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Original

Morphometric and Seminal Characterization of *Clarias gariepinus* Breeding Stock in their Pre-Breeding and Breeding Seasons

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ABSTRACT

Background: Artificial breeding of *Clarias gariepinus* entails sacrificing the male, which is necessary to know the semen's physical features and variations for preservation during the pre-breeding stage, and its utilization for the breeding stage. **Aim.** To characterize the morphometric changes and seminal quality of *Clarias gariepinus* during the pre-breeding season (PBS) and breeding (BS) season. **Methods:** A number of 42 breeding males were evaluated in the two stages, using weight (LW) (kg), length (FL) (cm), gonadal weight (GW), and seminal vesicles (SVW) (g), condition factor (CF), gonadosomatic index (GSI), and seminal-somatic vesicles index (S-SVI). Samples from testicle's semen (TS) and seminal plasma/testicle's semen (SP-TS) were collected. Their color, testicle seminal volume (TSV), and seminal plasma volume (SPV) (ml), motility, sperm activation time (SAT) (sec), sperm concentration (SC) (x10^9 sptz/ml), and spermatocrits (SPCTO) (5), were evaluated. An analysis of variance of the variables was conducted, considering the time as the variation source, along with a Pearson correlation analysis. **Results:** The LW, GW, and GSI increased significantly (P<0.05) during the BS). The TSV and SPV were lower in the PBS, with a higher SC. The creamy semen showed high SC (21.14 ± 2.90 x 10^9 sptz/ml) and SPCTO (19.50 ± 1.24 %), whose correlation was moderate (r=0.58, P<0.001). **Conclusions**: The breeding males with low GSI and the aqueous semen should be rejected due to their low cellular density. SC can be predicted through the spermatocrit, so that high-quality semen can be available during the PBS through conservation.

Keywords: *Clarias gariepinus*, seminal features, stage, morphometry (*Source: MeSH*)

INTRODUCTION

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Aquaculture has undergone higher productivity levels worldwide. Species like the African catfish, *Clarias gariepinus*, has played an important role in this increase. This species replaced tilapia as the most commonly grown fish by 2004, in Africa (FAO, 2012), with a marked influence of this sector in Nigeria (Jimoh *et al.,* 2021). It is considered one of the most significant tropical catfishes in aquaculture in Asia and even in Europe (Viveiros, So and Komen, 2000; Okoye *et al.,* 2017). Today, it is grown in modern systems with water recirculation in The Netherlands (Fleuren, Nooijen and Roosendaal, 2013). In Cuba, it ranks second in fresh water productions due to its high growth rate, endurance to environmental conditions, and continuous reproduction (De Graaf, Galemoni and Banzoussi, 1995).

Induced hatching means that the male must be sacrificed to collect the semen from the testicles (Steyn and Van Vuren, 1987), which permits the collection of limited amounts of sperm (Viveiros, So and Komen, 2000; Mansour, Lahnsteiner and Patzner, 2002). Seminal collection through abdominal massaging is almost impossible today, as the finger pressure makes the semen moves to the seminal vesicle rathern than to the pore of the genital papillae (De Graaf, Galemoni and Banzoussi, 1995; Viveiros *et al.,* 2002). It is important to acquire quality semen to increase the efficiency of artificial fertilization in fish species, so it requires the utilization of male breeders with a high spermatic viability and high seminal volumes (Solomon *et al.,* 2015); testicle semen quality vary among the males (Mansour, Lahnsteiner and Berger, 2004). Therefore, the determination of sperm quality parameters is a pre-requisite for satisfactory evaluation of their reproductive capacity, and help with artificial insemination in fish farms (Alavi *et al.,* 2009).

The spermatozoa capacity to fertilize the oocyte is the main factor affecting the fertilization rates. The capacity depends on qualitative and quantitative indicators, such as volume, concentration, motility, viability, and sperm morphology (Bustamante-González *et al.,* 2016), of which the volume indicates the difference, while concentration and motility determine the fertilizing capacity of the spermatozoa (Cabrita *et al.,* 2014), as the chemical properties of the seminal fluid ensure fish spermatozoa to remain still inside the fluid (Coward, Campos and Parrington, 2008).

Accordingly, the aim of this paper is to characterize the morphometric changes and seminal quality of *Clarias gariepinus* during the pre-breeding (PBS) and breeding (BS) seasons.

