Hand stripping of Male *Clarias g* Ovaprim at Varying Levels.

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Abstract

Matured African catfish (Clarias gariepinus), size ranging from 200-1600g total body weight (TBW) were procured from a private fish farm and transported in perforated 50 litre water holding capacity jerry can to Federal University of Technology, (F.U.T.) Minna, Bosso campus indoor fish hatchery and maintained for 2 weeks. They were fed with 40 % crude protein commercial diet with good water quality management before being used for breeding. 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. Fecundity increased with body weight and hence larger fish had higher fecundity and differed significantly (P<0.05) from each other. Mean fecundity was (280744±302). 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±0.006[a]). CM gave the highest percentage survival (75.20 %) though not significantly different (P>0.05), and with ±SEM (2.232) and SD (2.923) of the bred fingerlings that were managed for 12 weeks. The male species of Clarias gariepinus could be re-used for further genetic studies after milt stripping. Milt can be stripped without killing the male but proper and 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 10 h latency period and temperature of 25-29[0] C. This treatment or dosage is hence recommended for hand stripping of male breeders of Clarias gariepinus for breeding.

Key words: Hand stripping, milt, *Clarias gariepinus*, Ovaprim, breeding.
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Introduction.

Production of African catfish in aquaculture system is to ensure mass production and sustainability above the level that would be produced naturally. The high demand for *Clarias gariepinus* fingerlings for culture world- wide necessitates induced artificial reproduction. One major factor for successful aquaculture practice is the availability of fish fingerlings but in an attempt to achieve this, male brood stocks of catfish has to be sacrificed to obtain the milt to fertilize eggs (Hetcht *et. al.*, 1982). According to Viveen *et. al.* (1986) it has not been possible to strip milt from male catfish *Clarias gariepinus* due to anatomical structure of the testis. Their milt is obtained by sacrificing the male and dissecting the testes. This view was however at variance with observation made by Nguenga *et al.* (1996) and Melo and Godinho (2006) 'who described technique for collecting milt from catfish *Heterobranchus longifilis* without sacrificing the male. Although studies have shown that 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 proven male for further breeding and genetic improvement studies. Sometimes fish breeders and hatchery managers have to kill at least 3 males before obtaining male that has good milt despite the fact that the reddish genital papilla which indicates matured male has been observed. It is in light of this that attempt is hereby made to hand strip male *Clarias gariepinus* to obtain milt to fertilize egg via inducement by application of Ovaprim at different dose on varying brood stock sizes.

Materials and Methods.

Eighteen (18) samples, (9 males and 9 females) of ripe and matured broodstocks of *Clarias gariepinus* with size range of 200-1600g Total Body Weight (ATBW) 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. Before stocking, the brood stocks were disinfected with 0.5 % salt bath (5 g NaCl/1 litre of water at temperature of 25-27[0] C) for fifteen minutes. 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) as reported by Yisa *et. al.* (2010). Males were examined for rigid and reddish infusion of the genital papilla and for females, genital papilla 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 fish were treated with a single dose of hormone (Ovaprim) according to the method of Goudie *et. al.* (1992) and hand stripped for eggs after a minimum latency period of ten hours at water temperature of between 25-29^0 C. Eight (8 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. Male fish were anaethesized after latency period of 10 hours at water temperature of 25^0-29^0 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 fish 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. Fertilized eggs were rinsed with distilled water and taken to the incubator. Incubator was made of incubating net hapa with kakabarns, placed inside glass aquarium tanks filled with clean water was used for the purpose. The eggs were spread in a monolayer on the kakabarns and aeration 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, were fed with 0.5 mm floating feeds (Coppens) at 40 % crude protein. Water quality parameters including temperature, Dissolved Oxygen, pH and conductivity were monitored and maintained at optimum level. Percentage mortality and survival rates were determined using these formulae:

$$\text{Percentage Mortality} = \frac{\text{Cumulative Mortality}}{\text{Total number stocked}} \times 100$$

And

$$\text{Percentage Survival} = \frac{\text{Cumulative Survival}}{\text{Total number stocked}} \times 100$$

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

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

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

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

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

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

Results.

