SEMEN QUALITY PARAMETERS OF CULTURED BROODSTOCK AFRICAN CATFISH (*CLARIAS GARIEPINUS*) OF DIFFERENT AGES

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ABSTRACT

This study investigated the age-related variations in the semen volume, sperm count, spermatocrit, testicular and genital papillae allometric values as well as testicular histomorphologies of cultured male broodstock Clarias gariepinus at 6, 9 and 12 months of age. Each of these months constituted an age group and had 10 fish for the study which was performed in duplicate. The semen samples in all the groups were observed to be viscid and milky in colour; and had over 80% sperm motility. The means of the left testes semen volume, left testes and right testes spermatocrit of the 6 months group were significantly lower (p<0.05) than those of the 9 and 12 months groups; while the 12 months group was significantly higher than those of 6 and 9 months. The means of the right testes, left testes and right testes sperm counts of the 6 months group were significantly lower (p<0.05) than those of the 9 and 12 months groups. Sections of the testes of these fish at 6, 9 and 12 month of age upon histological examination showed similar features of their germinal epithelia and the lumina of their seminiferous tubules. It was concluded that 12 months old broodstock male catfish appear to be better candidates for spawning.

Keywords: *Clarias gariepinus*, Age, Spermatozoa, Seminal vesicle, Testes, Semen, Spermatocrit

INTRODUCTION

Aquaculture has been recording increasing productivity, and the African sharp-toothed catfish (*Clarias gariepinus*) has replaced tilapia as the most cultured fish since 2004 in Africa (FAO, 2012); and is about the most widely distributed fish in Africa (Skelton, 2001). It natural habitat range covers the most of the African continent (Picker and Griffiths, 2013); though it is considered a potamodromous freshwater species, it thrives in waters with pH ranges of 6.5 to 8.0, a temperature of 8°C to 35°C and can tolerate high turbidity and low dissolved oxygen (Safriel and Bruton, 1984; Teugels, 1986; Van der Waal, 1998). These and other factors together with its wide acceptance across many cultures and good organoleptic perceptions makes *C. gariepinus* one of the most common fish in Africa and some other parts of the world (Adeyemo *et al.,* 2007).

Attempts at management of the finite natural fish biodiversity are augmented through aquaculture (Lovshin *et al.,* 1990) and other assisted reproductive techniques. Most of these are predicated upon manipulations of the reproductive physiology of the female fishes via alterations of the cascade of reproductive hormones consequent upon regulation and control of the environmental signals or administration of exogenous hormones. Induced spawning in *C. gariepinus* often involves either sacrificing the male to harvest the milt from the testes (Steyn and Van Vuren, 1987) resulting in the loss of the particular fish without ascertaining the suitability of it as a good candidate for semen collection or surgical resection of part of the testis to harvest semen from the fish (Bart and Dunham, 1990) due to the inability to digitally strip the milt as digital pressure will result in the semen moving into the seminal vesicle and not through the genial papillae pore (de Graaf *et al.,* 1995). Assessment and establishment of fish semen quality parameters would greatly facilitate adoption of various reproductive technologies in aquaculture (Alavi *et al.,* 2008) especially the determination of the optimum age for broodstock male fish candidates. Amongst the myriad reports available on the semen quality parameters of *C. gariepinus* there is dearth of information on these across different ages of broodstock males.

This study therefore evaluated semen quality parameters which include semen volume, sperm count, spermatocrit, testicular and genital papillae allometric values as well as testicular histomorphologies across 6, 9 and 12 months age of cultured male broodstock African catfish (*C. gariepinus*).

MATERIALS AND METHODS

Experimental Fish: A total of sixty apparently healthy male cultured broodstock African catfish (*C. gariepinus*) used in this study were procured from Livestock Specialist Fisheries, Oguta, Imo State, Nigeria within the month of March, 2015. The fishes were stocked in concrete ponds according to age at a stocking density of 750 per 12 x 12 x 1 m^2 and were point-fed fish feed (Vital®, GCOML. Jos, Nigeria). Oguta is located within the tropical rain forest zone on 5.71^o North and 6.81^o East, and lies about 121m above sea level, with average temperature of 34 ^{o}C and 75 % humidity for the month of March.

