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Reproductive Tract Morphometry and Evaluation of Fresh Wild African Catfish (*Clarias gariepinus*) Milt from Lake Alau, Maiduguri, Nigeria

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ABSTRACT

Reproductive morphometry of *Clarias gariepinus (C. gariepinus)* is essential in distinguishing different species that are closely related to it and adequate information is required regarding the seasonal variation of milt characteristic of wild species. This study was designed to document the reproductive tract morphometric dimensions and milt characteristics of wild *C. gariepinus* during spawning and non-spawning seasons in Lake Alau, Maiduguri, Nigeria. Sixty male brood stocks were used comprising thirty each for spawning and non-spawning seasons. The reproductive tracts were removed and their dimensions measured. The milt samples were collected, pooled and evaluated. Results of the study showed that the body weight, sperm concentration and testicular indices differed significantly ($p<0.05$) between the two seasons of evaluation. In addition, a positive correlation ($p< 0.05$) between body weight of fish and milt volume ($r = 0.656$ and $r = 0.646$) was also observed in both seasons. It was concluded that the left testis of *C. gariepinus* is heavier, longer and thicker than the right. Nonetheless, the *C. gariepinus* had larger body weights and higher spermatozoa concentration in spawning season than in non-spawning season; those with larger body weights had higher volume of milt than those with lower body weights.

Keywords: *Clarias gariepinus;* Lake Alau; Morphometry; Milt; Non-spawning; Spawning

INTRODUCTION

Wild African catfish *Clarias gariepinus* is of economic importance in the fisheries of inland water bodies of Africa, including Nigeria. They have been reared for decades and its suitability for aquaculture is attributable to its indiscriminate feeding habit, rapid growth, tolerance to high stocking density and adverse environmental conditions (Viveen *et al.,* 1985). *C. gariepinus* are tolerant to hypoxia due to its ability to use aerial oxygen via its accessory breathing organ within and outside waters. They have high fecundity and consumer preference ranking (DeGraaf *et al.,* 1995). In the past, Nigeria was reported as the largest producer of *C. gariepinus* in sub-Sahara Africa (FAO, 2000), however, most of the strains used in aquaculture have been subjected to inbreeding and its consequences (Megbowon *et al.,* 2013).

The wild *C. gariepinus* in their natural habitat shows a discontinuous reproductive cycle, influenced by the circannual changes in water temperature and photoperiodicity (Van Oordt and Goos, 1987) with rise in water level due to rainfall as the triggering mechanism for spawning. One of the most important factors necessary in the successful culturing of a fish species is obtaining a basic understanding of its key biological processes (Eunice *et al.,* 2017). The detailed characteristics of the morphometric features of African catfish may also be needed to distinguish the different species that are closely related (Hamad, 2014); it can show high plasticity in response to differences in environmental conditions such as temperature, turbidity, food availability, water depth and flow in the same species amongst the fish population (Turan *et al.,* 2005).

The body weight of brood stock plays a major role in sperm maturation period, and there is a wide range in the number of spermatozoa produced indicated by increase in testicular weight at the same time as body weight (Billard, 1986). Spermatogenetic production has been studied in detail in only a small number of cases but since sperm constitute the majority of cells in the mature testis, testicular weight is thought to be a good criterion of the quantity of sperm produced (Billard, 1986). It is therefore evident that spermatogenetic activities vary greatly from one species to another and the reason for these differences remains unclear (Nagahama, 1983). Other than aspect of quantitative production, it is necessary to consider sperm quality and the amount of sperm available for fertilization. Fresh sperm assessment is a useful step to indicate the success of cryopreservation technique as well as giving the baseline

information on the fecundity of African catfish (Azlina *et al.,* 2012).

From reproductive and genetic perspective, the male African catfish is the most important individual animal of breeding in Nigerian aquaculture and influences more progeny than the females. Milt is a critical input in any aquaculture program; hence a basic biological knowledge of indigenous feral species is essential to increase the selection intensity for males in order to achieve greater genetic progress in aquaculture. The aim of this research was to evaluate morphometric traits of the male reproductive tract and milt characteristics in the wild African catfish during the spawning and non-spawning periods.

