COMPARATIVE STUDIES ON THE EFFECTS OF CRUDE PITUITARY EXTRACT (CPE) of Clarias gariepinus, Toad (Bufo Temporia) AND SYNTHETIC HORMONE (OVAPRIM) ON INDUCEMENT AND HATCHABILITY OF Clarias gariepinus (BURCHELL, 1822)

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Abstract

A research was carried out to determine the effects of the use of Crude Pituitary Extract (CPE) of Clarias gariepinus, Toad and Synthetic hormone (Oraprim) on the hatchability of Clarias gariepinus Fifteen matured female Clarias gariepinus, were induced with three types of hormones, Crude Pituitary Extract (CPE) of Clarias gariepinus, Toad (CPE) and Synthetic Hormone (ovaprim) tagged treatment A, B and C respectively. Treatment A, B, and C were replicated five times. Corresponding number of each replicates, had the same weight of 500gm, 520gm, 540gm, 560gm and 580gm. Treatment A and B were given two of first and decisive doses of 1ml of supernatant at an interval of 6hrs. Treatment C was given one and decisive dose of 0.5ml. of ovaprim. The sacrificed males were injected with 1ml. and final dose of CPE of Clarias gariepinus and Toad and 1ml of ovaprim respectively. The flow through system was used for incubation and the dry-wet system of fertilization was adopted. Hatching commenced after 12hrs. and was completed within 24hrs. Treatment A had a total of 4,127 hatchlings and percentage (%) hatchlings of 44.12, Treatment B, 1711 hatchlings and 18.29% and Treatment C had 3,517 hatchlings and 37.59%. The use of CPE of Clarias gariepinus was significant at both 1% and 5%, because calculated F- value of 10.38 is greater than the F- tab., which are 6.93 and 3.89 respectively. Also the Least Significant Difference (LSD) of 3.70 was obtained. The mean difference between treatment A and B was 4.83, which is > LSD 3.70. Therefore, there is significant difference in the use of CPE of Clarias gariepinus and Toad. The mean difference between Treatment A and C is 0.82, which is < LSD, therefore, there is no significant difference between Treatment A and C. Mean difference between Treatment C and B is 4.01 which is > LSD, showing significant difference between Treatment C and B. Therefore, the use of CPE of Clarias gariepinus, Toad [PE] and synthetic hormone (ovaprim) were effective for inducing fish to spawn, but CPE of Clarias gariepinus and Ovaprim were more effective than Toad. This is aim at improving fish breeding and availability of "fish seed" through artificial propagation.

Keywords: *Female Clarias gariepinus, Toad, Synthetic hormone, Ovaprim, Supernatant, Dry-wet, Flow- through, Hatchlings.*

Introduction

Fish constitutes a major food item in the diet of an average Nigerian, and with the continuous increase in population, fish demand had increased considerably. Fish nutritionally, is equivalent to meat in protein, with a good amino acid profile, high essential minerals and low saturated fatty acid. Thus the culture of fish has become an innovative technology aimed at producing large quantity of food fish for consumers (Idoniboye and Ayinla, 1991). Nigeria needs approximately 1.5 million metric tons of fish annually, but her total annual domestic production is less than

0.45 million metric tons. Inadequate fingerlings of commercially and culturable species is the major constraint of fish culture (Ayinla, 1991 and Adekoya, 2001). Consequently, the practice of fish breeding (artificial propagation) becomes timely and universally accepted, since most fish cannot breed in captivity.

Miller, (2000) and Butler, (2006) opined that fish grow best when they are conditioned to breed in a seemingly natural condition, that is really healthy, well fed, have no pickers or predators and have good water condition. Ugwu et al., (2006) opined that some kind of intervention by man in the course of natural propagation of culturable fishes is unavoidable, for better survival of offspring and for solving the problem of lack of "fish seed" of desirable quality and quantity. The choice of Clarias gariepinus, stems from its popularity, acceptability, taste, hardiness, disease resistance and adaptability to natural environment which renders it attractive to culturist. A lot of substances had been used to induce fish to spawn. This includes manipulation of the natural environment, use of Crude Pituitary Extracts (CPE) and the use of synthetic hormones (ovaprim). Others include Human Chronic Gonadotropin (HCG), Pituitary Extract from Toad and Bullfrog, pituitary of purified salmon gonadotropin, carp pituitary homogenate mixed with synahorin, Rabbit Pituitary Extract SG 100 (Housay, 1930; Von Ihering, et al., 1937; Rugh, 1937; Gerbilskii, et al., 1937). Recent reviews on induced fish breeding include, Shehadeh, (1975), Chandhuri, (1976), Fontaine, (1976), Harvey and Hoar, (1979), Aguigwo ,(1991), Nwuba, (1998), Gardner, (2006), FAO,(2006), Moody et al., (2009) and Ogunsina,(2010).

Aims and objectives:

(a) To improve fish breeding through fish propagation.

(b) To determine the most and best cost effective hormone for catfish breeding

(c) To make necessary recommendations to catfish breeders, for future fish seed propagation.

