PHENOTYPIC AND GENETIC EVALUATION OF FARMED AND WILD *Clarias gariepinus* BROODSTOCKS IN NIGERIA

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ABSTRACT

Clarias gariepinus accounts for more than a half of all Nigerian aquaculture production. The industry growth is limited due to poor seed quality which is suspected to be due to inbreeding within the broodstocks used by the industry. This study was conducted to explore the phenotypic and genetic variation among farmed and wild broodstocks of *C. gariepinus* in Nigeria. Nigeria was stratified into two, based on aquaculture intensity: High Aquaculture Area (HAA) and Low Aquaculture Area (LAA). Samples were collected from 10 farms (five farms each from HAA and LAA) and three rivers (wild). A total of 220 live broodstock samples (n = 20 per location) were collected for this study. Phenotypic characters such as morphometric and meristic characteristics, egg diameter, and fecundity were evaluated using standard procedures. *C. gariepinus* genetic characteristics were analyzed using Restriction site associated DNA sequencing (RADseq) marker to investigate the genetic relatedness and possible signals of inbreeding. The results of the morphometric, meristic, egg diameter and fecundity data showed that the farmed fish samples were significantly different (*one-way ANOVA, $P<0.05$*) from each other and from the wild samples. The cluster analysis of morphometric and meristic parameters showed high divergence among the fish sampled. One of the farms sampled in HAA clustered with the wild sample. The result of the genetic data showed that the relatedness within the farms varied from 0.14±0.18 to 0.67±0.09 and genetic relatedness between the farms ranged from -0.12±0.04 to 0.01±0.05. it therefore suggested a high level of relatedness within farm samples but not between farm samples. The PCA analysis revealed strong genetic variabilities among samples. A total of 21.17% of the genetic variability was accounted for PC1, separating strongly Farm 8 sample from the rest of the samples. A total of 17.94% of the genetic variability was observed on PC2 which differentiated strongly Farm 7 from the rest of the samples. The inbreeding coefficients (F_{IS}) was above the value of 1 in all farms, suggesting high inbreeding levels. Observed morphological differences could be due to genetic differences and environmental factors. The high level of inbreeding and relatedness within each farm sample could be due to poor broodstock management practices.

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1 INTRODUCTION

1.1 Background of the study

Nigeria is Africa's second-largest producer of fish from aquaculture, and it has recorded a rapid growth in aquaculture from 40,000 metric tonnes in 2006 to 296,191 metric tonnes in 2017 (Worldfish, 2018) (Figure 1). There are several species with high culture potential in Nigeria. However, the major cultured species are catfishes, carp and tilapia. The cultured catfish species include: *Clarias gariepinus, C. anguillaris, C. submarginatus, C. isheriensis, Heterobranchus bidorsalis, H. longifilis, Chrysichthys nigrodigitatus, Bagrus* spp. and *Synodontis* spp.

Nevertheless, *C. gariepinus* is undeniably the fish of choice for farmers and is therefore the most cultured fish in Nigeria. The growth of aquaculture in Nigeria is now largely being boosted by a steady rise in the culture of this species accounting for over 50% of total production, as shown in Figure 1 (FAO, 2019), with more potential to boost the country's production. However, inadequate availability of quality seed is a major problem limiting the fish production in the country.

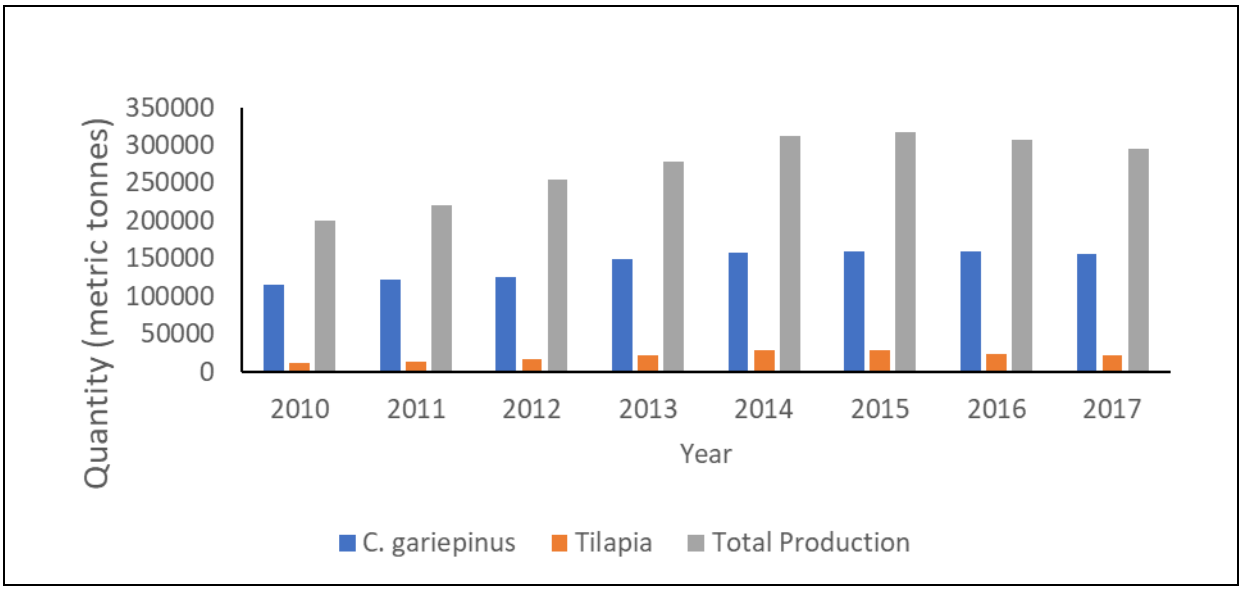

Figure 1: Status of aquaculture production in Nigeria (2010-1017) *(FAO, 2019)*.

Fish production in Nigeria originates from three sources: artisanal (inland rivers, lakes, coastal and brackish waters), aquaculture (fish farms) and industrial fishing. Catches from the wild (artisanal and industrial fishing) have been declining as a result of overfishing (Akinrotimi, Abu & Aranyo, 2011), which creates good opportunities for aquaculture development in Nigeria.

In attempts to improve aquaculture production and increase productivity, the Federal Government of Nigeria introduced in 2014 a Growth Enhancement Support Scheme which distributes inputs such as catfish seed and feed at a subsidized price to fish farmers across the country. Another project was set up by West Africa Agricultural Productivity Programme (WAAPP) in collaboration with Federal government research institutions and private hatcheries in 2017 to enhance broodstock and seed production in order to increase the quality of seed for aquaculture in the country (Ibiwoye & Thorarensen, 2018).

Despite all the strategies adopted to support the development of aquaculture in the country, the aquaculture industry is still confronted with challenges such as poor broodstock management, inadequate supply of quality seed, high cost of feed, disease outbreaks and faulty data collection (Adewumi & Olaleye, 2011). The poor management practices of broodstock includes inadequate feeding and handling stress (Shourbela, El-latif & El-Gawad, 2016), and lack of record keeping of the source, age and family line of the broodstock (De Silva, Ingram & Wilkinson, 2015). These practices could lead to inbreeding and, as a result, poor-quality fish seed.

Seed production is an important aspect of aquaculture that has been the subject of continuous research and innovation for increased fish production. Artificial propagation is a good means of providing enough quality seed for rearing in confined environments. Increased productivity of fry and fingerlings with attributes of fast growth and high environmental tolerance is *sine qua non* to ensuring food security in Nigeria. However, the scarcity of genetically improved fish seed is one of the major constraints to the rapid development of aquaculture industry in the country. Many fish hatcheries in Nigeria use catfish of the same limited parental lineage which is likely to result in inbreeding over several generations, leading to reduction in overall production (Olaleye, 2005).

1.2 Problem and Rationale

Aquaculture production has been continuously growing over the last decades. In Nigeria, *Clarias gariepinus* has gained popularity and attracted the interest of the aquaculture industry because of its high resistance to disease, fast growth rate (it reaches market size of 1 kg in 5–6 months under intensive management conditions), high fecundity, palatability, high stocking densities and ability to tolerate a wide range of environmental conditions (Eyo, Ekanem & Obiekezie, 2012; Olubunmi, Olateju, Latifat & Oluniyi, 2009). It is highly cherished as food in Nigerian homes and hotels and commands a high price (Olaleye, 2005). Further increase in the farming of this species relies on the ability of the hatcheries to produce fish seed in high quantity which is to a large extent dependent on the quality of the brood stock used.

Small scale farmers, making up 80% of aquaculture producers in Nigeria, are faced with insufficient seed supply which may be due in part to reduced reproductive success and poor-quality seeds. This has led to low quantity of fingerlings produced in the country: out of 4.3 billion required annually to meet estimated needs, only 85 million fingerlings are produced in the country (FDF, 2018). The current high need calls for proper broodstock management and quality control to ensure production of high quantity and quality fish seed. Most broodstocks used by these farmers are obtained from the wild or from hatcheries. Unfortunately, reduction in abundance of this species in the wild has been reported (Garg, Sairkar, Silawat & Mehrotra, 2009). As a consequence, most farms/hatcheries in Nigeria use effective breeding size (*N*e) for their broodstock (usually around 20 fish or even lower), which is far below the recommended number of 50 fish (Ibiwoye & Thorarensen, 2018). These stocks are often not renewed. This has the potential to lead to inbreeding over several generations.

One possible solution to prevent inbreeding in these stocks might be to increase the size of each broodstock by collecting more breeders from wild stocks and renewing the broodstocks regularly in order to increase their genetic variability. Another possibility would be to crossbreed the broodstocks of the different farms in Nigeria if the wild population of *C. gariepinus* is declining to a level limiting its current exploitation as potential breeder. These solutions can only be achieved if basic knowledge on the genetic variability of *C. gariepinus*

broodstocks in Nigerian's farms is known. Presently, there is a lack of information on the phenotypic and molecular characteristics of farmed and wild *C. gariepinus* broodstocks in Nigeria, hence this study.

1.3 Targeting the problems with the development of a large team project

The story of the Nigerian aquaculture is essentially linked to the catfish farming, especially to the predominant species *C. gariepinus* (Adewumi & Olaleye , 2011). As stated above, the fish farmers in Nigeria encounter many problems during the production process which lead to little or no profit. The effects of these problems are reflected in the constant low productivity of the farms which could lead to the collapse of the farms' productions.

In order to address these challenges, the UNESCO GRÓ - Fisheries Training Programme sponsored a series of studies. The present study is particularly addressing one of these challenges which is to investigate the quality of broodstocks used in the aquaculture industry using both phenotypic and genetic characters. This study will evaluate the quality of the broodstocks used in Nigeria using both morphological and genetic indicators of potential inbreeding signals. It will then recommend the best procedure to improve the farming practices based on the presence/absence of any inbreeding signals in the different broodstocks used in the Nigerian's farms.

1.3.1 General objectives

One of the main concerns of Nigeria presently is to assess the potential reason for the low productivity of fish farms. In this context, the present study will focus on detecting any signs of inbreeding potentially present in the different broodstocks currently used in the country.

1.3.2 Specific objectives of the study

The specific objectives of this study are:

i. To assess the phenotypic variations between farmed and wild populations of *C. gariepinus* broodstocks in Nigeria.

ii. To evaluate potential signals of inbreeding in the farmed *C. gariepinus* broodstocks in Nigeria.

2 LITERATURE REVIEW

2.1 Broodstock management

Fish seed production efficiency of many fish farms in Nigeria is mostly low due to poor handling of broodstock (Aiyelari, Adebayo & Osiyemi, 2007). Maintenance of quality broodstock in adequate number to produce large numbers of high-quality seed throughout the year is a primary requirement for the successful development of aquaculture. Mass production of high-quality seed depends on the availability of good quality egg and sperm. Therefore, broodstock collection, rearing and management are the most important part of aquaculture activities. Appropriate broodstock management is needed to ensure gonad quality, timely supply of strong and disease-resistant larva and fry. Unknown and known genetic changes, and possible loss of genetic variation in broodstock should be monitored to prevent inbreeding. Deoxyribonucleic acid (DNA) markers are a good monitoring tool for this purpose. The

information generated by these markers can be utilized in maintaining genetic variability and effective population size in broodstock and their progeny (Perez-Enriquez, Vega, Avila & Sandoval, 2001).

2.2 Inbreeding and inbreeding depression in fish

Inbreeding in an infinitely large population is defined as the mating of individuals that are more closely related to each other than individuals mating at random within a population. However, the populations used in most aquaculture programs are limited in number, which can lead to a reduction in heterozygosity (H_e) level, and therefore to inbreeding. It could undermine genetic gains and production performance when not controlled and monitored in production systems and breeding programs (De Donato, Manrique, Ramirez, Mayer & Howell, 2005). Reduction in reproduction activity has been reported in several inbred fish species (van Oosterhout, *et al*., 2003; Frommen, Luz, Mazzi & Bakker, 2008).

The loss of fitness and productive performance in inbred individuals is referred to as inbreeding depression (Gjedrem, Robinson & Rye, 2005). Factors contributing to inbreeding include small effective population size and the high fecundity of the breeders that lead to an increased probability of mating among relatives. Inbreeding is a common scenario in fish hatcheries where offspring are produced from broodstock whose genetic relatedness are unknown (Simonsen, Hansen, Mensberg, Sarder & Alam, 2005). The general consequence of inbreeding is the reduction of genetic variability where the inbred individual carries deleterious recessive alleles that are not expressed when they are masked in the heterozygous state. In their homozygote state these alleles may negatively impact the reproductive fitness of the carrier by decreasing gonad quality, larval viability, reducing survival, decreasing growth rate, lowering fecundity and reproductive ability of the fish and may increase the frequency of abnormalities in the population (De Donato, Manrique, Ramirez, Mayer & Howell, 2005).