MATERIAL AND METHODS

Study Area

This study was conducted in the Department of Theriogenology, University of Maiduguri, Maiduguri, Nigeria. Maiduguri is located between latitude 11º and 50ºnorth and longitude 13º and 36º east. The annual rainfall average 320mm, humidity of about 49% and evaporation of 203mm per year. The rainfall is generally been heaviest in August and the annual temperature average is 35.4 ºC. Lake Alau is a natural water storage formed by River Ngada, situated off Maiduguri-Bama Road, some 14km away from Maiduguri. This study was conducted in spawning season (June to August) and non-spawning season (November-February) (Bruton and Smith, 1988; Mayom and Mohammed, 2014).

Experimental Fishes and Management

Sixty adult males (≥250 g body weight) of wild African catfish (*C. gariepinus*) were used for this study with 30 in each season (spawning and non-spawning). All fish were bought live and transported in a plastic container from Lake Alau. Each fish was acclimatized in a tank containing clean water and allowed to rest over night before any evaluations were carried out.

Morphometric Data Collection

Each fish was placed in plastic bag at the point of purchase and weighed with a handheld digital electronic weighing scale (WeiHeng® 145.00) to determine the weight of fish (WF). Each male brood stock was then cleaned and dried using a clean paper towel and then made unconscious as described by Ofelia *et al.* (2012) and their body morphometry were evaluated. A measuring tape was used to determine the length of the fish (LF) and the abdominal circumference of the fish (ACF) while placed on flat surface at ventral recumbency, using a vernier calliper (Tricle brand®) the length of genital papillae (LGP) and basal diameter of genital papillae was measured at a dorsal recumbency. After which a mid-ventral incision was made using scalpel blade, and the reproductive organs were exposed and removed with a pair of scissors and forceps (Figure 1). The number of extensions of the seminal vesicle (NESV) were counted, weight of the right (WRT) and left testes (WLT) were determined using a sensitive digital electronic weighing balance (YP600©, 0.00001-g, China). The length of the testes and the diameter of the testes of both right and left sides were determined using the vernier callipers as (LTR, LTL and DTR, DTL respectively).

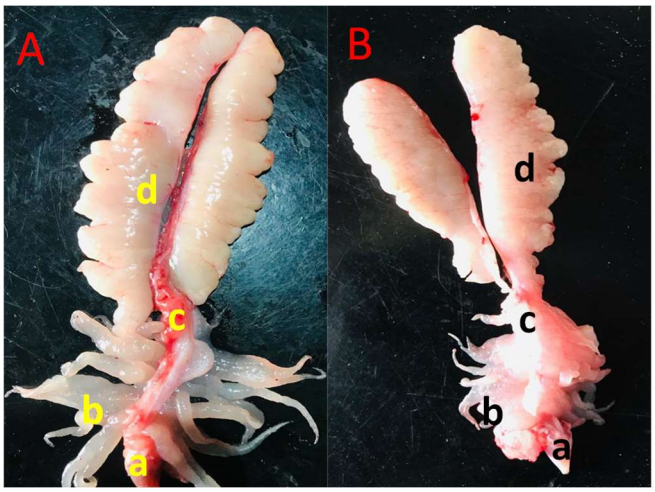

Figure 1: The reproductive organs of male African catfish *C. gariepinus* showing the conical papillae (a), seminal vesicles (b) bulbourethral gland (c) and testis (d) that are whitish in color during spawning (A) and pinkish color appearance during non-spawning (B) seasons

Milt Collection

The testes were incised and milt sample was collected after dissecting the testes with a surgical blade; the milt was gently stripped by squeezing out and collected into a graduated glass tube to measure the volume.

