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COMPARATIVE STUDY OF PISCINE AND NON-PISCINE PITUITARY EXTRACT AND OVULIN FOR INDUCING SPAWNING IN CATFISH *(Clarias gariepinus)*

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ABSTRACT

The study examined the effect of synthetic hormone (Ovulin), piscine hormone (*Clarias gariepinus*), pituitary extract and non-piscine; frog *(Haplobutrachus occipitalis)* pituitary extracts. The study was carried out in the Department of Marine Sciences, University of Lagos, Lagos state Nigeria. The broodstocks (*Clarias gariepinus*) were purchased from a private fish farm at Bariga in Lagos state and were transported in plastic can containing water. The temperature of the water was reducing by addition of ice block which helped to minimize stress on the fish. Hatchery raised 12 months gravid broodstocks were selected. All broodstocks were selected by external morphological characteristics. Female fish were selected on the basis of ovarian biopsy. The study lasted 65 days. Twelve gravid female and twelve mature male of *C. gariepinus* with body weight range of 500g to 1100g were used. The spawn weight was determined by estimating the mean weight of eggs used to achieve percent (%) fertilization. The ovulation rate was estimated from the weight of eggs released as a percentage of the total weight of the ovary. The percent fertilization was estimated from the surviving embryos 10 hours after fertilization. The percent (%) hatching was the number of hatched fry relative to the fertilized eggs, while the percent (%) survival was the number of surviving fry after 14 days of feeding with mixed artemia and artificial diets. The result showed that ovulin performed significantly better ($p< 0.05$) in all the parameters investigated. The randomized analysis of Variance (ANOVA) for the parameters investigated in the three hormonal materials shows percentage fertilization of 67.00±3.61%, 60.70±4.49% and 56.25±5.44%, percentage hatchability of 90.61±6.43%, 60.70±4.50%, and 56.26±5.46% and 69.33±5.13%, 61.67±10.41% and47.67±2.52% of survival rate were recorded for ovulin, fish pituitary and frog pituitary, respectively. Comparative cost benefit analysis shows that ovulin, which recorded better results was higher in terms of cost effectiveness compare to fish pituitary and especially that of frog which is both cheaper and available in our environment. Although for ease of handling and better result in terms of hatchability, fertilization and survival rate ovulin is highly recommended to hatchery users.

Key words: Piscine, Non piscine, Ovulin, *Clarias gariepinus*

INTRODUCTION

The demand for fish and fishery products is steadily increasing while natural fish populations have declined during the past decades due to environmental degradation and over-fishing [1]. This has resulted in increased efforts in the development of techniques for hatchery production of fish using unconventional sources of hormones for inducing spawning to ameliorate the impacts of soaring prices of alternative meat protein sources and dwindling catches from the wild. The expected high revenues coupled with the advantages of investment diversification have attracted many fish farmers to the industry [2]. According to Huisman and Richter [3], the average growth rate for aquaculture has been 8.9% per year since 1970 compared to just 1.2% for capture fisheries and 2.8% for terrestrially farmed meat production over the same period. In Nigeria, fish alone contributes between 20% and 25% per caput animal intake and could be as high as 80% in coastal and riverine communities [4]. Based on the current world per caput fish consumption of about 13.0 kg per year, approximately 97 million metric tonnes would be required by Nigerians by the year 2020 [2]. A 1992 United Nations DevelopmentProject (UNDP) assisted baseline study showed that the total annual fingerlings requirements for Nigeriawas 250,000 million compared to the domestic production of 7.2 million [5].

