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Full Length Research Paper

Hatchability of *Clarias anguillaris* eggs induced with frog pituitary gland and dosage recommended for breeding *Clarias anguillaris*

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Ripe *Clarias anguillaris,* with a size ranging from 350 to 600 g total body weight (TBW) (mean weight of 434.44 ± 79.39 g), were treated with frog (*Xenopus laevis*) crude pituitary glands at three treatment levels of 1 pituitary, 2 pituitaries and 3 pituitaries per broodstock and the control treated with Ovaprim. Each treatment was replicated three times. The frogs' weight ranged from 29.40 to 105.59 g. The latency period before successful stripping was observed to range from 9 to 14 h according to the hormone dosage. Eggs were incubated at a temperature range of 26 to 27°C. Hatching started after 24 h and was completed by 36 h of incubation. Egg yield or fecundity was observed to vary according to the dosage of pituitary glands administered. Two pituitary glands injection yielded the highest egg number with mean fecundity of 47081 ± 10516 followed by one pituitary gland treatment with mean fecundity of 34785 ± 7537. Three pituitary glands treatment gave the least fecundity of 26007 ± 4359. Percentage fertilization and hatching of the eggs were also higher in two pituitary glands treatment with 98% fertilization and hatching. This was followed by one pituitary (75%) and three pituitary glands with 62% fertilization and hatching. The fries were reared for 8 weeks. Mortality was observed to be highest during the second week in all the three treatments. Treatment two (2 pituitaries) still had the highest survival of 68.40% followed by treatment one (1 pituitary) with 29.60% survival and treatment three (3 pituitaries) with only 4.80% survival. The stock stabilized and no mortality (0%) was recorded from 3rd to 8th weeks of rearing. Results indicated that 2 pituitary glands treatment was most effective followed by 1 and 3 glands treatments respectively. Weight of frogs and their respective pituitaries and weight of fish appeared to have relative effects on their productivity. Three frogs (T_3) of mean weight 67.52 ± 33.70 were observed to be an over dose to a fish of 434.44 g mean weight.

Key words: *Clarias anguillaris*, breeding, frog pituitary.

INTRODUCTION

The demand for fish fingerlings for aquaculture is on the increase in Africa and has made hatchery propagation of culturable fish species important. Many fish species have been induced to spawn using different inducing hormones, as reported by Manickan and Joy (1989), Ayson (1991) and Young et al. (1989). Some of these inducing agents include carp pituitary gland (Janseen, 1985), human chorionic gonadotropin (HCG) (Lengendre, 1986), progesterone and LHRHa (Richter et al., 1987), de-oxycorticosterone acetate (DOCA) (Solar et al., 1990), Ovaprim and Ovatide. In many developing African countries, these materials are not always available due to marketing problems. This scarcity has led to the search for locally available alternatives such as the pituitary gland of the frogs. Nwadukwe (1993) used pituitary extract of frog *Dicroglossus occipitalis* to induce oocyte maturation, ovulation and spawning in *Heterobranchus longifilis*. Adeyemo and Fagbenro (2008) used Bull frog pituitary extract to induce spawning in *Clarias gariepinus*, while Adeyemo and Popoola (2004) used frog pituitary gland to induce ovulation in *C. gariepinus*. Mustafa et al. (1984) spawned the Asian catfish *Hetewropneustes fossilis* with frog *Rana tigrina* pituitary gland. While the condition of the brood fish and the environmental condition are important , the administration of the

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appropriate hormone is also equally important. The large number of failures in induced breeding can often be traced to inappropriate hormone inducement, poor condition of the brood fish, including their health, nutrition and stage of gonad development as well as environmental condition in spawning tanks or enclosures (Kutty, 2005). The scarcity of genetically improved fish seed constitutes the major constraint to the rapid development of aquaculture industry and stock management in Nigeria. The mud fish C*larias anguillaris* species are species of economic importance in Nigeria. They are widely cultured owning to their hardiness, early maturity and good market price.

