

EFFECT OF ALBINO GENE ON INTRASPECIFIC HYBRIDIZATION OF NORMAL AND ALBINO AFRICAN CATFISH (*Clarias gariepinus*)

ABSTRACT

The effect of albino gene on intraspecific hybridization of African Catfish and Albino *Clarias gariepinus* was carried out to determine their latency period, fecundity, fertility, hatchability, survival, growth performance, some morphometric and meristic characteristics and genetic characterization of parents and offspring of F1 generation. A total of four (4) mating combination experiments were conducted using eight (8) ripened, matured normal and albino *Clarias gariepinus* with average body weight of 1.64kg. The broodstock were purchased from Abdulfana fish farm Yola and transported in 50 litres jerrican to the Department of Fisheries and Aquaculture Adamawa State University Mubi. They were acclimatized for 10 days, vital feed were fed daily at 5% of their biomass. Artificial breeding was carried out using Ovaprim hormone at a dose of 0.50ml/kg and 0.25ml/kg for female and male respectively. After hand stripping of the female brooders, nine hundred and sixty (960) eggs were used for fertilization in each mating combination, 320 eggs replicated three times using Randomized Complete Design (RCD) to determine the percentage Fertility and Hatchability of the eggs. Two hundred and forty fry of two weeks old from each treatment were randomly selected and stocked in same culture system and fed with Coppens feed at 10% body weight, the experiment lasted for the period of 18 months for both F1 and F2 generations, where weight, length and survival/mortality rate, in each treatment and replications were recorded weekly. Water quality parameters were monitored weekly. Gene characterization (DNA extraction, Electrophoresis, Selection of Microsatellite Markers, Polymerase Chain Reaction (PCR) Amplifications and Electrophoresis for PCR, was carried out on the parent stock and all the offspring of mating combination in F1 generation. It was observed that female albino *Clarias gariepinus* had the highest latency period of 13.50 ± 0.22 hours at $27.50 \pm 0.20^\circ\text{C}$ and less fecundity of 4032 ± 0.12 , while the normal *Clarias gariepinus* had the least latency period of 12.00 ± 0.21 hours and high fecundity of 7020 ± 0.23 at similar temperature. Similarly crossed between $\text{NN}^\sigma \times \text{NN}^\omega$ had the highest fertility and hatchability of $44.79 \pm 0.32\%$ and $72.6 \pm 0.24\%$, while the least value of $27.92 \pm 0.31\%$ and $46.3 \pm 0.43\%$ were recorded in $\text{AA}^\sigma \times \text{NN}^\omega$. Also crossed between $\text{NN}^\sigma \times \text{AA}^\omega$ had the highest final mean weight gain of $912.90 \pm 0.00\text{g}$, followed by $\text{NN}^\sigma \times \text{NN}^\omega$ with $900.75 \pm 0.00\text{g}$, while $\text{NN}^\omega \times \text{AA}^\sigma$ and $\text{AA}^\sigma \times \text{AA}^\omega$ had the least value of $893.88 \pm 0.00\text{g}$ and $852.98 \pm 0.00\text{g}$ respectively. High survival rate of $41.25 \pm 0.00\%$ was recorded in crossed

between AA♂ x AA♀, while NN♂ x AA♀ had the least value of 12.50±0.00%. The Head width, eye diameter and occipital fontanelle of the F1 generation was higher than that of the parents. All the water quality parameters measured during the course of the research were within the normal range for tropical catfish cultured. The two loci of normal *Clarias gariepinus* (Cg01) and albino *Clarias gariepinus* (Cg02) showed amplification. The size of the two (2) microsatellite loci ranged from 0.92 to 1680 bp, with low estimates of null allele frequencies across all populations for the parent stock and 0.93 to 1.00 for F1 generation. The number of Allele observed from the genetic analysis of parent stock and F1 generation of Cg01 showed heterozygosity and the second allele on the second band Cg02 shows homozygosity. However, a homozygote excess was estimated in the parents of Cg02 and the result was 100% in both the parent and F1 generation of the normal and albino *Clarias gariepinus*. Normal male and female Albino *Clarias gariepinus* can be use by fish farmers to produced hybrid that have high survival rate and can grow faster. Further study should be carriedout to determined the causes of had skin, large head width, large eye diameter and wide occipital fontanelle of the F1 generation.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The demand for high quality protein especially from aquatic resources is rising, *Clarias gariepinus* is the most common and have received much attention because of its economic importance and high rate of success in rearing them (Adewolu *et al.* 2009). Albinism in catfish is the best known potentially valuable qualitative traits and inherited as a single homozygous recessive trait (Bondari, 1981). The term albino catfish can refer both to an albino specimen of any catfish species or to a particular species of catfish the Albino *Clarias gariepinus* Catfish (Westerman and Birge, 1978). Albino specimens of all catfish species including the Albino *Clarias* dramatically, increased agricultural production is clearly needed to meet this demand in the future, because capture fisheries is showing precipitous disclose due to over fishing habitat, destruction and increasing population and increased in capture fisheries are not anti-coopted under the current global condition (Dunham *et al.* 2001) the most current advance in aquatic production have been achieved through the application of genetic principles which includes selective breeding, hybridization, chromosome manipulation, sex reversal and gene transfer (Aliah and Taniguchi 1999). Framed sources are economically important food fish which have gained much prominence species of

Claria and *Heterobranchus* are widely cultured (Huisman and Richter, 1987). They are characterized by hardiness, resistance to diseases, high yield potentials and ability to grow on a wide range of natural and low cost artificial food and can withstand low oxygen and wide pH levels (Fagbenro and Sydenham, 1991). Genetic techniques have been applied to other animals to improve their quality but that of fish is not yet fully exploited. Failure to pay attention to the genetic aspect of fish husbandry may result to decline in productivity as inbreeding or other genetic consequences of mis-management may result in slower growth, decreased viability and disease susceptibility as well as decreased egg production (Bartly *et al.* 1990). Hybridization has been found to be a breeding programme that tries to find mating combination between different populations of fish which produce hybrid vigor (Tave, 1993). Hybridization has been used to increase growth rate, manipulate sex ratio, produce sterile animals, improve flesh quality, increase resistance and to tolerate environmental extremes as well improve a variety of other traits that make other aquatic animals production more profitable (Dunham *et al.* 2001). Intraspecific hybrids are produced when useful traits in parent fishes are transferred to the offspring, this is because for back cross hybrid produced from the fertile hybrid could not be easily differentiated from the pure parents (Aluko, 1996). The hybrid cross between *Heterobranchus* and *Clarias* species is receiving attention in Africa particularly Nigeria this hybrid has been reported to show that *Heterobranchus* species usually grow larger than the *Clarias* species, therefore shows better percentage survival (Madu

1995). This calls for the need to carry out more details investigation on hybridization among the *Clarias species* in order to assess the aquaculture potentials of their hybrid s in terms of growth performance and survival compared to the putative parents at the fry, fingerlings and adult stage (Onyia, 2010). *Clarias gariepinus* is an economically important fresh water species of the *Clariidae* family that contribute immensely to the annual fresh water fish production in Nigeria. They are also readily acceptable among Nigerians fish farmers and consumers, hence command high commercial value (Adebayo *et al.* 2001). They are commonly referred to as mud fishes or African catfish in various parts of Nigeria and are important source of animal's protein. Among the freshwater species for culture in Nigeria *gariepinus* is a natural occurring phenomena and that there has always been albino *Clarias gariepinus* even if only a few survived to become adults. An albino specimen of any of the larger catfish species can be a truly magnificent sight, such as large albino catfish (Groblan *et al.* 1992). The inheritance of albinism has been analyzed in many aquarium fish and several aquaculture species-Rainbow trout, channel catfish and Grass carp (Delgado *et al.*, 2009). In all the traits of the regular *clarias gariepinus* plate i and ii. They have the same well testing meat, and they grow up to the same size (Britton and Davie, 2006). However, the albino form of this catfish has some additional values. The Albino from of the catfish is an appreciated aquarim fish and can be sold in large quantities to aquarium stores (Galib *et al.*, 2012). Albino catfish are also preferred by some to stock fishing lakes with, since the albino catfish are

more easily spotted and allows the fishermen to see that there are fish in the lake even if they are unsuccessful in catching any (Clark, 2002). There is no doubt that commercial breeding of catfish in captivity has increased the amount of Albino catfish available both in the aquarium trade and in the wild as a result of different restocking efforts (Piorski, 2010). Albinism is passed genetically from parents to offspring (Dobosz *et al.* 1999). Each cell contains numerous pairs of genes, one from each parent. These genes transmit traits through generations. An albino offspring results from a specific combination of genes (Rothbard and Wohlfarth 1993). Albinos are not in frequent because the genes for that trait are recessive, while the genes for normal pigmentation are dominant (Rothbard and Wohlfarth, 1993). If both are present, normal pigmentation occurs. If only recessive genes occur, albinism may result. Only a small percentage of animals carry the recessive gene, so the chance of the pairing of recessive genes in an individual animal is slight. In natural environment, there are several reports of total or partial albinism in freshwater fishes and marine fishes (Piorski, 2010). Some cases of total or partial albinism in siluriformes have been reported, e.g., *Ictalurus punctatus* (Westerman and Birge, 1978), *Schizolecis guntheri* (Brito and Camaraschi, 2005). Intraspecific hybrids are produced when useful traits in parent fishes of the same genus are mated. Intergeneric hybrids on the other hand are generated when parents from two genera are crossed. All of the genetic information in an individual fish is contained in molecules of deoxyribonucleic acid. Molecules of DNA are composed of sub units called

nucleotide. Each nucleotide contains a compound called a nucleotide. The bases are four kinds of nucleotides in DNA because there are four different bases (adenine, guanine, thymine and cytosine). DNA molecules consist of a long ladder of paired nucleotides. The helix in the ladder gives the DNA molecule a double helix structure. Nucleotides form base pair in the double helix in a specific manner where thymine is found in one strand of the helix, only adenine will be found in the same position of the opposite strand. Similarly, where guanine is found in one of the chromosome strands, only cytosine will be found in the same position of the opposite strand. The two strands of the helix are said to be complementary because of the way nucleotides form base pairs (Westerman and Birge, 1978). Whenever a cell divides, the DNA must be replicated in order to provide each daughter cell with a complete set of genes. An advantage of complementary base pairing is evident during replication of the DNA molecule. During replication, the two strands of the DNA helix are separated by enzymes so that each strand is available to serve as a template for a new molecule. Individual nucleotides are affixed to each template. Two complete and identical DNA molecules resulted, the complementary pairing of bases ensures that the replication of DNA is essentially error free. Microsatellites represent another type of repetitive DNA (Westerman and Birge, 1978).

Microsatellites, also called Simple Sequence Repeats (SSRs), are composed of tandemly repeated 2-6-base units (motifs)., the allelic variability of microsatellites is based on the difference in the length of fragments caused by the different number of repeated units. The isolation of microsatellite loci from a genomic library for a new species or group of species is the first step for the investigation of microsatellite polymorphism (Calcagnotto *et al.* 2001). The sequence information for the flanking DNA is needed to allow the synthesis of specific PCR primers. Following primer design, products can be amplified and separated through capillary electrophoresis. Microsatellites are locus-specific and co dominant markers (Calcagnotto *et al.* 2001).

Microsatellites are also highly variable, and the number of alleles for one locus may be more than 30. Heterozygote for microsatellite loci has two bands on a gel which represent two alleles while homozygote has one band (one allele). Microsatellite alleles are designated by numbers which indicate the size of corresponding PCR product in base pairs (Taniguchi *et al.* 1999) the first studies on application of microsatellites as genetic markers in fish were published in the beginning of the 1990s. From this time, microsatellites were identified and analyzed in many important fisheries and aquaculture species (Sirol and Britto, 2006). Funkenstein *et al.* (1990) documented that very high variability, discrete character of inheritance, co dominance, and established standard techniques for their analysis, microsatellites are regarded now as the most popular and powerful type of DNA markers in fish genetics.

1.2 Statement for the Research Problem

In Nigeria and other developing country, there is little information on the effect of hybridization of Albino and Normal catfish *Clarias gariepiunus* the hybrids have been reported to show heteroses (Salami, *et al.* 1993, Aluko, 1999) but their suitability for aquaculture has not been thoroughly evaluated. There is also little information in literature on the growth and viability, hatchability, latency period, fecundity, fertility, fertilization, morphometric and meristic characters and colour ratio of the mating combination as well their gene characterization of the intraspecific hybridization of normal and albino *Clarias gariepinus*, The work of Onyia *et al.*

(2016) documented the growth and survival of Normal and Albino *Clarias gariepinus* that the normal *Clarias gariepinus* grow faster than the Albino *C. gariepinus*, hence there is need to assess the viability, latency period, fertility, fecundity, morphometric and meristic character, color and sex ratio as well their genetic characterization of the intraspecific hybrids of normal and Albino *Clarias gariepinus* in F1 and F2 which were lacking in their reports.

1.3 Justification for the Study

Production and culture of species of the catfish belonging to the *Clariidae* family is fast growing globally according to (Adewolu *et al.* 2008). This study will be useful in assessing viability, growth performance, latency period, fertility, fecundity, fertilization, colour and sex ratio of the various intraspecific mating combination of Albino and normal *Clarias* both in F1 and F2.

This study was initiated to provide detailed phenotypic appearance (morphometric and meristic character) description of the intraspecific combination of Albino and normal *Clarias gariepinus* in order to know the distinguish features for identification of their hybrids and their putative parent.

This study on intraspecific hybridization of *Clarias gariepinus* Albino and normal and their reciprocal hybrids is of great importance in ensuring a proper understanding of their gene characterization in both parent and F1 (Betiku and Omitogun, 2006) to create a good foundation for producing a genetically improved hybrid fish.

1.4 The Aims of the Study

The Aim of this study was to determine the effect of albino gene on intraspecific hybridization of normal and albino african catfish (*Clarias gariepinus*).

1.5 The Objectives of the Study

- i. to determine the latency period of the female parent stocks of normal pigmented, albino *Clarias gariepinus* and the female of hybrids in (F1) generation of their crosses.
- ii. to evaluate the fecundity, fertility, and hatchability of eggs of different mating combination in (F1 and F2) generation of *Clarias gariepinus*.
- iii. to determine the percentage survival of F1 and F2 generation of *Clarias gariepinus*.
- iv. to determine the growth performance and feed utilization of F1 and F2 generation of the hybrids.
- v. to determine the morphometric and meristic characters of the F1 and F2 generation of *Clarias gariepinus*.
- vi. to access the genetic characterization of the parent stocks and F1 generation

1.6 Research Hypotheses

- i. There is no significant differences ($P>0.05$) between the latency period and fecundity, of

the parent stock and F1 of normal and albino *C. gareipinus*.

- ii. There is no significant differences ($P>0.05$) between the percentage fertility, fertilization, hatchability and survival of normal and albino *Clarias gareipinus* in F1 and F2.
- iii. There was no significant differences among the percentage survival of the offspring in F1 and F2.
- iv. The growth performance and feed utilization of normal and albino *Clarias gareipinus* in F1 and F2 doesnot differ.
- iv. The morphometric, meristic and genetic characterization of the parent stock and their hybrid of normal and albino *Clarias gareipinus* doesnot differ.
- vi. The phenotypic and genotypic appearance of hybrid of normal and albino *Clarias gareipinus* do not differ.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy of *Clariid* Catfish

Catfishes of the genus *Clariidae* together with various tilapine species (*Cichlidae*) are at present the most important freshwater fishes used in fish culture in Africa. The taxonomy of both groups had been very confusing and only recently have detailed systematic revisions become available, enabling the correct identification of the species used; (Trewavas, 1983) for *Talipiine Cichild* fishes; (Teugels, 1996) for *Clarias gariiepinus*.

There is a need for proper identification and classification of the species for any successful genetic improvement production programme. Teugels (1996) carried out a systematic revision of the genus *Clarias* in Africa and treated five species as *C. anguillaris*, *C. senegalensis*, *C. gariepinus*, *C. lazera* and *C. mossambicus*, it was concluded that all the three are synonymous and that *C. gariepinus* and *C. anguillaris* are the only two mudfish species that belong to the “large species” and are therefore of high economic importance in Nigeria (Ezenwaji, 1985). Incidentally, these are the two species that are most common in the Nigerian natural waters (Ita *et al.* 1985; Sagua, 1978). Debouche *et al.* (1979); Teugels (1982); Benech *et al.* (1993) have published on the identification problems between *Clarias gariepinus* and the closely related and partially sympatric, among the *C. anguillaris* (Linnaeus, 1758).

2.2 African Mud Catfish (*Clarias gariepinus*)

Clarias gariepinus also known as African sharp-tooth catfish, is a typical air-breathing catfish with a scaleless, bony elongated body, long dorsal and anal fins as well as helmet like head. According to Picker and Griffiths (2011), *Clarias gariepinus* native range covers most African countries though with exception of Maghreb, Upper and Lower Guinea as well as Cape provinces of South Africa. However, though presumably, it is believed to be most widely distributed fish species in Africa (Skelton, 1993). *Clarias gariepinus* is the second most important freshwater fish, after

tilapia, in Africa. This is with exception of Nigeria, where *C. gariepinus* production exceeds that of tilapia, accounting for 70-80% of the total freshwater fish production (Woynoravitch, 1980).

This notwithstanding, the industry in Africa is not well established due to several limiting factors, which could include, development of catfish production in relation to environmental protection, genetic diversity and the future sustainability of the industry (Danish *et al.* 2012). Despite these surmountable problems facing fish industry, Danish *et al.* (2012) observed that *C. gariepinus* cultivation has the potential towards livelihood development, employment generation as well as ensuring nutritional enrichment in the regular diet among the populace.

2.3 Morphometric and Meristic Characters in (*Clariidae family*)

Morphometric and meristic characters in fish species have been commonly used to identify fish stocks, (Turan *et al.* 1998) and as such, these characters remain the simplest and most direct way among methods of species identification. According to Mamuris *et al.* (1998), Bronte *et al.* (1999) and Hockaday *et al.* (2000), analysis of phenotypic differences in morphometric characters or meristic counts is the method most commonly used to delineate stocks of fish. According to Avsar (1994) this is often being used in discrimination and classification studies by statistical techniques but despite the advent of techniques which directly considers the biochemical or molecular genetic

variation, these conventional methods still play vital functions in stock identification even to date (Swain and Foote, 1999).

The differences in the morphometric and meristic characters of a species between sexes of a particular environment or regions may result from differences in genotypes, or environmental factors operating on one genotype, or both of these acting together (Parish and Sharman, 1958).

The morphometric characteristics of this species are significant for easy assessment of the pure strain stocks because both the interspecific, intraspecific and intergeneric hybrids of the *Clarias* are also cultured with the pure strains of the fish. Also, the more closely related species like *C. anguillaris* or *C. macrocephalus* even *Heterobranchus* species are cultured with *Clarias gariepinus* on a large scale, thus the morphometric features study may be used for proper identification of the *C. gariepinus* pure strains and the hybrids from other genus or similar species (Food and Agricultural Organisation, 1996). It is also used in comparing fish of the same species just like it has been used for other fishes from different populations. The comparative study of the morphological and meristic traits of *Clarias gariepinus* collected for this study from two different pigmented fishes will be able to reveal if there is similarities or differences or possibly to determine the source(s) of their variation (Hockaday *et al.* 2000).

2.4 Morphometric and Meristic Characters of *Clarias gariepinus*

The literature on the morphometric characteristics of catfish, *C. gariepinus* is very scarce. The morphometric characteristics of this species are significant for easy assessment of the pure strain stocks because both the interspecific, intraspecific and intergeneric hybrids of the *Clarias* are also cultured with the pure strains of the fish. Also, the more closely related species like *C. anguillaris* or *C. macrocephalus* even *Heterobranchus* species are cultured with *C. gariepinus* on a large scale, thus the morphometric features study may be used for proper identification of the *C. gariepinus* pure strains and the hybrids from other genus or similar species Food and Agriculture Organization, (FAO, 1996). It is also used in comparing fish of the same species just like it has been used for other fishes from different populations. The comparative study of the morphological and meristic traits of *C. gariepinus* collected for this study from two different pigmented fishes will be able to reveal if there is similarities or differences or possibly to determine the source(s) of their variation.

2.5 Genetic Characterization in *Clarias gariepinus*

Conservation of genetic resources entails several activities, many of which may greatly benefit from knowledge generated through applying molecular marker technologies (Roll-Hansen, 1980). This is the case for activities related to the acquisition of germplasm (locating and describing the diversity), its conservation (using effective procedures) and evaluation for useful traits (Langholz

et al. 1987). In all, the availability of sound genetic information ensures that decisions made on conservation will be better informed and will result in improved germplasm management of the activities related to genetic resources, those involving germplasm evaluation and the addition of value to genetic resources are particularly important as they help identify genes and traits, and thus provide the foundation on which to enhance use of collections (Agbebi and Adebambo, 2013). ‘Characterization’ is the description of a character or quality of an individual (Allendorf and Waples, 1988). The word ‘characterize’ is also a synonym of ‘distinguish’, that is, to mark as separate or different, or to separate into kinds, classes or categories. Thus, characterization of genetic resources refers to the process by which accessions are identified or differentiated. This identification may, in broad terms, refer to any difference in the appearance or make-up of an accession (Guerra, 2000). In the agreed terminology of gene banks and germplasm management, the term ‘characterization’ stands for the description of characters that are usually highly heritable, easily seen by the eye and equally expressed in all environments (Avsar, 1994). In genetic terms, characterization refers to the detection of variation as a result of differences in either DNA sequences of specific genes or modifying factors (Nachtomy *et al.* 2007).