MATERIALS AND METHODS

Location

The materials were collected from the African catfish (*Clarias gariepinus*) (Burchell 1822) breeding stock, at El Dique Development and Innovation Unit (UDI), the Company for the Development of Aquaculture Technologies (EDTA).

Breeding management

The breeding animals were in a concrete tank with continuous water circulation and daily consumption of commercial feedstuff (36% crude protein, CP and 2 964 Kcal/kg digestible energy, DE), at 1% biomass.

Experimental design and sampled animals

A total of 42 male breeding animals were selected at random, 2 years of age, on average. Of them, 23 were in their pre-breeding season, and 19 were ready for reproduction. The semen from testicles (TS) and testicle semen plus seminal plasma (TS-SP) were collected in the two stages, as described in *Collection of testicle semen and seminal plasma*.

Morphometry

The morphometric parameters were evaluated, namely live weight (LW, kg), full length (FL, cm), gonad weight (GW, g), and seminal vesicle weight, (SVW, g). The Fulton (1902) condition factor (CF) was used to determine the general status of fish development, through the formula:

$$CF = \frac{LW}{FL^3} \times 100$$

The gonadosomatic index (GSI) (Ferrer, 1988) was calculated to determine gonad maturity and the somatic-seminal vesicle index (S-SVI) (Viveiros, Eding and Komen, 2001), through the formula:

$$GSI\ (\%) = \frac{GW}{LW} \times 100 \quad \text{and} \quad S\text{-}SVI\ (\%) = \frac{SVW}{LW} \times 100$$

Two 60 kg and 1 kg digital balances (GRAM) were used for weight determinations. Fish length was measured using an ichthyometer.

Collection of testicle semen and seminal plasma

Seminal collection was performed in graduated plastic tubes upon animal sacrifice, through abdominal dissection and testicle removal (Elizarde *et al.*, 2007). Multiple incisions were practiced to the testicles and seminal vesicles, in the form of finger extensions, to collect the semen from the testicles and seminal vesicles, respectively. The material collected was filtered with a zooplankton mesh (200 µm). The samples were stored at 4°C until macro and microscopic evaluation was performed, three hours following the extraction.

Physical characterization of the semen

The samples were characterized as to color, consistency, testicle seminal volume (TSV, ml), and volume of the seminal plasma (SPV, ml). A subjective evaluation of motility (%) was made, following a five-point scale (0- +4), suggested by Ninhaus-Silveira *et al.* (2006), mixing a drop of

fresh semen with distilled water; then it was observed through the microscope at a 10x/40x magnification. Then the spermatic activation time (SAT, sec) was measured using a semen drop mixed with running water to activate motility (López-Hernández *et al.*, 2018). The spermatic concentration was determined (SC, $x10^9$ sptz/ml), in a 1:800 dilution (0.01 ml of semen in 8 ml of 3% saline solution). The counts were done by duplicate in the Neubauer chamber with a 40X magnification. The two readouts were averaged according to the traditional methodology used for hematological counts described by Oppenheim (1973). Lastly, the spermatocrit (SPCTO, %) value was determined by sample centrifugation by duplicate for 20 minutes, at 3500 rpm. in a microhematocrit centrifuge (Hawkley) (Portales *et al.*, 2021).

Statistical analysis

The general statistical data were analyzed for the morphometric and physical features of the semen and the seminal plasma. Analyses of variance were performed using the general linear model (GLM) for these traits; the variation source established was the sample collection time. The natural logarithm of the motility and spermatocrit data were estimated for transformation prior processing. A Pearson correlation analysis was performed as well. All the statistical data were processed using MINITAB (2019).

RESULTS AND DISCUSSION

Morphometric indicators

Significant differences were found ($p<0.05$) in LW, GW, and GSI of the male breeding animals of *Clarias gariepinus*, in favor of the breeding season (Table 1).

Zacariah *et al.* (2016) also detected smaller reproductive organs in animals within the pre-breeding period, while other researchers did not find differences in testicle weight between the periods in wild *C. gariepinus* (Idahor *et al.*, 2014; Yusuf *et al.*, 2015; Ali *et al.*, 2022).

Although the seasonal differences are not well-defined, they are associated with environmental factors like temperature, feed availability, precipitations, and photoperiod. This fish has a discontinuous reproductive cycle in their natural habitat, influenced by circadian changes in water temperature and photoperiodicity (Olaleye, 2005), with rising water levels due to precipitations, as an activation mechanism for spawning. The weight of breeding males plays a major role in the period of sperm maturation, with a broad range in the number of spermatozoa, which is linked to the increase of testicle weight and body weight, and it is considered a good indicator of efficiency in spermatogenesis (Bromage and Roberts, 1995; Jimoh *et al.*, 2021), and the amount of sperm produced (Billard, 1986; Ali *et al.*, 2022).

