African Journal of Agriculture and Food Science

Vol.1, No.1, pp.19-26, 2018

www.abjournals.org

COMPARATIVE ASSESSMENT OF MILT QUALITY OF THREE POPULATIONS OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) BROODSTOCK

Odo S.N[1], Alozie E.C[1] and Anyanwu E.D[2]

[1]Department of Fisheries and Aquatic Resources Management, Micheal Okpara University of Agriculture Umudike, Nigeria.
[2]Department of Zoology and Environmental Biology, Micheal Okpara University of Agriculture Umudike, Nigeria.

ABSTRACT: *This study was aimed at assessing the semen characteristics of both wild and cultured Clarias gariepinus broodstocks and associated determinants. Fifteen (15) male broodstocks were selected from 3 study populations; with weight ranges of 500 to 900g. The fish were dissected and the gonads were removed. The gonads were assessed for pH, motility, volume, morphology, microbiological characteristics and sperm concentration. The data were analysed using ANOVA while Duncan Multiple Range Test was used to separate the means. The sperm characteristics (tapering head, long tails, weight of gonads and progressive motility) assessed had the best results from Joshua Fish Farm, followed by Michael Okpara University Fish Farm and the Wild Fish Stock. The microbial load was highest among the Wild Fish Stock compared to other populations. This study has shown that broodstock fed consistently with good quality feed had best sperm quality. Other environmental factors such as pH, temperature, and water quality may also affect the quality of the sperm.*

KEYWORDS: Clarias Gariepinus, Milt, Sperm, Motility, Morphology, Gonads, Broodstock

INTRODUCTION

The culture of the African catfish, *Clarias gariepinus* in Nigeria is bedevilled by the problem of high mortality in the young stages and the resulting problem of seed scarcity (Adewumi *et al*., 2005). The availability of gametes throughout the year is important to ensure a constant supply of fish (Yusuf *et al.*, 2015). In captivity (25^{o}C, 12h light per day), *Clarias gariepinus* gametogenesis is continuous once sexual maturity is reached (Huisman and Richter, 1987). The fish farming industry has been more focused on the quality of eggs or larvae rather than that of sperm, even though the quality of both gametes may affect fertilisation success and larval survival.

Sperm quality in farmed fish maybe affected by different components of broodstock husbandry; collection and storage of sperm prior to fertilisation or the fertilisation procedure. The quality of sperm is highly variable and depends on various external factors such as feeding regime, the quality of the feed and the rearing temperature of the fish (Billard *et al*., 1995). According to Rurangwa *et al* (2004), any quantifiable physical parameter that directly correlates with the fertilisation capacity of sperm could be potentially used as a measure of sperm quality. Different approaches for quantification of sperm quality has been suggested but motility is most commonly used since high motility is a prerequisite for fertilisation and correlates strongly with fertilisation success (Rurangwa *et al.*, 2004).

Sperm motility is the most evaluated criteria for sperm quality assessment in fish due to its correlation with fertility (Rurangwa *et al*., 2001; Adewumi *et al*., 2005) and it is affected by several parameters like temperature, pH, ions and their concentrations, dilution and the parasites affecting their environment. The recruitment of wild fish as well as the controlled production in aquaculture is a biological event strongly linked to the reproductive success and in particular, with the fertilization of mature oocytes ((Karouni *et al*., 2011). The brain is stimulated by environmental cues like water rise, temperature, feeding, rainfall, and photoperiod to release gonadotropin releasing hormones (Zohar *et al*., 2010). According to Fauvel *et al* (2010), fertilization is an integrative response to multiple factors which may hide variations of sperm intrinsic quality. In fish reproduction under controlled conditions, attempts are made to obtain sperm of the highest quality and hence to produce the highest possible numbers of good quality fingerlings (Ochokwu *et al*., 2015; Islam and Akhter, 2011).

Knowledge of the effects of broodstock nutrition on egg production and quality is an important factor in spawning, good health and growth of the progeny (Adewumi *et al*., 2005; Ochokwu *et al*., 2015); it is therefore necessary to have adequate knowledge of the physical characteristics of the semen of both wild and cultured catfish (*C. gariepinus*). This study was aimed at investigating and comparing some semen characteristics of both cultured and wild broodstocks and associated determinants.

MATERIALS AND METHODS

Study Area

This study was carried out at the Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike, Nigeria. It is located on the latitude of 05º2' North and longitude 07º32' East.

