Comparative Study of Inducing Broodstock with Natural and Artificial Hormones on Reproductive Performances of *Clarias gariepinus*

Abstract

This study was conducted to investigate the effects of artificial and natural hormones (ovaprim and pituitary extract) on the reproductive performances of *Clarias gariepinus*(African catfish). A total of twenty brood stock, 12 males and 8 females with mean weight of 2.0 kg each were selected for breeding following the external morphological characteristics and standard breeding procedure. The water parameters for indoor culture tanks during the study were optimal for breeding:pH 7.31; temperature, 27.6˚C. Dissolved oxygen, 5.18 mg L^{-1}. The result revealed no significant($P > 0.05$) differences in water quality parameters between the culture tanks for artificial and natural hormonal applications during the study. The percentage fertilization, hatchability, survival and fry production success were: 89.23%, 93.31%, 87.16% and 69.41%; and 79.02%, 72.56%, 67.32% and 34.67% respectively, for pituitary extract and Ovaprim®. Pituitary extract recorded higher values and significantly performed better ($P < 0.05$) between the two treatments. This indicates that natural hormone(pituitary extract) is equally effective and therefore recommended for the artificial propagation of *Clarias gariepinus***.**

Key Words: Catfish breeding, Fertilization, Fry survival, Hatchability, Induced ovulations and spawning.

1.0 Introduction

Aquaculture played and continue\ to playvital roles in the provision of the world protein, for human sustainability. Importance of fish in human diet and economic development of any society have been established. African cat fish such as Heterobranchus and Clarias species remained the most culturable species of significant in Nigeria and beyond (Otoh and Udoh, 2018;Oyeleye*et. al* (2016). This is due to the unique characteristic of the species such as fast growth rate, good taste, generally accepted forconsumption, high stocking density, high market price and high resistance to disease and ability to reproduce in captivity (Nlewadim*et. al*., 2011; Nya et. al., 2017;Otoh*et. al*., 2020; Otoh, *et. al*., 2022;Otoh, et. *al*., 2023 a, b). Although the growth of fish depends on availability of good feed of which a single feed stuff component cannot achieve (Ekanem *et. al.*, 2000).Heterobranchus and clarias species readily accept any supplementary feed and their growth rate is unique within a short period of culture (Otoh and Udoh,2019; Udoh and Otoh, 2017).

The process of spawning in natural habitat becomes very difficult inartificial environment. Collection of fingerlings from the wild, attracts disease infections, limited supply of seeds and high level of cannibalism (Olumuji and Mustapha, 2012). This attracts human interventions through hormonal inducement to facilitate egg maturity for breeding. Hormones are known to be chemical messengers released by the endocrine glands of the body directly into the bloodstream to carry out expected function at the target cells (Otoh and Udoh, 2018). Administration of hormones to induce ovulations and spawning in fish is achieved through artificial propagations with either natural or synthetic hormones (Nwokoye*et al.*, 2007; Ndimele and Owodeinde, 2012; Ngueku, 2015; Natea *et. al.*, 2017).

African catfish pituitary hormones are available at all time and is cheaper than any other hormones (Adebayo and Popoola, 2008). Two types of hormones used for this propagation are artificial hormones (Ovaprim®) and natural hormones which are collected from the pituitary gland located at brain region of the catfish. The pituitaries are whitish globule-like organs located at the ventral side of the brain of the fish skull and extracted from under the palate after sacrificing and decapitating the donor fish (De Graaf *et al*., 1995). Freshly collected pituitaries are preserved and dried in acetone (1ml acetone per pituitary) until used.

They are applied by macerating in a mortar containing 2 ml of 0.9% physiological salt solution (9 g NaCl L^{-1} of water) and the pituitary suspension drawn into a syringe for injection into the fish. Hormones are used to stimulate internal condition of fish to induce ovulation, spawning and gonad development; particularly, during dormant phase of the gonad development when the level of gonadotropin in the fish blood is low. Hormonal induction increases the hormonal level in the fish, thereby enhancing ovulation and spawning (De Graaf *et al*., 1995).

The knowledge and method of processing natural hormones for inducement is not common among fish breeders. Scarcity and high cost of artificial hormones; seasonal occurrence, time-consuming, and unreliable seed collection from the wild, impose problem of insufficient supply of fingerlings and fish seed (Dadebo,*et al.*, 2014). More so, government restriction on importation of goods and services could ultimately impinge mass production of African catfish. An attempt to overcome above situations necessitated investigations into the comparative studies on effect of natural and

artificial hormones on inducing broodstock and reproductive performances of *Clarias gariepinus.* This will afford enlightenment and promotion of the use of natural hormones as cheaper alternative.

