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Full Length Research Paper

Induced breeding of *Clarias gariepinus* using non-conventional method of abdominal incision

Yisa, T.A., S.L. Lamai, Tsadu, S.M. and R.J. Kolo

Department of Water Resources, Aquaculture and Fisheries Technology, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State, Nigeria.

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The experiment was conducted using ripe and matured African catfish (*Clarias gariepinus*), size ranging from 400-650g average total body weight (TBW). They 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 hatchery fish farm. The fish samples were maintained for 2 weeks and observed for ripeness and maturity under optimum temperature and fed with 40% crude protein commercial diet with good water quality management before being used for breeding. Incision was made on the dorso-ventral part of the body through Abdominal Incision Method (AIM) to extract milt to fertilize eggs. Fecundity increased with body weight and hence larger fish had higher fecundity and significantly different (P<0.05) from each other. The spent spawners after operation survived on gradual recuperation post- surgery. The variation in incision length 3.40 cm was most effective and healing and recuperation was within 14 days post-surgery. The hatchlings bred from Conventional Method (CM) and AIM was maintained to determine survival and mortality rates for 12 weeks. Volume of milt extracted, percentage fertility and hatching differed significantly (P<0.05) between CM and AIM with highest volume of milt extracted from CM to be (0.86 ± 0.006^{a}). The recovery and re-use time for male *C. gariepinus* was 45 days post-surgery. Male *C. gariepinus* could be used up to 6 times in a year for the purpose of breeding. CM gave the highest percentage survival (75.20 %) though not significantly different (P>0.05), and with ±SEM (0.516) and SD (1.789) of the bred fingerlings that were managed for 12 weeks. The specie of *C. gariepinus* could be re-used for further genetic studies after abdominal incision breeding method and milt can be extracted without killing the male brood stock but proper and adequate feeding is necessary to hasten the maturation and development of the gonads. Variation in incision length 3.40 cm is recommended for abdominal incision to extract milt from male *C. gariepinus* for breeding.

Key words: Incision, milt, *Clarias gariepinus*, recuperation and breeding.

INTRODUCTION

African catfish is one of the highly priced food fish in Nigeria and most parts of the world. They are widely cultured in Nigeria owing to their high market value, fast growing rate and ability to withstand adverse pond conditions especially low oxygen content. One of the constraints in expanding *Clariid* culture in Nigeria was inadequate quality fish seed. Pandey (2004) stressed that fish breeders who play a vital role in aquaculture practice need to make use of brood fish that is viable with good vigor and disease free and disease resistant strain that produces healthy off-springs. In Carp species milt in mature ripe running males is released by a slight pressure on the abdomen and the semen hand stripped on to the eggs, but the male spawners of *Clarias gariepinus* cannot be handled in this way as pressure on the gonads releases the milt into lobes of the seminal vesicles and not directly through the genital papilla.

Male of *C. gariepinus* therefore has to be sacrificed and have their gonads removed for fertilization. In view of the above an attempt is hereby being made to reverse the practice by adopting a simple scientific technique (abdominal incision method) where the life of genetically

*Corresponding author. E-mail: atyisa@yahoo.com Tel: 08068169704.

viable male brood fish is spared after extracting milt from gonads for further breeding and genetic improvement studies.

MATERIALS AND METHODS

Twelve (12) live samples, (6 males 6 females) of ripe and matured brood stocks of *C. gariepinus* with varying size range measured in grams were purchased from private fish farm at New-Bussa and transported in perforated 50 liter jerry can water holding capacity to the indoor concrete ponds in the hatchery of Federal University of Technology (F. U. T.) Minna.