Table 1. Morphometric traits, somatic indexes, and changes in the reproductive apparatus of males between the pre-breeding period (N=23) and breeding period (N=19).

Traits	Pre-breeding period (February-March)			Breeding period (June)			p
	M ± SE	SD	VC	M ± SE	SD	VC	
LW (kg)	2.14 ± 0.11	0.51	24	2.47 ± 0.10	0.45	18	p<0.05

FL (cm)	71.3 ± 1.90	9.09	13	73.05 ± 0.79	3.43	5	ns
GW (g)	23.42 ± 2.58	12.37	53	34.74 ± 3.37	14.67	42	p<0.05
SVW (g)	13.73 ± 1.74	8.37	61	17.37 ± 1.85	8.06	46	ns
CF	0.61 ± 0.04	0.17	28	0.64 ± 0.03	0.12	19	ns
GSI	1.06 ± 0.08	0.37	35	1.41 ± 0.12	0.53	38	p<0.05
S-SVI	0.65 ± 0.08	0.36	56	0.70 ± 0.07	0.30	42	ns

Legend: LW: live weight; FL: full length; GW: gonad weight (testicles); SVW: seminal vesicle weight; CF: Fulton's condition factor; GSI: gonadosomatic index; S-SVI: somatic-seminal vesicle index; p: significance level: ns: insignificant statistical differences.

In male *Clarias gariepinus* breeding animals grown in Nigeria, weighing 2.54 ± 0.12 kg, Jimoh *et al.* (2021) found a total gonad weight similar to the findings of this research (27.94 ± 8.17 g and 1.15 ± 0.38, respectively), though the condition factor was higher (0.84 ± 0.07). The authors considered that the GSI and the reproductive traits of the males would explain the difference in reproductive performance and the production of seeds in captivity. Kumari (2014) demonstrated the existence of a positive correlation between the testicle weight and seminal production, so GSI is used to estimate the reproductive performance of the fish. In the two seasons, this index was above 1, so the results of this research do not match the findings of Urbányi *et al.* (1999), who reported that quite a few siluridae, including the *Clarias gariepinus* are oligospermic (low spermatozoa count) (GSI<1), and that the volume collected is normally low, even after hormonal stimulation.

Moreover, the SV increase was not significant, neither was the SVI (0.70 ± 0.07%), which had a positive correlation with the testicle secretory activity of the species (Singh and Joy, 1999). The S-SVI was higher than the findings of Viveiros, Eding and Komen (2001) (0.29 ± 0.06 %) in males with well-developed seminal vesicles, having long extensions like fingers on either side of the spermatic conduits. It was also better than the values reported by Amer *et al.* (2005) (0.2 - 0.4 %).

Seminal quality

During the removal of testicles, their lobular appearance, roundness, and whitish color were exposed (Figure 1A), associated with the type I mature testicle (Mansour *et al.,* 2004), which produce high quality semen. A similar appearance was observed in the male gonads of wild *C. gariepinus* in a Nigerian lake, during the spawning season (Ali *et al.,* 2022). The accessory genital glands (seminal vesicles) showed multiple lobules and produced a highly viscous and pink fluid, corresponding to the common observations of family Clariidae (Van der Hurk, Resink and Peute, 1987; Singh and Joy, 1999).

Additionally, the testicles were atrophied, especially in the PBS (Figure 1B), and a pathological case in BS (Figure 1C). The pathological testicles showed a size increase (60 g) and grayish coloring, with a SVW of 30 g. The semen collected was aqueous and transparent, with a TSV of 40 ml and SPV of 8 ml, above the average, and poor quality (20% motility, FC 2.4 x 10^9 sptz/ml,

and SPCTO 2.4%). In turn, the fish's biochemical profile showed high triglyceride values (36.28 mg/dL) and glucose (13.69 mg/dL) in the blood, showing metabolic alterations.

