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Microscopic observation of spermatozoa in milt collected with syringe without sacrificing the male African Catfish (*Clarias anguillaris* B. 1911)

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Abstract
Some fish species will readily spawn in captivity while others will not, such as catfishes thus hormones are administered to stimulate spawning. Unfortunately, the males are being killed or at best operated on to harvest the milt before successful breeding in captive African Catfish (*C. anguillaris*). To possibly put an end to this inhumane practice, this study was aimed at collecting milt with syringe without killing the fish. The milt was collected from 90 African Catfish at 10 – 12 months of age and was viewed in the microscope to check for viable spermatozoa. Results showed that spermatozoa were found in 3(representing 3.3%) out of the 90 fish used in the study and only in Faceup (Ventral) position and $\Delta 90^{o}$ syringe inserted point that spermatozoa were found in both testes. These findings somewhat demonstrated that milt harvesting using syringe without sacrificing the male *C. anguillaris* may be possible in captive breeding.

Keywords: abdominal region, catfish, milt harvesting, testicular lobes, viable spermatozoa.

1. Introduction
Catfishes belong to the monophyletic group in the order *Siluriformes*. The body is cylindrical without scales and the head is flat and bony with barbells. There are chemoreceptors across the entire body with Weberian apparatus that allow for improved taste, smell, respiration, hearing and sound production [1]. Most catfishes are about 20 – 30 cm long, but could be as long as 1 – 3 m. They are heavy and are reduced in the gas bladder, hence negatively buoyant making them bottom dwellers. The fins which could be single or paired are used for movement, stability, nest-building, spawning and as tactile organs. The pectoral fins are located near the gill cover and have been adapted for defense and maneuvering of the fish [2]. Catfishes dwell in freshwater like a lake and swamp and feed basically on earthworms as well as insects. They are most diverse in the tropical South America, North America, Africa and Asia with more than half of all the species in the Americas [1].
Catfishes are of considerable commercial importance. Many of the larger species are farmed or fished for food while the smaller species are often used in aquarium hobby. Although some people described catfish dishes as watery without flavour yet others consider it as excellent food [3]. In central Europe, catfish dishes are often viewed as a delicacy to be enjoyed on feast days and holidays. In the United States of America, catfish became such a staple of the diets that National Catfish Day was established. Catfish is eaten in various forms such as cooked, grilled, smoked and fried. In Malaysia, it is fried with spices and eaten with tamarind or steamed rice. In Indonesia, it is grilled and eaten with vegetables [3]. In Nigeria, catfishes are often smoke-dried or sun-dried and they are used in cooking soup and stew. Meanwhile, it could be prepared in broth, which is served as “pepper soup” in most restaurants, eateries and social gatherings. Among the Bini tribe, it is called “Ehẹnbevbariẹ” which is so relished that it is a measure of one’s economic status in Ẹdo Kingdom.
In most fishes, the gonads which are usually similar in size are paired except in sharks where the right testis is normally larger and in primitive Jawless fish that has single testis [4]. The genital papilla is a small fleshy tube where the viable spermatozoa or eggs are released. According to [5] the testes in male African Mudfish (*C. gariepinus*) at 8mths old could be as long as 5cm with an average of 11 fringes/lobes. In the natural habitat, the catfishes release

eggs and milt into the water where fertilization takes place resulting in larvae or fry. Meanwhile, some male catfishes carry the eggs in their mouths until hatching and during this period they are unable to eat [6]. While some fish species reproduce freely in community tanks, most require spawning triggers. Sexual dimorphism has been reported in catfishes as well as the modification of the anal fin into an intromittent organ (an internal fertilizer) and accessory structures of the reproductive apparatus in both internal and external fertilizers [7, 8].

In captive *C. anguillaris*, spawning requires administration of hormones and expertise in stripping the females and the males are often sacrificed or rarely operated on (with low survival rates) to get the milt. This breeding technique has resulted in colossal economic losses with particular emphasis on loss of valuable time to rear another brood stock and genetic unpredictability. Even at that, there is still little or no other established artificial reproduction approach so far to end this brutal act in captive *C. anguillaris*. Thus, use of syringe in milt harvesting from live male fish for artificial spawning, could be a Mary Slessor's strategy to end this dastardly practice.

2. Materials and Methods

2.1 Study Location Description

The investigation was conducted in the months of October and November at the Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus Nigeria. Lying between latitude 8^o 35′N and longitude 8^o 32′ E on altitude 181.53 m above sea level, with a temperature range of 32–34 ^{o}C, 40–60% relative humidity and mean day light of 11 hrs [9].