Fecundity, volume of milt extracted, percentage fertilization and hatchability from the brood stocks *Clarias gariepinus* are presented in Table 1. It showed that CM gave the highest fecundity (number of egg release) 280744±302.171^a. The number of egg released in CM and OOI was significantly different (P<0:05) from each other. Similarly, CM recorded highest percentage hatching. This is probably because the net hapa withholds normal size fish eggs and egg shell but allowed the hatchlings swim out into the incubation tank hence successful hatching. The fecundity, volume of milt extracted and percentage 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 *Clarias gariepinus*. Out of nine (9) fish that were injected and stripped, only 3 mortality was recorded in experiment II. Average weight of eggs stripped from *Clarias gariepinus* in

relation to percentage fertilization, hatchability, water temperature and incubation are presented in Table 3. Experiment II (OOI) had the highest percentage fertilization of 71.50 % while experiment I had highest percentage hatching of 93.55 %.

Table 1: Fecundity, Volume of milt extracted, Percentage Fertilization and Hatchability from *Clarias gariepinus* for Induced breeding.

Parameters	EXPERIMENTS I (CM)	II (OOI)
Fecundity	280744±302.171[a]	276446±284.800[b]
Volume of milt extracted (ml)	0.86±0.006[a]	0.41±0.015[b]
Percentage fertilization	71.91±0.641[a]	71.74±0.155[a]
Percentage hatching	93.68±0.182[a]	69.79±0.299[b]

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

Table 2: Mean morphometric measurements of injected and stripped male *Clarias gariepinus*.

Experiments	Ave.TBW before stripping (g)	Ave.TBW after stripping (g)	Standard length (cm)	Total length (cm)	No. of fish injected	No. of fish stripped	Mortality after stripping
I (CM)	400	370	32.60	37.60	9	9	-
II (OOI)	550	520	35.00	40.10	9	9	3

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

The cumulative mean mortality/survival rates and their percentages for *Clarias gariepinus* 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 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 of 25.51 and 27.55^0 C, pH 6.87 and 6.89, and Dissolved Oxygen 7.62 and 7.72 mg/l for experiment I and II respectively. The inducement by Ovaprim at 1 ml, 1.25 ml and 1.5 ml to different sizes of *Clarias gariepinus* brood stock stimulated them to release milt. Three (3) experimental fish 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 0.12 ml (200-350 g) (1 ml dose), 0.15 ml (400-550 g) (1.25 ml dose) and 0.14 ml (600-1600 g) (1.5 ml dose) from *Clarias gariepinus* giving a total milt volume of 0.41 ml.

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

Expts.	ABW FS (g)	AW ES (g)	AN ES	No. of EF	% F	ANFH	% H	AWT 0C	LP (h)	IP (h)
I (CM)	400	23.00	280,306	201,600	71.92	188,600	93.55	25.51	11	29
II (OOI)	550	22.00	276,780	197,898	71.50	136,890	69.17	27.55	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.

Table 4: Cumulative Mean Mortality/Survival rates and percentages for *Clarias gariepinus* fingerlings produced through conventional induced breeding method (CM) and reared in indoor glass aquaria tank for 12 weeks.

Period (Weeks)	Initial Stock Per Tank 250 Mortality	% Cumulative Mortality	Survival	% Cumulative Survival
1	6	2.40	244	97.60
2	13	5.20	237	94.80
3	18	7.20	232	92.80
4	25	10.00	225	90.00
5	28	11.20	222	88.80
6	32	12.80	218	87.20
7	39	15.60	211	84.40
8	46	18.40	204	81.60
9	54	21.60	196	78.40
10	58	23.20	192	76.80
11	61	24.40	189	75.60
12	62	24.80	188	75.20
Mean		14.73		85.26
±SEM		±1.211		±2.232
SD		1.973		2.923

Table 5: Cumulative Mean Mortality/Survival rates and percentages for *Clarias gariepinus* fingerlings roduced through Ovaprim Overdose Inducement breeding method (OOI) and reared in indoor glass aquaria tank for 12 weeks.

