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Original Research Article

ASSESSMENT OF MORPHOLOGICAL INDICATORS FOR TESTICULAR MATURITY IN MALE AFRICAN CATFISH (CLARIAS GARIEPINUS) FOR ENHANCED GAMETE SELECTION IN CAPTIVE BREEDING

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ABSTRACT

The use of high quality gamete of male African catfish in captive breeding is of great importance to achieve fertilizability. The male African catfish possess two type of testis in which the milky white testes is always larger in size than the grey testis. This study was designed to identify other morphological indicators for testicular maturity in male *Clarias gariepinus* (*C. gariepinus*) rather than using only the reddening of the tip of the genital papillae for identification of maturity in male African catfish which is not always right. A total of 24 male and 4 female African catfish were used, with a focus on determining correlations between various morphometric parameters and testicular maturity. The weight, length of the fish, length of genital papillae, length and width of all fins of 24 males were measured. Fish were categorized based on testicular colour (grey or milky white) and evaluated for reproductive characteristics, including milt volume, sperm concentration, motility, and fertilizability. Results showed no significant correlation between testicular size and most morphological parameters in fish with grey testes. However, a significant positive correlation was found between total body length and tail fin width with testicular size in fish with milky white testes. Fish with milky white testes exhibited significantly better milt quality, with higher volume, sperm concentration, and fertilizability compared to those with grey testes. These findings suggest that certain morphometric features, particularly body length and tail fin width, may serve as useful indicators of testicular maturity and reproductive potential in African catfish.

Keywords: African catfish, testicular maturity, morphological indicators, milt quality, breeding efficiency, gamete selection

INTRODUCTION

African catfish, *Clarias gariepinus* (*C. gariepinus*) is an important source of animal protein in Nigeria and most African countries (Aremu & Ekunude, 2008). Because of their value and high marketability, they are widely cultured in Nigeria. They have fast growing rate and withstand adverse pond conditions such as hypoxia. Scarcity of good brood stock for the breeding of Clariids has been the major factor limiting the growth of catfish farming in Nigeria.

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 (Ali *et al.*, 2022). Anatomically, the testes of African catfish are located dorsally in the abdominal cavity lying within the long axis of the body with a whitish and lobular appearance (Zakariah *et al.*, 2016).

Testicular semen quality has great variability among different male individuals kept under similar conditions (Oteme, *et al.*, 1996; Mansour *et al.*, 2004; Ali *et al.*, 2022). Mansour *et al.* (2004) distinguished two types of testicular semen in African catfish depending on the maturation grade of the testes and period of collection. Testicular semen from

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mature testes (type I) was viscous in consistency and creamy white in colour while testicular semen from males with grey mature testes was watery in consistency and greyish white in colour (Mansour *et al.*, 2004). These two testicular semen types differed in several semen parameters, e.g. motility and sperm density, and behaved differently during short-term storage and during invitro incubation (Mansour *et al.*, 2004). Genital morphometrics (reddening of the tip of genital papillae) in determining sexual maturity in the male is not always right as such farmers/breeders usually sacrifice 2-3 males before they can get milt from brood stock (Viveiros *et al.*, 2001). The sacrificing of male African catfish is a huge waste that leads to inadequate availability of male brood stock. Hence, the current study was conducted to assess other morphological indicators for testicular maturity in male African catfish (*Clarias gariepinus*) for enhanced gamete selection in captive breeding.

MATERIALS AND METHODS

STUDY AREA

This study was conducted at the Andrology and Artificial insemination Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. Sokoto lies between 5º and 6º E longitude and between 13 and 14ºN latitude with an average annual temperature of 40ºC and mean annual rainfall of 300 mm – 1200 mm (Abdulrahim *et al.*, 2013). This research was carried out between the months of June to August (spawning period).

RESEARCH FISH AND MANAGEMENT

A total of twenty eight sexually mature C. *gariepinus;* twenty four males weighing between1.8–2.5 kg and four females weighing between1.5–2.0 kg were sourced from National Institute for Freshwater Fisheries Research (NIFFR) New Bussa, Niger state. The fish were acclimatized in 3.5 m x 3.5 m x 1.5 m outdoor tarpaulin tank for 2 weeks with mean temperature, pH and dissolved oxygen of 29.20 ± 1.89°C, 7.00 ± 0.79 and 4.95 ± 0.29 mg/l respectively. They were fed with a commercial diet containing 40% CP (blue crown®). Ethical approval for the study was obtained from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto with reference number UDUS/IACUC/2020/AUP-R0-20.