(d) To ensure that the lack of fish seed of Clarias gariepinus, which is a delight to most fish farmers and consumers is a thing of the past.

(e) To ensure sustainability and development of Aquaculture production as a business.

Materials and methods

40 matured Clarias gariepinus with weights ranging from 500gm to 580gm, comprising of 15 gravid female Clarias gariepinus and 25 male were carefully selected from Aquafish Farm, Awka, Anambra State. The 15 female were divided into three groups, bearing in mind their weight and each female was placed in a 25 litre plastic bowl, half filled with water. The inducing hormones were obtained from the Crude Pituitary Extract (CPE) of Clarias gariepinus (Treatment A), Pituitary Extract (PE) of Toad (Treatment B) and synthetic hormone (ovaprim) (Treatment C) imported from China. Each treatment was replicated five times with the corresponding weights of 500gm, 520gm, 540gm, 560gm and 580gm.

Extraction of Pituitary Gland from clarias gariepinus and Toad: The selected Clarias gariepinus, were sacrifice by cutting the fish spinal cord at the back region between the neck and head to demobilized it. The gap of the mouth was widened by further slitting the lower jaw to the operculum. The mouth was washed with clean water, dried and turned up-side down. The voma bone was carefully cut open to expose the pituitary gland, a creamy globule like organ at the centre of the brain case. The CPE was carefully removed with a spatula and put in a clean small mortar. The

some process was used to open the brain case of the Toad to obtaining the Toad (PE).

Injection of 1st Dose : The administration of the hormone began by 12 midnight. The Pituitary Extract was thoroughly grinded in a mortar and 1m1. of physiological water (0.9gm of table salt in 1 litre of boiled, cool, water) was added. The hypodermic syringe was used to obtain 1ml. of Clarias gariepinus hormone (Trt. A) (CPE) and Toad (PE) (Trt. B) was grinded and 1ml. was also obtained, which were injected according to their group Trts. A and B respectively. 0.5ml. each of ovaprim vial (Trt. C), was injected into the five female of Clarias gariepinus of the group. The injection was given at an angle of 45o and neddle between the dorsal fin and lateral line pointing towards the caudal fin. Care was taken not to empty the supernatant into the fish bowel. The point of injection was massage to aid even distribution of the hormone.

2nd and Decisive Dose: After 6hrs, 1ml. of corresponding hormones of treatment A and B were administered to their respective female fish specimens. No second administration of Trt. C. hormone. At the same time the first and decisive dose of 1ml. of hormone of (Trt. A and B) and 0.5ml.of Trt. C, were given to male fish specimens that will provide the milt for fertilization.

Fertilization process started six hours after the final and decisive dose were administered to the male fish specimens.

Procurement of Milt (sperm) from Male Clarias gariepinus: 15 matured males of Clarias gariepinus were sacrificed to obtain the milt for fertilization of the eggs. One male to one female of equal weight. The milt was obtained 30- 40 minutes before stripping of the female for eggs. The gut of the male fish was cut open with a scissors to expose the two lobe testis. The testis were removed carefully and dried with a blotting paper. Small incisions were made at the tip of the testis and the milt was squeeze out and washed into a container that is dry with a 0.9% saline solution. The stripping of the female continued until trace of blood was seen.Eggs were collected in a clean bowl.

Preparation of Incubation Trough: The method used in this research was the flow through system. The source of water was from a bore hole. The overhead tank let in water into the incubator and excess water was allowed to leave the incubator via the outlet pipe.The outlet pipe was used to regulate water level in such a way that a constant water level covering the eggs was maintained in the incubator.

Procurement of Eggs from the Female Fishes Induced with Treatment A, B and C (Hormone): The latency and incubation period of this experiment was between 27-28oC, with a latency time of 8hrs. and incubation period of between 22 24hrs.

At the end of the latency period by 12noon the next day, the female fish were stripped to obtain the eggs. This was done by catching the female fish with a hand net, and blindfolds it with a wet towel. The head was griped firmly and the belly pressed towards the genital opening. The eggs were collected in a dry bowl. Stripping was stopped when blood was noticed.

Fertilization and Incubation of Eggs: The eggs obtained from each female specimen were fertilized by the milt obtain from a male induced with the same hormone. The milt obtained was poured into the bowl containing the stripped eggs. The bowl was gently shaken to ensure proper mixing of eggs and milt Dry fertilization. Some quantity of saline solution (0.9%) was added to enhance mixing. Later, enough water to cover the eggs was added (Wet fertilization). This was stirred carefully with a clean feather to ensure maximum fertilization.

The fertilized eggs were poured evenly on the incubation net or substrate. The net was weighted down with clean stones. The incubation tank was well aerated because of the flow through system used. The

temperature was maintained between 2728oC and hatching commenced after 12hrs., and was completed after 24hrs. The healthy larvae clustered at the dark corners of the incubator. The larvae were quickly separated from the dead eggs by removing the incubation net. Care was taken not to remove healthy larvae. The first 3 4 days, the larvae fry fed from the yolk sac containing nutrient. Counting was done manually for each replicate.