2.6 Molecular Genetics and Genomic Character in Fish

Molecular genetics is an emerging field in fish breeding programs. It is the study of genetic material (genotypes) to help determine if fish possess certain traits of interest (phenotypes) (Nachomy *et al.* 2007). One such method of genetic testing that will soon become reality for the aqua culturist is DNA marker-assisted selection. When a certain trait of interest is studied, and a genetic marker found for this trait, a DNA test can determine which fish in the population will be the best to use in a breeding program (Taniguchi *et al.* 1999). Some agricultural programs such as beef and poultry have implemented these technologies in their selection and breeding programs already. As our knowledge of the genetics of aquaculture species increases, genetic testing will become a reality for this industry (Langholz *et al.* 1987).

Genome sequences for many important aquaculture species should be known in the very near future because of advances in sequencing technologies, but the complete DNA sequence of most aquaculture species is presently unknown (Denton, 1973). Genomic programs may still be useful for any breeding program. Maps of useful traits (their position along the chromosomes) will be valuable in the integration of genomic data with traditional selection programs. Long-term goals of a genomics program would be to identify sets of genes and be able to map multiple production traits to their chromosomes to assist in selection (Hallerman *et al.* 2003).

Albino individuals typically experience much higher levels of selection in the natural environment than normal individuals and as a consequence albino in the wild are usually rare. Pigment cells of vertebrates are derived from the dorsal neural crest during embryonic development. So far, at least seven types of pigment cells have been reported, including, black or dark brown, yellow, red, reflective silver, blue, or gold, white, blue and violet (Lapedriza *et al.* 2014). Only bony fishes possess all seven types of pigment cells. These cells are concerned because of their medical importance since a number of human diseases result from abnormal melanocyte development, such as piebaldism, vitiligo, albinism, and abnormal growth and dispersal of melanoma. And the studies of colour pattern formation, neural crest development, and the cell biology and biochemistry of melanin formation and distribution in animals are also performed. (Nordlund and Gullstrom, 2006). Yellow catfish, a teleost belonging to the family Bagridae, is one of the most important economic freshwater species in China due to its excellent meat quality.

Due to its biological and medical significance, the molecular mechanism of melanin biosynthesis has been extensively studied and the conserved melanin formation pathway has been elucidated in vertebrates (Liu *et al.* 2012). Melanin is produced in the melanosome of melanocytes, which originate from melanoblast precursors. These precursor cells were developed from the dorsal neural tube during embryonic development, and migrate to their targeted destinations along

defined pathways where they mature into functional melanocytes (Spritz and Hearing, 2013). These processes are strictly controlled by many genes, among which tyrosinase is the most important melanogenesis-related enzyme involved in the tyrosine metabolism pathway. Tyrosinase can catalyse the conversion of L-tyrosine to L-DOPA and regulate both the speed and specificity of melanogenesis (Hofreiter and Schöneberg, 2010). In addition, tyrosinase-related protein 1 and dopachrome tautomerase are also important enzymes in melanin synthesis (Braasch *et al.* 2009) and mainly regulate the eumelanin pathway. Recently, (encoding solute carrier family 7 member 11) gene has been reported to be a critical genetic regulator for pheomelanin synthesis in hair and melanocytes (Xu *et al.* 2014).

2.7 Protein Electrophoresis in Fish

The need for species identification is very important most especially in the developing countries where diverse but unidentified species are found (Spritz and Hearing, 2013). This enables the genetic materials or biological potentials of individual species to be fully understood and used in future breeding program. Electrophoresis is useful technique for studying the genetic composition of individuals and populations at the level of genes (Xu *et al.* 2014). Proteins usually disperse in solution as particles of colloidal size which possess electric charges, when passed under the influence of an electric field. The charged particles always migrate either to the cathode or anode depending on the net charge (Gaal *et al.* 1980). These differences in mobility of protein

particles provide the basis for differentiating fish species. Gordon and Marie (1987) defined electrophoresis as a separation technique closely associated with chromatography, that operates on a basis that focuses on the mobility of the charged molecules under the influence of an electric field towards the electrodes and the migration of molecules of different sizes or charges at different velocities.

2.8 An Inherited Trait

Albinism is passed genetically from parents to offspring. Each cell contains numerous pairs of genes, one from each parent. These genes transmit traits through generations (Cavaco *et al.* 2009).

An albino offspring results from a specific combination of genes. Albinos are infrequent because the genes for that trait are recessive, while the genes for normal pigmentation are dominant (Agbebi and Adebambo, 2013). If both are present, normal pigmentation occurs. If only recessive genes occur, albinism may result. Only a small percentage of animals carry the recessive gene, so the chance of the pairing of recessive genes in an individual animal is slight. In humans, for example, about one in 70 people carry a recessive gene for albinism, and about one in 20,000 humans are albinos (Ekpeme *et al.* 2015). At least 300 species of animals in North America have albino individuals (Pioski *et al.* 2010), In Missouri, people have photographed or witnessed albinism in

turtles, catfish, salamanders, deer, frogs, snakes, bluebirds and raccoons (Agbebi and Adebambo, 2013).

The degree of albinism varies among animal groups. Mammals estimate that true albinos occur in about one in 10,000 births (Sazima and Pombal, 1986). Normal- or random-breeding usually decreases the chance for albino offspring. Inbreeding among small isolated populations, or among closely related individuals, can increase the chances for albinism. Even among humans, albinism rates vary with geographic location (Britton and Davies, 2006). Animals in some areas have extremely high rates of albinism. The number of these partial albinos remains high because people living there feed and pamper their white squirrels and have passed ordinances to protect them from hunters and motorists (Kaplan, 2008).

2.9 Perils of Albinism

Lacking protective coloration, albino animals are more likely to be seen by both predators and prey. It's easy, for example, to spot Marionville's albino squirrels against the dark trunks of the trees they climb. According to Marceniuk (2005) it seems logical that albinos would have a survival disadvantage, some studies suggest that albino animals may not be as conspicuous to other predators as they are to us. Predators such as hawks, for example, may rely on a search image for prey that primarily involves shape and movement (Edwards, 1990). The colour of the prey may

make little difference, as long as the prey looks and acts like a food item. A lack of pigmentation can, however, affect the vision of albino animals, making it hard for them to find food and avoid danger (Edwards, 1990).

2.10 Albinism in Fish

Albinism is the lack of body and eye pigmentation due to absence of melanin in chromatophores, and has been observed in many species (Bridges and Von Limbach, 1999). It seems to be normally inherited as a simple autosomal recessive character (Tave, 1993). The distinctive appearance of albinos has made them of interest for commercial aquaculture (Bondari, 1984), aquarium breeding, and genetic studies as well (Thorgaard *et al.* 1995). There seems to be a disagreement among the scientist on the pleiotropic effect of the colour genes controlling the albino phenotype. According to some authors Bridges and von Limbach, (1999) and Bondari, (1984) albinism in commercial aquaculture species does not appear to have a significant pleiotropic effect on the mutant gene. However, there seems to be the possibility of some kind of pleiotropic effects. Tave (1993) cited that Wohlfarth and Moav, (1970) found that blue and gold common carp, which are similar in the inheritance to albino trout, have lowered growth rates as a pleiotropic effect. Bondari (1984) reported that normally pigmented catfish are superior to albino fish in growth when they were reared in tanks, ponds, and cages, and also suggested that the albinos seem somewhat more

lethargic, but were not substantially different from normally pigmented cousins in qualities such as fecundity, survival, or growth rate.

Dobosz *et al.* (1999) found that the genes controlling the palomino and albino phenotype in the spring spawning rainbow trout and catfish strain have strong detrimental pleiotropic effects on growth and vitality, the study confirmed the growth rates and daily feed consumption, condition factor, and consumer preference of the albino along with normally pigmented catfish which were cultured in tanks as all normal, all albino, and mixed or duo-culture groups were compared. In natural environment, there are several reports of total or partial albinism in freshwater fish (Westerman and Birge, 1978; Sazima and Pombal-Jr 1986). Ueda *et al.* (2007), Jeffery (2009) and marine fishes Béarez (2002); Simon *et al.* (2009); Piorski (2010) reported some cases of total or partial albinism in Siluriformes, e.g., *Ictalurus punctatus* (Westerman and Birge 1978), *Trichomycterus itacarambiensis* (Trajano, 1997), *Rhamdella minuta* (Sazima and Pombal-Jr. 1986) *Schizolecis guntheri* (Brito and Caramaschi 2005), *Ameiurus catus* (Britton and Davies 2006) and *Phreatobius cisternarum* (Shibatta *et al.* 2007). Frequently captured albino fish are only reported in aquarium magazines and local newspapers (Sazima and Pombal-Jr. 1986). All the species inhabit coastal zones of South America, *G. barbatus* and *G. planifrons* occupy coastal and

estuarine areas and are occasionally found in lowland rivers covering the southernmost portion of South America (Marceniuk and Menezes, 2007).

Adult fishes of the Ariidae family migrate from the ocean to the estuaries to reproduce. Juveniles may stay in the estuary nursery areas for several years, before they return to the ocean (Reis, 1986a; Reis, 1986b). All the species are intensively exploited as fishery resources due to their high commercial value and large size. *G. barbatus* may reach 1,200 mm of standard length and *G. planifrons* up to 711 mm (Marceniuk 2005; Milani and Fontoura, 2007). This study describes the first record of partial albinism in three specimens of marine catfish of *Genidens* genus in an estuary in southern Brazil. Albinism as the lack of body and eye pigmentation due to absence of melanin in chromatophores, has been observed in many species (Bridges and Von Limbach, 1999). It seems to be normally inherited as a simple autosomal recessive character (Bridges and Tave, 1993). The distinctive appearance of albinos has made them of interest for commercial aquaculture (Bondari, 1984), aquarium breeding, and genetic studies as well (Thorgaard *et al.* 1995).

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common carp, which are similar in the inheritance to albino trout, have lowered growth rates as a pleiotropic effect.

2.11 Genotype and phenotype

The distinction between phenotype and genotype is fundamental to the understanding of heredity and development of organisms. The genotype of an organism is the class to which that organism belongs as determined by the description of the actual physical material made up of DNA that was passed to the organism by its parents at the organism's conception (Nachtomý *et al.* 2007). For sexually reproducing organisms that physical material consists of the DNA contributed to the fertilized egg by the sperm and egg of its two parents. For asexually reproducing organisms, for example bacteria, the inherited material is a direct copy of the DNA of its parent. The *phenotype* of an organism is the class to which that organism belongs as determined by the description of the physical and behavioural characteristics of the organism, for example its size and shape, its metabolic activities and its pattern of movement.

Genotype refers to the genetic traits in an organism. It has to do with the genetic coding of an organism, Such coding is inheritable (Allandoff and phelp, 1988). The genotype is the genetic load that is copied every time a cell divides, and therefore is inherited down to the next generation.

Phenotype referred to observable, physical manifestations of an organism. The phenotype includes

physical characteristics, behaviors corresponding to such species, structures, organs, behaviors, reflexes, etc. Phenotype are things that can be seen with your eyes. (ex. colours, growth) The genotype is the genetic programming that provides the phenotype (Roll-Hansen, 1980).

Phenotypes can fall under the category of qualitative traits, or traits that can be simply described as one or the other (Bondari, 1984). A trait is qualitative if it can be sorted into one of at least two categories. These traits can't be measured numerically as is the case with quantitative traits. An example of a qualitative trait is fish pigmentation; the fish is either normally pigmented or albino. Qualitative traits are often the simplest to characterize because they are likely to be controlled by only one or a few genes, unlike quantitative traits. Chevassus and Dorson, (1990) Since an aquaculture species can be categorized by these traits, the population can be described by the ratios of its members with these traits the development of improved animals through selective breeding methods and quantitative genetic analyses relies heavily on statistical variables and procedures, knowledge and understanding of some basic concepts are required. The following points are not comprehensive, but provide an understanding of the selective breeding process. As stated in the above paragraph, quantitative traits or important catfish production traits such as weight, length, and fecundity are recorded as measurements on individuals (Bookstein, 1991). These measurements are referred to as the phenotype, which provides the actual physical record of the genes controlling the trait. When a large number of phenotypic measurements are recorded on

individual fish, a number of statistical variables such as the mean, variance, standard deviation can be calculated for the trait. Quantitative genetics and selective breeding can be used to determine which animals have the best performance for fastest growth) and determine the appropriate way to select for these animals in a breeding Programme (Clark, 2002).

The mean phenotype and variants are two of the most important variables quantitative geneticists first evaluate. For example, most catfish culturists know that fish sizes at harvest will vary greatly even when juveniles or fingerlings are stocked into ponds at the same size. Some fish will appear to have not grown at all, while others will have grown extremely rapidly (Braasch *et al.* 2009).

The growth of individual fish is controlled by the combination and interaction of the genes and the environment. Some fish grow fast because they have a good genetic basis; some grow fast because they have a favorable environment, perhaps providing success to more food, an environmental effect not related to genetics (Britton *et al.* 2006). In reality, the genetic control of traits like growth, reproduction, and disease resistance is extremely complicated (Kaplan, 2008).

Implementation of a breeding program for catfish genetic improvement therefore requires measurement of many economically important traits and understanding the genetic and environmental control over those traits (Chevassus and Dorson, 1990). After recording and analyzing the data on individual fishing a breeding program, specific fish are then selected with the best genetic merit to improve traits from one generation to the next. The method used to select

the best fish in the breeding Programme is also based on information obtained from the statistical analysis and determines whether commonly used breeding methods such as individual or family selection, hybrid crossbreeding, or a combination are used to genetically improve the line (Mia *et al.* 2005).

2.12 Phenotypic ratio

A ratio that shows the varied outcomes that results from a genetic cross for example, yellow flowers, round seeds, brown hair and green eyes (Tave, 1999). The genetic makeup of an organism is called genotype and the external appearance or expression of the genetic makeup is called phenotype (Colour, height, and shape.). The ratio indicates the number of heterozygote and homozygote with reference to the genotypic ratio and to the number of phenotypes expressed as phenotypic ratio. The concept was given by Sir Gregor Johann Mendel, Father of Genetics who worked on pea plant with reference to 7 different characters. The result obtained for a cross between single characters is called monohybrid cross and the ratio is referred to as monohybrid ratio which is 1:2:1 for genotypic ratio and 3:1 for phenotypic ratio. In the dihybrid cross the phenotypic ratio is 9:3:3:1 (Nachatomy *et al.* 2007).

The number of fish with each trait are simply added up and described, such as 3:1, 1:1, and so on. For example, a population of 20 fish has either a fan tail or a round tail, a qualitative trait. If 15

fish have fan tails and five fish have round tails, the ratio of fan tails to round tails is 3:1. Described another way, one-quarter of the fish population has round tails and three-quarters of the population has fan tails (Tave, 1993). Qualitative phenotypes are the same in different environments. A number of factors influence these ratios, including the number of genes needed to produce the phenotype and gene action. The genetic mechanisms described here are typically referred to as classical genetics or Mendelian genetics. Gene action can be characterized as single or multiple genes producing a qualitative phenotype (Kaplan, 2008). Classic qualitative traits are dominant or recessive. If a single allele is expressed over the other at the same locus, then the mode of action is termed dominance. Complete dominance mode of action describes the expression of alleles at the same locus where one copy (the dominant allele) masks the effect of the other copy (the recessive allele) (Nachatomy *et al.* 2007).

The phenotype expressed is termed the dominant phenotype, and the other is the recessive phenotype. When dominant alleles occur, there are three genotypes possible, while only two phenotypes can be produced. A classic example of complete dominance of a single locus is the gene for albinism in the channel catfish (Chevassus and Dorson, 1990).

The dominant genotype that produces normally pigmented catfish can be labeled as AA or Aa, where the capital (A) is the dominant allele and the lower case (a) is the recessive allele. Both AA and Aa fish produce normal pigmentation. Only fish with the complete recessive pigment gene

(aa) will be albino (Liu *et al.* 1992). Hence, with dominance there are three combinations of genotypes (AA, Aa, aa), while only two phenotypes are produced—normal (AA or Aa) or albino (aa) colouration. Fish that are homozygous dominant (AA) will obviously be normally pigmented, but so will fish with one dominant (A) and one recessive (a) allele (heterozygous dominant, Aa). With complete dominance, only parents that carry recessive alleles (either Aa or aa) can produce offspring with a recessive trait (Liu *et al.* 2012).

2.13 Reproductive biology of *Clariid* catfishes

In the wild, *clariid* catfishes breed during the rainy season (Elliot, 1976) and (Ezenwa *et al.* 1985). Sexual dimorphism exists between the male and female. As illustrated by De Kimpe and Micha, (1974), the male has an elongated conical urogenital papilla while in the female, the vent is more rounded and with a longitudinal cleft/groove. At maturity, a gravid female showed a deeper and more rounded soft abdomen, a reddish vent, prominence of blood vessels in the abdomen and the appearance of a few eggs upon slight pressure on the abdomen (Carreon, *et al.* 1973; Hogendoorn, 1979). Ripe males were characterized by a slightly vascularized genital papilla. Unlike the female, the males did not respond to stripping or the ejection of milt on slight manual pressure on the abdomen. Hogendoorn (1979) described the testes as kidney shaped and with lateral lobes; thus

accumulating the milt along the convex edge in the lobes and thereby impeding stripping or ejection of milt on manual pressure around the abdomen.

The fecundity of the clariid catfish is very high (Micha, 1989) found 3,000 to 328,000 eggs per female *C. anguillaris* while 4,020 to 19,030 were obtained in *C. gariepinus* with a mean of 10,528 eggs (Ufodike *et al.* 2003). Similar high fecundity was reported for *H. longifilis* (Legendre *et al.* 1992), *H. bidorsalis* (Fagbenro *et al.* 1991).

A study on the reproductive biology by Legendre *et al.* (1992) indicated that female *C. gariepinus* matures earlier (5-7 months) than female *H. longifilis* (12-24 months). In the reciprocal hybrids, female first maturity was attained at 20-21 months but they further observed that both reciprocal hybrids and parental species displayed an equilibrated sex ratio. Legendre *et al.* (1992) further recorded that gonad somatic index and fecundity in female hybrids were considerably lower than those found in the parental species. However, in hybrid males, gonadosomatic index was higher than those found in pugnacious sutshi (Tarnchalanukit, 1986) and *C. batrachus* x *Hereropheustes fossilis* (Mukhopadathy and Dehadrai, 1987). while studying production and gonad development of *H. longifilis* in tropical outdoor ponds observed that the genital papilla become morphologically distinguishable between the sexes in 8 months for all groups studied but at 9 ½ months, the male from all groups had attained sexual maturity, while the females were still sexually immature.

2.14 Latency for African catfish (*Clarias gariepinus*).

Fisheries have been recognized as one of the fastest growing sectors in the world. Fish is the most heavily traded food commodity in the market; with the continuous declining of natural fish production, it is crucial to improve fish production from aquaculture as it is one sector that can significantly contribute to World Fish Production (Gupta and Acosta, 2001). The production of marketable fish begins with the stocking of fry or juvenile into a rearing environment. These fish can come from wild capture, however the fish cannot be guaranteed that adequate numbers can be captured and stocked in the time corresponding to optimum production conditions; the fish farmer then naturally turns to other means of obtaining his stock which is invariably an artificial method (Oyelese, 2006).

African catfish was one of the most suitable species in aquaculture it has been considered to hold a great promise for fish farming; the African catfish having a high growth rate, resistant to handling and stress, being very well appreciated and having a high market value. One key constraint to its culture is the limited availability of quality fingerlings as seed material (Sahoo *et al.* 2007). Induced breeding may be a dependable alternative for obtaining high quality seed material. The species of induced breeding of *Clarias gariepinus* depends largely on the Latency period (Hogendoorn and Vismans, 1980; Zonneveld *et al.* 1988). The ripening of the ovary after injection

depends on the type of hormone used to introduce the female fish (Crandell *et al.* 1995). For example, a fish injected with Hormone Chorion Gonadotrophine (HCG) will be ready to be stripped after 14-17 hours of injection (Sahoo *et al.* 2007) whereas it is not so for other types of hormone. The amount of hormone used to inject fish is also important. The higher the dosage, the faster the time of stripping. The fish breeder must properly monitor the exact latency period of this specie to avoid over-ripeness and under-ripeness of their eggs in order to achieve maximum fertilization, hatchability and survival of the hatched ones (Hogendoorn and Vismans, 1980; Zonneveld *et al.* 1988). The effect of latency period on the spawning performance of the females have not been carefully studied, hence the study aims at achieving the best performance (fertilization, hatchability and survival), the viability of fresh and/or delayed gonad products in relation to the latency period of the fish species (Crandell, 1999).

2.15 Fecundity in Catfish

Knowledge of the fecundity of fishes is important for the comprehension of their life history (King, 1997). Fecundity assessments have been useful in racial distinction, progeny survival studies, stock evaluation and aquaculture-based induced spawning and egg incubation (Bagenal, 1978, Marcus, 1982; Coates, 1988). Colour, shape and size of eggs are also important parameters used in reproduction studies to characterize fish species and can also be used to predict the spawning

frequency of fish (Wootton, 1990). Gonadosomatic index (GSI) is one of the parameters used in reproduction studies of fish. The use of GSI to detect hydrated ovaries and therefore detect reproductive period from increase in weight has been established by (Hunter and Macewicz, 2001).

2.16 Fecundity in African Catfish (*Clarias gariepinus*)

The African catfish *Clarias gariepinus* which belongs to the *Clariidae* family continues to remain the most popular fish species in the Nigerian aquaculture. This could be due to several favorable physicals, biological and socio-economic characteristics exhibited by this species. Comparative studies have been carried out on Aqua feed with other imported feed such as Coppens with respect to growth performance of *C. gariepinus*, egg and sperm quality of *C. gariepinus* fecundity of *C. gariepinus* in earthen pond, gonad gross morphology and gonadosomatic index of *C. gariepinus* (Eyo, 2015). Meanwhile, no study has been reported on the fecundity of *C. gariepinus* fed with Aqua feed and Coppens feed in concrete tanks. Therefore, this study was carried out to determine the Latency Period, Fecundity, Fertility, Hatchability of *C. gariepinus* and their morphometric indices such as total length (TL) and total weight. high quality high market value, (Eyo, 2015). However, the growth rate of fisheries and aquaculture sector in Nigeria has been slow because of major challenges such as the availability of a cheap but high quality feed. This has led to the search for alternative and cheaper fish feed that will supply all the nutrients required by fish for optimal

growth (FAO, 2014). Fecundity is an index which measures the number of eggs carried by a gravid female fish or shrimp (Eyo *et al.* 2013). During selection of species for aquaculture, fecundity is one of the important characteristics considered by scientists (Shinkafi, and Ipinjolu, 2012). Fecundity is also used to evaluate the performance of aquaculture feed especially when a new feed is benchmarked. Aquafeed is known to influence fecundity and gonadal development in both shell and fin fishes (Ekanem, 2010; Ekanem *et al.* 2012; Ekanem *et al.* 2013).

