

HYPOPHYSATION IN *Clarias gariepinus* USING CRUDE PITUITARY GLAND EXTRACT OF BULL FROG (*Ranas catesbiana*).

¹Fatokun, B.O., ²Adedeji, O.B., ¹Aguihe, P.C ¹Adigun, O., ⁴Akinbosoye, O. and ³Aguihe E.O
¹Federal College of Wildlife Management, Forestry Research Institute of Nigeria, PMB 268, New-Bussa.
²Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan.
³National Institute of Freshwater and Fishery Research, New Bussa.
⁴Federal College of Forestry, Jericho, Ibadan
Corresponding author: niyifatokundr@yahoo.com

Abstract

Mature *Clarias gariepinus* ranging between 860-970g total body weight (TBW) were treated with crude pituitary gland extract obtained from the Bullfrog (*Ranas catesbiana*) at four treatment levels of two pituitaries, four pituitaries and six pituitaries per brood stock of mean weight 814 ± 81.62 g and Ovaprim® for control. Each treatment was replicated three times and the frog's weight ranged between 47.28-58.63g. The latency period before successful stripping was 14hours with two pituitary hormone dosage, 12hours with four pituitaries and 9hours with six pituitaries. Eggs were incubated at a temperature that ranged from 26-27°C. Hatching was noticed after 24hours and was completed after 36hours of incubation. It was observed that the fecundity varies with the dosage of administered pituitary. The highest number of egg yield was observed in (T₂) fishes administered with the four doses of pituitary gland (52168 ± 3623) closely followed by (T₁) fishes administered with two doses of pituitary gland (46218 ± 5142). The least fecundity was observed in (T₃) fish administered with six doses of pituitary gland (27360 ± 4250). Percentage fertilization and hatching of the eggs were also highest in T₂ (98%) and this was followed by the fishes in T₁ having 98% fertilization and 70% hatching ratio. The fishes in T₃ had the lowest value of 96% fertilization and 58% hatching. The fries produced were then reared for eight weeks and mortality observed was highest during their 2nd week of life in all the three treatments. Fries produced in T₂ (four pituitaries) was observed to have the highest survival rate (72.61%) followed by T₁ with 33.62% survival rate and T₃ with only 6.78% survival rate. Four pituitary gland treatments were found to be the most effective.

Keywords: Fecundity, hatching, incubation, hypophysation Pituitary.

Introduction

The progressive increase in the aqua cultural farming in Nigeria has led to an increase in demand for fish fingerlings and this has made the hatchery propagation of Culturable fish species an important and viable venture. Manickan and Joy (1989) and Ayson (1991) reported that many species have been induced to spawn using different inducing hormones some of these inducing agents include Carp's pituitary gland (Janseen, 1985), Human Chorionic Gonadotropins (HCG) (Legendre, 1986), progesterone and LHRHa (Richter, et al., 1987), De-oxy Corticosterone Acetate (DOCA) (Solar, et al., 1990). Due to scarcity and increase in price, some of these synthetic hormones are not always available in developing countries in Africa and this has led to the search for alterative materials such as pituitary glands of frogs. Nwadukwe, (1993) used pituitary extract of frog *Dicroglossus occipitalis* to induce oocyte maturation, ovulation and spawning in *Heterobranchus longifilis*. Adebayo and Popoola (2004) also used frog pituitary gland to induce ovulation in *Clarias gariepinus*. Asian catfish (*Heteropneustes fossilis*) was stimulated to spawn with frog (*Rana*

tigrina) pituitary by Mustafa et al., (1984). The administration of appropriate hormone is as important as the creation of conducive environment for the brood fish. Kutty (2005) opined that the large number of failures in induced breeding can often be traced to an inappropriate hormone inducement, poor condition of gonad development as well as to environmental condition in spawning tanks or enclosures. The effect of storage period on the efficacy of the pituitary extract of African bull frog for induced spawning was studied by Adebayo and Fagbenro (2008). The scarcity of genetically improved fish seed constitutes the major constraint to the rapid development of aquaculture industry and stock management in Nigeria.