Before stocking, the live sample brood fish was disinfected with 0.5 % salt bath 5 g NaCl / 1 litre water temperature of 25-28^0 C according to the method of Tonguthai et al., (1993). Bathing was done by dipping the fish into the solution for fifteen minutes. Salt bath is effective against ecto parasites, bacteria and fungi and also reduces stress (Tonguthai et al., 1993). After disinfection, the fish were acclimatized in the nursery concrete ponds in the fish farm of F. U. T. Minna for 2 weeks. They were maintained under optimum temperature and fed with 40 % crude protein commercial diet. Ripe and matured broodstock were carefully selected and examined for gonad development according to the method of Blythe et al., (1994). 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 by ensuring good water quality management and adequate feeding before being used for breeding. The ripe and matured brood fish were treated with a single dose of hormone (Ovaprim) according to the method of Goudie et al., (1992). Injection was given intraperitoneally. Females were hand stripped for eggs after a minimum latency period of ten hours with water temperature of between 25-27^0 C. Surgical operation was carried out on the matured male fish on the dorso-ventral part of abdominal cavity where testes is situated. To reduce stress during the surgical operation, each fish was injected intraperitoneally using general anaethesia Ketamine HCl according to fish size and weight (0.1ml/kg body weight) before operation. Each fish became inactive and unconscious within 30 seconds and remained so until after 25-30 minutes. The fish were placed dorsally on a wet disinfected white cloth spread on clean table with the head covered with a piece of wet clean towel. The surface of the abdomen was disinfected with methylated spirit (40 %) before incision was made on the dorso-ventral side of the abdomen. The incision was extended towards the head with sterilized surgical scissors 3-5 cm long for all replicates and assisted by a person to expose the internal organs including the testes which remained intact. The digestive tracts were pushed aside to reveal the testes. Milt was extracted from the testes using sterilized 2 ml size syringe and needle. Milt volume was weighed and then observed under Binocular Olympus microscope to determine their motility before been used to fertilize the eggs. About 8g of total eggs stripped were used to fertilize eggs. The incisions were sutured using simple interrupted suture pattern with catgut chromic 2/0 stitch. The surgery on each fish lasted for 25-30 minutes. Each incised fish was placed in a plastic trough with well aerated fresh water to enable them recover. After recovery from the anesthesia, each fish was placed into separate concrete tank (2 m x 2 m x 1 m) containing Ox tetracycline at dosage of 50 mg/L for 8 days without food under intensive care and monitored for recuperation, recovery and re-use time. The surgery and milt collection were performed in triplicates. Sterile procedure and aseptic technique was observed to avoid infection. The recovery and re-use time was determined by regularly examining the genital papilla for ripeness, rigid and reddish infusion. The milt and eggs were mixed together gently with a plastic spoon for 2-3 minutes. Small quantity of saline solution was then pour onto the eggs to avoid sticking together. The fertilized eggs were rinsed with distilled water and introduced into the incubation chamber for incubation. Incubating hapa with kakabarns, placed inside incubation tanks that contain 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. The net withholds normal size fish eggs and egg shell but allowed the hatchlings swim out into the incubation tank. 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 at 25 litre water measuring 0.6 m length, 0.3 m width and 0.2 m depth and reared for 12 weeks. The hatchlings were fed with hatched artemia cysts after yolk absorption thereafter the fry were fed with floating feeds (Coppens) at 40 % crude protein. Water quality parameters such as temperature, Dissolved Oxygen, pH and conductivity were monitored and maintained at optimum level. Mortality and survival rates were determined.

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

And

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

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

The morphometric measurement of the hatchlings was determined using sensitive electronic scale (P.E. Balance mx Rady 300 g max).

Also, percentage fertilization, hatchability and fecundity were determined according to method described by

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

Parameters	EXPERIMENTS I (CM)	II (AIM)
Fecundity	280744±302.171[b]	324400±539.259[a]
Volume of milt extracted (ml)	0.86±0.006[a]	0.63±0.015[b]
Percentage fertilization	71.91±0.641[b]	93.32±0.535[a]
Percentage hatching	93.68±0.182[b]	96.39±0.492[a]

ab :means denoted by different superscripts along the same row for each specie differ (P<0.05) significantly.

Key: CM- Conventional Method, AIM- Abdominal Incision Method.

Table 2. Mean morphometric measurements of injected and stripped *Clarias gariepinus* used for induced breeding.

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	12	12	2
II (AIM)	620	600	39.00	44.20	12	12	3

Key: Ave. TBW = Average Total Body Weight, CM-Conventional Method, AIM- Abdominal Incision Method.

(Oyelese, 2006) using the formulae:

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

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

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

The hatchability rates of eggs were determined based on the method of percentage in hatched eggs as described by Aluko and Ali (2001).

Experimental Design

Completely Randomized Design (CRD) was used for the experiment.

Statistical analysis

One wayAnalysis of Variance (ANOVA) was used as statistical package for the analysis. Standard deviation and standard error of mean was calculated per treatment. Also Duncan Multiple range Test was used for mean separation. All differences in mean values of parameters was determined at P = 0.05 level of significance.