Experimental Fish

A total number of 15 male broodstock were used; five from each of the 3 sampling locations. The cultured fish were collected from the Broodstock Section of Michael Okpara University of Agriculture, Umudike (MOUAU) Research Fish Farm; fed with different brands of fish feed and Joshua Fish Farm (JFF), Umudike; fed specifically with coppens fish feed during the study (June – August, 2017). The Wild Fish Stock (WFS) were collected from Onuimo, a fish landing site on Imo River. *C. gariepinus* was identified using taxonomic guide presented by Teugels (1986). Matured male samples were identified following FAO (2006) using redness of the genital papilla as a guide.

Milt Collection

Male reproductive behaviour does not take place spontaneously even after hormonal therapy. To obtain spermatozoa, it is necessary to sacrifice male brood fish or surgically removed part of their testes (Adeyemo *et al*., 2007). The male broodstocks of African Catfish, *C. gariepinus* both wild and cultured population were sacrificed by using a standard laboratory method called percussive stunning, after which the abdomen was dissected and the gonads were removed (Ogbonna, 2016); blood clots and other tissues were rinsed away. The gonads

were placed in the buffer solution prior to maceration to maintain its potency (Adeyemo *et al.,* 2007). Gonads were macerated in Petri dish and semen was transferred into freshly labelled sample bottles.

Post Collection Examination

The collected gonads of *C. gariepinus* were evaluated for gonad weight using a sensitive weighing balance (Exthech instruments: SC600) in grams (g). The pH of the semen was determined using a pH colour paper. The Sperm volume, concentration, appearance, morphology, colour, motility and microbial assessment were determined as follows:

Volume Determination

The semen volume was determined using calibrated syringe after gentle maceration of the milt sac and the semen were taken up slowly (Oguntuase and Adebayo, 2014).

Motility Determination

Spermatozoa motility was assessed by placing the slides containing a drop of semen on CELESTRON LABS microscope at 10x magnification and adjusted with 20x objective lens. Estimation of spermatozoa motility was started immediately after the semen was gently touched on the slide and smeared with crystal violet with a drop of a diluted Sulphuric acid (H_2SO_4) on the slides. The movement was observed for duration of 2minutes. Each motility determination was performed in triplicate for each semen sample. Motility were analysed by using descriptive and numerical scales for evaluation of microscopic wave pattern of semen. Only forward-moving sperm were judge motile, those simply vibrating or turning on their axes were considered immotile (Oguntuase and Adebayo, 2014).

Semen Concentration Determination

The standard method for determining sperm density (sperm cells/ml milt) in fish milt is to count spermatozoa generally using a haemocytometer or a similar counting chamber. The drop of the semen sample was smeared on the heamacytometer counting chamber; the ultra violet was smeared and then covered with the cover slit. The smeared sample was viewed with a CELESTRON LABS light microscope.

Morphology

The morphology was determined using a CELESTRON LABS microscope at 10x magnification and adjusted with 20x objective lens. The large oval head, swollen head, small oval head, tapering head, duplicate head, and amorphous head, mid piece defect, tail defect, immature cell, and length of tail observed.

Microbial Identification

The microbiological characteristics were evaluated by checking the total viable microbial count (TVC), total fungal count (TFC), total coliform count (TCC), total staphylococci count (TSC), using the appropriate culture media as Tryptone Soya Agar (for TVC), Potato dextrose Agar (for TFC), Macconkey Agar (for TCC), Mannitol salt Agar (for TSC) (Adenike, 2014). A drop of the sperm sample was stained, diluted (10^3 or 1/1000) and inoculated in a petri dish of appropriate culture media and then cultured for 48hours at

ambient temperature. The counting was done by mere viewing with eyes and then counted (Adenike, 2014).

Statistical Analysis

The means and standard deviations were determined using Descriptive Statistics of Microsoft Excel while one-way analysis of variance (ANOVA) test was used to determine the significant difference (P=0.05) among the samples. The significant means were separated using Duncan Multiple Range Test (DMRT) using SPSS 16.0 full version.

RESULTS

The result of the spermatozoa differential morphological characteristics of the 3 populations of *C. gariepinus* from MOUAU Fish Farm, Joshua Fish Farm and Wild Fish Stock is presented in Table 1. MOUAU Fish Farm recorded the highest mean value of tapering head (53.67%) while the lowest value was recorded in wild *C. gariepinus* (16.67%). There was a significant difference (P<0.05) in tapering head among the 3 populations with wild *C. gariepinus* being the source of the variation. The Wild Fish Stock had the highest mean value of large oval head (24%) while the lowest value was recorded in Joshua Fish Farm (8%). The Wild Fish Stock also showed a high significance difference (P<0.05) compared with other populations. The highest mean for long tails was recorded in Joshua Fish Farm (35%) while the lowest value was recorded among the wild *C. gariepinus* (11.33%). There was a significant difference (P<0.05) in long tail among the populations with wild *C. Gariepinus* being the source of the variation. The wild *C. gariepinus* recorded mean values of 10.33% in small oval head, 10.67% in mid piece defect and 14.33% in immature cells, which were all absent in the other populations. There were no amorphous heads, duplicate heads and short tails in the 3 populations.