Ten fish of 6, 9 and 12 months of the age groups studied were randomly selected and transported to the Laboratory of the Department of Veterinary Obstetrics and Reproductive Disease, Faculty of Veterinary Medicine, University of Nigeria where the study was done. Catfishes of the same age were cultured in the same pond and their ages were gotten from the farm record. The study was done in duplicate.

Ethics: Applicable institutional and international guidelines for the welfare and use of fish for research were strictly observed in the conduct of this study (AVMA, 2013).

Tissue Sample Collection: Following stunning, the live weight and length of the fishes were measured as well as the length of the genital papillae. The fish were sacrificed (AVMA, 2013) and then a ventral midline incision was made into the body cavity, and the testes were resected. The weights of the left and right testes were taken. These data were used to calculate the allometric indices for each fish according to the formula of Roff (1983).

Measurement of Semen Volume and Colour: Once testicular weights were obtained, a longitudinal incision was made on the left testes and the milt was then collected into calibrated glass tubes. The colour and volume were noted.

Determination of Sperm Motility, Count and Spermatocrit: This was derived using the haemocytometric method of Amann and Almquist (1961). Spermatocrit determination was by the microhaematocrit method of Ciereszko and Dabrowski (1993). Motility of the sperm cell in the milt sampled was observed under the microscope after activation using a hypertonic solution.

Histological Preparation: The right testes were promptly fixed in Bouin's fluid for 48 hours, the tissues were then dehydrated in graded concentrations of ethanol (70, 80, 90 % and absolute alcohol), then cleared in xylene in two changes at one hour interval, infiltrated with paraffin and then 5µm thick sections were cut using a rotatory microtome. The sections were stained with Haematoxylin and Eosin for light microscopy. Photomicrographs were captured using a Moticam Digital Camera (Motic China Group Company Limited, Xiamen, China).

Data Analysis: The collected data were subjected to one-way analysis of variance (ANOVA). The statistical analyses were done using SPSS 16.0 (for Windows SPSS 16.0 Inc., Chicago, IL, USA). The variant means were separated using least significant difference, and significant difference was accepted at probability level less than 0.05 ($P<0.05$).The results for all the parameters were presented as mean ± standard error (SE).

RESULTS

The mean live fish weight and length of the 6 months group was significantly lower ($p<0.05$) than those of the 9 and 12 months catfishes; while the 12 months group was significantly higher than those of 6 and 9 catfishes. The mean genital papillae length, mean left and right testicular weights of the 6 months group were significantly lowers ($p<0.05$) than those of the 9 and 12 months, but there were no significant variations ($p>0.05$) in these values in the 9 and 12 months old catfishes (Table 1).

There were no significant variations ($p>0.05$) in the means of the right and pooled testicular allometric weights across the groups. The mean genital papillae allometric length and left testicular allometric weight of the 6 months group were significantly lower ($p<0.05$) than those of 9 and 12 months age groups (Table 2). The semen samples were viscid and milky in colour; and had over 80% sperm motility.

The means of the left testes semen volume, left testes and right testes spermatocrit of the 6 months group were significantly lower ($p<0.05$) than those of the 9 and 12 months groups; while the 12 months group was significantly higher than those of 6 and 9 months. The means of the right testes, left testes and right testes sperm counts of the 6 months group were significantly lower ($p<0.05$) than those of the 9 and 12 months groups (Table 3).

