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APPLICATION OF OVAPRIM ON VARYING SIZES OF MALE *Heterobranchus bidorsalis* TO HAND STRIP FOR MILT

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ABSTRACT

Matured African catfish (*Heterobranchus bidorsalis*), size ranging from 500-1600g total body weight (TBW) were procured from private fish farm to Federal University of Technology, (F.U.T.) Minna indoor fish hatchery, maintained for 2 weeks and fed with 40 % crude protein commercial diet. The male breeders were hand stripped after application of (Ovaprim Overdose Inducement (OOI) at 1 ml, 1.25 ml and 1.5 ml to obtain milt to fertilize eggs. Mean fecundity was (402473 ± 479.575^{a}). .The hatchlings bred from Conventional Method (CM) and .OOI were maintained for 12 weeks to determine survival and mortality rates. Percentage hatching and volume of milt extracted differed significantly ($P<0.05$) between CM and OOI with highest volume of milt extracted from CM (0.86 ± 0.017^{a}). The male species of *H. bidorsalis* could be re-used for further genetic studies after milt stripping without killing but adequate feeding is necessary to hasten recovery and development of the gonads. The inducement by application of ovaprim at 1.25 ml was most effective at 12 h latency period and temperature of 24-28^{0} C. This dosage is recommended for hand stripping of male breeders of *H. bidorsalis* for breeding.

Key Words: Artificial spawning, semen, *Heterobranchus bidorsalis*, synthetic hormone, breeding.

INTRODUCTION

African catfish is widely and easily cultured in Nigeria because of its adaptability to environmental factors such as low oxygen content. It is hardened, fast growth and widely distributed hence preferred by many which gave rise to its high demand. The demand-supply gap could be bridged by availability of fish seeds to stock the production ponds. According to Van Der Waal and Polling (1984), in the past

when *Clarias gariepinus* was artificially spawned, males had to be sacrificed to obtain semen for fertilization. Experiments have been conducted to strip milt from male *C gariepinus* by Nguenga *et al.* (1996) and Melo and Godinho (2006) but with limited success. This is because the reproductive system of *C. gariepinus* has accessory glands and seminal vesicles, which empty into the spermatic ducts and secret a viscous fluid consisting of mucopolysaccharide and protein (Eduardo *et al.*, 2001). They asserted that the anatomical structure of testis and other internal organs prevent the possible stripping of milt from male *Clariid.* Viveiros *et al.* (2001) attempted stripping of milt from male *C. gariepinus* after inducement with *Clarias* pituitary suspension (*Clarias*-PS), nGnHa or nGnHa+PS, it was not possible. It was reported that internal inspection of the fish reveals that the testes were still small (9.5±7 g), despite the male fish was 9 months old with a mean body weight (BW) of 1.5±0.2 g. Intra testicular semen volume was only 3.6±1.3 ml. However, stripping of milt from male *C. gariepinus* was possible after treatment with *Clarias*-PS (n-1) at 1 ml/kg and Ovaprim at 0.5 ml/kg (n-2) but milt was watery and bloody with no motile sperm cells after addition of water (Viveiros *et al.*, 2003). They reported that hatching rate was poor (4 %) with stripped fluid from *Clarias*-PS+Ovaprim treated fish after sampling 24 h latency time.

Milt cannot be collected by stripping the male African catfish, but could be obtained by sacrificing the fish and dissecting the testes (Viveen *et al.*, 1986). The authors stressed that male African catfish do not release milt (semen) under abdominal massage in captivity and need to be killed in order to obtain milt for induced breeding exercise. They asserted that though milt collection from male African catfish after killing is effective for breeding purposes, it reduces the number of males in the population which may bring shortage of male for further breeding and genetic improvement studies. Sometimes fish breeders have to kill 2-3 males before obtaining quality milt to fertilize egg. It is in light of this challenge that attempt was hereby made to hand strip male *H. bidorsalis* to obtain milt to fertilize egg after inducement with Ovaprim overdose on varying sizes of male and thereafter save live.

MATERIALS AND METHODS

Eighteen (18) (9 males and 9 females) matured brood stocks of *H. bidorsalis* with size range of 500-1600 g Total Body Weight (TBW) were purchased from private fish farm at New-Bussa and transported to the indoor hatchery of Federal University of Technology, (F.U.T.), Bosso Campus Minna, Niger State. Before stocking, the brood stocks were disinfected with 0.5 % salt bath (5 g NaCl/1 litre of water at temperature of 26-29 °C) according to the method of Tonguthai *et al.* (1993). After disinfection, the fish were acclimatized in the indoor nursery concrete ponds for 2 weeks. They were maintained under optimum temperature and fed with 40 % crude protein commercial diet. The brood stocks were examined for gonad development according to the method of Blythe *et al.* (1994) and reported by Yisa *et al.*

