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**Storage Period; its Effect on Efficacy of Non-piscine (Frog)
Hormone used in Inducing Ovulation in African Catfish
(*Clarias gariepinus* Burchell, 1822)**

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Abstract: The study examined the effect of varying storage period on the efficacy of frog pituitary with the aim of determining the optimum period required for effectively enhancing induction ovulation, maturation and artificial propagation of *Clarias gariepinus*. Acetone dried pituitary extract obtained from non piscine source, the African bullfrog (*Rana adspersa*), were stored over a period of 16 weeks and used to induce spawning in the female *Clarias gariepinus*. Fresh pituitary extract was used as control having been previously worked upon and confirmed to be effective. There were significant differences in percentage fertilization of the eggs with the fresh hormone giving the highest yield and the hormone stored for 3 and 4 months giving least. The hormone stored for 4 weeks gave relatively high percentage fertilization and can therefore be used as optimum storage period.

Key words: African bullfrog (*Rana adspersa*), acetone, fertilization, pituitary

INTRODUCTION

The major pre-requisite for a necessary successful fish farming enterprise is the availability of fingerlings (fish seeds) so that as the adults are sold out for food, young ones are provided to replenish the stocks.

Historically, the source of fingerlings has been collection from the wild (natural waters) but the difficulties inherent in this method of fingerlings procurement is enormous such as it is time consuming, labour intensive and uncertainty in the species collected (Dada *et al.*, 1994).

The more reliable and surer way of fingerling production is a recent development in aquaculture especially in Nigeria. Before then, fish seeds are usually collected from the wild or imported. But as interest increased, the need for a steady and reliable source of fingerlings became very necessary. Induced breeding was then introduced in Nigeria specifically for crops species and was practiced at Oyo fish farms, Plateau state (In-Bac, 1982). Later the need for the reproduction of the popular local and commercially important species like the mud fishes e.g., *Clarias gariepinus*, in the eighties and the preliminary research to adopt mud fishes were carried out in the research institutes (Madu and Ita, 1988).

According to Harvey and Hoar (1979), FAO (1985), Viveen *et al.* (1986) and Madu (1989) fish fingerling production involves a series of breeding and feeding activities which result in the mass production of fish seeds under controlled environmental conditions, usually in a hatchery. One of these activities is induced spawning which involves the use of hormones (synthetic or non-synthetic). The hormone is meant to hasten the ripening of the eggs with few hours usually 12 h. The various hormones in use include pituitary extract or hypophysis from similar or different fish, Deoxycontilosterone Acetate (DOCA), Ovaprim, Ovotide and Human Chronic Gmadotropin (HCG) (produced in the placenta and extracted from the urine of pregnant women).

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Hypophysation, the use of pituitary extracts is presently the most popular and most economical (Madu and Ita, 1988). The pituitary can be extracted from both male and female fish (Viveen *et al.*, 1986).

Meanwhile, apart from the fish pituitary which is the popular choice, the frog pituitary can also be used (Fagbenro *et al.*, 1992) bearing in mind that frogs often constitutes nuisance in ponds and around wetlands particular during the rainy season, they could therefore be put to a reasonable use by during the dry season when their population drastically reduces, if only adequate information abound concerning the viability of the pituitary extracts after being stored. This will prevent farmers from spending so much on ovaprim and fish which are usually sacrificed (killed) when their pituitary extract is needed.

Researches had been carried on the potency of pituitary extract of frog (Fagbenro *et al.*, 1992; Mustafa *et al.*, 1984) and the various researches have proved that the pituitary extract of frog is viable for induced breeding of fish.

However, the effect of storage on the potency of the extract of frogs, pituitary has been hitherto unexplored. This was therefore aims at finding the effect of storage period on the efficacy of pituitary of frogs for breeding of *Clarias gariepinus*.

MATERIALS AND METHODS

Acetone dried pituitary extract of African bull frog (*Rana adspersa*) was used as hormone (Viveen *et al.*, 1986) Mature African bull frog (>120 g) were collected over a period of 4 months within the Federal University of Technology, Akure fish farm from January to April, 2005.

