Spawning of stripped eggs with milts collected using syringe from live African Catfish (*Clarias anguillaris*)

Kingsley Omogiade IDAHOR

ABSTRACT

In quest for alternatives to end the killing or operating on the male African Catfish (*C. anguillaris*) in captive breeding, syringe was used in milts collection from 180 live African Catfish at $\Delta 45^{o}$ caudally, $\Delta 45^{o}$ cranially and $\Delta 90^{o}$ vertically. Milts collected were used in fertilizing eggs stripped from 30 female African Catfish following hormone administration. Pectoral fin length taken as the starting point in this study were 5.25 cm, 5.38 cm and 5.61 cm in left and 5.26 cm, 5.39 cm and 5.59 cm in right recorded for 0.6 – 0.7 kg, 0.8 – 0.9 kg and ≥1.0 kg treatment groups respectively. The results showed that only 8 (representing 2.2 %) were successfully fertilized that is, 3 (representing 0.8 %) in the right testis and 5 (representing 1.4 %) in the left testis. This observation probably demonstrated the possibility of milts collection with syringe without killing the male fish and using the milts collected in captive fish reproduction. Unfortunately, it was somewhat observed that the fish position during milt collection, angle of syringe insertion, chances of spermatozoa alteration, tiny needle with small hollow centre and slanted end, may have drastically reduced the effectiveness and prospects of this technique. This was evidently reflected in the little or no milts harvested using syringe recorded in the experiment.

Key words: African Catfish, morphology, testes, spawning strategy.

INTRODUCTION

Fishes live in most natural aquatic habitats on earth except very salty water like the Dead Sea and the Great Salt Lake of Utah. Fishes are distributed across the sunny surface of the ocean down to the darkest depths where light never penetrates. Some can live in the hot desert pools at temperature of more than 38^{o}C where other animals may not thrive (Compton's Encyclopedia, 1998). In the tropics, some fishes are able to flop and crawl across mud flats and wet fields in search of food. While some species burrow into the mud when the water pools dry up, others could be dormant until favourable conditions are restored for active live (Bruton, 1996). According to Ferraris et al. (2007), more than 20,000 each of living and fossil kinds of fishes are known and new species are being discovered every year. This is more than all the other kinds of backboned animals combined. The sizes of these fishes differ as much as their shapes and there are over 2,000 species of Catfishes. Thus, they are one of the largest fish orders of the *Siluriformes* belonging to 34 families. These include the Old-world Catfish, Thorny Catfish, electric Catfish, naked Catfish and African Catfish – *Clarias gariepinus* (Sullivan, 2006).

African Catfish belongs to the family – *Claridae* characterized by poor swimming capability, hardiness and ability to withstand poor water quality and unfavourable climatic conditions that other species may not withstand (Okunsebor and Idahor, 2009). These attributes have perhaps made it possible for them to thrive well in captivity except for their inability to reproduce yet artificial reproduction techniques (i.e. captive spawning and brooding) have slowly improved. The reproductive organs of fish are the testes in males and ovaries in females. While the testes produce spermatozoa contained in fluid called milts, the ovaries produce eggs also referred to as "roe" or "spawn". Notably, all fish

Department of Animal Science, Nasarawa State University, Keffi, Shabu-Lafia Campus,
P.M.B. 135, Lafia, 950101, Nigeria. Email: omokingida @yahoo.com

hatch from eggs and usually the females and males release their eggs and milts respectively into the water where they are fertilized and hatched resulting in fry or larvae (Mazzoldi et al., 2007). Meanwhile, there are some species that are livebearers, where the eggs are formed and hatched within the female (ovoviviparous) before birth. Some other species do not lay eggs but the young ones obtain nourishment through an umbilical-like cord or secretions (viviparous) before birth (Brito and Bazzoli, 2003).

Although some fish species reproduce freely in community tanks, most species require spawning triggers. In the difficult-to-spawn species like African Catfish (*C. anguillaris*), the natural conditions are simulated and hormones (such as Phenyl, Human Chorionic Gonadotropin and Puberogen) are often administered with skillfulness before stripping. Unfortunately for the males, they are often killed or at best operated on to strip the milts with attendant low recovery rate and death. This unkind act will definitely result in colossal economic wastages, loss of valuable time to rear another brood stock and genetic unpredictability yet, there is little or no proven artificial reproduction techniques so far to end this cruelty practice. Hence, this novel scientific advancement using syringe to collect milts for spawning stripped eggs in order to stop killing the male fish was propounded.

