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

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**THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY MINNA, NIGER STATE NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE IN OF DOCTOR OF PHILOSOPHY (Ph. D) IN FISH GENETICS AND BREEDING**

**OCTOBER, 2019**

## DECLARATION

I hereby declared that this Thesis titled: "**Effect of albino gene on intraspecific hybridization of normal and albino African Catfish (*Clarias gariepinus)***" is a collection of my original research work and it has not been presented for any other qualification anywhere, information from other sources (published or unpublished) has been duly acknowledged

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## CERTIFICATION

The thesis Titled "**Effect of albino gene on intraspecific hybridization of normal and albino African Catfish (*Clarias gariepinus)***" by SHETTIMA, Aishatu PhD/SAAT/2014/645 meet the regulations governing the award of the degree of doctor of philosophy of the Federal University of Technology, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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## DEDICATION

This project is dedicated to God Almighty. Also to my late father Mr. Shettima Madu Wakawa and to my beloved mother Mrs. Halima Dudu for their prayers and encouragement towards this achievement. This thesis is also dedicated to my Husband Ephraim Suleiman, to my beloved children Hyelhirra, Sheila, Shobal and Abbati. To my sisters/brothers Fatsuma, Salamatu, Jummai, Asabe, Paulina, Adamu, Mamman, Ladi, Stephen and Alpha. I thank God Almighty.

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## ABSTRACT

The effect of albino gene on intraspecific hybridazation of African Catfish and Albino *Clarias gariepinus* was carriedout to determined their latency period, fecundity, fertility, hatchability survival, growth performance, some morphometric and meristics characteristics and genetic characterization of parents and offspring of F1 generation. A total of four (4) mating combination experiments were conducted using eight (8) ripped, 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 jerican to the Department of Fisheries and Aquaculture Adamawa State University Mubi. They were acclimatised for 10 days, vital feed were fed daily at 5% of their biomass. Artificial breeding was cariedout 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 ans F2 gerations, 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±0.20$^{o}$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 NN♂ x NN♀ had the highest fertility and hatchability of 44.79±0.32% and 72.6±0.24%, while the least value of 27.92±0.31% and 46.3±0.43% were recorded in AA♂ x NN♀. Also crossed between NN♂ x AA♀ had the highest final mean weight gain of 912.90 ±0.00g, followed by NN♂ x NN♀ with 900.75 ±0.00g, while NN♀ x AA♂ and AA♂ x AA♀ had the least value of 893.88±0.00g and 852.98±0.00g respectively. High survival rate of 41.25±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.

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# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background to the Study

The demand for high quality protein especially from aquatic resources is rising dramatically, increased agriculture production is clearly needed to meet this demand in the future, because capture fisheries is showing precipitous discloses 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 advances in aquatic production have been achieved through the application of genetic principles which includes selective breeding, hybridisation, chromosome manipulation, sex reversal and gene transfer (Aliah and Taniguchi 1999). Farmed sources are economically important food fish which have gained much prominence species of *Clarias* and *Heterobranchus* are widely cultured (Huisman and Richter, 1987). They are characterised by hardiness, resistance to disease, high yield potentials and ability to grow on a wide of natural and low cost ratification food and can withstand low oxygen and wide pH levels (Fagbenro and Sydenham, 1991). Genetic techniques have been applied to other animal to improve their qualify 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 productive as inbreeding or other genetic consequences of mismanagement may result in slower growth, decreased viability and disease susceptibility as well as decreased egg production (Bartly *et al.*, 1990). Hybridisation 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). Hybridisation has been used to increase growth rate, manipulate sex ratio, produce sterile animals, improve flesh quality, increase resistance and to tolerant to 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 produce 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 connotation attention in Africa particularly Nigeria this hybrid had been repeated to show heterosis (Madu *et al.,* 1992, Salamin *et al.,* 1993). Research result from mud catfish farmers shows 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 hybridisation among the *Clarias species* in order to assess the aquaculture potentials of their hybrids in terms of growth performance and survival compared to the putative parents at the fry, fingerlings and adult stages (Onyia, 2010). *Clarias* gariepinus *is* an economically important fresh water fish species of the *Clariidae* family that contribute immensely to the annual fresh water fish production in Nigeria. They are also readily acceptable among Nigerian fish farmers and consumers, hence command high commercial values (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 protein. Among the freshwater species for culture in Nigeria, *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 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 cases the albinism was controlled by autosomal recessive mutation. Albino *Clarias gariepinus* has all the traits of the regular *Clarias gariepinus* plate i and ii. They have the same well tasting meat, and they grow to the same size (Britton and Davie, 2006). However, the albino form of this catfish has some additional values. The Albino form of the catfish is an appreciated aquarium 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 chrosome 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 tenderly 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 electrophosatellites 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 hybridisation of Albino and Normal catfish *Clarias gariepiunus* the hybrids have been reported to show heterosis (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, fertilisation, morphometric and meristic characters and colour ratio of the mating combination as well their gene characterisation of the intraspecific hybridisation 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 characterisation 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 *Claridae* 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, fertilisation, colour and sex ratio of the various intraspecific mating combination of Albino and normal *Clarias* both in F1and 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 hybridisation of *Clarias gariepinus* Albino and normal and their reciprocal hybrids is of great importance in ensuring a proper understanding of their gene characterisation in both parent and F1 (Betiku and Omitogun, 2006) to create a good foundation for producing a genetically improved hybrid fish.

## 1.4 The Aim of the Study

The Aim of this study was to determine the effect of albino gene on intraspecific hybridisation 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 utilisation 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 assess the genetic characterisation 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, fertilisation, 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 utilisation of normal and albino *Clarias gareipinus* in F1 and F2 doesnot differ.

iv. The morphometric, meristic and genetic characterisation of the parent stock and their hybrid of normal and albino *Clarias gariepinus* doesnot differ.

vi. The phenotypic and genotypic appearance of hybrid of normal and albino *Clarias gariepinus* 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 gariepinus*.

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. gariepiunus 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, 1958).

## 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 (*Claridae 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 Oganisation, 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 Organisation, (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 Characterisation 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). 'Characterisation' 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, characterisation 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 'characterisation' 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, characterisation 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) (Nachtomy *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 dispense 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 QR1e 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. barbus* and *G. planifrons* occupy costal 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. barbus* 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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### 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 (Nachtomy *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 refered 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 (Nachtomy *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) (Nachtomy *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 pangasius 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 recognised 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 Choronion 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 fertilisation, 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 (fertilisation, 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 characterise 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.*

*gariepiunus* 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 determined 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 Fertilisation

External fertilisation 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 fertilisation and colonisation of new environments. The presence of the fertilised 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 Fertilisation 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 ($25^0$c) appears to enhance ovarian activity (Richter *et al.,* 1987), (Huisman and Richter, 1987), whereas temperature greater than $30^0$c 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-steriod estrogen anatagonist "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 fertilisation, incubation and hatching in fishes.

Fertilisation 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 fertilisation 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 $30^0$c (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-hetotest (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 Hybridisation 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 hybridisation 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 hybridisation 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 hybridisation 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 maximising 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. mossambiccus* (high salinity tolerance) and *O. niloticus* (low salinity tolerance) and their hybrids in comparative growth trials (Villegas, 1990b). 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 hybridisation 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.

Hybridisation 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 (Wolhfarth, 1994). Conversely, the cross between striped bass has 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. Hybridisation 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 intraspecifichybrid 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 bleeding strategy arrests the deteriorating genetic potentials of our clariid fish stocks and consequently increases genetic diversity.

Legendre *et al.*, (1992) made successful hybridisation 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 hybridisation 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 fertilisation 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 homorne-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 fertilisation 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 hybridisation 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 intraspecificcrossing 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 Hybridisation

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 intraspecificand 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 stadti* 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 hybridisation, 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 hybridisation experiments have been utilised 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-fertilisation 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*). Fertilisation, 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.

Hybridisation between giant catfish (*Pangasiodon gigas*) and giant pangas (*Pangasius sanitwongsei*) are now been practicing 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*), hybridisation 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 Production

Inter-specific hybridisation 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 hybridisation 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 (Faturo, 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* fertilisation 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 hybridisation among different species of fishes are described below.

## 2.23 Overall Improvement

The principal aim of hybridisation 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 stripped 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. luscious*) 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 Hybridisation on Disease Resistance and Environmental Tolerances

Hybridisation 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 hybridisation was followed with triploidisation. *et al.*, (1991 Dorson ) stated that the rainbow trout (*O. mykiss*)xchar (*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.

Hybridisation 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 Polyploidisation

Hybridisation 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, triploidisation of the hybrids may confer increased viability, growth and survival (Grey *et al.*, 1993). Triploidisation 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 acclimatisation times (*et al.,* 1993 Seeb ).

### 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 intraspecificcrossing 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 *(gariepinus)*

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 fertilisation 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 Utilisation 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 utilisation 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 brasiliensi*s, 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 combing with biofloc has never been reported previously. Hence, the aim of the study was to evaluate the growth performance and feed utilisation of African catfish fed experimental diet reared in the biofloc cultured system and enhanced with probiotic**.**

## 2.30 Growth Performance, Survival and Feed Utilisation 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 (it roots taste like horseradish) and ben oil or benzoil 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 strive.

The use of *M. oleifera* leaf meal as a non-conventional and cheap 8protein 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 Bocurtis 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 crappiexwhite crappie (*Pomoxis nigromaculatus x P. annularis*) stocked in small ponds and impoundments (Hooe *et al.,* 1994), silver carpxbighead carp (*Hypophthalmichthys molitri 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 $5^0$C sudden change in temperature will stress or even kill fish, and this has formed the basis for

acclimatisation 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 $30^0$C as at $20^0$C (Adeniji and Ovie, 1990). Auta (1993) reported that temperature range of $25^0$C – $30^0$C 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 µS/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 (µS/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 – 2,000µS/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 $50^0$C 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 $9^{o}$ 55' and $10^{o}$ 45' North and longitute$13^{o}$ 0' and $15^{o}$ 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.

1) 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.
2) 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 $20m^2$ (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).

