic hormone.

**Keywords:** African catfish, artificial propagation, pituitary hormone, synthetic hormone

### 1. Introduction

Aquaculture in Ethiopia remains more potential than actual practice despite the fact that the country's environmental and socio-economic conditions support its development [1]. The consumption and demand of fish as a cheap source of protein is increasing in the country, but the fish supplies mostly come from the major lakes and rivers [5]. Consequently, capture fisheries from those natural environments presently causes over exploitation and seem to have reached their natural limits. As the report of [2] indicates the fisheries production of the major lakes of Ethiopia is declining in an alarming rate. As a result of high population growth in the country, there is high competition to be engaged in fisheries activities around the lakes area. Thus, as the experience of different countries in the world shows, if aquaculture developed in a sustainable way, it can be the best alternative to tackle this kind of problems [3].

Nile tilapia is benchmark of the country fish farm production hence, narrowing Ethiopian production diversity of aquaculture [1]. Though its farming is not well practiced, African catfish has great economic importance next to Nile tilapia in country [4]. Fortunately, African catfish farming has witnessed an increased production and gained a considerable importance recently in several African countries, turned it from just an undesirable species in tilapia ponds or a 'police-fish' to control over breeding in mixed-sex tilapia culture in earthen ponds to an important and potential species for aquaculture [5]. However, the seed availability which is hindered by the scarcity of natural spawning in captivity, photophobic behavior the species and shortage of high quality fingerlings are the bottleneck for successful culture of this species [4]. In addition, the dependence on natural resources for seed collection is seasonal reliant, restricted, unreliable, time-consuming and uneconomic [5,6]. As a result, spawning induction of captive fish becomes the best scenario to overcome such problems through injection of one of possible hormones.

The uses of synthetic and non-synthetic hormones have been reported in different regions with recommendations of different doses. Others have also reported the potency of pituitary extract of non-piscine extract from frog (*Hoplobatrachus occipitalis*) in induced breeding of African catfish. However, not much has been reported on comparative studies on the latency period,

fertility, spawning, and hatchability rate of synthetic and non-synthetic hormones. Therefore, the aim of the present study was to evaluate spawning fecundity, fertility rate. Latency period and hatchability rate of African catfish exposed to piscine pituitary and synthetic hormone.

## 2. Materials and Methods

### 2.1. Description of the experiment site

The experiment was conducted from March to May 2015 at Batu Fishery and Aquatic Life Research Centre (BFALRC) located at 160 km south of Addis Ababa in Batu Town, Oromia Regional State, Ethiopia. The research center is situated near the shore of Lake Zeway within the mid Ethiopian Rift Valley system (7.919 °N and 37.727 °E) at an elevation of 1638 meters above sea level [7].

### 2.2. Broodstock selection and management

Adult females and males of African catfish were collected from Lake Zeway and transported to BFALRC and kept in concrete ponds having size of 7x5x1m. Female brooders having a well distended abdomen and weigh more than 500 g were selected. For male broodstock selection color of genital papilla (reddish) and also weight greater than 200 g were considered [7, 8]. Prior to the experimental setup, the broodstocks were acclimatized in separate concrete pond for a month. The stock was fed twice daily at 7-9 am and 4-6 pm with pellet feed prepared from Noug cake and wheat bran (2:1) at the rate of 5% of their body weight. A seine net was used to gently capture the brood fish. After collection of the fish from the conditioned pond, fish were treated with formalin (25 ml/ L for 30 minutes) in bath to prevent the transfer of pathogens from fish to hatching system, eggs and fry [9].

### 2.3. Experimental design

The experiment was laid in completely randomized design (CRD) with three treatments each with three replications. The treatments were;

- T1 (CG) = Pituitary extracted from African catfish
- T2 (CC) = Pituitary extracted from Common carp
- T3 (SH) = Synthetic hormone(LHRH-A<sub>2</sub> + Domperidone)

### 2.4. Hormone injection

#### 2.4.1. Piscine pituitary treatment

The extracted pituitary glands were homogenized in a tissue grinder (mortar and pestle) with 2 ml of 0.9% salt solution. The solution was administered to matured female African catfish by intra-muscular injection into the dorsal muscle above the lateral line just below the posterior part of the dorsal fin with a syringe (5 ml volume) at a dose of 2 mg/kg body weight [10]. The injected area of each fish was massaged gently with a finger in order to distribute the hormone evenly into the muscle while slowly retracting the needle.