Thus, amongst the most significant advancements in the field of aquaculture to surmount this challenge, during recent decades, is the development of techniques to induce reproduction in fish. These techniques have allowed farmers to profitably breed and raise species that do not naturally reproduce in captivity, and to manipulate the timing of reproduction to suit production cycles. In the past, fish farmers collected their fingerlings mostly from the wild. However, this method is labour intensive and unpredictable and also inadvertently introduces unwanted species of fish into the ponds. Fish in captivity may not always reproduce at the most advantageous time, and alteration of the spawning cycle may be desirable. This allows a farmer to obtain fish outside of the normal spawning season either to lengthen time for grow-out or to produce hybrids with other species. It also improves efficiency by getting fish to spawn on a predetermined date, to maximize survival by fertilizing and incubating eggs under hatchery conditions where successful techniques for altering the spawning cycle of fish have become valuable too. Amongst the culturable food fishes in Nigeria, catfish is the most popular commercial species with fish farmers and consumers. This is because it commands a good commercial value in Nigerian markets [6, 7, 8]. It belongs to the family *Clariidae*, and is found in most African countries including Nigeria [2, 9]. It is also distributed throughout the world although. The family, *Clariidae*, is divided into two genera namely: *Clarias* and *Heterobranchus*. At various geographical locations, it bears different names, for example *C. lazera* in North and Central Africa, *C. senegalensis* in East Africa, *C.mossambicus* in West Africa, and *C. gariepinus* in South Africa.

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 *(Haplobutrachus occipitalis)* in induced breeding of *C. gariepinus*. However, not much has been

reported on comparative studies on the ovulation rate, fertilization, hatchability of the egg, availability and cost implications of non-piscine hormones. Such information, however, is needed for the farmer to make a choice regarding which hormonal materials to use on the farm to maximal profit. The results from this research shall guide farmers to make informed choices regarding hormonal material to use for better results in artificial reproduction by induced breeding. Therefore, the purpose of the study was to evaluate the level of ovulation inducement of the hormonal materials (piscine, non-piscine extract), and ovulin and their effects on spawning efficacy, percentage hatchability of the fertilized eggs, and cost-benefits analysis of the different hormonal materials.

MATERIALS AND METHODS

This study was carried out at the Department of Marine Sciences, University of Lagos. The broodstocks *(Clarias gariepinus)* were purchased from a private fish farm at Bariga in Lagos State, and were transported in plastic cans containing water. The temperature of the water was reduced by addition of ice block as this helps to minimize stress on the fishes.

Experimental Design

Hatchery raised 12 months gravid broodstocks were selected. All broodstocks were selected by external morphological characteristics, according to earlier procedures described by Ayinla *et al*. [8]. Female fishes were selected on the basis of ovarian biopsy of Legendre [10]. Twelve female and 12 male catfishes with weight ranges of 800 g to 1 kg were selected. The broodstocks were kept singly in aerated 50 litres aquarium with 25 litres of water for 12 hrs in glass tanks measuring 118 cm x 83 cm x 58 cm) in dimension at the Aquaculture Unit of Department of Marine Sciences, University of Lagos,. The 12 female catfishes were divided into four groups with one as control marked as T_1 (control with saline solution), T_2 (Ovulin), T_3 (fish pituitary extract of *C. gariepinus*), and T_4 (Fresh pituitary extract of Frog – *Haplobutrachus occipitalis*) and placed in tanks.

Preparation of Pituitary Glands

Pituitary glands were removed from the mature fishes and toads, respectively. Each fish or toad was weighed to the nearest gram and immediately killed. Careful removal of the roof of the buccal cavity exposed the round whitish mass, pituitary gland, located ventrally at the base of the cerebrum. This was then removed with fine forceps, weighed and stored in saline solution of 0.9% prior to use. The glands were later ground with one fish pituitary gland used per kg of gravid female fish while, six to eight glands of common toad were ground, using glass mortar and pestle, and homogenised in 2 mL saline solution of 0.9%. Each hormonal concentrate (the supernatant) was then collected in 2 mL syringe for use.

Ovulin composition

Ovulin is a compound S-GnRHa liquid injection with trade name “Ovulin” (Ningbo Sansheng Pharmaceutical Company Ltd. China. Specification: 10 mL of Ovulin contains Dom 100 mg; S-GnRHa 0.2 mg. No side effect has been recorded in the use

of the hormone. It is stored in a cool and dry place with temperature below 25^{o}C and has a shelf life of two years. The compound is used to induce breeding in both freshwater and marine brood fish.