2.17 Fertilization

External fertilization in an aquatic environment protects the eggs from drying out (Bondari, 1984). Broadcast spawning can result in a greater mixture of the genes within a group, leading to higher genetic diversity and a greater chance of species survival in a hostile environment. For sessile aquatic organisms such as sponges, broadcast spawning is the only mechanism for fertilization and colonization of new environments. The presence of the fertilized eggs and developing young in the water provides opportunities for predation, resulting in a loss of offspring. Therefore, millions of eggs must be produced by individuals. The offspring produced through this method must mature rapidly. The survival rate of eggs produced through broadcast spawning is low (Shibatta, 2007).

2.18 Hormone Induced Spawning and Artificial Fertilization in Fishes

Self-propagation in the wild or semi natural environment, as well as hormone induced spawning in ponds did not prove to be a reliable method for adequate fingerling production (Micah, 1975). Sometimes, river spawners like the *Clariid* catfishes may fail to spawn in confined waters because the gonads will develop up to a certain stage and remain dormant until re-absorption sets in. hypophysation, the injection of hormone extracts is the most popular technique for the induced spawning of *Clariid* catfishes (Micah, 1975).

High and constant water temperature (25⁰c) appears to enhance ovarian activity (Richter *et al.* 1987), (Huisman and Richter, 1987), whereas temperature greater than 30⁰c was shown to disturb fish reproduction (Huisman and Richter, 1987). Several hormones have been used in induced spawning of the *Clariid* Catfishes, all with varying level of success and efficacy. Adigun (2005) compared the use of carp pituitary extracts and deoxycorticosterone acetate (DOCA) and human gonadotropin in induced spawning of *Clariid lazera*. Their results showed that DOCA was more potent than carp pituitary extract though the difference was not significant. The effectiveness of ovaprim (domperidone and luteinizing hormone) in inducing ovulation and spawning of *C. gariepinus* was studied in females weighing between 280-600g and males weighing 670-1800g (Ugwumba and Ugwumba, 1998). Ayinla *et al.* (1989) and Nwadukwe (1995) carried out hormonal induction in *H. longifilis* using various doses of acetone-dried powered carp pituitary extracts and the best result was obtained using 6gm/kg of female fish body weight. Tan-Fermin *et*

al. (1997) studied the primozide-induced spawning of Asian catfish, *C. macracephalus* (Gunther) at different times during an annual reproductive cycle. Not all the natural/biological hormones or their synthetic analogue used in these studies gave promising results. Nwoko (1991) studied the effects of intramuscular administration of a potent non-steroid estrogen antagonist "Tamoxifen" on gravid *Clarias gariepinus* and the result indicated that Tamoxifen might not be effective in inducing ovulation and spermiation in the species. Nwoko (1991) in his study on the comparative efficacy of human chorionic gonadotropin (HCG), toad and *Clarias* pituitary homogenate on spawning in *Clarias lazera* and *Clarias anguillaris*, shows that HCG failed to induce spawning in *C. lazera*, although effective in *C. anguillaris* while the toad pituitary was not successful in both *Clarias* species, even at a high dosage level. However according to Fagbenro *et al.* (1991) and Salami *et al.* (1992) using pituitary extracts from non-piscine sources such as common toad (*Bufo regularis*), the African bullfrog (*Rana adspersa*) and domestic chicken (*Gallus domesticus*) could induce ovulation and spawning in catfish, *C. isheriensis*. Salami *et al.* (1992) also showed that HCG was not as effective as the frog pituitary extract in inducing ovulation in *C. gariepinus*. (Woynarovich and Horvath, 1980) extensively reviewed on the artificial fertilization, incubation and hatching in fishes.

Fertilization period is usually limited. There is hydration, because the eggs start to swell immediately it is contact with water which results in the closure of the micro lye.

Various factors affect the viability and hatching success of fish eggs. According to Kuo and Shoa (1974), insufficient development of eggs before hypophysation is an important cause of poor hatching results. Hypophysation is normally undertaken when gonadal development is already complete. Latency period; the time between hypophysation and ovulation/stripping is another important factor affecting hatching of eggs. Once ovulation or final ripening has taken place, it cannot be reversed and the eggs must be spawned or stripped failing which they over ripe and can no longer be fertilized. The knowledge of the latency period for any breeder is thus very important so that ovulation, spawning or stripping and fertilization could be adequately programmed and timed. In *H. longifilis*, the latency period is fifteen hours (Olufeagba,1998) while that of *C. anguillaris* is twelve hours (Madu *et al.* 1992).

The effect of varying latency period on the quantity and quality of ova after CG-induced ovulation in *H longifilis* has also been studied by (Legendre and Oteme, 1995). They observed that in individual female, ovulation of post vitellogenesis oocytes is not synchronous and takes 3 to 4 hours to be completed, between 7-8hrs and 11hrs after HCG injection at 30⁰c (Nunez – Rodriguez *et al.* 1995) made a comparative study of vitellogenesis of two African species, *Chrysichys nitrodigitatus* and *H. longifilis* and recorded that if maturation is not artificially stimulated, the females of *H. longifilis* remain at the same stage of vitellogenesis while large oocytes undergoes

atresia. These are then continuously replaced by growing vitellogenesis oocytes. Cavaco *et al.* (1998) in a study of sex steroids and the initiation of puberty in male African catfish (*C. gariepinus*) suggested that 11-tetotest (KT) is physiologically the most relevant androgen for the initiation of spermatogenesis.

Hussan and Mazid (2001) observed stock deterioration in hatchery population due to poor brood stock management and inbreeding depression. This was attributed to the fact that with the rapid expansion of fish culture operations, farmers solely depend on hatcheries for their seed requirement, whereas in the past, a major portion of the seed was collected from rivers. There is therefore every possibility of inbreeding in these hatcheries where female and male breeds are chosen from a finite population for mating with a greater choice of crossing sib or closely related fish. Moav and Wolfharth (1976) had earlier stated that a single full sib mating of a particular fish might result in 10-20% depression in growth while a considerable proportion of individual might show physiological abnormalities.

2.19 Hybridization in Fishes

Any breeding activity should start with a very careful selection of the foundation stock on which production and further breeding shall be based. This concerns the selection of both the most promising species and the productive strain within the species, either from the wild or from

hatcheries with different production environments and/or foundation stocks (Langhoiz, 1985).

Purdom (1993) noted that more logical approaches to hybridization address specific problems such as the clarification of taxonomic relationship, the development of experimental tools for studying physiology, the production of genetic variance for selection programme, the control of sex ratio and production of superior fish for culture.

Intraspecific hybridization and intraspecific cross breeding may improve a farm animal by non-additive genetic effects. However, the maximum dominance advantage is present in the first generation (F_1) and will be partially lost in future generations. Also, there is loss of the dominance superior from the pure breeds due to segregation and recombination of gametes from crossbred parents. This is known as epistatic recombination loss (Dickerson, 1973).

However, the new additive genetic variance introduced from hybridization may be two or three times greater than that introduced by mutation and may be used by fish breeders in a selection program (Grant, 1994).

Heterosis is the biological phenomenon whereby an F_1 hybrid of two genetically dissimilar parents shows an increased vigor, at least over the mid parents (means of both parental species). Studies have been conducted on several fishes with a view of producing individuals with hybrid vigor (positive heterosis) for maximizing productivity. F_1 hybrids of female channel catfish (*Ictalurus*

punctatus) x male blue catfish (*Ictalurus furcatus*) exhibits superiority for growth rate, disease resistance, tolerance of low oxygen levels, dressing percentage and harvestability (Smitherman and Dunham, 1985). However, producing hybrids on a commercial scale has not been practical because of reproductive isolating mechanisms between these two species. The hybrids between the white bass (*Morone chrysops*) and striped bass (*M. saxatilis*) referred to as the sunshine bass, grows faster with better overall characteristics than either parent species (Smith, 1988). In addition, crosses of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) also show faster growth than the parent species.

Numerous crosses of common carp with Rohu, mrigal (*Cirrhinus cirrhous*) and catla (*Catla catla*) (Khan *et al.* 1990); green sunfish (*Lepomis cyanellus*) crossed with blue gill (*L. macrochirus*) (Tidwell *et al.* 1992; Will *et al.* 1994) and gilthead seabream (*Sparus aurata*) with red seabream (Knibb *et al.* 1998), have also resulted in improved overall performance for aquaculture systems. Increased environmental tolerance may also be imparted to hybrids when one parent species has a wide or specific physiological tolerance or due to increased heterozygosity (Nelson and Hedgecock 1980) Noy *et al.* 1987). Villegas (1990_a) evaluated the salinity tolerance of *O. mossambicus* (high salinity tolerance) and *O. niloticus* (low salinity tolerance) and their hybrids in comparative growth trials (Villegas, 1990_b). The hybrid had combined traits of good growth

and survival. The development of hybrids between superior stocks of *O. niloticus* and *O. mossambicus* has the potential to combine the greater growth potential of the former with the saline tolerance of the latter and thus does appear to represent a potential commercial application for hybridization in brackish water aquaculture. Hybrids of Florida red-strain hybrid (*O. mossambicus* x *O. Urolepis hornorum*) (Kim, 2007) and *O. niloticus* x *O. aureus* hybrids (Lahav and Lahav 1990; Wohlfarth, 1994) also show enhanced salinity tolerances.

Hybridization between some species, such as *Oreochromis niloticus* and *Oreochromis aureus*, result in predominately male offspring (Rosenten and Hulata, 1994). Other tilapia crosses that produce mainly male offspring include, *O. niloticus* x *O. urolepis hornorum* or *O. macrochir* and *O. mossambicus* x *O. urolepis hornorum* (Wohlfarth, 1994). Conversely, the cross between striped bass and yellow bass produced 100% females (Wolters and Demay, 1996). This can be desirable for culture purposes where there are; growth differences between the sexes; sex-specific products such as caviar are wanted, or reproduction needs to be controlled like in overpopulation and stunting in tilapia production ponds. Hybridization between species can also result in offspring that are sterile or have diminished reproductive capacity (Dunham, 2001). As with mono sex production, the production of sterile hybrids can reduce unwanted reproduction or improve growth by energy diversion from gametogenesis. The more distantly related the two species, the greater likelihood of their hybrid being sub-viable or sterile (Chevassus, 1983). Examples of sterile

hybrids or those that have reduce fertility include various catfishes (Le Grande *et al.* 1984; Smitherman and Dunham, 1985) and cyprinids (Chevassus, 1983). Sometimes an intraspecific hybrid does not exhibit heterosis for specific traits, but may still be important for aquaculture if it expresses other useful traits from the parent species (Chevassus, 1983). The hybrid between African (*Clarias gariepinus*) and Thai (*C. macrocephalus*) catfish combines the fast growth of the African catfish and the desirable flesh characteristics of the Thai catfish (Na-Nakorn, 1999). Likewise, the rohu (*Labeo rohita*) x catla (*Catla catla*) hybrid grows almost as fast as pure catla, but has the small head of rohu considered desirable in Indian aquaculture (Reddy, 2000). *Catla catla* x *Labeo fimbriatus* (fringed-lipped peninsula carp) hybrids have the small heads of *labeo fimbriatus*, plus the deep body and growth rate of *Catla catla* (Bassavaraju *et al.* 1995).

Intraspecific backcrossing has also been useful to successfully introgress (merge) genes from one closely related species into another. Cold tolerance and colour genes have been transferred among tilapia in this manner. The hybrid cross between *Heterobranchus* and *Clarias* is receiving considerable attention in Africa particularly, Nigeria. These hybrids have been reported to show heterosis (Madu *et al.* 1992; Salami *et al.* 1992; Aluko, 1998). Eight fast growing and viable intergeneric hybrids have been produced (Aluko, 1999), by crossing four catfish species belonging to two genera; *Clarias* and *Heterobranchus*. These intergeneric hybrids which have been developed

up to fingerlings stage showed superior growth advantage over the parent species. This breeding strategy arrests the deteriorating genetic potentials of our clariid fish stocks and consequently increases genetic diversity.

Legendre *et al.* (1992) made successful hybridization between *C. gariepinus* and *H. longifilis* in three experiments and recorded that the reciprocal hybrid was viable with their survival rates being similar to those found in the maternal species. They further recorded that the hybrid morphology was immediate to that of the parents while no difference in external morphology is found between the reciprocal hybrids. True hybrids produced between grass carp and silver carp failed to acquire the positive traits of the parent species and thus proved useless for aquaculture. Hence any hybridization program should take into account all those aspects and careful evaluation has to be made with regards to that of hybrid progeny that results from a particular hybrid cross (Reddy *et al.* 1997).

Physical abnormalities occur both in wild and cultured fish population. In cultured populations, it is important to describe such phenotypes, analyze their effects on economically important criterion and discover their causes so as to control the frequencies. Smitherman *et al.* (1996) recorded that if deformities have a genetic basis and are recessive, crossbreeding should eliminate the abnormalities, but if the deformities were dominant, all individual possessing a copy of the gene

would express it and recognized after which they could easily be culled merely eliminating the gene from the population in a single generation. Aluko *et al.* (2001) investigated the genetic basis for pectoral fin deformities in *C. gariepinus*, *H. longifilis* and their hybrids. It was recorded that the level of aberration in some of these crosses indicates the involvement of genotypes rather than the influence of environment.

2.20 Producing Catfish Hybrid

The primary constraint to commercial production of most catfish hybrids has been the lack of reliable, cost-effective methods for producing large quantities of fry (Reddy *et al.* 1997). However, refinements of techniques for producing hybrids and general superiority of hybrids catfish have spurred renewed interest in use of hybrids for commercial production. Traditional pond-spawning which is effectively used to produce some catfish fry, is ineffective for consistent, large-scale production of hybrid fry. Therefore, production of hybrid fry in most catfishes depends on the use of hormones to induce ovulation in females, manual 'stripping' of eggs and manual fertilization of the eggs with catfish sperm (Dunham *et al.* 2000). Hormone often used includes pituitary extract: Common carp-CCP or catfish CP, lutenizing hormone releasing hormone analog-LHRHa, human chorionic gonadotropin and synthetic hormone-ovaprin, ovatide). Strategies for hormone-induced production of hybrids can be classified into two main categories: Pair-spawning and group-

pawning. Important factors for successful production of hybrid fry include: Good broodstock quality, proper calculation and administration of hormone dosage, proper testes collection and sperm preparation, accurate determination of the time of ovulation in females, good stripping and fertilization techniques and aggressive egg treatment, (Bondari, 1984).

Great variations in the reproductive ability of hybrids are observed: from complete fertility of intergeneric hybrids of some Cyprinodontidae to complete or incomplete fertility of intraspecific and intergeneric hybrids of the sturgeon (Acipenseridae), the carp (Cyprinidae) sunfishes (Centrarchidae) and others (Bartley, and Bentley, 1990). The degree of fertility of hybrids does not always correspond to the degree of taxonomic relationship of the species crossed. In fact, intergeneric hybrids may be more fertile than intrageneric ones. Sometimes, even reciprocal hybrids produced by crossing the same species, but by substitution of a female of one species by a male of another, and vice versa, may be different in fertility. Individual hybrids may vary in fertility. In fact, some individuals of so-called sterile hybrids can produce offspring. Therefore, one of the methods of eliminating complete sterility in such hybrids is to carry out hybridization on a larger scale to provide a larger number of hybrid individuals, some of which may be fertile. Fertility may be expected to increase in the second hybrid generation (Chevassus, 1983). This is true of such hybrids as *Cyprinus* x *Carassius* which, as a rule, are sterile; but among them some

exceptional individuals may be fertile. Investigations carried out by Kuzema have shown possibilities of successful selection work on Ukrainian hybrids of *Cyprinus* x *Carassius*. Fertility of most hybrids is disordered to a certain extent, and they are sometimes sterile (Clark, 2002). In the process of evolution of various species, they became gradually isolated ecologically, geographically and in other respects. Due to isolation a sort of barrier appeared which prevented them from free crossing. However, such isolation was not always reliable; the possibility of intraspecific crossing still remained. This resulted in the development of a second barrier - disorder in hybrid fertility, preventing them from mixing and destroying specific differences Colombo *et al.* 1998). But where the first barrier acts without failure, fully eliminating the possibility of two species crossing, the second barrier might not appear. If such species are crossed artificially, the first barrier is removed, and a fertile hybrid is produced. Charles Darwin meant these very cases when he wrote "there exist species which are not easy to cross, but their hybrids, once produced, are highly fertile". The experiments have shown that man, having succeeded in crossing such species, may find access to reproductive potentials that do not occur in nature (Reddy *et al.* 1997).

2.21 Artificial Hybridization

It has been possible to produce fertile hybrids of beluga (*Huso huso*) and sterlet (*Acipenser ruthenus*), two species which do not cross in nature due to marked ecological differences.

The sturgeons are remarkable for their ability to cross and for their hybrids that are fertile to various degrees. For many years we crossed the sturgeons in most specific combinations in order to produce intraspecific and intergeneric hybrids, not only in the first generation, but also in the second and even the third generation (also back-cross and triple-cross). In our work we did not come across any instance in which it was impossible to produce viable offspring (Krasnai, 1987).

Without detailed cytogenetic analyses of the causes of fertility and sterility of fish hybrids, there are more chances of producing completely fertile hybrids if the species crossed have equal chromosome number, which must be homologous and able to conjugate in the course of gametogenesis. When there is unequal chromosome number and violation of the conjugation process, sterility occurs in various degrees (Rahman *et al.* 1995). Until recently the sturgeons and their hybrids were not studied cytogenetically. There was research work on chromosome complexes of parent species and their hybrids, which has already given interesting results. Therefore, crossing of the first four species in any combination is likely to produce fertile hybrids; crossing each of the four with *A. gulden stadtii* produces hybrids which are either of limited fertility or are completely sterile, the results of abnormal gametogenesis (Salamin *et al.* 1993). Hence, before starting work on hybridization, it is necessary to study chromosome complexes of the species to be crossed; this will permit prediction, with a greater degree of probability as to whether or not the hybrids will be fertile or sterile, and will avoid waste of labour and money (Smith, 1988).

Laboratory hybridization experiments have been utilized extensively to confirm the probable hybrid nature of certain individuals by demonstrating that two taxa will interbreed when provided with the opportunity to do so, or that gametes from two taxa can be artificially cross-fertilized (Sahoo *et al.* 2009). Seeb *et al.* (1993) repeated that Hybrids produced from appropriate cross-fertilization techniques among commercially important fish species have been tested for their growth performance, viability and fertility. Hybrid recently produced experimentally between Sheim (*Acanthopagrus latus*) and sobiaty (*Sparidentex hasta*) in Kuwait appears to have good growth, flesh quality and is fertile (Khaled Al-Abdul-Elah Kuwait Institute of Scientific Research, comm.). Hybrids resulting from crossing several sunfish species have been used for the past three decades to improve pond fishing (Wolfarth, 1994). The most desirable hybrids result from crossing the female green sunfish (*Lepomis cyanellus*) with males from one of three other species. These include the bluegill (*L. macrochirus*), the redear, or shellcracker (*L. microlophus*) and the warmouth, or goggleye (*L. gulosus*). Rapid and superior growth is one-way hybrid sunfish exhibit hybrid vigor. Experimental hybrids between dusky grouper (*E. marginatus*) and the white grouper (*E. aeneus*) were evaluated, but all the hybrids died within 10-day post-hatching (Glamuzina *et al.* 1999). The camouflage grouper (*Epinephelus polyphekadion*) is more resistant to environmental stress and disease than the marbled grouper (*E. fuscoguttatus*). Experimental hybrids (marbled

grouper x camouflage grouper) exhibited faster growth performances and increased conversion efficiency (James *et al.* 1999).

Hybrid between the Beluga (*Huso huso*) and Russian sturgeons (*Acipenser gueldenstaedtii*) was evaluated and appeared to have a wide salinity tolerance to both fresh and seawater as well as good growth rate (Gorshkova *et al.* 1996) and these hybrids now are being considered for culture in Russia and Iran (Shilat, Iranian Fisheries Company). Two loach (*Misgurnus* sp.) are cultured both for food and for ceremonial purposes by Buddhists in Korea (Kim *et al.* 1994): the mud loach (*M. mizolepis*) and the cyprinid loach (*M. anguillicaudatus*). The mud loach grows to a larger size, has a faster growth rate and is more resistant to diseases, while the cyprinid loach has a more desirable body colour. These two species of loach were hybridized to combine the fast growth and large size of the mud loach (*Misgurnus mizolepis*) with the desirable body color of the cyprinid loach (*M. anguillicaudatus*). Fertilization, hatching, survival and karyology of the hybrids were very similar to the parents (Kim *et al.* 1995). These hybrids are now being culturing commercially and continued studies are planned to combine other desirable characteristics of the hybrids and their fertility. Hybrids produced using the eggs of Asian catfish (*Clarias batrachus*) and African catfish (*C. gariepinus*) performs as well as either parental control during spawning stage and better in fry and advanced fry stage, while the reciprocal hybrids are inferior in all performance traits (Khan *et al.* 2000). During the different experiments, this hybrid group showed highest survival from post-

larval stage to market size fish (Rahman et al. 1995; Khan et al. 2000). Growth performance was always better than maternal control and in some cases better than or close to paternal control. Preliminary observations of organoleptic testing revealed that the hybrid showed superior taste performance compared to parental groups (Rahman et al. 1995). Further researches are needed to examine other desirable traits of the hybrids and their sterility.