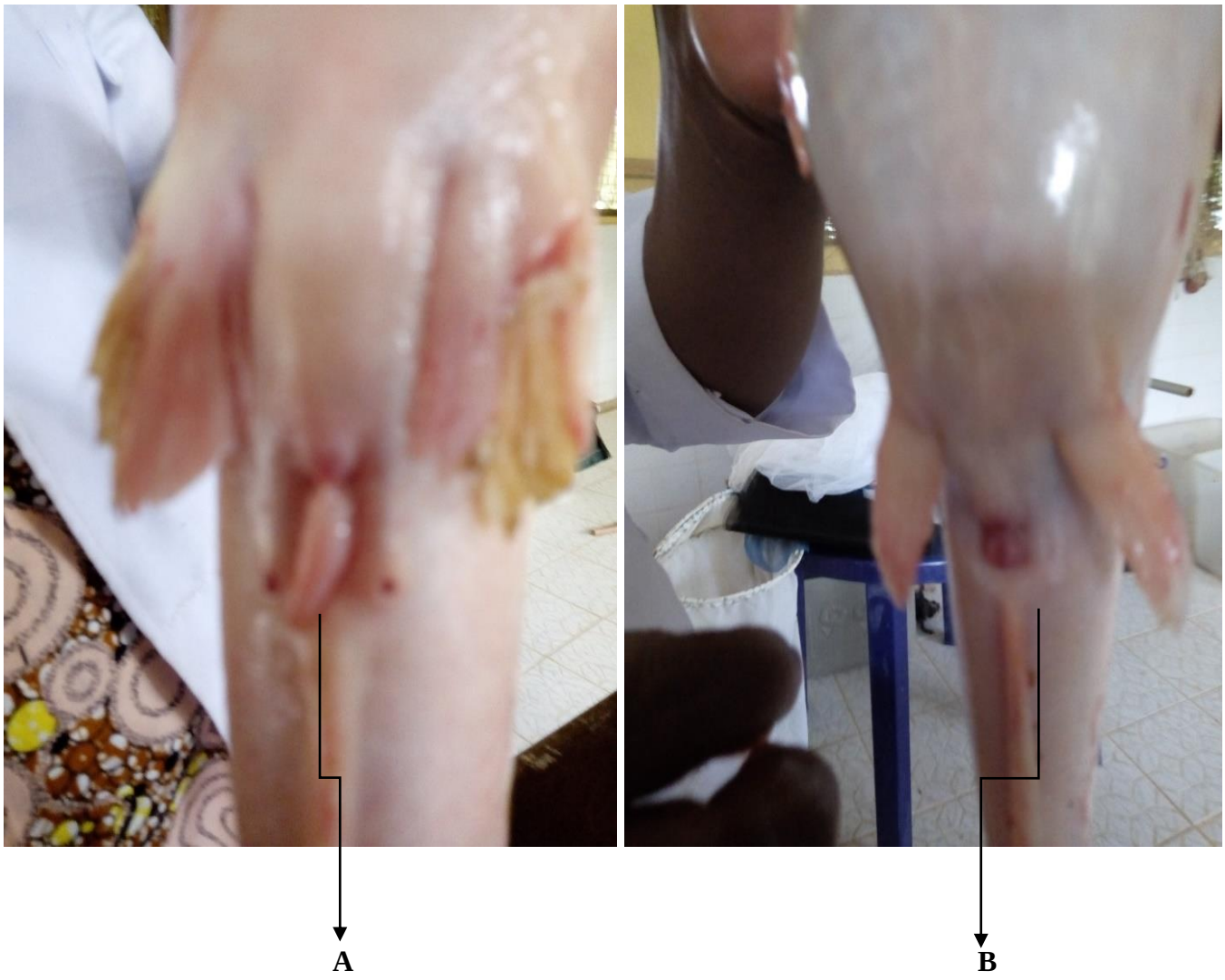



**Plate III: The Genital Papilla of Broodstock of Male and Female Albino *Clarias gariepinus.***

**KEYS**:

**A**= Male Albino Papilla
**B=** Female Albino

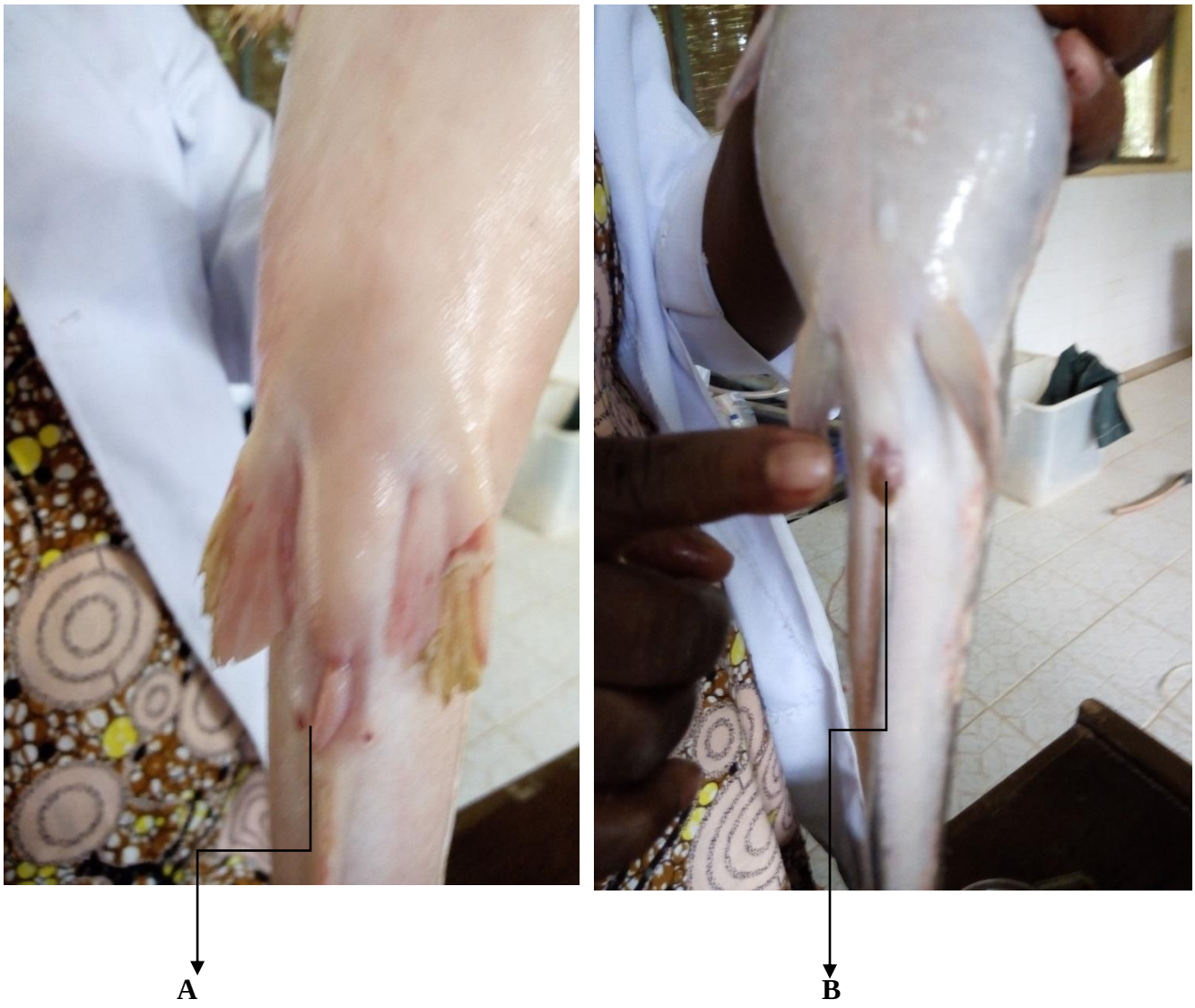



**Plate IV: Matured Male albino (A) and normal Female (B) *Clarias gariepinus* Broodstocks.**

**KEYS**:

**A=** Male Albino papilla

**B**= Female Normal normal


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

**KEYS:**

**A=** Female Albino *Clarias*

**B=** Male Normal Papilla

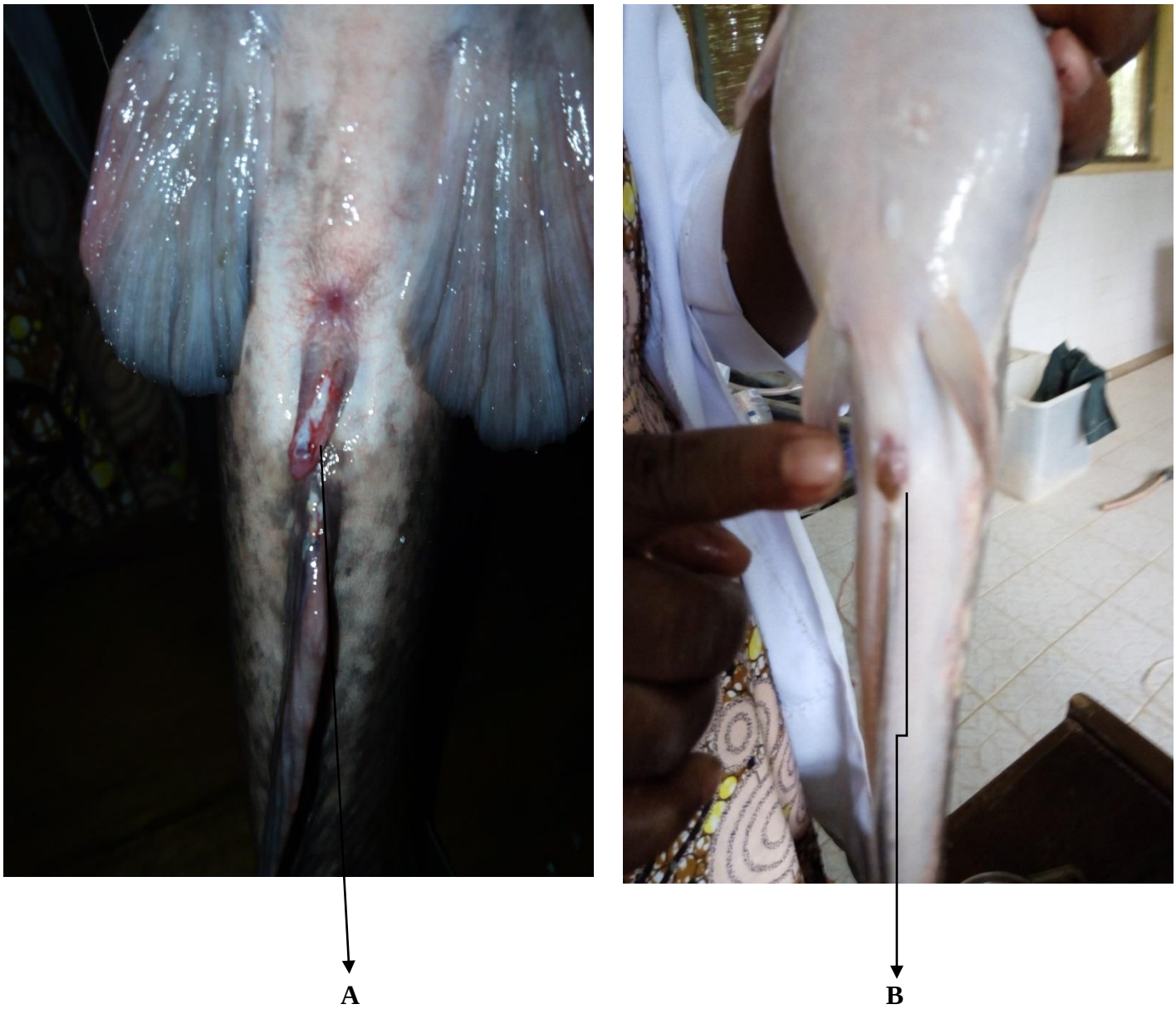



**Plate VI: Matured normal Male (A) and normal Female (B) *Clarias gariepinus* Broodstocks.**

**KEYS:**

**A=** Male *Clarias gariepinus* Papilla

**B=** Female *Clarias gariepinus* opening

### 3.7 Collection, Transportation and Acclimatisation 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 jerican (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).

### 3.8 Hypophysation and Artificial Hybridisation

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.

### 3.9 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 $F_{2.}$

### 3.10 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 $27^0$c to generate four different crosses (genetic crosses) as follows:

### 3.11 Hybridisation 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**(♂) |

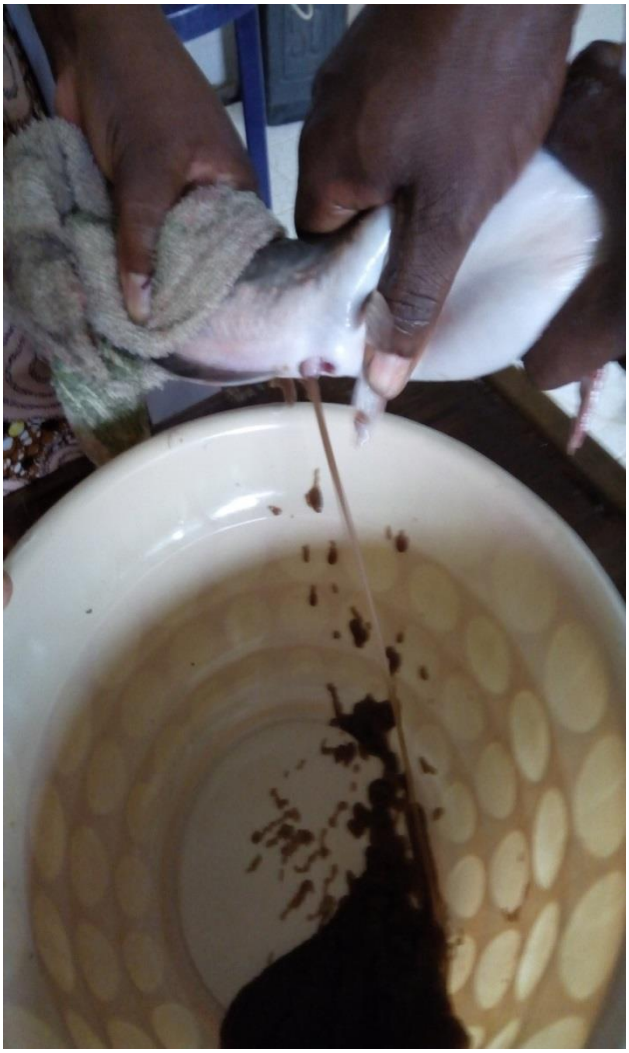
**KEYS:**

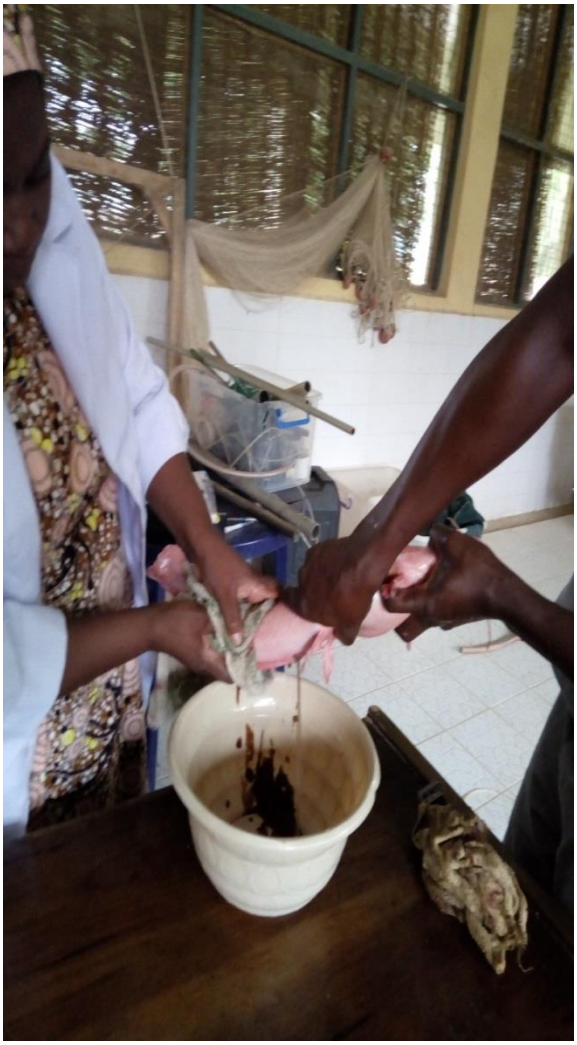
NN----NORMAL *Clarias gariepinus*

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

### 3.12 Incubation and Hatching

The Fertilized eggs from each mating combination were incubated in well aerated rectangular fibre plastic trough (3x0.4x0.2$m^2$) under ambient temperature of $27^0$c. 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).

### 3.13 Estimation of Percentage 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 completetly 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).

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

### 3.14 Estimation of Percentage Fertility

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 Palikova1 *et al.*, (2011) as shown below;

Percentage fertility (%) was calculated $= \frac{\text{Number of fertile eggs}}{\text{Total Number of eggs}} \times 100 \quad (2)$

### 3.15 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).

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;

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

### 3.16 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.

### 3.17 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) where 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)

$$\text{Specific Growth Rate (\% day)} = \frac{\ln W_1 - \ln W_0}{T} \times 100 \qquad (4)$$

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

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

T = Time

### 3.18 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).

### 3.19 Determination of Feed Conversion Ratio

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).

$$\text{Feed Conversion Ratio (FC R)} = \frac{\text{Total feed consumed (g)}}{\text{Weight gain (g)}} \quad (5)$$

### 3.20 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;

$$\text{Survival rate (\%)} = \frac{N_i - Nf}{N} \times 100 \quad (6)$$

Where $N_f$ *final* number of fish

$N_i$ *initial* number of fish

### 3.21 Determination of the Morphometric and Meristic Character of the Various Combination

The phenotypic characterisation 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.

### 3.22 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).

### 3.23 Gene Characterisation

#### 3.23.1 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 Rahman *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 votexing and incubated in a water bath at $55^0$c 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 votexing 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 $-20^0$ C for further use.

### 3.23.2 Selection of microsatellite markers

The process of electrophorosses 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).

### 3.23.3 Polymerase chain reaction (PCR) amplifications and primers for electrolysis

Amplification of the extracted DNA was done through Polymerase Chain Reactions (PCRs) carried out in 10µl reaction volumes containing primers used as 20 mg - 40 mg genomic DNA, 2µl 5× 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), 0.3µl each of the forward and reverse primer sets and 5.9µl 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.

### 3.23.4 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 × 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. 5µl of the DNA ladder was loaded in the first well while 4µl 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) Rahman *et al.,* (1995).

### 3.23.5 Statistical 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 AND DISCUSSION

### 4.1 Results

#### 4.1.1 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 $27.5^0$C respectively.

**Table 4.1.1 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 ($^0$C) |
|---|---|---|---|---|---|---|
| NN♀ | 1.56 | 0.78 | 9:00 | 9:00 | $12.00\pm0.21^b$ | $27.50\pm0.18^a$ |
| NN♀ | 1.76 | 0.88 | 9:08 | 9:14 | $12.60\pm0.32^b$ | $27.50\pm0.13^a$ |
| AA♀ | 1.68 | 0.84 | 9:11 | 10:06 | $13.40\pm0.22^a$ | $27.50\pm0.20^a$ |
| AA♀ | 1.62 | 0.81 | 9:15 | 9:51 | $13.30\pm0.32^a$ | $27.50\pm0.16^a$ |

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.*

#### 4.1.2 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 $28.32^O$C respectively.

**Table 4.1.2 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 ($^0$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 .1.3 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 significanct 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 4.1.3 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 | $44.79\pm0.32^{a}$ | $7020.00\pm0.12^{a}$ | $72.6\pm0.24^{a}$ |
| AA♂ x AA♀ | 960 | $37.18\pm0.13^{b}$ | $5460.00\pm0.12^{c}$ | $68.3\pm0.12^{b}$ |
| NN♂ x AA♀ | 960 | $42.92\pm0.31^{b}$ | $6240.00\pm0.23^{b}$ | $56.9\pm0.13^{c}$ |
| AA♂ x NN♀ | 960 | $27.92\pm0.31^{c}$ | $4032.00\pm0.34^{d}$ | $46.3\pm0.43^{d}$ |

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*

### 4.1.4 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 4.1.4 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 ($P<0.05$).

### 4.1.5 Growth and feed utilisation parameters for F1

Table 4.5 show the growth and feed utilisation 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 4.1.5 Growth and Feed Utilisation 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±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 FRC(g) | 1.48±0.50[d] | 1.52±0.50[c] | 1.59±0.79[b] | 1.69±0.75[c] |
| Specific Growth Rate SGR(%/day) | 1.35±0.24[b] | 1.31±0.50[a] | 1.32±0.87[a] | 1.38±0.02[a] |
| 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*

### 4.1.6 Growth and Feed Utilisation Parameters for F2

Table 4.6 Showed the Growth and Feed Utilisation 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 4.1.6 Growth and Feed Utilisation 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±070[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*

### 4.1.7 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 – 32.00$^0$C this fall within the tropical fish temperature range of 25.0 – 32.0$^0$C 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 4.1.7 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 ($^0$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♂ 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*

### 4.1.8 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$^0$C this fall within the tropical fish temperature range of 25.0 – 32.0$^0$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 4.1.8: 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($^{0}$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*

### 4.1.9 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 mahalnob is square distance ($D^2$) between male Albino and female normal (AA ♂ x NN ♀) female Albino and male normal (NN♂ x AA♀), Albino male and Albino female. (AA♂ x AA♀), and normal male and female (NN♂ x NN♀) *C.* gariepinus including their offspring in both f1 and f2 of the intraspecifichybrid were not significantly different ($P<0.05$). This clearly indicate how extremely difficult it could be to distinguish this species from their intraspecific hybrid using their morphological and meristic features. There are varying pattern of inheritance of some character by offspring of the various mating combination, the hybrid for the offspring of mating combination of Normal and Albino in F1 and F2 shows positive heterosis in the inheritance of brown eyes colour, head width, premaxillary width, and vomemine width in which case they possess different body color (brown) compared to the both parental width pink and black colored eyes. The head of all the mating combination shows the same flattered like the positive parent of *C. gariepinus species*. Therefore, this external features characteristic of both male and female used for each hybridisation 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 intraspecifichybrids 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♀ x NN♂), male Albino and female normal (AA♂ x NN♀) 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 4.1.9 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 4.1.10 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 fin length | 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).