Hormone Injection

The gravid fishes were injected during the cool hours of the night around 9.30 G.M.T Treatment 1 (The control fish) was injected 2 mL of 0.6% saline solution. Treatment 2 (Ovulin) which is in liquid form was administered at 0.5 mL/kg body weight of female fish [9, 10]. Treatments 3 were injected with freshly prepared pituitary gland of fish (pituitary was removed from 1 kg of fish and was injected to 1 kg of female broodstock) in 2 mL saline solution and Treatments 4 were injected with freshly prepared pituitary gland of 4-6 mg toad (pituitary was removed from toad and was injected into 1 kg of female brood stock) in 2 mL saline solution, respectively. It involves the pulverizing of the fresh pituitary gland of fish (*C. gariepinus*) and that of common toad in 2 mL of normal saline solution. The pituitary suspension was drawn with 2 mL hypodermic syringe with 0.6 mm gauge needle. The weighed fish was then covered with towel and injected intramuscularly above the lateral line towards the dorsal section and pointed towards the ventral side. After withdrawal of the needle, the fish was finger-rubbed to avoid back flow of the injected fluid. The injected fishes were then returned, separately, into their respective tanks.

Spawning Procedure of the Injected Fish

The fish were checked for ovulation by gently pressing the abdomen using the method adopted by Haniffa and Sridhar [11]. The males were randomly selected and lacerated through the abdominal region to remove the pair of sperm sacs (per male). Sperm/milt collected from each of the selected males was used to fertilize the already stripped eggs by washing the raw sperm from the sperm sacs on the egg mass with 0.9% saline solution.

Preparation of Milt

One male fish per female fish was killed, dissected, and the milt sac removed prior to artificial spawning. The sac was cut open with a sharp scissor and the milt washed into a vial with 0.9% saline solution. For this study, vials with milt were prepared to cater for fish spawned with triplicates of 3 treatments for each hormone.

Stripping, fertilization and Incubation

Stripping took place 10 h after injection at a mean temperature of 28^{o}C. This was carried out by holding the fish at the head and tail by an assistant. The ovulated eggs oozed out on slight pressure by thumb onto the plastic bowl. Incisions were then made on the sperm sac which was collected minutes prior to stripping by sacrificing the mature male. Milt was squeezed over the eggs. The two sex products were then mixed with plastic spoon. To this, 0.6% Saline solution was added and further agitated. During stripping, the oocytes (eggs) were extruded at the slightest pressure, and they appeared brownish in colour. When a few oocytes were placed in a Petri dish containing little water and examined under light microscope, the cytoplasm appeared shifted to the periphery. In all hormonal treatments, dead eggs appeared whitish and opaque within 8 to 10 hours of fertilization.

Spermatozoa from one mature male were used to fertilize eggs stripped from two females. After ovulation, the females were stripped and the eggs weighed and fertilized. The process from stripping to fertilization took three minutes to accomplish. Incubation of the fertilized eggs was carried out in (118 x 83 x 58 cm^3) glass tank. It was equipped with RESUN LP- 100 low noise air-pump (aerator) facilities. A net was suspended above the water for spreading of the fertilized eggs. The eggs were spread in single layers on the suspended net. Water parameters were monitored. Temperature was measured with centigrade thermometer, pH was monitored using Hanna pH meter and optimum oxygen level was maintained with RESUN LP- 100 low noise air-pump. A sample of 200 g eggs was taken from each of the treatments at random and incubated in aerated aquaria (118 x 83 x 58 cm^3). Hatching started in 24 hrs and at 26 hrs, the percentage of hatched embryos were calculated for each female. The Dead eggs were removed [12] while, percentage hatchability and larvae deformity were calculated [10]. The number of eggs released was calculated following the gravimetric method [10, 13]. The net was removed with the egg shells while, the hatched larvae clustered at dark corners of the incubation tank. The spawn weight was determined by estimating the mean weight of eggs used to achieve percent fertilization. The ovulation rate was estimated from the weight of eggs released as a percentage of the total weight of the ovary. The percent fertilization was estimated from the surviving embryos 10 hours after fertilization. The percent hatching was the number of hatched fry relative to the fertilized eggs while, the percent survival was the number of surviving fry after 14 days of feeding with mixed artemia and artificial diets.