(2010). Males were examined for rigid and reddish infusion of the genital orifice and for females, genital orifice for reddish infusion, distension of the belly and release of eggs when gentle pressure was applied on the abdomen. The selected samples were properly maintained separately before being used for breeding. Ripe and matured female brood fishes were treated with a single dose of Ovaprim according to the method of Goudie *et al.* (1992) and hand stripped for eggs after a minimum latency period of twelve hours at water temperature of between 24-28^0 C. Eight (8.5 g) of total eggs stripped was used to fertilize eggs. Nine male brood stocks were treated with Ovaprim at a dose of 1 ml, 1.25 ml and 1.5 ml respectively to stimulate them to release milt through stripping technique. Two factors were considered-Conventional Method (CM) (sacrificing the male brood stocks to obtain milt) and Ovaprim Overdose Inducement (OOI) with three treatments (1 ml, 1.25 ml and 1.5 ml) each replicated three times.

Injection was administered intraperitoneally. The male fishes were anaethesized after latency period of 12 hours at water temperature of 24^0-28^0 C. When performing the stripping, an assistant held a clean and dry 20 ml glass beaker under the genital papilla to collect the milt. The stripped fishes were then left in fresh water to recover from the effect of the anaesthesia. The stripped volume of milt was weighed, observed under Binocular Olympus microscope to determine their motility before being used to fertilize eggs. The milt and eggs were mixed together gently with a plastic spoon for 2-3 minutes. Small quantity of saline solution was then poured onto the eggs to avoid sticking together. The fertilized eggs were rinsed with distilled water and taken to the incubator for incubation. Incubator made of net hapa with kakabarns, placed inside glass aquarium tanks filled with clean water was used for the purpose. Fertilized eggs were spread in a monolayer on the kakabarns in the incubator. Aeration was maintained by flow through system. The hapa was constructed from a coated nylon net with 1.5 mm mesh size.

When hatching was completed, the hapa with un- hatched eggs and shells was lifted out of the incubation tank and washed. 250 fry were stocked per glass aquaria tanks and reared for 12 weeks. After yolk absorption, the hatchlings were fed with decapsulated artemia for 8 weeks and thereafter they were fed with 0.5 mm 40 % crude protein Coppens fish feed. Water quality parameters including temperature, dissolved oxygen, pH and conductivity were monitored and maintained at optimum level. Percentage mortality and survival rates were determined with the following formula:

$$\text{Percentage Mortality} = \frac{\text{Cumulative Mortality}}{\text{Total number stocked}} \times 100 \quad (1)$$

$$\text{Percentage Survival} = \frac{\text{Cumulative Survival}}{\text{Total number stocked}} \times 100 \quad (2)$$

(After Bargenal, 1978) as adopted by Yisa *et al.* (2010)

The weights of the hatchlings were determined using sensitive electronic balance (P.E. mx Rady). Fecundity, percentage fertilization and hatchability were determined according to method described by (Oyelese, 2006) using the formulae:

$$\text{Fecundity} = \frac{\text{Total weight of stripped eggs}}{\text{Weight of eggs in sub-sample}} \times \text{Total No. of eggs in sub-sample} \quad (3)$$

$$\text{Percentage Fertilization} = \frac{\text{No. of fertilized eggs}}{\text{No. of eggs stripped}} \times 100 \quad (4)$$

$$\text{Percentage Hatchability} = \frac{\text{No. of fry}}{\text{No. of fertilized eggs}} \times 100 \quad (5)$$

Statistical analysis

One way Analysis of Variance (ANOVA) was used as statistical package for the analysis. Data obtained were pooled from the replicates and mean values, standard deviation as well as standard error of mean were calculated per treatment. Also Duncan Multiple Range Test was used for means separation. All differences in mean values of parameters were determined at P = 0.05 level of significance.

RESULTS

The fecundity, volume of milt extracted, percentage fertilization and hatchability from the brood stocks *H. bidorsalis* are presented in Table 1.

Table 1: Fecundity, volume of milt extracted, percentage fertilization and hatchability from *Heterobranchus bidorsalis* for induced breeding

Parameters	EXPERIMENTS	
	I (CM)	II (OOI)
Fecundity	359255±309.545^{b}	402473±479.575^{a}
Volume of milt extracted (ml)	0.86±0.017^{a}	0.37±0.010^{b}
Percentage fertilization	61.01±0.206^{b}	87.31±0.127^{a}
Percentage hatching	69.71±0.196^{b}	71.53±0.142^{a}

ab:means denoted by different superscripts along the same row for each specie differ (P<0.05) significantly.
Key: CM- Conventional Method, OOI: Ovaprim Overdose Inducement.