African clawed frog (*Xenopus laevis*) is a giant species of frog that grows up to between 100 and 130 mm in length, with adult males generally 10 to 30% smaller. They are air breathing aquatic frogs that occur in virtually every water body in its native range of Sub-Saharan Africa. This frog is most commonly found in stagnant or still waters of ponds or sluggish streams but may also inhabit fast flowing waters (Gampper, 1995).

The sacrifice of male Clarias species for milt often leads to depletion of male brood stock from fish farms or hatcheries. The use of frog pituitary to breed this species has not been a common practice in Nigeria. Evolutionary evidence from morphological and physiological characteristics has indicated that frogs (Amphibian) and fish (Pisces) have the same ancestral origin. They share the same habitat and many workers have found that frog pituitary gland can induce ovulation and spawning in fish. There is no known standard dosage of frog pituitary for fish breeding. This work is also a pioneer work on breeding of *Clarias anguillaris* using frog crude pituitary. The objectives are: to investigate the effectiveness of frog (*Xenopus laevis*) pituitary gland in induced breeding of *Clarias anguillaris* with the view to cut down the cost of fingerling production from rather expensive second generation hormone compounds; to determine the hatchability of *Clarias anguillaris* eggs induced with frog pituitary gland and to establish a dosage of frog crude pituitary gland to be recommended for breeding of *Clarias anguillaris*.

MATERIALS AND METHODS

The experiment was conducted at the hatchery complex of school of Agriculture and Agricultural Technology Fish farm unit, Federal University of Technology, Minna. Twelve female and four male *Clarias anguillaris* broodstocks, with size ranging from 350 to 700 g in weight, were used for the experiment. Eighteen live frogs (*Xenopus laevis*), with size ranging from 29.40 to 105.59 g collected from River Gadu in Minna were used for the experiment. Four treatments including control were carried out in the following experimental design. One frog pituitary gland to one female fish, replicated three times, was used as treatment 1; two frog pituitaries to one female fish, replicated three times, was used as treatment 2; and three frog pituitaries to one female fish, replicated three times, was used as treatment 3. The control group were treated with Ovaprim at 0.5 mg/kg.

The frogs' weights and weights of pituitary glands extracted were recorded. The pituitary glands were mass rated and homogenised in 0.5 ml saline solution in a laboratory crucible and administered intraperitonially to the fish with 1 ml syringe and needle. After injection, the fish were placed in hatchery tanks and observed for latency period during which ovulation was tested for by test stripping at every two hours. After the latency period, eggs were stripped and fertilized normally by wet technique with milt from the male donors. Four males were sacrificed for their milt, one for each set of treatment. Fecundity of each female treated was determined by the method of Bagenal (1978) and adopted by Yisa et al. (2010) which is number of eggs in a subsample of 0.5 g multiplied by total weight of eggs. After fertilization, the eggs were incubated for 24 h. Percentage fertilization and hatching were estimated from the number of unhatched and hatched eggs. The four treatments were carried out separately as four breeding trials with two days interval for incubation and hatching of each set. After hatching, the fry were reared in glass aquaria tanks for 8 weeks during which mortality, survival and growth rates (increase in length and weight) were recorded. The data were used to determine percentage mortality, survival and growth rates. Results were compared by Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Induced breeding of *C. anguillaris* with frog pituitary gland was successfully carried out at four treatment levels including control. Table 1 shows the number and weight of frog, weight of pituitary gland, number and weight of fish, fecundity, percentage fertilization and hatching. The latency period before successful striping ranged from 9 to 14 h at ambient temperature of 26 to 27°C. This fall within the range observed by Nwadukwe (1993) which was 11 h at 25°C and 7 h at 29°C. Sule (1999) observed that the optimum latency period before stripping *Clarias* species in arid zones of Nigeria is 9 h. Hatching started after 24 h and was completed by 36 h at 26 to 27°C. Commencements of hatching at 24 h of incubation have been observed by others including Manikan and Joy (1989) and Nwadukwe (1993). Fecundity varied according to the hormone dosage and treatment. Two pituitaries combined (T2) gave the highest egg release and mean fecundity of 46081 ± 10516.36 followed by one pituitary treatment (T1) with mean fecundity of 34785 ± 7537.76. Three pituitaries combined (T3) gave the least mean fecundity of 26007 ± 4359.58. Two pituitaries combined weighing 0.5 g was the most effective dosage followed by one pituitary weighing 0.42 g. Three frog pituitaries (T3) with mean weight of 0.62 g appeared to

Table 1. Number and weight of frog per treatment, weight of frog pituitary, weight of broodfish, fecundity, % fertilization and % hatching.