Determination of Water Parameters

Water pH was measured with a Phillip pH meter (model pH-009 111), with glass electrode. Dissolved oxygen (DO) was measured with DO meter (model EUTECH DO 600) while water temperature was determined by simple mercury-in-glass thermometer. The physico-chemical parameters of the water are presented in Table 1.

Statistical Analysis

All percentage data were calculated prior to analysis. Data obtained were pooled for each treatment and compared by one-way analysis of variance (ANOVA) test to determine significant differences ($P<0.05$) and Turkey's post-hoc test was used to determine differences among treatment means.

RESULTS

There was slight fluctuation of temperature based on four- hour check during the experiment. The values ranged from 28.5^oC to 30^oC. During this period the brooders were restless with observable aggressiveness irrespective of the type of the hormone. The control (Treatment 1) that was injected with 2 mL of 0.6% saline solution showed no sign of restlessness and there was no change in the development of the ovary. The temperature ranges during the experiment are shown in Table 2.

There was statistical difference (P<0.05) in the number of ovulated eggs in all three hormonal materials used in this study as shown in Table 3. However, physical observation indicates higher ovulation in the frog pituitary treated group. There was no significant difference (P>0.05) in the quantity of eggs spawn in the various hormones and likewise, the quantity of ovulation observed. However, the mean weights of the various eggs were highest for frog pituitary and least in fish pituitary.

There was significant difference (P<0.05) in the percentage fertilization in the three treatments with the highest percentage of fertilization (67.00 ± 3.61) observed in ovulin-injected *C. gariepinus* (Table 3). The graphical representation of the percentage fertilization is shown in Figure 1.

Both percentage hatchability and survival rate was also significantly different, with the highest percentage hatchability and survival rates (90.61±6.43) and (69.33±5.13) (figures 2 and 3), respectively.

The results of the effect of various hormones on the spawning of C. gariepinus are shown in Table 3. The oocytes maturation occurred in all the female hypophyzed with the different hormones, Ovulin (0.5ml/1kg), one fish pituitary in 1ml of saline solution/1kg, seven (7) frog pituitary of 6mg/ 2ml solution of saline solution/500g of female gravid *C. gariepinus*. Spawning was observed at 9hrs Latency period at temperature of 29^{oC} ± 0.76 with ovulin, 11hrs at temperature of 29^{o}c ±0.76, the same latency period was observed in frog pituitary. At the end of all the experimental observation the latency period was longer on *C.gariepinus* injected with both pituitary extract with 2hrs interval than Ovulin injected breeder.

The hatching period took 21hrs, 23hrs and 23hrs in Ovulin, fish and frog extract, respectively.

Cost benefit of hormonal treatment

The comparative cost of the hormonal materials was determined. Gravid fish *C. gariepinus* which weighed 2.7kg was injected with ovulin worth $6 (N742.50), while *C. gariepinus* which weighed 2.7kg was injected with fish pituitary from sacrifice fish worth $8 (N1080.00) and frog which cost *C. gariepinus* $4(N500.00).