2.2 Experimental Design

Using [5] report on description of African Mudfish (*C. gariepinus*) testes anatomical position as a guide, syringe insertion to harvest milt was done at Δ45^o caudally, Δ90^o vertically and Δ45^o cranially as treatment groups. A total of ninety live male *C. anguillaris* of about 10 – 12 mths old were procured from commercial fish farms outlets in Lafia metropolis. These were randomly allotted such that each treatment group had 30 fish each. These were further replicated given a total of 5 fish per replicate based on the testis.

2.3 Data Collection and Statistical Analysis

2.3.1 Body measurements

All the fishes were weighed individually using table scale (Five Goats Brand®) the distance between the mouth and caudal fin base as well as the end of caudal fin was measured using measuring tape (Butterfly Brand®) to obtain values for body weight, standard body length and total body length respectively as described by [10]. Also, the papilla length, right and left pectoral fin length were determined using measuring tape.

2.3.2 Microscopic slide preparation

With the assistance of Laboratory Personnel, the fish were held at Facedown (Dorsal), Sideways (Lateral) and Faceup (Ventral) positions. Then syringe was inserted Caudally, Vertically and Cranially at an angle of Δ45^o, Δ90^o and Δ45^o respectively on both testes (i.e. right and left) at different trials. Amounting to six syringe insertion times per fish and the end of pectoral fin was taken as the starting point (Δ90^o Vertically). After each milt collection attempt, the fish was returned to water bath containing clean water. In each case, the syringe was emptied into a test tube containing physiological saline to obtain diluted milt as described by [11]. Although the essence was to obtain high egg hatchability, in the present study, it was rather aimed at spermatozoa low density and high motility on the microscope slide. An applicator was used to take a drop of the diluted milt on microscopic slide for observation at x10, x40 and x100 magnification in the microscope (Olympus Microscope® Tokyo, Japan) as described by [12].

The sets of data collected were subjected to one way analysis of variance for treatment mean values comparison according to [13] software package. The mean values were separated by Duncan multiple range tests of the same software package. Where applicable, the values were subjected to simple descriptive statistics according to [14].

3. Results

Table 1. Body parameters measured in male *C. anguillaris*

	Syringe insertion point (Mean ± SEM)			
Parameters	**Δ45^o Caudally**	**Δ90^o Vertically**	**Δ45^o Cranially**	**P-values**
Number of specimen	30	30	30	-
Body weight (kg)	1.01±0.05	1.18±0.07	1.11±0.08	0.15
Total body length (cm)	55.4±0.95	56.0±0.84	56.3±1.43	0.83
Standard body length(cm)	45.5±0.84	50.1±0.82	49.6±1.79	0.58
Papilla length (cm)	1.31±0.04^b	1.37±0.09^b	1.55±0.02^a	0.03
Left pectoral fin length (cm)	7.07±0.25	7.02±0.28	6.26±0.08	0.06
Right pectoral fin length (cm)	7.05±0.25	6.88±0.39	6.28±0.09	0.19

^{a,b,c}: Means along the same row with different superscripts differ significantly at 5% probability;
$^{\pm SEM}$: Standard error of mean; $^{P\text{-values:}}$ Probability; $^{\Delta}$: Angle of syringe insertion.

Presented in Table 1 is the body parameters measured in male *C. anguillaris*. There were no significant differences (P>0.05) among the treatment mean values except in papilla length where the value (1.55±0.019cm) of fish in Δ45^o Cranially group was significantly longer (P<0.05) than 1.27±0.092cm) observed in Δ90^o Vertically group. This value was however statistically similar (P>0.05) to 1.31±0.043cm recorded in Δ45^o Caudally inserted fish.

Table 2 shows the microscopic observation of milt collected with a syringe from live *C. anguillaris*. Out of a total of 90 male fish used in the experiment, only in 3 cases (representing 3.3%) recorded in Δ90^o Vertically inserted syringe group in Faceup (Ventral) position that viable spermatozoa were observed (See Plate 4). It was observed that 2 of the 3 cases representing 66.7% were recorded in the right testis while a case only was recorded in the left testis.

