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Determination of Testes Regeneration Period for African Catfish (*Clarias anguillaris*) after Milt (Semen) Collection Through Ablation

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Abstract: The testes regeneration period of male African catfish (*Clarias anguillaris*) after milt collection through ablation was studied at 30 days intervals for the period 120 days with the intention to determine the testes regeneration period. Milt was collected from twelve matured males of *Clarias anguillaris* and the testes were left to regenerate. Testes weight, milt volume, Gonadosomatic Index (GSI), percentage fertilization and hatching rates increased with increase in testes regeneration periods. The highest testes weight, milt volume, gonadosomatic index, percentage fertilization and hatching rates were obtained at 120 days (4 months) regeneration period. There was no significant differences ($p>0.05$) between 120 and 90 days testes regeneration periods. Milt from African catfish *Clarias anguillaris* can be obtained without killing the male. The economical time to re-use male *Clarias anguillaris* after testes regeneration is 90 days (3 months). Fish breeders, hatchery operators as well as fish geneticist in Nigeria can therefore use male African catfish up to three times in a year for breeding purposes and for further genetic studies.

Key words: Ablation, male *C. anguillaris*, milt collection, testes regeneration

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. They are endemic to African and have wide geographical distribution from the Middle East in the North Orange river in South Africa and Northern Africa (Teugels, 1984). Due to their hardiness and adaptability, they thrive well in variety of climatic conditions including Europe Netherlands, Germany and Belgium, (Verreth, 1993). Clariids are potamodromous, which means they migrate within streams and rivers (Teugels, 1984). One of the major constrains in expanding Clariid culture in Nigeria was inadequate quality fish seed. Techniques of induce breeding have now been developed to produce fast growing Clariid fingerlings. Female are induced with various hormones and stripped 9-12 h latency period. To obtain milt for fertilizing female eggs, males are generally killed before testes are removed and squeezed to release the milt Steyn and van Vuren (1987) and Legendre (1996), such practice compromise attempts for selective breeding and other genetic studies.

The testes of African catfish (Clariids) are situated in the dorsal part of the abdominal cavity. They are lobular and appear whitish in colour. They are covered by the intestine in such a way that application of pressure cannot easily release milt (semen). Several techniques have been developed by several authors Nguenga *et al.* (1996) Viveiros *et al.* (2001), Hiemstra *et al.* (2005) and Pavlov (2006) to preserve fish semen so as to preserve the genetic quality of valued fish. Experiments have also been carried out to increase milt volume and to strip milt from male by Nguenga *et al.* (1996) and Melo and Godinho (2006) but with limited success. This is because their reproductive system 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). The seminal vesicle and other internal organs prevent the possible stripping of milt from Clariid male testes.

Viveiros *et al.* (2001) attempted stripping of milt form male *Clarias gariepinus* after inducement with Clarias pituitary suspension (*Clarias*-PS), nGnHa or nGnHa+PS, it was not possible. Internal inspection of the fish reveals that the testes were still small (9.5 ± 1.7 g), despite the male fish was 9 months old and a mean body weight (BW) of 1.5 ± 0.2 g. Intratesticular semen (IT) volume was only 3.6 ± 1.3 mL. However, stripping of 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

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after addition of water (Viveiros *et al*., 2003). Hatching rate was also very poor (4%) with stripped fluid from *Clarias*-PS + Ovaprim treated fish after sampling 24 h latency time. Viveiros *et al*. (2003) reported the possibility of stripping male Catfish injected with Carp-PS (Carp pituitary suspension), after 12 h latency time (*n*-4).

The absence of seminal vesicle extensions induced by treatment with 20 mg/kg 17-α-methyltestosterone through feeding facilitated the collection of milt by stripping from *C. gariepinus* (Viveiros *et al*., 2001). Technique for collecting milt from *Heterobranchus longifilis* male without sacrificing the male have been described by (Nguenga *et al*., 1996). According to them, cicatrization of the cut occurred within 4 weeks and regeneration was within 2-3 months. Majorities of anesthetic procedures in fish are accomplished by a dip or bath treatment in a static bath or with flowing water. Tranquil (Tricaine) MS-222, a sodium channel blocker is the only anesthetic approved for use in food fish Bowser (2001), because it has a wide margin of safety in a number of species including *Clariid* species.