Period (Weeks)	Initial Stock Per Tank 250 Mortality	% Mortality	Cumulative Survival	% Cumulative Survival
1	4	1.60	246	98.40
2	9	3.60	241	96.40
3	13	5.20	237	94.80
4	22	8.80	228	91.20
5	27	10.80	223	89.20
6	35	14.00	215	86.00
7	40	16.00	210	84.00
8	47	18.80	203	81.20
9	55	22.00	195	78.00
10	67	26.80	183	73.20
11	75	30.00	175	70.20
12	77	30.80	173	69.20
Mean		15.70		84.30
±SEM		±1.244		±2.231
SD		1.984		2.901

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

Experiment	Dissolved Oxygen (mg/l)	Temperature (°C)	pH	Conductivity (µs/cm)
I(CM)	7.62	25.51	6.87	260.83
II(OOI)	7.72	27.55	6.89	278.00

CM-Conventional Method, OOI-Ovaprim Overdose Inducement.

Discussion.

Fecundity increases with body weight and size hence larger fish has higher fecundity. The *Clarias gariepinus* brood stocks used in this study had an average Total Body Weight (TBW) of 550 g hence higher fecundity (280744±302.171[a]). A similar observation was made by Tsadu *et. al.* (2009) where they obtained fecundity from three different brood stock sizes 42,972, 50,925 and 80,878 eggs for 250g, 350g and 500g brood stocks respectively. The highest percentage fertilization and hatching (Table 1) might be attributed to egg and milt quality and viability. Eggs were dark brown in colour and were not watery, an indication of good quality and viability. Volume of milt extracted (0.86ml±0.006[a]) was higher in CM, suggestive that the brood stocks were sacrificed to remove testis to fertilize eggs thus much milt was squeezed out unlike in OOI, where milt was hand stripped hence little quantity was obtained.

The decrease in total body weight after stripping indicated that eggs inside body cavity contribute to body weight of fish and when stripped the weight reduces. 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 2). The report of Aiyelari *et. al.* (2007) indicates that brood stock mortality after stripping can result due to stress. It was observed that as temperature decreases latency and incubation period increases (Table 3). 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 quality milt. Oyelese (2006) stressed the importance of water temperature as a determinant of fertilization and hatchability rates in artificially induced breeding of *Heterobranchus bidorsalis*. 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) was as a result of cannibalism by fast growers (shooters) among the hatchlings which was not noticed in good time. This was because the carcasses of the dead fry were not always found in the tanks hence assumed to be cannibalized. This was similar to observation made by Tsadu *et. al.* (2009) who recorded high mortality in their study on effects of net hapa on the survival of *Clarias gariepinus* (Burchell, 1822) fry from different brood stock sizes. 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 *Clarias gariepinus* to

release milt after a latency period of about eleven hours gave a desired result. The sum volume of milt hand stripped from male *Clarias gariepinus* was 0.41 ml. The volume of milt hand stripped at the dose Ovaprim 1.25 ml was higher probably because it enhances 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 that of Viveen *et. al.* (1986), Nguenga *et. al.* (1996) and Melo and Godinho (2006) who had indicated that milt cannot be collected by hand stripping male African catfish, but could be obtained by sacrificing and dissecting the testes. However, the result agrees with that of Van Der Waal and Polling (1984) who recorded successes achieved in hand stripping male African catfish after administration of hypophysis extract.

Conclusion.

It could be concluded that 1.25 ml of Ovaprim administered as inducement on male *Clarias gariepinus* released 0.15ml milt after hand stripped to fertilize egg was most effective and therefore recommended for use.

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