Histological examination of sections of the testes at 6, 9 and 12 month of age showed that the germinal epithelia of their seminiferous tubules had spermatogenic cell lines at different stages of development. These spermatogenic cells were all enclosed in cyst within the germinal epithelia. These cysts had clones of spermatogenic cell lines at identical stages of development (Figure 1). The lumina of the seminiferous tubules contained clusters of free spermatozoa (Figure 1). An incidental finding in this study was that the temperature (which was not measured) of the stage of the light microscope alone was observed to induce motility of the sperm cell after some time.

DISCUSSION

The significantly higher mean allometric testicular weight of the left testes at 12 months of age compared to that of the 6 months old age groups indicated that there was further growth of the testes of the fishes in these age groups resulting in the increased mass. Though at 9 and 12 months of age the testicular allometric weights were higher than those of 6 month and might translate to enhanced spermatogenic bioactivity. This observation was in line with earlier reports of Okoye *et al.* (2016) which indicated positive correlation between testicular weight and spermatogenesis in *C. gariepinus* and Kumari (2014) that reported the importance of testicular allometric weight as a good index for estimation of the reproductive performance in *Heteropneustes fossilis*. Higher left testicular allometric weights compared to the right from 6 months have also been reported in *C. gariepinus* (Okoye *et al.,* 2016).

Table 1: Morphometrical parameters of cultured male broodstock African catfish (*Clarias gariepinus*)

Morphometric Parameters	6 months	9 months	12 months
Fish weight (g)	1366.67 ± 33.33^{a}	1766.67 ± 33.33^{b}	2050.00 ± 28.87^{c}
Fish length (cm)	57.07 ± 1.03^{a}	63.40 ± 0.67^{b}	67.00 ± 1.06^{c}
Genital papillae length (cm)	1.73 ± 0.07^{a}	2.23 ± 0.32^{b}	2.23 ± 0.13^{b}
Left testicular weight (g)	2.77 ± 0.41^{a}	4.67 ± 1.12^{b}	6.76 ± 1.74^{b}
Right testicular weight (g)	3.01 ± 0.49^{a}	4.35 ± 0.75^{b}	5.67 ± 1.08^{b}

[a,b,c] Different superscripts in a row indicates significantly different (p<0.05) means across the groups

Table 2: Genital papillae and testicular allometric values of cultured male broodstock African catfish (*Clarias gariepinus*)

Parameters	6 months	9 months	12 months
Genital papillae length	3.03 ± 0.07^{a}	3.51 ± 0.47^{ab}	3.33 ± 0.19^{b}
Left testicular weight	0.20 ± 0.03^{a}	0.26 ± 0.06^{ab}	0.33 ± 0.08^{b}
Right testicular weight	0.22 ± 0.03^{a}	0.24 ± 0.04^{a}	0.28 ± 0.06^{a}
Pooled testicular weight	0.42 ± 0.06^{a}	0.51 ± 0.10^{a}	0.61 ± 0.13^{a}

[a,b] Different superscripts in a row indicates significantly different (p<0.05) means across the groups

Table 3: Semen quality parameters of cultured male broodstock African catfish (*Clarias gariepinus*)

Semen parameters	6 months	9 months	12 months
Colour	Milky	Milky	Milky
Left testes semen volume (ml)	1.02 ± 0.16^{a}	1.93 ± 0.57^{b}	3.35 ± 0.49^{c}
Right testes semen volume (ml)	1.10 ± 0.06^{a}	1.90 ± 0.47^{b}	2.20 ± 0.48^{b}
Progressive motility (%)	> 80	> 80	> 80
Left testes sperm count (cell/ml)	$3.38 \times 10^{10} \pm 8.33 \times 10^{9a}$	$3.93 \times 10^{10} \pm 9.84 \times 10^{9a}$	$9.72 \times 10^{10} \pm 2.01 \times 10^{9b}$
Right testes sperm count (cell/ml)	$2.58 \times 10^{10} \pm 4.55 \times 10^{9a}$	$2.61 \times 10^{10} \pm 4.62 \times 10^{9a}$	$6.76 \times 10^{10} \pm 2.61 \times 10^{9b}$
Left testes spermatocrit (%)	6.67 ± 0.33^{a}	13.67 ± 0.67^{b}	16.00 ± 1.53^{c}
Right testes spermatocrit (%)	7.00 ± 0.58^{a}	12.33 ± 1.20^{b}	20.33 ± 1.86^{c}

[a,b,c] Different superscripts in a row indicates significantly different (p<0.05) means across the groups

Higher genital papillae allometric length at 9 and 12 month compared to that of the 6th month may not serve as a good meristic index of reproductive status.