The African catfish (*Clarias gariepinus*) are species of economic importance in Nigeria and are widely cultured owing to their hardiness, early maturity and good market price. The use of frog pituitary to include breeding in these species has not been a common practice in Nigeria. Roberts (1975) opined that evolutionary evidence from morphological and physiological characteristics has indicated that frogs (Amphibians) and fish (Pisces) have the same ancestral origin and it has

been established that the pituitary hormone of frogs can be used in spawning of fish to improve their fecundity and no known standard dosage of frog's pituitary for fish breeding has been ascertained. Thus the objective of this work is to ascertain the effectiveness of African bullfrog's (*Ranas Catesbiana*) pituitary gland in induced breeding of *Clarias gariepinus* with the view to cut down the cost of fingerlings production from rather expensive second generation hormonal compounds; to determine the hatchability of *Clarias gariepinus* eggs induced with frog's pituitary gland and to establish a dosage of frog's Pituitary gland to establish a dosage of frog's crude pituitary gland to be recommended for breeding of *Clarias gariepinus*. African bullfrog (*Ranas catesbiana*) is medium sized specie that grows up to between 90 - 110mm in length, with adult females usually larger in size than the males (Gampper, 1985). They are air-breathing aquatic frog that occurs in virtually every water body in Nigeria. They are commonly found in stagnant or still waters of ponds or sluggish streams but they may also inhabit fast flowing waters.

Materials And Methods

Experimental site and management of brood stock: The research was conducted at the experimental unit of the Federal College of Wildlife Management New-Bussa, Niger State, Nigeria which covers a land mass of about 16,200,00km² and situated between Latitude 9° 00' - 11° 36'N and Longitude 3° 00' - 43° 9'E. The study area was bounded to the coast by River Niger (Kainji Lake) to the Northern and Eastern boundaries by Kebbi State and to the West is Republic of Benin. Twelve (12) females and four (4) male *Clarias gariepinus* brood stocks, size ranging from 860 - 970g to be used for this research work were sourced from a reputable farm in New-Bussa, Niger State, Nigeria and were each kept in 1m³ plastic tanks for acclimatization to full sexual maturity for twenty-four hours. Thirty six (36) live frogs (*Ranas catesbiana*) with sizes ranging from 47.28 - 58.63g collected from Oli River in New-Bussa, Niger State, Nigeria were used for the experiment.

Harvesting of pituitary from African bull frog: The pituitary gland which is located on the vertical side of the brain below the lower hypothalamus and olfactory lobe of the brain was extracted by first killing the donor frog and removing the lower jaw to reveal the bony capsule in the center of the underside of the upper jaw. This capsule was carefully fractured and pressed to reveal the small white pituitary gland in the center of the

underlying tissue which was then picked by forceps. The extracted pituitary was then macerated and homogenized in 0.5ml saline solution.

Experimental designs and pituitary extract administration: Twelve female brood stocks of *Clarias gariepinus* used for this research were randomly allocated into four experimental treatments (T₁, T₂, T₃ and T₄) and each treatment replicated thrice (T₁R₁, T₁R₂, T₁R₃,.....T₄R₁, T₄R₂, T₄R₃) using Complete Randomized Design (CRD). The fishes in T₁ were injected intramuscularly at the dorsal part of the caudal peduncle using 1ml needle and syringe with two frogs' pituitary gland per replicate. Four frogs' pituitaries were injected into each replicate in T₂ and finally, six frogs' pituitaries were injected through the same route into each replicate of fishes in T₃. The control group which was designated as T₄ was injected with Ovaprim® hormone (Syndel, Canada) at a dose rate of 0.5mgL⁻¹/Kg per replicate. The weight of the pituitary gland extracted were recorded and reconstituted to make 0.5mgL⁻¹. After injection, the fishes in each treatment were placed in separate hatchery tanks 1x1.5m in size and were observed for latency period during which ovulation was tested for by stripping two hourly.

After the latency period, eggs were stripped and fertilized normally by wet technique with milt from four male donors. Four male *Clarias gariepinus* were sacrificed for their milt, one for each set of treatment. Fecundity of all the treated female was determined by the method of Bagenal (5). The eggs were incubated for 24hours after fertilization. Percentage fertilization and hatching were estimated from the number of un-hatched and hatched eggs using the formula:

$$\text{Percentage (\%) Hatchability} = \frac{NH \times 100}{NF}$$

Where: NH= Number of eggs hatched and NF= Number of eggs fertilized

After hatching the fries produced by each treatment were separately reared in different glass aquaria tanks for eight weeks during which mortality, survival and growth rates were recorded for each treatment. The percentage survival was calculated by using the formula: Percentage (%) survival =

$$\frac{(M - y)}{M} \times 100$$

Where; y = Number of mortality recorded throughout the rearing period
M = Number of hatchlings 24 hours post fertilization

Statistical Analysis: The data obtained was used to determine percentage mortality, survival and

growth rates and one way Analysis of Variance (ANOVA) was used to compare the results as shown on Table 2. All the water quality parameters of the water used in raising the hatched fries were monitored throughout the experimental period and this is shown in Table 1.

Results

Table 1 shows the water quality parameters of the water used in rearing the fries produced from *Clarias gariepinus* induced with frog's (*Ranas catesbiana*) pituitary gland extract.