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. 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 farmers have to kill 2-3 males before obtaining male that has good milt despite the fact that the reddish genital papillae which indicates matured male has been observed. This is a major constraint in the catfish farming sub-sector. Stripping of milt from African catfish (*C. gariepinus*) was not possible due lack of gonadotrophin surge and presence of seminal vesicle and internal organs that blocks the flow of milt (Viveiros *et al*., 2001).

In aquaculture, the availability of matured male and female throughout the year is important to ensure constant supply of fingerlings. There is need for alternative way(s) for milt collection from the male so as to avoid constant killing during fish seed production. Milt collection from Clariids through ablation (without sacrificing the male) has not been reported in Nigeria. There is also paucity of information on ideal regeneration time of African catfish testes after milt collection through partial removal of the testes (ablation). The regeneration period of African catfish male testes after milt collection through ablation is necessary to save male population from dwindling sequel killing for the purpose of obtaining milt. The purposed of this study was to: investigate the possibility of obtaining milt from male African catfish (*C. anguillaris*) through ablation, determination of the testes regeneration period, and assessment of its potency of the milt after regeneration. This is with view to determine the number of times in which the male African cat fish (*C. anguillaris*) could be used in a year for breeding purposes in Nigeria.

MATERIALS AND METHODS

The experiment was conducted between July to September 2009 at the Fish hatchery complex of Fisheries Department Alau, University of Maiduguri, Nigeria located between latitude 13º86′ N and 14º N and longitude 12º E and 13′ E. Raining season begins in July and last in October in the study area.

Experimental fish: Sexually matured males of *C. anguillaris* (517-570 g) from the wild were procured from fisherman in the Lake Alau. They were acclimatized in 10 m x 9 m x 1.2 m out door concrete tank. The male broodstocks were fed with 40% CP. commercial diet. The fish were observed regularly for features associated with ripeness. Sexually matured males were identified when the tip of the genital papilla was reddish. Twelve (12) matured males were selected from the concrete pond after draining. The ablation of the testes was carried out after surgical operation. To reduce stress during the surgical operation, each fish from each group was anaesthetized using Tranquil-MS222 (Syndel International Inc. Canada) following the manufacturer's instruction (2-3 drops/L of water) before operation. Each fish was dipped in the anesthesia until the fish was no longer active. The fish were removed from the anesthesia and placed on dorsal decumbency 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% alcohol) before an incision was made on the ventral side of the abdomen. The incision was extended towards the head with surgical scissors 3-5 cm long to expose the internal organs but remained intact. The digestive tract were lifted out and pushed aside to reveal the testes. About three quarter of the testes were removed and placed into a petri-dish containing physiological saline solution (0.9% NaCl) and preserved in refrigerator at 4ºC.

The incisions were sutured using simple interrupted suture pattern with catgut chromic 2/0. The surgery on each fish lasted for 10-15 min. Each 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 x2 m x1 m indoors) containing Oxtetracycline at dosage of 50mg/L for 5 days. The surgery and milt collection were performed in triplicates.

Experimental design: After healing, the fish were grouped randomly into four (4) treatment groups 30, 60, 90 and 120 days. The fish were fed with 40% crude protein commercial feed fourteen (14) days post surgery. All the instruments used during the operations were disinfected with detol antiseptic.

Testes regenerations were examined surgically by re-opening each treatment group. Testicular weight, milt volume and gonadosomatic index (GSI) before testes ablation and after regeneration from each fish were recorded. The Gonadosomatic Index (GSI) of each male fish after testes regeneration was calculated according (Render *et al*., 1995): GSI = (GW/BW) × 100; where; GW = gonad weight and BW= body weight. Potency of the milt was tested out by fertilizing eggs from matured female. Before fertilization, the females were induced with 0.5 mL/kg ovaprim hormone. The females were stripped after 10 h latency period at 27-31ºC water temperature. Milt from each male after testes regeneration was used to fertilize stripped eggs from each female. One teaspoon (about 1680) of the fertilized eggs using the milt from each of the treatment was incubated in plastic trough (0.8 m diameter x 0.45 m deep) under flow- through system. Fertilization and hatching rates of the eggs were recorded.

Water quality parameters: Dissolved oxygen (DO) and water temperature (ºC) were recorded with digital DO/temperature analyzer Model: JPB-608 DO while pH was recorded using digital pen pH meter Model: ATC pH/ºC both in the morning (08.00 h) and evening (18.00 h) local time daily.