MATERIALS AND METHODS

Climatic description of the experimental location

The experiment was performed in the month of November at the Hatchery Unit, Department of Fisheries, Nasarawa State University Keffi, Shabu-Lafia Campus. Situated on latitude 8^{o} 35′ N and longitude 8^{o} 32′ E, altitude 181.53 m above sea level with temperature range of 32 – 34^{o}C, relative humidity varying between 40 and 60 % and mean day light of 11 hrs (NIMET, 2013).

Experimental design, data collection and analysis

Although a total of 210 African Catfish comprising 180 males and 30 females were used in this study, only the males of about 11mths old were randomly distributed into three treatment groups:- 0.6 – 0.7 kg, 0.8 – 0.9 kg and ≥ 1.0 kg respectively with 60 replicates per treatment for morphological parameters measurements. The males were further redistributed randomly into three sub-treatment groups according to the syringe insertion points with 20 replicates each per $\Delta 45^{o}$ caudally, $\Delta 90^{o}$ vertically and $\Delta 45^{o}$ cranially. The females were only sources of the needed eggs for the investigation thus each female was stripped using hormone (Ovaparim® Syndel Laboratories Ltd, Canada) according to the manufacturer's prescription. The eggs stripped from one female were divided into thirty-six separate spawning bowls such that it will match up with the milts collected from six males (i.e. one female provided the eggs for milts processed from 6males). Syringe was inserted into the right and left testes at $\Delta 45^{o}$ caudally, $\Delta 90^{o}$ vertically and $\Delta 45^{o}$ cranially taken the end of pectoral fins on the abdominal region as the starting point as reported by Idahor (2013). After every attempt to collect milts, the fish was immediately returned to water bath containing clean water. The inserted syringe was emptied into a beaker containing physiological saline to obtain diluted milts as described by de Graaf (1989). Although the stripped eggs and the milts volumes were not measured in this study, the milts so-collected were mixed with the divided stripped eggs in each of the spawning bowls, using aspirator and aerator at room temperature and were incubated according to de Graaf et al. (1995). See plates I – V.

Prior to allotment of the male fish to the three treatments, they were weighed using table scale (Five Goats Brand®). The distance between the mouth and the caudal fin base and end of the caudal fin were estimated as standard body length and total body length respectively using measuring tape (Butterfly Brand®). The right and left pectoral fins length as well as the papilla length were determined using measuring tape (Butterfly Brand®) as described by Idahor (2013). The values gathered were subjected to analysis of variance according to SPSS (2007) software package. Meanwhile, the data on spawn fertilization were subjected to simple descriptive statistics according to Adesoye (2004).

RESULTS

The morphological characteristics of male African Catfish are given in Tab. 1. There were no statistical differences ($P>0.05$) among the treatment groups. Meanwhile, the live weight values ranged between 0.72 – 1.04 kg, standard body length (44.31 – 46.46 cm), total body length (50.2 – 52.51 cm) whereas, the papilla length values ranged from 1.29 cm in 0.6 – 0.7 kg fish to 1.38 cm in ≥1.0 kg fish. Similarly, the left pectoral fin length was least (5.25 cm) in 0.6

– 0.7 kg fish and highest (5.61 cm) in ≥1.0 kg fish and the right pectoral fin length ranged from 5.26 – 5.59 cm.

Shown in Tab. 2 is the fertilization of eggs with milts collected with syringe from African Catfish. It was observed that out of the 180 males in which syringe was inserted six times (i.e. $\Delta 45^o$ caudally, $\Delta 90^o$ vertically and $\Delta 45^o$ cranially in both right and left testes) only 8 representing 2.2 % were successfully fertilized (See Plate V). Three (representing 0.8 %) of the fertilized eggs were recorded in the right testis at $\Delta 90^o$ vertically collected milts from 0.6 – 0.7 kg and 0.8 – 0.9 kg fish respectively. Five (representing 1.4 %) were recorded in the left testis also at $\Delta 90^o$ vertically collected milts but in 0.8 – 0.9 kg and ≥ 1.0 kg respectively.