2.0 Material and Methods

2.1 Location of Study

The research was conducted at the Fish Farm Complex (Fig. 1b) of Akwa Ibom State University, Obio Akpa campus (Fig. 1a), located between latitude 5°17′N and 7°27′N, Longitude 7°27′E and 7°58′E. The area has an annual rainfall ranging from 3500– 5000 mm and average monthly temperature of 25°C in the tropical rainforest zone of southeast Nigeria (Otoh and Udoh, 2018).

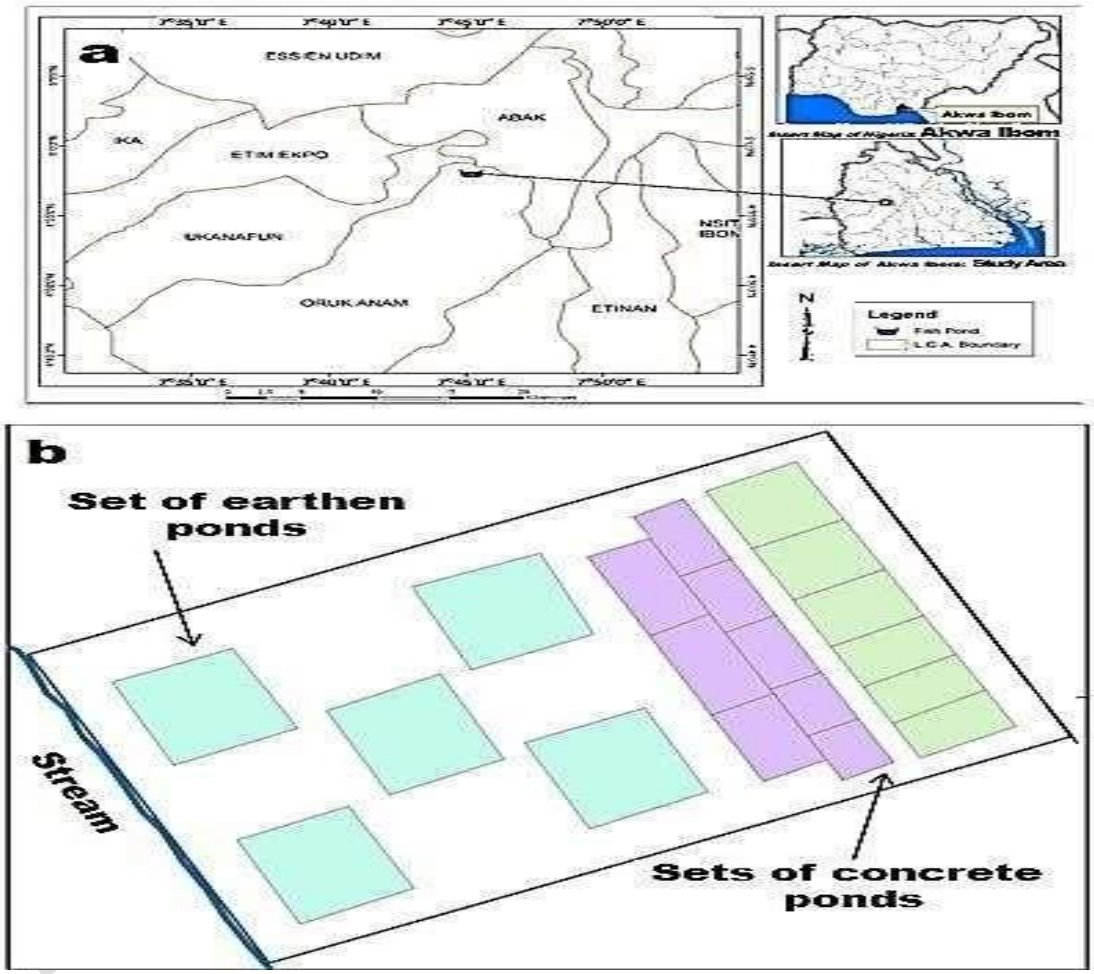

Fig. 1: A cross section of map of Nigeria showing Akwa Ibom with an inset showing the location (a) and layout (b) of the University Fish Farm Complex

2.2 Brood Stock Acquisition and Care

Twenty (20) farm-raised broodstock: 12 males and 8 females, averagely (2 kg each) were sourced from a private fish farm in line with pre-determined selections conditions according to Otoh, *et. al*., (2020). Eight indoor concrete breeding tanks of equal dimension, 1 x 1 x 1 m^3, were utilized for this study. Water level and temperature were maintained at 5$cm^{3 \text{ and}}$ 26 ˚C, respectively, in the hatchery all through the study.