### 4.1.10 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 4.1.11: 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 ± standard deviation for Cg01 and Cg02 loci are: na = 1.5 ± 0.71, ne = 1.14 ± 0.20 and I = 0.19 ± 0.27.

**Table 4.1.12: 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 ± 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 4.1.13 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 4.1.14 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 4.1.15 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 4.1.15 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 4.1.16 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 4.1.16 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*

### 4.1.11 Genetic analysis of the offspring in $F_1$

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. 1.17 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*

### 4.1.12 The observed allele frequency

The observed number of allele (na) 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 4.1.18: Summary of Genetic Variation Statistics for all Loci of Offspring F1**

| Locus | * na | ne* | ne* | 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(±) | | 0.7071 | 0.0939 | 0.1653 |

**Keys:**

* na = Observed number of alleles

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

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

### 4.1.13 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 4.1.19: 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. 1.14 The summary of f-statistics and gene flow

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

**Table 4. 1.20: 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 = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

### 4.1.15 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 4.1.21: 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♂ 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 Female Normal *Clarias gariepinus*

### 4.1.16 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 4.1.22 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♂ 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*

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.

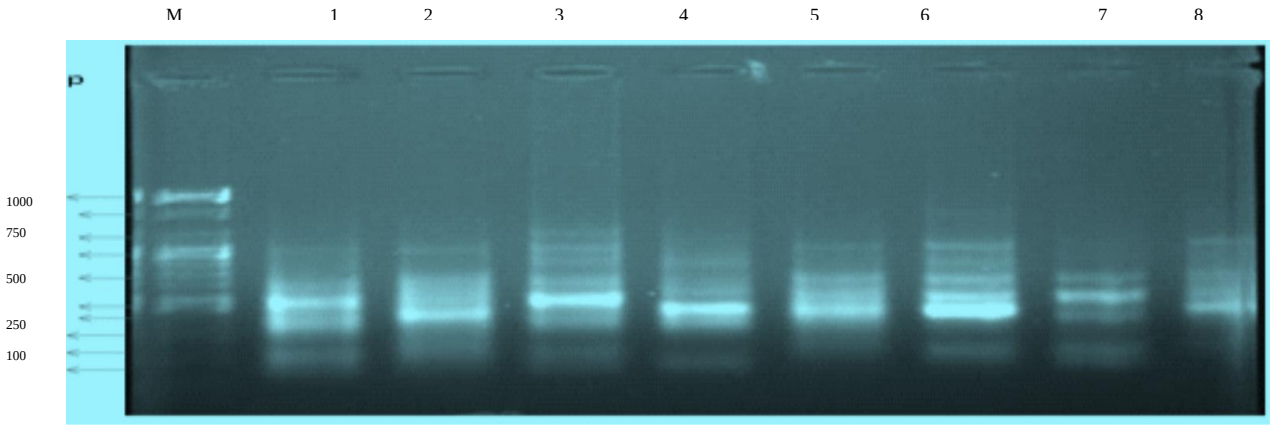
**Table 4.1.23: Overall Nei's Original Measures of Genetic Identity and Genetic Distance for parents and offspring's F1 and F2**

| Pop. ID | AA♂ | AA♀ | NN♂ | NN♀ | NN | AA | AA♂ x NN♀ | NN♂ x |
|---|---|---|---|---|---|---|---|---|
| AA♂ | **** | 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 | **** |

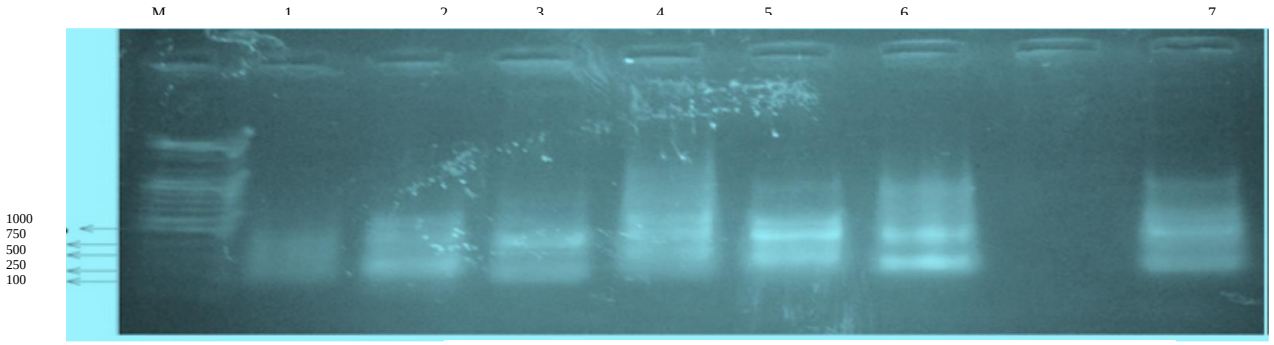
**Keys:**
Nei's genetic identity (above diagonal) and genetic distance (below diagonal).
AA♂ = Albino male
AA♀ = Albino female
NN♂ = Normal male *Clarias gariepinus*
NN♀= Normal female *Clarias gariepinus*
NN = Normal
AA = Albino
AA♂ x NN♀= Albino male and Normal female *Clarias gariepinus*
NN♂ x AA♀= Normal male and Albino female *Clarias gariepinus*

### 4.1.17 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*




Albino *Clarias gariepinus*

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

**Table 4.1.24 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*

### 4.1.18 Colour variation among the offspring of the various mating combination F1 and F2.

A chi square (xe) 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♂ x NN♀), female albino and male normal (AA♀ x NN♂) 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♂ x NN♀), female albino and male normal (AA♀ x NN♂) 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 4.1.25 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 *Clarias* | | 129 | 146 | 208 |
| ALBINO *Clarias* | 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 4.1.26: 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 *Clarias* | | 135 | 164 | 218 |
| ALBINO *Clarias* | 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).

## 4.2 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 $27.5^0$C and the Latency period for F1 generation showed 13.25 hours and the normal female had 12.15 hours at $28.30^O$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$^{o}$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 fertilisation 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 fertilisation and hatchability rates in comparison with the other combinations. Lower fertilisation 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 had more rigid requirement for spawning and lower tolerance for deviation from optimum condition compared to Normal pigmented female.

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 with 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 difference in growth performance between Albino and Normal *Clarias gariepinus* crosses, the Normal *Clarias gariepinus* exhibited a higher growth performance then 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 74.7cm and 41.7g vs 35.5cm). The result from the present study also similar with 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 areof similar features to the parents in terms of caudal peduncle length in the various mating combination as shown in table 4.6. The litle 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 biger 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♂ x NN♀), female albino and male normal (AA♀ x NN♂) 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 (He) and homozygosity (Ho) 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 FST estimates between parent and offspring populations gave similar results.

The RAPD markers were reported to be higly 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 Ne'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 – 32.00$^0$C this fall within the tropical fish temperature range of 25.0 – 32.0$^0$C. Boyd, and Folorunsho (2007) recorded the range of 26.0 -30.0$^0$C 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.

# CHAPTER FIVE

## 5.0 CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Conclussions

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 $27.5^0$C that is 13.40 and 12.00 hour respectively.

The Hybrid crossses 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.

Crosess 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.

Crosess 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.

## 5.2 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.*

## REFERENCES


Adah, P. M. Onyia, L.U. and Obande, R. A. (2014). Fish Hybridization in Some Catfishes: A. *Biotechnology* (13) 248-251.

Adebayo, O.T. (2006) Reproductive performance of African clariid cat fish *Clarias gariepinus* broodstock on varied maternal stress. *Journal of fisheries international* 1 (1-2) 17 -20.

Adebayo, A.H., Aliyu, R.., Gatsing, D. and Garba, I.H. (2006). The effect of ethanolic leaf extract of *Chrysophyllum albidum* G. on biochemical and haematological parameters of albino wistar rats. *African Journal on Biotechnology* **(**9) 2145-2150.

Adebayo, O.T and Popoola, O.M. (2008). Comparative Evaluation and Efficacy and Cost of Synthetic and nonsynthetic Hormones for Artificial Breeding of African Catfish (*Clarias*gariepinus). *Journal of Fish Aquatic Science* (3) 66-71.

Adebayo, A.A. (2004). Mubi region, a geographical synthesis. *Paraclete Publisher*, Yola – Nigeria.

Adebayo, O.T. & Alasoadura, T.A**. (**2001). Dietary protein requirements of *Clariid* catfish hybrid, *Clarias gariepinus* x *Heterobranchus bidorsalis*, fry fed practical diets. *Journal of Applied Aquaculture*, 11(4) 93–101.

Adeniji, H.A. and S.I. Ovie (1982): Study and approisol of the water quality of the Asa, Oli And Niger rivers *NIFFR Annual Report* 1982 pp. 15-20

Adewolu, M.A. & Aro, O.O. (2009). Growth, feed utilization and haematology of *Clarias gariepinus* (Burchell, 1822) fingerlings fed diets containing different levels of vitamin C. *American Journal of Applied Sciences*, 6(9): 1675–1681.

Adewolu, M.A., Ogunsanmi, A.O and Yunusa, A. (2008). Studies on growth performance and feed utilization of two clarid catfish and their hybrid reared under different culture system, *European journal of scientific research.* 23 (2): 252 – 260.

Adigun, B.A., (2005). Water Quality Management in Agriculture and Freshwater Zooplankton Production, for use in Fish Hatcheries. *National Institute for Freshwater, New Bussa*, Niger State, pp: 26.

Adikwu, L A (1991): The Development of complete diets from local feedstuffs and Industrial wastes for the culture of Tilapia. *Oreochmrnis niloticus* in Nigeria. Technical Report for the *International Foundation for Science* 5-114 Grey 1 urcgatan, Stockholm/SWEDEN.

Adikwu; I.A. (2003). A review of Aquaculture Nutrition in Aquaculture Development in Nigeria. National Workshop on Fish Feed Development and Feeding Practices in Aquaculture Organized by Fisheries Society of Nigeria (FISON) in Collaboration with *National Institute of Fresh Water Fisheries Research (NIFFR) and FAO National Special Programme for Food Security* (FAO-NSPFS). 34 – 42.

Agbebi, O.T., D.E. Ilaboya and A.O. Adebambo, (2013). Preliminary characterization of genetic strains in clariid species, *Clarias gariepinus* and *Heterobranchus bidorsalis* using microsatellite markers. *African Journal of Biotechnology* 12: 364-369.

Alabaster, J.S. and R. Lloyd, (1980). Water Quality Criteria for Freshwater Fish. Butter w orth and Company. Ltd., London, pp 147-152.

Al-Dohail, M.A, Hashim R, and Aliyu-Paiko M (2009) Effects of the probiotic, *Lactobacillus acidophilus*, on growth performance, haematology parameters and immunoglobulin concentration in African catfish (*Clarias*gariepinus, Burchell 1822) *fingerling.*40(14): 1642–1652.

Aliah R,. S Taniguchi S (1999). Comparison of genetic variability in nine domesticated stock of Indonesian common carp by using microsatellite DNA markers. *Fish Genetic Breeding, science* 28: 121-130.

Aluko, PO, Awopetu JI & Adeola AO (2001). Genetic basis for pectoral fin deformities in the African catfish *Clarias gariepinus* (Burchell, 1822), *Heterobranchus longifilis* (Valenciennes, 1840) and their hybrids. *Aquaculture Research,* **32**(1): 21-27.

Allen, S.K. and R.J. Wattendorf, (1987). Triploid grass carp: Status and management implications. Fisheries, 12: 20-24.

Allendorf, F.W. and Waples, R.S. (1988). Conservation and Genetics of Salmonid Fishes. In: Conservation Genetics: Case Histories from Nature, Avise, J.C. and J.L. Hamrick (Editions.). Springer, New York, USA., ISBN: 9780412055812, pp: 238-280.

Aluko, P.O., (1999) Variation in dorsal and adipose fin length of the $F_1$, $F_2$ and backcros hybrids involving parental *Heterobranchus longifilis* and *Clarias anguillaris*. *National Institute for Freshwater Fisheries Research Annual Report,* New Bussa, Nigeria, pp: 64-73.

Aluko, P.O., (1998). Growth characteristics of the parental, $F_1$, $F_2$ and backcross generation of the hybrids between *Heterobranchus longifilis* and *Clarias anguillaris*. *West African Journals of Biological Sciiences,* 8: 16-21.

Anon, (2001) What is so healthy about seafood a guide for seafood marketers. Fisheries Research Development Report, Australia.

Anyanwu, P.E., B.C. Okoro, A.O. Anyanwu, M.A. Matanmi and B.I. Ebonwu *(*2007). Length-weight relationship, condition factor and sex ratio of African Mudcatfish (*Clarias*gariepinus) reared in indoor water recirculation system tanks. *Resource Journal of Biological Science 2: 780-783.*

Arthur, P.F, Archer JA, Johnston DJ, Herd RM, Richardson EC, Parnell PF (2001). Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. *Journal of Animal Science*.;79(11):2805–11.

Asaduzzaman, M, Wahab MA, Verdegem MC, C.N. (2008) ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. *Aquaculture*; 280(1–4): 117–123.

Association of Official Analytical Chemists International. Official methods of analysis, 17th edition (2000) AOAC *International, Gaithersburg*, MD, USA; 2000.

Austin, M (1999). Morphometric separation of animal cohorts within mid-Atlantic bluefish, Pomatomus saltatrix, using disciminant function analysis. *Fisheries Bulletin US*.; 97:411-420.

Auta, J. (1993): Water Quality Management in Fish Ponds. *Proceedings of National Workshop on Fisheries Extension Delivery.* 2P.

Avsar, D., (1994). Diel diet and feeding behavior of scaldfish (*Arnoglossus laterna* Walbaum, 1792) in the bay of Mersin. 34(1/2):89-101.

Awom, I.E, Eyo VO (2016). Comparative study of growth performance, food utilization and survival of African catfish *Clarias gariepinus* (Burchell, 1822) Fingerlings Fed Live Maggot (*Musca domestica*) and the Coppens Commercial Feed. *International Journal of Scientific Research in Science, Engineering and Technology*.;2(2): 379-386.

Avise, J.C. and van den Avyle, M.J. (1984). Genetic analysis of reproduction of hybrid white bass x striped bass in the Savannah River. *Transitional. Fish. Sociology*., 113: 563-570.

Bagenal, T.B. (1978) Methods for assessment of fish production in freshwaters. Handbook No. 3, Blackwell *Scientific Publication, 3rd Edition, London*, 300 pages.

Baker, D.(1972) Quantity of protein required by rainbow trout International *journal of fisheries and Aquacukture* 2(4) 21-34

Baker, C.S. and S.R. Palumbi (1992). Which whales are hunted, A molecular genetic approach to monitoring whaling. 265,1538–1539.

Bartley, D.M., Gall G.A.E. and Bentley, B. (1990). Biochemical genetic detection of natural and artificial hybridization of Chinnook and Coho Salmon in Northern California. *Transmission American Fish Society*., 119: 431-437.

Bartley, D.M and Hallerman E.M. (1995) Global perspective on the utilization of genetically modified organisms in aquaculture and fisheries. *Aquaculture*, 1–7p.

Baski and Means (1988) Proximate composition of and mineral contents of pebbly fish, Alestes baremoze (joannis, 1835) fillets in relation to fish size Ugandan *Journal of Agricultural Science* 15,(1) 41-50

Basavaraju, Y., Deveraj K.V. and Ayyar S.P. (1995) Comparative growth of reciprocal carp hybrids between *Catla catla* and *Labeo fimbriatu. Aquaculture* 129, 187–191.