Inbreeding is estimated by a value called the inbreeding coefficient (F) (Wright, 1965). It represents the rate of inbreeding which is the probability that two alleles at any locus are identical and originated from a common ancestor, and this expresses the increase in average inbreeding level in a population from one generation to the next. The rate of inbreeding per generation can be calculated as follow: $\Delta F = (F_1 - F)/(1 - F)$ where F and F_1 are the average inbreeding in the population at the beginning and the end of the interval (García-Dorado, Wang & López-Cortegano, 2016). It can be measured either using pedigree analysis when detailed information on the relationships of individuals over generations and the pathways of inheritance are available or using genetic markers to study inbreeding depression without the need to conduct parentage analysis over many generations. The latter is widely used because of poor record keeping in the aquaculture systems (Gjedrem, 2005), especially in a developing country like Nigeria.

2.3 Phenotypic studies in fish

A variety of phenotypic traits have been shown to be affected by inbreeding (De Rose & Roff, 1999). Besides the reduction of productive performance, changes in morphometric and meristic characters, and other traits associated with reproductive capacity (egg and sperm quality), or physiological efficiency have also been reported as consequence of inbreeding (Wright, et al., 2008). Those traits are therefore often used to detect signals of inbreeding (Wang, Huang, Fang, Yang & Li, 2012).

2.3.1 Studies on effect of inbreeding depression on egg quality

Gamete quality is a major issue for the aquaculture industry. This determines the success of reproduction (Ilmonen, Stundner, Thoß & Penn, 2009). The quality of a gamete can be defined as its ability to fertilize (sperm) or to be fertilized (oocytes), and subsequently develop into a normal embryo (Bobe & Labbe, 2009). The relationship between the level of individual inbreeding and specific fitness traits, such as gamete quality, hatchability and survival of animals to a given age, is one of the methods to evaluate the severity of inbreeding depression in populations for which complete pedigree information is available. Inbreeding depression leads to compromised fertility, low hatchability and survival (Keller & Waller, 2002; Asa *et al*., 2007; Ilmonen, Stundner, Thoß & Penn, 2009; Fitzpatrick & Evans, 2009).

Several parameters have been used to explain egg quality such as the size and floatability of the egg. For example, sinking eggs of marine fish or white eggs of *C. gariepinus*, can be used to identify non-viable eggs. Egg size may vary both within a species and between populations of the same species within the limits set by their genes (Beacham & Mubray, 1985). Ecological explanations for differences in egg size of fish in different populations include temporal and spatial changes in food supply and quality available to larvae, as well as predation. Size of fish affect egg size, with a larger body size often resulting in the production of larger eggs and a better egg survival (Heinimaa & Heinimaa, 2004).

2.3.2 Morphological markers

Morphometric and meristic features are often used to describe the phenotype of fish species. Fish morphometry refers to the measurement of total length of fish and other parts of its anatomy. Linear distances such as lengths of different fins and ratios to total lengths are measured on the fish (Erguden & Turan, 2005). Morphometric characters are used to describe aspects of shape of a fish's body, thus enabling their identification. Meristic characters are counts made on the body of the fish such as number of fin rays and number of barbels to differentiate species/populations. Both measurements have also been used to assess intra-specific differences in sampled populations as well as inter-specific differences in various fish species. Due to the presence of many variables in morphology, multivariate analysis is usually employed in morphological studies. The study of differences in morphometric and variability of stock is important in phylogenetics which can be further used in providing information for subsequent studies on the genetic improvement of stocks (Ola-Oladimeji, Oso, Oladimeji, & Idowu, 2017).

2.4 Studies on molecular markers used in fish genetics

The study of molecular markers is usually based on polymerase chain reaction (PCR) for amplification of the DNA which has been a revolutionary tool in genetics studies. There are several molecular markers available for the genetic characteristics of species like restriction-site associated DNA sequencing, (RADseq), restriction fragment length polymorphism (RFLP), isozyme electrophoresis and microsatellites (Dunham *et al*., 2001), and more recently Single Nucleotide Polymorphisms (SNPs) and Genome sequencing (Vignal, Milan, SanCristobal & Eggen, 2002). Different techniques have been used in analyzing genetic diversity of a multitude of species and breeding programs involving fish (Ellegren, 2014). This is due to the fact that application of molecular markers based on relative difference in DNA sequences between individuals would detect more polymorphism than morphological and protein-based markers (Oyebola, Omitoyin, Salako & Awodiran, 2013). More attention has

been paid to the detection of genome-wide, high output sequencing-based molecular markers such as Restriction-site-associated DNA sequencing (RADseq), which will be used in this project because it is cost effective and efficient especially in setting up a breeding program.

2.4.1 Restriction-site-associated DNA sequencing (RADseq)

In this study, Restriction-site-associated DNA sequencing (RADseq) is used because of the following benefits over other markers mentioned earlier. It produces thousands of loci (Figure 2) that provide an appealing alternative to other markers particularly for species without prior genetic information.

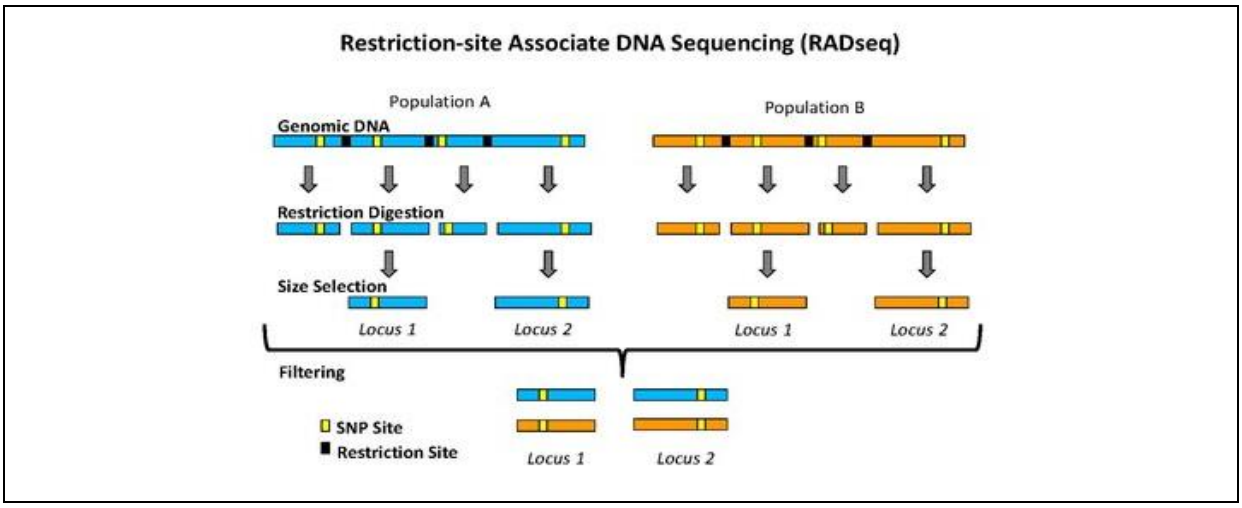

Figure 2: RADseq examples, from the genomic DNA to restriction digestion and identification of locus of interest**.**

The RADseq approach has rapidly gained popularity as it can produce large amounts of data (like Single Nucleotide Polymorpshims, SNPs) at reasonable costs (Andrews, Good, Miller, Luikart & Hhenlohe, 2016). The advantages of RADseq-derived markers are their suitability for comparing both strongly and weakly distinct populations, and reveal ancestral patterns of genetic structuring due to the slow mutation rate of SNPs produced (Lemopoulos, et al., 2019). In addition, the RADseq approach can provide more reliable information on population structure than other markers (Bruneaux *et al*., 2013) and improved resolution for data sets with fewer individuals (Jeffries *et al*., 2016), which is the case in the present study. It helps to identify locus of interest, allows generation of population-specific genotype data (i.e. no ascertainment bias) and offers flexibility in terms of desired marker density across the genome (Robledo, Palaiokostas, Bargelloni, Martınez & Houston, 2018). It has been used in selective breeding programs especially in the era of genetic relatedness of species (Bradbury *et al*., 2015). It has been demonstrated that SNPs obtained by RADseq were more accurate than microsatellites for characterizing introgression between Atlantic salmon (*Salmo salar*) from the East and West coasts of the Atlantic Ocean. It has also been demonstrated that SNPs obtained by RADseq produce more precise estimates of relatedness than other genetic markers (Thrasher, Butcher, Campagna, Webster & Lovette, 2018).

3 MATERIALS AND METHODS

3.1 Study sites

The study was carried out on the University of Ibadan fish farm, Ibadan, Oyo State. The laboratory activities were done in the Department of Aquaculture and Fisheries Management fish farm Laboratory and Agresearch Laboratory in New-Zealand

3.2 Experimental design

Stratified random sampling technique (EPA, 2002) was adopted for the collection of *C. gariepinus* samples. Nigeria was divided into two strata, based on aquaculture intensity (Figure 3; Table 1). The area with high aquaculture intensity was Southern Nigeria while the Northern Nigeria formed the area with low aquaculture intensity (Omitoyin, 2007; Ajani, Akinwole & Ayodele, 2011).

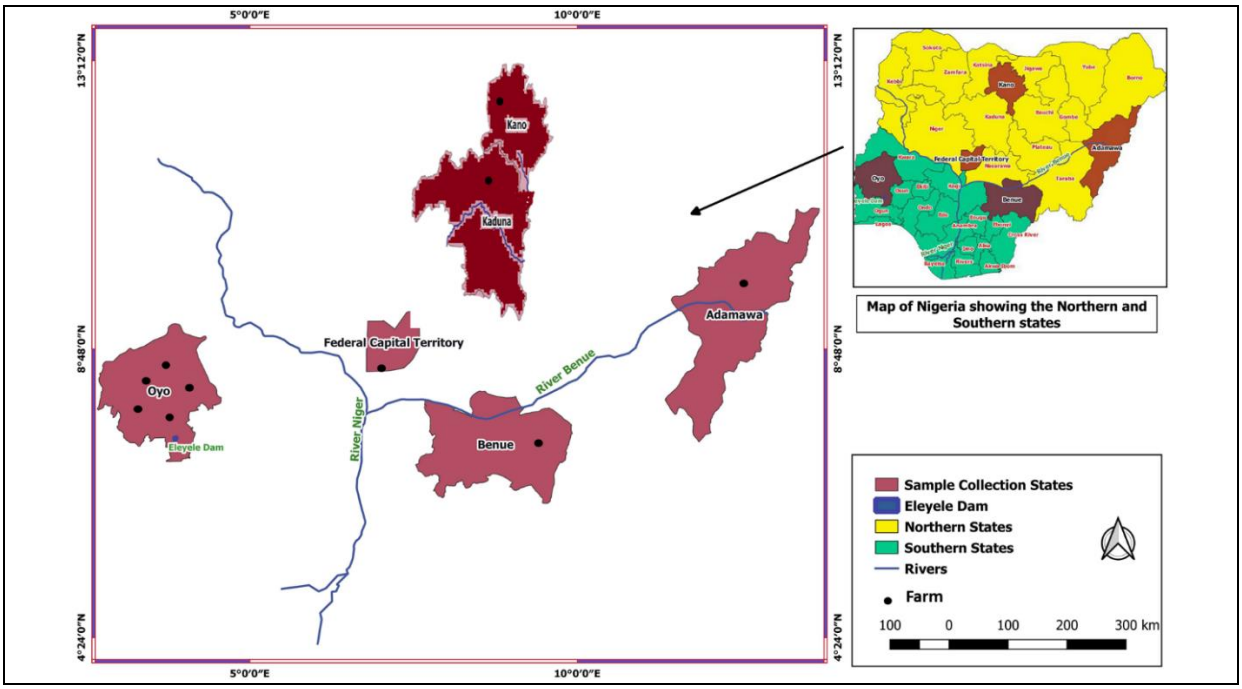

Figure 3: Map representing the *Clarias gariepinus* samples collected in the different farms (black dots) in Nigeria.

3.3 Sample collection procedure

Samples of *C. gariepinus* broodstocks were collected from five reputable fish farms from high aquaculture areas and from low aquaculture areas (Table 1). For the morphometric and meristic analysis, a total of 200 individuals (n = 20 per location) were collected from five farms from each region (North and South). In addition, a total of 20 wild individuals of *C. gariepinus* were collected from three major rivers in Nigeria, making a total of 220 samples from farmed and wild environment of both areas. A total of 110 individuals of these populations were used for genetic analysis.

Table 1: Code name, location, and number of samples (n) of *Clarias gariepinus* collected in 10 different farms and one wild population in Nigeria.