Hybridization between giant catfish (*Pangasiodon gigas*) and giant pangas (*Pangasius sanitwongsei*) are now being practiced in Thailand (Pongthana, National Aquaculture Genetics Research Institute, Thailand). Both of these catfishes are extraordinarily large, reaching 3m and 30kg and the giant catfish is considered as an endangered species whose trade is restricted under the Convention on International Trade in Endangered Species of Wild Flora and Fauna. Hybrids between these two catfish species shows good growth performances and should be used to reduce pressure on the giant catfish so as not to endanger it through excessive catch of brood fish from the wild or through genetic introgression of the two parental species. Due to the wide geographical distribution of yellow bass (*Morone mississippiensis*), hybridization tests with striped bass and comparisons with the sunshine bass have been conducted. The yellow bass hybrid exhibited 65% survival to harvest as compared to 45% for the sunshine bass, but poorer growth rate and condition factor when raised in tanks continuously supplied with pond water (Wolters and de May, 1996).

Further research has been undertaken to explore the possibility of combining other desirable traits in the above hybrid progeny.

2.22 Use of Intraspecific Hybrids in Aquaculture

Inter-specific hybridization has long been practiced in various species of fishes to increase growth rate, improve flesh quality, and produce sterile animals, increase disease resistance and environmental tolerance and to improve other quality traits to make fish more profitable (Bartley et al. 1991). Majority of the earlier works on hybridization was conducted for salmon fishes, but these species did not produce hybrids of commercial importance. For this reason, hybrids in these fishes do not draw attention to fish culturists (Faturu, 2003). Due to the increased expansion of fish farming throughout the world, hybrids produced from inter-specific crosses play substantial role for global aquaculture production (FAO, 1996). The increased use of artificial breeding and in-vitro fertilization techniques and increased knowledge of reproductive biology encourage the aquaculturists to produce hybrids in a view to improve the quality traits over their pure parental siblings. Evaluation of some of the important traits and performances that have been improved through hybridization among different species of fishes are described below.

2.23 Overall Improvement

The principal aim of hybridization is to combine desirable traits from different species to increase the overall production or marketability of a cultured species (Padhi *et al.* 1997). The major hybrid catfish cultured in Thailand is a cross between African (*Clarias gariepinus*) and Thai (*C. macrocephalus*) catfish, which combines fast growth rate of the African catfish with the desirable flesh characters of the Thai catfish (Nwadukwe, 1995). The overall product is improved and the flesh is still acceptable to Thai consumers, although it does not grow as fast as the pure African catfish. The roux-catla hybrid grows almost as fast as pure catla, but has the small head of the rohu and is therefore useful in Indian aquaculture (Reddy, 2000). Catlaxfringed-lipped peninsula carp (*Labeo fimbriatus*) were reported to have small heads of the fringed-lipped peninsula carp and deep body and nearly equal growth rate to the catla; dressing percentage also improved in this hybrid (Basavaraju *et al.* 1995).

The sunshine bass hybrid (white bass x striped bass) has a suite of advantageous traits including good osmoregulation, high thermal tolerance, resistance to stress and disease, high survival in culture and modified water-bodies and ability to utilize soy beans as a protein source (Smith, 1988; Colombo *et al.* 1998). The overall growth performances of hybrids (*C. catla* x *L. rohita*) fed on

wheat bran was consistently higher followed by rice broken and blood meal (Um-E-Kalsoom et al.2009). Among the cultivatable hybrids, red tilapia is more desirable than darker skinned tilapia in Cuba, Venezuela, Thailand, Europe and the United States of America. Most red tilapia are descended from the Nilexblue tilapia cross (Verdegem et al. 1997), but red tilapia also result from the cross of Wami tilapia (*O. urolepis hornorum*) with Mozambique tilapia (Earnest et al. 1991). It has been reported that red tilapia from Nile tilapia x Mozambique tilapia and Nile tilapia x Wami tilapia are being farmed in central Thailand. The latter cross is also salt tolerant and used for coastal aquaculture in parts of South East Asia (Bhikajee, 1997). Stability of the skin coloration is often a problem in successive generations and studies have been undertaken to understand the genetic mechanisms of colour inheritance (Koren et al. 1994; Hussain, 1994).

Hybrids between different species of North American catfish have been researched for more than 30 years. Among the intraspecific catfish hybrids, crosses between channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) exhibits good culture characters of the channel catfish with the ease of harvesting characters of the blue catfish such as better angling and increased seinability (Dunham and Argue, 1998). Once breeding problems are worked out, these hybrids may be useful in culture as they show heterosis for growth rate and are superior to channel catfish in low oxygen tolerance, disease resistance uniformity in body shape, angling vulnerability, seinability and dress-out percentage (Dunham and Argue, 1998). Hybrid produced from the crosses between the

muskellunge (*Esox masquinongy*) and the pike (*E. luciosus*) is sterile and well-adapted to intensive culture systems. However, the hybrid has the similar sport fish characteristics of the pure parental muskellunge, but higher protein requirements than both parental species (Brecka *et al.* 1995).

2.24 Effect of Hybridization on Disease Resistance and Environmental Tolerance

Hybridization may be used to improve disease resistance by breeding a higher resistant species with a less resistant one. Dorson *et al.* (1991) reported that crosses of coho salmon (*Oncorhynchus kisutch*) with other species, e.g., rainbow trout had increased disease resistance to a variety of salmonid viruses, but other culture characteristics were poor. Viability was increased when hybridization was followed with triploidization. Dorson *et al.* (1991) stated that the rainbow trout (*O. mykiss*) \times char (*Salvelinus* spp.) triploid hybrids had increased resistance to several pathogenic salmonid viruses and early sea water tolerance. Hybrids may have increased environmental tolerances when one parental species has a wide range of tolerance (e.g., euryhaline species), a specific tolerance (cold tolerance species), or because of increased heterozygosity sometimes being associated with a broad niche (Nelson and Hedgecock, 1980; Noy *et al.* 1980). Mozambique tilapia and Wami tilapia can reproduce in saline waters; however Nile tilapia has improved culture performance in many aquaculture systems.

Hybridization between Mozambique and Nile tilapia yields a red tilapia with salinity tolerance (Lim et al. 1993). Hybrids between Mozambique and Wami tilapia, called the Florida red strains, have high growth rates and can reproduce in salinities of 19 ppt (Earnst et al. 1991). Crosses between Nile tilapia and blue tilapia also resulted in progeny with good salinity tolerances (Lahav and Lahav, 1990; Wohlfarth, 1994). Hybrids also may be used to exploit degraded aquatic environments. Lakes affected by acid rain may not be suitable for native salmonids, but splake, a hybrid between lake trout (*Salvelinus namaycush*) and brook trout (*S. fontinalis*) can tolerate reduced pH levels of 4.9-5.4 of acid lakes of Ontario; lake trout reproduce successfully only in waters with pH values above 5.5 (Snucins, 1993). The splake also was shown to have higher survival and growth than both brook and lake trout in lakes with pH in the range of 5.5-7.2 (Inssen et al. 1982).

2.25 Hybrid Polyploidization

Hybridization combined with chromosome manipulation may increase the viability and developmental stability of hybrid fishes during early life history stages (Wilkins et al. 1995).

Polyploid hybrid salmon appear to be better suited for a variety of culture situations than either polyploid or hybrid salmon are on their own. Although many diploid salmonid hybrids are not used for culture, triploidization of the hybrids may confer increased viability, growth and survival

(Grey et al. 1993). Triploidization of Atlantic salmon (*Salmo salar* x brown trout (*S. trutta*) hybrids increased survival and growth rate to a level comparable to Atlantic salmon (Galbreath and Thorgaard, 1995). General disease resistance was improved by triploidizing the cross between rainbow trout and char; rainbow trout and coho salmon triploid hybrids had increased resistance to infectious disease, but the latter hybrids grew more slowly (Dorson et al. 1991). Triploid Pacific salmon hybrids between chum salmon (*Oncorhynchus keta*) and chinook salmon (*O. tshawytscha*) have earlier seawater acclimatization times (Seeb et al. 1993).

2.26 Heredity of Hybrids

Many scientists, Muchlisin *et al.* (2015) in particular, have shown that development of some characteristics of hybrids, to a great extent, depends on the environment in which the hybrids were bred. This environmental selection of characters is possible due to the great heterozygosity of the hybrid. Study of morphological characteristics of certain hybrids of the sturgeons, the carps and others (as compared to their parent species) shows that hybrids have an intermediate heredity not only in the first generation, but also in the second, as well as in the generations produced by back-crossing and triple-crossing (Adah *et al.* 2014). In comparison to the first generation, the following generations, often but not always, are characterized by greater variability; however, no typical morphologic segregation followed by return to the initial species has been observed. Hence it was

believed that intraspecific hybrids possess the so-called permanent intermediate heredity, which does not obey Mendel's law of segregation. But recent experimental research has proved that the type of heredity observed in crossing different species does obey the law of segregation. But the law, revealed by the behaviour of genes, is impossible to observe visually in morphological characteristics. The species crossed are different in many characteristics, and consequently in a great number of genes. In intraspecific crossing high polymeric inheritance occur when numerous genes affect the development of some definite characteristics in a similar way. This is why in the second generation a much greater number of combinations is inevitable; and among the mass of intermediate specimens, only a few of them are homozygous, which, in all characteristics show a return to the parental form (Bondai, and Dunham, 1987).

2.27 Hatchability of African Catfish

The technique described by Janssen (1995) for artificial reproduction of *Clarias gariepinus* requires an ample flow of good quality water, a reliable supply of electricity and a stable optimal temperature. These requirements lie beyond the socio-economic reach of most rural smallholders, they cannot adopt the technique and continue to lack catfish fingerlings for on-growing. As part of a participatory catfish reproduction research project in Cameroon, farmers were assisted in the development of methods by which they could reproduce *Clarias gariepinus* using only available

materials, that is, without needing to buy any tools or to set up any costly systems. Some farmers chose to incubate fertilized eggs in hapas installed directly in earthen ponds while others chose to do so in backyard basins. It was subsequently observed that in-hapa hatchability was often reduced by heavy rainfall during incubation while in-basin hatchability was equally inhibited at high incubation densities in contained stagnant water. These authors found no information on the effects of rainfall or turbidity on egg hatchability. On the other hand, Haylor (1992) found that incubation of *C. gariepinus* eggs was not only possible, but even faster in stagnant than in flowing water, but did not report any effects of egg density on hatchability. The objective of their work was to investigate how rainfall and water stagnation affect incubation and thus adapt technique to guarantee reliable and satisfactory hatchability of *C. gariepinus* eggs under on-farm conditions (Hogendoorn and Koops 1983).

2.28 Hatchability and Survival of Hatchlings in Catfish

The success of any fish farming operation depends on the availability of a ready supply of fish larvae for on growing to market size (Rottmann *et al.* 2003). The rearing of the larvae to the fry stage is most critical in the cycle of fish seed production in hatcheries, therefore, the rearing of the larvae under controlled hatchery condition requires the development of specific culture techniques.

Reproduction technique is one of the factors that affect the performance of any fish farm as it can either be natural or artificial (Krasnai, 1987).

The output of natural propagation in fish is very low and cannot meet the protein requirement of its consumers (FAO, 1996). In view of this an artificial propagation technique under more controlled conditions has been discovered to produce reliable sources of fish fries and fingerlings distribution centre (FAO, 1996). Artificial breeding otherwise known as hypophysation is practiced with the involvement of reproductive hormones. Induced breeding through hormone treatment and artificial incubation of fertilized egg has advantages of better rate of fertilization and hatching, better conditions for growth and survival of larvae to fingerling and better protection of larvae against unfavorable environmental condition and predators (Woynarovich and Horvath, 1980). However, most of the hormones that are generally used for induced breeding are deficient in various ways, such as Deoxycorticosteroid Acetate (DOCA) causes severe ulcer on the injected female; Human Chronic Gonadotropin (HCG) is very expensive; Common carp (*Cyprinus carpio*) pituitary gland material are not easily accessible to small scale fish farmers, although Ovaprim (Salmon Gonadotropin Releasing Hormone) had recorded numbers of success but the price is very high.

The report by Hill *et al.* (2009) revealed average success rates of 50% ovulation, 54% spermiation and 1.3% mortality were recorded after injection of different species with ovaprim. Also, ovaprim has been used successfully for hypophysation in different families of fish like cyprinidae Hill *et al.* (2005) Characidae and Cobitiidae (Yanong *et al.* 2009). However, the price of ovaprim increased indiscriminately due to import duties, therefore, to reduce the cost of production arising from purpose of ovaprim, there is need to find an alternative cheaper spawning aid (Yanong *et al.* 2009). African Catfish pituitary hormone (a non - synthetic hormone) is said to be readily available and cheaper than any other hormone (Adebayo and Popoola, 2008) and can be prepared in a suspension (Fagbenro *et al.* 1991).

2.29 Growth Performance and Feed Utilization in Catfish

Feed is one of the important agro-inputs in aquaculture production system that contributes to approximately 40–60% of production cost (Sahwan, 2016), and it has direct effect on the growth rate of the fish (Muchlisin *et al.* 2015; Karina *et al.* 2015). The aquaculture activity is commonly produced waste, for example, feed remains and Feed is one of the important agro-inputs in aquaculture production system that contributes to approximately feces which changes into ammonia and nitrite once the oxygen level is low (Putra *et al.* 2016). In the closed culture system, the concentrations of ammonia (NH₃) and nitrite (NO₂) are increasing rapidly and would be toxic

to organisms (Sidik *et al.* 2002; Sakala and Musuka 2014). According to Asaduzzaman *et al.* (2008) the intensive application of commercial feed in the aquaculture (Asaduzzaman *et al.* 2008) causes environmental pollution and increases the possibility of the disease outbreak. Therefore, the water quality management is crucial in the aquaculture system.

The objective of water quality management is to provide the comfortable environment and meet the optimum requirements for cultured organisms. According to Gunadi and Hafsaridewi (2007), the microbial activities can be used to improve water quality and reduce the burden of contamination by fish farming waste. Therefore, the heterotrophic bacteria have promising potency to be applied in the utilization of fish feeds (Gunadi and Hafsaridewi, 2007). Waste ammonia in the fish culture. Beside, these bacteria are formed as a floc (clumps) in the cultures media; hence it can be used as an alternative (Nurhatijah *et al.* 2016) feed source for cultured fish. Crab *et al.* (2007). Biofloc has abilities to suppress the toxic compounds such as ammonia and harmful bacteria (pathogenic) so that the cultured organisms grow well. Muhammadar *et al.* (2014). Application of biofloc in the cultures system has been reported by several researchers, for example, in the culture of channel catfish, (Schrader *et al.* 2011; and Green *et al.* 2014), in the South American catfish *Rhamdia quelen*, in Nile tilapia *Oreochromis niloticus*, *Farfantepenaeus brasiliensis*, and in (Schveitzer *et al.* 2015) cultured system of the shrimps *Litopenaeus vannamei* and *Penaeus monodon*. However, application of biofloc on African catfish *Clarias gariepinus*

cultures has never been reported previously (Nurhatijah *et al.* 2016; Furtado *et al.* 2014). African catfish is the popular species for aquaculture business in Southeast Asian countries. This species has several advantages, for example, resistance to diseases and handling stress and high growth rate, thus (El- Naggar *et al.* 2006) accounting for its commercial importance worldwide. According to Muchlisin *et al.* (2010) The protein requirement for African catfish ranges from 25% to 40%, lipid 9.5 to 10%, carbohydrates 15 to 30%, vitamins 0.25 to 0.40%, and minerals 1.0%¹, with energy level of 2000 cal/g to 3000 cal/g. Suhendra (1988) in addition, the application of probiotic into African catfish diet has been reported by several researchers, for example, (Al-Dohail, 2009; Ige, 2013; Dennis and Uchenna, 2016). However, application of probiotic combining with biofloc has never been reported previously. Hence, the aim of the study was to evaluate the growth performance and feed utilization of African catfish fed experimental diet reared in the biofloc cultured system and enhanced with probiotic.

2.30 Growth Performance, Survival and Feed Utilization of *Clarias gariepinus*

In Nigeria, the present growth of aquaculture industry has impacted positively on the recessed economy since it has become a source of livelihood for over hundreds of thousands of the employed and unemployed populations. Nigeria is one of the highest fish consuming nation in Africa with an annual fish consumption record of 1.5 million tons (Emmanuel *et al.* 2014). The

African Catfish *Heterobranchus longifilis* belonging to the family *Clariidae* is one of the most important aquaculture species in Nigeria because of its fast growth rate, appreciable size, disease resistance, high fecundity, ease of artificial breeding, tolerance of high stocking densities in captivity, tolerance of harsh of environmental conditions, acceptability of farm made feed, good market value, good taste and meat quality (Eyo, 2014). According to Elkanem *et al.* (2017), catfish is valuable by fish consumers because of its excellent taste and meat quality especially when presented in smoked, fried or dried form.

Apart from its acceptability and affordability, the African Catfish is rich in omega-3- fatty acid, thiamine, riboflavin, phosphorus, vitamins A, vitamin D, iron and calcium which is required for good health and tissue development (Katersky and Carter, 2005). Despite the growth of aquaculture industry in Nigeria, scarcity and expensive nature of high quality feed such as Coppens, Aller Aqua, Multi feed, Skrettings etc. especially in rural areas where some fish farms are located have slowed down the growth rate of this industry (Eyo, 2015), this challenge has resulted in searching for cheaper and alternative feed ingredients that will boost fish growth without any negation.

The use of leaf meals becomes not just an alternative feed ingredient but a cheaper and nutrient rich ingredient which is available all year round. *Moringa oleifera* belonging to the family

Moringaceae is one of such plants that is rich in nutrients required by fish for optimal growth and health. According to Olson *et al.* (2010), *Moringa oleifera* is commonly known as drumstick tree (because of its long, slender, triangular seed-pods), horseradish tree (its roots taste like horseradish) and ben oil or benzoin tree (because of the oil derived from the seeds). *M. oleifera* which is widely distributed in Africa and Asia is documented by (Francis *et al.* 1999; Kake, 2003) to contain 29.7% CP, 86% DM, 29.9% EE, 4.38% CF, 3056 kcal/kg energy, 0.26% calcium, with phosphorus and tannin (1.23 g/kg) in negligible amounts. Ochang *et al.* (2015) opines that *Moringa oleifera* which has a good potential for forage also has a good coppicing ability and can be grown easily. *Moringa oleifera* is economical to produce in commercial quantities requiring inexpensive inputs to thrive.

The use of *M. oleifera* leaf meal as a non-conventional and cheap protein source in aquafeed for different fish species has yielded positive results in relation to growth performance, survival and economic evaluations. Chabi *et al.* (2015) reported a positive effect of *M. oleifera* on the development of juvenile *Clarias gariepinus*. Richter *et al.* (2003) recommended 30% substitution of *M. oleifera* leaf meal for fish meal in the diet of Nile tilapia (*Oreochromis niloticus*). Bundit *et al.* (2014) reported similar observation for Bocourti catfish (*Pangasius bocourti*). Ochang *et al.* (2015) recommended 20% *Moringa oleifera* leaf meal (MLM) for optimal growth of *C. gariepinus*. However, limited studies have been conducted on the effect of *Moringa oleifera* leaves meal on the African catfish *Heterobranchus longifilis* which is one of the popular aquaculture

species in Nigeria. Therefore, the objective of this study is to evaluate the effects of varying inclusion.

2.31 Improved Growth Performances

Increased growth rate is the most desirable trait for stock improvement in aquaculture. Growth increase may result from dominant variance (Tave, 1986) or from increasing the number of polymorphic loci in an individual. Increased heterozygosity has been implicated in improved growth and other desirable characters in a variety of species such as developmental compatibility (Leary *et al.* 1983) food conversion efficiency and oxygen metabolism (Danzmann *et al.* 1985, Koehn and Gaffney 1984). A hybrid between white bass (*Morone chrysops*) and the striped bass (*M. saxatilis*) is called sunshine bass, exhibits faster growth and has many good culture characteristics than either parents under captive culture system (Smith, 1988). Crosses of the black crappie x white crappie (*Pomoxis nigromaculatus* x *P. annularis*) stocked in small ponds and impoundments (Hooe *et al.* 1994), silver carp x bighead carp (*Hypophthalmichthys molitrix* X *Aristichthys nobilis* (Krasnai, 1987) in polyculture systems and catfish hybrids between the African catfish (*Clarias gariepinus*) and the Vundu (*Heteropneustes longifilis* or *H. bidorsalis*) in intensive concrete tanks. Salamin *et al.* (1993 and Nwadukwe 1995) reported the fastest growth as heterosis than conspecific parents. Improved growth performances were also obtained from crosses of

mrigal (*Cirrhinus mrigala*) and catla (*Catla catla*) and common carp (*Cyprinus carpio*) with rohu (*Labeo rohita*) in pond culture system in India (Khan *et al.* 1990). Intergeneric hybrids between catla (*Catla catla*) and fimbriatus (*Labeo fimbriatus*) were observed to be combine desirable qualities such as the small head of the fimbriatus and the deep body of the catla and exhibited heterosis in terms of meat yield with higher flesh content than either of the parents (Basavaraju *et al.* 1995). Hybrids between tambaqui (*Colossoma macropomum*) and pacu in Brazil and Venezuela raceways and ponds grew faster than either parent (Senhorini *et al.* 1988). Crosses of the green sunfish (*Lepomis cyanellus*) with bluegill (*L. macrochirus*) (Tidwell *et al.* 1992; Will *et al.* 1994) and crosses of the gilthead sea bream (*Sparus aurata*) with red sea bream (*Pagrus major*) also had positive heterosis in growth and other culture characteristics (Hulata, 1995). Several hybrids have been produced in the Mediterranean with the cross between red sea bream and common dentex (*Dentex dentex*), being especially fast growing in cage culture management (Colombo *et al.* 1998).