Discussion

Induced breeding of *Clarias gariepinus* (Burchell 1857) with pituitary gland obtained from *Ranas catesbiana* was carried out at four treatment levels including control. The latency period before successful stripping ranged from 9-14 hours at a temperature range of 26°C-27°C. This explains the fall within the range observed by Nwadukwe, F.O. (1993) which was eleven hours at 25°C which was reduced to seven hours at 29°C. Sule (1999) observed that the optimum latency period before stripping *Clarias* species in arid zones in Nigeria is about nine hours. Hatching commenced after twenty four hours and was completed by thirty six hours at 26°C-27°C. Manickan and Joy (1989) and Nwadukwe, (1993) had also observed the commencement of hatching after twenty four-hours of incubation. It was observed that fecundity varied with the dosage of hormones in the treatments. Administration of combined four pituitaries (T₂) gave the highest egg release and mean fecundity of 52168 ± 3623 this is closely followed by two pituitary treatments (T₁) with mean fecundity of 46218 ± 5142. Administration of six combined pituitary (T₃) gave the least mean fecundity of 27360 ± 4250. Four pituitaries combined weighing 0.98g was the most effective dosage followed by two pituitaries weighing 0.52g, combined six pituitaries weighing 2.43g appeared to be an overdose to fish of 915g mean weight. Nwadukwe, (1993) achieved oocyte maturation, ovulation and hatching with frog's pituitary dose of 7mg/kg fish weight. Percentage fertilization was ninety-eight percent (98%) in Treatments 1 (T₁), Treatment 2 (T₂) and Treatment 3 (T₃) and was ninety-six percent (96%) in control treatment. Percentage hatching was highest in T₂ ninety-eight percent (98%) followed by T₁ seventy-five percent (75%), control seventy-five percent (75%) and T₃ sixty-two percent (62%). Nwadukwe, (1993) obtained mean percentage fertilization of 7350 ± 9.30% and mean hatching of 63.08 ± 7.08%. Table 2 above shows the

analysis of variance (ANOVA) for comparison of fecundity, percentage fertilization and hatching, initial and final weight of fries produced after raising for eight weeks. Results of water quality parameter analysis are also presented in Table 1. The values are within the tolerance range for hatching, survival and growth of fish as reported by Marilyn, (1976), Viveen et al (1986) and Ayinla, (1991).

Conclusion and recommendations.

This experiment indicated that hypophysation with frog's (*Ranas catesbiana*) pituitary gland can successfully induce breeding in *Clarias gariepinus* (Burchell 1857) at a dosage of four pituitary at a dose rate of 1.0mg pituitary/kg fish.

References

- Adebayo, O.T. and Fagbenro, O.A. 2008: Effect of storage period on the efficacy of African bull frog pituitary extract for induced spawning of *Clarias gariepinus*. International Journal of Zoological Research 4 Pp77-80.
- Adebayo, O.T. and Popoola, O.M. 2004: Storage period; its effect on efficacy of non-piscine (Frog) hormone used in inducing ovulation in African catfish (*Clarias gariepinus*). International journal of Zoological Research 4(2): 124-128.
- Ayinla, O.A. 1991: Feed requirement and fattening of Broodstock. Proceedings of fish seed propagation Course. African Regional Aquaculture Centre. (ARAC) Aluu Port-Hacourt Nig p20
- Ayson, F.G. 1991: Induced spawning Rabbit fish, *Siganus guttatus* (Bloch) Using Human Chorionic Gonadotropins (HCG). Aquaculture 95, 133-137.
- Bagenal, T.B. 1975: Methods for assessment of fish production, Blackwell Scientific Pub Ltd, Oxford OX2OEL, 155-160
- Gampper, T. 1995: (September 21, 2001) Natural History of the upland Clawed Frog, Nebraska Herpetological Society: <http://www.sonic.net/~melissk/xenopus.html>. P1-10.
- Janseen, J.A.L 1985: Elevage du poisson-chat Africain, *Clarias lazera* (Cuv & Val 1840) en Republique Centafricain. 1: Propagation Artificielle. FAO Project GCP/CAR/007/NET Banui; C.A.R. DOC. Tech. 20, 100pp
- Kutty, M.N. 2005: Aquaculture Principles and Practices Second Edition. FAO/NACA, Aquacultural Expert and Black Well