Code name	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9	Farm 10	Wild
Location	Oyo State (South)	Oyo State (South)	Oyo State (South)	Oyo State (South)	Oyo State (South)	Abuja (North)	Adamawa (North)	Benue (North)	Kaduna (North)	Kano (North)	Wild
Aquaculture Areas	HAA	HAA	HAA	HAA	HAA	LAA	LAA	LAA	LAA	LAA	LAA/ HAA
Morphometric	n=20	n=20	n=20	n=20	n=20	n=20	n=20	n=20	n=20	n=20	n=20
Meristic	n=20	n=20	n=20	n=20	n=20	n=20	n=20	n=20	n=20	n=20	n=20
Egg Analysis	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10
Genetic Analysis	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10	n=10

N/B: HAA: High aquaculture area; LAA: Low aquaculture area

3.4 Phenotypic studies

3.4.1 Morphometry studies

In the laboratory, 220 samples of *C. gariepinus* were measured for morphometric characters to the nearest centimetre (1 cm) using a wooden measuring board. A total of 26 morphometric (Plate 1; Table 2) and six meristic parameters (Table 3) were recorded for each fish following the standard methods of biometric characterization commonly used in the morphological characterization studies of the African catfish (Agnese, Teugels, Galbusera, Guyomard, & Volckaert, 1997; Teugels, 1998; Fagbuaro, Oso, Ola-Oladimeji, & Akinyemi, 2016; Ola-Oladimeji, Oso, Oladimeji, & Idowu, 2017).

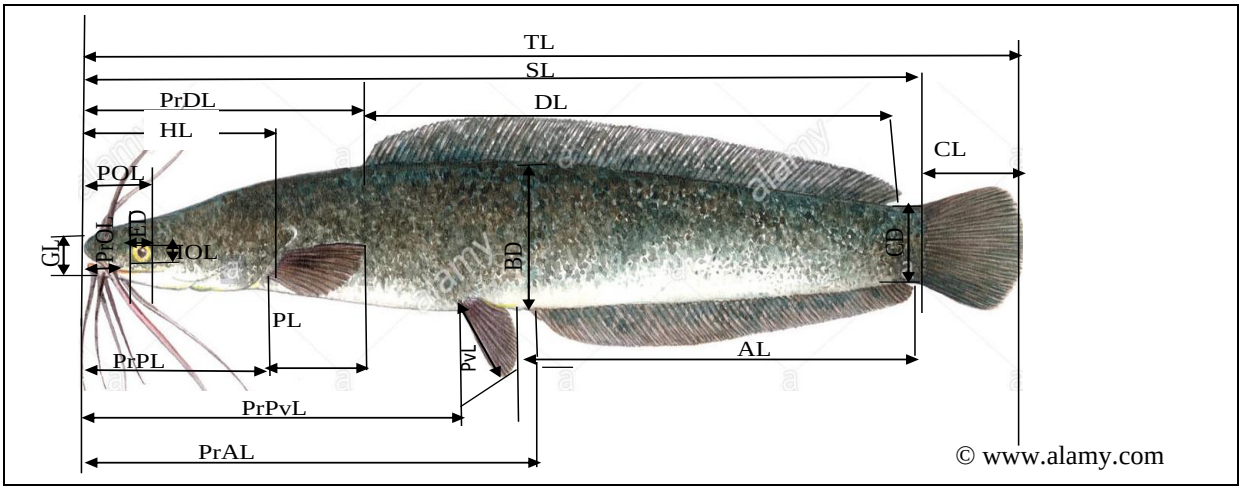

Plate 1: Schematic illustration showing morphometric characters measured in *Clarias gariepinus* samples. See Table 2 for acronyms.

Table 2: Definitions of morphometric measurements taken on *Clarias gariepinus* broodstock samples.

Character	Acronym	Reference point for body measurement
Total length	TL	Tip of the snout to the end of tail
Standard length	SL	Tip of the snout to the tail base
Head length	HL	Front of the upper lip to the posterior end of the opercula membrane
Pre orbital length	PrOL	Length from the tip of the snout to the anterior margin of the orbit
Post orbital length	POL	Distance from the posterior margin of the orbit to the end of the two orbitals
Eye diameter	ED	The greatest bony diameter of the orbit
Inter Orbital Length	IOL	Length from the upper margin of right orbit to the upper margin of the left orbit as measured from the dorsal surface.
Pre dorsal length	PrDL	From base of first dorsal spine to base of last dorsal ray
Pre pectoral length	PrPL	Front of the upper lip to the origin of the pectoral fin
Pre pelvic length	PrPvL	Front of the upper lip to the origin of the pelvic fin
Pre anal length	PrAL	Front of the upper lip to the origin of the anal fin
Pectoral fin length	PL	From base to tip of the pectoral fin
Dorsal fin length	DL	From tail base to tip of the dorsal fin
Pelvic fin length	PvL	From tail base to tip of the pelvic fin
Anal fin length	AL	From tail base to tip of the anal fin
Caudal fin length	CL	From tail base to tip of the caudal fin
Caudal depth	CD	The least depth of the tail base
Body depth	BD	Maximum depth measured from the base of the dorsal spine
Gape length	GL	Length between the angles of the mouth
Dorsal fin base length	DFBL	From base of first dorsal spine to base of last dorsal ray
Pectoral fin base length	PFBL	Length of the base of pectoral fin
Pelvic fin base length	PFBL	Length of the base of pelvic fin
Anal fin base length	AFBL	Length of the base of anal fin

Sources: Adapted from Agnese, Teugels, Galbusera, Guyomard, & Volckaert (1997); Teugels (1998); Fagbuaro, Oso, Ola-Oladimeji, & Akinyemi (2016); Ola-Oladimeji, Oso, Oladimeji, & Idowu (2017).

Table 3: Definitions of meristic counts of *Clarias gariepinus* broodstock samples.

Character	Acronym	Description
Anal fin ray count	AFR	Number of soft fin rays in anal fin
Dorsal fin ray count	DFR	Number of soft fin rays in dorsal fin
Pectoral fin ray count	PFR	Number of soft fin rays in pectoral fin
Caudal fin ray count	CFR	Number of caudal fin rays
Pelvic fin ray count	PVFR	Number of soft fin rays in the pelvic fin
Number of barbels	BB	Numbers of barbels on both sides of the jaw

Source: Adapted from (Oyebola , Omitoyin , Salako , & Awodiran, 2013)

3.4.2 Condition factor

The total specimen obtained will be used to calculate the condition factor. The condition factor which measures the relative wellbeing of the fish will be calculated for both sexes using (Gomiero & Braga, 2005);

$$K = 100\ \frac{W}{L^b}$$

K = Condition factor; W = Weight in (g); L = Total Length in (cm); b = Slope from the regression of log(weight) on log(length)

3.4.3 Fecundity estimation and oocyte diameter

Fecundity was determined based on a method described by Bagenal (1978). The fully liberated eggs were then counted by gravimetric sub-sampling (wet method). A small portion of the eggs was weighed and counted. From this, the number of eggs in a whole ovary was extrapolated. Twenty eggs from each ovary were picked at random, and the diameter of the eggs were measured using a calibrated micrometer mounted on the eyepiece of a monocular microscope (1 division = 0.05 mm).

3.5 Genetic characterization and diversity using RAD sequencing

Fin clips from the caudal fin of each of selected *C. gariepinus* were taken and each piece put in appropriately labelled 1.5 ml microcentrifuge tube. Absolute ethanol was added to fill the tube to the brim and the lid was affixed. The tubes were sealed and later transported to the Agresearch Laboratory in New-Zealand. The genetic part (RADseq) of the research was conducted in this laboratory following the procudures of Lu *et al*., (2013) and Dodds *et al*., (2015) as described below.

One GBS library was prepared using a PstI-MspI double-digest, and included negative control samples (no DNA). Libraries underwent a Pippin Prep (SAGE Science, Beverly, Massachusetts, United States) to select fragments in the size range of 193-318 bp (genomic sequence plus 123 bp of adapters). Single-end sequencing (1x101bp) was performed on an Illumina HiSeq2500 utilizing v4 chemistry. Raw fastq files were quality checked using a custom qc pipeline (available at https://github.com/AgResearch/DECONVQC). As one of the qc steps raw fastq files were quality checked using FastQC v0.10.1 Within the QC pipeline demultiplexing and sequence clean-up was taken care of via UNEAK (Tassel version 3.0.173). Raw reads were processed through a reference-free SNP detection pipeline, UNEAK (Tassel version 3.0.173). Allelic depth values were processed through KGD v0.8.2 (available at https://github.com/AgResearch/KGD).

3.6 Data and statistical analyses

3.6.1 Phenotypic data

i. Morphometric data

To ensure that data variations in this study were only attributed to body shape differences, and not to the relative sizes of the fish, a transformation was applied according to the allometric formula developed by Elliott *et al*. (1995): $Madj = M \times (Ls \times Lo^{-1})^b$;

where M is the original measurement, Madj is the size-adjusted measurement, Lo is the TL of the fish, and Ls is the overall mean of the TL for all fish from all samples. Parameter b

was estimated for each character from the observed data as the slope of the regression of log M on log Lo using all the fish in all the groups. Meristic data were not transformed because it has been established that meristic characters are independent of fish size (Strauss 1985).

Statistical analyses in the present study included descriptive statistics using Rstatistical package 2.6.1 as well as univariate analysis of variance. Data on the morphometric measurements and merristic counts were analyzed using ANOVA with means separated using Tukey test. Morphometric and meristic data were subjected to discriminant function analysis (Yakubu, 2011).

ii. Fish gonad quality

The raw data for egg quality analysis were subjected to one way analysis of variance (ANOVA), using R. The means were separated with the use of a Tukey post hoc test. Regression analysis was used to determine the following relationships: Fecundity with total weight (TW) and Egg size (ES) with total weight (TW).

3.7 Genetic data analyses

3.7.1 RADseq data

Relatedness and inbreeding coefficients (F_{IS}) were estimated from genetic relatedness matrix (GRM) using method described in Dodd *et al*., 2015. Minor allele frequency (MAF) was estimated for 37,671 putative SNPs after filtering (Hardy-Weinberg disequilibrium>-0.05). Principal component plot is based on GRM matrix and plotting using ggplot2 in R version 2.6.1.

4 RESULTS

4.1 Morphometric and Meristic data

4.1.1 Morphometric data

The correlation matrices indicating the relationship between various morphometric parameters of wild and cultured *C. gariepinus* samples prior to data transformation are shown in Figure 4. Almost 90% of the characters show a strong positive correlation value, above 0.50. Head length, standard length and dorsal fin length exhibited a correlation coefficient of more than 90% (r=0.9) with total length while eye diameter and anal fin base length which were below 0.5 showed the lowest correlation coefficient with size. Overall, eye diameter and dorsal fin base had the lowest correlation coefficient of 0.471 while total length and head length exhibited the highest correlation (r=0.965).

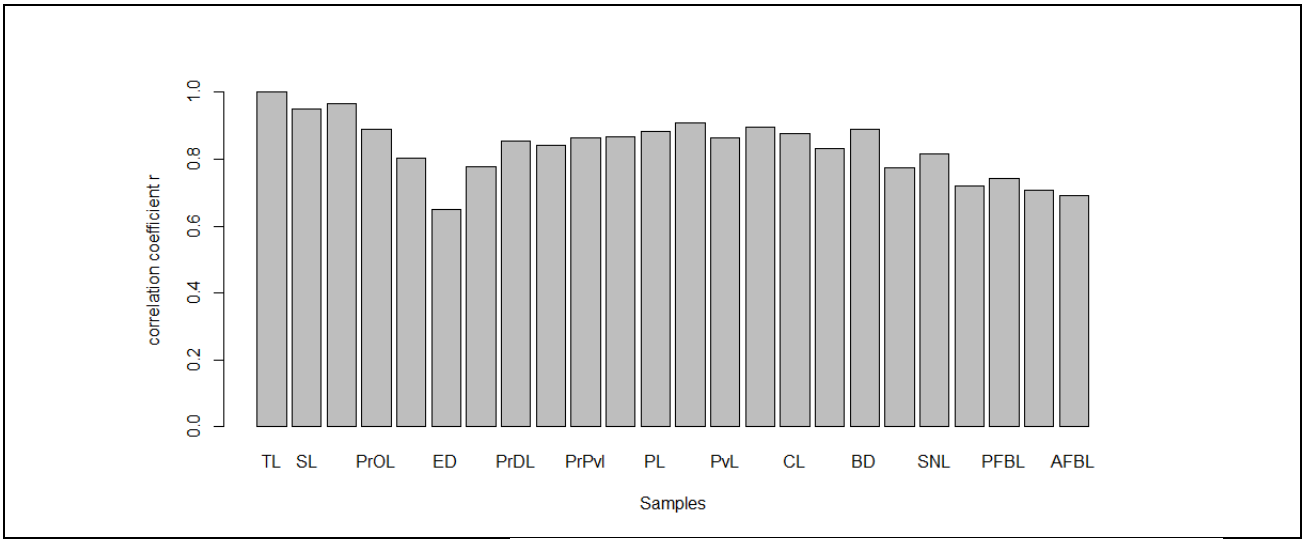

Figure 4: Correlation coefficient of initial morphometric characters of *Clarias gariepinus* broodstock samples from farmed and wild samples collected in Nigeria

No significant correlation (*P<0.05*) was observed between morphometric measurements and total length (Figure 5) after the data were log transformed. After all the parameters except total length and standard length were log transformed, *r* values of zero and below were observed. This implies that the log transformed data were free from the influence of size.