2.32 Water Quality

The availability of good quality water is important for all fish farming systems. This is because productivity of any given body of water is determined by its biotic and abiotic properties (Haruna, 2003). The environment of the fish must be conducive for optimum growth and development, therefore, an ideal water condition is necessary for the survival of fish. All life processes of the

fish wholly depend on the quality of its environment. Water quality parameters become more critical in intensive culture systems where fish are raised in artificial ponds with reduced self-purification capacities as compared with natural systems. Water quality assessment is usually aimed at pollution control and planning of water resource management. The physical properties consist of the total dissolved solids (TDS), and total suspended solids (TSS) (measured as conductivity), temperature and turbidity, are important in digestibility because outdoor tanks are directly exposed to sun light; therefore, wide temperature values may occur while total suspended solids affect turbidity and water transparency (Apollos and Galyson, 2011).

2.33 Dissolved Oxygen

In the absence of pollution this is the single most important critical water quality parameters for fish in pond culture system (Boyd, 1990). All aquatic organisms with the exception of some bacteria require oxygen to survive (Apollos and Galyson, 2011). Most of these organisms extract their oxygen from the water. Thus, the extraction of oxygen and its addition to the water are critical factors to the survival of fish. Oxygen can be a limiting factor in aquatic systems. It tends to affect primary and secondary production. Two major sources of oxygen for water are through photosynthetic activities which produce oxygen and free atmospheric oxygen. The amount of oxygen in water is increased by primary production which takes place in out-door ponds and by

wind action which aerates the water surface. Wheaton (1977) states that the rate of oxygen transfer from air to water depends on water temperature, degree of saturation and turbulence of the air-water interface. At night photosynthetic process is minimized or stopped due to absence of light. But living organisms (plants and animals) continue to respire and consume oxygen. As a result, dissolved oxygen levels fall at night especially before dawn. The oxygen content of natural and pond waters usually reaches its daily maximum just at or slightly after daybreak. Dissolved oxygen levels rise from morning through the afternoon as a result of photosynthesis, reaching its peak in late afternoon. Maximum dissolved oxygen concentrations are usually observed in the mid to late afternoon. Inadequate dissolved oxygen has many effects on fish: fish stops feeding, growth is impaired and fish become stressed thereby becoming more susceptible to diseases, parasites and easy preys.

2.34 Total Dissolved Solids (Tds)

Total Dissolved Solids (TDS), refers mainly to inorganic substances that are dissolved in water. These substances could include carbonate, bicarbonate, chloride, sulphate, phosphate, nitrate, calcium, magnesium, sodium, and other ions. A certain amount of these ions in water is necessary for aquatic life (Stone and Thomforde, 2006). Total dissolved solids (TDS) measured in mg/l (or PPM) are solids in water that can pass through a filter (usually with a pore size of 0.45 micrometers) (Stone and Thomforde, 2006).

Alterations in TDS concentrations can be harmful to fish because the density of water determines the flow of water into and out of an organism's cell. Therefore, too low or too high TDS concentrations can impair growth and sometimes may even lead to death of aquatic organisms. Total suspended solids (TSS) is similar to TDS, high concentrations of TSS may reduce water clarity, contribute to decrease in photosynthesis, combine with toxic compounds and heavy metals, and may lead to increased temperature (Murphy, 2005).

2.35 Temperature

This is another important factor affecting the wellbeing of cultured fish. Fish are poikilotherms and water temperature plays a tremendous role in their feeding. Temperature affects metabolic activities, feeding potential, growth, survival, and reproduction in all fishes (Dupree and Hunner, 1984), and efficiency of food conversion. Adeniji and Ovie (1990) observed that a 5⁰C sudden change in temperature will stress or even kill fish, and this has formed the basis for acclimatization in fish research. Temperature has a profound effect on the rate of chemical and biological processes in water. For instance, fish require twice oxygen as much at 30⁰C as at 20⁰C (Adeniji and Ovie, 1990). Auta (1993) reported that temperature range of 25⁰C – 30⁰C is ideal for fish production in the tropics.

2.36 Hydrogen Ion Concentration

The pH of water is a measure of hydrogen ions that cause acidity and alkalinity on a scale of

0 – 14, with 7 being the natural state. In ponds, the time of the day in which water sample is taken often influence the pH value because of variations in the carbondioxide concentration. During the day, plant remove carbondioxide for photosynthesis, then pH will increase. At night, the pH will decrease as carbondioxide accumulates. Increasing the total alkalinity concentration in water helps buffer against pH changes (Stone and Thomforde, 2006).

Very low and high pH levels may reduce reproduction in fish and this could be associated with death (Stone and Thomforde, 2006). Adeniji and Ovie (1990) found out that acid and alkaline death points are approximately at pH 4 and 11 respectively. They also reported that pH values ranging from 6.5 – 9.0 were observed to be the most suitable for fish production.

2.37 Electrical Conductivity (Ec)

This is a measure of how well a solution conducts electricity and is correlated with salt content. Conductivity is typically reported in units of $\mu\text{S}/\text{cm}$ (Micro Siemens per centimeter). Freshwater fish generally thrive over a wide range of electrical conductivity. Electrical conductivity (EC) also can be used to give an estimate of the total amount of dissolved solids (TDS) or total amount of dissolved ions in water. Total dissolved solids (TDS) value is measured in mg/l and is about half of the EC ($\mu\text{S}/\text{cm}$). High conductivity is an indication of the presence of large amounts of

dissolved salts which may be detrimental to fish. The desirable range is 100 – 2,000 μ S/cm (Stone and Thomforde, 2006).

2.38 Ammonia

Ammonia is harmful to fish life especially the unionized fraction. The harmful effects on fish are related to pH and temperature values. The unionized fraction increases with rising pH and temperature values. Alabaster and Lioyd (1980) reported that the ammonia criterion could be applied, provided the temperature was neither bellow 50⁰C nor pH above 8.5.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The research was conducted at the Department of Fisheries and Aquaculture Teaching and Research Fish Farm, Adamawa State University Mubi. The entire area is located approximately between latitude $9^{\circ} 55'$ and $10^{\circ} 45'$ North and longitude $13^{\circ} 0'$ and $15^{\circ} 5'$ East. It occupies the North Eastern part of Adamawa State (Adebayo, 2004).

3.2 Experimental Site

The field studies were carried out at the Department of Fisheries and Aquaculture Adamawa State University Mubi Hatchery Complex and the laboratory analysis was done at Biotechnology Centre, Modibbo Adama University of Technology, Yola, Adamawa State.

3.3 The Fish Hatchery Complex

This was the main site of the field experiment. The complex consisted of an indoor and outdoor hatchery management system with sections that includes; the overhead storage tank system, the indoor hatchery with 30 plastic tanks, the outdoor concrete pond with 40 experimental tanks,

nursery ponds and outdoor concrete brood stock tanks plate i and ii. The different sections were systematically interconnected to form a large unit complex.

3.4 The Indoor Hatchery

Plate 1: was the breeding site in the complex. It was well equipped with facilities for water flow through, mechanical aeration, spawning, incubation and hatching of eggs and the early nursery management of hatchlings the source of water was through the overhead tanks which are connected to a borehole system. Facilities in the indoor hatchery include.

The mini flow through system which consists of a series of small plastic tanks linked together with same water inlets but individual separate outlets. Mechanical aeration system by means of mechanical air blower which distributes air from blower to the indoor concrete tank and incubating troughs through a network of pipes, hoses and diffusers. Additional overhead water shower system was also provided for aeration when there is no electricity to supply the mechanical blower.

Horizontal water flow through incubating/hatching troughs for egg incubating and hatching. Water was allowed to flows horizontally on top of the incubating troughs and drained at the base. It also has provisions for both mechanical aeration and water shower system plate I.



Plate I: Indoor hatchery experimental setup for crosses between normal pigmented and albino *C. gariepinus* broodstocks.

3.5 The Outdoor Concrete Nursery Ponds

Plate ii: is the outdoor concrete pond consisted of series of 40 small sized 1m x 1m x 1m length, width and depth concrete ponds which were used exclusively for the nursery management of the hatchlings. Each nursery pond has its own water inlet and outlet devices. The outdoor concrete ponds consisted of a set of 40 concrete ponds with surface area of 20m² (4m x 5m) plate II.



Plate II: Outdoor Experimental Tanks

3.6 Brood Stock Selection

Broodstocks used for artificial breeding were composed of two males, an albino and a normal pigmented catfish, and two females, normal pigmented and an albino from each tank they were

randomly selected for the breeding excises, Ripe and matured broodstock were carefully selected and examined for gonad development according to the method of Blythe *et al.* (1994). Males were examined for rigid and reddish infusion of the genital orifice while for females, the genital orifice for reddish infusion, distension of the belly and release of eggs when gentle pressure was applied on the abdomen were obtained (plate iii,iv, v and vi).







A

B

Plate III: The Genital Papilla of Broodstock of Male and Female Albino *Clarias gariepinus*.

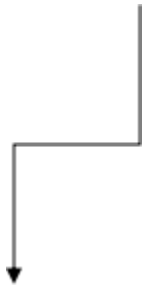
KEYS:

A= Male Albino Papilla

B= Female Albino







A

B

**Plate IV: Matured Male Albino (A) and normal Female (B) *Clarias gariepinus*
Broodstocks.**

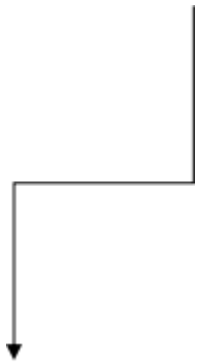
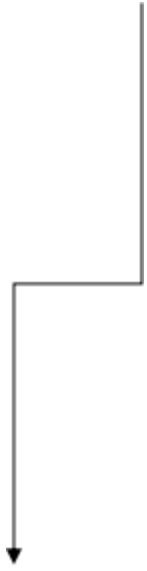
KEYS:

A= Male Albino papilla

B= Female Normal normal







A B

**Plate V: Matured Female albino (A) and normale Male (B) of *Clarias gariepinus*
Broodstocks.**

KEYS:

A= Female Albino *Clarias*

B= Male Normal Papilla







A

B

Plate VI: Matured normal Male (A) and normal Female (B) *Clarias gariepinus* Broodstocks.

KEYS:

A= Male *Clarias gariepinus* Papilla

B= Female *Clarias gariepinus* opening

3.7 Collection, Transportation and Acclimatization of Breeders

The parent broodstocks used in this study are; *Clarias gariepinus* normal and Albino which were obtained from earthen pond of Abdulfana fish farm Yola and transported live in an open plastic jerrican (50litre) to the Department of Fisheries and Aquaculture, Adamawa State University Mubi,

Male and female *Clarias* species were separated from one another during transportation. Sexually matured and ripe breeders of each species (male and female) with weight of 1.2kg were collected.

The broodstock were acclimatized in 8 x 5 x 3m outdoor concrete tanks for two weeks in the

Broodstock Tanks of the Department of Fisheries and Aquaculture Adamawa State University

Mubi they were fed daily at 5% of their biomass with 40% protein diet (6mm size Vital feed).

3.7 Hypophysation and Artificial Hybridization

The male and female fish were weighed separately with sensitive weighing balance (Ohaus).

Ovaprim injection was given intra-muscularly at the recommended dose of 0.5ml per kg of female fish and 0.25ml per kg of male fish (Madu, 1995, Yisa *et al.* 2016). The injected breeders were kept separately in four plastic bowls containing water. The bowls were covered with netting material in order to ensure that the fish did not jump out from the bowls during latency period of about 12 hours.

3.8 Determination of the Latency Period

Latency period were determined by gentle pressing on the the swollen abdomen of both the normal and albino female *Claria gariepinus* at 12 hours after injected with the hormone Ovaprim, the normal female *Clarias gariepinus* respond by oozing out gravid eggs, the albino female's eggs couldn't respond at the 12 hours, then 1-hour interval were added at 13 hours before the stripping of the female albino commence. The Ovaprim injection was given 2 hours' interval to the female albino *Clarias gariepinus* follow by the normal female *Clarias gariepinus* were injected, this were done in order to strip the eggs at the same time with the normal female and albino female in F₂.

3.9 Milt and Egg Collection

Plate VI: showed the and stripping of females was carried out after a latency period, this was done by gentle hand stripping into dry clean bowls. The females were first cleaned with towel to avoid water getting in contact with the eggs. The males were dissected at the abdomen using a pair of scissors to expose the testis plate vii, the testis were crushed to remove the milt, the milt was mixed with 0.9% saline (NaCl solution) (Woynarovich and Horvath, 1980; Yisa *et al.* 2016). The eggs and milt of the parents were mixed together using chicken feather under normal laboratory temperature of about 27⁰c to generate four different crosses (genetic crosses) as follows:

3.10 Hybridization Process of Normal Pigmented and Albino Fish

Experiment 1 Experiment 2

Intraspecific (f1)	Intraspecific (f2)
AA(♂) x AA (♀)	AA (♂) x AA(♀)
AA(♀) x NN (♂)	AA(♀) x NN(♂)
NN(♂) x AA (♀)	NN(♂) x AA(♀)
NN(♀) x NN (♂)	NN (♀) x NN(♂)

KEYS:

NN----NORMAL *Clarias gariepinus*

AA----ALBINO *Clarias gariepinus*

AA/NN---- ALBINO/NORMAL *Clarias gariepinus*

NN/AA----NORMAL/ALBINO *Clarias gariepinus*



A

B

Plate VII: Hand Stripping of female normal (A) and female Albino (B) *Clarias gariepinus*.

KEYS:

A= Stripping of normal female eggs

B= Stripping of Albino female eggs





A

B

Plate VIII: Removal of Normal Male (A) and Albino Male (B) Gonad of *Clarias gariepinus*.

KEYS:

A= Normal Male dissected

B= Albino Male dissected



A

B

Plate IX: Separated Normal Male Gonad (A) and Albino Male Gonad (B) of *Clarias gariepinus*.

KEYS:

A= Normal Male gonad

B= Albino Male gonad

3.11 Incubation and Hatching

The Fertilized eggs from each mating combination were incubated in well aerated rectangular fibre plastic trough (3x0.4x0.2m²) under ambient temperature of 27⁰c. in each trough, there was tray constructed from a coated nylon net with 1.5 mm mesh size. The net size allowed the hatchlings swim out into the incubation tank and the tray with un- hatched eggs and shells which were lifted out of the incubation tank and washed (Brooks, 1994).

3.12 Estimation of Fecundity

Eggs were washed with water and blotted dry and then counted, fecundity estimation was carried out using sampling methods of(Simpson, 1959) cited in (Yeldan and Avsar, 2000).

Four replicates batch of 1g each was completely counted under a Zeiss dissecting microscope using a mechanical counter. Means of the count from mating group was used in estimation of fecundity for each fish as total number of eggs per female was determined using the formula bellow adopted from as below; (Yeldan and Avsar, 2000).

$$\text{Fecundity} = \frac{\text{Total weight of eggs} \times \text{Number of eggs in Subsamples}}{\text{Weight of subsample}} \quad (1)$$

3.13 Estimation of Percentage Fertility

$$= \frac{\text{Number of fertile eggs}}{\text{Total Number of eggs}} \times 100 \quad (2)$$

Nine hundred and sixty (960) fertilized eggs were used for each of the experiment. Fertility percentage was determined by counting the number of fertile eggs against the total number of the eggs, estimation was carried out using sampling methods of Palikova *et al.* (2011) as shown below;

Percentage fertility (%) was calculated

3.14 Estimation of Percentage Hatchability

Percentage hatchability was estimated using both numerical and volumetric methods. The mean number of hatchlings in each mating combination was obtained by direct counting of unhatched eggs as well as the number of hatchlings in the incubating troughs, El-Gamal *et al.* (2008).

$$= \frac{\text{Number of hatchlings}}{\text{Total Number of fertile eggs}} \times 100 \quad (3)$$

The percentage numbers of hatchlings in each crosses combination were obtained by direct counting of number that was hatched as well as the number of fertile eggs in the incubating tray. Percentage number of hatchlings among the crosses combination El-Gamal *et al.* (2008) is as follows;

Percentage hatchability

3.15 Rearing in the Hatchery

Thirty-six (36) hours at the end of the hatching, two hundred and forty fry (240) at seven days old were randomly placed in the four crosses and stocked in twelve plastic bowl per each containing 25ml of water in triplicate. The fry was fed with hatched Artemia cysts after yolk absorption, the fry was fed with Coppens feed of 0.2- 0.3mm,0.5-.8mm and 2mm. The experiment at 14 days (2weeks), weight is determined by using sensitive weighing balance, length is measured by the aid of Ruler, and survival rate in each bowl was determined weekly for eight weeks.

Water quality parameters such as temperature, Dissolved Oxygen and pH were monitored and maintained at level of 0-14, with 7 being the natural state as recommended by (Stone and Thomforde (2006); Adeniji and Ovie (1990) reported that pH values ranging from 6.5-9.0 were observed to be the most suitable for fish production. All the parameters were determined by using Aqua-testing kit.

3.16 Growth Performance

Data of weight and length collected during the experimental period were used subsequently for the determination of growth rate, the growth performance of the fry and fingerlings in terms of Mean Weight Gain (MWG) Specific Growth Rate (SGR) and Mean Length Gain (MLG). The mean final weight and mean final length were taken at each development stages of the experiment in order to distinguish between the growth rates by each group of the crosses. Measurements were carried out

for weight with electric weighing balance and total length with measuring scale for fry and fingerlings from crosses. Length gain weight gain and specific growth rate (SGR) were determined by formula adopted from Adebayo, (2006).

Weight gain (g) = mean final body weight (g) (MFW)- Mean initial body weight(g) (MIW);

Length gain (cm) (MLG) = mean final length (cm) (MFL) – Mean initial length (cm) (MIL)

$$= \frac{\ln W_1 - \ln W_0}{T} \times 100 \quad (4)$$

Specific Growth Rate (% day)

$\ln W_1$ = Log of Final weight (g)

$\ln W_0$ = log of Initial weight in (g)

T = Time

3.17 Determination of Feed Intake

Daily feed intake was determining from each experiment by weighing the quantity of feed consumed by each experimental and recorded for future use, Arthur *et al.* (2001).

3.18 Determination of Feed Conversion Ratio

$$= \frac{\text{Total feed consumed (g)}}{\text{Weight gain (g)}} \quad (5)$$

Feed conversion ratio was determined

by calculating the total quantity of feed consumed divided by the weight gain as recommended by

Willems *et al.* (2013).

Feed Conversion Ratio (FC R)

3.19 Estimation of Percentage Survival

The survival rate was calculated at the end of the research by estimating the final number of fish

in each experiment at the end of the research as described by Onyia *et al.* (2016) as follows;

$$= \frac{N_i - N_f}{N_i} \times 100 \quad (6)$$

Survival rate (%)

Where N_f *final* number of fish

N_i *initial* number of fish

3.20 Determination of the Morphometric and Meristic Character of the Various Combination

The phenotypic characterization of the various mating combination were carried out using some of their morphometric and meristic character, ten (10) Morphometric and meristic characters were examined in samples from each mating combination. A total of 40 fishes were used. The morphometric and meristic characters were measured using the convectional method described by Turan, (2004). The characters examined are: Total Length Standard Lengths, Weight, Head Width, girths, eye diameter, inter orbital distances, nasal barbell lengths, maxillary barbell length. The other Morphometric character are dorsal fin length, dorsal fin height, caudal peduncle length, gap between adipose and dorsal fins, and fin length, and fin height, pectoral fin to pelvic fin, pelvic fin to anal fin, frontal fontanelle length, occipital fontanelle width.

The Measurement were taken to the nearest 0.1mm with the use of caliper, the meristic count was made using hand lens and a dissecting microscope. The characters counted includes dorsal fin rays, pectoral fin rays, pelvic fin rays, and fin rays and caudal fin rays.

3.21 Colour Ratio of the Offspring of the Mating Combination

The determination of albino ratio in the crosses between normal pigmented and albino pigmented as well as albino x albino were carried out by pun net square. When crosses occur between the parents that differ in only one characteristic known as a monohybrid crosses that the recreation of offspring is monohybrid Castillo *et al.* (2012). The punnet square was drawn by applying the rule

of probability combination to predict the possible outcome of genetic crosses. The total number of colour of albino and normal *Clarias gariepinus* in the nursery ponds were counted and recorded, this is done in the crosses between albino male and normal female, then normal male and albino female among the experiments Magalhaes *et al.* (2010).

3.22 Gene Characterization

3.23 DNA Extraction

The work bench was first sterilized by swabbing the samples with a cotton soaked in methylated spirit, Genomic DNA was extracted using the Zymo-research kit (California, USA G589E) from fish tissue samples preserved in alcohol following the procedure of Islam *et al.* (1995) below:

Preserved fish tissue sample of the dorsal fins were collected from the both parent of Normal and Albino *Clarias* and washed using distilled water and put into 1.5ml microcentrifuge tubes. This was done separately for each sample. Added to the tissue sample (25mg) in a microcentrifuge tube a solution of water 2x digestion buffer (951) and proteinase K (101) were done, these were mixed thoroughly by vortexing and incubated in a water bath at 55⁰c for 1-3hours. 31 of RNase A was added to the tube and incubated at room temperature for 5mins. 7001 of Genomic lysis buffer was added to the tube and mixed by vortexing and then centrifuged at 10,000xg for one minute. The supernatant was transferred to a zymo-spin- column in a collection tube and centrifuged at

10,000xg for 1 minute. 2001 of DNA pre-wash buffer was added to the pin-column in a new collection tube. It was centrifuged at 10,000xg for 1 minute. 4001 of g-DNA wash buffer was added to the spin column and centrifuged at 10,000xg for 1 minute. The spin-column was transferred to a clean microcentrifuge tube. 1001 of Elution buffer was added to the spin column and incubated for 2-5 minutes at room temperature. It was then centrifuged at top speed for 30 seconds to elude the DNA. The eluted DNA was stored at -20°C for further use.