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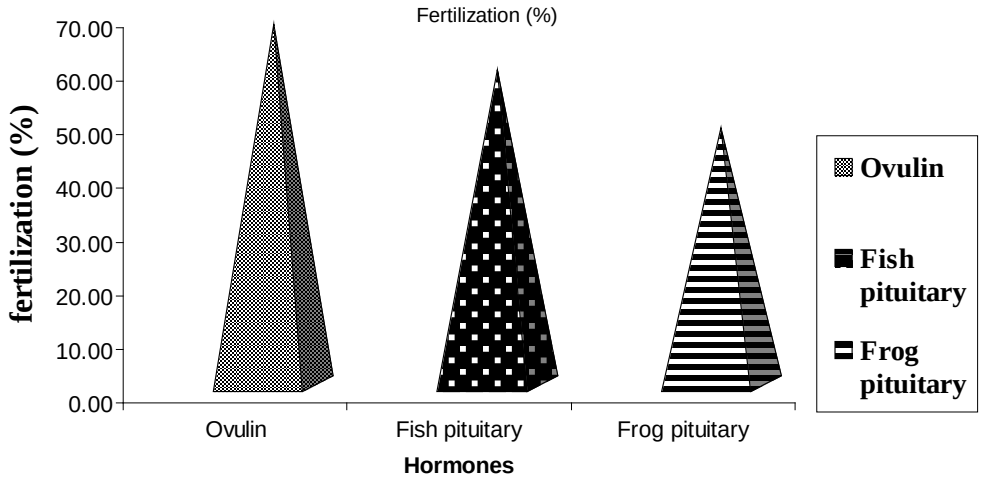

Figure 1: Percentage fertilization of artificial spawning of *C. gariepinus* injected with Ovulin, fish pituitary and frog pituitary glands

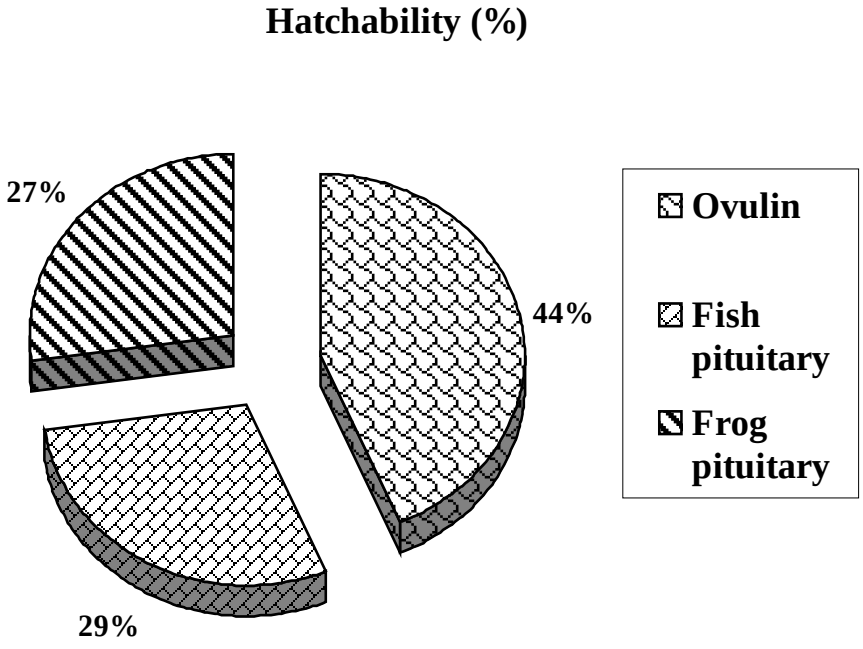

Figure 2: Percentage Hatchability of artificial spawning of *C. gariepinus* injected with Ovulin, fish pituitary and frog pituitary glands