The Table showed that Ovaprim Overdose Inducement (OOI) gave the highest fecundity (number of egg released) 402473±479.575 eggs. The quantity of number of eggs released in CM and OOI was significantly different (P<0.05) from each other. The fecundity, volume of milt extracted, percentage fertilization and hatchability differed significantly (P<0.05) between CM and OOI as indicated in Table 1. Table 2 shows the morphometric measurement of injected and stripped male *H. bidorsalis*.

Table 2: Mean morphometric measurements of injected and stripped male *Heterobranchus bidorsalis*

Experiments	Ave.TBW before stripping (kg)	Ave.TBW after stripping (kg)	Standard length (cm)	Total length (cm)	No. of fish injected	No. of fish stripped	Mortality after stripping
I (CM)	2.5	2.3	55.60	61.00	9	9	-
II (OOI)	1.7	1.5	52.10	57.00	9	9	2

Key: Ave. TBW -Average Total Body Weight, CM- Conventional Method, OOI- Ovaprim Overdose Inducement.

Out of nine in number of fish that were injected and stripped, only two mortalities were recorded in experiment II. Average weight of eggs stripped from *H. bidorsalis* in relation to percentage fertilization, hatchability, water temperature and incubation are presented in Table 3.

Table 3: Average weight of eggs stripped in relation to percentage fertilization, hatchability, temperature, and latency and incubation periods of *Heterobranchus bidorsalis*

Expts.	ABW FS (kg)	AW ES (g)	AN ES	No. of EF	% F	ANFH	% H	AWT °C	LP (h)	IP (h)
I (CM)	2.5	24.00	358,973	218,720	60.93	152,600	69.77	26.73	12	29
II (OOI)	1.7	22.90	401,562	350,602	87.31	250,781	71.53	27.45	10	25

Key: Expts.-Experiments, ABW-Average Body Weight of Female Spawners, AWES-Average Weight of Egg Stripped, ANES-Average Number of Egg Stripped, NEF-Number of Egg Fertilized, % F-Percentage Fertilization, ANFH-Average Number of Fry Hatched, % H-Percentage Hatchability, AWT-Average Water Temperature, LP-Latency Period, IP-Incubation Period, CM-Conventional Method, OOI- Ovaprim Overdose Inducement.

Experiment II (OOI) had the highest percentage fertilization and hatchability of 87.31 % and 71.53 % respectively of the hatchings. The cumulative mean mortality/survival rates and their percentages for *H. bidorsalis* fingerlings reared for 12 weeks in indoor glass aquaria tank for experiment I (CM) and II (OOI) are presented in Table 4 and Table 5 respectively.

Table 4: Cumulative mean mortality/survival rates and percentages for *Heterobranchus bidorsalis* fingerlings produced through conventional induced breeding method (CM) and reared in indoor glass aquaria tank for 12 weeks

	Initial Stock Per Tank 250			
Period (Weeks)	**Mortality**	**% Cumulative Mortality**	**Survival**	**% Cumulative Survival**
1	8	3.20	242	96.80
2	16	6.40	234	93.60
3	29	11.60	221	88.40
4	54	21.60	196	78.40
5	73	29.20	177	70.80
6	92	36.80	158	63.20
7	96	38.40	154	61.60
8	98	39.20	152	60.80
9	99	39.60	151	60.40
10	100	40.00	150	60.00
11	101	40.40	149	59.60
12	102	40.80	148	59.20
Mean		28.93		71.06
±SEM		±2.010		±2.701
SD		2.843		2.989

Table 5: Cumulative Mean Mortality/Survival rate and percentage for *Heterobranchus bidorsalis* fingerlings roduced through Ovaprim Overdose Inducement breeding method (OOI) and reared in indoor glass aquaria tank for 12 weeks

	Initial Stock Per Tank 250			
Period (Weeks)	**Mortality**	**% Cumulative Mortality**	**Survival**	**% Cumulative Survival**
1	9	3.60	241	96.40
2	17	6.80	233	93.20
3	42	16.80	208	83.20
4	70	28.00	180	72.00
5	101	40.40	149	59.60
6	111	44.40	139	55.60
7	116	46.40	134	53.60
8	117	46.80	133	53.20
9	118	47.20	132	52.80
10	119	47.60	131	52.40
11	120	48.00	130	52.00
12	120	48.00	130	52.00
Mean		35.33		64.66
±SEM		±3.444		±3.944
SD		3.563		3.734