Fresh Milt Evaluation

A macroscopic evaluation of milt such as volume, pH, consistency, and colour were recorded as described previously by Buyukhatipoglu and Holtz (2001) and Dominic and Abiodun (2014). Microscopic evaluation of the sperm count (concentration) was determined with a Neubauer counting chamber. Sodium citrate buffer solution (2.94%) was used as a diluent in a ratio of 1:100 (v/v) as described by Brar *et al*. (2000) and Bustamante *et al*. (2016). A drop of semen-buffer solution ratio and 1% formal saline solution was mixed with a vortex (5 µL of milt and 50 µL of 1% formal saline solution). The Neubauer chambers were filled and allowed to rest for one minute, two counts of 0.2 mm^2 was conducted viewed under ×100 objectives lens power microscope. Concentration (Conc.) was calculated by the number of sperm per ml of milt as described by Hafez and Hafez (2000).

Concentration was calculated as Conc. =ΣSC × nsq × DF ×10^3

Key
ΣSC: average number of sperm cells counted in the large central area
nsq: number of squares counted in the central area (5)
DF: dilution factor (100)
10^3: factor of number per cubic centimetre (10,000cc).

Other microscopic milt evaluations such as mass motility and progressive individual motility in all samples collected were estimated by a technique described by Rurangwa *et al.* (2004); Fauvel *et al.* (2010); Dominic and Abiodun (2014) with litle modifications (addition of suitable activation medium for motility evaluation and dilution ratio) by microscopic examination at ×10 to ×40viewed on a glass slide with or without cover slip. Livability and morphologic abnormalities were determined with the aid of Eosin-

Nigrosin stain as described by Rurangwa *et al.* (2004); Fauvel *et al.* (2010).

Statistical Analyses

Data obtained were analysed using SPSS® software (version 16.0). The analyses done were Student sample t-test and Correlation Coefficient *(r)*. Results were expressed in Mean ± Standard Deviation and p-values less than 0.05 were considered significant.

Ethical Statement

The ethics governing the use and conduct of experiment on animals were strictly observed.

RESULTS

The body weight of the fish measured during spawning season was 994.8 ± 394.3 g which was significantly higher ($p<0.05$) than that observed during the non-spawning season with a mean body weight of 898.6 ± 172.6 g (Table 1). Similarly, the morphometric investigations among testicular indices (weight, length and diameter of testes) measured were observed to be significantly different ($p<0.05$) between the left and right testes in both spawning and non-spawning seasons (Table 1). However, there were no statistical differences ($p>0.05$) in the values recorded in length of fish, abdominal circumference, length and diameter of genital papillae and number of seminal vesicles between spawning and non-spawning seasons.

The Macroscopic parameters of some milt characteristics freshly collected from *C. gariepinus* in spawning and non-spawning seasons (Table 2) showed that the viscosity, consistency and colour of milt were mucoid, thick and creamy coloured and watery to mucoid, thin and milky in spawning and non-spawning periods, respectively. The microscopic evaluations such as progressive individual sperm motility, livability and morphologic abnormalities were observed to have no significant difference ($p>0.05$) between spawning and non-spawning seasons similar to the volume and pH of milt measured. However, the mean concentration is 2.9 ± 0.4 and 2.6 ± 0.4 billion sperm cells per ml in spawning and non-spawning season and differed significantly ($p<0.05$) between the seasons. A photomicrograph of fresh undiluted *C. gariepinus* milt stained with eosin-nigrosin are shown in Figurez 2 and 3. All milt pooled in this study contains few numbers of dead and abnormal sperm (Figure 2 and 3) but these cells in large number would show impaired fertility or sterility. The dead sperm ranges from 2-18 % in both spawning and spawning season. In this study, a baseline spermiogram from the fresh wild *C. gariepinus* milt was established.

The relationship between body weight of fish and some milt parameters (milt volume, concentration and progressive motility) were correlated during spawning and non-spawning seasons (Table 3) which revealed that there was a positive correlation ($r = 0.356$ and $r = 0.346$) between body weight of fish and milt volume and were significant ($p<0.05$) in spawning and non- spawning seasons respectively. On the other hand, the relationship between weight of testes (WT) and some milt parameters (milt volume, concentration and progressive motility) was not significant ($p>0.05$) during spawning and non- spawning seasons.