3.24 Selection of Microsatellite Markers

The process of electrophoreses were conducted by selecting two microsatellite markers and used in *Clarias gariepinus* DNA extraction as described by Galbusera *et al.* (2000). the markers cg01 and cg02 were selected based on their ease of use in the amplification of diversity indices (allelic richness, polymorphic information content, observed and expected heterozygosities).

3.25 Polymerase Chain Reaction (PCR) Amplifications and Primers for Electrophoresis

Amplification of the extracted DNA was done through Polymerase Chain Reactions (PCRs) carried out in 10 μl reaction volumes containing primers used as 20 mg - 40 mg genomic DNA, 2 μl 5 \times Taq master mix of Taq DNA Polymerase, G1R and G1F, G2R and G2F, G3R and G3F dGTP, dTTP, (NH₄) SO₄, MgCl₂, Tween-20, Nonidet P-40, red dye, gel loading buffer, stabilizers (Jena bioscience, LObstedter, Germany), 0.3 μl each of the forward and reverse primer sets and 5.9 μl of

PCR grade water (Jena Bioscience, LObstedter, Germany). Amplifications were carried out using GeneAmp PCR thermocycler programmed as follows: 30 cycles each of denaturation, annealing and extension temperatures at 94°C for 30seconds, 58°C-60°C for 30 seconds and 72°C for 30 seconds respectively and a final extension temperature of 72°C for 10 seconds to complete the amplifications.

The PCR condition for each marker was optimized and differs from those described by Galbusera *et al.* (1996). The initial denaturation time was not included and the thermocycler was loaded at 94°C and the annealing temperature for each marker was adjusted to yield clear bands.

3.26 Electrophoresis For PCR

Electrophoresis was conducted on 1.2% Agarose gel and scored by comparison to 8-bp (base pairs) standard DNA ladder as (Jena Bioscience, LÖbstedter, Germany) with the following values 75, 154, 220, 298, 344, 396, 504 and 1632 bp. 1.2%. Agarose gel was prepared by weighing 1.0g of Agarose powder on a mini sensitive scale and transferred into a conical flask, 100ml of distilled free water was added to it and the mixture was heated in a microwave for two minutes. The conical flask was brought out and left to cool for few seconds without solidifying. 10µl of Ethidium bromide solution was added with a micropipette and shaken gently, then poured into the electrophoresis tray containing already inserted electrophoresis combs used to create wells. The

mixture was left to solidify while the electrophoresis tank was filled with $10 \times$ TBE solution prepared by adding 490ml of water to 10ml TBE solution.

The comb was removed after the mixture had solidified, and the tray was placed into the electrophoresis tank. $5\mu\text{l}$ of the DNA ladder was loaded in the first well while $4\mu\text{l}$ of each sample was loaded in consequent wells. The electrophoresis set was then operated for 60 minutes at 80volts. After a maximum time of 60 minutes, the gel was removed and viewed under a UV Trans-illuminator. The DNA bands were scored with Gel-analyzer in comparison to the 8-bp standard DNA ladder (Jena Bioscience, LÖbstedter, Germany) Islam *et al.* (2012).

3.2.7 Data Analysis

Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA).

The difference between the means were determined using Least Significant Difference (LSD) at 95% confidence level ($P < 0.05$). The statistical analysis was based on the population sample.

The Genetic distance, similarity matrix, and input data for the phylogenetic was computed using the online version of Unweighted Pair Group Method with Arithmetic mean (UPGMA).

CHAPTER FOUR

4.0 RESULTS

4.1 Latency Period of Female Parent Stocks

The Table 4.1 showed the Latency period of the female parent stocks of all the crosses. The female albino *C. gariepinus* had the highest latency period of 13.4 hours, While normal *Clarias gariepinus* had the least latency period of 12.0 hours at temperature of 27.5°C respectively.

Table 4.1 Weight, Quantity of Ovaprim Injected and Latency Period of Parent Stock .

Type of Female	Weight of female (Kg)	Quantity of Ovaprim(ml)	Time of injection (pm)	Time of stripping (am)	Latency period (hours)	Water temperature (°C)
NN♀	1.56	0.78	9:00	9:00	12.00±0.21 ^b	27.50±0.18 ^a
NN♀	1.76	0.88	9:08	9:14	12.60±0.32 ^b	27.50±0.13 ^a
AA♀	1.68	0.84	9:11	10:06	13.40±0.22 ^a	27.50±0.20 ^a
AA♀	1.62	0.81	9:15	9:51	13.30±0.32 ^a	27.50±0.16 ^a

Mean in the same column with the same super script do not significantly differ (P>0.05).

KEY

NN♀ = Female Normal *Clarias gariepinus*

AA♀ = Female albino *Clarias gariepinus*

4.2 Latency Period of Female F1 Generation

Table 4.2 showed the Latency period of the female fish for F1 of all the crossing. The female albino *Clarias gariepinus* had the highest Latency period of 13.25 hours, While normal *Clarias gariepinus* had less Latency period of 12.10 hours at temperature of 28.32°C respectively.

Table 4.2 Weight, Quantity of Ovaprim injected and Latency period of F1 Generation .

Type of Female fish	Weight of female fish (Kg)	Quantity of Ovaprim injection (ml)	Time of injection (pm)	Time of stripping (am)	Latency period (hrs)	Water Temperature (°C)
NN	1.21±0.00 ^b	0.60	9:02	9:03	12.10±0.00 ^b	28.20±0.00 ^a
NN	1.13±0.00 ^b	0.56	9:03	9:06	12.15±0.00 ^b	28.10±0.00 ^a
AA	1.30±0.00 ^a	0.65	8:20	9:21	13.20±0.10 ^a	28.30±0.00 ^a
AA	1.11±0.00 ^b	0.55	8:23	9:24	13.25±0.10 ^a	28.32±0.00 ^a

Mean in the same column having the same super script do not differ significantly (P<0.05).

AA = Albino *Clarias gariepinus*

NN= Normal *Clarias gariepinus*

4.3 The Percentages Fertility, Fecundity and Hatchability of the Parent Stocks

The percentage fertility showed the normal *Clarias gariepinus* (NN♂ x NN♀) had the highest fertility of 44.79±0.32, followed by crossing between female albino and normal male *Clarias gariepinus* (AA♀ x NN♂) with value of 42.92±0.13. (NN♀ x AA♂) had the least of 27.92±0.31.

The values of Normal Pigmented *C. gariepinus* (NN♂ x NN♀) and Albino (AA♂ x AA♀) were not significantly different ($P < 0.05$) from the parent crosses. On the other hand, there were significant differences ($P < 0.05$) among the hybrids.

The mean number of eggs (fecundity) produced by the offspring of the parental intraspecific mating combinations at the end of 16 months of growth studies is shown in Table 4.3. The parent of normal female *Clarias gariepinus* had the highest fecundity with a value of 7020±0.23/kg, followed by albino female *Clarias gariepinus* whose value was 4032±0.12kg bodyweight. Mean fecundity was significantly different ($p < 0.05$) among the offspring of the parental mating combinations.

Hatching rate were also recorded high in NN♂ x NN♀ (72.6%) followed by pure breed of AA♂ x AA♀ had (68.3 %) as shown in table 4.3. The values of hatching rate in the hybrids for AA♂ x

NN♂ (56.9%) and for NN♂ x AA♀ (46.3%) were the least. The result for Normal Pigmented *C. gariepinus* for the hatching rate was significantly different ($P>0.05$) from the others.

Table 4.3 Percentage Fertility, Fecundity and Hatchability of Crosses Between *Clarias gariepinus* normal and Albino *Clarias* Parent Stock

Mating combination	Number of eggs fertilized	Fertility %	Fecundity	Hatchability %
NN♂ x NN♀	960	44.79±0.32 ^a	7020.00±0.12 ^a	72.6±0.24 ^a
AA♂ x AA♀	960	37.18±0.13 ^b	5460.00±0.12 ^c	68.3±0.12 ^b
NN♂ x AA♀	960	42.92±0.31 ^b	6240.00±0.23 ^b	56.9±0.13 ^c
AA♂ x NN♀	960	27.92±0.31 ^c	4032.00±0.34 ^d	46.3±0.43 ^d

Mean in the same column with the same super script do not differ significantly ($P<0.05$).

NN♂ x NN♀ = Male and Female Normal *Clarias gariepinus*

AA♂ x AA♀ = Male and Female Albino *Clarias gariepinus*

NN♂ x AA♀ = Male Normal and female Albino *Clarias gariepinus*

AA♂ x NN♀ = Male Albino and normal Female *Clarias gariepinus*

4.4 Percentage Fertility, Fecundity and Percentage Hatchability of F1

Table 4.4 showed the results of the Percentage Fertility of the normal *Clarias gariepinus* (NN♂ x NN♀) had the highest Fertility of 92.12±0.23 followed by crossing between female albino and normal male *Clarias gariepinus* (NN♂ x AA♀) with 89.33±0.37, (AA♂ x AA♀) had the least of 72.11±0.34.

The mean number of eggs (Fecundity) produced by the offspring of the Parental. intraspecific mating combinations at the end of 16 months of growth studies is shown in Table 4.4. Among the offspring, the normal *C. gariepinus* has the highest Fecundity with a value of 8688.0±0.00/kg and

7900.0±0.00/kg, followed by Albino *Clarias gariepinus* whose value was 6240.0±0.00/kg and 3360.0±0.00/kg. Mean Fecundity was significantly different ($p>0.05$) among the offspring of the mating combinations. The values of Normal Pigmented *C. gariepinus* (NN♂ x NN♀) and Albino (AA♂ x AA♀) were not significantly different ($P < 0.05$) from the hybrid crosses.

Table 4.4 Showed the Hatching Rate was also recorded high in NN♂ x NN♀ (79.32%) followed by crosses between NN♂ x AA♀ (68.43 %) as shown in table 4.4. The values in the hybrids for AA♂ x AA♂ (52.12%) and for AA♂ x NN♀ (57.57%) were the lowest. The result for Normal Pigmented *C. gariepinus* for both fertility and Hatching Rate were not significantly different ($P<0.05$) from the others.

Table 4.4 Percentage Fertility, Fecundity, Hatchability and Survival of Crosses of *Clarias gariepinus* normal and Albino F1

Mating combination	Number of eggs fertilized	Fertility %	Fecundity	Hatchability %
NN♂ x NN♀	960	92.12±0.23 ^a	7900.0±0.00 ^b	79.32±0.24 ^a
AA♂ x AA♀	960	72.11±0.34 ^c	8688.0±0.00 ^a	52.12±0.45 ^c
NN♂ x AA♀	960	89.33±0.37 ^a	6240.0±0.00 ^b	68.43±0.67 ^b
AA♂ x NN♀	960	79.43±0.45 ^b	3360.0±0.00 ^c	57.57±0.56 ^c

Mean in the same column having the same super script do not differ significantly (P<0.05).

4.5 Growth and Feed Utilization Parameters for F1

Table 4.5 show the growth and feed utilization of F1 crossed between AA♂ x NN♀ gives the highest mean weight value of 912.90 ±0.00g Taable 4.5 followed by NN♂ x NN♀ with 900.75 ±0.00g and NN♂ x AA♀ with 893.88±0.00g and AA♂ x AA♀ 852.98±0.00g was the least after 14 months of culture. The specific growth rate SGR (%/day) recorded highest among the crosses in AA♀ x NN♂ with value of (1.38±0.02) follow by NN♂xNN♀ with value of (1.35±0.24). The least recorded in AA♂ x AA♀ with (1.31±0.50). The crosses from all the breeding trails increased in length during the rearing period. The maximum values for mean final length (29.95±0.00cm) was recorded in AA♂ x NN♀ followed by NN♂ x NN♀ (29.75±0.00cm), NN♂ x AA♀ (28.00±0.00cm) whereas the minimum size for mean final length (27.35±0.00 cm) was observed in AA♀ x AA♀. There was no significant difference (P> 0.05) in final mean weight gain of the crosses. Survival rate was high in crossing between the pure albino *Clarias* AA♂xAA♀ with 41.25±0.00% while crossing between albino female and normal *Clarias* NN♂ x AA♀ has the least value of 12.50±0.00% respectively table 4.5.

Table 4.5 Growth and Feed Utilization Parameters for F1 Crosses Between Normal *Clarias* and Albino *Clarias gariepinus*

Parameters	Mating		Combination	
	NN♂xNN♀	AA♂xAA♀	NN♂ x AA♀	AA♀ x NN♂
Mean initial weight(g)	3.30±0.00 ^a	2.81±0.33 ^b	3.51±0.00 ^a	2.92±0.00 ^b
Mean final weight (g)	900.75±0.00 ^b	852.98±0.00 ^c	912.90±0.00 ^b	993.88±0.00 ^a
Mean weight gain(g)	897.45±0.00 ^b	850.17±0.00 ^d	823.54±0.00 ^b	990.96±0.00 ^a
Mean initial length (cm)	2.85±0.00 ^a	2.75±0.00 ^b	2.72±0.50 ^b	2.75±0.00 ^b
Mean final length (cm)	29.87±0.50 ^b	27.35±0.00 ^b	29.95±0.00 ^b	30.00±0.00 ^a
Mean length gain (cm)	27.02±0.50 ^d	24.60±0.50 ^b	27.23±0.50 ^c	27.25±0.00 ^a
Feed intake (g)	16.75±0.00 ^c	46.50±0.00 ^a	14.50±0.00 ^d	23.50±0.00 ^b
Feed Conversion ratio	1.48±0.50 ^d	1.52±0.50 ^c	1.59±0.79 ^b	1.69±0.75 ^c
FRC(g)				
Specific Growth Rate	1.35±0.24 ^b	1.31±0.50 ^a	1.32±0.87 ^a	1.38±0.02 ^a
SGR(%/day)				
Condition Factor(K)	0.54±0.12 ^a	0.58±0.91 ^a	0.47±0.99 ^b	0.36±0.00 ^c
Survival Rate	15.00±0.00 ^c	41.25±0.00 ^a	12.50±0.00 ^d	33.75±0.00 ^b
Mortality Rate	85.00±0.00 ^b	58.75±0.00 ^d	87.50±0.00 ^a	66.25±0.00 ^c

Mean in the same row with the same super script do not differ significantly (P<0.05)

NN♂ x NN♀ = Male and Female Normal *Clarias gariepinus*

AA♂ x AA♀ = Male and Female Albino *Clarias gariepinus*

NN♂ x AA♀ = Male Normal and female Albino *Clarias gariepinus*

AA♂ x NN♀ = Male Albino *Clarias* and Female Normal *Clarias gariepinus*

Table 4.6 Growth and Feed Utilization Parameters for F2

Table 4.6 Showed the Growth and Feed Utilization of F1 crossed between NN♂ x NN♀ gives the highest mean weight value of 809.98±0.00g, followed by NN♂ x AA♀ with 804.13 ±0.00g and AA♂ x AA♀ with 731.08±0.00g and AA♂ x NN♀ 716.03±0.00g was the least after 16 months of culture. The Specific Growth Rate (SGR %/day) recorded highest among the crosses in AA♀ x NN♂ with value of (1.37±0.07) follow by NN♂xNN♀ with value of (1.31±0.16). The least recorded in AA♂ x AA♀ with (1.23±0.14). The maximum values for mean final length (37.87±0.40cm) was recorded in AA♂ x NN♀ followed by NN♂ x AA♀ (35.20 ±0.00cm), NN♂ x NN♀ (33.80±0.00cm), whereas the minimum size for mean final length (32.67±0.00 cm) was observed in AA♀ x AA♀. There was no significant difference (P> 0.05) in final mean weight gain of the crosses. Survival rate was high in crossing between the pure albino *Clarias* AA♂xAA♀ with 56.25±0.00% while crossing between albino female and normal male *Clarias Gariepinus* NN♂ X AA♀ has the least value of 38.75±0.00% respectively.

Table 4.6 Growth and Feed Utilization parameters of the F2 Crosses of *Clarias normal* and albino *Clarias gariepinus*

Parameters	Matting Combination			
	NN♂ x NN♀	AA♂ x AA♀	NN♂ x AA♀	AA♂ x NN♀
Mean initial weight(g)	4.81±0.00 ^a	4.44±0.33 ^b	3.82±0.00 ^c	3.04±0.33 ^d
Mean final weight (g)	809.98±0.00 ^b	731.08±0.00 ^c	804.13±0.00 ^b	916.03±0.00 ^a
Mean weight gain(g)	805.17±0.00 ^b	726.64±0.00 ^d	800.18±0.00 ^b	912.99±0.00 ^a
Mean initial length (cm)	2.62±0.50 ^a	2.40±0.00 ^c	2.72±0.50 ^b	2.62±0.50 ^b
Mean final length (cm)	33.80±0.00 ^b	32.67±0.00 ^c	35.20±0.00 ^b	39.87±0.40 ^a
Mean length gain (cm)	33.42±0.50 ^b	30.27±0.50 ^b	32.50±0.00 ^c	37.25±0.00 ^a
Feed intake (g)	20.50±0.00 ^c	15.25±0.00 ^c	9.00±0.00 ^d	27.75±0.00 ^a
Feed Conversion ratio FRC(g)	0.020±0.75 ^b	0.021±0.25 ^c	0.011±0.50 ^d	0.039±0.50 ^c
Specific Growth Rate SGR(%/day)	1.23±0.68 ^c	1.23±0.14 ^c	1.31±0.16 ^b	1.37±0.70 ^a
Condition Factor(K)	0.27±0.83 ^a	0.18±0.48 ^c	0.20±0.00 ^b	0.19±0.00 ^c
Survival Rate	51.25±0.00 ^b	56.25±0.00 ^a	38.75±0.00 ^c	56.25±0.00 ^a
Mortality Rate	48.75±0.00 ^d	43.75±0.70 ^b	61.25±0.00 ^a	43.75±0.70 ^a

Mean in the same row having the same super script do not differ significantly (P<0.05).

NN♂ x NN♀ = Male and Female Normal *Clarias gariepinus*

AA♂ x AA♀ = Male and Female Albino *Clarias gariepinus*

NN♂ x AA♀ = Male Normal and female Albino *Clarias gariepinus*

4.7 Water Quality Parameters of the F1 Crossing Between Normal and Albino Clarias gariepinus

Table 4.7 present the result of mean water quality parameter of the F1 crosses between C. gariepinus Normal and Albino. The temperature recorded ranges from 26.00 – 32.00⁰C this fall within the tropical fish temperature range of 25.0 – 32.0⁰C Auta, (1993) is ideal for fish production in the tropics. The dissolved oxygen range from 4.00 - 12.00mg/l, these ranges are all found within the optimum range. The mean pH values ranges from 6.12 – 8.13 was within the recommended range. Means values of water quality parameters of Clarias gariepinus normal and albino among the experiments at 18 months as shown on Table 4.7:

Table 4.7 Water Quality parameters of F1 Crosses Between *Clarias gariepinus* normal and Albino *Clarias*.

Parameters	Mating Combination			
	NN♂ x NN♀	AA♂ x AA♀	NN♂ x AA♀	AA♂ x NN♀
Ammonia (Mg/l)	0.30±0.25 ^c	0.33±0.25 ^b	0.32±0.50 ^b	0.34±0.00 ^a
Temperature (°C)	27.72±0.50 ^a	26.52±0.50 ^c	26.12±0.50 ^b	27.25±0.00 ^a
Conductivity (µm/l)	0.62±0.50 ^a	0.51±0.00 ^d	0.56±0.75 ^c	0.58±0.00 ^b
Dissolved Oxygen (Mg/l)	5.27±0.75 ^c	6.15±0.00 ^b	6.20±0.00 ^a	6.20±0.00 ^a
Ph	7.87±0.50 ^a	8.00±0.00 ^a	8.00±0.00 ^a	8.00±0.00 ^a

Mean in the same row having the same super script do not differ significantly ($P < 0.05$).

$NN♂ \times NN♀$ = Male and Female Normal *Clarias gariepinus*

$AA♂ \times AA♀$ = Male and Female Albino *Clarias*

$NN♂ \times AA♀$ = Male Normal *Clarias gariepinus* and female Albino

$AA♂ \times NN♀$ = Male Albino *Clarias* and Female Normal *Clarias gariepinus*

4.8 Water Quality parameters of the F2 Crossing Between Normal and Albino Clarias gariepinus

The mean water quality parameter ranges are found within the optimum range. The temperature recorded ranges from 26.00 – 32.00°C this fall within the tropical fish temperature range of 25.0 – 32.0°C. The dissolved oxygen range from 4.00 - 12.00mg/l. Which is within the normal range required for fish growth? The mean pH values ranges from 6.12 – 8.13 was within the recommended range according to (Onuoha and Njoku 1997) were of the view that pH value in fish culture should range between 6.5 -9.0. Grand Means values of water quality parameters of Clarias gariepinus normal and albino among the experiments at 18 months.

Table 4.8: Water quality parameters of the F2 Crosses Between Normal and Albino Clarias gariepinus.

Parameters	Mating		Combination	
	NN♂ x NN♀	AA♂ x AA♀	NN♂ x AA♀	AA♂ x NN♀
Ammonia(Mg/l)	0.28±0.25 ^c	0.33±0.00 ^b	0.31±0.75 ^b	0.33±0.75 ^a
Temperature(°C)	27.65±0.00 ^b	27.35±0.75 ^c	27.97±0.00 ^a	27.65±0.75 ^b
Conductivity(µm/l)	0.53±0.50 ^a	0.46±0.50 ^b	0.46±0.50 ^b	0.46±0.00 ^b
Dissolved Oxygen(Mg/l)	5.5±0.00 ^a	5.75±0.00 ^a	5.55±0.00 ^a	5.52±0.50 ^a
pH	7.5±0.00 ^a	7.50±0.00 ^a	7.47±0.50 ^b	7.47±0.50 ^b

Mean in the same row having the same super script do not differ significantly (P>0.05)

NN♂ x NN♀ = Male and Female Normal Clarias gariepinus

AA♂ x AA♀ = Male and Female Albino Clarias

NN♂ x AA♀ = Male Normal Clarias gariepinus and female Albino

AA♂ x NN♀ = Male Albino Clarias and Female Normal Clarias gariepinus

4.9 Morphometric and Meristic Characters of Offspring of Various Mating Combination for Clarias gariepinus normal and Albino for F1 and F2.