Table 6 shows the water quality parameters during the experiment. It showed that values of all the water quality parameters measured were within the tolerance range of warm water fishes. The fingerlings that were reared and managed, survived well within temperature range of 26.39 °C and 26.17 °C, pH range of 6.81 and 6.73, and dissolved oxygen 6.38 and 7.17 mg/L and conductivity 261.33 and 277.00µs/cm for experiment I and II respectively. The inducement by Ovaprim at 1 ml, 1.25 ml and 1.5 ml to different sizes of *H. bidorsalis* brood stock stimulated them to release milt. Two (2) experimental fishes that were induced with Ovaprim dose at 1.5 ml died after hand stripping. This might be attributed to stress as a result of over dose. The volume of milt released from hand stripping according to size and dosage were 0.10 ml (500-650 g, 1 ml dose), 0.14 ml (700-950 g, 1.25 ml dose) and 0.13 ml (1100-1600 g, 1.5 ml dose) from *H. bidorsalis* giving a total milt volume obtained to be 0.37 ml.

DISCUSSION

Fecundity increases with body weight and size hence larger fish has higher fecundity. The observed higher fecundity with increased body weight and size of brood stocks concurred with observations made by Anene and Keke (2009). The highest percentage fertilization and hatching might be attributed to eggs and milt quality and viability. The eggs were dark brown in colour and were not watery, an indication of good quality and viability. The volume of milt extracted was higher in CM, suggestive of the fact that the brood stocks were sacrificed to remove testis to fertilize eggs thus much milt was squeezed out unlike in OOI, milt was only hand stripped hence only little quantity was obtained. The decrease in total body weight after stripping indicated that eggs inside body cavity contributed to body weight of fish and when stripped the weight reduced. The ease of stripping might be responsible for the less mortality of brood stocks recorded during the study indicating less stress on the fish before and after the stripping exercise.

Table 6: Grand Mean values of water quality parameters of the reared *Heterobranchus bidorsalis* fingerlings in indoor glass aquaria tank for 12 weeks

Experiments	Dissolved Oxygen (Mg/l)	Temperature (^{0}C)	pH	Conductivity (µs/cm)
I (CM)	6.38	26.39	6.81	261.33
II (OOI)	7.17	26.17	6.73	277.00

KEY: CM-Conventional Method, OOI-Ovaprim Overdose Inducement.

The report of Aiyelari *et al.* (2007) indicated that brood stock mortality after stripping can result due to stress. It was observed that as temperature decreased latency and incubation period increased. This observation agrees with observation made by Janssen (1987). Hence the high fertilization and hatchability recorded in this study could be attributed to optimum water temperature, egg viability and good milt quality. Oyelese (2006) stressed the importance of water temperature, quality eggs and milt as some determinant of fertilization and hatchability rates in artificially induced breeding of catfish.

Survival rate was higher in conventional breeding method probably because the eggs were more viable and qualitative than non-conventional Ovaprim Overdose Inducement method and the stock stabilized with weeks. The relative high percentage mortality observed in experiment II (OOI) might be due to transition from yolk sac feeding to exogenous feeding as observed by Nlewadin and Madu (2004). Although 0.5 ml of Ovaprim per body weight of fish is the recommended dose to inject into fish body to stimulate maturation, ovulation and spawning (Aluko and Aremu, (1997) and adopted by Tsadu (2002), the 1 ml, 1.25 ml and 1.5 ml used in this study to stimulate the male *H. bidorsalis* after a latency period of about twelve hours released sum volume of milt to be 0.37 ml. The volume of milt hand stripped at the dose Ovaprim 1.25 ml was higher probably because it enhanced sperm maturity and release. The mortality after hand stripping might be as a result of stress emanated from over dosage. The result of this study was at variance with the report of Viveen *et al.* (1986), Nguenga *et al.* (1996) and Melo and Godinho (2006) that milt cannot be collected by hand stripping the male African catfish, but could be obtained by sacrificing the fish and dissecting the testes. The result also was at variance to the work of Viveiros *et al.* (2001) and (http://cdserver2.ru.za/cd/catfish/pictures/slo.jpg 1/1/2002 who made attempt stripping milt from male *C. gariepinus* after inducement with *Clarias* pituitary suspension (*Clarias*-PS), nGnHa or nGnHa+PS but was not possible. However, the result concurred with the report of Van Der Waal and Polling (1984) who have recorded successes achieved in hand stripping male African catfish after administration of hypophysis extract, milt was successfully stripped and ova fertilized, resulting in hatching success of up to 98 percent. Also, the result corroborated the work of Lamai (1996) who successfully hand stripped male African catfish, *C. gariepinus* to obtain milt to fertilize eggs after applying inducing agents.

CONCLUSION

From the foregoing, it could be concluded that 1.25 ml of Ovaprim administered as inducement on male *H. bidorsalis* released 0.14 ml milt after hand stripped to fertilize eggs was most effective and therefore recommended.

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