Treatment	Weight of frog (g)	Pituitary/ treatment	Weight of pituitary (mg)	Weight of broodfish (g)	Fecundity	% Fertilization	% Hatching
T1R1	39.40	1	0.40	450	43474	98.5	75.5
T1R2	42.29	1	0.42	450	38500	98.9	75.6
T1R3	42.03	1	0.42	400	30882	98.7	75.4
T2R1	54.41 52.21	2	0.50	500	56888	98.9	98.5
T2R2	49.60 47.09	2	0.52	450	43474	98.8	98.9
T2R3	58.20 44.40	2	0.54	550	40882	98.5	98.6
T3R1	57.64 52.20 48.30	3	0.62	460	21355	98.2	62.0
T3R2	60.00 45.50 53.60	3	0.61	550	26666	98.1	62.5
T3R3	42.28 49.11 55.09	3	0.62	550	30000	98.3	62.6
T4 R1	Ovaprim			400	28000	96.5	78.1
T4R 2	Ovaprim			450	21333	93.6	75.8
T4R 3	Ovaprim			550	42000	98.5	80.0

Table 2. Analysis of variance (ANOVA) for comparison of fecundity, % fertilization, % hatching and initial and final weight of fry produced from *C. anguillaris* induced with frog pituitary and reared for 8 weeks.

Parameter	Treatment 1	Treatment 2	Treatment 3	Control	±S.E.
Fecundity	34786 ± 5386^{ab}	43749 ± 3005^{b}	26008 ± 4360^{a}	30444 ± 1054	6089.94
% Fertilization	98.90 ± 0.00^{a}	98.80 ± 0.00^{a}	98.20 ± 0.00^{a}	96.20 ± 2.46^{b}	1.422
% Hatching	75.50 ± 0.00^{b}	98.60 ± 0.00^{c}	62.30 ± 0.00^{a}	77.67 ± 2.55^{b}	1.453
Mean initial weight of fry (g)	3.66 ± 2.88^{a}	4.16 ± 2.89^{ab}	5.20 ± 2.11^{c}	4.50 ± 3.22^{b}	7.63
Mean final weight of fry (g)	33.90 ± 8.69^{a}	38.66 ± 9.62^{b}	32.33 ± 7.24^{a}	35.66 ± 32.11^{ab}	7.62
Mean weight gain (g)	30.24 ± 5.81^{b}	34.50 ± 6.73^{c}	27.13 ± 5.13^{a}	31.16 ± 28.89^{b}	1.45

be an overdose to fish of 550 g mean weight. Nwadukwe (1993) achieved oocyte maturation ovulation and hatching with frog pituitary dose of 7 mg/kg fish weight. Percentage fertilization was 98% in T1, T2 and T3 and from between 93 to 98.5% in control. Percentage hatching was highest in T2 (98.9%) followed by T1 (75%), control (75%) and T3 (62%). Nwadukwe (1993) obtained mean % fertilization of 73.50 ± 9.30% and mean hatching of 63.08 ± 7.08%. Table 2 shows the analysis of variance (ANOVA) for comparison of fecundity, % fertilization and hatching, initial and final weight of fry reared for eight weeks. Figures 1 and 2 show the survival