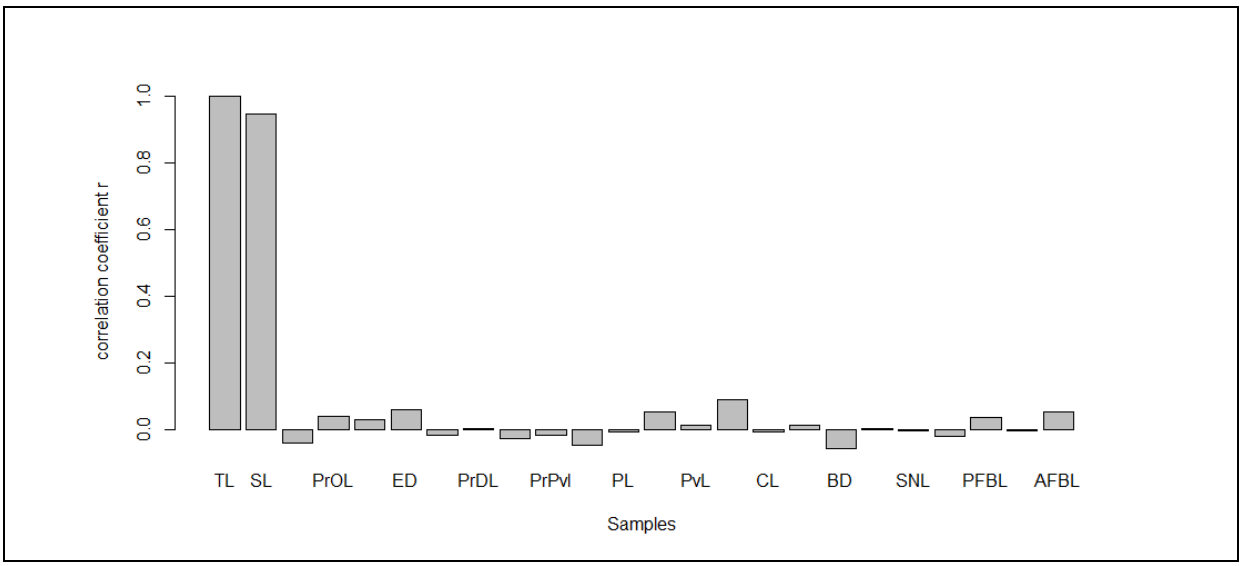

Figure 5: Correlation of log transformed morphometric characters of *Clarias gariepinus* samples from farmed and wild samples collected in Nigeria.

Most of the morphometric parameters measured (Appendix I, II and III) were significantly different (one-way ANOVA, *P< 0.01*). The fish sampled from Farm 4 were the highest in average size and this was reflected in most of the morphometric parameters taken as a total of 15 measured traits exhibited highest values in this farm when compared to the rest of the samples. On the other hand, the samples from the wild were the smallest in size and consequently in some of the morphometric parameters taken such as standard length, head length, pre-dorsal length, pre-pectoral length, pre-pelvic length, pre-anal length, pectoral length, dorsal length, anal length, body depth, gape depth, Pelvic fin base length and Anal fin base length.

There was a relationship between the samples in some of the morphometric measurements observed. However, sizes of fish obtained from Farm 1, Farm 5, Farm 6 and Farm 7 were similar in size as revealed by their total length and standard lengths which ranged between 70.7 ± 4.45cm to 35.1 ± 6.7cm and 59.4 ± 6.3cm to 29.8 ± 3.52cm respectively.

The head length ranged from 13.5 ± 0.69cm to 15.1 ± 0.47cm for Farm 8 and Farm 1 respectively. The post-orbital length was the highest (5 ± 0.19cm) for the fish collected in Farm 6 while Farm 7 fish exhibited the lowest value for this trait (3 ± 0.75cm). The Eye diameter (ED) ranged between 0.8 ± 0.14cm for the wild and 1.4 ± 0.42 for Farm 4.

The average inter-orbital length obtained for the Farm 1, Farm 5, Farm 6, Farm 7, Farm 8, Farm 9 and Farm 10 showed no significant different but differed significantly with the rest. This pattern was also observed in the values of pre-dorsal fin length and pre-pectoral fin length. Average values of pre-pelvic fin length and pre-anal fin length followed the same pattern of variation among the treatments.

Pre-pelvic fin length measured in Farm 1, Farm 7 and Farm 6 showed no significant difference but differed from other samples, also the pre-anal fin length showed significant difference across the samples with exception of Farm 1, Farm 6 and Farm 9 which showed no significant difference. Pectoral fin length appeared highest in Farm 2 (7.1 ± 0.77cm) and lowest in Farm 5 (6.1 ± 0.37cm).

Dorsal fin length was significantly longer in Farm 4 (34.3 ± 0.38cm) and Farm 6 (34.7 ± 1.68cm) and shorter in Farm 2 (28.9 ± 1.82cm)

The pelvic fin length ranged between 4.6 ± 0.26 cm and 5.8 ± 0.15 cm among all the samples with the lowest and highest values recorded in Farm 7 and Farm 4 respectively. The anal fin length showed similarities between the samples from Farm 2 and Farm 7, Farm 5 and Farm 8, Farm 6 and Farm 9. Caudal fin length ranged from (6.3 ± 0.25cm) to (7.6 ± 1.01cm) for Farm 9 and Farm 4 respectively.

The caudal fin depth was not significantly different ($P>0.05$) between Farm 2 (4.7 ± 0.17cm), Farm 5 (4.7 ± 0.44cm), Farm 8 (4.7 ± 0.81cm) and Farm 10 (4.6 ± 0.42cm) but exhibited significant differences with other samples. The wild population had the lowest body depth of 9 ± 1.1cm while Farm 7 had the highest (10.4 ± 0.51cm). Gape length also showed similarities between the samples from Farm 1, Farm 2, Farm 3, Farm 5 and Farm 8. These samples displayed respective gape length values of 4.3 ± 0.43cm, 4.4 ± 0.18cm, 4.3 ± 0.17cm, 4.1 ± 0.51cm and 4.3 ± 0.49cm. Snout length and Pectoral fin base length showed similar trend across all the samples. The longest dorsal fin base was found in Farm 4 (3.3 ± 1.3cm) while the shortest length was seen in Farm 1 (2.4 ± 0.05cm). Pelvic fin base length of the samples was significantly highest in Farm 5 with a value of 2.3 ± 0.28cm and lowest in the wild with the value of 1.5 ± 0.19cm. Anal fin base length varied from 1.2 ± 0.43cm to 2.5 ± 0.09cm among the samples.

4.1.2 Meristic counts

Appendix IV shows the summary of the meristic parameters measured on *C. gariepinus* samples from Nigeria farms and the wild population. The anal fin ray count averages ranged from 48 ± 3.91 to 4.2 ± 10.16, with the lowest and highest values recorded in the samples from Farm 9 and Farm 4 respectively. These samples were significantly different from all the other

samples. The dorsal fin ray count had on average 59.9 ± 7.67 observed in Farm 5 and a maximum value of 71.5 ± 5.43 recorded in Farm 8 sample. Pectoral fin ray count was similar for fish from Farm 1, Farm 2, Farm 3, Farm 8, Farm 9, which exhibited significant differences with all the other samples. Values of the caudal fin ray count were only significantly different between the wild population and all the other farmed samples. The Pelvic fin ray count and number of barbels showed no significant differences among the various samples.

4.1.3 Cluster Analysis

The dendrogram drawing based on Euclidean distances between the samples using an UPGMA displayed three main clusters and three outliers (Figure 6) based on morphometric and meristic characters. The group were composed as follow. Group 1 was composed of samples from Farm 7, Farm 1, Farm 3 and Farm 10; group 2 was composed Farm 2 and Farm 9 samples, wild and Farm 5 samples formed group 3; Farm 8, Farm 4 and farm 6 samples were separated from the groups.

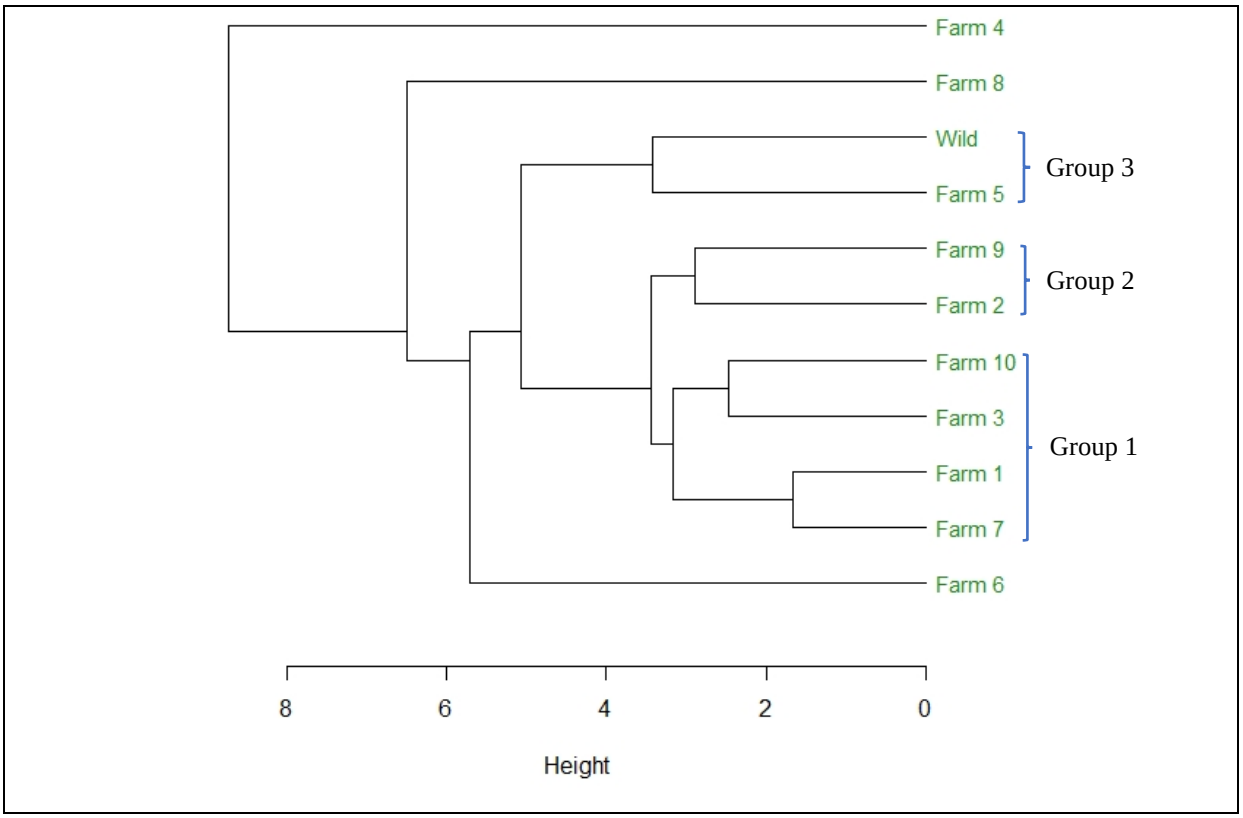

Figure 6: Cluster dendrogram performed on morphometric and meristic data of the 11 samples of *Clarias gariepinus* collected in Nigeria

4.2 Condition factor data

The condition factor (K) of the wild sample was significantly higher (One-way ANOVA, $P< 0.05$) than the farm raised sample (Figure 7). Most of the cultured samples were not shown to be significantly different from one another. For instance, samples from Farm 1, Farm 2, Farm 5, Farm 6, Farm 7, Farm 8 and Farm 9 showed similarity in their condition factors, also, Farm 3 and Farm 4 showed no variation from each other. However, the body weight showed

significant variation (One-way ANOVA, *P<0.05*) in the different samples (Figure 8), it was noticed that the weight of Farm 4 was higher than the rest of the groups which showed lower values. The wild sample had the lowest in body weight.

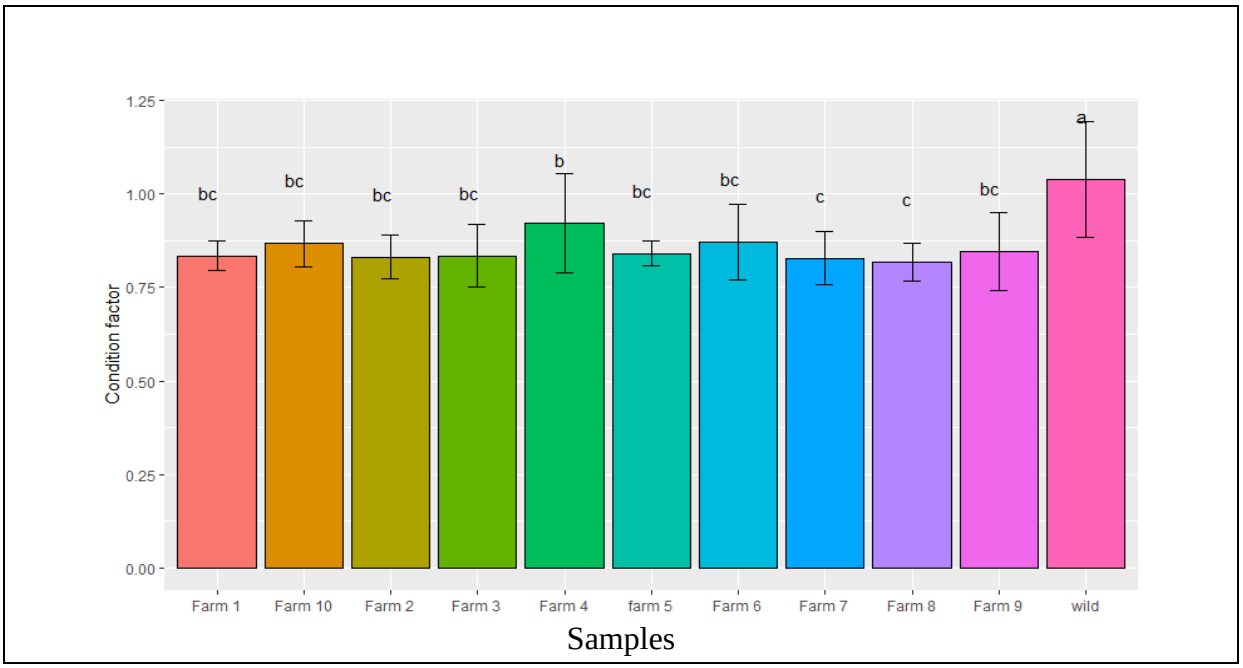

Figure 7: Condition factor (K) (Y-axis) of *Clarias gariepinus* broodstock samples (X-axis) in Nigeria. N/B: Whiskers represent 2 standard deviation (SD); different letters denote significant differences (one way ANOVA, *P< 0.05*; Where; a>bc>b>cd>…….f).