Table 2: Microscopy of milt collected with syringe from male *C. anguillaris*

Live fish position	Testis	Milt observed	Angle of syringe insertion									
			Δ45º Caudally		Δ90º Vertically		Δ45º Cranially		Total Milt observed			
			Freq	%	Freq	%	Freq	%	None	%	Yes	%
Facedown	Left	None	5	16.6	5	16.7	5	16.6	15	16.7	0	0.0
(Dorsal)		Yes	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Right	None	5	16.6	5	16.7	5	16.6	15	16.7	0	0.0
		Yes	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Sideways	Left	None	5	16.6	5	16.7	5	16.6	15	16.7	0	0.0
(Lateral)		Yes	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Right	None	5	16.6	5	16.7	5	16.6	15	16.7	0	0.0
		Yes	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Face up	Left	None	5	16.6	4	13.3	5	16.6	14	15.5	0	0.0
(Ventral)		Yes	0	0.0	1	3.3	0	0.0	0	0.0	1	1.1
	Right	None	5	16.6	3	10.0	5	16.6	13	14.4	0	0.0
		Yes	0	0.0	2	6.6	0	0.0	0	0.0	2	2.2
		Total	30	100.0	30	100.0	30	100.0	87	96.7	3	3.3

Freq: Frequency of occurrence; Δ: Angle of syringe insertion; %: Percentage of occurrence.

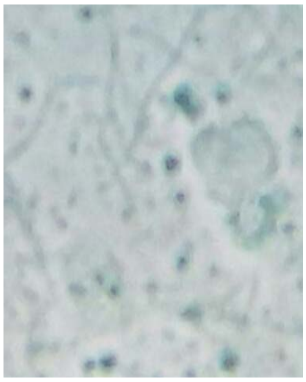

Plate 1: Δ90º Vertically inserted syringe	**Plate 2:** Δ45º Cranially inserted syringe	**Plate 3:** Δ45º Caudally inserted syringe	**Plate 4:** Micrograph of sperm cells as viewed in microscope at X 100 magnification

4. Discussion

The similarities in body parameters values recorded could probably be coincidental or purely due to possible phenotypic variability within animal species. Whereas, the differences in papilla length recorded may be solely due to the stage of fish growth and development as influenced perhaps by age, source and management practices adopted in the commercial farms where they were procured [15, 16, 17, 18]. In any case, all the values recorded were similar to those reported in healthy and matured catfish [16, 17, 19]. It implied therefore that at 10 – 12 months of age, African Catfish may be sexually matured as described by [18] The viable spermatozoa viewed and recorded in Δ90º Vertically inserted syringe fish at Faceup (Ventral) position may be solely attributed to ease of targeting the milt at the mid of the testis, suggesting that use of syringe in milt collection in live *C. anguillaris* may be possible in any of the testes. In the same vein, since [5] reported that the end of the pectoral fin is the mid of the testes, it is most likely where the milt is highly concentrated. Therefore, this observation could be partly linked to the shorter right pectoral fin recorded in the present study.

Also, the observation showed that it might probably be easier to collect milt with syringe from live *C. anguillaris* when restricted with the ventral side upwards, buttressing the report of [5] who recorded relatively lower testes depth at Faceup (Ventral) position. Meanwhile, the higher occurrence recorded in the right testis could be possibly due to the less testicular fringes (lobes) associated with the right testis, that probably have concentrated the milt at the mid testis lumen. This partially confirmed the report of [5] that left testis may have more testicular fringes (lobes) than the right testis. More significantly, it is possible that the syringe could not reach the lumen at Δ45º cranially and caudally inserted positions (See Plates 1 – 3) hence, there was no viable spermatozoon recorded among the fish in these treatments. Notably, the results signified that it is perhaps feasible to collect milt with syringe for spawning stripped eggs as artificial reproduction strategy in *C. anguillaris* without killing the male.

The findings indicated that male African Catfish at about ten months old perhaps exhibited morphological characteristics of mature and healthy fish. Also, it was discovered that use of syringe in milt harvesting at this stage of maturity was seemingly possible without killing the fish. However, the length of the syringe needle, the hollow centre and the slant pointed end constituted some limitations to milt harvesting during the study. Therefore, customized syringe with longer, wider and round end like the hypodermic (immunization) syringe may be required. The breakthrough recorded in this study could be hinged on the hindsight of the anatomical depth of fish testes. Also, knowledge of the end of pectoral fin at the abdominal region that essentially marked the mid of testes served as a veritable pathway to this outcome. Hence, it was the starting point (Δ90° Vertically) for syringe insertion at Δ45° Caudally and Δ45° Cranially respectively.

5. Conclusion
It was however observed that the fish position during milt collection as well as the angle of syringe insertion may have reduced the efficiency and potency of this novel approach to milt collection without killing the fish. Nevertheless, with this ground breaking novel approach to milt harvesting without male fish sacrifice. It is hopeful that in depth researches should be embarked upon to empirically establish a Mary Slessors principle, to end this dastardly practice of killing the male *C. anguillaris* in captive breeding.

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