Hypophysics extracted from frog were packed separately into seal vial and stored in a desiccator mature male and female *C. gariepinus* broodfish were purchased from reputable farm in Akure in May 2006. Mature adults (mean weight 500 g) were selected based on their morphological features. Each sex was stored into separate concrete pond and fed for one week with 40% crude protein diet.

Acetone dried pituitary extracts for each month were crushed in a fine powder, in a porcelain mortar after which 2 mL of 0.9% NaCl solution was added and thoroughly mixed. The females were intramuscularly injected while the males were not given any doses.

Fifteen spawning trials were carried out i.e., 3 trials per treatment (storage period as the treatment) over 4 month period. The injected female fish were removed from covered container after 12 h and eggs were stripped into a dry bowl. Testis were removed from dissected male fish and the milt was used to fertilized the eggs following the procedure describe by Viveen *et al.* (1986). A portion of the eggs from each fish for each month and the control was collected in a bowl and kept in the Federal University of Technology, Akure hatchery to determine the percentage fertilization, hatchability and survival. The larvae were fed shell free artemmia and for one week after yolk absorption.

All percentage data were arc sine transformed 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 treatment means were subjected to filcher Least Significant Differences (LSD) test. Line graph from excel package was also used to compare some variables

RESULTS AND DISCUSSION

Generally the value obtained for the ovulation and spawning responses in the hormone treatment over the various storage periods were significant different. It was observed that the storage length had effect on the ovulation of the fish sample used (Table 1) fertilization rate was high in the control hormone treatment and at 4 week-storage period (>60%). It was however very low in fish administered with the hormones stored for 8 weeks while it was negligible for fish treated with hormones store for the 12 and 16 weeks period.

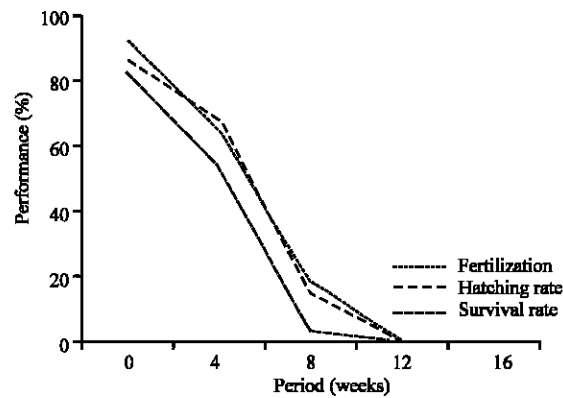


Fig. 1: Reductive performance of *C. gariepinus* induced with varying storage period of frog pituitary extract

Table 1: Summary of induced ovulation and spawning of *C. gariepinus* using non-piscine (frog) hormone stored over a period of 16 weeks

Parameters	0 weeks control	4 weeks	8 weeks	12 weeks	16 weeks
Body wt. (g)	530.00±16.33 ^b	395.00±69.40 ^f	580.00±16.33 ^{cb}	607.30±1.22 ^a	635.00±12.2 ^b
Fecundity	34250.00 ±1428.87 ^a	20300.00 ±1143.09 ^b	29400 ±286.19 ^a	21350.00 ±4286.61 ^b	16100.00 ±1143.09 ^b
Relative fecundity	64.43±0.54 ^e	53.00±6.53 ^b	57.00±5.72 ^b	35.35±6.94 ^f	25.33±2.05 ^c
Egg weight (g)	32.00±2.45 ^b	29.00±1.63 ^b	42.00±3.27 ^a	30.50±6.12 ^{bc}	23.00±1.63 ^c
Fertilization (%)	92.00±3.27 ^a	64.33±17.56 ^b	18.33±2.05 ^c	0±0.00	0±0.00
Hatching rate (%)	87.00±2.04 ^a	68.00±2.04 ^b	14.50±0.41 ^c	0±0.00	0±0.00
Survival (%)	83.00±2.45 ^a	53.00±3.67 ^b	3.00±1.00 ^c	0±0.00	0±0.00
Hatching period (h)	72.00±0.00	72.00±0.00	72.00±0.00	0±0.00	0±0.00
Latency period (h)	12.00±0.00	12.00±0.00	12.00±0.00	0±0.00	0±0.00