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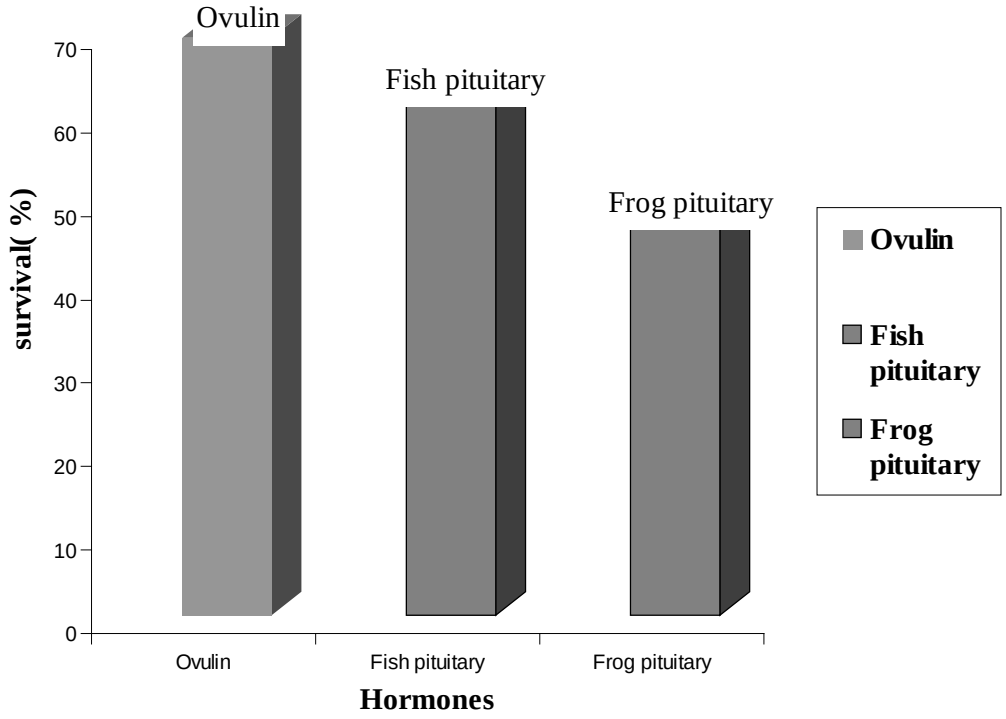

Figure 3: Percentage survival after 14 days of artificial spawning of *C. gariepinus* injected with Ovulin, fish pituitary and frog pituitary glands

DISCUSSION

The water parameters such as pH, Dissolved Oxygen (DO) and Temperature range recorded during the period of the study could be considered to be within the limit of aquatic life survival [14].The ovulation rate shows no significance difference statistically. The control showed no sign of restlessness of the brooders and there is was change in the development of ovulation. Physical observation, however, indicated high quantity of ovulation in frog pituitary. This could be due to the high effect of dosage in relation to its body weight. Eyo and Mgbenka [15] had earlier established linear relationship between fecundity, ovarian weight, length, GSI and Somatic weight of *C. gariepinus*. Also Ikenweiwe *et al.* [16] reported differences among the mean body weight in respect to weight of eggs. Highest percentage fertilization was recorded in ovulin injected *C. gariepinus*. This is similar with the report of Haniffa M A K and S Sridhar [11] in induced breeding of catfish, *H. fossilis* using ovaprim. Lowest percentage fertilization recorded in this study was found in frog pituitary injected *C. gariepinus*. This was different from the report of **Fagbenro** *et al.* [17]. He stated that *C. gariepinus* injected with frog pituitary hormone was successful and had a high percentage fertilization to the level of 98%. This may be due to the different species of frog used or the body different in the body weight in relations to the concentration of hormone used. The percentage hatchability as well as the percentage fertilization showed higher result in ovulin, followed by pituitary of fish with the

lowers recorded in frog pituitary [18] Adebayo and Fagbenro reported higher hatchability in fresh pituitary gland of frog and low hatchability in air dried frog pituitary. No comparison was made with either ovulin hormone or fish pituitary. The percentage survival was recorded best in ovulin and lowest in frog pituitary. This is similar with the observation of Haniffa M A K and S Sridhar [11] in induced spawning of *H. longitilis* using ovaprim. He also reported lower survival rate of fish at juvenile stage, which is similar to the observation made in this study. Generally, hormone products have correlating effect on animal growth, as most hormones are either growth promoters or inhibitors, depending on the dosage. The comparative cost of the hormonal materials used showed that ovulin, which recorded better performance in all the parameters evaluated, cost ($6) N742.50 for induce breeding of *C. gariepinus* of total body weight of 2.7kg. While the cost of pituitary could be said to be free or minimal since the farmer might sell fish used or consumed it after and such cost if calculated will almost goes back to the amount which he purchase it in the first place. The cost of getting frogs is zero since frogs are always available in the environment except for labour which could be incurred in employing children to run such errands or to set a trap for the frog. For this study, it cost $4(N500) to get the frogs used. Therefore, the cost of natural pituitary is cheaper compared to the synthetic pituitary ovulin. This is different from the report of Nwokoye *et al*. [19]. He reported that the cost of getting pituitary extract was higher than the cost of Ovaprim. This is because he did not put into consideration the fact that the fish *H. bidorsalis* was not discarded after the pituitary was removed. He properly converted it into other uses which he did not cost.