Table 1: Morphological Characteristics of the different Spermatozoa

Sample	% Tappering Head	% Large Oval Head	% Small Oval Head	% Swollen Head	% Mid Piece Defect	% Long Tails	% Immature Cells
MFF	53.67^{a}	3.67^{a}	7^{a}	-	-	33.67^{a}	-
JFF	41.67^{a}	8^{a}	10^{a}	-	-	35^{a}	-
WFS	16.67^{b}	24^{b}	9.33^{a}	10.33	10.67	11.33^{b}	14.33

Legend: MFF = MOUAU Fish Farm; JFF = Joshua Fish Farm; WFS = Wild Fish Stock
Means with the same superscript within column are not significantly different (P>0.05).

The results of other spermatozoa quality characteristics were presented in Table 2. Joshua Fish Farm recorded the highest mean value ($5.60 \times 10^{7} \pm 1.2$cells/ml) for spermatozoa concentration while the lowest mean value ($2.68 \times 10^{7} \pm 0.59$cells/ml) was recorded among the Wild Fish Stock. There was no significant difference (P>0.05) in sperm concentration (cells/ml) between the three populations. Joshua Fish Farm also recorded the highest mean value for gonad weights ($4.47 \times 10^{3} \pm 0.66$g) while the lowest mean value was recorded for the

Wild Fish Stock (1.25 x 10^3±0.17g). Joshua Fish Farm was significantly difference (P<0.05) among the populations.

Microbial assessment of the spermatozoa showed that Wild Fish Stock recorded the highest mean values for total viable microbial count (29.67 x 10^3 ±23.37), total fungal count (20.00 x 10^3 x 10^3 ±15.09) and total coliform count (16.00 x 10^3 ±3.46), followed by Joshua Fish Farm and the least recorded in MOUAU Fish Farm while no total staphylococci count was recorded in all the populations (Table 3).

In terms of spermatozoa physical quality characteristics, all the samples had pale milky white colour, were mildly mucoid, odourless and positive for methylene blue reduction test. On the other hand, Joshua Fish Farm recorded moderate (2) to very motile (3) for the sperm progressive motility, MFF recorded mild (1) throughout while WFS recorded from poor (0.5) to very motile (3).

Table 2: Other Spermatozoa Quality Characteristics (x 10^3)

Sample	Sperm Concentration (Cells/ml)	Sperm Volume (ml)	Sperm pH	Gonad Weight(G)
MFF	4.43 x 10^7 ±1.2^a	1.5±0.44 ^{a}	8.00±0.0 ^{a}	2.02±0.38^b
JFF	5.60 x 10^7±2.62 ^{a}	2.27±0.64 ^{a}	8.00±0.0 ^{a}	4.47±0.66 ^{a}
WFS	2.68 x 10^7 ±0.59 ^{a}	1.27±0.5 ^{a}	8.33±0.58 ^{a}	1.25±0.17^b

Legend: MFF = MOUAU Fish Farm; JFF = Joshua Fish Farm; WFS = Wild Fish Stock
Means with the same superscript within column are not significantly different (P>0.05).

Table 3: Microbiological Characteristics of Spermatozoa

Sample	TVC (Cfu/ml)	TFC(cfu/ml)	TCC(cfu/ml)	TSC(cfu/ml)
MFF	4.67 ± 4.16^a	0.00 ± 0.00^c	2.67 ± 4.62^a	0.00 ± 0.00^a
JFF	21.33 ± 17.24^b	12.67 ± 13.32^a	8.67 ± 7.57^b	0.00 ± 0.00^a
WFS	29.67 ± 23.37^c	20.00 ± 15.09^b	16.00 ± 3.46^c	0.00 ± 0.00^a

Legend: MFF = MOUAU Fish Farm; JFF = Joshua Fish Farm; WFS = Wild Fish Stock
Means with the same superscript within column are not significantly different (P>0.05).