The composition of fish semen and its physical characteristics have been found to vary with the fish species (Kruger *et al.,* 1984). Milt quality is a measure of the ability of the sperm cells to successfully fertilise an egg and such ability mostly depends on qualitative parameters of milt such as composition of seminal fluid, milt volume, sperm density and sperm motility (Rurangwa *et al.,* 2004). The semen quality also varies considerably among the individuals of the same species (Piironen, 1985).

The mean ranges of semen volume of fishes includes 0.2 – 2.2 ml in turbot (Suquet *et al.,* 1992), 1 – 60 ml in Atlantic halibut (Methven and Crim, 1991), 1.2 – 4.6 ml in rainbow trout (Büyükhatipoglu and Holtz, 1984), 0.1 – 1.5 ml in pike (Hulak *et al*., 2008), 0.2 – 2.0 ml in tench (Linhart and Kvaniscka, 1992), 1 – 9 ml in grass carp (Belova, 1981), 0.3 – 3 ml in tilapia (Chao *et al.,* 1987) and 0.2 – 1.3 ml in *C. gariepinus* (Solomon *et al.,* 2015). Results of the above reports were in line with the findings of this study. However, this study showed that the semen volume increased significantly with age across the groups.

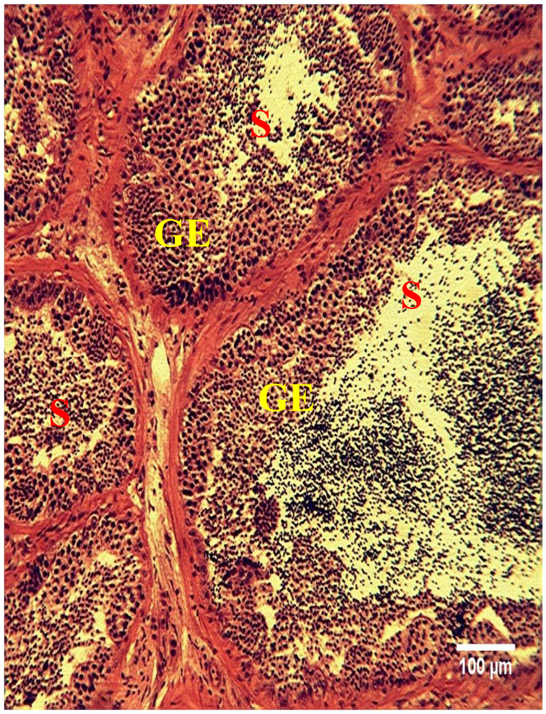

Figure 1: Photomicrograph of a section of the testis of a 6 months old African catfish (*Clarias gariepinus*) showing seminiferous tubules (S) germinal epithelia (GE) having clones of sperm cells in cysts while the lumen contains free spermatozoa. H & E, Mag. x 100