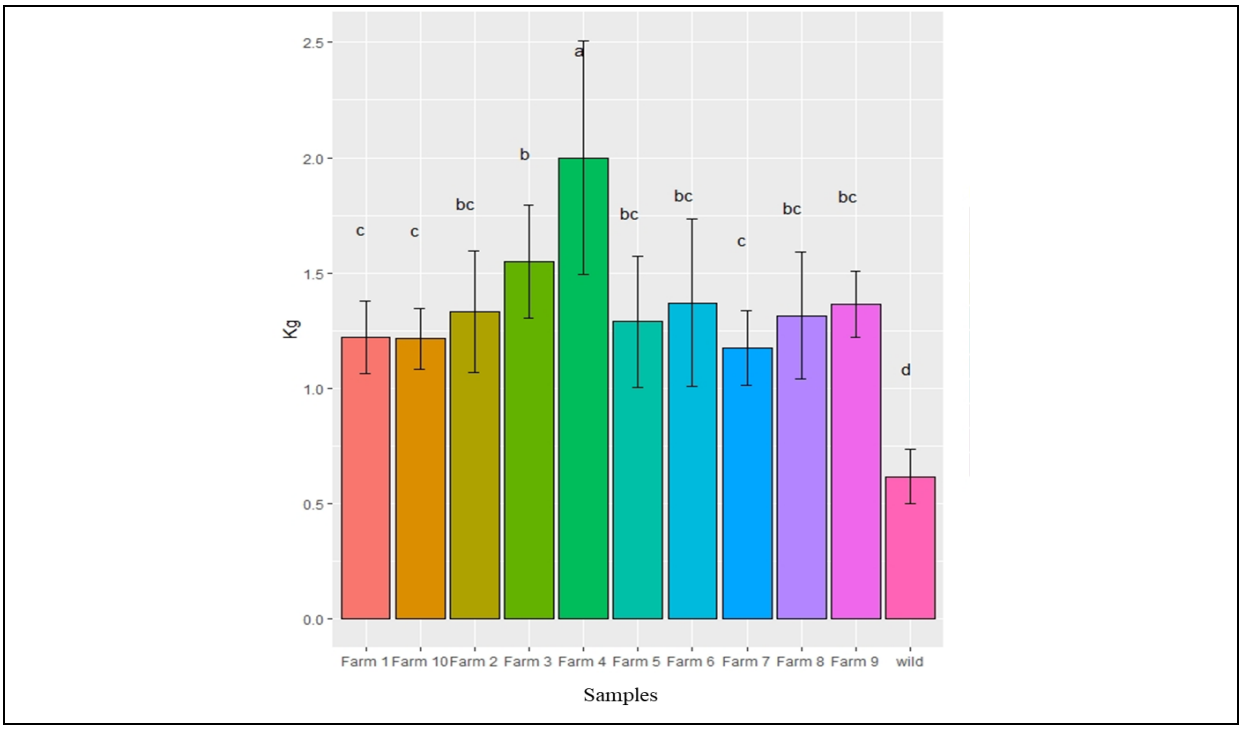

Figure 8: Body weight (Kg) (Y axis) of *Clarias gariepinus* broodstock samples (X-axis) from Nigeria. N/B: Whiskers represent 2 SD; different letters denote significant differences (one way ANOVA, *P< 0.05*; Where; a>bc>b>cd>…….f).

4.3 Gonad quality data

4.3.1 Egg diameter analysis

Significant differences were found in the egg diameter among the 11 groups. Farm 4 had the highest egg diameter values and it was significantly different (One-way ANOVA, *P< 0.05*) from all other samples while Farm 1 had the lowest value (Figure 9). Furthermore, Farm 5 was not significantly different from Farm 8 but was it was different from other samples. Similarly, Farm 6 showed similarity in their egg diameter with the wild population. Farm 2 and Farm 7 showed a similar trend.

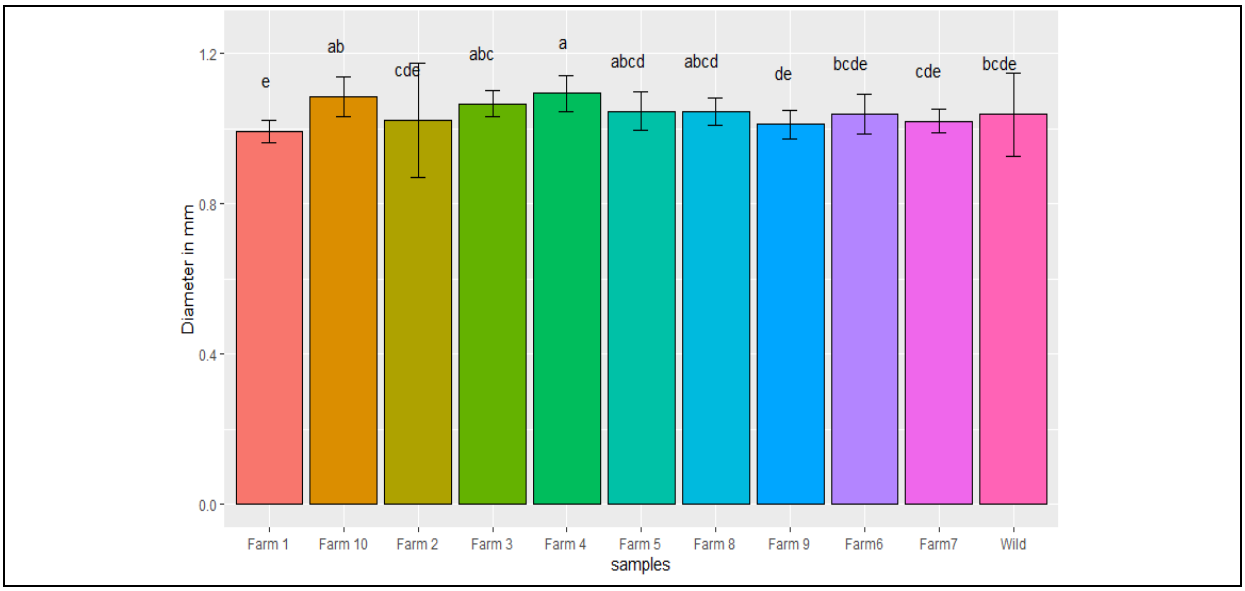

Figure 9: Mean egg diameter (Y-axis) of female *Clarias gariepinus* collected in the different broodstock samples (X-axis) from farmed and wild environments in Nigeria. N/B: Whiskers represent 2 SD; different letters denote significant differences (one way ANOVA, *P< 0.05*; Where; a>bc>b>cd>…….f).

4.3.2 Fecundity data

Significant differences (One-way ANOVA, *P<0.05*) seen in the fecundity between the samples (Figure 10).

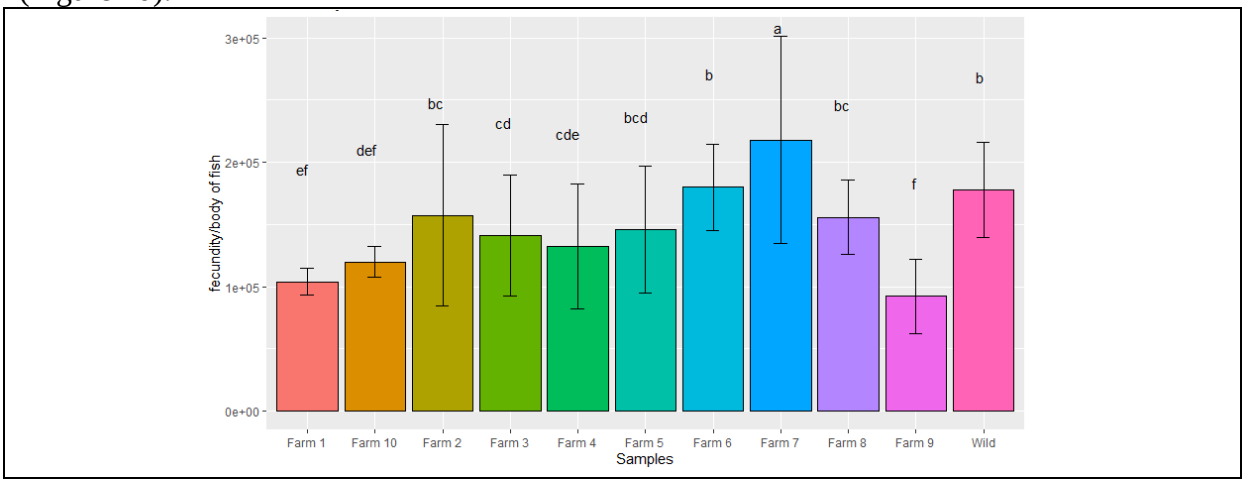

Figure 10: Relative fecundity (Y-axis) of *Clarias gariepinus* broodstock samples (X-axis) in Nigeria. N/B: Vertical whiskers represent 2 SD; different letters denote significant differences (one way ANOVA, P< 0.05; Where; a>bc>b>cd>…….f).

Broodstock samples from Farm 7 was seen to be significantly higher than others while Farm 9 showed the lowest difference across the samples. However, Farm 8 and Farm 2 showed no significant difference but were different from other samples. Similarly, fecundity of the fish from Farm 6 showed no significant difference from the wild sample.

4.4 Genetic variability and inbreeding

4.4.1 Genetic variability

The PCA analysis performed on farm samples revealed the presence of genetically distinct groups corresponding to the different farm samples (Figure 11). The observed groups were composed of: Group 1, Farm 9 and 10; Group 2, Farm 7; Group 3, Farm 8 Group 4, all the remaining farm samples (Figure 11). The first PCA axis (PC1) explained 21.17% of the genetic variability and mainly revealed a difference between Farm 3 and all the other samples. The variability between Group 1, Group 2 and Group 4 also existed but the level of differentiation was lower. The second PCA axis (PC2) explained 17.94% of the genetic variability and clearly distinguish the 4 different groups.

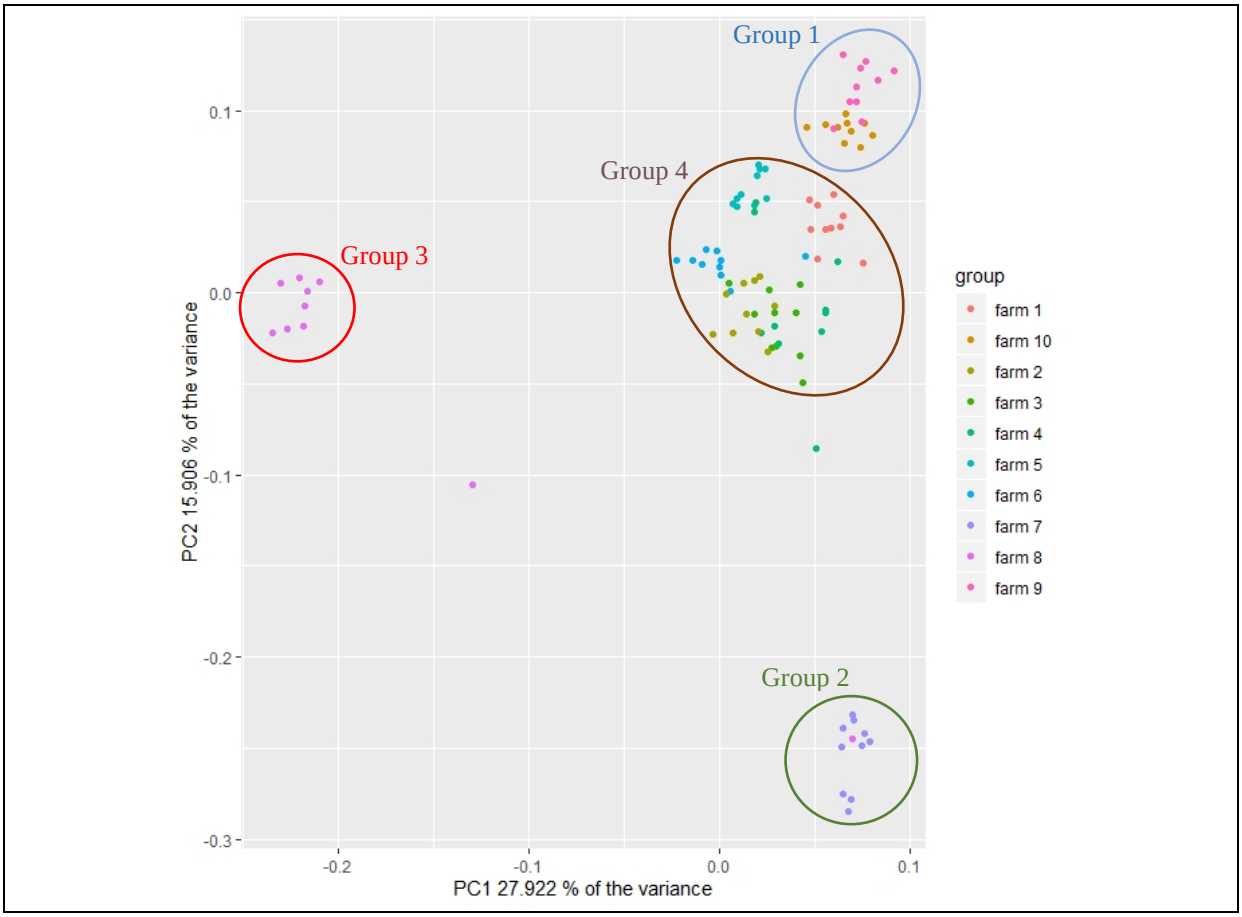

Figure 11: Principal Component Analysis (PCA) of *Clarias gariepinus* farm samples from Nigeria. Each dot represents an individual from each farm sampled. Colours depicting their origin are presented on the side of the graph.