CONCLUSION

Although considering the overall performance of ovulin and also as have been reported earlier by Zohar [20].The GnRH analogues are advantageous, because they resist enzymatic degradation when injected into gravid fish resulting in a more prolonged stimulation of hormone released, when compare to the native natural hormones**.** Just as confirmed in this study by the higher percentage of fertilization, hatchings and high survival rate obtained in ovulin injected C. *gariepinus*. Also the rigorous procedures involved in removing pituitary glands still recommend Ovulin as a better option in artificial breeding. It can substitute the pituitary with advantages of high estrualizing rate, short response time and lack of any side-effects. Farmers, however, could use fish pituitary and especially the frog pituitary in terms of cost as alternative hormone.

Table 1: Physico-chemical parameter of the water

Parameter	Result
Appearance	Clear with no visible particles
pH	6.7 ±0.1
Dissolved oxygen (DO) mg/l	7.5 ±0.05
Temperature	29.0^{o}C±0.1
Salinity	0.5‰±0.04

Table 2: Temperature (^{o}C) checks at 4 hour interval

Time	Control	Ovulin	Fish pituitary	Frog pituitary
10.30pm	28.5	29.5	29.9	28.8
2.30am	28.5	29.00	28.5	28.8
6.30am	28.5	28.5	29.7	29.5
Means	25.5±0.01	29.00±0.1	29.4±0.2	29.00±0.1

Table 3: Induced Ovulation and spawning of *C. gariepinus* using Ovulin, Fish pituitary extract and frog extract

Parameter	Hormones		
	Ovulin	Fish pituitary	Frog pituitary
Body weight (g)	900.00±100.00[a]	900.00±173.00[a]	550.00±50.00[b]
Egg weight (g)	25.94±4.71[a]	24.48±4.63[a]	26.57±2.97[a]
ovulation (g)	20924.88±16118[a]	29157.12±5516[a]	31646.43±3532[a]
Fertilization (%)	67.00±3.61[a]	60.70±4.49[ab]	56.25±5.44[b]
Hatchability (%)	90.61±6.43[a]	60.70±4.50[b]	56.26±5.46[b]
Hatching period	21.00±0.00[a]	23.00±0.00[a]	26.00±0.00[a]
Latency period	9.00±0.00[a]	11.00±0.00[a]	11.00±0.00[a]

*Mean values followed by the same superscript in each column are not significant different ($p<0.05$)

REFERENCES

1. **Rottmann R W, Shireman J V and FA Chapman** Introduction to Hormone-Induced Spawning of Fish. *SRAC Publication*, 1991; No. 421.

2. **Ayoola S O** Modern Fish Farming Techniques (Aquaculture) Glamour books, Dugbe, Ibadan, Nigeria. 2010; 180pp.

3. **Huisman E A and C J J Richter** Reproduction growth, health Control and aquacultural potential of the African Catfish *Clarias gariepinus*. (Burchell 1822).*Aquaculture*, 1987; **63**: 1 – 14.

4. **Ayoola SO** Relationships of chemical composition, quantity of milt to fertility and hatchability of *Clariesgariepinus* (burchell, 18822).*African journal for food Agriculture, Nutrition and Development.* 2009a; Vol: 4.9.

5. **Fish Network Fisheries Resources Management**. A Quarterly publication of the Fisheries Society of Nigeria Lagos.FISON.1994; **3(1**): 1-6.