Bearez, P. (2002) First record of albinism in a morey eel *Muraena clepsydra* (Muraenidae) from Ecuador *Cybium.* 26: 159-160.

Benech, V., Teugels, G. G. & Goure`ne, G. (1993). Critère pratique pour distinguer deux poissons-*chats africains, Clarias anguillaris C. gariepinus* (Siluriformes; Clariidae). *Cybium***,** 83–85p.

Betiku, O.C. and Omitogun, G.O. (2006): Genetic diversity of commercial *Clarias gariepinus* population revealed by Sodium dodecyl sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). *Proceedings of the 31st Annual Conference of the Genetics society of Nigeria*. 135-140.

Bhikajee, M. (1997) Mariculture of the red tilapia in enclosed bays and in cages –the Mauritian experience. In: Fitzsimmons, K. (ed.), *Tilapia Aquaculture*, *Northeast Regional Agricultural Engineering Service* 106, (2), New York, pp. 595–599.

Blaxhall, P.C. (1981). A comparison of methods used for the separation of fish lymphocytes. *Journal of Fish Biology,* 18: 177-181.

Bloom, Kligerman, A.D. and S.E. (1977). Rapid chromosome preparations from solid tissues of fishes. *Journals Fish Resources Board Canada* 34:266-269.

Blythe, WG, Helfrich LA, Libey G (1994) Induced maturation of stripped bass, Morone saxatilis exposed to 6, 9 and 12 months photothermal regimes. *Journal of World Aquaculture Society* 25: 183-192.

Bondari, K. (1981). Response to bidirectional selection for body weight in channel catfish Aquaculture, 33: 73-81.

Bondari, K. (1984). Comparative of albino and normal pigmented channel catfish in tanks, cages and ponds. *Aquaculture,* 37: 293-301.

Bondai, K. and Dunham, R.A (1987) effects of inbreeding on economic traits of channel catfish, *theoretical and Applied Genetics* 74, 1-9

Bondai, K., wave, G.O., Mullinir, B.G. Jr and Joyce, J.A (1985) influence of blood fish on the breeding performance of channel catfish. *Progressive fish Aquaculturist* 47, 21-26.

Bongers, A.B.J. Abarcia J.B. Zandieh Doulabi B. Eding E.H. Komen J and Richter CJ.J. (1995). Maternal influence on development of androgenetic clones of common carp, *Cyprinus carpio L. Aquaculture* 137:139-147.

Bookstein, FL. (1991) Morphometric tools for landmark data geometry and biology Cambridge. Cambridge University Press; pp. 153-168.

Boyd, C. E (2004). Farm-Level Issues in Aquaculture Certification: Tilapia. Report commissioned by WWF-US in 2004. Auburn University, Alabama 36831.

Boyd, Donald J. Hamilton Lankford, Susanna Loeb, Jonnah E. Rockoff, and James H. Wyckoff. (2007) The Narrowing gap in fish production in New York cityand its implication for fish farmers achievement in high – poverty area. – CALDER working paper 10. Washinton, DC

Boyd, C.E and Lichtkoppler F.(1979) Water quality management in pond fish culture research and development series No22 International centre for Aquculture Agricultural Experiement Station Auburn University, Auburn Alabama

Braasc, I.. Brunet F., Volff J.-N and Schartl M. (2009a). Pigmentation pathway evolution after whole-genome duplication in fish. *Genome Biology Evolution* 1(0): 479–493.

Braasch, I.. Liedtke D., Volff J.-N., Schartl M., (2009b) Pigmentary function and evolution of tyrp1 gene duplicates in fish. *Pigment Cell Melanoma Resources* 22(6): 839–850.

Brecka, B.J., Kohler C.C. and Wahl, D.H. (1995). Effects of dietary protein concentration on growth, survival and body composition of *Esox masquinongy* and tiger muskellunge, *Esox masquinongy* x *E. luscius* fingerlings. *Journal World Aquacultural Society,* 26: 416-425.

Bridge, and Tave, D. (1993). Genetics for Fish Hatchery Managers, 2nd edition Van Nostrand Reinhold, New York, New York. Pp 1339.

Bridges, W.R. and von Limbach. (1999). Inheritance of albinism in rainbow trout. *The Journal of Heredity*, 63: 152-153.

Bridge, W.R. and Bruno Von Limbach (1972)Inheritantnce of albinism in rainbow trout *Journal of Heridity* 63 (13) 152-153

Britz, P.J., Lee, B. & Botes, L. (2009). AISA 2009 Aquaculture Benchmarking Survey: Primary Production and Markets. *AISA report produced by Environmental-Fish Africa* (Pty) Ltd. 117 pp.

Brito, M.F.G. and Camaraschi E.P. (2005) An albino armored catfish *Schizolecis guntheri* (Siluriformes: Loricariidae) from an Atlantic forest coastal basin. *Neotropical Ichthyology*, 3:123- 125.

Britton, J.R. and Davies, G.D. (2006). First record of the white catfish *Ameiurus catus* in Great Britain. *Journal of Fish Biology*.

Bronte, C.R., Fleischer, G.W., Maistrenko, S.G. and Pronin, N.M. (1999). Stock structure of Lake Baikal omul as determined by whole body morphology. *Journal of Fish Biology* 54, 787-798

Brooks, G.B. (1994). A simplified method for the controlled production and artificial incubation of Orechromis eggs and fry. *The Progressive Fish Culturist* 56:58-59.

Buj, I. Podnar M, Mrakovcic M, Caleta M, Mustafic P, Zanella D, Marcic Z (2008). Morphological and genetic diversity of *Sabanejewia balcaniain, Crotia Folia zoology*. 57(1-2):100-110.

Bundit, Y. Rungkan K, Siripavie C, (2014). Partial replacement of protein in soybean meal by moringa seed cake (*Moringa oleifera*) in Bocurtis catfish (Pangasius bocourti). Songklana-Karin *Journal of Science and Toxicology*. 36(2):125-135.

Calcagnotto, D. Russello M and DeSalle R (2001) Isolation and characterization of microsatellite loci in *Piaractus mesopotamicus* and their applicability in other Serrasalminae fish. *Molicular Ecology Notes* 1:245-247.

Carpenter, KE. Sommer H.J and Marcus L.F (1996) Converting truss inter landmark distances to Cartesian Coordinates. In Marcus LF, Corti M, Loy A, Naylor G, Slice DE, editions. Advances in morphometrics. ATO Asi Series A.; *Life Sciences New York Plenum Publication.* 284:103-111.

Carreon, J.A Esttocapio, F.A and Enderez E.M (1973) Recommended procedure for induced spawning and fingerlings production of *Clarias macrocephalus* Gunther . *Aquaculture Neitherland* 8 267-281

Castillo, Cajas R.F., Selz O., Ripmeester E.A.P., Seehausen O., Maan M.E (2012). Species-specific relationships between water transparency and male coloration within and between two closely related Lake Victoria cichlid species. *International Journal of Evolutionary Biology*. :12.

Cavaco, L. M., Hasman H., Xia S., Aarestrup F. M. (2009). A novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrobial Agents Chemotherapy* 53; 603–608. 10.1128/AAC.00997-08

Chevassus, B., (1983). Hybridization in fish *Aquaculture*, 33: 245-262.

Chevassus, B. and Doson H. (1990). Hybridization in fish. Aquaculture, 33: 245-262. the first National symposium on fish nutrition and fiSh feed technology held at the Nigerian Institute for Oceanography and Marine Research (NIOMR), Lagos 26Th - 29'n October 1999, A Eyo. pp 89 - 94.

Clark, S. (2002). First report of albinism in the white-spotted bamboo shark, *Chiloscyllium plagiosum* (Orectolobiformes: Hemiscyllidae), with a review of reported colour aberrations in elasmobranches. *Zoo Biology* Campton, D.E., 1987. 1987. In: Population Genetics and Fishery Management, Ryman, N. and F.M. Utter (Edission.), University of Washington Press, Seattle, WA., USA., pp: 161-192.

Coates, D., (1988). Length-dependent changes in egg size and fecundity in females, and brooded embryo size in males, of fork-tailed catfishes (Pisces: Ariidae) from the Sepik River, Papua New Guinea, with some implications for stock assessments. *Journal of Fish Biology* 33:455-464.

Colombo, L., A. Barbaro, A. Francescon, A. Libertini and M. Bortolussi (1998). Towards an Integration between Chromosome Set Manipulation, Intergeneric Hybridization and Gene Transfer in Marine Fish Culture. In: *Genetics and Breeding of Mediterranean Aquaculture species, Bartley, D. And B. Basurco* (Editions.). Vol. 34, CHEAM Zaragoza, Spain, pp: 77-122.

Crab, R. Avnimelech Y and Defoirdt T, (2007). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture*.; 270(1–4): 1–14.

Crandell, P.A., M.P. Matsuoka and W.W. Smoker, (1996). Effects of timing of stripping and female on the viability of gynogenetic and non-gynogenetic diploid pink salmon (*Oncorhynchus gorbuscha*). *Aquaculture*, 137: 109-119.

Chabi, IB, Kayodé APP, Agassoussi OAS, Agbobatinkpo PB, Chikou A, Codjia JTC. (2015) Development and bioefficacy study of plant-based proteins diets for juvenile African catfish. *Journal of Applied Biosciences. 94:8801–8808*

Clay, D. (2002), sexual maturity and feaudity of the African catfish (*Clarias*gariepinus) with an obeservaion of the spawing behaiviour of the nile catfish (*Clariaslazera*). *Zodogyical journal of the linean society 68:351-365.*

Cuvier, G.; Latreille, P. A. (1829). Le règne animal distribué d'après son organisation, pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie comparée, par M. le cher, Cuvier. Avec figures, dessinées d'après nature.

Danish, Rask Moller P (2012). Atlas of Danish Fishes. Version 2.1. Zoological Museum, Natural History Museum of Denmark.*Occurrence dataset https:// organization /10.15468/gekzst accessed via GBIF.organisation on* 2019-08-0.

Danzmann, R.G., Ferguson, M.M. and Allendorf, F.W. (1985). Does enzyme heterozygocity influence developmental rate in rainbow trout. 56:417-425.

Debouche, C., Marquet, J.-P. & Teugels, G. G. (1979). Détermination de poissons du genre *Clarias*par une méthode généralisable aux poissons non écailleux. *Bulletinde l'Institut Fondamental de l'Afrique Noire* 41**,** A, 4, 844–862.

De Kimpe, P. and Micha, J. C. (1974). First Guidelines for the Culture of *Clariaslazera* in Central Africa. *Aquacultural technology* 4:227 – 248.

Delgado, J. Carvalho D., Sousa R., Ferreira S. and Aveiro M.J. (2009). First record of albinism in the deep water black scabbard-fish *Aphanopus carbo* (Trichiuridae) off Madeira. *Journal of Applied Ichthyology*, 25:483–484.

Dennis, EU and Uchenna OJ. (2016): Use of probiotics as first feed of larval African catfish *Clarias* gariepinus (Burchell 1822). *Annual Resources Revised Biology* 9(2): 1–9.

Denton, T.E. and W.M. Howell, (1973). Chromosomes of the African polypterid fishes, *Polypterus palmas* and *Calamoichthys calabaricus*. Experientia 29(1):122-124.

Dickerson, H.W., Clark, T.G., Findly, R.C., (1973). *Ichthyophthirius multifiliis* has membrane-associated immobilization antigens. *Journal of Protozoology* 36, 159–164.

Dillon, N., A.D. Austin and E. Bartowsky, (1996). Comparison of preservation techniques for DNA extraction from hymenopterous insects. *Institute of Molicular Biology*. 5: 21–24.

Dobosz, S., Kohlmann, K., Goryczko, K. and Kuzminski, H. (1999). Growth and vitality in yellow forms of rainbow trout. *Journal of Applied Ichthyology*, 16 (3): 117-120.

Dorson, M., Chevassus, B. and Torhy, C. (1991). Comparative susceptibility of three species of char and rainbow trout x char triploid hybrids to several pathogenic salmonid viruses. *Disease of Aquaculture Organisms*, 11: 217-224.

Dunham, R.A. and B.J. Argue, (1998). Seinability of channel catfish, blue catfish and other $F_1$, $F_2$, $F_3$ and backcross hybrids in earthen ponds. *Program Fish-Cult*., 60: 214-220.

Dunham, R.A., D.M. Lambert, B.J. Argue, C. Ligeon, D.R. Yant and Z.J. Liu, (2000). Comparison of manual stripping and pen spawning for production of channel catfish x blue catfish hybrids and aquarium spawning of channel catfish.

Dunham. R.A. Majundar, K. Hallerman., E. and Main G. (2001). Review of the status of aquaculture genetics. In K.R.P. subasinghe, P. Buemo, M.J millennium Philipa: Haugh; S.E. and J.R. Arthur (edition). *Aquaculture in the third Halzize proceedings of the conference on Aquaculture in the millinium*, Bangkok, Thailand, 20-25 February, 137-186.

Dupree, H. K. & Hunner, J. V. (1984). Third Report to the Fish Farmers. The status of warm water fish farming and progress in: *Fish Farming Research. U. S. Deparment. of the Interior Fish and Wildlife Services,* Washington D. C. 270.

Edwards, P. (1990) Environmental issues in integrated agriculture and waste water-fed fish culture system. *Paper presented at Bellagio Oonference on Environmental and Third Aquaculture.*Development September 1990, BeMagi, Italy.

Ekanem, AP. EyoVO, Udoh JP, Okon JA (2014). Endoparasites of food-fish landing from the Calabar River, Cross River State, *Nigeria. Journal Science of Research Republished;*3(6): 810-7

Earnst, D.H., Watanabe, W.O. Elington, L.J. Wicklund R.I. and Olla, B.L. (1991). Commercial-scale production of florida red tilapia seed in low-and brackish-salinity tanks. *Journal of World Aquacultural Society, 22: 36-44.*

Ekanem AP, and Ekanem IE (2010). Effects of phyto-protein and normal diet on the growth and gonadal development of *Clarias gariepinus*. *Tropical Environmental Resources*;9: 556-9.

Ekanem AP, Eteng SU, Nwosu FM, and Eyo VO (2012). Comparative study of the growth and gonad development of *Clarias gariepinus* (Burchell 1822) fed diets with plant and

animal-based ingredients in concrete tanks. *Journal of Agricultural Science Technology;* 2(10): 1203-10.

Ekanem, A.P, Eyo VO, James PU, and Udo NE (2013). Effects of Unical feed on fecundity and gonad development of *Clarias* gariepinus; a comparative study with Coppens commercial feed in Earthen pond. *International Journal Science Resources;* 2(10): 8-14.

Ekanem, SB, Eyo VO, Okon EE (2017) The effects of Brazilian tea (*Stachytarpheta jamaicensis*) and Bitter kola (*Garcinia kola*) seed meal on the growth and gonad development of the African catfish *Clarias*gariepinus (Burchell, 1822). *Ege Journal of Fisheries and Aquatic Sciences. 4(2):179-185.*

El-Gamal, A.H.E., El-Greisy, Z.A. (2008) Effect of removal of egg adhesiveness on hatchability and effect of different levels of salinity on survival and larval development in common carp, Cyprinuscarpio. *Journal of Applied Science Research* 4,1935-1945.

El- Naggar, GO, John G, Rezk MA (2006), Effect of varying density and water level on the spawning response of African catfish *Clarias*gariepinus: *implications for seed production. Aquaculture.* 261(3): 904–907.

Elliott, J.M. and W. Davidson. (1975). Energy equivalents of oxygen consumption in animal energetics. *Oecologia* 19: 195-201.

Emmanuel, O, Chinenye A, Oluwatobi A, and Peter K. (2014) Review of aquaculture production and management in Nigeria. *American Journal of Experimental Agriculture*.; 4(10):1137-1151.

Eyo, Andrew A. (2001): Chemical composition and Amino acid content of the common available feedstuffs used in fish feeds in Nigeria. In Fish Nutrition and fish feed technology, *Proceedings of the first National symposium on fish nutrition and fish feed technology held at the Nigerian Institute for Oceanography and Marine Research (NIOMR),* Eagos 261h - 29th October 1999 Editions, A. A Eyo. pp 14 - 25.