4.4.2 Inbreeding signals

Minor allele frequency (MAF), which represents the frequency of the second most common allele occurring in a given sample, is a good indicator of inbreeding. Analysis of this parameter

within the farm samples of Nigeria showed a very high frequency of MAF, suggesting a level of inbreeding (Figure 12).

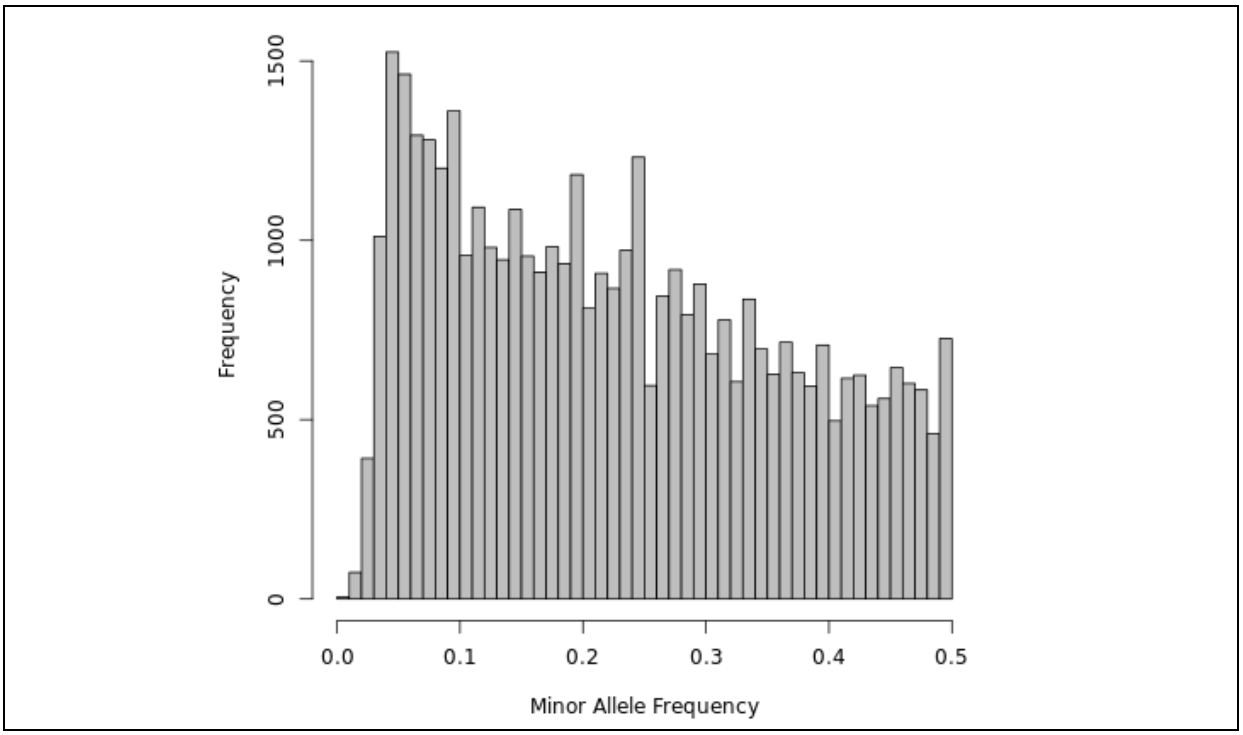

Figure 12: Minor Allele Frequency (MAF) of Single Nucleotide Polymorphisms (SNPs) of *Clarias gariepinus* farm samples from Nigeria.

Genetic relatedness analysis showed that within sample relatedness was high and ranged from 0.14±0.18 to 0.67±0.09 in Farm 3 and farm 7 respectively (Table 4). On the other hand, there is no genetic relatedness between the farm samples. The genetic relatedness (R) between the farm ranged from -0.12±0.04 between Farm 1 and Farm 10 to 0.01±0.05 between Farm 1 and Farm 10.

Table 4: Genetic relatedness (R) between farm samples of *Clarias gariepinus* collected in Nigeria.

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9	Farm 10
Farm 1	**0.53 ± 0.06**									
Farm 2	-0.03 ± 0.04	**0.27 ± 0.18**								
Farm 3	0.03 ± 0.05	0.03 ± 0.04	**0.14 ± 0.18**							
Farm 4	0.01 ± 0.09	0.01 ± 0.05	0.03 ± 0.07	**0.28 ± 0.4**						
Farm 5	0.00 ± 0.04	-0.02 ± 0.05	-0.04 ± 0.07	-0.04 ± 0.07	**0.59 ± 0.06**					
Farm 6	-0.01 ± 0.20	-0.04 ± 0.04	-0.01 ± 0.04	-0.01 ± 0.06	-0.03 ± 0.04	**0.46 ± 0.28**				
Farm 7	-0.09 ± 0.04	0.00 ± 0.04	0.02 ± 0.05	0.00 ± 0.09	-0.09 ± 0.04	-0.08 ± 0.03	**0.68 ± 0.08**			
Farm 8	-0.15 ± 0.04	-0.05 ± 0.04	-0.07 ± 0.05	-0.09 ± 0.05	-0.08 ± 0.04	-0.06 ± 0.04	-0.03 ± 0.26	**0.43 ± 0.30**		
Farm 9	-0.06 ± 0.03	0.00 ± 0.03	-0.02 ± 0.04	-0.02 ± 0.07	-0.05 ± 0.02	-0.09 ± 0.03	-0.1 ± 0.04	-0.14 ± 0.04	**0.58 ± 0.09**	
Farm 10	-0.09 ± 0.04	-0.03 ± 0.04	-0.01 ± 0.04	-0.02 ± 0.05	-0.08 ± 0.04	-0.07 ± 0.06	-0.08 ± 0.04	-0.13 ± 0.03	0.12 ± 0.04	**0.56 ± 0.08**

N/B: Bold values indicate relatedness within samples and normal font values indicate relatedness between samples.

The results of the relatedness were reflected in the inbreeding coefficient, too, as most of the farm samples exhibited a high level of inbreeding above the value of 1 (Figure 13).

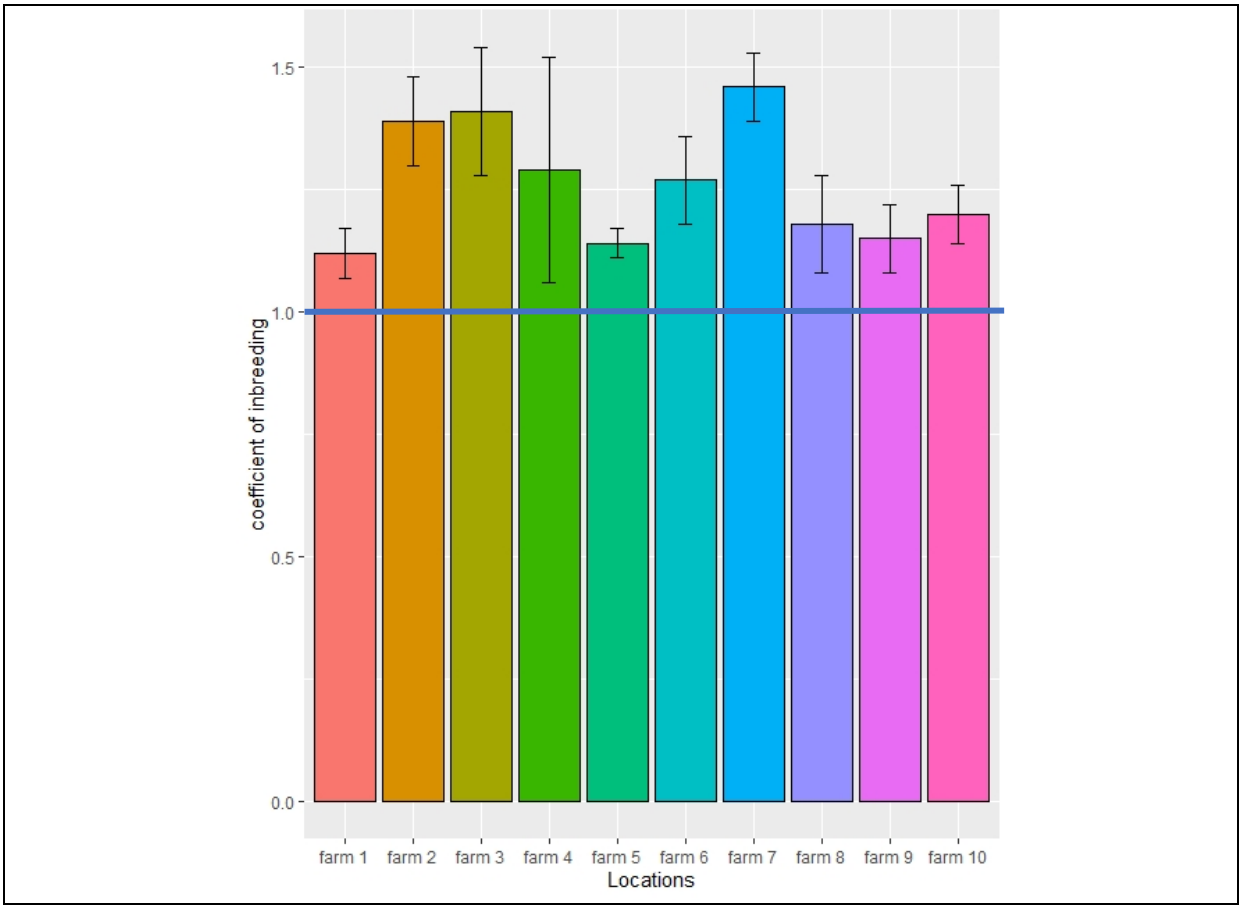

Figure 13: Coefficient of inbreeding (colored histograms) calculated for each farm samples of *Clarias gariepinus* collected in Nigeria. The Vertical whiskers represent 1 SD. Values above 1 (indicated by the blue line) shows inbreeding.

5 DISCUSSION

Management and maintenance of genetic diversity are very important for both wild and cultured fish groups because decline in genetic diversity levels impede survival and growth of offspring in an aquaculture system (Diyaware, Suleiman, Alade & Popoola, 2018). The study of genetic diversity of the Nigerian stocks of *C. gariepinus* is important because the species is an important source of animal protein, commands high commercials values due to its high fecundity, high palatability, resilience, disease resistance and rapid growth among other attributes (Olubunmi, Olateju, Latifat & Oluniyi, 2009). However, poor seed quality has been reported to be the main problem limiting the growth of this species in Nigeria, which is known to be in pari passu with the broodstock quality used (Ume, Ebeniro, Ochiaka & Uche, 2016; Digun-Aweto & Oladele , 2017). Poor broodstock management such as keeping a low effective number of parents, poor record keeping about the pedigree information, random mating design, among other factors in this economically important species have been reported in hatcheries (Ibiwoye & Thorarensen, 2018; Awodiran & Afolabi, 2018), and this could lead to loss of genetic variability. There is therefore a need to improve and maintain a healthy level of genetic variability in farming programme of this species in order to maximize productivity and profitability.

5.1 Morphometric characters

In order to evaluate morphological differences between wild and cultured fish of the same species, different authors have used morphometric and meristic variables (Solomon, Okomoda, & Ogbenyikwu, 2015; Fagbuaro, Oso, Ola-Oladimeji, & Akinyemi, 2016; Ola-Oladimeji, Oso, Oladimeji, & Idowu, 2017).

Results of the morphometric characterization in the present study revealed that the *C. gariepinus* obtained from the cultured and wild environment were morphologically different (Appendix I-III). The morphometric variability among the eleven samples in this study was mainly due to the variation of characters related to fins, and body characteristic because the effect of size was successfully eliminated by the allometric transformation and this was demonstrated by correlation analysis. The differences observed in these samples could be linked to both genetic differences and environmental factors in natural and farming conditions. This corresponds to the results obtained by Solomon, Okomoda & Ogbenyikwu (2015) who noted significant differences in all the morphometric characters measured on *C. gariepinus* collected from the cultured and wild environments in Benue State of Nigeria. Ola-Oladimeji, Oso, Oladimeji & Idowu (2017) also reported morphological differences between *C. gariepinus* strains obtained from both wild and cultured populations in Ekiti State, Nigeria. In addition, there was also variation between the samples from the farm (cultured environment). This could be attributed to the fact that domesticated fish has high adaptation to wide range of geographical locations, which leads to high morphometric variations with respect to their wild counterparts (Turan, Yalcin , Turan, Okur & Akyurt, 2005). However, breeding over several years may have diluted the initial gene pool of the domesticated fish leading to genetic variation which translates to higher morphometric differences found between them. Fagbuaro, Oso, Ola-Oladimeji & Akinyemi (2016) reported similarities in the morphometric composition of *Clarias gariepinus* collected from a fish pond in Emure- Ekiti (controlled population) and Ogbese River (uncontrolled population); however, this does not conform to the data obtained in the present study.