The mean sperm count was found to vary significantly among the different age groups. Most studies on fish species have also shown that there are variations in the sperm count among different species of fishes (Piironen, 1985). The mean range of sperm count values reported for other fishes were: 8 – 23 × 10^9 spermatozoa ml^{-1} in the red porgy (Mylonas *et al.*, 2003), 5 – 55 × 10^9 spermatozoa ml^{-1} in the European sea bass (Fauvel *et al.,* 1999; Rainis *et al.*, 2003), 32 – 44 × 10^9 spermatozoa ml^{-1} in the turbot (Suquet *et al.,* 1992), 38 – 45 × 10^9 spermatozoa ml^{-1} in the yellow perch (*Perca flavescens*) (Ciereszko and Dabrowski, 1993), 8.9 – 11.8 × 10^9 spermatozoa ml^{-1} in rainbow trout (*Oncorhynchus mykiss*) (Ciereszko and Dabrowski, 1993) and 14.1×10^9 spermatozoa ml^{-1} in brown trout (*Salmo trutta*) (Piironen and Hyvärinen, 1983). The findings of this study showed a higher range of sperm count of 2.58 – 9.72 × 10^{10} spermatozoa ml^{-1} which is higher than those cited above but within the upper range of 3.5 – 9.7 × 10^{10} spermatozoa ml^{-1} for *C. gariepinus* and 2.6 – 3.5 × 10^{10} spermatozoa ml^{-1}reported by Solomon *et al.* (2015) and Verma *et al.* (2009) for six different species of carp respectively. The significantly higher sperm count at 12 months of age compared to those of 6 and 9 months for *C. gariepinus* observed in this study is in tandem with findings a significantly higher semen volume at 12 months.

The results of this study showed a progressive significant increase in the spermatocrit across the broodstock fish. However, the range of spermatocrit observed in this study was lower than those reported by Suquet *et al.* (1994) for different marine, amphihaline and freshwater species; but was in agreement with the spermatocrit value of 11.2 ± 0.5 % reported by Munkittrick and Moccia (1987). The established positive linear relationship between spermatocrit and sperm density (Rakitin *et al.,* 1999; Tvedt *et al.,* 2001; Agarwal and Raghuvanshi, 2009) that made use of spermatocrit as an easy, rapid and reliable means of estimating the concentration of sperm cell may not be adoptable in *C. gariepinus*. The present study was in agreement with the report of Rurangwa *et al.* (2004) that spermatocrit was not very specific and also not reliable as an estimator of fertility potential.

Identical observations in the histomorphology of testicular sections, motility and colour indicated that they were all mature broodstock fish. However, since motility, progressive and duration of motility are very important markers of quality in spermatology (Verma *et al.,* 2009), similarity observed in motility across the age groups precludes it from being used in assessment of the semen quality of these age groups of male broodstock fish. Thus the significantly higher mean values obtained for the GSI, semen volume, sperm count and spermatocrit for the 12 months old broodstock males *C. gariepinus* implies that older broodstock may have better semen quality parameters and this may suggest that they are better candidates to be used for spawning. However, this has to be in consideration with the economics and risks associated in keeping them up to 12 months.

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REFERENCES

ADEYEMO, O. K., ADEYEMO, O. A., OYEYEMI, M. O. and AGBEDE, S. A. (2007). Effect of semen extenders on the motility and viability of stored African catfish (*Clarias gariepinus*) spermatozoa. *Journal of Applied Sciences and Environmental Management*, 11(1): 13 – 16.

AGARWAL, N. K. and RAGHUVANSHI, S. K. (2009). Spermatocrit and sperm density in snow trout (*Schizothorax richardsonii*): correlation and variation during the breeding season. *Aquaculture*, 291(1): 61 – 64.

ALAVI, S. M. H., PSENICKA, M., RODINA, M., POLICAR, T. and LINHART, O. (2008). Changes of sperm morphology, volume, density and motility and seminal plasma composition in *Barbus barbus* (Teleostei: Cyprinidae) during the reproductive season. *Aquatic Living Resources*, 21(1): 75 – 80.

AMANN, R. P. and ALMQUIST, J. O. (1961). Reproductive capacity of dairy bulls. I. Technique for direct measurement of gonadal and extra-gonadal sperm reserves. *Journal of Dairy Science*, 44(8): 1537 – 1543.

AVMA (2013). American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2013 Edition, American Veterinary Medical Association. 1931 N. Meacham Road Schaumburg, IL 60173.