Statistical analysis: Data obtained from the experiment were subjected to one-way analysis of variance, while means were compared for significant differences ($p<0.05$) using Duncan's multiple range test Duncan (1955) using SPSS 11 for windows.

RESULTS

All the fish that underwent surgical operation during 120 days testes regeneration trials healed 10-14 days post surgery (PS). No mortality was recorded. Water temperatures range was 28.37-32.80ºC, while dissolved oxygen and pH ranges were 4.08-5.88 mg/L and 6.94-7.66, respectively (Table 1). After ablation, *C. anguillaris* testes were able to regenerate throughout the trial period (120 days). Table 2 show testicular weight (g), milt volume and GSI of C. *anguillaris* after testes regeneration. The testicular weight, seminal volume and Gonadosomatic Index (GSI) increased with an increase in the regeneration periods (RGPs). The highest mean testicular weight (1.20 g) after testes regeneration was obtained in 120 days regeneration period followed by 90, 60 and 30 days RGPs respectively. There was no significant differences ($p>0.05$) between the testicular weight obtained during the 120 and 90 days RGPs, while significant differences ($p<0.05$) existed between testicular weight values obtained during the 60 and 30 days RGPs. Milt volume was higher (0.80 mL) during 120 days RGP period followed by 90, 60 and 30 (0.40, 0.40 and 0.20 mL) days respectively. There was no significant differences ($p>0.05$) between the milt volume obtained during 90, 60 and 30 days RGPs.

Table 1: Mean water quality parameters during testes regeneration after milt collection through ablation

	Water qualities					
	T(ºC)		DO_2		pH	
Regeneration periods (days)	Morning	Evening	Morning	Evening	Morning	Evening
30	28.98	28.58	5.25	5.88	7.66	7.00
60	32.80	27.88	4.08	5.24	7.23	6.94
90	32.77	30.03	5.17	4.87	7.33	7.00
120	31.52	28.65	4.74	5.36	7.40	6.98

Table 2: Testicular weight, milt volume and GSI (Mean±SD) of C. *anguillaris* after testes regeneration

	Regeneration periods (days)			
Parameters	30	60	90	120
Average Fish weight (g)	517.67	570.00	550.00	544.00
Average Total length(cm)	42.17±.0.72	41.80±0.92	42.23±2.89	43.83±1.69
Testes weight before regeneration(g)	1.53±0.64^{a}	1.83±0.50^{a}	1.47±0.31^{a}	1.90±0.36^{a}
Testes weight after regeneration(g)	0.43±0.15^{c}	0.80±0.20^{b}	1.13±0.21	1.20±0.10^{a}
Milt vol. before regeneration (ml)	0.63±0.38^{b}	0.67 0.31^{b}	0.53±0.25^{b}	1.80±0.82^{a}
Milt vol. after regeneration (ml)	0.20±0.10^{b}	0.40±0.10^{b}	0.40±0.10^{b}	0.80±0.26^{a}
GSI before regeneration (%)	0.22±0.15^{b}	0.39±0.06^{a}	0.28±0.08^{a}	0.29±0.04^{ab}
GSI after regeneration (%)	0.12±0.04^{b}	0.17±0.03	0.22±0.06^{a}	0.23±0.04^{a}

Means with different superscript letters within a row are significantly different ($p<0.05$)

Table 3: Percentage fertilization and hatchability (Mean±SD) of *C. anguillaris* milt after regenerated testes

	Regeneration periods (days)			
Parameters	30	60	90	120
No. of fert. eggs	1680	1680	1680	1680
No. of un-fert. eggs	440.33±260.90[a]	382.67±41.18[a]	358.33±59.00[a]	286.67±30.55[a]
Fertilization (%)	73.62±15.24[a]	76.37±4.29[a]	78.67±3.51[a]	83.93±1.82[a]
No. of hatchlings	813.33±193.28[b]	1181.70±129.45[a]	1024.7067.68[ab]	1241.00±42.58[a]
Hatchability (%)	63.76±3.55[c]	75.73±2.34[b]	77.65±6.75[b]	88.27±3.53[a]

Means with different superscript letters within a row are significantly different ($p<0.05$)

GSI in the male *C. anguillaris* also increased with increase in the testes regeneration periods. The highest GSI (0.23%) was observed in 120 days RGP followed by 90, 60 and 30 days with 0.22, 0.17 and 0.12%, respectively. No significant differences ($p>0.05$) was observed statistically among GSI values recorded in 120, 90 and 60 days RGPs.