The cluster analysis using unweighted pair group method (UPGMA) revealed (Figure 6) that the samples were further grouped into five clusters based on body shapes. Interestingly, the wild sample clustered with samples from Farm 5. This may suggest that the farmed fish samples and the wild samples were different, though they still exhibited similarity of trait between them. This is similar with the result of Fagbuaro, Oso, Ola-Oladimeji & Akinyemi (2016) who reported that the cultured *C. gariepinus* from Ado-Ekiti were grouped along with wild fish from Agbabu, and Esaodo in Nigeria. In their study, these authors stated that the similarity between farm and wild samples could be due to that fact that the cultured wild fish belong to an escapee group or have ancestral relationship.

5.2 Meristic characters

It has been established that meristic characters are independent of fish size; hence, they should not change during growth (Straus, 1985), therefore data from the meristic counts were not log transformed prior to further analysis. In this study it was observed that the meristic characters showed little or no variability between the farm raised samples and samples from the wild. There were significant differences in four out of six meristic characters studied (Appendix IV). Meristic counts are more primitive features than morphometric measurements and therefore usually provide stronger evidence for species differentiation (Fakunmoju, Akintola & Ijimakinde, 2014). This slight variation in the meristic characters could be as a result of the

difference in the genetic make-up between the farm raised samples and the wild. Furthermore, this variation could be environmentally related, since the fish samples were not drawn from the same distribution. This corresponds to the results of Solomon, Okomoda & Ogbenyikwu (2015) who noted significant differences recorded in three of five meristic counts made on *C. gariepinus* collected from the cultured and wild environments in Benue State of Nigeria, although the results of the study showed little or no variability in meristic counts. Ola-Oladimeji, Oso, Oladimeji & Idowu (2017) also reported slight differences in the meristic traits between *C. gariepinus* strains obtained from both wild and cultured populations in Ekiti State, Nigeria. However, the meristic result in this study is in contrast with the finding of Fagbuaro, Oso, Ola-Oladimeji & Akinyemi (2016) who noticed similar result while working with *Clarias gariepinus* collected from a fish pond in Emure-Ekiti (controlled population) and Ogbese River (uncontrolled population) in Nigeria.

5.3 Condition factor

Condition factor is an index used for monitoring feeding intensity, age and growth rate in fish. The condition factor defines the well-being of the fish in a particular environment at a time and it gives information on the physiological state of the fish in relation to its welfare (Abowei, 2010; Kumolu-Johnson, Ndimele & Akintola , 2010). It is strongly influence by both abiotic and biotic environment conditions and factors like sex, season, age and maturity stages of the fish (Anene, 2005).

The weight of the fish samples from the farm are significantly higher (Figure 8) than the wild samples. This is could be attributed to artificial feed provided to the farmed samples. In disagreement with Fagbuaro, Oso, Ola-Oladimeji, & Akinyemi (2016), the mean condition factor (K) (Figure 7) of the wild samples in this study, was significantly higher (one way ANOVA, $P<0.05$) than that of farmed fish samples. Values of K for the wild sample was higher than the value of 1 while the K values in the farmed fish samples were less than 1. However, this result is similar to González *et al*. (2016); Solomon, Okomoda, & Ogbenyikwu (2015); Lizama , De Los, & Ambrosio (2002) who recorded a low condition factor value in the big size cultured fish compared the small weighed wild fish. The high condition factor of the wild was likely due to the gonad condition of the female stocks (gravid), as observed in their fecundity data. The low K recorded in the farmed fish showed that the feed quality of the farmed broodstock samples may not be adequate.

5.4 Egg diameter and relative fecundity of the samples

Reproduction activities such as fertilization success, hatching rate and larval growth may be related to the size of the eggs and fecundity (Palumbi, 2004). For many fishes, female size may determine the size of the eggs, where larger fish produce bigger egg and smaller fish produce smaller egg size (Kamler, 2005). Therefore, size adjustment was done for the egg size to remove variation due to size of the female fish.

Eggs collected from farm raised fish samples had significantly larger eggs and greater number of eggs per kilogram of the female fish than the eggs collected from the wild samples (Figure 8 and Figure 9). Factors such as diet, environment, and genotype, could potentially contribute to the observed differences between egg quality collected from the cultured and wild stocks (Bobe & Labbe, 2009). In this study, it is easy to point out that the bigger egg size and higher fecundity discovered in the farmed fish as compared to the wild could be more related to

environmental than genetic factor, because the genetic results in this study showed high level of inbreeding in the farmed fish compared to the wild samples. This implies that no effect of inbreeding was found on the egg size and fecundity. The results of this study contrast to the results of some other fish studies that report inbreeding depression affecting females' fecundity (Gallardo, Garcia & Lhorente, 2004; Fessehaye, Bovenhuis, Rezk & Crooijmans, 2009). However, Naish, Seamons, Dauer, Hauser & Quinn, (2013) also detected no significant correlation between inbreeding coefficient and female fecundity, gonad mass or age at return in the rainbow trout (*Oncorhynchus mykiss*), similarly, Langen, Bakker, Baldauf & Shrestha, (2017) who researched on the effects of ageing and inbreeding on reproductive traits in a cichlid fish (*P. taeniatus*) discovered no effect of inbreeding on egg size, fecundity and growth of the fish as inbred female *P. taeniatus* were larger than outbred ones. They suggested that the deleterious alleles are probably purged from the population.

5.5 Genetic variability and inbreeding

Although genetic studies have been performed in recent years on the catfish *Clarias gariepinus* in Nigeria (Popoola, Fashakin & Awopetu, 2014; Diyaware, Suleiman, Alade & Popoola, 2018; Awodiran & Afolabi, 2018), there is a crucial lack of information on the genetic characteristics of farm samples versus wild population. The use of RADseq during this project was successful since most of the samples collected could be analyzed except for the wild sample; this was potentially due to bad DNA quality or contamination.

However, the genetic analyses performed on the farm samples during this project clearly demonstrated that all the samples collected either from high or low aquaculture area in Nigeria, exhibited a level of inbreeding. This was first apparent at the level of Minor Allele Frequency (MAF; Figure 12), which exhibited a typical frequency pattern of inbreeding, e.g. a considerable number of SNPs had a MAF below 25%.

This result was also confirmed by the observed level of inbreeding in all farm samples which were significantly higher than 1 (Figure 13), and even higher than 1.25 for some of the samples.

The combined results of relatedness (Table 6) and of the inbreeding (Figure 12 & 13) revealed that almost all farm samples, exhibited a level of inbreeding and high relatedness within the farms, which therefore may suggest that farming practices could have been more adequately controlled in the studied farms.

All these inbreeding signals detected, resulted in clear clustering of individuals from different farms in different groups using PCA analysis (Figure 11). These groups exhibited different levels of individual relationships, with for example Group 3 almost exclusively composed of Farm 3 samples and exhibiting low genetic diversity. Strangely, Farm 3 did not exhibit the highest level of relatedness and therefore one can foresee that the observed level of inbreeding in this farm is not due to relatedness among individuals. Although Farm 4 was distributed in many groups in the PCA analysis, suggesting that it was more diverse than Farm 3, it also exhibited a low level of relatedness and the same reasoning can therefore be applied.

The observed level of inbreeding in the farm samples can be explained by two hypotheses:

1) The wild population of *Clarias gariepinus* in Nigeria is in poor health condition and exhibit a high level of inbreeding reflected in the farm samples,

2) The farming practices in Nigeria are based on a too small number of parents which ultimately led to the use of highly related individuals as broodstock and hence led to inbreeding.

In recent years, the genetic variability and status of *Clarias gariepinus* has been studied in Nigeria rivers. These studies showed that the population of this species did not exhibit any signs of inbreeding (Popoola, Fashakin & Awopetu, 2014) and exhibit a large genetic diversity (Awodiran & Afolabi, 2018). In addition, these studies tend to indicate that farm and wild population of *Clarias gariepinus* were genetically highly differentiated (Awodiran & Afolabi, 2018; Diyaware, Suleiman, Alade & Popoola, 2018).

Therefore, one can conclude that the observed results during this project are not due to a poor health status of *Clarias gariepinus* wild population (hypothesis 1) but rather to the fact that farming practices are probably based on a too small number of breeders within each farm.

6 CONCLUSION

The present study analyses morphological and genetic differences between farmed and wild fish samples. The differences between the fish samples could be a result of their environment as well as their genetic makeup. This study showed that although the morphological and gonad quality analysis were useful in population differentiation, they were not suitable to determine inbreeding signs. However, the genetic markers revealed that farmed fish exhibited a high level of genetic relatedness and inbreeding. The most plausible cause is poor management practices such as lack of record keeping, maintaining the genetic diversity, and an uncontrolled random mating system.

7 RECOMMENDATIONS

The wild sample was not genetically analysed in this project, but few studies have shown that the wild samples from some rivers were not genetically related and exhibited high level of genetic variability. However, further tests should be performed to evaluate their genetic characteristics, their potential relatedness and potential signs of inbreeding. If the wild population cannot be used to renew the broodstocks in the farms, alternative process such as outcrossing farm samples should be envisioned. Furthermore, in order to remedy the observed challenge in *C. gariepinus* farming industry, the following recommendations can be made:

1. A breeding plan should be initiated for *Clarias gariepinus* in Nigeria.
2. Available genetic resources which could be used for breeding in Nigeria should be systematically explored, both from wild and farms. This can be done using the RAD sequencing tool used in this study or other genetic tools.
3. If the status of the wild population is poor, outcrossing farm samples which exhibit a level of differences (for example, Farm 8 and Farm 7) could be explored, and if necessarily, implemented.
4. Set up recording database for mating and performance information from all farms.
5. Fish farmers should be trained in the animal breeding and broodstock management.

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APPENDICES

Appendix I: Mean±standard deviation of mean (cm), F and P-values of morphometric measurement (MM) of *Clarias gariepinus*

	High Aquaculture Area					Low Aquaculture Area					Wild		
MM	Farm 1 n=20	Farm 2 n=20	Farm 3 n=20	Farm 4 n=20	Farm5 n=20	Farm 6 n=20	Farm 7 n=20	Farm 8 n=20	Farm 9 n=20	Farm 10 n=20	Wild n=20	F-val	P-val
TL	57.3 ± 5.03^{c}	60.1 ± 6.87^{bc}	65.3 ± 2.49^{ab}	**70.7 ± 4.45^{a}**	58.5 ± 6.53^{c}	59.3 ± 7.12^{c}	56.5 ± 6.06^{c}	60.1 ± 5.14^{bc}	60.7 ± 3.08^{bc}	56.1 ± 4.98^{c}	**35.1 ± 6.70^{d}**	50.26	0.00
SL	49.3 ± 4.07^{cd}	49.6 ± 7.18^{cd}	55.1 ± 1.71^{ab}	**59.4 ± 6.30^{a}**	48.8 ± 6.16^{cd}	52.1 ± 6.09^{bcd}	49.7 ± 5.17^{cd}	52.9 ± 4.89^{bc}	50.1 ± 2.65^{bcd}	47.2 ± 3.68^{d}	**29.8 ± 3.52^{e}**	44.42	0.00
HL	**15.1 ± 0.47^{a}**	14.8 ± 0.62^{ab}	14.8 ± 0.32^{ab}	14.2 ± 0.45^{cd}	14.5 ± 0.5^{bc}	14.1 ± 0.34^{cd}	14.6 ± 0.30^{bc}	**13.5 ± 0.69^{e}**	14.5 ± 0.17^{bcd}	15 ± 0.50^{ab}	14 ± 0.86^{de}	16.59	0.00
PrOL	3.5 ± 0.09^{d}	3.6 ± 0.31^{cd}	3.6 ± 0.23^{cd}	**4.2 ± 0.23^{a}**	3.6 ± 0.13^{cd}	3.6 ± 0.20^{cd}	3.1 ± 0.33^{e}	3.8 ± 0.36^{bc}	3.5 ± 0.11^{cd}	4 ± 0.18^{ab}	**2.8 ± 0.22^{f}**	54.95	0.00
POL	4.5 ± 0.10^{bc}	4.6 ± 0.36^{ab}	4.1 ± 0.85^{c}	4.8 ± 0.20^{ab}	4.5 ± 0.10^{bc}	4.8 ± 0.22^{ab}	4.5 ± 0.23^{bc}	**3 ± 0.75^{d}**	4.5 ± 0.15^{bc}	**5 ± 0.19^{a}**	4.6 ± 0.42^{ab}	36.00	0.00
ED	1.1 ± 0.18^{bc}	1.1 ± 0.18^{b}	1.1 ± 0.15^{ab}	**1.4 ± 0.42^{a}**	1.1 ± 0.18^{bc}	1.1 ± 0.25^{bc}	1.3 ± 0.45^{ab}	1.2 ± 0.13^{ab}	1.2 ± 0.14^{ab}	1.1 ± 0.14^{b}	**0.8 ± 0.14^{c}**	6.20	0.00
IOL	5.8 ± 0.63^{c}	6.9 ± 0.94^{ab}	3.5 ± 0.76^{d}	**7.2 ± 0.58^{a}**	5.7 ± 0.83^{c}	5.7 ± 0.69^{c}	5.7 ± 0.95^{c}	5.7 ± 1.05^{c}	5.9 ± 0.38^{c}	6.2 ± 1.2^{bc}	**5.7 ± 0.57^{c}**	25.40	0.00
PrDL	16.7 ± 0.37^{ab}	**17.9 ± 0.48^{a}**	16.9 ± 0.35^{ab}	17.3 ± 4.24^{ab}	16.1 ± 1.03^{b}	17.1 ± 0.36^{ab}	16 ± 0.63^{b}	17.4 ± 1.66^{ab}	17.1 ± 0.85^{ab}	17.7 ± 0.54^{a}	**13.9 ± 1.31^{c}**	10.32	0.00

broodstock samples from Nigeria.