BART, A. N. and DUNHAM, R. A. (1990).Factors affecting survival of channel catfish after surgical removal of testes. *The Progressive Fish Culturist*, 52(4): 241 – 246.

BELOVA, N. V. (1981). The ecological and physiological peculiarities of sperm in pond cyprinids. Communication I. Production and ecological and physiological peculiarities of the sperm of cyprinids. *Journal of Ichthyology*, 21(3):90 – 102.

BÜYÜKHATIPOGLU, S. and HOLTZ, W. (1984). Sperm output in rainbow trout (*Salmo gairdneri*): effect of age, timing and frequency of stripping and presence of females. *Aquaculture*, 37(1): 63 – 71.

CHAO, N. H., CHAO, W. C., LIU, K. C. and LIAO, I. (1987). The properties of tilapia sperm and its cryopreservation. *Journal of Fish Biology*, 30(2): 107 – 118.

CIERESZKO, A. and DABROWSKI, K. (1993). Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using a spectrophotometric technique. *Aquaculture*, 109(3-4): 367 – 373.

DE GRAAF, G. J., GALEMONI, F. and 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.

FAUVEL, C., SAVOYE, O., DREANNO, C., COSSON, J. and SUQUET, M. (1999). Characteristics of sperm of captive sea bass in relation to its fertilization potential. *Journal of Fish Biology*, 54(2): 356 – 369.

FAO (2012). *The State of World Fisheries and Aquaculture*. Food and Agricultural Organisation, Geneva.

HULAK, M., RODINA, M. and LINHART, O. (2008). Characteristics of stripped and testicular northern pike (*Esox lucius*) sperm: spermatozoa motility and velocity. *Aquatic Living Resources*, 21: 207 – 212.

KRUGER, J. D. W., SMIT, G. L., VUREN, J. H. J. and FERREIRA, J. T. (1984). Some chemical and physical characteristics of the semen of *Cyprinus carpio* L. and *Oreochromis mossambicus* (Peters).

Journal of Fish Biology, 24(3): 263 – 272.

KUMARI, S. (2014). Study on growth and reproductive behaviour in freshwater catfish (*Heteropneustes fossilis*). *Scholars Academic Journal of Bioscience*, 2(6): 380 – 383.

LINHART, O. and KVANISCKA, P. (1992). Artificial insemination in tench, *Tinca tinca* L. *Aquaculture Research*, 23(2): 183 – 188.

LOVSHIN, L. L., DA SILVA, A. B., CARNEIRO-SOBRINHO, A. and MELO, F. R. (1990). Effects of *Oreochromis niloticus* females on the growth and yield of male hybrids (*O. niloticus* X *O. hornorum* male) cultured in earthen ponds. *Aquaculture*, 88(1): 55 – 60.

METHVEN, D. A. and CRIM, L.W. (1991). Seasonal changes in spermatocrit, plasma sex steroids and motility of sperm from Atlantic halibut (*Hippoglossus hippoglossus*). Page 170. *In:* SCOTT, A. P., SUMPTER, J. P, KIME, D. E. and ROLFE, M. S. (Eds.). *Proceedings of 4th International Symposium of Reproductive Physiology of Fish,* Sheffield.

MUNKITTRICK, K. R. and MOCCIA, D. (1987). Seasonal changes in the quality of rainbow trout *(Salmo gairdneri)* semen: effect of a delay in stripping on spermatocrit, motility, volume and seminal plasma constituents. *Aquaculture*, 64(2): 147 – 156.

MYLONAS, C. C., PAPADAKI, M. and DIVANACH, P. (2003). Seasonal changes in sperm production and quality in the red porgy *Pagrus pagrus* (L.). *Aquaculture Research*, 34(13): 1161 – 1170.

OKOYE, C. N., IGWEBUIKE, U. M., UDOUMOH, A. F. and OKEREKE, C. T. (2016). Testicular morphology and sperm motility in cultured African catfish (*Clarias gariepinus*) at different stages of development. *Notulae Scientia Biologicae*, 8(3): 281 – 285.