#### 2.4.2. Synthetic hormone treatment

The preparation of the Synthetic hormone for injection was based on the direction of a manufacturer. A vial of LHRH-A<sub>2</sub> containing 125 µg powders was dissolved in 12.5 ml of saline solution which gives a concentration of 10µg/ml. Then a vial of Domperidone containing 100 mg was mixed with a 10ml saline solution to make a concentration of 10 mg/ml. Finally, equal volume of each solution was taken and mixed together to inject the fish [10]. The prepared mixture of synthetic

hormones was injected in to the experimental fish at a dose of 1 ml/kg body weight of the fish.

### 2.5. Checking for ovulation

Checking of ovulation started 11 h after injection of each stimulator and continued at one-hour intervals [11]. Females were tested for ovulation by hand stripping of the abdomen [12]. It was gently squeezed toward the genital opening. Broodfish was considered ovulated upon yielding an ample amount of green brown eggs. Immediately before stripping of females rated as ovulated fish, the males were sacrificed and the testes were removed, macerated and squeezed in physiological saline solution (0.9% NaCl) at a proportion of one g testis per 5 ml salt solution. The solution was immediately coarsely filtered and held under lower temperature nearly 6 °C [13]. Sperm suspension was checked under the microscope for detecting sperm motility. Females were stripped in the pre-weighted plastic bowl. The sperm suspension of three males was then poured over the egg masses evenly and clean water was added. Before this, about one gram of eggs from each female was mixed gently with 0.5 ml of sperm suspension. Total eggs were fertilized using 2.5 ml of sperm suspension per 100 g eggs. Water was added to activate sperm and the mix was allowed to stand for two minutes, then eggs were rinsed in 0.9% NaCl solution. After two minutes of fertilization, eggs were washed with water and additional fresh water was added.

### 2.6. Incubation of fertilized eggs

Incubation of eggs was carried out as described by [14]. For each female brood fish, one gram of eggs was taken randomly and incubated in an aerated plastic bowl with 10 cm water depth for the purpose of incubation. Microscopic examination was done 24 hours post incubation to check the unfertilized or dead white eggs. A sample of 100 eggs was taken from the central part of the plastic bowl of each female fish [11], transferred to a petridish and examined under a binocular microscope at 3x magnification. Opaque unfertilized eggs were separated from the transparent living ones and the number of fertilized one was counted. Unfertilized and dead white eggs were counted and removed immediately to avoid fungal infection. After hatching the embryos penetrated through the mosquito nets and gathered in the corner of the bowl (only dead eggs, egg shells and deformed embryos remained on the surface of the stretched nylon mesh).

### 2.7. Spawning performance parameters

Spawning performance parameters were calculated as described by [15, 16].

- Fertility Rate (FR %) = (Number of fertilized eggs/Total number of eggs collected)\*100
- Hatchability Rate (HR %) = (Number of hatchlings/Total number of eggs fertilized)\*100
- Relative fecundity = Total number of eggs/ Body weight
- Latency period (hrs): The period from injection till the onset of ovulation (hrs).

### 2.8. Data analysis

The mean differences in spawning fecundity (SF), fertility rate (FR) and hatchability rate (HR) were tested using one way analysis of variance (ANOVA) at 5% significance level. Welch F statistic was considered in order to elude the effect of lack of homoscedasticity in the data. The statistical analyses were conducted in software packages of SPSS

(version 16.00) and PAST (Version 2.17).