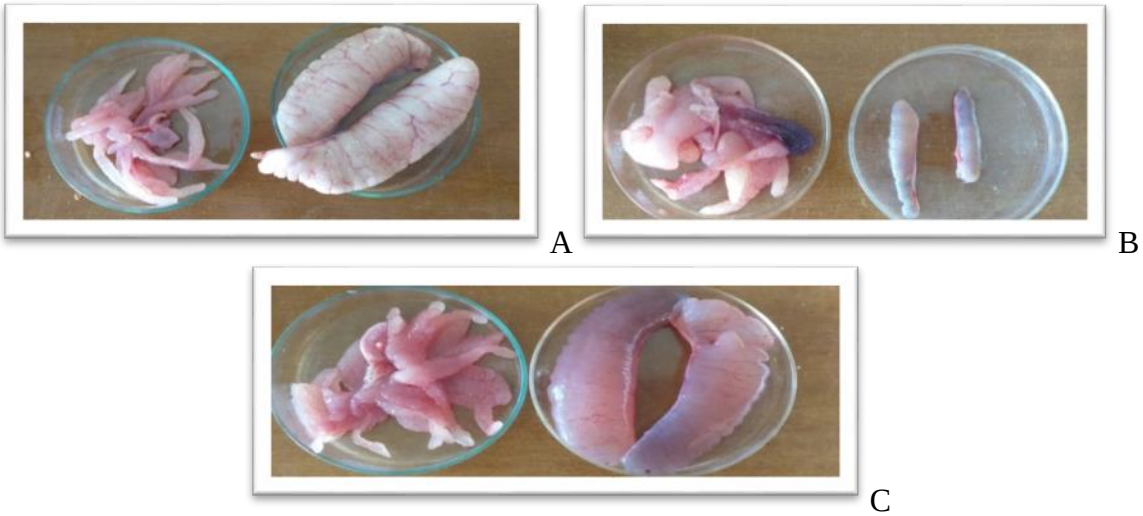

Figure 1. Testicles and seminal vesicles of *Clarias gariepinus* breeding animals: A. Type I mature testicles; B. Atrophied testicles and larger size vesicles; C. Testicles related to poor seminal quality (pathological case).

In general, the TS collected in the two seasons showed a white color with a milky consistency, through in the PBS some samples were creamy, coinciding with the parameters observed in non-spawning and spawning seasons of wild *C. gariepinus* (Ali *et al.*, 2022). The TSV produced was high, matching the ones obtained in Nigeria (17.69 ml), which indicated that fewer males are required to fertilize the eggs, thanks to their abundant production of semen (Jimoh *et al.*, 2021).

A physiological relation was observed between the seminal consistency and the TSV, SC and SPCTO (Figure 2). The TSV decreased as the consistency changed from aqueous to milky and creamy. The creamy semen was associated with greater cell density (21.14 ± 2.90 x 10^9/ml and 19.50 ± 1.24 % SPCTO), compared to the aqueous semen (3.27 ± 0.87 x 10^9/ml and 4.30 ± 1.90 %).

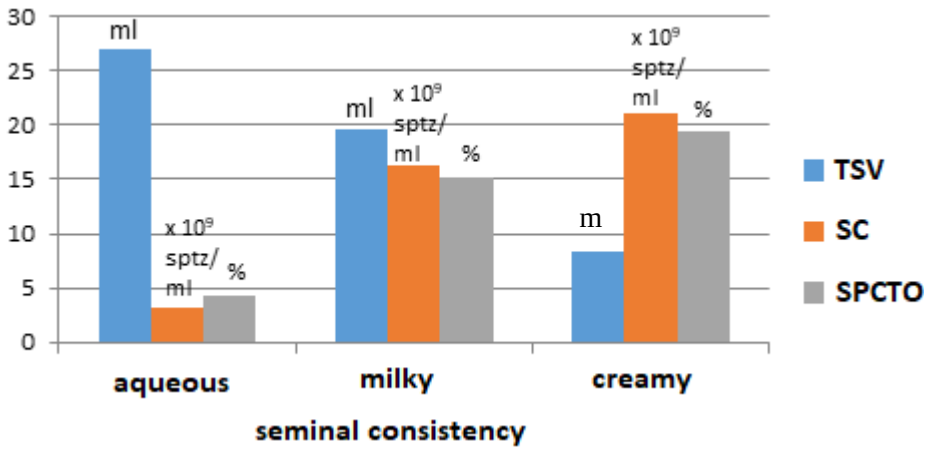

Figure 2. Indicators of seminal quality linked to the consistency of the testicle semen.