Table 1: Morphometric parameters of *Clarias gariepinus* during the spawning and non-spawning seasons in Maiduguri, Nigeria

Parameters	Spawning season	Non-spawning season
Body weight of fish (g)	994.80 ± 394.30^{a}	898.6 ± 172.6^{b}
Length of fish (cm)	51.08 ± 7.82	51.05 ± 6.98
Abdominal circumference (cm)	21.19 ± 2.62	22.58 ± 7.83
Length of genital papillae (cm)	1.48 ± 0.29	1.52 ± 0.29
Diameter of genital papillae (cm)	0.75 ± 0.21	0.78 ± 0.157
Number of seminal vesicles (NESV)	38.25 ± 4.85	37.65 ± 2.9
Weight of testes right (g)	1.69 ± 0.69^{x}	1.54 ± 0.62^{x}
Weight of testes left (g)	2.34 ± 1.53^{y}	2.31 ± 1.05^{y}
Length of testes right (cm)	5.25 ± 1.19^{x}	5.419 ± 1.19^{x}
Length of testes left (cm)	5.73 ± 1.46^{y}	5.553 ± 1.08^{y}
Diameter of testes right (cm)	1.79 ± 0.52^{x}	1.418 ± 0.50^{x}
Diameter of testes left (cm)	1.83 ± 0.62^{y}	1.666 ± 0.52^{y}

Values (Mean ± SD) on the same row with different superscripts$^{(a,b)}$ and within column with different superscripts$^{(x,y)}$ differs significantly at $p < 0.05$

Table 2: Some fresh milt characteristics (mean ± SD) of *Clarias gariepinus* in spawning and non-spawning seasons in Maiduguri, Nigeria

Milt characteristics	Spawning season	Non-spawning season
Volume (ml)	3.6 ± 1.1	3.3 ± 1.1
Viscosity	Mildly mucoid	Watery-to-mildly mucoid
Consistency	Thick	Thin–to-thick
Color	creamy	milky
Mass motility (%)	87.3 ± 6.6	86.3 ± 7.1
Progressive Motility (%)	84.4 ± 7.6	84.4 ± 7.3
Livability (%)	90.8 ± 7.6	89.8 ± 6.2
Morphologic Abnormalities (%)	3.6 ± 2.2	2.7 ± 2.1
Concentration ($x10^9$/ml)	2.9 ± 0.4^{a}	2.6 ± 0.4^{b}
pH	6.6 ± 0.3	6.6 ± 0.2

Values (Mean ± SD) on the same row with the different superscripts $^{a \text{ and } b}$ differed significantly at $p < 0.05$

Table 3: Relationship between body weight of fish, weight of testes and some milt parameters (milt volume, concentration and progressive motility) of *Clarias gariepinus* in spawning and non-spawning seasons

Parameters	Spawning season		Non-spawning season	
	Pearson's Correlation coefficient (*r)*	p-value	Pearson's Correlation coefficient (*r)* p-value	p-value
Body weight of fish – Milt volume	0.356	0.04	0.346	0.04
Body weight of fish – Concentration	0.311	0.12	0.290	0.22
Body weight of fish – Progressive motility	-0.263	0.26	0.024	0.92
Weight of testes – Milt volume	0.205	0.39	0.278	0.24
Weight of testes – Concentration	0.206	0.38	0.183	0.44
Weight of testes – Progressive motility	-0.073	0.76	-0.226	0.34

Correlation is significant at $p<0.05$ levels.

DISCUSSION

In the current study, the mean weight and length in spawning and non-spawning seasons respectively of *C. gariepinus* obtained was higher than what was reported by Zakariah *et al.* (2016) and Eunice *et al.* (2017), weight (532 ± 8.32 g and 510.8 ± 9.27 g) and length (43.2±4.72 cm and 42.1±2.48 cm), but agrees with Idahor *et al.* (2014) during spawning and non-spawning seasons, respectively. These differences in mean values might have been due to some environmental factors such as food, temperature, water pH and dissolved substances which generally affect fish growth as reported by Ben (2003). Disparity of individual growth may result from individual differences in feed intake or feed efficacy or a combination of both (Qiant *et al.,* 2002). Also, genetic factors may play a role in bringing about differences in the growth of individual fish (Qiant *et al.,* 2002). Although in general, as the fish grows older, the growth rate decreases, however, unlike other vertebrates that stop growth at certain ages due to heredity, fish do not really lose the capacity to grow (Pauly, 1992). However, the largest fish available in the market were purchased to increase the quality of milt that could be obtained and these may also be another reason why mean weight and length were higher than what was obtained by Zakariah *et al.* (2016) in Maiduguri.