- Publishing Limited, 9600 Gersington Road, Oxford Ox42DQ UK pp. 174-179.
- Legendre, M. 1986:** Seasonal Changes in sexual Maturity and fecundity and HCG Induced Breeding of the catfish, *Heterobranchus longifilis* Val. (Clariidae) Reared in Ebric Lagoon (Ivory-Coast). *Aquaculture* 55, 201-213.
- Manickan, P. and Joy, K.P. 1989:** Induction of Maturation and Ovulation by Pimozide-LHRH Analogue Treatment and Resulting Higher Quality egg Production in Asian Catfish (*Clarias barachus*). *Aquaculture* 83, 193-199.
- Marilyn, C. 1976:** Freshwater Fish Pond Culture and Management Appropriate Technology for Development Pp 47-50.
- Mustafa, S., Ahmed, Z., Murad, A. and Zofair, S.M. 1984:** Induced spawning of catfish by frog pituitary gonadotropins. *Progressive Fish Culturist*. 46: 43-44.
- Nwadukwe, F.O. 1993:** Induced oocyte maturation, ovulation and spawning in the African catfish, *Heterobranchus longifilis* using frog pituitary extract. *Journal of Aquaculture and Fisheries Management*. 24: 625-630.
- Richter, A.C.J.J., Eding, E.H, Goss, H.L.Th.De LeeuwR, Scott, A.P. and Van Oordt, P.G.W.J. 1987:** The effect of pimozide/LHRHa and 17a hydroxyprogesterone on Plasma Steroid Levels and Ovulation in the African Catfish (*Clarias gariepinus*). *Aquaculture* 63, 157-168.
- Roberts, M.V.B. 1975:** Biology: A Functional Approach. Reconstructing evolutionary pathway Pp 552-553.
- Solar, L.L., Mclean, E., Baker, I.J., Sherwood, N.M. and Donalson, E.M. 1990:** Induced ovulation of Sable Fish (*Anoploma fimbria*) (D-Ala⁶) LH-RH Ethylamide. *Fish physiology and Biochemistry* 8, 497-499.
- Sule, O.D. 1999:** Determination of Optimum Latency time for stripping *Clarias gariepinus* in the Arid zone of Nigeria, National Institute For Freshwater Fish Research (NIFFR) Annual Report. P.73.
- Viveen, W.J., A.R., Richter, C.J.J., Van Oordt 1986:** Practical Manual for the Culture of the African Catfish (*Clarias gariepinus*). Netherland Pub., Pp1-10.

Table 1: Weekly mean water quality parameters of water used to rear fry produced from *Clarias gariepinus* induced with frog's (*Ranas catesbiana*) pituitary gland extract.

WEEK	TEMPERATURE	PH	DISSOLVED OXYGEN	CONDUCTIVITY
1	28.2 ± 2.31	6.81 ± 0.50	6.2 ± 0.45	113.6 ± 11.6
2	28.1 ± 2.40	7.02 ± 0.66	6.0 ± 0.40	78.6 ± 6.22
3	26.2 ± 2.15	6.96 ± 0.58	5.7 ± 0.40	91.6 ± 7.90
4	26.1 ± 2.20	6.80 ± 0.48	6.3 ± 0.45	86.3 ± 6.92
5	27.2 ± 2.28	6.43 ± 0.32	6.4 ± 0.44	109.0 ± 10.82
6	26.0 ± 2.20	6.93 ± 0.56	5.7 ± 0.36	165.1 ± 14.90
7	25.0 ± 2.18	6.98 ± 0.52	6.1 ± 0.38	177.0 ± 16.23
8	26.2 ± 2.24	6.86 ± 0.54	5.9 ± 0.37	202.0 ± 17.82

Table 2: Fecundity, percentage fertilization, percentage hatching and weight of fry produced from *clarias gariepinus* induced with frog pituitary and reared for eight weeks.

Parameter	T1	T2	T3	T4
Fecundity	46218 ± 5142 ^{ab}	52168 ± 3623 ^a	27360 ± 4250	31634 ± 1096 ^b
% fertilization	98.90 ± 0.00 ^a	98.80 ± 0.00 ^a	96.20 ± 0.00 ^{ab}	96.32 ± 2.41 ^{ab}
% Hatching	75.40 ± 0.00 ^{ab}	98.60 ± 0.00 ^a	58.14 ± 0.00 ^b	75.49 ± 2.68 ^{ab}
Mean initial wt of fry (g)	3.35 ± 2.63 ^c	3.85 ± 2.76 ^b	4.89 ± 2.10 ^a	4.19 ± 3.19 ^{ab}
Mean final wt of fry (g)	33.59 ± 8.28 ^c	38.35 ± 8.63 ^a	32.02 ± 7.41 ^c	35.35 ± 33.16 ^{ab}
Mean wt gain	30.24 ± 4.63 ^b	34.50 ± 5.89 ^a	27.13 ± 6.81 ^c	31.16 ± 30.23 ^{ab}
Survival rate %	33.62 ^b	72.61 ^{ab}	6.78 ^c	88.83 ^a

Means with different superscript on the same horizontal rows are significantly different (P<0.05)