PICKER, M. and Griffiths, C. (2013). *Alien and Invasive Animals: A South African Perspective*. Random House Struik, Cape Town, South Africa.

PIIRONEN, J. (1985). Variation in the properties of milt from the Finnish landlocked salmon (*Salmo salar m.* Sebago Girard) during a spawning season. *Aquaculture*, 48(3-4): 337 – 350.

PIIRONEN, J. and HYVÄRINEN, H. (1983). Composition of the milt of some teleost fishes. *Journal of Fish Biology*, 22(3): 351 – 361.

RAINIS, S., MYLONAS, C. C., KYRIAKOU, Y. and DIVANACH, P. (2003). Enhancement of spermiation in European sea bass (*Dicentrarchus labrax*) at the end of the reproductive season using GnRHa implants. *Aquaculture*, 219(1): 873 – 890.

RAKITIN, A., FERGUSON, M. M. and TRIPPEL, E. A. (1999). Spermatocrit and spermatozoa density in Atlantic cod (*Gadus morhua*): correlation and variation during the spawning season. *Aquaculture*, 170(3): 349 – 358.

ROFF, D. A. (1983). An allocation model of growth and reproduction in fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 40(9): 1395 – 1404.

RURANGWA, E., KIME, D. E., OLLEVIER, F. and NASH, J. P. (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish. *Aquaculture*, 234(1): 1 – 28.

SAFRIEL, O. and BRUTON, M. N. (1984). A cooperative aquaculture research programme for South Africa. *South African National Scientific Programmes Report,* 89: 1 – 79.

SKELTON, P. (2001). *A Complete Guide to the Freshwater Fishes of Southern Africa*. Struik Publisher, Cape Town, South Africa.

SOLOMON, S. G., ATAGUBA, G. A., OKOMODA, V. T. and SOLOMON, P. (2015). Relationship between somatic, gonadal characteristics and semen quality in the male African catfish *Clarias gariepinus* broodstock. *International Journal of Aquaculture*, 5(29): 1 – 5.

STEYN, G. J. and Van VUREN J. H. J. (1987).The fertilizing capacity of cryopreserved sharptooth cattish (*Clarias gariepinus*) sperm. *Aquaculture*, 63(1-4): 187 – 193.

SUQUET, M., BILLARD, R., COSSON, J., DORANGE, G., CHAUVAUD, L., MUGNIER, C. and FAUVEL, C. (1994). Sperm features in turbot (*Scophthalmus maximus*): a comparison with other freshwater and marine fish species. *Aquatic Living Resources*, 7(4): 283 – 294.

SUQUET, M., OMNES, M. H., NORMANT, Y. and FAUVEL, C. (1992). Assessment of sperm concentration and motility in turbot (*Scophthalmus maximus*). *Aquaculture*, 101(1-2): 177 – 185.

TEUGELS, G. G. (1986). The nomenclature of African *Clarias* species used in Aquaculture. *Aquaculture*, 38(4): 373 – 374.

TVEDT, H. B., BENFEY, T. J., MARTIN-ROBICHAUD, D. J. and POWER J. (2001). The relationship between sperm density, spermatocrit, sperm motility and fertilization success in Atlantic halibut *Hippoglossus hippoglossus*. *Aquaculture*, 194(1): 191 – 200.

VAN DER WAAL, B. C. W. (1998). Survival strategies of sharptooth catfish *Clarias gariepinus* in desiccating pans in the northern Kruger National Park. *Koedoe*, 41(2): 131 – 138.

VERMA, D. K., ROUTRAY, P., DASH, C., DASGUPTA, S. and JENA, J. K. (2009). Physical and biochemical characteristics of semen and ultrastructure of spermatozoa in six carp species. *Turkish Journal of Fisheries and Aquatic Sciences*, 9(1): 67 – 76.