Values with different letter(s) are significantly different at p<0.05

The same result was also observed with the other parameters like hatchability which was relatively high for the control 0 week to the 4 week storage period (>60%) and low for 8 week-storage period and negligible mL for the 12 and 16 weeks storage period (Fig. 1). Hatching time and the latency for all the treatment were however not significant different (p<0.05) from each other.

The man weight of eggs spawned (per kg of fish) using hormones stored for 12 and 16 weeks were low in relation to the weight of the fish used. Fecundity was also low relative to the weight of fish used.

The results show that the pituitary stored for 4 weeks gave the best result. Its yield was significantly different from that gotten using hormones stored over the 8 to 16 weeks period. It shows that the frog pituitary extract is still effective when stored for not more than 4 weeks. Since pituitary stored for 8 weeks gave field (e.g., 18% fertilization) it can be implied that a range exists within the 4 and 8 weeks period within which stored pituitary will still give a yield relatively high. But from this study the optimum storage period obtained is 4 weeks.

Fish administered with treatment 3 and 4 (12 and 16 weeks) spawned very little with highly negligible fertilization hatchability and survival rate.

Fresh pituitary treatment shows fertilization of 92%. This spawning response for fresh pituitary treatment is comparable with that reported by Fagbenro *et al.* (1992). They reported that *Clarias gariepinus* were successfully spawned using frog pituitary hormone with an average fertilization of 98%. The result obtained for fish given the fresh pituitary extract for ovulation induction were significantly different from the rest generally.

However, the results for the various parameters obtained for fish treatment with month 1 hormone was encouraging fertilization >60%, hatchability, >60%. Month 2 hormone however was significantly low for such parameters as fertilization, hatchability and survival rate from figures above, fish administered the 16 weeks old pituitary extract gave the highest body weight followed by fish given the 12 weeks old hormone, then 8 weeks old hormones; fresh hormones and finally 4 weeks old hormone. However, the highest eggs weight yields were by the 12-week-old hormone followed by 8-week-old hormone with the 16-week-old hormone yielding the least. The significant difference obtained in the weight of the egg of the fish given the different treatment in spite of the body weight must have been due to the effect of the storage period. Also, although the fecundity yield of 4-weeks old hormone was not the highest, its relatively fecundity gave the second highest still confirming that the fish injected with fresh pituitary extract and 4 weeks old pituitary extract gave the optimum result.

The hatching time ranged between 24 to 26 hours under the prevailing temperature of 25-27°C. This is in line with Viveen *et al.* (1986) who reported hatching time of 23-27 h for the temperature range. The latency period of 12 h was also observed for all the fish treatment that ovulated.

Also from the Table 1, the results show that parameters such as relative fecundity, fertilization and hatchability and survival rate were highest for fresh frog pituitary. This may be due to the fact that all the chemical constituents of the hormone have not in any way been altered. This is very much unlikely to be due to the weight of the fish used at the week 0 treatment which is the control treatment using the fresh pituitary was not the highest. The highest being week 16 and yet gave no significant result in terms of fertilization, hatchability, survival and relativity fecundity. This could be due to the effect of storage period on the potency of the hormone. The result suggest that time factor will play a major role in the continued efficacy or otherwise of the frog pituitary hormone.

CONCLUSIONS

This result is important as it shows that although adhere is still a significant different in the overall result obtained over the various storage periods. Hormone stored for 4 weeks still gave a fairly yield in terms of fertilization, hatchability and survival. Farmers can therefore afford to still store their pituitary for a maximum of 4 weeks for optimum result.

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