Percentage fertilization and hatchability also increased with increase in testes RGPs (Table 3). Higher mean percentage fertilization and hatchability (83.330% and 88.27%, respectively) were recorded in 120 days RGP followed by 90, 60 and 30 days RGPs, respectively. There was no significant differences ($p>0.05$) between percentage fertilization observed throughout the RGPs. Percentage hatchability during 120 days RGP was higher and it is statistically different ($p<0.05$) from that of 90 and 60 days. The percentage hatchability values observed during 30 days of RGP was also statistically different ($p<0.05$) from the values obtained during 120, 90 and 30 days.

DISCUSSION

The water quality parameters recorded during this study was within the recommendations of Viveen *et al.* (1985) for rearing of African catfish fish. Healing of the cuts occurred within 14 days post surgery in this study, it was earlier than the work Nguenga *et al.* (1996) who reported that cicatrization (healing) of the cut occurred within 4 weeks (30 days) in male *Heterobranchus longifilis*. This could be due to differences in climatic condition and species of fish.

The increase in testicular weight, milt volume and GSI with increase in the regeneration periods observed in this study could be due to longer feeding time of the male broodstock after ablation might have given more times for the development of the gonads. The testes weight regenerated up 28.10, 43.1, 76.87 and 63.12% in 30, 60, 90 and 120 days RGPs, respectively. The higher milt volume (0.8 mL) obtained 120 days during this study is similar to the values reported by Aral *et al.* (2007) in *Oncorhynchus mykiss* and lower than that of *Cyprinus carpio* (0.3 mL) as reported by (Yuehi and Chang, 1997). According to them milt volume is one the features reflecting the milt yield and spermatozoa concentration. Milt volume was able to develop to 31.75, 59.70, 75.47 and 44.44% in 30, 60, 90 and 120 days regeneration times, respectively.

The highest GSI (0.23%) recorded during 120 days testes regeneration during this study was higher than the one recorded by El-Greisy and Shaheen (2007) for *Mugil cephalis* control during comparative studies on the effect of different hormones on GSI of the fish. All the GSIs observed during 30 to 120 days testes RGPs were higher than the GSI values reported by Viveiros *et al.* (2001) from stripped male *C. gariepinus* treated with 17-α-methytestosterone 12-47 days after hatching. This is due to the fact that the stripped males were unable to produce much milt after injecting male *C. gariepinus* with male sex based hormone compared to those that regenerated naturally after ablation.

The high percentage fertilization and hatching rate (82 and 88.27%, respectively), observed in this study is higher than 4% reported by Viveiros *et al.,* (2003) in stripped milt from *C. gariepinus* male after inducement with Clarias pituitary suspension (Clarias PS) and combination of *Clarias* PS and ovaprim. It is also higher than 63.1% hatching rate observed by Viveiros *et al.,* (2003) from stripped milt of male *C. gariepinus* treated with 17-α-methyltestoterone. These great differences in fertilization and hatching rates could be attributed to fact that stripped milt (semen) from male testes after treatment with 17-α-methyltestoterone could not have enough spermatozoa to fertilize the eggs from one female compared to those obtained after testes regeneration.

CONCLUSION

Testes of male African catfish (*C. anguillaris*) were able to regeneration after milt (semen) collection through ablation from 30-120 days post surgery. The economical time to re-use male African catfish (*C. anguillaris*) after testes regeneration through ablation is 90 days. This means that it possible to obtain milt from male *C. anguillaris* 3-4 times in one year through ablation for breeding purposes and later the spent males can be sold live. Male African catfish could also be re-used several times for further genetic studies in Nigeria through ablation owing to the erratic power supply that might hinder cryopreservation. Milt can be collected from a

male African catfish without killing the fish. This will depend on the weather condition of the year. Proper and adequate feeding of the male after ablation is very necessary to hasten the development of the gonads. Hatchery operator in Nigeria can obtain milt from male African catfish three (3) months after ablation. This will give an opportunity of using one male 3-4 times in year.

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