DISCUSSION

The morphology of the samples was differentiated based on the shape of the head, mid piece, tails, and roundish cells. MOUAU Fish Farm had the highest mean for tapering head while the Wild Fish Stock had the lowest. This could be attributed to the nature of the environment, temperature or the quality of nutrition (Bobe and Labbé, 2010). Joshua Fish Farm recorded highest mean for long tails while the Wild Fish Stock recorded the lowest mean value. There were incidences of deformed sperm in the form of large oval head, roundish head, amorphous sperm and mid piece defect; this is in line with Jakab *et al* (2003) that reported the presence of deformities such as increased size of the sperm head, roundish sperm head and increased incidence of amorphous head.

The pH values for both cultured and wild male broodstock ranged from neutral to alkaline. The recorded pH values were higher than those recorded by Ingerman *et al* (2002). Williot *et al* (2000) reported a range of pH values of 6.0 – 7.0 for *Acipenser transmontanus* in Kootenai River, USA. The mean semen volumes were lower than values reported by Lahnsteiner *et al* (1993), Geffen and Evans (2000) and Atasever and Bozkurt (2015). The semen volume value for both cultured and wild male broodstock did not show any significant different. This is at variance with Adenike (2014), that reported a significant different ($p<0.05$) in the mean semen volume both fish cultured and wild samples of *C. gariepinus*; attributed to feeding and environmental conditions. The gonad weight values for both cultured and wild male populations were in line with Yusuf *et al* (2015) that recorded (1.60 – 6.30g) and (1.12 – 3.76g) for two populations (culture and wild *C. gariepinus*) respectively. The mean gonad weight values for both wild and cultured fish also showed a significant different ($P<0.05$) as in this study. This could be attributed to sperm allocation tactics which can vary according to the size and status of a male or its amount of available sperm in gonads (Atasever and Bozkurt., 2015). These differences may likely be due to feed condition, water quality, and different environmental conditions and spawning season (Yusuf *et al.,* 2015).

The microbiological characteristic of the sperm is also an important parameter to consider when assessing the sperm quality. This agreed with Ochuko *et al* (2015) that reported that the exposure of maturing males and females to pollutants and contaminants may affect the egg, sperm, and fry production. Moretti, *et al* (2009) observed that bacterial contamination is quite frequent and could contribute to the deterioration of the sperm quality.

The poor progressive motility of (0) recorded for wild African catfish agreed with Alavi and Cosson (2006); they asserted that motility is induced after the spermatozoa are released into the aqueous during natural reproduction or into the diluents during artificial reproduction. Most studies on fish also show that duration and motility of semen may vary seasonally (Akcay *et al*., 2004; Yusuf *et al* 2015).

CONCLUSION

This study has shown that broodstock fed consistently with good quality feed had best sperm quality. Other environmental factors such as pH, temperature, and water quality may also affect the quality of the sperm. The consideration of a good sperm quality based on the parameters assessed is of paramount importance in aquaculture production. When all these factors are considered, monitored, and properly managed, there will be improvement and sustainability in aquaculture system in Nigeria.

REFERENCES

Adenike, O.M. (2014). The Effect of Different Processing Methods on the Nutritional Quality and Microbiological Status of Catfish (*Clarias lazera). Journal of Food Processing and Technology,* 5: 333.

Adewumi, A.A, Olaleye, V.F. and Adesulu, E.A. (2005). Egg and Sperm Quality of the African catfish, *Clarias gariepinus* (Burchell) Broodstock Fed Differently Heated

Soybean-based Diets. *Research Journal of Agriculture and Biological Sciences*, 1(1): 17-22.

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 Science and Environmental Management*, 11(1):13-16.

Akcay, E., Bozkurt, Y., Tekin, N. and Secer, S. (2004). Cryopreservation of mirror carp spermatozoa. *Turkish Journal of Veterinary and Animal Science*, 28(5):1-7.

Alavi, S.M. and Cosson, J. (2006). Sperm motility in fishes. II. Effects of ions and osmolality: a review. *Cell Biology International*, 30(1):1-14.

Atasever, M. and Bozkurt, Y. (2015). Effect of different photoperiod regimes on sperm quality, fecunduty and fertilization in rainbow trout (Oncorhynchus mykiss). *Journal of Applied Science and Environmental Management*, 11(1):13-16.

Billard, R., Cosson, J., Crim, L.W. and Suquet, M. (1995). Sperm physiology and quality. In: Bromage, N.R. and Roberts, J. (Eds), *Broodstock Management and Egg and Larval Quality*. Blackwell Sc. Ltd, Oxford. pp. 25-52.

Bobe, J. and Labbé, C. (2010). Egg and sperm quality in fish. *General and Comparative Endocrinology*, 265(3):535 – 548.