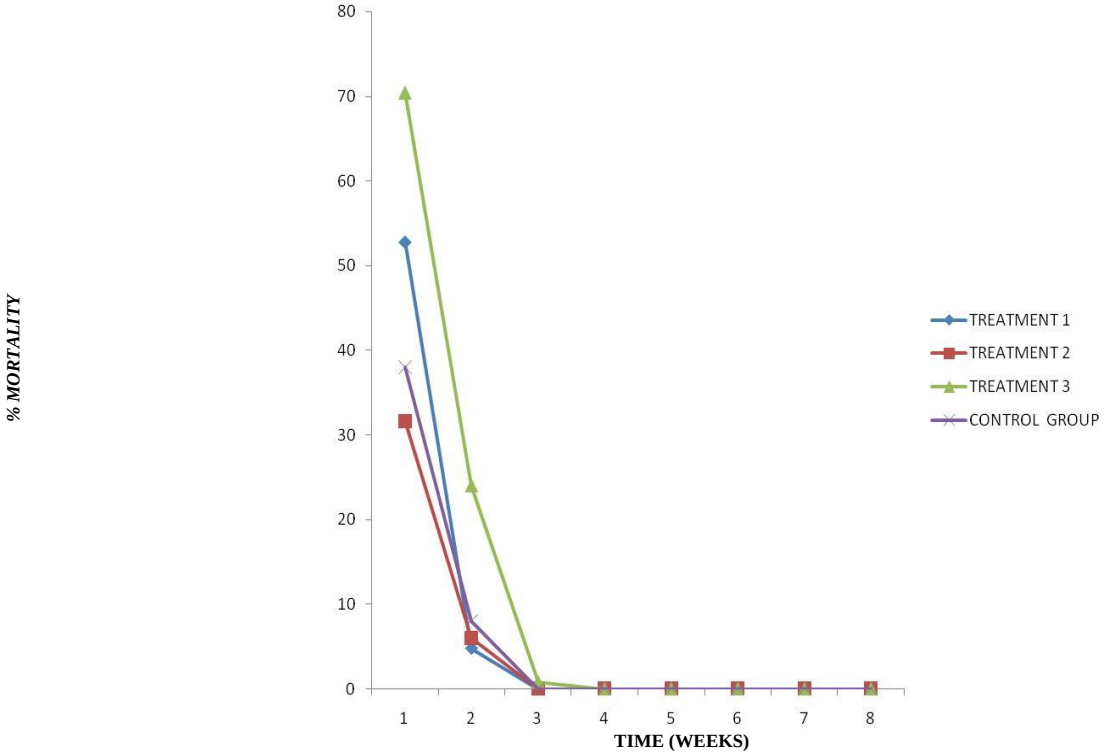

Figure 1. Percentage mortality of fry from *Clarias anguillarias* treated with frog pituitary extracts and reared for eight weeks.