DISCUSSION

It was observed that all the values of parameters measured, were within the values reported for healthy fish at similar age range (Okunsebor and Idahor, 2009; Viveen *et al*., 1996). More interestingly, it was observed that all the morphological characteristics measured, were increasing with increase in live weight indicating that the fish were probably still growing as reported by Okunsebor and Idahor (2009) and Gilbert (1994). The results perhaps reflected that there were no morphological differences in the male African Catfish (*Clarias anguillaris*) at 10 – 11 months of age and weighing between 0.6 and 1.0 kg live weight.

Also, it was observed that some of the incubated eggs were fertilized with the milts collected with syringe. The hatchlings recorded in this trial probably demonstrated the possibility of spawning stripped eggs with milts collected using syringe as proposed by Idahor (2013) from any of the testes without killing the fish. However, the low fertility rate recorded could be solely attributed to the short, slanted end and small hollow centre needle used in the study and partially due to possible distortion of the spermatozoa morphology during the study. Also, it could be probably due to the water quality and Hatchery Units environmental conditions that de Graaf et al. (1995) reported could affect incubation and hatching of spawned eggs.

In this novel scientific procedure to stop killing the male fish before milts harvesting, end of the pectoral fin length in the abdominal region, testicular depth and angle of syringe insertion were keenly considered. It was however observed that the fish restrictive position during milt collection, angle of syringe insertion, chances of spermatozoa alteration, tiny needle with small hollow centre and slanted end may have drastically reduced the effectiveness and prospects of this artificial reproduction technique in African Catfish. This was evidently reflected in the little or no milts harvested with the syringe during the experiment. Consequently, for better outcome in subsequent studies, fish restrictive position, syringe insertion points, longer needle with wider hollow centre and round end should be considered. This will immensely enhance the adoption or otherwise of this artificial reproduction technique in captive African Catfish.

REFERENCES

ADESOYE P.O. 2004. *Practical Guides to Statistical Analysis for Scientists*. A Primer edition.

Debo Prints, Ibadan. 189 pp.

BRITO M.F.G. & BAZZOLI A. (2003) Reproduction of the Surubim Catfish (Pisces,

Pimelodidae) in the Sao Francisco River, Pirapora Region, Mines Gerais, Brazil Arquivo

Brasileiro de Medicina. *Veterinaiale Zootecnia*, **55**(5): 624.

BRUTON M.N. 1996. Alternative live-history strategies of Catfishes. *Aquatic Living Resourses,* **9**:

35– 41.

COMPTON'S ENCYCLOPEDIA. 1998. Online V3.0. The Learning Company, Inc.

DE GRAAF, G.J. 1989. La reproduction artificielle et l'alevinage de *Clarias gariepinus* au centre de production d'alevins de Loka en Côte-d'Ivoire, Rapport d'une mission effectuée du 28/10/1989 au 10/11/1989. Projet du développement de la pisciculture rurale, FAO/IVC/87/007.

DE GRAAF, G.J., GALEMONI, F. & BANZOUSSI B. 1995. The artificial reproduction and fingerling production of the African Catfish *Clarias gariepinus* (Burchell 1822) in protected and unprotected ponds. *Aquaculture Research,* **26**: 233–242.

FERRARIS C.J.JR., MIYA M., AZUMA Y. & NISHIDA M. 2007. Checklist of catfishes, recent and fossil (Osteichthyes: *Siluriformes*) and catalogue of *Siluriforme* primary types. Zootoxin **141**:8:5.

GILBERT S.F. 1994. *Developmental Biology* 4th edition. 18BN 0–8789 –249–6 Sunderland, Massachusetts: Sinauer Associates, Inc., 781 pp.

IDAHOR K.O. 2013. Anatomical determination of testes position in African Mudfish (*C. gariepinus*) as a guide to harvest milts using syringe without sacrificing the fish. *Journal of Animal Production Advances*, **3**(9): 265–270.