### 3. Result and Discussion

#### 3.1. Spawning fecundity

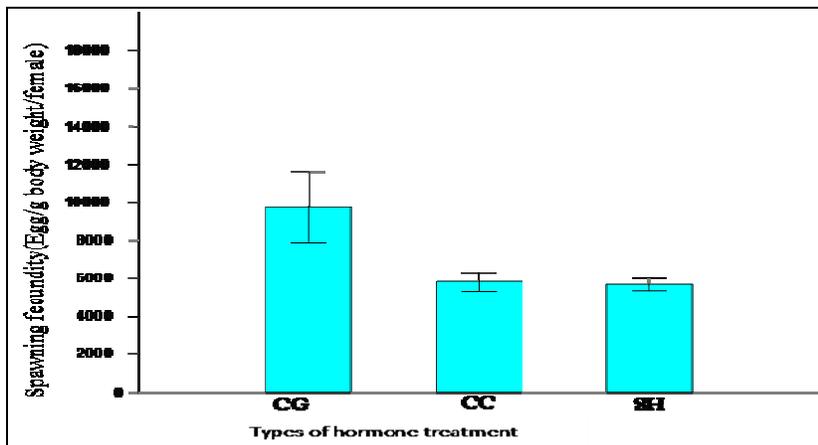
The best spawning fecundity result was recorded from the treatment group whose ovulation was stimulated with injection of African catfish pituitary extract with a mean of 9,731.6 eggs/g body weight/female. The mean values for spawning fecundity of females African catfish injected with pituitary extracted from Common carp and synthetic hormone were 5,813.8 and 5,666.6 eggs/g body weight/female respectively. Despite the lack of statistically significant variations in spawning fecundity of the three treatment groups (Welch F statistic = 0.27, p = 0.073), the effect of size measured as partial Eta squared ( $\eta^2$ ) was reasonably large (58.2%) (Table 1; Fig .1) [17]. The lack of statistical significance among the treatment groups could be due to the poor power of ANOVA test (50.3%), which in turn is due to the small sample size (n = 3) per each treatment group [17]. Therefore, based on the justification of the poor power of ANOVA test and the very large effect size, the spawning fecundity of the group treated with the African catfish pituitary can be considered as a better response than that of the other two treatment groups (common carp pituitary and synthetic hormone).

The absolute mean spawning fecundity results of the natural hormone treatments (African catfish and Common carp) in the

present study (63,653.66 and 49,150.66 eggs per female, respectively) were less than the findings of [7] (69,939.00 and 54,633.00 eggs per female, respectively). The lower values in the present study could be attributed to the hormone dosage differences where higher dosage (3 ml/kg body weight) was used in the past study versus lower dosage the present study (2 ml/kg body weight). Moreover, despite the higher values reported previously [7], the body weight of the brooder fish, which is the most important factor affecting the spawning fecundity, was not mentioned. For instance, [8] reported that brooder fish can spawn 5% of their body weight. Similarly, the values of spawning fecundity of the present experiment with synthetic hormone recipients were less than the values reported by [18]. The differences could be attributed due to the types of synthetic hormone and dose applied. The present experiment used the combination of LHRH-A<sub>2</sub> + Domperidone at dose of 1 ml/ kg, but this report used only LHRHa at dose of 2 ml/ kg.

**Table 1:** The mean and range of the standardized spawning fecundity (eggs/g body weight) among the three treatment groups (Mean ± SE)

Treatments	N	Mean	SE	Range
CG	3	9,731.6	1.860	7,098.31-1,3325.89
CC	3	5,813.8	5.028	4,896.99-6,630.17
SH	3	5,666.6	3.264	5,131.25-6,257.80



**Fig 1:** Spawning fecundity (mean ± SE) among the three treatment groups; CG = African catfish; CC = Common carp; SH = Synthetic hormone

#### 3.2. Fertility Rate

The mean value of fertility rate among the three groups was higher in CG than CC and less in SH recipient female African catfish, respectively (Table 2; Fig. 2). The One-way ANOVA result for fertility rate among the three hormone treatment groups were statistically not significant (p = 0.069). However, the effect of size measured as partial  $\eta^2$  was 59% and the power of ANOVA test was 51.7%. The fertility rate recorded in the present experiment was high with a mean of 84.3% in African catfish females injected with pituitary extracted from African catfish and followed by Common carp pituitary recipients with a mean fertility rate of 80.6%. The lower mean fertility rate 74.9% was recorded in African catfish female's injected with synthetic hormone. The One way ANOVA for fertility rate among the three hormone treatment groups was statistically not significant (p = 0.069). Leven's statistic was applied to check test of homogeneity of variance among the groups and shows greater than 5% level of significance (p =