RESULTS

The fecundity, volume of milt extracted, percentage fertilization and hatchability from the brood stocks of *C. gariepinus* is presented in Table 1. The table showed that Abdominal Incision Method (AIM) gave the highest fecundity (number of egg release) 324400±539.259[a]. The quantity of number of egg released in CM and AIM was significantly different (P<0.05) from each other. Similarly, AIM recorded highest percentage fertilization and hatchability. The fecundity, volume of milt extracted, percentage fertilization and hatchability differs significantly (P<0.05) between AIM and CM as observed in Table 1. Table 2 shows the morphometric measurement of injected and stripped *C. gariepinus*. Out of twelve numbers of fish that were injected and stripped, only 2 and 3 mortality were recorded in experiment I and II. Average weight of eggs stripped from *C. gariepinus* in relation to percentage fertilization, hatchability, water temperature and incubation are presented in Table 3. Experiment II (AIM) had the highest percentage fertilization and hatchability of 92.36 % and 97.08 % of the bred *C. gariepinus* hatchlings respectively.

The Incision Point (IP) on the dorso-ventral side of the abdomen (gonad position) was carried out to expose the testis which is situated in the dorso-ventral part of the abdominal cavity. The milt was extracted from the testis using 2 ml syringe and needle tilted at an angle of about $30\text{-}40^0$ to extract the milt.

The variation of incision length made on the dorso-ventral side of the abdomen on the position of testis and length of day for healing are presented in Table 4. The variation

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

Expts.	ABW of FS (g)	AW of ES (g)	AN of ES	No. of EF	% F	AN FH	% H	AWT (^{0}C)	LP (h)	IP (h)
I (CM)	400	23.00	280,306	201,600	71.92	188,600	93.55	25.51	11	29
II (AIM)	620	25.00	323,420	298,700	92.36	289,980	97.08	27.09	10	26

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, AIM-Abdominal Incision Method.

Table 4. Variation of incision length on male brood stock of *Clarias gariepinus.*

Species type	Incision Length Variation (CM) Length I	Length II	Length III	No. of fish incised	Length of Day for Healing
Clarias gariepinus	2.50	3.40	4.00	12	14

Plate I: The healed stitched incision point on the body of survived male brood stock *Clarias gariepinus* (three weeks after). Key: HSIP: The Healed Stitched Incision Point.

in incision length was to determine the best length that could reveal the testes properly to extract milt. Plate I shows the gradual and progressive healing and recuperation process of the incision point 3 weeks post-surgery. The eight days hunger post-surgery that the fish were subjected to facilitate the healing process because pressure on the incision point and water pollution was reduced.

The recovery and re-use time was 45 days post-surgery. The genital papilla of the male spent spawners became reddish particularly at the tip and turgid at 45 days indicating that it was ripe and mature to spawn again.

Table 5. 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.

Initial Stock Per Tank 250				
Period (Weeks)	**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 6. Cumulative Mean Mortality/Survival rates and percentages for *Clarias gariepinus* fingerlings produced through abdominal incision induced breeding method (AIM) 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	15	6.00	235	94.00
3	23	9.20	227	90.80
4	29	11.60	221	88.40
5	39	15.60	211	84.40
6	47	18.80	203	81.20
7	57	22.80	193	77.20
8	64	25.60	186	74.40
9	74	29.60	176	70.40
10	92	36.80	158	63.20
11	102	40.80	148	59.20
12	108	43.20	142	56.80
Mean		21.93		78.06
±SEM		±1.967		±2.010
SD		2.423		2.789

Healing of the incision point occurred within 14 days post-surgery. The cumulative mean mortality/survival rates and their percentages for *C. gariepinus* fingerlings reared for 12 weeks in indoor glass aquaria tank for experiment I (CM) and II (AIM) are presented in Table 5 and Table 6 respectively. Table 7 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 25.51 and 27.09^0 C, pH range 6.87 and 6.89, and Dissolved Oxygen range 6.38 and 7.62 and 7.59 mg/l for experiment I and II respectively.

Table 7. Grand Mean values of water quality parameters of the reared *Clarias gariepinus* fingerlings in indoor glass aquaria tank for 12 weeks.

Experiments	Dissolved Oxygen (Mg/l)	Temperature (^{0}C)	pH	Conductivity (µs/cm)
I (CM)	7.62	25.51	6.87	260.83
II (AIM)	7.59	27.09	6.89	118.52

Key: Cm-Conventional Method, Aim-Abdominal Incision Method.