MAZZOLDI C., LORENZI V. & RASOTTO M.B. 2007. Variation of male reproductive apparatus in relation to fertilization modalities in the Catfish families *Auchemipteridae* and *Callichthyidae* (*Teleostei*: *Siluriformes*). *Journal of Fish Biology*. **70**:243–256. DOI: 10.1111/j.1095-8649.2006.01300.x.

NIMET. 2013. Nigerian Metrological Agency, synoptic Office, Lafia, Nasarawa State, Nigeria.

OKUNSEBOR S.A. & IDAHOR K.O. 2009. Use of morphological and haematological indices of African Catfish (*C. gariepinus*) to assess Doma Dam water quality. Proc. 14th Ann. Conf., ASAN, LAUTEECH, Nigeria, pp. 559–561.

SALLIVAN J.P., LUNDBERG J.G. & HARDMAN M. 2006. A Phylogenetic analysis of the major-groups of Catfishes (*Teleostei*: *Siluriformes*) using rag 1 and rag 2 nuclear gene sequences. *Molecular and Phylogenetic Evolution*, **41**(3): 636 – 62.

SPSS 2007). Programming and Data Management: A Guide for Statistical Package for Social Sciences. SPSS and SAS Users, Fourth Edition Inc., Chicago III.

VIVEEN W.J.A.R., RICHTER C.J.J., VAN OORDT P.G.W.J., JANSEN J.A.L. & HUISMAN E.A. 1996. *Practical Manual for the culture of the African Catfish* (*C. gariepinus*) *2500 E.B*. The Hague,The Netherland 94 pp.

Table 1: Morphological characteristics of male African Catfish (*Clarias anguillaris*)

Measurements	Live weight					
	0.6–0.7 kg	**0.8–0.9 kg**	**≥1.0 kg**	**Mean**	**SEM**	**Prob.**
No. of fish	60	60	60	-	-	-
Age (months)	10.90	11.00	11.25	11.05	0.191	0.416
Live weight(kg)	0.72	0.91	1.04	0.96	0.072	0.213
Standard body length (cm)	44.31	45.97	46.46	45.58	1.446	0.547
Total body length (cm)	50.20	51.77	52.51	51.49	1.455	0.522
Left pectoral fin length (cm)	5.25	5.38	5.61	5.42	0.150	0.242
Right pectoral fin length (cm)	5.26	5.39	5.59	5.41	0.152	0.332
Papilla length (cm)	1.29	1.35	1.38	1.34	0.043	0.296

Values on the same row without superscripts did not differ statistically at 5% probability test; ^{SEM}: Standard error of means; $^{Prob.}$: Probability.

Table 2: Spawning of eggs with milts collected with syringe from the male African Catfish (*Clarias anguillaris*)

Treatment	Right testis (††)				Left testis (††)			
	Fertilized		Unfertilized		Fertilized		Unfertilized	
	Freq	%	Freq	%	Freq	%	Freq	%
0.6 – 0.7 kg								
Δ45° caudally	0	0.0	20	5.6	0	0.0	20	5.5
Δ90° vertically	1	0.3	19	5.3	0	0.0	20	5.6
Δ45° cranially	0	0.0	20	5.5	0	0.0	20	5.5
0.8 – 0.9 kg								
Δ45° Caudally	0	0.0	20	5.5	0	0.0	20	5.6
Δ90° vertically	2	0.6	18	5.0	4	1.1	16	4.4
Δ45° cranially	0	0.0	20	5.6	0	0.0	20	5.5
≥1.0 kg								
Δ45° caudally	0	0.0	20	5.6	0	0.0	20	5.6
Δ90° vertically	0	0.0	20	5.5	1	0.0	19	5.5
Δ45° cranially	0	0.0	20	5.5	0	0.3	20	5.5
Total	3	0.9	177	49.0	5	1.4	175	48.7

Freq: Frequency; (††): Volumes of the stripped eggs and the milts were not determined.

Step 1

Plate I: Spawned eggs

Step 2

Plate II: Milts collection

Step 3

Plate III: Incubation

Step 4

Plate IV: Hatching

Step 5

Plate V: Fertilized eggs

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