Nine variables were used to compare some morphometric and meristic characters of the parent stock. The nine variables namely; head width, eye diameter, occipital fontanelle length, frontal fontanelle length, pre-maxillary width, vomerine length, vomerine width. Pectoral fin length, and caudal peduncle length. Table 4.9.

*The low mahalanobis square distance between the two different pigmented Clarias species indicated their level of similarities to each other. The mahalanobis square distance (D^2) between male Albino and female normal ($AA \text{ ♂} \times NN \text{ ♀}$) female Albino and male normal ($NN \text{ ♂} \times AA \text{ ♀}$), Albino male and Albino female. ($AA \text{ ♂} \times AA \text{ ♀}$), and normal male and female ($NN \text{ ♂} \times NN \text{ ♀}$) *C. gariepinus* including their offspring in both *f1* and *f2* of the intraspecific hybrid were not significantly different ($P < 0.05$). This clearly indicates how extremely difficult it could be to distinguish this species from their intraspecific hybrid using their morphological and meristic features. There are varying patterns of inheritance of some characters by offspring of the various mating combinations, the hybrid for the offspring of mating combinations of Normal and Albino in *F1* and *F2* shows positive heterosis in the inheritance of brown eye colour, head width, premaxillary width, and vomerine width in which case they possess different body colors (brown)*

compared to the both parental with pink and black colored eyes. The head of all the mating combination shows the same flattened like the positive parent of C. gariepinus species. Therefore, this external features characteristic of both male and female used for each hybridization exercise seemed to have little to no influence to the external features of the resulting hybrid offspring except for the brown eyes and brown body observed in some offspring at the end of the research. The intraspecific hybrids however also show some level of positive heterosis in the inheritance of frontal fontanelle length similar to their parents as shown in (table 4.6) and also as a reflection of head length as shown in the same table which there were no significant differences in other cephalic traits between the intraspecific hybrids and the parental. There has not been any previous documented morphological description of the intraspecific hybrids of these species.

In the inheritance of the adipose fin length, the dorsal fin, shows no significant difference ($P < 0.05$) in the hybrid of F1 and F2. The intermediate morphological traits of the two different coloured hybrids suggest that they are product of true fission of the genome of two different colored fishes, except for some of the hybrid especially the reciprocal hybrids of female Albino and normal male ($AA^{\ominus} \times NN^{\oplus}$), male Albino and female normal ($AA^{\oplus} \times NN^{\ominus}$) had brownish color when observed phenotypically as discussed earlier. Morphological abnormalities observed in some offspring of the mating combination. The skin of the albino cat fish tends to be harder than the normal colour

C. gariepinus, this was observed during breeding when ovaprim was injected to the females both in F1 and F2 generation, and this probably is due to environmental condition.

Table 4.9 Morphometric and Meristic Characters of Offspring of Various Mating Combination for Normal and Albino *Clarias gariepinus* for F1 generation.

Mating combination	Number sampled	Head width	Eye diameter	Occipital fontanelle width	Frontal fontanelle length	Pre maxillary width	Vomerine length	Vomerine width	Pelvic fin length	Caudal peduncles length
NN♂ x NN♀	10	4.21±0.32 ^b	0.61±0.31 ^a	0.61±0.31 ^a	2.56±0.31 ^a	2.61±0.32 ^a	0.61±0.31 ^a	1.80±0.43 ^a	2.30±0.30 ^a	1.20±0.32 ^a
AA♂ x AA♀	10	5.20±0.31 ^a	0.52±0.31 ^b	0.40±0.61 ^b	2.13±0.22 ^b	2.41±0.13 ^a	0.32±0.34 ^b	1.60±0.31 ^b	2.10±0.30 ^b	1.32±0.36 ^a
NN♂ x AA♀	10	4.80±0.35 ^b	0.72±0.31 ^a	0.60±0.83 ^a	2.47±0.23 ^a	2.00±0.23 ^b	0.36±0.32 ^b	1.5±0.23 ^b	2.00±0.30 ^b	1.10±0.82 ^b
AA♂ x NN♀	10	5.30±0.34 ^a	0.51±0.33 ^b	0.50±0.13 ^a	2.12±0.21 ^b	2.20±0.34 ^a	0.52±0.35 ^a	1.8±0.34 ^a	2.41±0.31 ^a	1.00±0.31 ^b

Mean in the same Column with the same super script do not differ significantly (P<0.05).

Table 4.10 Morphometric and Meristic Characters of Offspring of Various Mating Combination for Normal and Albino *Clarias gariepinus* for F2 generation.

Mating combination	Number sampled	Head width	Eye diameter	Occipital fontanelle width	Frontal fontanelle length	Pre maxillary width	Vomerine length	Vomerine width	Pelvic length	fin	Caudal peduncles length
NN♂ x NN♀	10	4.20±0.32 ^b	0.59±0.31 ^a	0.60±0.31 ^a	2.56±0.31 ^a	2.61±0.32 ^a	0.51±0.31 ^a	1.70±0.43 ^a	2.30±0.30 ^a		1.20±0.32 ^a
AA♂ x AA♀	10	5.18±0.31 ^a	0.52±0.31 ^b	0.40±0.61 ^b	2.13±0.22 ^b	2.41±0.13 ^a	0.42±0.34 ^b	1.60±0.31 ^b	2.10±0.30 ^b		1.32±0.36 ^a
NN♂ x AA♀	10	4.80±0.35 ^b	0.72±0.31 ^a	0.60±0.83 ^a	2.47±0.23 ^a	2.00±0.23 ^b	0.36±0.32 ^b	1.5±0.23 ^b	2.00±0.30 ^b		1.10±0.82 ^b
AA♂ x NN♀	10	5.30±0.34 ^a	0.51±0.33 ^b	0.50±0.13 ^a	2.12±0.21 ^b	2.20±0.34 ^a	0.52±0.35 ^a	1.8±0.34 ^a	2.41±0.31 ^a		1.00±0.31 ^b

Mean in the same column having the same super script do not differ significantly (P<0.05).

4.11 Genetic Analysis of the Parent Stock

The result of the analysis is shown in table 4.11. There were slight significant different between the parent stock. For the genetic analysis, two microsatellite loci were used for population structure analysis of normal pigmented and albino *Clarias gariepinus*. The two loci (Cg01 and Cg02) showed amplification and as a result, the locus could not be scored. The size of the microsatellite loci ranged from 92 to 168 bp, with low estimates of null allele frequencies across all populations for the parent stock includes: male and female normal pigmented *Clarias gariepinus*, male and female albino *C. gariepinus*. The population Descriptive Statistics of the parent stocks show that, the allele frequencies for Cg01 locus are 87.5% and 12.5% for alleles A and B respectively. The locus Cg02 is 100% for A allele as shown in Table 4.11.

Table 4.11: Allele Frequency of Parent Stock

Allele \ Locus	Cga01	Cga02
Allele A	0.875	1.000
Allele B+	0.125	0.000

Cg01 Locus of Normal Clarias gariepinus

Cg02 Locus of Albino Clarias gariepinus

Table 4.1.2 Showed the observed number of alleles (na), effective number of alleles (ne), Shannon's Information index (I) for the two loci Cg01 and Cg02 are 2.00, 1.28, 0.38 and 1.0, respectively were shown in Table 4.12. The values of the mean \pm standard deviation for Cg01 and Cg02 loci are: $na = 1.5 \pm 0.71$, $ne = 1.14 \pm 0.20$ and $I = 0.19 \pm 0.27$.

Table 4.12: Summary of Genetic Variation Statistics for All Loci of Parent Stock

Locus	*na	ne*	ne*	I*
Cg01	16	2.0000	1.2800	0.3768
Cg02	14	1.0000	1.0000	0.0000
Mean	15	1.5000	1.1400	0.1884
St. Deviation		0.7071	0.1980	0.2664

* na = Observed number of alleles

* ne = Effective number of alleles [Kimura and Crow (1964)]

* I = Shannon's Information index [Lewontin (1972)]

Cg01 of Normal *Clarias gariepinus*

Cg02 of Albino *Clarias gariepinus*

Table 4.13 Showed the summary of heterozygosity for all loci in the parent stock. The locus Cg01 has an average heterozygosity of 0.1406 with Nei's (1973) expected heterozygosity of 0.2188. The locus Cg02 does not show any heterozygosity (indicating 100% homozygosity). The mean \pm standard deviation Nei's (1973) and average heterozygosity are 0.1094 ± 0.1547 and 0.0703 ± 0.0994 respectively. The percentage polymorphic loci which measures and quantify genetic diversity in the population is 50%..

Table 4.13 Summary of Heterozygosity Statistics for All Loci of Parent Stock

Locus	Sample Size	Obs. Hom.	Obs. Het.	Exp. Hom.*	Exp. Het.*	Nei**	Ave. Het.
Cg01	16	0.7500	0.2500	0.7667	0.2333	0.2188	0.1406
Cg02	14	1.0000	0.0000	1.0000	0.0000	0.0000	0.0000
Mean	15	0.8750	0.1250	0.8833	0.1167	0.1094	0.0703
St. Dev.		0.1768	0.1768	0.1650	0.1650	0.1547	0.0994

KEY:

* Expected homozygosity and heterozygosity were computed using Levene (1949)

** Nei's (1973) expected heterozygosity

The number of polymorphic loci is: 1

The percentage of polymorphic loci is: 50.00 %

Cg01 of Normal *Clarias gariepinus*

Cg02 of Albino Clarias gariepinus

Table 4.14. Showed the mean Fixation index (Fst) which is a measure of population differentiation due to genetic structure is (Fst = 0.1429, p<0.05), indicating no considerable divergence in the population as shown in Table 4.13. A value of zero in the Cg02 locus implies complete panmixis that is the two populations are interbreeding freely.

Table 4.14 Summary of F-Statistics and Gene Flow for All Loci of Parent Stock

Locus	Sample Size	Fst(i)	Nm*
Cg01	16	0.1429	1.5000
Cg02	14	0.0000	****
Mean	15	0.1429	1.5000

Keys:

** Nm = Gene flow estimated from $Fst = 0.25(1 - Fst)/Fst$.*

Cg01 of Normal Clarias gariepinus

Cg02 of Albino Clarias gariepinus

Table 4.15 Showed the Nei's Original Measures of Genetic Identity and Genetic Distance of the parent stock. The level of genetic identity ranged from 0.9707 to 1.0000, while the genetic distance ranged from 0 to 0.0297. A similar result is seen in Table 4.15, where Nei's unbiased measure of genetic identity and genetic distance showed no variation between the albino and normal pigmented *Clarias gariepinus*. The dendograms based on the genetic distance from the parents are shown in appendix 1 and appendix 2 respectively

Table 4.15 Nei's Original Measures of Genetic Identity and Genetic Distance of the Parent Stock

Pop. ID	AA male	AA Female	NN Male	NN Female
AA Male	****	0.9707	0.9707	1.0000
AA Female	0.0297	****	1.0000	0.9707
NN Male	0.0297	0.0000	****	0.9707
NN Female	0.0000	0.0297	0.0297	****

Keys:

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

AA = Albino *Clarias gariepinus*

NN = Normal *Clarias gariepinus*

Table 4.16 Showed the Nei's Unbiased Measures of Genetic Identity and Genetic Distance of the parent stock. The level of genetic identity ranged from 0.9871 to 1.0341, while the genetic distance ranged from 0 to 0.0130. A similar result is seen above in Table 4.14. The Nei's unbiased measure of genetic identity and genetic distance showed no variation between the albino and normal pigmented *Clarias gariepinus*. The dendograms based on the genetic distance from the parents are shown in appendix 1 and appendix 2 respectively

Table 4.16 Nei's Unbiased Measures of Genetic Identity and Genetic Distance of Parent Stock

Pop. ID	AA male	AA Female	NN Male	NN Female
AA Male	****	0.9871	0.9871	1.0000
AA Female	0.0130	****	1.0341	0.9871
NN Male	0.0130	0.0000	****	0.9871
NN Female	0.0000	0.0130	0.0130	****

Keys:

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

AA = Albino *Clarias gariepinus*

NN = Normal *Clarias gariepinus*

4.17 Genetic Analysis of the Offspring in F₁

Population Descriptive Statistics for the offspring shows that the allele frequencies of the offspring from the two loci are 93.75% of A allele and 6.25% of B allele for the Cg01 locus. The Cg02 locus does not show any level of polymorphism with an allele frequency of 100% of A allele as shown in

Table 4.16.

Table 4. 17 Allele Frequency of Offspring F₁

Allele \ Locus	Cg01	Cg02
Allele A	0.9375	1.000
Allele B	0.0625	0.000

Keys:

Cg01 Normal Clarias gariepinus

Cg02 Albino Clarias gariepinus

4.18 The Observed Allele Frequency

The observed number of allele (n_a) is 2.0 and 1.0 for locus Cg01 and Cg02 respectively. The effective number of allele (Kimura and Crow, 1964) is 1.1327 and 1.0 for locus Cg01 and Cg02 respectively. The Shannon's Information index is 0.2338 for locus Cg01 as shown in Table 4.17.

Table 4.18: Summary of Genetic Variation Statistics for all Loci of Offspring F1

Locus	* n_a	n_e^*	n_e^*	I^*
Cg01	16	2.0000	1.1327	0.2338
Cg02	10	1.0000	1.0000	0.0000
Mean	13	1.5000	1.0664	0.1169
Standard. Deviation(\pm)		0.7071	0.0939	0.1653

Keys:

* n_a = Observed number of alleles

* n_e = Effective number of alleles [Kimura and Crow (1964)]

* I = Shannon's Information index [Lewontin (1972)]

4.19 The Summary of Heterozygosity

Table 4.19 showed the summary of heterozygosity statistics for all loci. The observed heterozygosity ranged from 87.5% (Cg01) to 100% (Cg02), the observed heterozygosity ranged from 0 to 12.5%.

Table 4.19: Summary of Heterozygosity Statistics for All Loci of Offspring F1

Locus	Sample Size	Obs. Hom.	Obs. Het.	Exp. Hom.*	Exp. Het.*	Nei**	Ave. Het.
Cg01	16	0.8750	0.1250	0.8750	0.1250	0.1172	0.1406
Cg02	10	1.0000	0.0000	1.0000	0.0000	0.0000	0.0000
Mean	13	0.9375	0.0625	0.9375	0.0625	0.0586	0.0703
St. Dev.		0.0884	0.0884	0.0884	0.0884	0.0829	0.0994

Keys:

* Expected homozygosity and heterozygosity were computed using Levene (1949)

** Nei's (1973) expected heterozygosity

The number of polymorphic loci is: 1

The percentage of polymorphic loci is: 50.00 %

Exp. Hom = Expected homozygosity

Exp. Het = Expected heterozygosity

4. 20 The summary of F-Statistics and Gene Flow

Table 4.20 showed the mean Fixation index ($F_{st}=0.20$, $p<0.05$), indicating no considerable divergence in the population.

Table 4. 20: Summary of F-Statistics and Gene Flow for all Loci of Offspring F1

Locus	Sample Size	Fst	Nm*
Cg01	16	0.2000	1.0000
Cg02	10	0.0000	****
Mean	13	0.2000	1.0000

Keys:

* $Nm = \text{Gene flow estimated from } F_{st} = 0.25(1 - F_{st})/F_{st}$.

4.21 The Nei's Original Measures of Genetic Identity/Distance

Table 4.21 Showed Nei's original measures of genetic identity/distance and Nei's unbiased measures of genetic identity/distance respectively. The albino X albino offspring showed no genetic diversity, likewise the hybrid (albino X normal).

Table 4.21: Nei's Original Measures of Genetic Identity and Genetic Distance of Offspring F1

Pop. ID	Normal	Albino	Alb. X Norm.	Norm. X Alb.
NN♂ x NN♀	****	0.9707	1.0000	1.0000
AA♂ x AA♀	0.0297	****	0.9707	0.9707
NN♂ x AA♀	0.0000	0.0297	****	1.0000
AA♂ x NN♀	0.0000	0.0297	0.0000	****

Keys:

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

$NN♂ \times NN♀ = \text{Male and Female Normal Clarias gariepinus}$

$AA♂ \times AA♀ = \text{Male and Female Albino Clarias gariepinus}$

$NN♂ \times AA♀ = \text{Male Normal and female Albino Clarias gariepinus}$

$AA♂ \times NN♀ = \text{Male Albino and Female Normal Clarias gariepinus}$

4.22 The Nei's Unbiased Measures of Genetic Identity/ Distance of Offspring F1

The positions of the offspring are shown as similarities with table 4.20, on the Nei measures of original and unbiased genetic identity and genetic distance in Table 4.21.

Table 4.22 Nei's Unbiased Measures of Genetic Identity and Genetic Distance of Offspring F1

Pop. ID	Normal	Albino	Alb. X Norm.	Norm. X Alb.
NN♂ x NN♀	****	0.9871	1.0000	1.0000
AA♂ x AA♀	0.0130	****	0.9871	0.9871
NN♂ x AA♀	0.0000	0.0130	****	1.0000
AA♂ x NN♀	0.0000	0.0130	0.0000	****

Keys:

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

$NN♂ \times NN♀ = \text{Male and Female Normal Clarias gariepinus}$

$AA♂ \times AA♀ = \text{Male and Female Albino Clarias}$

$NN♂ \times AA♀ = \text{Male Normal Clarias gariepinus and female Albino}$

$AA♂ \times NN♀ = \text{Male Albino Clarias and Female Normal Clarias gariepinus}$

The overall genetic identity and genetic distance is shown in Table 4.22. The genetic distance ranged from 0 to 0.029, while the level of genetic identity ranged from 0.9707 to 1.

Table 4. 23: Overall Nei's Original Measures of Genetic Identity and Genetic Distance for Parents and Offspring's F1 and F2

Pop. ID	AA♂	AA♀	NN♂	NN♀	NN	AA	AA♂ x NN♀	NN♂ x AA♀
AA♂	****	0.9707	0.9707	1.0000	1.0000	0.9707	1.0000	1.0000
AA♀	0.0297	****	1.0000	0.9707	0.9707	1.0000	0.9707	0.9707
NN♂	0.0297	0.0000	****	0.9707	0.9707	1.0000	0.9707	0.9707
NN♀	0.0000	0.0297	0.0297	****	1.0000	0.9707	1.0000	1.0000
NN	0.0000	0.0297	0.0297	0.0000	****	0.9707	1.0000	1.0000
AA	0.0297	0.0000	0.0000	0.0297	0.0297	****	0.9707	0.9707

AA♂ x NN♀	0.0000	0.0297	0.0297	0.0000	0.0000	0.0297	****	1.0000
NN♂ x AA♀	0.0000	0.0297	0.0297	0.0000	0.0000	0.0297	0.0000	****

Keys:

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

AA♂ = Albino male

AA♀ = Albino female

NN♂ = Normal male *Clarias gariepinus*

NN♀ = Normal female *Clarias gariepinus*

NN = Normal

AA = Albino

AA♂ x NN♀ = Albino male and Normal female *Clarias gariepinus*

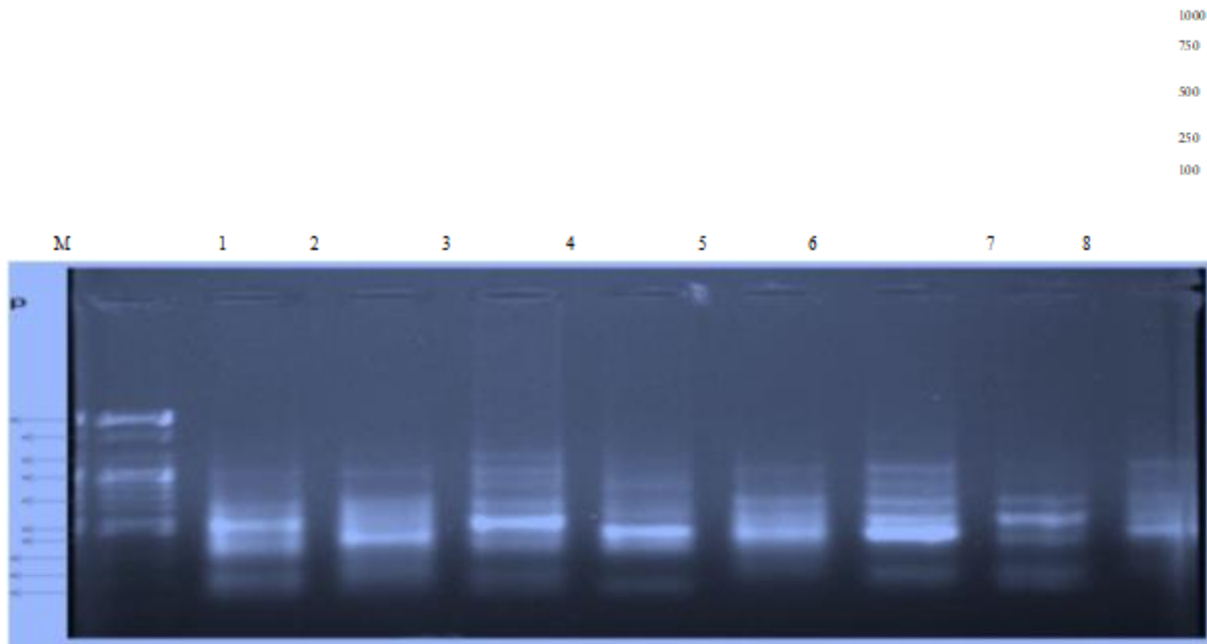
NN♂ x AA♀ = Normal male and Albino female *Clarias gariepinus*

4.24 The RAPD Analysis

*Plate IX: The Rapid profiles and polymorphism generated by PCR: Among the 2 primers screen, normal *Clarias gariepinus* yield comparstively larger number of bans with good resolution though, normal *Clarias gariepinus* Plate ix showed more discriminating bands than albino *Clarias gariepinus* each primer produced a unique fragrament pattern of amplified DNA with varied*

number of bands plate xiii, both fish have resulted to 50.00% polymorphism, it was observed that the gene flow among the two Clarias gariepinus was 1.0000.