STUDY DESIGN AND DATA COLLECTION

This study employs an observational study with convenient sampling technique. The 24 males of C. *gariepinus* were tagged with different colours of bead by passing thread and needle through the hole of the bead and a knot was made on the dorsal fin for ease of identification. Morphometric measurements of each fish such as body weight, body length, head length, fins length and width, genital papillae length were recorded before the opening of the fish. The fish body weight was obtained by placing the fish on a weighing balance (Camry with product code; 3008286 made in China). Total body length was obtained by placing a tape-line from the snout to the tip of the tail. Head length was measured from the tip of the mouth to the posterior end of the head. Fins length was measured from the base to the tip of the fins. Fins width was measured by placing the thumb and index finger from one end of the fin to the other and spread it while the tape-line was placed at the widest part and the width was recorded. Genital papillae length was measured from the base to the tip of the genital papillae.

DETERMINATION OF TESTICULAR FEATURES

Following surgical opening of the before milt collection, gross testicular features were observed such as testicular colour either grey (1) or milky white (2) testes. Testicular size was assessed base on fullness; turgid (full), not turgid ("half" full), and flaccid (empty-looking), thickness (thick and thin) and length (medium and long) according to (Prista *et al.*, 2014) and on a scale 1-5 as shown below.

Scale 1; flaccid, thin and medium; Scale 2; not turgid, thin and long; Scale 3; not turgid, thick and medium; Scale 4; turgid, thick and medium; Scale 5; turgid, thick and long

MILT QUALITY ASSESSMENT

Milt quality (volume, colour, consistency, concentration and motility) were evaluated at initial immediately after. Milt volume was measured using 5 ml syringe. Milt colour was assessed as either colourless (1) or milky white (2) from grey and milky white testes respectively. The milt consistency was graded as watery (1) and creamy (2). Sperm motility was evaluated by adding normal physiological saline to the milt to activate the sperm cells and to reduce the concentration by serial dilution at the rate of 1:20 then a drop was placed on glass slide and viewed under microscope × 40 magnification objectives. Sperm concentration of the milt was determined using the nenbaur impured haemocytometer. Cover slip was placed on the haemocytometer after supporting the rails with water. This helps to hold down the cover slip while loading the sperm. Using a microtitre pipette 10-15 µl of the diluted sperm (1:20) was dropped under the cover slip on each side of the haemocytometer. The haemocytometer was carefully placed in the pre-wetted chamber, the bottom of the haemocytometer was dried and was placed under microscope without tilting it, the haemocytometer was viewed at × 40 Objective, making sure to count 5 squares on each side of the haemocytometer and then the average was calculated. Each side of the haemocytometer has an identical grid system consisting of 25 large squares in which each large square is divided into 16 smaller squares. The sperm heads was counted in the 5

large squares (top right, top left, bottom right, bottom left and the centre) of which each are containing 16 smaller squares.

The formula for sperm count = Total no of sperm counted from the 5 squares× dilution factor / volume × 1000

Sperm per ml = the number of sperm counted $\times 10^9$

DETERMINATION OF FERTILIZABILITY

The female were injected with Ovaprim® intramuscularly at dose rate of 0.5 ml/kg to induce ovulation manufactured by Western Chemical Inc. After 10 hrs latency period the females were stripped by abdominal massage, the eggs were collected from the genital opening of the female by gentle massage in to a bowl. Potency of milt obtained from the experiment was tested by fertilizing one table spoon of eggs and mixed with milt in a container and was rock for 15 seconds then was incubated in conventional sieves for 12 hours in plastic tank under flow-through system as described by Diyaware *et al.* (2010). The fertilization success was determined based on the appearance of the eggs in which the fertilized eggs appeared greenish in colour while the unfertilized eggs appeared whitish in colour according to Oyebola & Awodiran (2015). An imaginary line was drawn to divide the sieve into four and the ratio of fertilized eggs were estimated in each quarter and then added to get the estimated total fertilization rate in percentage.

STATISTICAL ANALYSIS

Morphometric measurements were analysed using correlation analysis with testicular maturation. The data obtained for comparison between grey and milky white testes were analyzed unpaired t-test using invivo stat version 3.7.0.0 with P value < 0.05 was considered as significant, results are presented in tables.

RESULTS

The results in Table Ia indicate the morphometric measurements of C. *gariepinus* in correlation with testicular size were not significantly correlated (P > 0.05) between all the indices measured for fish having grey testes with testicular size. Similarly, in Table Ib, there were no statistical significance correlation (P > 0.05) between all the morphometric measurement for fish having milky white testes with testicular size except the total body length and width of tail fin that is positively correlated significantly (P < 0.05) with a correlation coefficient of 0.60 and 0.46 respectively.