2.3 Hormone Preparation

a. **Artificial Hormone.** Four (4) female brood fish (spawners) were selected (64.1 - 64.2 cm and 2.00 – 2.10 kg), and initiated to spawn with single dose of ovaprim® (0.5 ml kg^{-1} female body weight; allowed 16-hour latency at 26-27°C water temperature; before manual stripping to obtain eggs) according to Otoh and Udoh, (2018).

b. **Natural Hormone**: Four (4) male brood fish (spawners) were selected (64.0 - 64.1 cm and 2.00 – 2.10 kg), and decapitated for pituitary extraction (Asangusung *et al*., 2020). The pituitaries suspensions obtained after macerating in saline solution were administered as one fish pituitary per female broodstock of equal weight (64.2- 64.4 cm and 2.00 – 2.10 kg). The four females induced broodstock were also allowed a 16-hour latency period at the same water temperature before stripping.

2.4 Gamete Collection, Fertilization and Incubation

Milt from eight (8) separate male broodstock were collected and pooled together into one volume after sacrificing the males and extracting the testes. The pooled milt was diluted with 16ml saline solution and then divided into eight equal portions (approximately 2 ml each) and preserved at below 7 ˚C temperature in a refrigerator, until utilized. At the end of the latency period, each of the four female brood stocks, induced with ovaprim® and pituitary extracts, were separately stripped and pooled into two labeled plastic containers, respectively. Thereafter, the pooled gametes were split into four sets each (4 replicates of equal volume of oocytes, each approximately 2000 oocytes).

The four replicates (2g of eggs containing 2000 oocytes), each from ovaprim® and pituitary extracts treatments, were inseminated and fertilized with pooled milt (2 ml of pooled semen per replicate). Each fertilization was done in a bowl and activated with about 100 ml of 0.9% saline solution, then after 5 minutes the saline solution was decanted and the fertilized eggs uniformly spread in a monolayer on a kakaban (shredded nylon sack) and incubated in aerated indoor concrete breeding tanks, 1 x 1 x 1 m^3 at water temperature of 26°C. During incubation, water levels were maintained at 10 cm depth. Six hours after fertilization, the colour variation between the eggs was observed. Clear and transparent eggs were considered fertilized while dead/white and opaque ones were regarded as unfertilized (Udoh, 2000). Based on the counts, the reproductive performances of the hormone sources were assessed as percent fertilization, hatchability, survival and fry production success rates:

Fertilization rate (%) of eggs = (No. of eggs fertilized /total number of eggs) x 100.

Hatchability (%) = (number of hatchlings / total number of fertilized eggs) x 100. Percentage hatchability was obtained by direct counting of unhatched eggs as well as the numbers of eggs hatched in each incubating tank. Hatching rate = (No of healthy fertilized eggs / No of fertilized eggs used) x 100 (Udoh, 2000).

Survival rate (Ks) was calculated during initial feeding according to the following formula:

Survival rate = (number of live larvae / total number of larvae hatched) x 100 (Udoh, 2000). Efficiency of hatching was evaluated by the method of Rana (1995);

Fs (%) = Kf.Kh.Ks/10,000

where:

Fs = Success rate (%) of fry production at 10-day post hatching.

Kf = Fertilization rate (%) of eggs

Kh = Hatching rate (%) of fry

Ks= survival rate (%) of 10-day-old swim-up fry.

2.5 Statistical Analysis

The trials were replicated four times for each treatment (ovaprim® and pituitary extract) and all values subjected to two-sample T-test to determine significant differences at 95 % level. Replicates were treated as random effect. T-test was also deployed to determine significant differences in water quality owing to hormonal applications.

3.0 Results

3.1 Physico-Chemical Parameters of Culture Media

The reproductive performances in this study were considered under similar optimal environments for both artificial and natural hormonal applications. The culture system was within the range of 26 - 27 °C, 7.3 - 7.4, and 5.1 - 5.3 mg L^{-1}, for temperature, pH and dissolved oxygen, respectively (Table 1).