Eyo, V.O and Ekanem AP (2011) Effect of feeding frequency on the growth, food utilization and survival of African catfish (*Clarias gariepinus*) using locally formulated diet. *African Journal of Environmental Pollution and Health*.; 9 (2):11–17.

Eyo, V.O Ekanem AP, Eni GE, Asikpo PE, Jimmy UU. (2013;) Comparative study of growth performance, food utilisation and survival of hatchery bred and wild collected fingerlings of African catfish *Clarias*gariepinus. *Greener Journal of Oceanography*. 1(1):1-10.

Eyo, V.O Ekanem AP, Eni G, (2013) Edet AP. Relationship between fecundity and biometric indices of the silver catfish *Chrysichthys nigrodigitatus* (Lacepede) in the Cross River estuary, Nigeria. *Croat Journal Fish*; 71: 131-5.

Eyo, V.O Ekanem AP, and Jimmy UU. A (2014) comparative study of the gonado-somatic index (GSI) and gonad gross morphology of African catfish (*Clarias gariepinus*) fed unical aqua feed and coppens commercial feed. *Croatian Journal of Fisheries*.72 :63–69.

Eyo, V.O Ekanem AP, Ajom VA. (2015) Fecundity studies of the African catfish *Clarias* gariepinus (Burchell, 1822) fed coppens and unical aqua feed in circular concrete tanks. *Journal of Coastal Life Medicine* pp 137.

Ezenwa, B. (1985). Culturable fish seed in Nigerian Water, a research note on Swamp Fisheries Management, *Ministry of Agriculture* Kaduna, p.17

Fagbenro, O.A and Sydenham, D.H.J, (1988). Evaluation of *ClariasIsheriensis* under semi – intensive management in ponductions. *International journal of Aquaculture Research* 74: 287-291

Fagbenro, O.A and Arowosoge, A.I. (1991b). Growth response and nutrient digestibility by *Clarias isheriensis* (Sydenham 1980) fed varying levels of dietary coffee pulp as replacement for maize in low-cost diets. *Bioresources Technology*. (37): 253 – 258.

Fagbenro, A.A., Adepanusi, E.O., Fapohunda, O.O. (2003). Feed Stuffs and Dietary Substitution for Farmed Fish in Nigeria. National Workshop on Fish Feed Development and Feeding Practices in Aquaculture Organized by Fisheries Society of Nigeria (FISON) in Collaboration with *National Institute of Fresh Water Fisheries Research (NIFFR) and FAO National Special Programme for Food Security* (FAO-NSPFS.). 60 - 72.

Fadri S, Muchlisin Z, and Sugito S. (2016): Growth performance, survival rate and feed utilization of Nile Tilapia, *Oreochromis niloticus* fed experimental diet contains jaloh leafs, Salix *tetrasperma* Roxb at different levels of EM-4 probiotic. *Journal Ilmiah Mahasiswa Kelautan dan Perikanan Unsyiah; 1(2): 210–221*.

Faturoti, E. O., Balogun, A. M. and Ugwu, L. L. C. (2003). Nutrient utilization and growth responses of *Clarias*(*Clariaslazera*) fed different dietary protein levels. *Nigeria Journal of Applied fisheries and Hydrobiology* 1:41-45.

FAO, (1996). Artificial Reproduction and Pond Rearing of the African Catfish *Clarias gariepinus* in Sub-Saharan Africa-A Handbook. *Food and Agriculture Organization of the United Nations*, Rome, Italy, ISBN-13: 9789251039182, Pages: 73.

FAO, (2003). Fishstat plus, Version 2.30. Food and Agriculture Organization of the United Nations, Rome, Italy. Farahvash, T., J. Shodja, A. Rafat and A. Keshtkaran, 2011. The effect of bilateral crossbreeding between arkhamerino and ghezel sheep on the quality of wool of their $F_1$ crosses. *Asian Journal of Animal Veterinary Advanced.,* 6: 397-400.

Ferraris, Jr CJ (2003) Genus and species *incertae sedis* in Siluriformes. In: Reis RE, Kullande S, O and Ferraris Jr. CJ (eds) Check List of the Freshwaters of South and Central America. *EDIPUCRS 1, Porto Alegre*, pp 254.

Food and Agriculture Organization of the United Nations. (2014) The state of world fisheries and aquaculture. Rome: Food and Agriculture Organization of the United Nations 2014. *Available from: http://www.fao.org/3/a-i3720e/index.html*.

Francis, G. Makkar HPS, and Beckar K. (1999) Plant toxins and detoxification methods to improve and Extract Moringa oleifera leaves. *Pakistan Journal of Nutrition* 1999;7(1)13-16.

Fujii, (2002): Tracking released Japenese flounder Paralichthys olivacous by Mitochondrial DNA sequence. In: chill, K. (Eds.), Ecology and Aquaculture Species and enhancement of stocks. *Proceeding 13th US-Japan meeting on aquaculture*. Pp 51-53.

Fulton, T. W. 1902. The rate of growth of fishes. 20th Annual Report of the Fishery Board of Scotland 1902 (3):326-446. 1904. The rate of growth of fishes. *22nd Annual Report of the Fishery Board of Scotland* 1904 (3):141-241

Funkenstein, B., Chen, T.T., Powers, D.A. and Cavari, B. (1990). Cloning and sequencing of the gilthead seabream (*Sparus aurata*) growth hormone-encoding cDNA. Gene 103: 243- 247.

Furtado, P.S Gaona CA, and Poersch LH, (2014), Application of different doses of calcium hydroxide in the farming shrimp *Litopenaeus vannamei* with the biofloc technology (BFT). *Aquaculture International*. 2014; 22(3): 1009–1023.

Gaal, J., Z. Horvath and I. Eross (1980) The effect of organophosphorous compound on the cholinesterase activity of one summer old carps. *Aquaculture Hungerica* II: 117-123.

Galbreath, P.F. and G.H. Thorgaard, (1995). Sexual maturation and fertility of diploid and triploid Atlantic salmon x brown trout hybrids. *Journal Aquaculture ionResearch*, 137: 299-311.

Galbusera, P., Van Dongen, S. and Matthysen, E. (1996)). Cross-species amplification of microsatellite primers in passerine birds. *Conservation Genetics study*, 1(2): 163-168.

Galib SM**,** Naser SMA, Mohsin ABM, Chaki N and Fahad MFH (2012) Fish diversity of the River Choto Jamuna, Bangladesh: Present status and conservation needs. *International Journal of Biodiversity and Conservation* 5(6): 389-395. doi: 10.5897/IJBC2013.0552

Garg, S.K., Jana, S.N. & Arasu, A.R.T. (2006). Determination of fertilization rate for optimum pond productivity and fish growth in inland saline groundwater ponds: monoculture of grey mullet and milkfish. *Asian Fisheries Science*, 19: 97–183.

Garg, R.K., P. Sarkar, N. Silawat, N. Vijay, N. Batav and N.N Mehrotra (2010). Assesment of genetic diversity of *Clariasbatracus* using RAPD markers in three water bodies of *Bhopal Journal of Environmental biology* 31: 749- 753.

Goudie, C.A., Simco, B.A., Davis, K.B., Liu, Q., (1995). Production of gynogenetic and polyploidy catfish by pressure-induced chromosome set manipulation. *Journal of Aquaculture Research* 133, 185–198.

Glamuzina, B., V. Kozul, P. Tutman and B. and Skaramuc, (1999). Hybridization Of Mediterranean groupers: *Epinephelus marginatus* x *E. aeneus* andearlydevelopment *Aquaculture Resources* 30:625-628.

Gold, J.R., W.J. Karel and M.R. Strand, (1980). Chromosome formulae of North American fishes. *Progress Fish Culture* 42:10-23.

Gomelsky B., Cherfas N.B., Ben-Dom N. and G. Hulata, 1996. Colour inheritance in ornamental (koi) carp (Cyprinus carpio L.) inferred from color variability in normal and gynogenetic progenies. *Israelite Journal of Aquaculture - Bamidgeh*, 48:219-230.

Gomelsky, Boris, S. D. Mims and Richard. J. Onders. (2000). Induced gynogenesis in Black Crappie. North American 461 *Journal of Aquaculture* 62: 33-41.

Gorshkova, G., S. Gorshkova, H. Gordin and W. Knibb, (1996). Karyological studies in hybrids of Beluga, *Huso huso* (L.) and the Russian *Acipenser guldenstati* Brante Israelites. *Journal of Aquaculture Bamidgeh,* 48: 35-39.

Grant, J. R. H. (1994). Historical Shoreline Response to Inlet Modifications and Sea Level Rise. Masters of Science Thesis. Gainesville: University of Florida.

Grande, M., Murua, H., Zudaire, I., Korta, M. 2012. Oocyte development and fecundity type of the skipjack, Katsuwonus pelamis, in the Western Indian Ocean. *Journal of Sea Research.* 73, 117-125.

Green, B.W Schrader KK, and Perschbacher PW (2014) Effect of stocking biomass on solids, phytoplankton communities, common off-flavors, and production parameters in a channel catfish biofloc technology production system. *Aquacultural Resources* 45; (9): 1442–1458.

Grey, A.K., Evans, M.A. and Thorgaard, G.H. (1993). Viability and development of diploid and triploid salmon hybrids. *Journal of Aquaculture Research,* 112: 125-142.

Griffiths, A. J.F., Miller J. H., Suzuki D.T., Lewontin, R.C. and Gelbart, W.M. (2011). An Introduction to Genetic Analysis. *7th edition. New York*: W.H. Freeman and Company.

Groblan, J.P, du perez HH, vanderbank FH, (1992). A compassion of growth performance and genetic triats between four sselected groups of afican catfish (*Clawes gariepinus*) Buchell (1822). *Comparative Biochemistry Physiology* l102:373-377.

Guerra, M. (2000) Patterns of heterochromatin distribution in plant chromosomes. *Genetic Molicular Biology*, 23:1029–1041.

Gunadi, B, and Hafsaridewi R (2007) Waste utilization aquaculture of catfish (*Clarias gariepenus*) intensive maintenance system with heterotrophic for tilapia. Final Report of Research Activity in *Research and Technology for Freshwater Aquaculture Stattion*.pp, 83-86.

Gupta, M.V. and B.O. Acosta, (2001). Fish Genetics Research in Member Countries and Institutions of the International Network in Genetics in Aquaculture. *The World Fi Center, Orissa, India, ISBN*-13: 9789832346050, Pages: 179.

Guerra, R. R. , N. P. Santos , P. Cecarelli , A. J. Mangetti , J. R. M. C. Silva , F. J. Hernandez-Blazquez (2006). Stratum adiposum, A Special Structure of the African Catfish Skin (*Clarias gariepinus*, Burchell 1822) *Anatomia histologia ebryologia* 35:3.

Hallerman, E.M. and Kapuscinsky, A.R. (1995). Incorporating risk assessment and risk management into public policies on genetically modified finfish and shellfish. *Journal of Aquaculture Research*, 137:9-17.

Hallerman, E.M. (2003). Coadaptation and outbreeding depression. In E.M. Hallerman, edition Population genetics: *principles and applications for fisheries scientists*. pp. 239–259.

Haruna, A.B., K.A. Abubakar and B.M.B. Ladu, (2006). An assessment of physico-chemical parameters and productivity status of Lake Geriyo, Yola, Adamawa State, Nigeria. *Biology of Environmental Science Journal Tropics*, 3: 18-23.

Haruna, A.B. (2003). Aquaculture in the tropics theory and practice. Alhassan publisher Kano Ni geria pp 22.

Hartly, S.E and Horne MT, (1985) Cytogenetic techniques in fish genetics. *Journal fish Biology* 26: 575-582

Haylor, G.S., (1992), Controlled hatchery production of *Clarias gariepinus* (Burchell): growth and survival of larvae at high stocking density. *Aquaculture and fisheries management*, 23, 303-314.

Head, W.D., A. Zerbi and W.O. Watanabe, (1994). Preliminary observations on the marketability of saltwater-cultured Florida red tilapia in Puerto Rico. *Journal of World Aquaculture Sociology 25: 289-296.*

Heglern, ex Englem, in the Kainji area Aquatic Botnany 29:351-366. Mohanty. S. N. and Dashm S.P. (1995): Evaluation of Azolla caroliniana for inclusion in carp die. *Journal of Aquaculture in the Tropics Vol. 10(4) pp 343-353.*

Heisman, EA, and Richter CJJ (1987). Reproduction, Growth, Health Control and Aquaculture Potential of the African Catfish, *Clarias gariepinus* (Burchell 1822). *Journal of Aquaculture Research* 63: 1-14.

Hill, Hora, S. L. & Mukherji, D. D. (2005) Fishes of Naga, Assembling and Records of fish *Indian Museum* 37: 381-404.

Hill, J.E., Kilgore, K.H., Pouder, D.B., Powell, J.F.F., Watson, C.A. (2009) Survey of Ovaprim use as a spawning aid in ornamental fishes in the United States as administered through the University of Florida. *Tropical Aquaculture Labouratory* 71, 206-209.

Hockaday, S., Beddow, T.A., Stone, M., Hancock, P. and Ross, L.G. (2000). Using truss networks to estimate the biomass of Oreochromis niloticus and to investigate shape characters. *Journal of Fish Biology* 57, 981-1000.

Hofreiter, M and Schoneberg T, (2010) The genetic and evolutionary basis of colour variationin vertebrates' *journal of Cellular and Molecular Life Sciences* 67:2591 6203

Hogendoorn, H., (1979). Controlled propagation of the African catfish, *Clarias lazera* (C&V). I. Reproductive biology and field experiments *in Aquaculture*, 17 (4): 323-333.

Hogendoorn, H. and M.M. Vismans, (1980). Controlled propagation of the African catfish, *Clarias lazera* (C and V). II. *Artificial reproduction in Aquaculture*, 21: 39-53.

Hogendoorn, H. & Koops W.J. (1983), Growth and production of the African catfish, *Claria lazera* (C&V). I: Effect of stocking densities, pond size and mixed culture with tilapia (*Sarotherodon niloticus L*.) under extensive field conditions. *Journal of Aquaculture science,* 4, 227-248.

Hooe, M.L., Buch, D.H. and Wahl, D.H. (1994). Growth, survival and recruitment of hybrid crappies stocked in small impoundments. *Journal of Fish Management*., 14: 137-142.

Howell WM and Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: *a 1-step method Experiments* 36:1014-1015.

Huang, X. X., X. Q. Zeng, and C. Zhang. (2010). Study on the fishery biology of Pholis fangi in the inshore waters of Qindao. *Periodical of Ocean University of China* 40:55–59.

Hung, S.S.O., Fynn-Aikins, F.K., Lutes, P.B. and Xu, R.P. (1989). Ability of juvenile white sturgeon (*Acipenser transmontanus*) to utilize different carbohydrate sources. *Journals of Nutritions*, 119: 727-733.

Hunter, J.R. and Macewicz, B.J. (2001). Improving the accuracy and precision of reproductive information used in fisheries: modern approaches to assess maturity and fecundity of warm- and cold-water fish squids. *Fishery bulletin*, 90(1): 101-128.

Huisman, E.A and Richter, C.J (1987). Reproduction, growth, heat control and aquacultural potential of the African catfish. *Clariass gariepinus* (Buchell, 1822). *Journal of Aquaculture science* 63:1-14.

Hussain, M.G., (1994). Genetics of body color inheritance in Thai and Egyptian red tilapia strains. *Asian Fish Science.,* 7: 215-224.

Hulata, G., (1995). The history and current status of aquaculture genetics in Israel. *Israllite Journal of Aquaculture Bamidgeh,* 47: 142-154.

Hussain, M.G. and Mazid, M.A. (2001). Genetic Improvement and Conservation of Carp Species in Bangladesh. *Bangladesh Fisheries Research Institute,* Dhaka, Bangladesh, ISBN: 9789843113092, Pages: 74.

Ige B.A (2013) Probiotics use in intensive fish farming. *African Journal of Microbial Resources*. ; 7(22) 2701–2711.