The comparison between some morphometric measurement and milt characteristics of C. *gariepinus* with grey and milky white testes are listed in Table II. Out of 24 males of C. *gariepinus* used for the experiment, 4 (16.6%) had grey coloured testes while 20 (83.4%) had milky-white testes. Fish with grey testes (figure 1B) had higher mean live body weight, total body length and width of tail fin while those with milky-white testes (Figure 1A) had a lower mean live body weight, total body length and width of tail fin , however, there were no significant difference (P > 0.05). Fish with grey testes had lower mean value of genital papillae length, testicular size, milt volume, milt concentration, fertilizability and with non-motile spermatozoa while fish with milky white testes had higher mean value of genital papillae length, testicular size, milt volume, milt concentration, fertilizability and with motile spermatozoa, which are statistically significant (P < 0.05) except for the length of genital papillae (P > 0.05).

TABLE IA: SOME MORPHOMETRICS MEASUREMENTS OF C. *GARIEPINUS* WITH GREY TESTES IN CORRELATION WITH TESTICULAR SIZE (1.50 ± 1.00)

Morphological Structures	Mean ± SD	Correlation coefficient
Anal fin length left/ right (cm)	4.50 ± 0.58	-0.58
Anal fin width left/ right (cm)	3.88 ± 0.69	-0.93
Dorsal fin (cm)	38.63 ± 1.13	-0.62
Total body length (cm)	66.63 ± 5.79	-0.65
Fish weight (kg)	2.08 ± 0.51	-0.75
Genital papillae length (cm)	1.83 ± 0.24	-0.92
Length of head (cm)	16.50 ± 0.71	-0.94
Length of tail fin (cm)	8.00 ± 0.82	0
Pectoral fin length (cm)	5.88 ± 0.85	-0.58
Pectoral fin width (cm)	5.38 ± 0.48	-0.87
Ventral fin (cm)	27.25 ± 4.03	-0.37
Width of tail fin (cm)	8.75 ± 0.87	-0.19

All values are expressed as mean ± SD

TABLE IB: SOME MORPHOMETRIC MEASUREMENTS OF C. *GARIEPINUS* WITH MILKY WHITE TESTES IN CORRELATION WITH TESTICULAR SIZE (3.66 ± 0.99)

Morphological Structures	Mean ± SD	Correlation coefficient
Anal fin length left / right (cm)	4.65 ± 0.46	-0.15
Anal fin width left / right (cm)	3.93 ± 0.77	-0.28
Dorsal fin (cm)	37.55 ± 2.11	0.41
Fish length (cm)	64.34 ± 3.50	0.60[a]
Fish weight (kg)	1.82 ± 0.26	0.37
Genital papillae length (cm)	1.92 ± 0.17	-0.11
Length of head (cm)	15.76 ± 0.84	0.21
Length of tail fin (cm)	7.75 ± 0.72	0.07
Pectoral fin length (cm)	5.88 ± 0.43	0.25
Pectoral fin width (cm)	5.28 ± 0.66	0.02
Ventral fin (cm)	26.18 ± 1.66	0.06
Width of tail fin (cm)	8.750 ± 1.63	0.46[a]

TABLE II: COMPARISM BETWEEN SOME MORPHOMETRIC MEASUREMENTS, MILT CHARACTERISTICS OF C. *GARIEPINUS* WITH GREY TESTES AND MILKY WHITE TESTES

Observations	Grey Testes (n=4)	Milky-White Testes (n=20)
Experimental fish (%)	16.6	83.3
Fish weight (kg)	2.15±1.19[a]	1.82±0.26[b]
Fish length (cm)	66.62±5.79[a]	64.34±3.50[b]
Genital papilae length (cm)	1.82±0.24[a]	1.92±0.17[b]
Width of tail fin (cm)	8.75±0.87[a]	8.70±1.16[b]
Testicular size	1.50±1.00[a]	3.60±0.99[a]
Milt volume (ml)	0.10±0.12[a]	1.12±0.55[a]
Milt colour	1	2
Milt consistency	1	2
Concentration ($\times 10^9$ ml^{-1})	7.00±10.40[a]	67.20±27.70[a]
Motility (%)	0	79
Fertilizability (%)	16.67±28.87[a]	71.25±20.89[a]

(Milt colour : colourless = 1, milky white = 2) (milt consistency: watery = 1, creamy = 2), Rows with the same superscript showed statistically significant difference (P < 0.05), Some values are expressed as mean ± SD

Rows with superscript are statistically correlated at(P < 0.05)
All values are expressed as mean ± SD

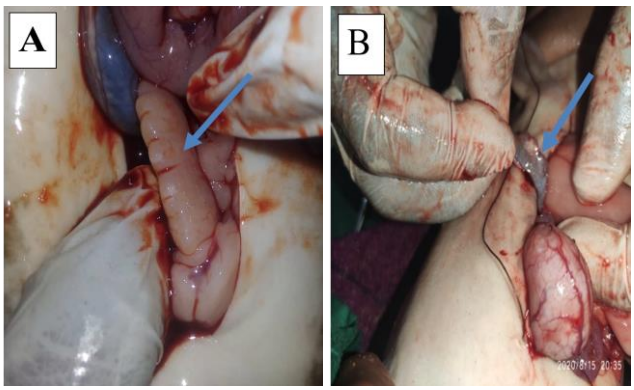

Figure I: Testes of the male *Clarias gariepinus* showing with blue arrow (A) milky white testes and (B) grey testes.