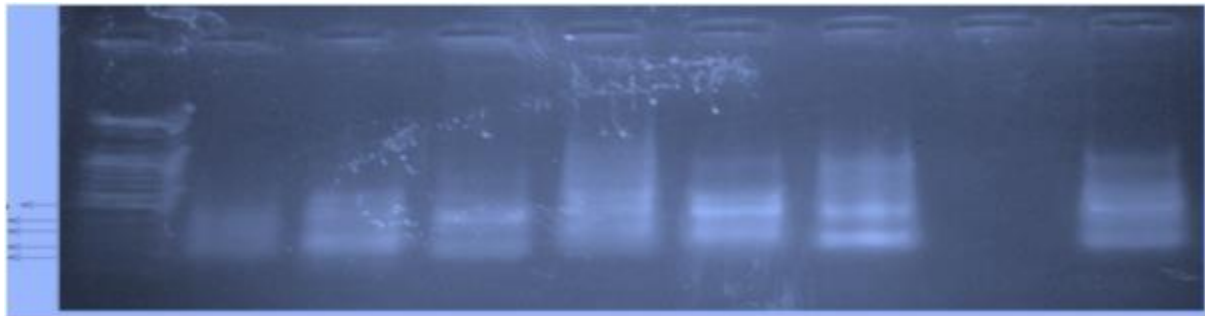
Normal *Clarias gariepinus*



M 1 2 3 4 5 6 7

1000
750
500
250
100

Albino *Clarias gariepinus*



**Plate X: RAPD profile of Normal and Albino *Clarias gariepinus* primer M: 0.92 - 1680bp
DNA ladder**

Table 4.25 Estimates of Genetic Variation: Number and proportion of Polymorphic loci, Gene Diversity, Number of Allels, Effective Number of Allele and Gene Flow.

Parameters	AA	NN
Number of polymorphic loci	1.00	1.00
Polymorphic loci (%)	50.00	50.00
Gene diversity	0.000	0.140
Observed number of allele	10.000	16.000
Effective number of allele	1.000	2.000
Gene flow	1.000	

Where AA = Albino *Clarias gariepinus*

NN = Normal *Clarias gariepinus*

4.26 Colour Variation among the Offspring of the Various Mating Combination F1 and F2.

A chi square (χ^2) analysis of the number of colours of offspring in F1 and F2 at (2 months) indicated that there was a significant difference ($P < 0.05$) at phenotypic appearances of male albino and female normal ($AA^{\text{♂}} \times NN^{\text{♀}}$), female albino and male normal ($AA^{\text{♀}} \times NN^{\text{♂}}$) respectively. This is presence in both F1 and F2 in the mating combination of the hybrid offspring produced, an analysis to evaluate if there was any departure from the expected 1:2 Colour ratio is shown in Table 4.25 and 4.26, this revealed that there was two different colour appearance of the mating combination of albino and normal (AA x NN) except for the brown colour observed in some combination of F1. The male albino and female normal ($AA^{\text{♂}} \times NN^{\text{♀}}$), female albino and male normal ($AA^{\text{♀}} \times NN^{\text{♂}}$) in the two offsprings of F1 and F2 shows a ratio 1:2:20, 1:2:24 and 1:2:20, 1:2:29 respectively. All the other mating combination has offspring that did not differ from 1:1 colour ratio of the offsprings.

Table 4.26 Variation in Colour Ratio among the Offspring of the Various Mating Combination F1.

COMBINATIONS	AA♂ x AA♀	AA♀ X NN♂	AA♂ X NN♀	NN♀ X NN♂
NORMAL <i>Clarias</i>		129	146	208
ALBINO <i>Clarias</i>	104	53	82	
Color ratio	1:1:10 ^b	1:2:20 ^a	1:2:24 ^a	1:1:13 ^b

Mean in the same row with the same super script do not differ significantly ($P < 0.05$).

Table 4.27: Variation in Colour Ratio among the Offspring of the Various Mating Combination F2.

COMBINATIONS	AA♂ x AA♀	AA♀ X NN♂	AA♂ X NN♀	NN♀ X NN♂
NORMAL <i>Clarias</i>		135	164	218
ALBINO <i>Clarias</i>	147	52	82	
COLOUR RATIO	1:2:20 ^b	1:2:24 ^a	1:2:20 ^a	1:1:29 ^b

Mean in the same row having the same super script do not differ significantly ($P < 0.05$).

CHAPTER FIVE

5.0 DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussions

5.1 The latency period of Normal and Albino female Clarias gariepinus in parent stock and F1 generation.

The latency period for the parent stock of female albino Clarias gariepinus had the highest latency period of 13.50 hours, While normal female Clarias gariepinus had less latency period of 12.0 hours at temperature of 27.5°C and the Latency period for F1 generation showed 13.25 hours and the normal female had 12.15 hours at 28.30°C respectively, this agreed with Crandell (1996), who reported that the higher the temperature, the lower the latency period, and the optimum temperature to keep the fish is at 25 and the fish will be ready in about 11-13 hours, while according to FAO (1996), latency period of 10 hrs are unarguable, but the best latency period for Clarias gariepinus is at 29.5°C.

This study observed some rigid and hard skin of the female albino during administration of the hormone ovaprim in the combination of parent stock and the F1, this is different from the normal female Clarias gariepinus skin as described by Ugwumba and Ugwumba (1998) who reported the effectiveness of ovaprim in inducing ovulation and spawning of C. gariepinus studied in female weighing 280-600g and male weighing 670-1800g.

The mean fertilization and hatchability rate for the four crosses investigated in their study were quite low. The pure bred Normal X Normal, and crossed between normal male C. gariepinus and albino female (44.79% and 42.92%) respectively gave the highest fertilization and hatchability rates in comparison with the other combinations. Lower fertilization and hatchability in Albino X

Albino and Normal female x Albino male (27.29% and 37.18%). The finding agreed with the work of Bondari (1984), who reported that Albino are more difficult to spawn than the Normal pigmented. However, the author reported in another study that there were no significant differences as a result of pigmentation in percentage hatchability, between albino (60%) and normal pigmented (61%) Channel catfish respectively. The temperature might account for some of the differences in brood stock performance during this study. Albino female may have had more rigid requirements for spawning and lower tolerance for deviation from optimum conditions compared to Normal pigmented females.

*The mean weight gain of the four genetic crosses under study for 18 months was between 809.98g and 726.64g. The hybrid of the Normal pigmented showed the highest final mean weight gain of 897.45g. This is due to the combination of the two characters from the parents. This is similar to the work of Onyia et al. (2016) who studied the growth and survival of normal coloured and Albino *Clarias gariepinus* and reported that the highest growth rate was 11.82g in the same crosses. There was a difference in growth performance between Albino and Normal *Clarias gariepinus* crosses, the Normal *Clarias gariepinus* exhibited a higher growth performance than the Albino in the subsequent crosses. This was similar to earlier studies which reported Normal pigmented channel catfish (*Ictalurus punctatus*) having body weight and total length (77.8g vs 74.7g and 41.7cm vs 35.5cm). The result from the present study is also similar to the work of (Karatas and Kocaman, 2014) who reported that Normal Coloured Rainbow trout had the highest growth in weight and specific growth rate (19.3g and 2.2) when compared to the Albino Rainbow trout (17.2g and 2.0). The study differed from the work of Gaudie et al. (1995) who reported that growth of albino catfish was similar to that of Normal *C. gariepinus* catfish. The difference in*

growth performance of albino and normal pigmented catfish may be attributable to the pigmentation and its possible pleiotropic effect.

The results showed that the survival of larvae up to the first feeding stage were similar in all the genetic groups investigated. The survival rate was high in the crosses between albino and normal C. gariepinus than the both individual parents. This study was as well similar with the work of Bondari, (1984) who reported that Albino catfish had lower survival rate at fry stage than Normal fish, while cross between the two has the highest survival rate, though dress-out percentage are nearly equal. The study shows the morphometric and meristic character of the whole four mating combinations of both in f1 and f2, Albino (AA♂ x AA♀), Albino male x normal female (AA♂ x of pure albino and pure normal (NN♀), Albino female x normal male (AA♀ x NN♂), normal male and normal female (NN♂ x NN♀) showed the same resemblance in terms of the various characters measured. Most of the characters of interest among the combination were not significantly different ($p > 0.05$).

The F1 generation are of similar features to the parents in terms of caudal peduncle length in the various mating combination as shown in table 4.6. The little variation was found in head width, eye diameter and occipital fontanelle of the F1 generation which was higher than that of the parents. This shows that the hybrid has high potential character of growing bigger than both parent. The results showed the colour ratio of the offspring in F1 and F2 at (2 months) this indicated that there was significant ($P < 0.05$) difference at phenotypic appearances of the crosses between male albino and female normal (AA♂ x NN♀), female albino and male normal (AA♀ x NN♂) respectively. An analysis to evaluate if there was any departure from the expected 1:2 Colour ratio is shown in Table 4.19 and 4.20, this revealed that there was two different colour appearance of the mating combination of albino and normal (AA x NN) except for the deep brown

colour observed in some combination in F1 of this study. The male albino and female normal ($AA^{\text{♂}}$ x $NN^{\text{♀}}$), female albino and male normal ($AA^{\text{♀}}$ x $NN^{\text{♂}}$) in the two offsprings of combination had offspring that did not depart from 1:1 colour ratio of the offsprings.

The results from the studies showed that the ratio of normal pigmented breed and albino normal hybrid were not significantly different with the phenotypic ratio and genotypic ratio between the crosses, The genetic control of these colour traits in normal pigmented parents had only normal pigmented offspring; these results showed that a single gene controls normal colour, as the correlation and duplicated nature of this locus is, it was observed that allele was linked to a dominant alleles with homozygote, since both parents had normal pigmented colour and the progeny segregated for this trait. Albino breed and normal breed were significantly different from other crosses.

*The crosses between the albino broodstock resulted in phenotypical ratio of 4:1 with genetically alleles of homozygote recessive and heterozygote which consists of two different colour offspring, albino and lightly normal pigmented. The likelihood of these two parents producing an offspring with albino phenotype was 80%. Similarly, the offspring of this cross has 20% chance of expressing the lightly normal coloured phenotype and deep brown coloured. The findings differed from report by Rothbard and Wohlfarth (1993), who reported that inheritance of albinism in grass carp, as a results of a cross between wild-type heterozygote male and albino female. Among the resulting progeny 52.0% fish were of wild-type colour while 48.0% were albino. The crosses of normal pigmented and albino *Clarias gariepinus* produces normal pigmented heterozygote with deep brown colour observed among the offspring in F1 and F2 generations. The findings were relevant with the co-dominant and incomplete dominant cases reported by Tave (1993). One allele is not completely dominant over the other. There is a blending with the heterozygous offspring or*

*both alleles contribute to the phenotype. Co-dominant is a system in which alleles are from each homozygote parents combine in the offspring and the offspring simultaneously demonstrates both parent phenotypes. The finding agreed with the work of Maliszewski (1987) who stated that progeny from a wild female and yellow albino male were half wild and half brownish yellow. These reports do not conform with work of Gomelsky et al. (1996) on dihybrid crosses that investigated the colour ratios in progenies obtained after crossing of two-color and tri-colour. Result show from that study that the white-red colour complex and the presence of black patches in koi were inherited independently and the presence of black patches was controlled by the dominant mutation of one gene. Unlike this study which deal with mono hybrid crosses. This study showed that cross breeding of normal and albino *C. gariepinus* could produce colours of both alleles.*

Albino male heterozygote was crossed with the normal pigmented homozygote; the offspring were heterozygote normal pigmented. Complete dominant gene action occurs when the dominant allele is so strong that it produces its phenotype, regardless of the genotype. Only a single dominant allele is needed to produce the dominant phenotype. This means that the homozygous dominant and heterozygous genotypes both produce the dominant phenotype.

The determination of the genotypic variation of four parents and their different crosses of offspring in populations using two microsatellite loci was considered, Microsatellites have been increasingly used as molecular markers, because their polymorphisms have shown high efficiency for many studies (King et al. 2001). In the present study, all selected microsatellites were polymorphic, fourteen and sixteen different alleles were observed among all populations. The mean number of alleles per locus within populations ranged from 1.1400 to 1.500. The lowest allele number within populations was 1, and the highest was 2. In general, there was significant correlation between genetic diversity and the number of alleles. Therefore, the number of alleles

can be used for the evaluation of genetic diversity (Huang et al. 2002). Average observed heterozygosity (H_e) and homozygosity (H_o) within populations varied from 0.7500 to 0.7633 and 0.2500 to 0.2668 respectively. In these studies, heterozygote's for microsatellite loci Cg01 the PCR result has two bands on a gel which represented two alleles from $AA♂$ and $AA♀$ offspring, while homozygote's have one band (one allele) from $AA♀$ and $NN♂$, $NN♀$, and $NN♂$ offspring in the population. Similar study by Taniguchi et al. (1999) reported that variation of 14 red sea bream individuals for locus electrophoresis gel represents genotype of one individual fish. The numbers under each gel show that the genotype of the fish was heterozygous with the size of two band allele and the second allele on the gel two homozygous fish whose profiles have only one band. However, a homozygote excess was estimated in the parents and the result was 100% in both the parent and offspring of the Cg01, these might be caused by the effect of parents of the brood stock which was collected at different locations in the hatchery. Each sub-population tends to increase the homozygosity, and consequently excessive homozygosity which was detected in the parents formed in the hatchery station. On the other hand, the deficiency of heterozygosity was recorded in the locus Cg01 as against the high value recorded in Cg02. Genetic differentiation detected by F_{ST} estimates between parent and offspring populations gave similar results.

The RAPD markers were reported to be highly reproducible in a size ranging between 200 and 1500 bp (Liu et al. 1999). Good quality and reproducible PCR product of 92 – 168bp was found in this study. This is in line with the study of Ikpeme et al. (2015) who reported good quality and reproducible PCR products of 100 bp- 1600 bp in *Clarias gariepinus*. This was also similar to those found in *Clarias batracus* (100 bp- 1200 bp) Garg et al. (2010) in India where the results show that from two primer screen, both normal and albino *Clarias gariepinus* show 50.0% polymorphism.

*There were significant genetic differences between parent and offspring populations. This genetic divergence may be caused by artificial selection, founder effects, and random genetic drift in the offspring stock or in the breeding program. The present results could also be due to the random changes in the allelic frequencies because of the sampling in small population. In comparison to the present study, the high level of heterozygosity in some loci and low level in others was reported by (Calcagnotto et al. 2001). The effective number of contributing parents was reduced (N_e from 19.8 to 12.9) but it was sufficient to maintain the offspring heterozygosity of 0.0994 consistent with the parental average of 0.0994. This indicated that the parental level of genetic variability was maintained in the progeny. The loss of genetic variability could be predicted when there was significant reduction in the N_e 's; such a loss was reported by (Ponte et al. 2006). The mean water quality parameter ranges as summarized were found within the optimum range. The temperature recorded during the study was in the range of 26.00 – 32.00⁰C this fall within the tropical fish temperature range of 25.0 – 32.0⁰C. Boyd, and Folorunsho (2007) recorded the range of 26.0 - 30.0⁰C as ideal for aquatic survivals. The dissolved oxygen range from 4.00 - 12.00mg/l. Which is within the normal range required for fish growth? The mean pH values ranges from 6.12 – 8.13 was within the recommended range according to Onuoha and Njoku (2010) were of the view that pH value in fish culture should range between 6.5 -9.0. Grand Means values of water quality parameters of the reared *Clarias gariepinus* fingerlings in indoor glass aquaria for 12 weeks.*

5. Conclusions

The following conclusions were drawn from the results of the experiments conducted;

The latency period of female albino Clarias gariepinus was higher than that of female normal Clarias gariepinus with one hour differences at the same temperature of 27.5⁰C that is 13.40 and 12.00 hour respectively.

The Hybrid crosses from the female normal Clarias gariepinus had higher fecundity of 8688.0±0.00 than the parents stock. This is because of proper feeding and management.

Crosses between normal Clarias gariepinus male and female had the highest fertility and hatchability of 44.79±0.32 and 72.60±0.24, and has low survival rate of 15.00±0.00 at the end of the research.

Crosses between albino female and normal male Clarias gariepinus had the highest specific growth rate of 1.38±0.02 and 1.37±0.75 at F1 and F2 generations.

There were significant differences ($p < 0.05$) among the head width, eye diameter and occipital fontanelle of the F1 generation which was higher than that of the parents, also it was observed that there is different in colouration of deep brown in F1 and F2 generations.

The number of allele observed from the genetic analysis of parent stock and F1 generation showed heterozygosity and the second allele on the second band shows homozygosity. However, a homozygote excess was estimated in the parents and the result was 100% in both the parent and F1 generation of the normal and albino Clarias gariepinus. The parental level of genetic variability was maintained in the progeny.

5.3 Recommendations

Based on the aforementioned the following recommendations are made;

- 1. Selection of parent stock for crossing should be made from albino Clarias gariepinus and normal Clarias gariepinus male because of their high survival rate and high specific growth rate.*
- 2. Fish farmers should be encouraged to propagate albino Clarias gariepinus for genetic research purpose.*
- 3. Further studies should be carried out on the causes of neutral colour (deep brown) observed from the offsprings and the causes of hard skin in albino Clarias gariepinus.*
- 4. Further study should attempt on the causes of differences in the latency period of female albino and normal female Clarias gariepinus.*

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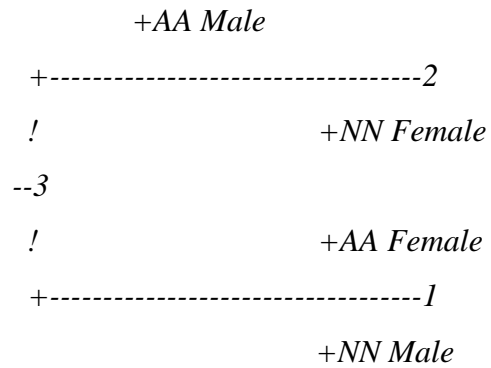
Appendix 1



Albino Clarias gariepinus (Burchell 1822). The subject of the study showing external morphology



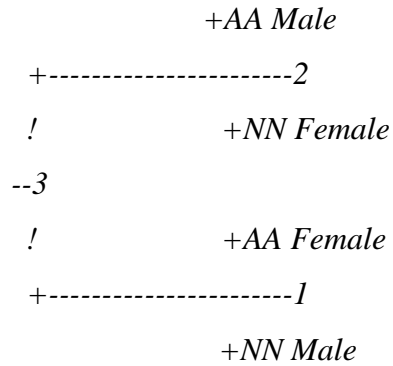
Normal Clarias gariepinus (Burchell 1822). The subject of the study showing external morphology



<i>Between</i>	<i>And</i>	<i>Length</i>
3	2	1.48558
2	<i>AA Male</i>	0.00000
2	<i>NN Female</i>	0.00000
3	1	1.48558
1	<i>AA Female</i>	0.00000
1	<i>NN Male</i>	0.00000

Original Genetic distance Dendrogram of Normal Pigmented and Albino C. gariepinus parents

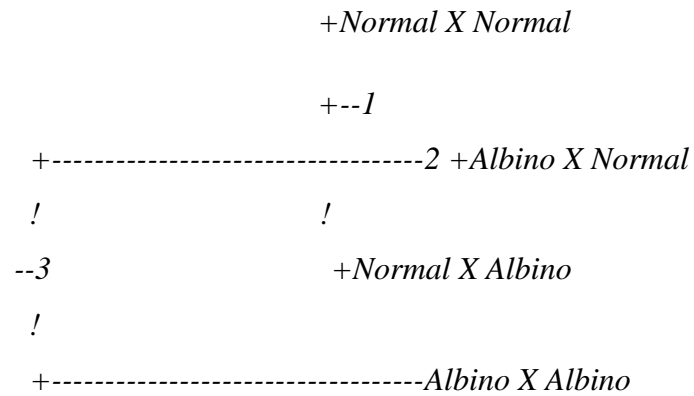
Appendix 2



<i>Between</i>	<i>And</i>	<i>Length</i>
-----	----	-----
3	2	0.64752
2	AA Male	0.00000
2	NN Female	0.00000
3	1	0.64752
1	AA Female	0.00000
1	NN Male	0.00000

Unbiased Genetic distance of Normal Pigmented and Albino *C. gariepinus* based on Nei's (1978).

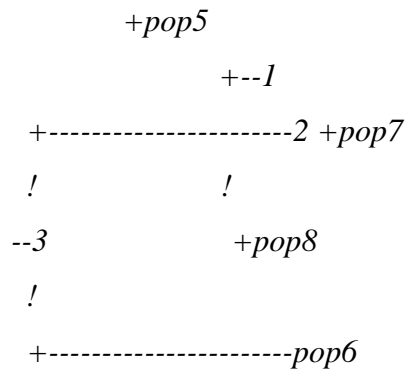
Appendix 3



<i>Between</i>	<i>And</i>	<i>Length</i>
3	2	1.48558
2	1	0.00000
1	Normal X Normal	0.00000
1	Albino X Albino	0.00000
2	Normal X Albino	0.00000
3	Albino X Albino	1.48559

Original Genetic distance Dendrogram of Normal Pigmented and Albino *C. gariepinus*.

Appendix 4



<i>Between</i>	<i>And</i>	<i>Length</i>
-----	---	-----
3	2	0.64752
2	1	0.00000
1	Normal X Normal	0.00000
1	Albino X Albino	0.00000
2	Normal X Albino	0.00000
3	Albino X Albino	0.64752

Unbiased Genetic distance of Normal Pigmented and Albino Clarias gariepinus based on Nei's (1978).