DISCUSSION

Fecundity increases with body weight and size hence larger fish has higher fecundity. The *C. gariepinus* brood stocks used in this study had an average Total Body Weight (TBW) of 620 g hence higher fecundity (324400). This observation is in accordance with observations made by Anene and Keke (2009). The highest percentage fertilization and hatching (Table 1) might be attributed to egg 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 (0.86ml±0.06) 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 AIM, milt was only extracted hence only 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 similar observation made by Janssen (1987). Hence the high fertilization and hatchability recorded in this study could be attributed to optimum water temperature and egg and milt quality and viability. Oyelese (2006) stressed the importance of water temperature as a determinant of fertilization and hatchability rates in artificially induced breeding of *C. gariepinus*.

The incision made on the abdominal region (gonad position) was aimed at exposing the testes to extract milt using 2 ml size syringe and needle to fertilize egg without sacrificing the male. This was similar to the work of Diyaware et al., (2010) when the ablation of the testes was carried out after surgical operation on *Clarias anguillaris* to collect milt to fertilize egg, determination of the testes regeneration period and assessment of potency of the milt after regeneration. The essence of the use of anaesthetic agent was similar to the report of Diyaware et al., (2010) that fish were anaesthesized to reduce stress and ensured calmness during the surgical operation.

The length variation of incision on the male brood stock was aimed at determining the appropriate length that would expose testes properly to extract milt from it (Table 4). This method was however at variance with the method adopted by Diyaware et al., (2010) where in their study determination of testes regeneration for *C. anguillaris* after milt collection through ablation, three quarter (3/4) of the testes were removed and placed in petri-dish containing saline solution (0.9 % NaCl) and preserved in refrigerator at 4^0 C. Out of the three length variation incision (Table 4) 3.40 cm length revealed the testes enough and properly to extract milt. Healing of the incision point (cut) in *C. gariepinus* occurred within 14 days post-surgery (Table 4). This concurs with result of Diyaware et al., (2010) who reported that healing of the cut occurred within 14 days in male *C. anguillaris* but contrary to the work of Nguenga et al., (1996) who reported that cicatrization (healing) of the cut occurred within 30 days in male *Heterobranchus longifilis*. This could be due to differences in climatic condition and species of fish. The hunger that the fish were subjected to for 8 days was contrary to the work of Nguenga et al., (1996) and Diyaware et al., (2010). This however, reduces pressure on the incision point and healing process was facilitated.

The economical recovery and re-use time (45 days) for *Clarias gariepinus* brood stock that underwent surgical operation to extract milt to fertilize egg was at variance with the result of Diyaware *et al.,* (2010), study on testes ablation and regeneration which they reported to be 90 days in *C. anguillaris*. The difference in recovery and re-use time in male African catfish might be due to factors such as technique to collect milt, difference in fish species, climatic condition and feed after post-surgery.

Survival was higher in CM (Table 5) probably because the eggs were more viable and milt qualitative than AIM and the stock stabilized with weeks. Egg quality and viability resulted in vigour hatchlings which increases chances of high survival. The relative high percentage mortality (43.20 %) observed in AIM (Table 6) might be due to transition from yolk sac feeding to exogenous feeding as observed by Nlewadin and Madu (2004). Values of all the water quality parameters measured were within the tolerance range of warm water fishes (Table 7).

According to Huisman and Richter (1987), *C. gariepinus* maintained at 25-27^0 C in the Netherlands showed uninterrupted gonad development. Fish are able to survive in waters with pH range of 3.5-10 but the desirable range for good growth is from 6.5-9.00 (Ofojekwu, 1990). Pandey (2004) reported that pH range 6.5-9.5 is suitable for fish growth and production. The Dissolved Oxygen pattern corroborates that reported by (Adekoya *et al.,*, 2004) that dissolved oxygen less than 3ppm causes discomfort to fish and lead to death.

CONCLUSION

From the study it could be concluded that the incision made on the abdominal cavity (gonad position) on the body of *C. gariepinus* for the purpose of extracting milt to fertilize egg was successful. The recovery and re-use time for male *C. gariepinus* was 45 days post-surgery. This implies that it is possible to obtain milt from male *C. gariepinus* 5-6 times in one year through abdominal incision method for breeding purpose and later the spent males can be sold live. Male African catfish (*C. gariepinus*) could be re-used several times for further genetic studies through abdominal incision breeding method and milt can be collected without killing the fish.

RECOMMENDATION

Further study should be conducted to attempt to extract milt from the testes with syringe and needle without incision into body cavity.

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