There is previous information that confirm the variation of the spermatocrit and viscosity (consistency) of semen among males, species, and through the reproductive season (Rakitin, Ferguson and Trippel, 1999; Ali *et al.*, 2022). In keeping with these authors, this species can produce a large number of seeds, and it is also possible to collect quality semen during spawning and non-spawning seasons, then use consistency in the evaluation of semen throughout the breeding campaigns to discard faulty semen, as a *priori* criterion.

In natural settings, in Nigeria, this species showed lower SC and SV (2.9 ± 0.4 and 2.6 ± 0.4 x 10^9/ml, 3.6 and 3.3 ml) in spawning and non-spawning seasons, respectively. It was linked to a lower breeding animal weight (994.8 and 898.6 g), since fishes with higher weights produced the greatest seminal volumes (Ali *et al.*, 2022). Overall, there are variations in the number of spermatozoa among species (2 x 10^6 at 5.3 x 10^{10}), which is related to the breeding season, hormonal treatment, fertility percentages, availability and the nutritional properties of the feed and water quality (Murakami *et al.*, 2014; López-Hernández *et al.*, 2018).

In this study, new spermatozoa count was higher than the ones reported by Viveiros, So and Komen (2000); Yusuf and Ilker (2017), and Okoye *et al.* (2017), and lower than the findings of Solomon *et al.* (2015) (35 - 97 × 10^9 sptz/ml), the latter case attributed to the peak of the mating season. Ali *et al.* (2022) also collected sperm in the non-spawning season; even the milky semen collected had a mean concentration within the normal range (1.8 - 7.2 x 10^9/ml), according to Viveiros, So and Komen (2000), and Yusuf and Ilker (2017) in the African catfish, in Türkiye. It can be attributed to the fact that the testicles of male *Clarias gariepinus* are completely developed when they reach approximately 200 g of weight (De Graaf and Janssen, 1996; Ali *et al.*, 2022).

Moreover, no differences were found in motility, SPCTO and SAT between the two periods, with high quality semen. The lowest TSV and SPV were observed in the PBS animals, associated with higher SC (Table 2). Increased SV in the BS animals was attributed to the presence of type I mature testicles and an increase in GW and SVW. The secretory activity of the SV of *Clarias gariepinus* is stimulated by testosterone (Singh and Joy, 1997), and its mating is seasonal. The

testicular activity and spermatogenesis depend largely on the photoperiod and temperature (Garg and Sundararaj, 1985).

Table 2. Season comparison of the physical characteristics of the semen and seminal plasma of *Clarias gariepinus* breeding animals

Seminal quality	Pre-breeding season	Breeding season	p
	M ± SE	M ± SE	
TSV (ml)	11.10 ± 1.82	23.57 ± 2.25	p<0.001
SPV (ml)	0.86 ± 0.28	1.93 ± 0.35	p<0.05
Motility (%)	66.72 ± 6.17	79.74 ± 4.92	ns
SC (x10^9 sptz/ml)	19.08 ± 1.43	13.39 ± 1.60	p<0.05
SPCTO (%)	16.9 ± 1.07	13.8 ± 1.21	ns
SAT (sec)	40.1 ± 2.54	35.6 ± 2.86	ns

Legend: TSV: testicle semen volume; SPV: seminal plasma volume; SC: spermatic concentration; SPCTO: spermatocrit; SAT: spermatic activation time.

In *Clarias gariepinus* different stages of gonadal maturation can be identified among the males, and also in the same testicle of a fish during the natural spawning. It affects consistency, volume, and sperm density, though motility remains unchanged (Mansour *et al.*, 2004). This indicator did not show significant differences between the two seasons, and it is considered the best semen quality biomarker, associated with the fertilization capacity (Oguntuase and Adebayo, 2014).

Contrary to other indicators, SAT coincides with the duration of sperm motility (40 seconds) reported by Mansour *et al.* (2004), which diminished about 50% after half of that time had elapsed, coinciding with the time reported in the striped catfish *Pseudoplatystoma metaense* (39.5 seconds), by Ramírez-Merlano, Medina-Robles, and Cruz-Casallas (2011). Its brevity is associated with the short time available for immotile spermatozoa to get in contact with the water, by acquiring motility and movement speed progressively to ensure fertilization, before the ova released into the water hydrate and close their microvilli, the oocyte orifice through which the spermatozoa penetrate for fertilization. This is explained by the absence of acrosome in the spermatozoa of teleostan fishes, contrary to those of mammals (Quagio-Grassiotto *et al.*, 2001). Therefore, both gametes are released into the water in a synchronized manner, where they activate, and fertilization takes place (Dumorne *et al.*, 2018).