DISCUSSION

In this study, male *C. gariepinus* exhibited variation in testicular maturation stages, even though they were of the same age and rose under similar conditions. Despite the reddening of the genital papillae, which indicates maturity, these findings align with Piironen (1985), who reported that milt quality varies significantly among individuals of the same species. The live weight (2.15 kg) and total body length (66.62 cm) of fish with grey testes showed no correlation with testicular maturation. This is consistent with Akter *et al.* (2019), who found no significant correlation between length-weight relationships (272.3 g, 27.8 cm) and testicular maturation. In contrast, the total body length of fish with white testes (64.34 cm) did show a significant correlation with testicular maturation, contradicting the findings of Akter *et al.* (2019), which did not observe such a correlation.

The length of the genital papillae in fish with grey and milky white testes (1.82 ± 0.24 cm and 1.92 ± 0.17 cm, respectively) did not show a significant correlation with testicular maturation. This is in agreement with Neves *et al.* (2019), who also found no significant correlation between the length of the genital papillae (2.43 cm) and the maturity of the testes. Therefore, the length of the genital papillae isn't a dependable maturity marker indicator in male *C. gariepinus*, although it is evident that larger genital papillae often correspond to more advanced sexual maturity. This agrees with the study conducted by Jamie-Lee *et al.* (2019) who reported that urogenital papillae do not provide an accurate or reliable indication of the maturity stage of *C. gariepinus* fish.

Additionally, there was no significant correlation between the length and width of any fins, except for the width of the tail fin of fish with white testes (8.70 ± 1.16 cm), which did show a significant correlation with testicular maturation. Furthermore, the pectoral fin was found to be a reliable landmark for locating the position of the testes, which is in agreement with Idahor (2014), who noted that the end of the pectoral fin corresponds to the midpoint of the testes.

When comparing the morphometric measurements, such as weight, total body length, width of the tail fin, and genital papillae length between fish with grey testes and those with white testes, no statistically significant differences were observed. This may be due to the fact that fish with grey testes may exhibit poor reproductive performance, thus diverting energy towards growth rather than reproduction. In contrast, fish with white testes require more energy for reproductive functions, which could explain the observed differences in reproductive characteristics.

In addition, a study by Ali *et al.* (2022) reported a positive correlation between body weight of fish and milt volume in wild *C. gariepinus*; indicative that those with larger body weights had higher volume of milt and spermatozoa concentration than those with lower body weights, our findings in this research contradicts this report perhaps due to the fact that cultured strain was used in our study.

Milky white and grey testes (shown in Figure 1 'A and B') were observed to produce creamy and watery milt, respectively. The creamy milt exhibited higher values in milt volume, sperm concentration, and fertilizability with motile

spermatozoa (1.12 ml, 67.20×10^9 ml^{-1}, 71.25%) compared to the watery milt, which had lower values in all three parameters (0.10 ml, 7.00×10^9 ml^{-1}, 16.67%). These findings align with those of Mansour *et al.* (2004) and Mansour *et al.* (2005), who also reported that type I milt (creamy) had greater milt volume, sperm concentration, and fertilizability with motile spermatozoa (2.2mls, 109.84×10^9 ml^{-1}, 90.30%), while type II milt (watery) showed lower values (1.3mls, 3.6×10^9 ml^{-1}, 87.5%). However, this study disagrees with the findings of Mansour *et al.* (2005), who suggested that grey testes milt contained motile spermatozoa. The discrepancy between the two studies may stem from differences in methodology, specifically the use of motility analysis software in Mansour *et al.*'s work, which adjusts particle recognition to the exact size of African catfish spermatozoa. Additionally, this study found that creamy milt exhibited higher fertilizability than watery milt, contrasting with Mansour *et al.* (2005), who reported similar fertilization rates for both milt types. Moreover, Mansour *et al.* (2005) observed a significantly higher hatching percentage in type I (creamy) milt compared to type II (watery) milt, which is not consistent with the results of the present study.

CONCLUSION

There were no significant correlations found between testicular size and most morphological parameters in fish with grey testes. However, significant correlations were observed between body length and tail fin width with testicular size in fish with milky white testes. Furthermore, fish with milky white testes exhibited superior milt quality, including higher volume, sperm concentration, and fertilizability. The results suggest that certain morphometric indicators, particularly body length and tail fin width, can be useful in identifying mature males with higher reproductive potential.

RECOMMENDATIONS

During artificial breeding the width of tail fin and total body length should be considered for selection of matured male African catfish

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