NB: Values with same superscripts across the rows are not significantly different at P>0.01. All the morphometric characters were adjusted except total length (TL). TL = total length; SL = standard length; HL = head length; PROL = pre orbital length; POL= post orbital length; ED eye diameter; IOL = inter orbital length; PRL pre-dorsal fin length.

Appendix II: Mean±standard deviation of mean (cm), F and P-values of morphometric measurement (MM) of *Clarias gariepinus* broodstock samples from Nigeria continued.

	High Aquaculture Area				Low Aquaculture Area						Wild		
MM	Farm 1 n=20	Farm2 n=20	Farm 3 n=20	Farm 4 n=20	Farm5 n=20	Farm 6 n=20	Farm 7 n=20	Farm 8 n=20	Farm 9 n=20	Farm 10 n=20	Wild n=20	F-val	P-val
PrPL	11.4 ± 0.32^{b}	11.6 ± 0.62^{b}	11.1 ± 1.75^{bc}	10.8 ± 0.17^{bc}	11.4 ± 0.42^{bc}	11 ± 0.54^{bc}	11.3 ± 0.31^{bc}	$\mathbf{13.6 \pm 1.58^{a}}$	$\mathbf{10.5 \pm 0.88^{c}}$	11.5 ± 0.46^{b}	10.9 ± 0.97^{bc}	16.93	0.00
PrPvL	22.8 ± 0.26^{bcd}	22.1 ± 1.67^{cd}	$\mathbf{21.5 \pm 2.97^{d}}$	$\mathbf{25.1 \pm 0.29^{a}}$	22.2 ± 0.71^{cd}	23.2 ± 1.09^{bc}	22.8 ± 0.64^{bcd}	22.8 ± 2.14^{bcd}	23 ± 0.98^{bc}	24.2 ± 0.84^{ab}	22.1 ± 1.56^{cd}	10.01	0.00
PrAl	28.3 ± 0.71^{bc}	27.8 ± 0.61^{cd}	29.5 ± 0.4^{ab}	$\mathbf{30.9 \pm 2.82^{a}}$	26.3 ± 1.45^{de}	28.1 ± 1.13^{bc}	27.8 ± 0.58^{cd}	25.7 ± 3.18^{e}	27.6 ± 1.35^{cd}	28.4 ± 0.30^{bc}	$\mathbf{25.5 \pm 0.66^{e}}$	22.29	0.00
PL	6.9 ± 0.15^{abc}	$\mathbf{7.1 \pm 0.77^{a}}$	6.9 ± 0.22^{abc}	6.6 ± 0.86^{bc}	$\mathbf{6.1 \pm 0.37^{d}}$	7.1 ± 0.27^{ab}	6.5 ± 0.13^{cd}	7.1 ± 0.29^{ab}	6.7 ± 0.63^{bc}	6.8 ± 0.27^{abc}	6.5 ± 0.32^{cd}	9.64	0.00
DL	31.2 ± 0.70^{c}	$\mathbf{28.9 \pm 1.82^{e}}$	30.6 ± 1.56^{cd}	34.3 ± 0.38^{a}	29.5 ± 1.93^{de}	$\mathbf{34.7 \pm 1.68^{a}}$	31.6 ± 1.40^{bc}	32.7 ± 1.20^{b}	29.2 ± 2.19^{de}	30.5 ± 0.93^{cd}	31.8 ± 1.30^{bc}	34.94	0.00
PvL	5.2 ± 0.28^{bc}	5.2 ± 0.50^{bc}	4.8 ± 0.16^{cd}	$\mathbf{5.8 \pm 0.15^{a}}$	5.5 ± 0.68^{ab}	4.7 ± 0.66^{d}	5.1 ± 0.55^{bcd}	$\mathbf{4.6 \pm 0.26^{d}}$	5.1 ± 0.48^{bcd}	5.4 ± 0.34^{ab}	5.3 ± 0.55^{bc}	11.71	0.00
AL	21.1 ± 0.62^{def}	21.5 ± 0.74^{cde}	21.7 ± 0.37^{cd}	$\mathbf{27 \pm 0.51^{a}}$	22.1 ± 0.78^{c}	23.5 ± 1.58^{b}	21.5 ± 0.59^{cde}	22.1 ± 0.38^{c}	23.1 ± 1.19^{b}	20.6 ± 0.95^{ef}	$\mathbf{20.4 \pm 1.56^{f}}$	75.68	0.00
CL	6.9 ± 0.14^{bcd}	6.7 ± 0.47^{cd}	7.2 ± 0.44^{abc}	$\mathbf{7.6 \pm 1.01^{a}}$	7.3 ± 0.70^{ab}	7 ± 0.704^{bc}	6.7 ± 0.26^{bcd}	7 ± 0.30^{bc}	$\mathbf{6.3 \pm 0.25^{d}}$	7 ± 0.59^{bc}	6.9 ± 0.70^{bc}	6.72	0.00

NB: Values with same superscripts across the rows are not significantly different at P>0.01. PrPL = pre-pectoral fin length; PrPvL = pre-pelvic fin length; PrAL = pre-anal fin length; PL = pectoral fin length; DL = Dorsal fin length; PvL = pelvic fin length; AL = Anal fin length; CL = caudal fin length.

Appendix III: Mean±standard deviation of mean (cm), F and P-values of morphometric measurement (MM) of *Clarias gariepinus* broodstock samples from Nigeria continued.

	High Aquaculture Area					Low Aquaculture Area				Wild		
MM	Farm 1 n=20	Farm2 n=20	Farm 3 n=20	Farm 4 n=20	Farm 5 n=20	Farm 6 n=20	Farm 7 n=20	Farm 8 n=20	Farm 9 n=20	Farm 10 n=20	Wild n=20	F-val	P-val
CD	4.3 ± 0.48^{bcd}	4.7 ± 0.17^{ab}	**4.9 ± 0.12^{a}**	4.3 ± 0.94^{bcd}	4.7 ± 0.44^{ab}	4.1 ± 0.48^{cde}	**3.8 ± 0.15^{e}**	4.7 ± 0.81^{ab}	4.6 ± 0.42^{ab}	4.4 ± 0.26^{abc}	3.9 ± 0.24^{de}	11.11	0.00
BD	9.9 ± 0.19^{abc}	9.2 ± 0.65^{cd}	9.5 ± 0.14^{bcd}	9.2 ± 1.66^{cd}	9.8 ± 0.51^{abcd}	9.5 ± 0.48^{bcd}	**10.4 ± 0.51^{a}**	10.2 ± 1.17^{ab}	10.1 ± 0.89^{ab}	9.9 ± 0.76^{abcd}	**9 ± 1.10^{d}**	5.80	0.00
GL	4.3 ± 0.43^{cd}	4.4 ± 0.18^{cd}	4.3 ± 0.17^{cd}	**5.3 ± 0.51^{a}**	4.1 ± 0.51^{cd}	4.7 ± 0.14^{b}	4.4 ± 0.14^{bc}	4.3 ± 0.49^{cd}	4.1 ± 0.14^{d}	4.1 ± 0.14^{d}	**3.7 ± 0.40^{e}**	29.25	0.00
SNL	3.1 ± 0.17^{cd}	3.3 ± 0.34^{bcd}	3.3 ± 0.29^{bcd}	3.5 ± 0.39^{bcd}	3.6 ± 0.42^{bc}	**4 ± 0.99^{a}**	3.4 ± 0.17^{bcd}	3.7 ± 0.49^{ab}	**3 ± 0.34^{d}**	3.4 ± 0.15^{bcd}	3.4 ± 0.36^{bcd}	7.84	0.00
DFBL	**2.4 ± 0.05^{c}**	2.6 ± 0.21^{bc}	2.7 ± 0.26^{bc}	**3.3 ± 1.30^{a}**	2.7 ± 0.34^{bc}	2.6 ± 0.08^{bc}	2.6 ± 0.07^{bc}	3.1 ± 0.57^{ab}	2.4 ± 0.45^{c}	2.6 ± 0.18^{bc}	2.6 ± 0.30^{c}	6.54	0.00
PFBL	2.8 ± 0.19^{ab}	2.5 ± 0.12^{b}	2.4 ± 0.29^{b}	3.0 ± 0.82^{a}	**1.9 ± 0.39^{c}**	**3 ± 0.12^{a}**	2.6 ± 0.25^{ab}	2.6 ± 0.54^{ab}	2.8 ± 0.29^{ab}	2.5 ± 0.25^{b}	2.8 ± 0.23^{ab}	12.16	0.00
PVBL	1.7 ± 0.07^{cde}	1.6 ± 0.12^{de}	1.8 ± 0.39^{cde}	2.0 ± 0.63^{c}	**2.3 ± 0.28^{a}**	2.3 ± 0.11^{ab}	1.6 ± 0.24^{de}	1.9 ± 0.7^{cd}	2 ± 0.25^{bc}	1.8 ± 0.15^{cd}	**1.5 ± 0.19^{e}**	14.21	0.00
AFBL	2.0 ± 0.22^{c}	1.9 ± 0.23^{cd}	2.3 ± 0.22^{abc}	**2.5 ± 0.09^{a}**	1.6 ± 0.31^{de}	2.3 ± 0.70^{abc}	2.2 ± 0.44^{abc}	2.3 ± 0.34^{abc}	2.5 ± 0.45^{ab}	2.1 ± 0.32^{bc}	**1.2 ± 0.43^{e}**	21.44	0.00

NB: Values with same superscripts across the rows are not significantly different at P<0.01. CD = caudal fin depth; BD = body depth; GL = gape length; SNL= Snout length; DFBL = dorsal fin base length; PFBL= Pectoral fin base length; PVBL= Pelvic fin base length; AFB = Anal fin base length.

Appendix IV: Mean±standard deviation of mean (cm), F and P-values of meristic counts (MC) of *Clarias gariepinus* broodstock samples from Nigeria.

	High Aquaculture Area (HAA)					Low Aquaculture Area					Wild		
MC	Farm 1	Farm2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9	Farm 10	wild	F-val	P-val
AFR	51.5 ± 4.88^{ab}	50.9 ± 3.57^{ab}	50.6 ± 3.62^{ab}	**54.2 ± 10.16^{a}**	49.9 ± 6.08^{ab}	51.2 ± 3.88^{ab}	50.7 ± 4.81^{ab}	53.2 ± 2.97^{a}	**48 ± 3.91^{b}**	50 ± 5.06^{ab}	53.1 ± 1.94^{ab}	2.43	0.009
DFR	65.8 ± 4.78^{bc}	65.2 ± 3.91^{bc}	62.1 ± 3.91^{cd}	66.5 ± 6.75^{abc}	**59.9 ± 7.67^{d}**	66.7 ± 3.38^{abc}	65.7 ± 3.96^{bc}	**71.5 ± 5.43^{a}**	63.3 ± 5.82^{bcd}	64.1 ± 5.28^{bcd}	68.5 ± 2.89^{ab}	7.49	0.000
PFR	8.7 ± 0.47^{a}	8.9 ± 0.83^{a}	**9.0 ± 0.73^{a}**	8.4 ± 0.51^{ab}	8.6 ± 0.5^{ab}	8.5 ± 0.51^{ab}	8.6 ± 0.51^{ab}	8.8 ± 0.37^{a}	8.7 ± 0.47^{a}	8.7 ± 0.47^{a}	**8.1 ± 0.32^{b}**	4.35	0.000
CFR	18.4 ± 0.60^{a}	18.4 ± 0.60^{a}	18.6 ± 0.51^{a}	18.4 ± 0.75^{a}	18.4 ± 0.51^{a}	18.1 ± 0.88^{a}	18.2 ± 0.62^{a}	18.5 ± 0.51^{a}	18.6 ± 0.49^{a}	**18.7 ± 0.47^{a}**	**17.4 ± 0.49^{b}**	7.79	0.000
PvFR	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{a}	1.05	0.403
BB	8.0 ± 0.00^{a}	8.0 ± 0.00^{a}	8.0 ± 0.00^{a}	8.0 ± 0.00^{a}	8.0 ± 0.00^{a}	8.0 ± 0.00^{a}	8.0 ± 0.00^{a}	8.0± 0.00^{a}	8.0 ± 0.00^{a}	8.0 ± 0.00^{a}	8.0 ± 0.00^{a}	3.06	0.001

AFR =anal fin ray; DFR = dorsal fin ray; PFR = pectoral fin ray; CFR = caudal fin ray; PvFR = pelvic fin ray; BB= Number of barbels
NB: Values with same superscripts across the rows are not significantly different at P<0.0