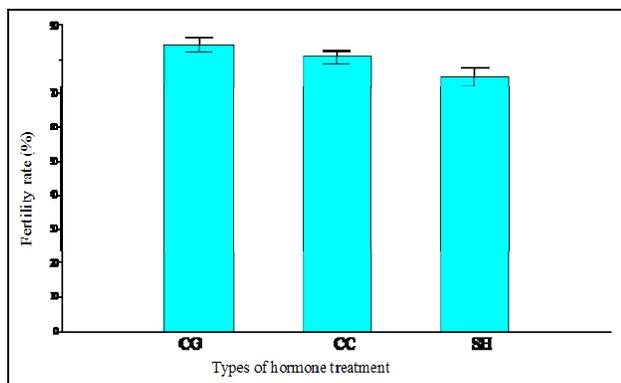
0.063). The effect size (partial  $\eta^2$ ) shows 59%, which was considerably large [17]. Additionally, the power of test was also poor i.e. 51.7% [17]. The lack of statistical significance among the treatment groups could be due to the poor power of ANOVA test. This means that the fertility rate among the groups was practically large, but it could not be detected by ANOVA, which could be because of the small sample size (n = 3) per each treatment group. Therefore, based on the justification for the poor power of ANOVA test and the very large effect size, the fertility rate of the group treated with the African catfish pituitary can be considered as a better reaction than that of the other two treatment groups (CC pituitary and SH).

The fertility rate of fishes that were injected with African catfish and CC (84.3% and 80.6%, respectively), in the present study, were fairly higher than the report of [7] (76.9% and 80.5% fertility rate, respectively). The difference in fertility rate between this report and the present study could

be due to differences in the condition at which eggs incubated and hatchery facilities. Similarly, the fertility rate of the present experiment was higher than the work reported by [19] in Nigeria. This report showed pituitary extracted from African catfish and synthetic hormone (Ovulin) was applied for the inducing of female African catfish. From both treatments fertility rate yielded was 60.7% and 67%, respectively. On the other hand, the fertility rate of the present experiment with the synthetic hormone recipients of the female African catfish was similar with the reports of [18, 20] which showed 75% fertility rate. These reports used synthetic hormone (LHRHa) and (Ovaprim) at a dose of 2 ml/kg and 0.5 ml/kg body weight of the female African catfish respectively. In contrast, the present experiment showed poor fertility rate with the synthetic hormone treatment, which appears to be reasonable when compared to other reports [5, 21, 22], which reported FR as 83.17%, 88.4% and 88.7%, respectively. The appeared variation between the present experiment and those reports on the fertility rate result could be due to the synthetic hormone type. This shows that GnRha + Dompridone for the former one and Ovaprim for the last two respectively. Additionally incubation of eggs, hatchery facilities and fertilization time and situation might also play the role.

**Table 2:** Comparison of the fertility rate (FR %) among the three treatment groups (Mean ± SE); CG = African catfish; CC = Common carp; SH = Synthetic hormone

Treatments	N	Mean	SE	Range
CG	3	84.3	2.112	80.50-87.80
CC	3	80.6	1.778	78.30-84.10
SH	3	74.9	2.818	70.80-80.30



**Fig 2:** Fertility rate (Mean ± SE) for the three treatment groups; CG = African catfish; CC = Common carp; SH = Synthetic hormone

### 3.3. Hatchability rate

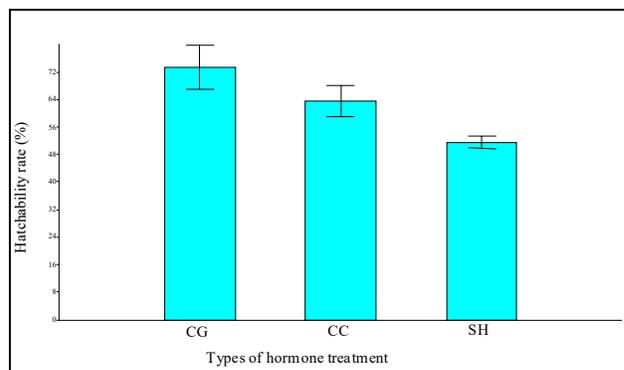
The highest mean hatchability rate was recorded in recipients of pituitary extract from African catfish followed by CC pituitary recipient and less in synthetic hormone recipient female African catfish (Table 3; Fig. 3). In the present experiment, hatchability rate among the three hormone treatment groups was significant ( $p = 0.04$ ). The mean hatchability rate recorded with the natural pituitary extract i.e. pituitary from African catfish and CC was 73.3% and 63.5% respectively. This record was better when compared to similar report that used the same source of pituitary for inducing female African catfish. For instance, the report of [7] showed that, the mean hatchability rate was 45.3% and 42.9% from

females African catfish which were injected by African catfish and Common carp pituitary extract respectively. The enhanced result recorded by the present study could be due to the hatchability facilities. Even if, the other parameters does not reported by this study the temperature at which eggs incubated was 23 °C, which is less than the optimum water parameters sited for the eggs and larvae incubation. But, in the present study eggs were incubated at temperature of 27 °C, which is reported as the optimum temperature for egg and larvae incubation by [23]. Similarly, the hatchability rate recorded by the present experiment specifically when pituitary was used from African catfish (73.3%) was higher as compared to the result (60.7%) of [19], using the same source of pituitary extract.