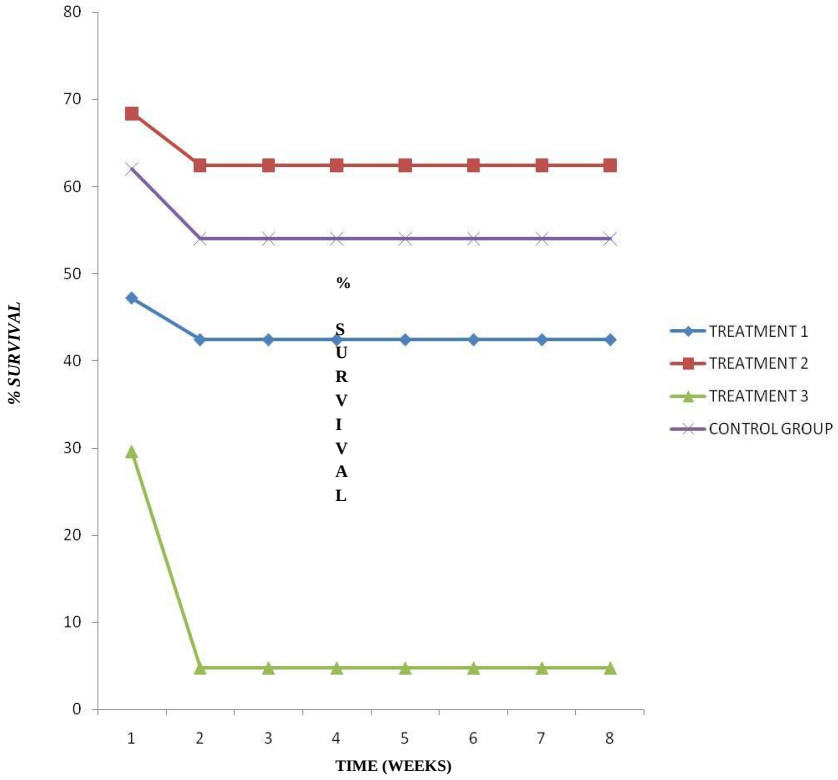

Figure 2. Percentage survival of fry from *Clarias anguillarias* treated with frog pituitary extracts and reared for eight weeks.

Table 3. Weekly percentage survival of fry from *Clarias anguillaris* treated with frog pituitary extracts and reared for eight weeks.

Weeks	T1	T2	T3	T4
1	47.20	68.40	29.60	62.00
2	43.50	64.50	5.00	55.00
3	43.50	64.50	5.00	55.00
4	43.50	64.50	5.00	55.00
5	43.50	64.50	5.00	55.00
6	43.50	64.50	5.00	55.00
7	43.50	64.50	5.00	55.00
8	43.50	64.50	5.00	55.00

T1 = 1 pituitary gland to 1 broodfish; **T2** = 2 pituitary glands to 1 broodfish; **T3** = 3 pituitary glands to 1 broodfish; **T4** = Control group treated with Ovaprim hormone.

Table 4. Weekly mean water quality parameters of water used to rear fry produced from *C. anguillaris* induced with frog pituitary gland extract.

Weeks	Temperature (°C)	pH	Dissolved oxygen (mgL^{-1})	Conductivity (µohm/s)
1	28.1 ± 2.40	6.81 ± 0.52	6.2 ± 0.45	111.7 ± 12.4
2	28.0 ± 2.40	7.02 ± 0.68	6.0 ± 0.41	79.5± 6.02
3	26.0 ± 2.25	6.98 ± 0.55	5.6 ± 0.38	90.1± 8.50
4	26.0 ± 2.25	6.82 ± 0.51	6.4 ± 0.48	85.2± 7.03
5	27.0 ± 2.35	6.42 ± 0.25	6.2 ± 0.45	108 ± 11.02
6	26.0 ± 2.25	6.94 ± 0.53	5.6 ± 0.38	165.1± 15.20
7	25.0 ± 2.05	6.97 ± 0.55	6.0 ± 0.41	174 ± 15.51
8	26.0 ± 2.25	6.85 ± 0.52	5.8 ± 0.39	201 ± 18.05

and mortality rates of the hatchlings reared for eight weeks. Mortality was highest during the second week in all the treatments after which the stock stabilised and no mortality was recorded again from third to eighth week. Survival was highest in treatment 2 (T2) which recorded 68.40%, followed by the control (T4) which recorded 62%, then treatment 1 (T1) which recorded 47.20% and treatment 3 (T3) which recorded 29.60% (Table 3). Nwadukwe (1993) obtained less than 50% survival in induced natural spawning of *H. longifilis* treated with frog pituitary gland. Results of water quality parameters analysis during incubation, hatching and rearing of the hatchlings are presented in Table 4. The values are within the tolerance range for hatching, survival and growth of fish as similarly reported by Marylin (1976), Viveen (1986) and Ayinla (1991).

CONCLUSION AND RECOMMENDATION

The experiment has indicated that hypophysation with frog pituitary gland can successfully induce breeding in *C. anguillaris* at a dosage of 0.5 mg/Kg of fish body weight. The dosage was obtained by combining two frog pituitaries for one broodfish of 450 to 550 g average weight. Frog pituitary gland treatment did not show any negative effect in induced breeding of *C. anguillaris*. Induced breeding of *C. anguillaris* with pituitary gland of African clawed frog (*Xenopus leavis*) at 2 pituitaries to one broodfish of 450 mean weight is hereby recommended.

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