In the present study, the weight, diameter and length of the testes was not significant between spawning and non-spawning periods. These, findings agree with those of Idahor *et al*. (2014) and Yusuf *et al*. (2015), but have slightly lower values in comparison to the study by Zakariah *et al.* (2016), this could be due to the higher number of fish used in the current study. The testes and reproductive organs of *C. gariepinus* during spawning period as observed in this study were lobular and whitish as compared to during non-spawning season that appears smooth and pinkish (figure 1).

The testicular indices (weight, length and diameter of testes) obtained in this study agrees with that of Yaseer *et al*. (2013). However, the numbers of extensions of seminal vesicles (38.3 ± 4.9 and 37.7 ± 2.9) in spawning and non-spawning seasons respectively were similar to the findings by Zakariah *et al.* (2016). The number of seminal vesicles corresponds with the report of Van den Hurk *et al.* (1987), who reported a wide range in the number of seminal vesicles in Clariid species and disagrees with the report by Fishelson *et al.* (1994) who observed up to 50 numbers of extensions of seminal vesicles surrounding the sperm duct. The length of genital papillae (LGP) was in the same range to the findings of Idahor *et al*. (2014).

In the present study that sperm was also obtained in the non-spawning season, even with the corresponding thin and milky milt obtained, the mean concentration was within normal range ($1.8 – 7.2\times10^9$/ml) as reported by Viveiros *et al.* (2000). This might be attributed to the fact that the testis of a male *C. gariepinus* is fully developed once they reach a weight of approximately 200g, similar to a previous finding reported in Congo Brazzaville by DeGraaf and Janssen (1996). Some of the primary morphologic abnormalities seen in this study might have resulted from damage by xenobiotics, altered genetic mutation or handling and processing of the milt sample as reported by Fauvel *et al.* (2010).

The fresh milt quality assessment shows that only sperm concentration (in spawning; 2.9 ± 0.4 and in non-spawning; 2.6 ± 0.4 $x10^9$) showed a statistical significance ($p<0.05$) between spawning and non-spawning seasons and the mean values were within standard range (1.8 to 7.2 $x10^9$/ml) similar to previous observation made by Viveiros *et al.*(2000) and to that observed by Yusuf and Ilker (2017) also in wild African catfish in Turkey. This may be due to the fact that spermatocrit and viscosity of milt vary between specific males, species and across reproductive seasons. Rurangwa *et al*. (2004) in their study reported that the sperm concentration in Rainbow trout declines as the spawning season advances and this could be a reason for its decrease in the non-spawning season. Other variation in milt traits were not significant, but in comparison to other studies, all mean characteristics of pre-diluted milt measured (progressive individual sperm motility, livability and morphologic abnormalities) agrees with the findings (≥85%progressive motility) reported by Adeyemo *et al.* (2007), the mean progressive individual motility was higher than what was obtained by Ofelia *et al.* (2012), which may be attributed to the fact that sperm motility for the freshly collected semen from healthy brood stock is usually between 90-100% motility provided that the sperm sample was not activated or contaminated by water, urine or any external medium while sampling was carried out as reported by Saeed *et al*. (2010). The mean volume (3.6 ± 1.1 ml and 3.3±1.1) of milt and the mean pH (6.6 ± 0.3 and 6.6 ± 0.2 pH) which was slightly acidic in spawning and non-spawning periods obtained were similar to the observation made by Orlu and Ogbalu (2011) and Yusuf *et al*. (2015) in wild African catfish species. The sperm quality index is

predictive of fertility and hatchability among females. Thus, wild *C. gariepinus* can produce large numbers of seeds and good quality milt can be collected and used in artificial breeding in spawning and non- spawning seasons.