TABLE 1: Diurnal reading of water parameters for indoor culture tanks for both artificial and natural hormonal applications during the study

Timing/Mean	Dissolved Oxygen (mg/l)	Temperature ^{o}C	pH
Morning	5.10 -5.30	27.50-27.60	7.30-7.40
Evening	5.10 -5.30	27.50-27.60	7.10-7.40
Mean ± SE	5.18±0.01	27.55±0.01	7.31±0.02

3.2 Reproductive performance of *Clarias gariepinus* broodstock reared in Concrete Aquaculture Ponds and induced with Artificial and Natural Hormones

Table 2 summarizes the significant differences ($P < 0.05$) in all the reproductive parameters considered in the study. Results indicate use of artificial and natural hormones successfully induced spawning in *Clarias gariepinus*broodstock with varying reproduction performances. The percentage fertilization, hatchability, survival and fry production success of brood stock induced with natural hormones: 84.25%, 92.32%, 88.14% and 68.42%, respectively, were significantly ($P< 0.05$) higher than 75.01% fertilization, 69.57% hatchability, 64.33% survival and 33.66% fry production success observed in brood stock induced with artificial hormones. The induction of *C. gariepinus* broodstock with natural hormones elicited higher reproductive performances (Table 2) compared to treatment with artificial hormones.

TABLE 2: Reproductive performance of *Clarias gariepinus* broodstock reared in Concrete Aquaculture Ponds and induced with Artificial and Natural Hormones

Reproductive Performance	Mean (±SE)		Min – Max		T-test	P-value	inference
	Artificial	Natural	Artificial	Natural			
% Fertilization	79.02±2.52^{a}	89.23±0.52^{b}	74.00 – 86.00	88.0 – 90.46	3.5586	0.117*	Significant
% Hatchability	72.56±4.05^{a}	93.31±2.29^{b}	63.40 – 81.60	88.30 – 98.10	4.8971	0.0028*	Significant
% Survival	67.32±2.40^{a}	87.16±3.38^{b}	62.30 – 72.33	78.12 – 96.10	5.7471	0.0014*	Significant
% Fry Production Success	34.67±2.84^{a}	69.41±1.91^{b}	26.57 – 42.97	64.31 – 74.60	10.0130	0.0001*	Significant

^{a,b}treatments with different superscript in a row are significantly different, $P < 0.05$. *Significant difference ($P < 0.05$)

4.0 Discussion

Human intervention for sufficient supply of fish seed in aquaculture sector through hormonal spawning is a welcome development. It has been observed that any direction that have no alternative, stands a chance of stagnation and diminishing.The results on the comparative effects of inducing broodstocks with artificial and natural hormones on the reproduction performances of *Clarias gariepinus*showed significant differences ($P < 0.05$) in all the reproductive parameters considered in this study. This is in agreement with the observation of Natea *et. al.*,(2017). The observed differences in broodstock performance could be attributed to the comfort of the body cells in the entire systems for proper development of eggs since the broodstocks were induced with their body component to stimulate ovulation. The success of fry production depends on the health condition of the eggs (viability) which promote fertilization, hatchability and survival of larvae. It could also be attributed to management system at the hatcheries levels such as space, feeding level and water quality (Otoh *et al*., 2020, George and Atakpa, 2015, Jonah, *et. al*., 2020).

Toxic substances in artificial hormones could have influenced the reproductive performances of the species as observed in the study. This result is in agreement with Mohammad and Mojgan, (2017) who observed that the high mortality observed in larvae stage is due to the presence of toxic substances in Ovaprim. Ajah (2007) stated that larvae survival depends not only on diets and water parameters but also on the type of hormones which means that low survival of larvae in this study could be a function of hormone type. Tioguéet *al*. (2018) reported that the superiority of the Ovaprim at the beginning of the reproduction is offset by the poor survival rate of the larvae which is better with the pituitary gland. This result also revealed that apart from forceful maturity of eggs for spawning, natural hormones (pituitary) also performed other functions in the reproductive system of *Clarias gariepinus*different from that of artificial hormones which lead to differences observed in all the reproductive parameters considered in this study. However, Asangusung *et al.* (2020) highly recommend ovaprim to hatchery managers for optimum economic performance in the artificial propagation of *Clarias gariepinus.*

Calmness of broodstock induced with natural hormones were observed throughout the latency period while the broodstock induced with artificial hormone were aggressive and restless which could strain both the broodstock and the eggs. Although natural hormone performed better in this study, the use of artificial hormone is also as effective in the artificial propagation of catfish.

5.0 Conclusion

Based on the result of this study, both artificial and natural hormones induced propagation of *Clarias gariepinus* effectively but the natural hormones show better reproductive performances than the artificial.Considering the biological compatibility behaviors of the broodstock in captivity during spawning, availability and cost effective, the natural hormone (pituitary extract is recommended for use at farm levels).

6.0 References

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