Inssen, P.E., Powell, M.J. and Miller, M. (1982). Survival and growth of matched plantings of lake trout (*Salvelinus mamaycush*), brook trout (*S. fontinalis*) and lake x brook $F_1$ splake hybrids and backcrosses in Northeastern Ontario lakes. *Ontario Ministry of Natural Resources, Ontario Fisheries Technical Report Series 6, Toronto, Canada.*

Ikenweiwe**,** N.B, Bolaji, B.O, and Bolaji, G.A. (2010) Fabrication and Performance Assessment of a Locally Developed Fish Smoking Kiln. *Ozean Journal of Applied Sciences;* 3(4):363-369.

Ikpeme, E.V., O.U. Udensi, U.B. Ekaluo, M.E. Kooffreh, C.M. Okolo, P.B. Ekpo and N.C. Ogbonna, 2015. Unveiling the genetic diversity in *Clarias gariepinus* (Burchell, 1822) using Random Amplified Polymorphic DNA (RAPD) finger printing technique. *Asian Journal of Animal Science.,* 9: 187-197.

Ita, E.O., Sado, E.K., Balogun, J.K., Pandogari, A. and Ibitoye, B. (1985). Inventory Survey of Nigerian Inland Waters and their Fishery Resources: a preliminary checklist of inland water bodies in Nigeria with special reference to ponds, lakes, reservoirs and major rivers. *Kainji Lake Research Institute Technical Report Series* no. 14.

Ita, O (1985). A preliminary checklist of inland water bodies in Nigeria with special reference to ponds, lakes, reservoirs and major rivers. *Kainji Research Institutes Technology Reports.*, (14).

James, C.M., Al-Thobaiti, S.A. Rasem, B.M. and Carlos, M.H (1999). Potential of grouper hybrid (*Epinephelus fuscoguttatus* x *E. polyphekadian*) for aquaculture. ICLARM Q., 22: 19-23.

Janssen J., (1995), Elevage du poisson chat africain *Clariaslazera* (C&V) en République centrafricaine: I. Propagation artificielle Document Technique 20, GCD/CAF/007/NET. *Food and Agricultural Organisation of the United Nations.*

Jeffery, W. R. (2009). Emerging systems in evo/devo: Cavefish and mechanisms of microevolution. *Evolution Development* 10, 265–272.

Jothillakshmana, N. and KK. Marx, (2013). Hybridization between indian catfish, Heteropneustes fossilis (Bloch) and Asian catfish, *Clarias batrachus* (Linn.). *12: 976-981*.

Katersky, S. & Carter, C.G. (2005). Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. *Journal of Aquaculture science*, 250: 775–780.

Karataş, T and Kocaman EM (2009). Farklı sıcaklıklardaşokuygulamalarının Gökkuşağıalabalığı (*Onchorhynchus mykiss*) yumurtalarıü zerineetkisi. Atatürk Üniversitesi Fen BilimleriEnstitüsü Su Ürünleri Anabilimdalı, YüksekLisansTezi, Erzurum. (ın Turkish).

Karatas, T. and Kocaman, E. M. (2014). Growth and feed conversion ratios of Albino and Normal pigmented Rainbow Trout (Oncorhynchus mykiss). *International Journal of Fisheries and Aquatic Studies* 2(2):64-66.

Karina, S, Akbar M, Supriatna A, (2015) Replacement of soybean meal with *Moringa oleifera* leaf meal in the formulated diets of tilapia (*Oreochromis niloticus*) fingerlings. *AACL Bioflux.* 8(5):790–795.

Kakengi, A.M.V., Shen, M.N., Sarvert, S.V. and Fujihara, T. (2003), "Can Moringa oleifera be used as aprotein supplement to ruminant diet". *Asian – Australian Journal of Animal Science* 18 (1): 42 – 47.

Kaplan, (2018) Interspecific Interactions May Influence Reef Fish Management Strategies in the Gulf of Mexico. *American fishery society* 10: pp 1-5.

Khan, H.A., Gupta, S.D. Reddy, P.V.G.K. Tantia, M.S. and Kowtal, G.V. (1990). Production of sterile intergeneric hybrids and their utility in aquaculture and stocking. Procedure of the Carp Seed Production Technology, Septembet 2-4, 1990, *Asian Fisheries Society,* Mangalor, India, pp: 41-48.

Khan, M.M.R., Mollah, M.F.A. and Ahmed, G.U. (2000). Mass production of hybrid magur and its culture potential in Bangladesh *Aquaculture Resources*, 31: 467-472.

Kim, D.S., Jo, J.Y. and Lee, T.Y. (1994). Induction of triploidy in mud loach (*Misgumus mizofepis*) and its effect on gonad development and growth. *Journal of Aquacultural science*, 120: 263-270.

Kim, D.S., Y.K. Nam and I.S. Park, (1995). Survival and karyological analysis of reciprocal diploid and triploid hybrids between mud loach (*Misgurnus mizolepis*) and cyrinid loach (*Misgurnus anguillicaudatus*). *Journal of Aquacultural science*, 135: 257-265.

Kim, M.-K. (2007) Fish distant hybridization of chromosome map. *China Agricultural Science and Technology Publishing House*, 151 pp.

King, R.P., (1997). Growth performance of Nigerian fish stocks. ICLARM Q. 20(2):31-35.

King, TL, Lubinski BA, Spidle AP. (2001) Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae. *Journal of Conservation on Genetic science*. ;2:103–119.

Knibb, W., Gorshkova, G. & Gorshkov, S. (1998). Genetic improvement of cultured marine finfish: case studies. In S. De Silva, educational Tropical mariculture, pp 111-149. *New York, Academic Press Ltd.*

Koehn, R.K. and P.M Gaffney, (1984). Genetic heterozygocity and growth rate in *Mytils edulis*. *Marine Biology of fish., 82: 1-7.*

Koren, A., Pruginin Y. and Hulata G. (1994) Evaluation of some red tilapia strains for aquaculture. *Israeli Journal of Aquaculture* 46, 9–12.

Krasnai, Z.L., (1987). Intraspecifichybridization of warm finfish. Proceedings of the World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture, June 27-30, 1986, Bordeaux, France, pp: 35-45.

Kuo, S.-R. and K.-T. Shao, (1974). Species composition of fish in the coastal zones of the Tsengwen estuary, with descriptions of five new records from Taiwan. *Zoology Studies of fish* 38(4):391-404.

Kushwaha, N.S. Mani, I., R. Kumar, M. Singh, B. Nagpure, P.K. Srivastava, K. Murmu, D.S.K. Rao and W.S. Lakra, (2009). Karyotypic diversity and evolution of seven mahseer species (Cyprinidae) from India. *Journal Fish Biology* 75:1079-1091.

Lahav, M. and Lahav E. (1990) The development of all-male tilapia hybrids in Nir David. *Israellite Journal on Aquaculture Bamidgeh* **42**, 58–61.

Lapedriza, A., Petratou K., and Kelsh R. N., (2014). Neural crest cells and pigmentation, pp. 287–311 in *Neural Crest Cells*. Elsevier.

Langholz, H.-J. and G. Hörstgen-Schwark, (1987) Family selection in rainbow trout. p. 375-385. In K. Tiews (edition) Selection, hybridization and genetic engineering in aquaculture. *Vol. 1. Proceedings of a World Symposium sponsored and supported by (EIFAC), and ICES, Bordeaux. H. Heenemann GmbH & Co., Berlin, Germany*.

Levan, A., K. Fredga and A.A. Sandberg, (1964). A Nomenclature for centromeric position of chromosomes. *Journal of genetic and Heredity,* 52: 201-220.

Leary, R.F., Allendorf, F.W. and Knudsen, K.L. (1983). Developmental stability and enzyme heterozygocity in rainbow trout. 301: 71-72.

Legendre, M. and Oteme, Z. (1995). Effect of varying latency period on the quantity and quality of ova after hCGinduced ovulation in the African catfish, *Heterobranchus longifilis* (Teleostei, Clariidae). *Journal of Aquatic Living Resources,* 8: 309-316.

Legendre, M., Teugels, G.G., Cauty, C. and Jalabert, B. (1992). A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus* (Burchell, 1822), *Heteropneustes longifilis* Valencinnes, 1840 and their reciprocal hybrids (Pisces, Clariidae). *Journal Fish Biology,* 40: 59-79.

Li, J.; Huang, L.; Sato, T.; Zou, L.; Jiang, K.; Yahara, T.; Kano, Y (2013). Distribution pattern, threats and conservation of fish biodiversity in the East Tiaoxi, China. *Environmental Biology in Fisheries 96*, 519–533.

Lim, C., B. Leamaster and J.A. Brock, 1993. Riboflavin requirement of fingerling red hybrid tilapia grown in seawater. *Journal World Aquaculture Sociology,* 24: 451-458.

Linnaeus, C. (1958)). Systema naturae per regna tria naturae secundum classes, ordinus, genera, species cum characteribus, differentiis synonymis, locis. *10th edition Volume. 1. Holmiae Salvii*, 824 p.

Liu, Q., Goudi, C.A., Simco, B.A., Davis, K.B. & Morizot, D.C. (1992). Gene-centromere mapping of six enzyme loci in aynogenctic channel catfish. *Journal Heredity* 83: 245-248. *Aquaculture* 174:59-68.

Liu, D., Y. Guo, Z. Wang and C. Liu, (2012). Phylogenetics inferred from mitogenome and control region of Silver Sillago, *Sillago sihama*. *Mitochondrial DNA sequence* 23(4):255-263.

Madu, C.T., S. Mohammed, A. Mezie, J. Issa and E.O. Ita, (1992). Comparative growth survival and morphormetric characteristics of *Clarias anguillaris*, *Heterobranchus bidorsalis* and their hybrid fingerlings. *National Institute for Freshwater Fisheries Research Annual Report,* New Bussa, Nigeria, pp: 56-61.

Madu, C.T., Mohammed, S. Issa, J. and Ita, E.O., (1993). *National Institute for Freshwater Fisheries Research Annual Report,* New Bussa, pp: 23-29.

Madu, C.T. (1995). Status of Fish Hatcheries and Fish Seed Fingerlings Production in Nigeria. IN: *Report of National Aquaculture Diagnostic Survey,* May-July 1994 (edited by J.S.O. Ayeni), pp.13-34. New Bussa: NIFFR.

Madu, C. T. and Akilo K. T. (2001): The Use of live maggot and live Tilapia fry as unconve ntional diets cryoprotectant on the motility after short-term storage. Pp14-16.

Macewicz, B.J. and Hunter, J.R (2001). Improving the accuracy and precision of reproductive information used in fisheries: modern approaches to assess maturity and fecundity of warm- and cold-water fish squids. *Fishery bulletin*, 90 (1**):** 101-128.

Magalhaes I.S., Seehausen O (2010). Genetics of male nuptial colour divergence between sympatric sister species of a Lake Victoria cichlid fish. *Journal of Evolutionary Biology*. 23:914–924.

Mair, G.C., (1999). Genetics of broodstock management: Basic principles and practices. A.A.R.M. Newslett., 4: 4-6.

Maliszewski, R. (1987). Yellow-orange rainbow trout (in Polish). Gosp Ryb 4:12–13

Mamuris, Z, Apostolidis AP, Panagiotaki P, Theodorou AJ, and Triantaphllidis C (1998). Morphological variation between red mullet populations in Greece. *Journal of Fish Biology* 52:107-117.

Marceniuk, A.P. (2005a). Chave para a identificação das espécies de bagres marinhos (Siluriformes, Ariidae) da costa brasileira. *B Institute Pesca*. 31(2): 89-101.

Marceniuk, A.P. and N.A. Menezes, (2007). Systematics of the family Ariidae (Ostariophysi, Siluriformes), with a redefinition of the genera. *Journal of Zootaxa science*1416:1-126.

Marcus, O., (1982). The biology and fishery of *Sardinella maderensis* (1839) caught off the Nigerian coastal waters. *Annual Report, Nigerian Insttitute of Oceanography Marine Research Lagos*. p. 9-10.

Mgbenka, B.O. and Lovell, R.T. (1984). The progressive fish culturist. Aquaculture Fish service with U.S. Dept, IF & W. series. Vol. 46: 4

Mgbenka, B.O. Hya l. (1990): Digestibility of some agricultural by-products by African catfish (*Clarias*grapienus Burchell 1822) and preliminary investigation on effects of L-ascorbic acid (vitamin C) in an agricultural by-product based diet on the African catfish. Pages 30. In: Baring A. Costa Pierce, Clive Lightfoot, Kenneth Ruddel and Roger S. V. Pullin (editors). *Summary Report on the ICLARM-37 Finfish Feed Technology in Nigeria.*

Mia, M.Y., J.B. Taggart, A. Gilmour, E. Topan and K. Das (2005). Detection of hybridization between Chinese carp species (*Hypophthalmichthys molitrix* and *Aristichthys nobilis*) in hatchery broodstock in Bangladesh, using DNA microsatellite loci. Aquaculture, 247: 267-273.

Micha, J.C., (1975). Quelques données écologiques sur la grenouille africaine *Dicroglossus occipitalis* (Gunther). La Terre et la Vie, Revue d'Ecologie Appliquée, vol. 29, 307-327.

Micha, J.C., (1989). Intégration de la pisciculture dans le développement rural. Colloque C.I.U.F. Le petit paysannat dans les pays en voie de développement, 15 octobre 1987 à Bruxelles. EditionC.I.U.F.

Milani, P.C.C., and Fontoura, N.F. (2007). Diagnóstico da pesca artesanal na lagoa do Casamento, sistema nordeste da laguna dos Patos: *uma proposta de manejo Biociências.*; 15(1): 82-125.

Miller, J. (2003). Aquaculture Professional Organizations: the future of Nigeria's aquaculture industry is in your hands. IN: *Proceedings of the 18th Annual Conference of the Fisheries Society of Nigeria* (FISON*)* (educational. by A.A. Eyo and J.O. Ayanda. Ilorin), pp. 5-6.

Miranda, Ribeiro, A. (1918). Dois gêneros e três espécies novas de peixes brasileiros determinados nas collecções do Museu Paulista. *Revista do Museu Paulista*, 10: 787-793.

Moav, R., Wohlfarth, G.W., Hulata, G., (1975). Genetic differences between the Chinese and European races of the common carp. I. Analysis of genotype–environment interactions for growth rate. *Journal of Hereditыresearch* 34, 323– 340.

Mohanty. S. N. and Dashm S.P. (1995): Evaluation of *Azolla caroliniana* for inclusion in carp die. *Journal of Aquaculture in the Tropics Vol*. 10(4) pp 343-353.

Moutopoulos, D.K. and K.I. Stergiou, (2002). Length-weight and length-length relationships of Muchlisin ZA, Hashim R, Chong AS: Preliminary study on the cryopreservation of tropical bagrid catfish (*Mystus nemurus*) spermatozoa pp17-139.

Muhammadar, AA, Mazlan AG, and Samat A (2014) Growth, survival and feed conversion of juvenile tiger grouper *Epinephelus fuscoguttatus* in different salinity regimes. AACL Muchlisin ZA, Nadiya N, Nadiah WN, *et al. Preliminary study on the natural Bioflux* 7(4): 241–247.

Muchlisin, Z.A Musman M Siti-Azizah MN. (2010b). Length-weight relationships and condition factors of two threatened fishes, Rasbora tawarensis and Poropuntius tawarensis, endemic to Lake Laut Tawar, Aceh Province, Indonesia. *Journal of Applied Ichthyol* 26: 949-953

Muchlisin, Z.A, Nadiah WN, Nadiya N (2015), Exploration of natural cryoprotectants for cryopreservation of African catfish, *Clarias gariepinus,* Burchell 1822 (Pisces: Clariidae) spermatozoa. *Czech Journal of Animal Science*. 2015; 60(1): 10–15.

Murtala, A.G. (2002) Morphological variation of horse mackerel (*Trachurus trachulus*) in the Iberian and North African Atlantic; implications for stock identification. *Journal of Marine Science*.;57(4):1240-1248.