The characteristics evaluated corroborated that *C. gariepinus* is a continuous high-quality sperm-producing species for periods in which temperatures and photoperiods are unfavorable for female fertilization. These results support the rationale for conserving semen during this stage, since the asynchrony of spermatogenesis backs the continuous production of sperm of grown *C. gariepinus*, once they begin with the production of sperm, at 6 months of age, when the spermatozoa are reported to have the capacity for progressive unidirectional movement, indicating a fish's sexual maturity. However, genetic factors and culturing conditions, including nutrition, influence the maturation age of the grown fishes (Okoye *et al.*, 2016). Additionally, the highest SGI, seminal volume, SC, and SPCTO were found in 12-month-old males, with improved seminal quality in larger fishes (Okoye *et al.*, 2017), which explains the selection of breeding males at this time.

Correlation between the breeding and seminal features

The highest correlations between the morphometric and seminal traits were observed in the PBS (Table 3).

Table 3. Relation between live weight, testicle and seminal vesicle weight, and some parameters of the semen of *Clarias gariepinus* in the pre-breeding and breeding seasons

Parameters	Pre-breeding season		Breeding season	
	Pearson correlation coefficient (r)	P value	Pearson correlation coefficient (r)	P value
LW - GW	**0.73**	**<0.0001**	0.24	0.34
LW - SVW	0.32	0.13	0.40	0.09
LW - TSV	**0.71**	**0.0001**	0.16	0.52
LW - SPV	0.12	0.64	-0.21	0.58
GW - SVW	0.31	0.14	0.42	0.08
LW - TSV	**0.98**	**<0.0001**	**0.92**	**<0.0001**
GW - SPV	0.01	0.95	0.45	0.22
GW - SPCTO	0.10	0.64	-0.31	0.21
GW - SC	**0.55**	**0.0058**	-0.09	0.72
SVW - TSV	0.36	0.09	0.32	0.20
SVW - SPV	**0.86**	**<0.0001**	0.22	0.56
TSV - SPCTO	0.09	0.67	-0.45	0.06
TSV - SC	**0.55**	**0.0057**	-0.15	0.55
SPCTO - SC	**0.53**	**0.0073**	0.33	0.18

The correlation is significant for p<0.05

There was a high and significant correlation (p<0.001) between the LW and TSV (r=0.71), GW, and TSV (r=0.98), and between SVW and SPV (r=0.86). A moderate and significant correlation (p<0.01) was observed between the GW and SC (r=0.55), TSV and SC (r=0.55), and between SPCTO and SC (r=0.53). These findings coincide with the reports of Yusuf *et al.* (2015), who found positive, though higher, correlations (r=0.72, p<0.05) of SV and GW with SC, in breeding males grown in Nigeria, whereas the correlations were similar between the GW and TSV (r=0.98, p<0.01).

In the BS, there was a joint growth of the testicles and seminal vesicles, as a response to the favorable environmental conditions for breeding (increase of GW, TSV, SPV), and a reduction of SC. However, only the GW and the TSV showed a significant correlation (r=0.92; p<0.0001), whereas the association of sperm density indicators (SC and SPCTO) was low. The correlation between LW and TSV (r=0.16, p>0.05) in the BS contrasted the reports of Ali *et al.* (2022) in the spawning season of *C. gariepinus*, in natural settings, in Nigeria (r=0.36; p<0.05). Likewise, the findings of this study differ from those authors, who found low and non-significant relations of testicle weight with the SV (r=0.20) and SC (r=0.28).

The results achieved permit the utilization of SPCTO as a practical indicator of rapid measurement to predict SC in fishes only in the PBS, according to Rakitin *et al.* (1999) and

Portales *et al.* (2021). In other coldwater species, there are higher correlations (r=0.84), such as in the Himalayan fish *Schizothoraichthys progastusis* (Agarwal, Vandana and Raghuvanshi, 2013).

CONCLUSIONS

The breeding males of *Clarias gariepinus* with aqueous semen and a low gonadosomatic index should be discarded due to their low cellular density. The sperm concentration can be predicted through spermatocrit determination so that high-quality semen can be available, using conservation techniques, during the pre-breeding season.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: APG, MTGC, MVC, LIA; data analysis and interpretation: APG, MTGC, MVC, LIA; redaction of the manuscript: APG, MTGC, MVC, LIA.

CONFLICT OF INTEREST STATEMENT

The authors declare the existence of no conflicts of interests.