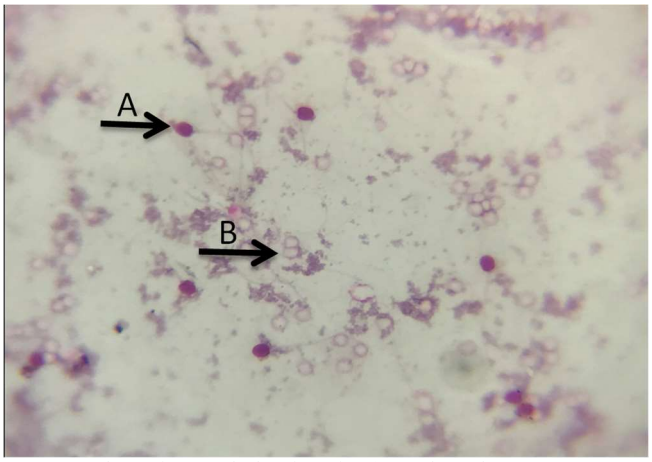

Figure 2: Photomicrograph showing Live (A) and Dead (B) Spermatozoa in C. *gariepinus* milt stained with Eosin-Nigrosin stain (×100 objective)

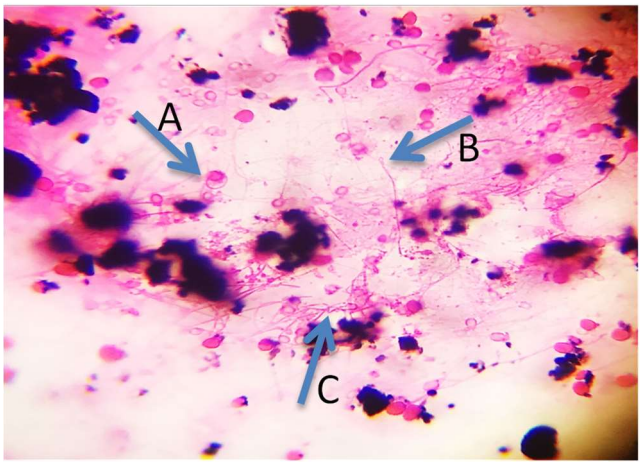

Figure 3: Photomicrograph showing some morphologic abnormalities (A) coiled mid piece, (B) small head and (C) coiled tail of sperm in *C. gariepinus* milt stained with Eosin Nigrosin stain (×100 objective)

The age and body weight of brood stock has a significant influence on the sperm quality and may affect the success of storing sperm (Vuthiphandchai and Zohar, 2007; Azlina *et al.,* 2011; Dominic and Abiodun, 2014). In this study, there was a significant ($p< 0.05$) positive correlation between body weight of fish and milt volume in spawning and non-spawning seasons which corresponded with a similar finding by Gjerde (1984) and Yusuf *et al.* (2015); who has also reported a positive correlation between volume of milt and body size (weight and length) in Atlantic salmon (*Salmosalar*) and rainbow trout and the later on cultured African catfish which signifies that higher body weight fish tends to have an increased in the volume of milt than lower body weight fish, or probably due to physiological constraints (Stockley *et al.,* 1996).

Conclusion

Wild *C. gariepinus* have larger body weight and higher sperm concentration in spawning season than in non-spawning season. However, those with larger body weight produced higher volume of milt than those with lower body weight in both spawning and non-spawning seasons. The left and right testes differ significantly with the left testes being heavier, longer and thicker in both seasons studied. The result obtained from this study can be applicable as a selection criterion of male *C. gariepinus* and other Clariid families for artificial breeding and provided baseline information on spermiogram of *C. gariepinus* in Lake Alau.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analyses were performed by RIA, SOA, AA, UMB, MAW and MMB. The draft manuscript was prepared by RIA and SOA. MMB and MAW read, corrected and approved the manuscript for publication. All authors have read and approved the final manuscript.

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