Mukhopadathy, SM,and Dehadrai PV. (1987). Survival of hybrids between airbreathing catfishes *Hetero pneustes* fossilis (Bloch) and *Clarias batrachus* (Linn.). Matsya, 12-13: 162-16

Murphy, W. J., and Collier G. E. (2005) A molecular phylogeny for aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): The role of vicariance and the origins of annualism, *Molicular Biology Evolusion*, volume. 14 pg. 790-799.

Naeem, M, and Salam A. (2005) Morphometric study of freshwater bighead Aristichthys nobilis from Pakistan in relation to body size Pakistan *Journal of Biological Science. ;*8(5):759-762.

Nachtomy, O, Shavit A, and Yakhini, Z, (2007) Gene expression and the concept of the phynotype; *Journal of Historicaal philosophy in Biological Biomolecular Science* 38; 238-254.

Na-Nakorn, U. & Legrand, E. (1993). Induction of triploidy in Puntius gonionotus (Bleeker) by cold shock. *Kasetsart University of Fisheries Resources Bullgate* 18, 10 pp.

Nelson, K. and Hedgecock, D. (1980). Enzyme polymorphism and adaptive strategy in the decapod crustacean. *Amphibian National,* 116: 238-280.

Njoku, DC. Onuoha L. (1997). Age composition and growth of the large-scaled mullet, *Liza grandisquamis* (Pisces: Mugilidae) Valencinnes 1836 onnthe New Calabar estuary off the Nigerian coast. *Fisheries Research, n*26: 67 – 73.

N.R.0, (1993): Nutrient Requirement of Fish. *National Research Council. National Academic Press Washington.* Pp 140.

Noy, R., B. Lavie and E. Nevo, (1980). The niche-width variation hypothesis revised: Genetic diversity in the marine gastropods *Littorina punctata* and *L. neritoides*. *Journal Experts of Marine Biological Ecology*. 109: 109-116

Nordlund, L. M., and Gullström, M. (2006). Biodiversity loss in seagrass meadows due to local Invertebrate fisheries and harbour activities. *Estuarine, Coastal and Shelf Science*, 135, 231– 240.

Nunez – Rodriguez, Ion R, Telvi L, Chaussain JL, Barbet JP, Nunes M, Safar A, and Rethore MO, (1998) Failure of testicular development associated with a rearrangement of 9p24.1 proximal to the SNF2 gene. *Journal of Human Genetic* 102:151–156.

Nurhatijah, N. Muchlisin ZA, and Sarong MA (2016) Application of biofloc to maintain the water quality in culture system of the tiger prawn (*Penaeus monodon* 9(4): 923–928.

Nwadukwe, F.O. (1995). Hatchery propagation of five hybrid groups by artifical hybridization of *Clarias gariepinus* (B) and *Heterobranchus longifilis* (Val.) (*Clariidae*) using dry, powdered carp pituitary hormone. *Journal of Aquacultureof the Tropics*. 10: 1-11.

Nwoko, C.U, Umeham, S.N and B.C Azionu (1991) Changes in limnology and water resources of lake chad. The eefect of aridity and drought in arid zone, hydrology and water resources pp441-449

Ochang, SN, Peter BM, Ugbor ON, Egor A. (2015) Effect of diets with moringa leaf meal on growth, carcass composition and haematology of *Clarias gariepinus*. *International Journal of Fisheries and Aquatic Studies;3(2):397-401.*

Ojima, Y., K. Maeki, S. Takayama and S. Nogusa, (1963). A cytotaxonomic study on the *Salmonidae*. 6:91-98.

Omitogun, O. G, Olanyan, O. F, Oyeleye, O.O,Ojiokpota,C.Aladele,S.E., and Odofin, W.T. (2010). Potentials of short term and long term cryopreservation sperm of African giant catfish *Clarias gariepinus* Burchell (1822). For aquaculture, *African journal of Biotechnology Vol. 9 No 41, PP. 6973-6982, ISSN 1684-5315.*

Olson, ME. (2010) Flora of North America Committee, ed. eFlora summary: Moringaceae: Drumstick Family. *Flora of North America, North of Mexico.* New York and Oxford. 7:167–169.

Olufeagb, S. O. and Aluko P. O. (1998). Growth and survival of triploid *Heterobranchus longifil is (Volume 1840). NIFFR Annual Report*, 102-109.

Onuoha, G.C, Ekpo IE, Chude LA, Isangedighi IA (2010). Composite preliminary ichthyofaunal survey of Ntak Inyang Stream, Ikpa River, Nigeria. *Nigerian Journal of Agriculture, Food and Environment* (*NJAFE*), 6(1&2): 82-89.

Oyelese, O.A., (2006). Fry survival rate under different anoxic conditions in *Clarias gariepinus*. *Resources Biology Science*, 1: 88-92.

Onyia, L.U., Ladu, B.M.B. and Olufeagba, S. (2016). Evaluation of hatchability, survival and growth of *Clarias anguillaris*. *World Journal Biotechnol*., 11: 1662-1667.

Padhi, B.K. and Mandal, R.K. (1997). Inadvertent hybridization in a carp hatchery as detected by nuclear DNA RFLP. *Journals of Fish Biology*, 50: 906-909.

Palikova1, M., Krejci, R. (2011) "Artificial Stripping and Embryonic Development of the Common Gudgeon (GobiogobioL) and Its Use in Embryo-Larval Tests- A Pilot Study."*Czech Journal of Animal Science* 51, 174-180.

Parish, B.B., and Sharman D.P. (1958). Some remark on method used in herring 'racial investigations, with special reference to otolith studies – Rapp. P.-V. Réun.

Pepple, P.C.G. and C.O. Ofor, (2011). Length-weight relationship of Heterobranchus longifilis reared in earth ponds. *Nigerian Journal Fish.*, 8: 315-321.

Pinheiro, AG. Teixeira CM, Rego AL. Marques JF, Cabral HN. (2005) Genetic and morphological variations of Solea lascaris Risso, (1810) along the Portuguese Coast. *Fish Resources* ;73 (12):67-78.

Picker, M.D. & Griffiths, C.L. (2011). Alien and Invasive Animals – A South African Perspective. *Randomhouse/Struik, Cape Town, South Africa.* 240 pp.

Piorski, N.M. (2010) Diversidade genética elogeogra a das espécies

Pisano, E, Ozouf-Costaz C, Foresti F, and Kapoor BG (2007). *Fish Cytogenetics*. Enfield, NH: *Science Publishers, International.* 502 pages.

Ponte, S. (2006). Eco-labels and fish trade: Marine Stewardship Council Certification and the South African hake fishery, Working paper 9/2006, Danish Institute for international studies, Denmark pp 102.

Prasad. R.and Patro, R.(1994) Chromosomes of six marine percoids from the Indian Sea. *Journal of Indian Biological science.* 11(1-2):9-12

Purdom, C.M., (1993). Genetics and Fish Breeding. *Chapman and hall London*. p.277.

Putra, D.F Fanni M, Muchlisin ZA, (2016) Growth performance and survival rate of climbing perch (*Anabas testudineus*) fed Daphnia sp. enriched with manure, coconut dregs flour and soybean meal. 9(5): 944–948.

Rahman, M.A., Bhadra, A. Begum, N. Islam M.S. and Hussain, M.G. (1995). Production of hybrid vigor through cross breeding between *Clarias batrachus* Lin. and *Clarias*gariepinus Bur *Aquaculture*, 138: 125-130.

Raturoti, E O. (2003) Commercial fish feed Development and Aquaculture pp 88-94. In Proceedings of National Workshop on Fish Feed Development and Feeding Practices in Aquaculture held at N1FFR 15pp.

Reddy, H.R.V., Ganapathi Naik, M., & Annappaswamy, T.S. (1997). Evaluation of the dietary essentiality of vitamins for Penaeus monodon. *Journal of Aquaculture Nutrition,* 5(4): 267–276.

Reddy, P.V.G.K., (2000). Captive breeding of *Osteobrama belangeri* (Val.) - a threatened food fish. p.122-123. In A.G. Ponniah and U.K. Sarkar (eds.) Fish biodiversity of north east *Indian Publication*. 2,228p.

Reis, E.G. (1986b) Reproduction and Feeding Habitats of the Marine Catfish *Netuma barba* (Siluriformes, Ariidae) in the Estuary Lagoa dos Patos, Brazil. *Atlântica*, 8:35-5.

Roll-Hansen, Nils. (2009). "Eugenics before World War II: The Case of Norway." *History and Philosophy of the Life Sciences,* 2, 269-98.

Richter, N. Siddhruraju A, Becker K. (2003) Evaluation of nutritional quality of moringa (*Moringa oleifera Lam.*) leaves as alternative protein source for Tilapia (*Oreochromis niloticus* L.). *Journal of Aquaculture* .2003;217:599-611.

Rosenstein, S. and Hulata, G. (1994). Sex reversal in the genus *Oreochromis*: Optimization of feminization protocol. *Journal of Aquaculture*, 89: 329-339.

Rottmann, R.W., Shireman, J.V, and Chapman, F.A. (2003). Hormonal Control of Reproduction in Fish for Induced Spawning. SRAC Publication, *Southern Regional Aquaculture Centre.* Pp 424-431.

Rothbard, S. and Wohlfarth, G.W. (1993). Inheritance of albinism in the grass carp, *Ctenopharyngodon idella*. *Journal of Aquaculture,* 115: 13-17.

Ricker, W.E, (1975). Computation and interpretation of biological statistics of fish populations. Bull. *Fish Research Board Canada* 191: 1-382.

Sahwan, M.F: (2016) The feed fish and shrimp. *Penebar Swadaya. Jakarta* P.172.

Salami, A.A., O.A. Fagbenro and D.H.J. Sydenham, (1993). The production and growth of *clariid* catfish hybrids in concrete tanks. *Israeli Journal Aquacultulture Bamidgeh,*45:18-25.

Sakala, M.E, and Musuka CG: (2014) The effect of ammonia on growth and survival rate of tilapia rendalli in quail manured tanks. *International Journal of Aquaculture*; 22(4): 1–6.

Sahoo, S.K., S.S. Giri, S. Chandra and A.K. Sahu, (2007). Spawning performance and egg quality of Asian catfish *Clarias batrachus* (Linn.) at various doses of human chorionic

gonadotropin (HCG) injection and latency periods during spawning induction. *Journal of Aquaculture*, 266: 289-292.

Sagua, V. O., (1978). The effect of Kainji Dam, Nigeria on fish production in the River Niger below the dam at Faku. *CIFA Technology Paper/Doc Technology CPCA*, (5): 209–294.

Sazima, I. and Pombal J. Jr (1986) Um albino de Rhamdella minuta, com notas sobre comportamento *Osteichthyes, Pimelodidae*). *Revista Brasileira de Biologia* 46, 377–381.

Seeb, J.E., Thorgaard, G.H. and Tynan, T. (1993). Triploid hybrids between chum salmon female x chinook salmon male has early sea-water tolerance. *Journal of Aquaculture*, 117: 37-45.

Senhorini, J.A., G.M. Figueiredo, N.A. Fontes and J. Carolsfeld, (1988). Larval and fry culture Of pacu, *Piaractus mesopotamicus*, tambaqui, *Colossoma macroponum* and their reciprocal hybrids. 1: 19-30.

Scheerer, P.D. and Thorgaard, G.H. (1983). Increased survival in salmonid hybrids in induced triploidy. *Canadian. Journal of Fisheries and Aquatic. Science.*, 40: 2040-2044.

Schveitzer, R, Poli MA, De Oliveira Nuñer AP (2015): The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. *Aquacultural Engineering.*; 66:17–21. 10.1016/ *journa of aquacultural engineering* 2015.01.004

Shelton, W.L., Meriwether, F.H., Semmens, K.J. & Calhoun, W.E. (1983). Progeny sex ratios from intraspecific pair spawnings of *Tilapia aurea* and *T. nilotica*. In *L. Fishelson & Z. Yaron*, editions. *Proceedings of the International Symposium on Tilapia in Aquaculture*. p. 270-280. Tel Aviv University, Israel.

Schweizer, D (1980) Simultaneous fluorescent staining of R–bands and specific heterochromatic regions (DAPI bands) in human chromosomes. *Research Cell Genetics* 27:190–193.

Schrader, K.K Green BW, and Perschbacher PW: (2011) Development of phytoplankton communities and Comm on off-flavors in a biofloc technology system used for the culture of channel catfish (*Ictalurus punctatus*). *Aquaculture Engineering.*; 45(3): 118–126.

Schibatta, O.A., (2007), Reprodução do pirá-brasilia Simpsonichyhys boitonel Carvalho (Cyprinodontiformes, Rivulidae), e caracterização de seu habitat no Reserva Ecológica do Instituto Brasileiro de Geografia e Estatística, Brasília, Distrito Federal, Brasil. *Revolution Brasil Zoology* 22 (4): 1146-1151

Shinkafi, BA, Ipinjolu JK. (2012) Gonadosomatic index, fecundity and egg size of Auchenoglani

occidentalis (Cuvier and Valenciennes) in River Rima, North-Western Nigeria. Niger *Journal of Basic Apply Science*; 20(3): 217-24.

Shibatta, O.A, Muriel-Cunha J, and Pinna M.C.C(2007) A new subterranean species of *Phreatobius goeldi*, 1905 (*Siluriformes, Incertae sedis*) from the Southwestern Amazon basin. *Paper Avul Zoology*; 47(17): 191-20.

Snucins, E.J., (1993). Relative survival of hatchery-reared lake trout, brook trout and F1 splake stocked in low-PH lakes. *National Journal of Fish Management.*, 12: 460-464.

Sidik, A.S, Sarwono, and Agustina (2002) The effect of stocking density on nitrification rate in closed recirculating culture system. *Journal Akuakultur Indonesia.* 1(2): 47–51.

Simon, K. D., Bakar Y., Samat A., Zaidi C. C., Aziz A., and Mazlan A. G., (2009) Population growth, trophic level, and reproductive biology of two congeneric archer fishes (Toxotes chatareus, Hamilton 1822 and Toxotes jaculatrix, Pallas 1767) inhabiting Malaysian coastal waters. *Journal of Zhejiang University Science* 10(12):902–911.

Sirol, R.N., and S.G. Britto. (2006). Conservação e manejo da ictiofauna: repovoamento. Pages 275-284. In: M.G. Nogueira, R. Henry, and A. Jorcin (editions.). Ecología de Reservatórios: Impactos Potenciáis, Ações de Manejo e Sistemas em Cascatas. RiMA, São Carlos, Brasil.

Stevenson, R.D. & Woods, W.A. (2006) Condition indices for conservation: new uses for evolving tools. *Integrative and Comparative Biology*, 46, 1169– 1190.

Skelton, P.H. (1993). A complete guide to the freshwater fishes of southern Africa. Halfway House, South Africa, *Southern Book Publisher*, 388 pp.

Spritz, RA, Hearing VJ. (2013). Abnormalities of Pigmentation. Principles and Practice of Medical Genetics. 1–44

Steffens, W., Jaehnichen, H. and Fredrich, F. (1990). Possibilities of sturgeon culture in Central Europe. *Research on Aquaculture*, 89: 101-122.

Stone, NM and Thormforde HK (2006) Understanding your fish pond water analysis report. University of Arkansas Co-operative Extension 1-4pp

Suhendra, N(1988) Grouth performance of working catfish *Clarias batracus* fed an experimental diet with varying levels of protein and energy.; 7(2) 16–23.

Smith, T.I.J., (1988). Aquaculture of striped bass and its hybrids in North American. *Journal of Aquaculture Resources*, 14: 40-49.

Smitherman, R.O and R.A Dunham (1985) Genetics and breeding pages 283-316 in C.S Tucker *Edition, Channel catfish. Amsterdam*.

Smitherman, R.O., Dunham R.A., Whitehead P.K. (1996) Selection, hybridization and genome manipulation in Siluroidei –*Research on Aquatics. Living Resources*. 9: 93-102

Spritz, RA, and Hearing VJ. (2013). Abnormalities of Pigmentation. *Reasearch on Principles and Practice of Medical Genetics.* 1–44

Swain, D.P. and C.J. Foote, (1999). Stocks and chameleons: The use of phenotypic variation in stock identification. *Research on Fish Resources*, 43: 1123-1128.