Data Analysis

The total hatchlings of all the replicates in Treatment A, B and C were counted manually, and percentage hatchability was also calculated. The mean hatchlings of treatment A, B and C were subjected to Analysis of Variance (ANOVA), for significant difference probability. The Least Significant Difference (LSD) of Treatment A, B and C were calculated.

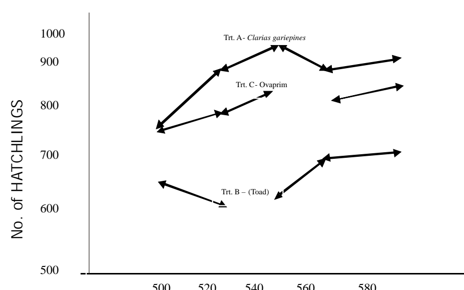
Result and Discussion: Table 1: No. of Hatchlings in Treatment A, B and C

Treatment Hormone	I 500gm	II 520gm	III 540gm	IV 560gm	V 580gm	Total
CPE *Clarias gariepinus*	630	801	944	901	881	4,127
Toad (PE)	330	225	262	441	453	1,711
Ovaprim (Synthetic)	618	660	745	711	783	3,517
					GRAND TOTAL	9,355

Table 1. Shows that the five replicate induced with treatment A (CPE) produced the highest number of hatchlings of 4.127 followed by Trt. C. treated with synthetic hormone (ovaprim) with 3,517 hatch lings and Trt. B. treated with the Toad (PE) 1,711 hatchlings. This experiment also showed that the group induced with Clarias gariepinus (CPE), gave the highest percentage (%) hatchlings of 44.12 than those treated with synthetic hormone (ovaprim) 37.6% and Toad (PE) 18.3% respectively.

Jalabert, et al., (1971) observed that the techniques used to preserve the synthetic hormone affect the potency of the extract or hormone. This agrees with the result of this experiment because Clarias gariepinus CPE was used in-situ and produced the highest hatchlings than the two other hormones. Peter, et al., (1988) used the method called Limpo method to induce ovulation in female fish by injecting them with a combination of synthetic gonadotropin releasing hormone analogue (LHRN A) and the drug doperidone. The hormone stimulate the sex organ of fish, while, the drug inhibits the action of dopermine produced by fish that inhibits ovulation.Unlike the use of CPE, the ovaprim does not require two times injection to induce and the cost implication was said to be cheaper, contrarily to the present experiment, where the cost implication is quite high and ovaprim availability is not assured, because of the place the experiment was carried out. The fishes used in this experiment were locally available and cheap to procure. Although those injected with pituitary extracts were handled many times than those injected with ovaprim. The use of Toad pituitary extract (PE), induced fish to spawn in this study. Ayinla, et al.,(1988) succeeded in using Toad (PE) to successfully induce ovulation in fish, and this agrees with this work, but it produced the lowest hatchlings in this experiment. The cost implication of using toad (PE) is cheapest but the major constraint, is it physical nature and culturist choice, which affects peoples choice of using it. The three treatments (A, B and C) were subjected to statistical analysis. There is a significant difference ($p < 0.05$) at 1% and 5% levels because the calculated F value of 10.38 is greater than the F-tab which is 6.93 and 3.89 respectively. The Least Significance Difference LSD which is 3.70 showed that there is no significant difference $P > 0.05$ in the use of Clarias gariepinus CPE and ovarprim in this experiment. This is because the mean difference between the number of hatchlings of treatment A and C is 0.82 lower than the LSD(3.70). There is significant difference ($P < 0.05$) between treatment A and B with mean difference of 4.83 and significant difference

between treatment C and B with mean difference of 4.01.



Weight of Female (Clarias gariepinus) (gm)

Figure 1: Shows pattern and number of hatchlings of Treatments A, B and C

Treatment A, showed the highest point of hatchlings at the weight of 540gm. Also specimen treated with Trt. C showed, the same pattern of increase hatchlings at the same weight but, not as high as trt. A. Treatment B did not follow the pattern as Trt. A and C, but showed the lowest number of hatchlings in all the treatments.

Many species of fish does not spawn in captivity, breeders have used so many substances to induce ovulation in fish (Marioghae, 1991). Ball, et al; (1969) reported that frog pituitary extract has successfully induced final ovulation in Clarias gariepinus. Liao et al; (1973) used Mullet pituitary homogenate mixed with Synahorin. Shedadeh, et al; (1973) used purified Salmon gonadotropin, SEG 100, Kuo, et al; (1973 and 1975) used Human Chrorionic Gonadotropim HCG. Other substances that had been used to induce spawning in fish includes; Follicle Stimulating Hormones (FSH), Lutnfenizing Hormone (LH) and Deoxycorticosterone Accetate (DOCA). Teleost gonadotropins are inactive in most fish bioassays (Burzawa Gerard and Fontaine, 1972). Therefore, it is recommended that more research work should continue in this area, in order to dictate easy methods of induce ovulation in other culturable fish species other than Catfish, that does not breed in captivity.

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