Bromage, N.R. (1995). Broodstock management and seed quality, general consideration. In: Bromage, N.R. and Roberts, J. (Eds). *Broodstock Management and Egg and Larval Quality* Blackwell Sc. Ltd, Oxford. pp. 1- 24.

FAO (2007). The State of World Fisheries and Aquaculture 2006. FAO Fisheries and Aquaculture Department. Food and Agriculture Organization of the United Nations, Rome. pp. 5-22.

Geffen, A.J. and Evans, J.P. (2000). Sperm traits and fertilization success of male and sex-reserved female Rainbow trout. *Aquaculture*, 19: 12-20.

Huisman, E.A., and Richter, C.J.J. (1987). Reproduction, growth, health control and aquacultural potential of the African cattish, *Clarias garieninus* (Burchell 1822). *Aquaculture*, 63:1-14.

Ingerman, R.L., Holcomb, M., Robinson, M.L. and Cloud, J.G. (2002). Carbon dioxide and pH affect sperm motility of white sturgeon (*Aspencer transmontanus)*. *Journal of experimental biology*, 205:2885-2890.

Islam, M.S. and Akhter, T. (2011). Tale of Fish Sperm and Factors Affecting Sperm Motility: A Review. *Advances in Life Sciences*, 1(1):11-19.

Jakab, A., Kovacs, T., Zavaczki, Z., Borsos, A., Bray-Ward, P., Ward, D., and Huszar, G. (2003). Efficacy of the swim-up method in eliminating sperm with diminished maturity and aneuploidy. *Human Reproduction*, 18:1481–1488.

Karoui, S., Diaz, C., Serrano, M., Cue, R., Celorrio, I., and Carabano, M.J. (2011). Time trends, environmental factors and genetic basis of semen traits collected in Holstein bulls under commercial conditions. *Animal Reproduction Science*, 124:28 -38

Lahnsteiner, F., Patzne, R.A. and Weismann, T. (1993). Energy resources of spermatozoa of the rainbow trout, *Oncorhynchus mykiss* (Pisces: Teleostei). *Reproduction Nutrition Development*, 33:349-360.

Moretti, E., Capitani, S., Figura, N., Pammolli, A., Federico, M.G, Giannerini**,** V. and Collode, G. (2009). The presence of bacteria species in semen and sperm quality**.** *Journal of Assisted Reproduction and Genetics*, 26(1):47–56.

Ochokwu, I.J., Apollos, T.G and Oshoke, J.O. (2015). Effect of Egg and Sperm Quality in Successful Fish Breeding. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 8(8V.II):48-57.

Ogbonna, C.N. (2016). Comparative Study of Early Development, Hatchability and Growth 0f Pure and Reciprocal oF *Clarias gariepinus* and *Heterobranchus longifilis*. Unpublished Degree Project, Department of Fisheries, Michael Okpara University of Agriculture, Umudike, Nigeria.

Oguntuase, B.G. and Adebayo, O.T. (2014). Sperm Quality and reproductive performance of male *Clarias gariepinus* induced with synthetic hormones (Ovatide and Ovaprim). *International Journal of Fisheries and Aquaculture*, 6(1):9-15.

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* 234:1–28.

Rurangwa, E., Volckaert, F.A.M, Huyskens, G., Kime, D.E. and Ollevier, F. (2001). Quality control of refrigerated and cry preserved semen using computer assisted sperm analysis (CASA), viable staining and standardized fertilization in African catfish (*Clarias gariepinus*). *Theriogenology*, 55(3): 751-769

Teugels, G.G. (1996). Taxonomy, phylogeny and biogeography of catfishes (Ostariophysi, Siluroidei): An overview. *Aquatic Living Resources*, 9:9-34.

Williot, P., Kopeika, E.F. and Goncharov, B.F. *(2000).* Influence of testis state, temperature and delay in semen coll*ection on spermatozoa motility in the cultured Siberian sturgeon (Acipenser baeri* Brandt*). Aquaculture 189, 53-61.*

Yusuf, O.Y., Adeshina, I. and Adewale, A.Y. (2015). Comparative Studies of Some Semen Physical Characteristics of Cultured and Wild African Catfish (*Clarias gariepinus*, Burchell, 1822) Broodstock. *Gashua Journal of Irrigation and Desertification Studies*, 1(1&2):173- 180.

Zohar, Y., Muñoz-Cueto. J.A., Elizur, A., and Kah, O. (2010). Neuroendocrinology of reproduction in teleost fish. *General Comparative Endocrinology*, 165: 438–455