Tan-Fermin, J. D., R. R. Pagador and R. C. Chavez. (1997). LHRHa and pimozide-induced spawning of Asian catfish *Clariasmacrocephalus* (Gunther) at different times during an annual reproductive cycle. *Aquaculture* 148:323-331.

Taniguchi, N, Sumantadinata K, Iyama S. (1999). Genetic change in the first and second generations of hatchery stock of black sea bream. Aquaculture. 35:309-320

Taniguchi, N. Doyle R, W (1997) Isolation and characterization of microsatellite Loci from red sea beam *pagrus major* and detection in closely related specie. *Fish science* 63:199-204.

Tarnchalanukit, W., (1986). Experimental hybridization between catfishes of the families *Clariidae* and Pangasiidae in Thailand. *Environmental. Biology Fish* 16(4):317-320**.**

Tave, D., (1986). Genetics for Fish Hatchery Managers. AVI Publishing Company International, Westport Connecticut, ISBN: 9780870555329, Pages: 299.

Tave, D. (1993). Genetic for fish hatchery managers second edition, van nostrand reinhold, newYork, 415pp.

Teugels, G.G., (1982). A Systematic outline of the African species of the genus *Clarias*;*Clariidae*), with an annotated bibliography. Issue 236 of Annalen: Zoologische Wetenschappen, *Musee Royal de L'Afrique Central, Belgium,* pp: 145-146.

Theriogenology, (2004) Juveniles of the catfish (*Clarias anguilaris*) (E). In Fish Nutrition and fish feed technology, Proceedings of the first National symposium on fish nutrition and fish feed technology held at the Nigerian Institute for Oceanography and Marine Research (NIOMR), Eagos 26Ih - 29th October 1999, A. A Eyo. Pp 72-80.

Thorgaard, G.H., Spruell, P., Wheeler, P.A., Scheerer, P.A., Peek, A.S., Valentine, J.J. and Hilton, B. (1995). Inicidence of albinos as monitor for induced triploidy in rainbow trout. *Journal of Aquaculture science,* 137: 121-130.

Tidwell, J.H., Webster, C.D. and Clark, J.A. (1992). Growth, feed conversion and protein utilization of female green sunfish male bluegill hybrids fed isocaloric diets with different protein levels. *Progress Fish Culture*., 54: 234-239.

Trajano, E., (1997). Threatened fishes of the world: *Pimelodella kronei* (Ribeiro, 1907) (Pimelodidae). *Environmental Biology Fishery* 49:332.

Trewevas, E. (1983): *Tilapiine fishes of the General Sarotherodon, Oreochromis* and *Danakilia.* British Museum of Natural History, Publication Number. 878. *Comstock publishing associates.* Ithaca, New York. 583pp

Tseg, T.D. (2004) Morphological variation between populations of spotted mackerel (*Scomber australescus*) off Taiwan. *Fishery Resources* 68(13):45-55.

Torres, R.G.A, Gonzalez P.S, and Pena S.T. (2011) Anatomical, histological, and ultrastructural description of gills and liver of the Tilapia Fagbuaro *et al.* JAERI, 2(1): 39-45, 2015; Article no.005 *Oreochromis niloticus*). *International Journal of Morphology.* 2010; 28:703-712.

Turan, C., Carvalho, G.R. and Mork, J. (1998). Molecular genetic analysis of AtlantoScandian herring (*Clupea harengus*) populations using allozymes and mitochondrial DNA Markers*Journal Marine Biology Association* U.K. 78, 269-283.

Turan, C. D., Erguden, M., Gurlek, N., Basusta and Turan, F. (2004). Morphometric structuring of the anchovy (*Engraulis encrasicolus* L.) in the Black, Aegean and northeastern Mediterranean seas. *Turkish J. of Vetenary and Animal Sci*., 28:865-871.

Ueda, T, Ishnabe T, and Jeon S-R. (2007) Establishment of an Albino Strain of the Bitterling *Tanakia signifer* (*Pisces, cyprinidae*). *Journal of Heredity* 98(3): 277-279

Ufodikc, & C.T, E.B.C. (2003): Growth and survival of catfish (C/arias ungitllaris) juveniles fed live Tilapia and maggot unconventional diets *Journal of aquatic Science.*

Ugwumba, A. A and Ugwumba A.O (2003). Aquaculture options and the future of fish supply in Nigeria. *The Zoologist*: 2 95 – 98.

Ugwumba, O.A.; Ugwumba, A.A.A.; Sowumi, A.A.; Bubu, O.A. and Ofuani, F. (1998). Induced ovulation, artificial spawning, survival and early growth of larvae of the African catfish, *Clarias gariepinus* (Burchell, 1827). In sustainable utilization of aquatic/wetland resources. *Nigeria Association of Aquacultural Sceince. 83-97*

Um-E-Kalsoom, M. Salim, T. Shahzadi and A. Barlas, (2009). Growth performance and Feed Conversion Ratio (FCR) in hybrid fish (*Catla catla* x *Labeo rohita*) fed on wheat bran, rice broken and blood meal. *Pakistan Veterinary Journal*, 29: 55-58.

Verdegem, M.C.J., Hilbrands A.D. and Boon J.H. (1997) Influence of salinity and dietary composition on blood parameter values of hybrid red tilapia, *Oreochromis niloticus x O. mossambicus*. *Aquaculture Research* 28**,** 453–459.

Vidotto-Magnoni, A.P.; Carvalho, E.D (2004). Aquatic insects as the main food resource of fish. The community in a Neotropical reservoir. *7*, 701–708.

Viola, S., O. Rapport and G. Zohar, (1988): Animal protein free feeds for hydride Tilapia (*O. niloticus X O. aureus*) in intensive culture. *Journal of Aquaculture science* 75: 1 15- 125.

Villegas, C.T. (1990[a]). Evaluation of the salinity tolerance of *O. mossambicus, O. niloticus*, and their F1 hybrids. *Research of Aquacultural science* 85:281-292.

Villagas, C.T. (1990b). Growth and survival of *Oreochromis niloticus, O. mossumbicus* and them F1 hybrids in various salinitis. *Research of Aquacultural science* Pp 507-510.

Volckert, F.A M, Kayens G, Schaller R, (1999) Aerial surveillance of operational oil pollution in Belgium's maritime zone of interest. 40(11): 1051-1056.

Westerman, A.G. and W.J. Birge. (1978). Acceleratedrate of albinism in channel catfish exposed to metals. *Preceeding of Fish-cultural science*. 40:143.

Will, P.S., Paret, J.M. and Sheehan, R.J. (1994). Pressure induced triploidy in hybrid *Lepomis*. *Journal World Aquacultural Society*., 25: 507-511.

Willems, OW, Miller SP,Wood BJ (2013). Assessment of residual body weight gain and residual intake and body weight gain as feed efficiency traits in the Turkey (Meleagris gallopavo). *Genetic Sellection Evolutionary*; 45:26.

Wilkins, N.P., Gosling, E. Curatolo, A. Linnane, A. Jordan, C. and Courtney, H.P. (1995). Fluctuating asymmetry in Atlantic salmon, European trout and their hybrids, including triploids. *Journal of Aquacultural science,* 137: 77-85.

Wheaton, F.W. (1977). Aquaculture Engineering. Wiley Interscience, New York, 708 pp.

Wohlfarth, G. (1993). Heterosis for growth rate in common carp. Aquaculture, 113: 31-46.

Wohlfarth, G.W. (1994). The unexploited potential of tilapia hybrids in aquaculture. *Aquaculture Fisheries Management.* 25: 781-788.

Wohlfaarth, G. and Moav, R. (1970). Effect of variation in spawning time on *Clarias*gariepinus *and Heterobronchus longifera. Aquaculture and fisheries management* 25: 781-788pp.

Wootton, R.J., (1999). Ecology of Teleost Fishes. 1st Edn., Chapman and Hall, London, UK, ISBN-13: Pages: 404.

Wolters, W.R. and de May, R. (1996). Production characteristics of striped bass x white bass and striped bass x yellow bass hybrids. *Journal of world Aquaculture Society* 27: 202-207.

Woynarovitch, E. and Horvath, L. (1980). La Reproduction Artificielle des Poissons en eau Chaude: Manuel de Vulgarization, *Food and Agricultural Organization Document, Techpeches*. Volume 201, page 191.

Xu, C Bian, Q, Zan X, Xu X, Liu J, and Chen T. (2014) Mudskippers are amphibious fishes that have developed morphological and physiologica adaptations to match their unique lifestyles; p.12-54.

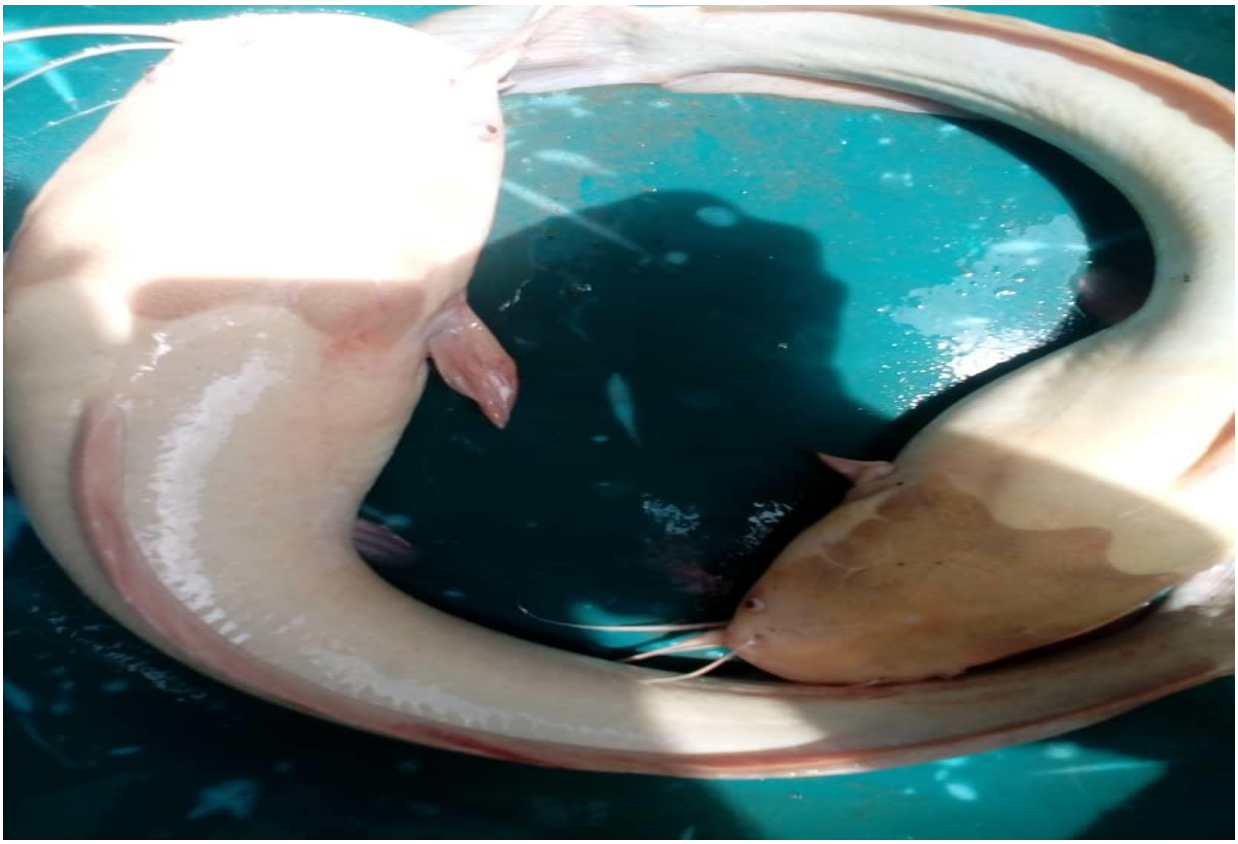
Yanong, R.P.E., Carlos, M and Craig, A.W. (2009). Use of Ovaprim in Ornamental Fish Aquaculture. Program in fisheries and Aquatic Sciences, *Institute of Food and Agricultural Sciences.* University of Florida. FA 161pp.

Yisa, TA, Lamai SL, Tsadu SM, Kolo RJ (2016) Effect of incision variation length on Heterobranchus bidorsalis male spawners to extract milt for induced breeding. *American Journal of Experimental Agriculture.* 10: 1-7.

Zhang, J.B., J.F. Cao, X.F. Yang and R.B. Yang, (2014). Length-weight relationships for three fish species from Yalong River in China. *Journal of Applied Ichthyology*. 30(1):210-211.

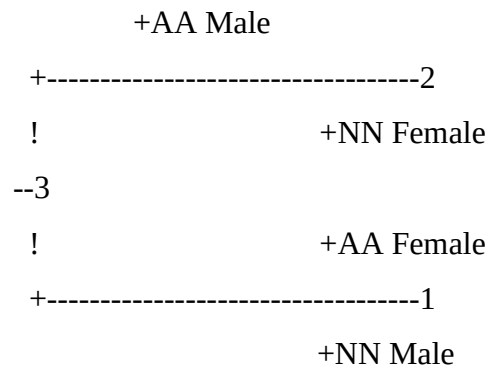
Zonneveld, N., W. J. Rustidja, A. R. Viveen and W. Maduna. (1988). Induced spawning and egg incubation of the Asian catfish, *Claria sbatrachus. Journal of Aquacultural science* 74:41-47.

## Appendix 1



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

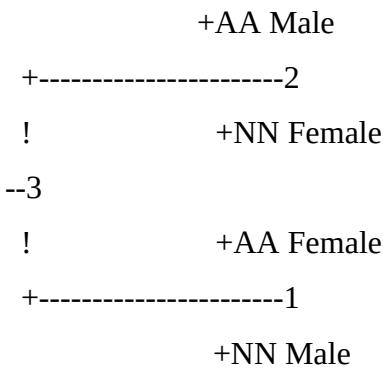
Normal *Clarias gariepinus* (Burchell 1822). The subject of the study showing external morphology




| Between | And | Length |
|---|---|---|
| 3 | 2 | 1.48558 |
| 2 | AA Male | 0.00000 |
| 2 | NN Female | 0.00000 |
| 3 | 1 | 1.48558 |
| 1 | AA Female | 0.00000 |
| 1 | NN Male | 0.00000 |

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

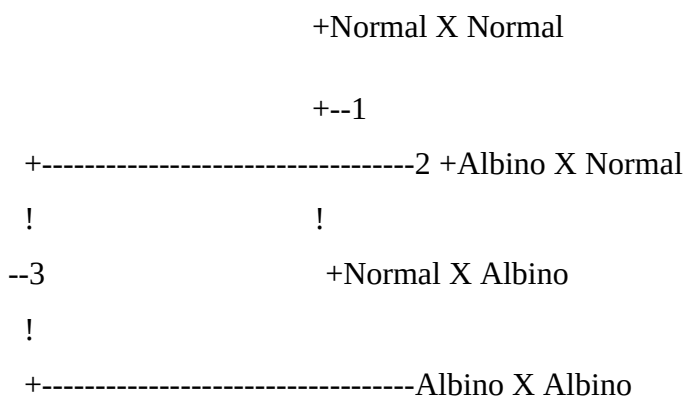
**Appendix 2**




| Between | And | Length |
|---|---|---|
| 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).**

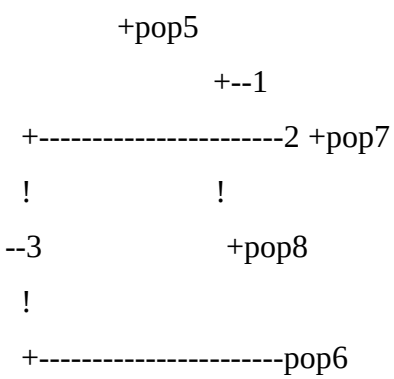
**Appendix 3**




| Between | And | Length |
|---|---|---|
| 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**




| Between | And | Length |
|---|---|---|
| 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).**