Hatchability rate with the SH treatment in the present study, though lower when compared to other studies [16, 20, 21, 22] that reported 89.1%, 71.7%, 57.7%, and 65%, is noteworthy as it demonstrated more than 50% success. These studies used female African catfish for treatment application purpose and the synthetic hormones used were Ovaprim for the first three studies and combination of GnRha and Dompridone for the last study, respectively. Thus, the differences in the hatchability rate between the present experiment and these reports could be ascribed to variation in the kind and dose of synthetic hormone used.

**Table 3:** Comparison of the hatchability rate (HR %) among the three treatments groups (Mean ± SE); CG = African catfish; CC = Common carp; SH = Synthetic hormone

Treatments	N	Mean	SE	Range
CG	3	73.3	6.24	60.90-80.80
CC	3	63.5	4.38	58.20-72.20
SH	3	51.5	1.79	48.60-54.80



**Fig 3:** Hatchability rate (%) (Mean ± SE) for the three treatment groups; CG = African catfish; CC = Common carp; SH = Synthetic hormone

### 3.4. Latency period

Long-lasting latency period has indirect implication on the quantity and quality of eggs produced as well as the quality of the larvae. This indicates that, the shorter the latency period, the better the reproductive output. In this study, latency period ranged from 11.5 to 12.5h (Fig. 4) for the ovulated three experimental groups, narrow hour difference among the groups. The longer latency period was recorded in CC (12.5 h) treatment group. In contrary, the shortest latency period was in SH treatment group without significant difference among them, while all incubated at temperature of 27 °C. Though, the induction time result of the present study was similar with that of [24] who reported that combination of

GnRha with Domperidone shows latency time of 13 hrs. There was a difference of 1.5 hrs between this report and the present experiment with the synthetic hormone applied on African catfish females. Additionally, from the same work [24] reported that, the latency time of 12 hours when CC pituitary extract was applied on CG females for induction. This was analogous with the present experiment when African catfish females were injected with the CC pituitary extract. In the same way, [16] reported that the latency time was 11.5 hrs in female African catfish injected with Domperidone +GnRha. Similarly, the report of [22] showed that female African catfish induction time was 11.5 hrs when injected with Ovaprim. Even though, the present experiment used both the natural and synthetic hormone for inducing reproduction of African catfish females the induction time was not shows this much variation. Although, other reports used different sources for the inducing purpose, but the induction time shows more or less similar with the present experiment.

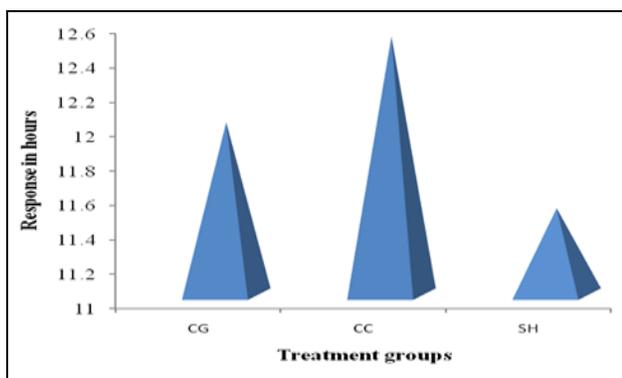


Fig 4: Induction responses (hours) for the three treatment groups; CG = African catfish; CC = Common carp; SH = Synthetic hormone

#### 4. Conclusion and Recommendation

The present study showed the possibility of artificial propagation of African catfish using various hormone treatments, which gives hope for the continued supply of fingerlings in the aquaculture of African catfish. The use of piscine pituitary hormone treatment from fish offal, particularly an extract from African catfish, is economically and environmentally preferable in the artificial propagation of African catfish. This is justified by the findings of the present study that better spawning performances in SF, FR and HR were obtained from a fish group treated with natural pituitary extracts, especially from African catfish. Furthermore, the use of pituitary extract from fish offal is more feasible as it is locally available at relatively cheaper price than synthetic hormones which are only hardily available and more expensive. Further study on feed preference and survival rate of larvae is recommended to augment the present study and thus for better insight about artificial propagation of African catfish.

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