6. **Ezenwaji H M G** African *Clarias* taxonomy implication for field work. Proceeding of the 4th Annual Conference of the Fisheries Society of Nigeria (FISON) Held at Port-Harcourt.1985; 191-196pp.

7. **Oladosu G A, Ayinla O A, Adeyemo A A, Yakubu A F and A A Ajani** A Comparative study of the reproductive capacity of the African catfish species *Heterobranchusbidorsalis*(Geoffrey) *Clarias gariepinus(*Burchell) and their hybrid "*Heteroclarias*" *ARAC Tech. Pap.*1993;**92**: 1 – 5.

8. **Ayinla OA, Kayode O, Idoniboye-Obu O I E, Oresegun A and V E Adidu** Use of tadpole meal as substitute for fish meal in the diet of *H.bidorsalis (*Geoffrey St Hillarie 1809). *J. Aqua. Trop*. 1994; **9(I**): 25-33.

9. **Viveen WJ A R, Richter C J J, Van Oordt P GW, Janssen J A L and EA Huisman** *Practical manual for the culture of the African catfish* (*Clarias gariepinus)* Section for Research and Technology, Ministry for Development Co-operation. Netherlands. 1985; 128pp.

10. **Legendre M** Seasonal changes in sexual maturity and fecundity and human chorionic gonadotropin (HCG) induced breeding of the Catfish *Heterobranchus longifilis*Val (Clariidae) reared in Ebrie Lagoon (Ivory Coast). *Aquacul.*1986; **55**: 201-213.

11. **Haniffa M A K and S Sridhar** Induced spawning of spotted murrel *Channapunctatus)* and catfish *Heteropneustesfossilis* using humanchorionic gonadotropin and synthetic hormone (*Ovaprim). Vet. Arhiv*. 2002; **72(1):** 51 – 56.

12. **Nwadukwe F O** Inducing oocyte maturation, ovulation and spawning in the African catfish *Heterobranchuslongifilis* (Valences Pisces: Clariidae) using frog pituitary extract. *Aquacul.Fish.Man*. 1993; **24**: 625 – 630.

13. **Lagler K F** *Freshwater Fishery Biology*. Iowa, 2nd (Ed) WM. C. Brown Company Publishers. 1982; 108-109pp.

14. **Boyd C E and F Lichtokoppler** Water *quality management in pond culture*. Research and development series.International Centre for Aquaculture.Agricultural experiment.Station Auburn University, Auburn, Alabama, 1979; 56 p.

15. **Eyo J E and B O Mgbenka** Aspect of the biology of *Clarias gariepinus* in Anambra river basin I: Oocyte diameter fecundity and sex ratio. *J. Agric. Sci. Tech.* 1992; 2(1): 47-51.

16. **Ikenweiwe N B, Idowu A A and S O Ayoola** Effect of Oven and Air dried pituitary hormone on the ovulation capacity of gravid *Clariesgariepinus*. *African Journal of Livestock Extension.* 2010; **8**: 1-3

17. **Fagbenro O A, Salami A A and D H J Syndenham** Induced Ovulation and spawning in the catfish,*Clariasisheriensis*, (Clariidae) using pituitary extracts from non piscine sources. *J. Applied Aquacult.*1992; **1**: 15-20.

18. **Adebayo O T and O AFagbenro** Induced Ovulation and spawning of pond African giant catfish, *Heterobranchus bidorsalis* by exogenous hormones.*Aquaculture*. 2004; **242**: 229-236

19. **Nwokoye C O, Nwuba L A and J E Eyo** Induced propagation of African clariidae catfish, (*Heterobranchus bidorsalis)* using synthetic and homoplastic hormones. *African Journal of Biotechnology*. 2007; **6 (23):** 2687-2693.

20. **Zohar Y, Sherwood N M, Rivier J F and N Zmora** Gonadotropin releasing potencies of three native forms of gonadotropin releasing hormones present in the brain of gill head sea bream *Sparusaurata.Gen. Comp. Endocrinol*. 1995; **97**: 288-299.