MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF TWO STRAINS OF CLARIID FISH SPECIES IN KANO STATE, NIGERIA USING MICROSATELLITE MARKERS

BY

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AHMADU BELLO UNIVERSITY

ZARIA, NIGERIA.

AUGUST, 2017

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE (ANIMAL GENETICS AND BREEDING)

DEPARTMENT OF ANIMAL SCIENCE,

FACULTY OF AGRICULTURE,

AHMADU BELLO UNIVERSITY,

ZARIA NIGERIA

AUGUST, 2017

DECLARATION

I declare that the work in this thesis entitled "**MORPHOLOGICAL AND GENETIC CHARACTERIZATION OFTWO STRAINS OF CLARIID FISH SPECIES IN KANO STATE, NIGERIA USING MICROSATELLITE MARKERS"** has been carried out by me in the Department of Animal Science, Ahmadu Bello University, Zaria – Nigeria. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree at this or any other Institution.

Ibrahim Onotu Suleiman ---------- ----------

Name of student Signature Date

CERTIFICATION

This dissertation entitled "**MORPHOLOGICAL AND GENETIC CHARACTERIZATION OFTWO STRAINS OF CLARIID FISH SPECIES IN KANO STATE, NIGERIA USING MICROSATELLITE MARKERS"**by Ibrahim Onotu SULEIMAN meets the regulations governing the award of the degree of Doctor of Philosophy in Animal Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

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Abstract

This study aim to investigate the morphological and genetic characterization of strains of Clariid fish species in some river bodies in Kano Stateusing microsatellite markers.One hundred and seventy seven Clariid fish samples (*Clariasgariepinus* and *Heterobranchuslongifilis*)were collected from six rivers (Thomas, Ghari, Tiga dam, Duddurun Gaya, Karaye and Bagwai) in Kano state. Body weight, twenty-two morphometric characteristics and four meristic counts were measured on each fish sample to determine the influence of river location, strain of fish and sex.Body weight was measured in grams using sensory scale, the morphometric measurements were measured in centimetres using flexible tapewhile meristic counts were counted visually.The morphometric characteristics taken on the body were; Body weight (BW), Total length (TL), Standard length (SL), Pre-dorsal distance (PDD), Pre-anal distance (PAD), Pre-ventral distance (PVD), Pre-pectoral distance (PPD),Caudal peduncle depth (CPD), Body depth at anus (BDA); measurements taken on the fin were; Dorsal fin length (DFL), Anal fin length (AFL), Pectoral fin length (PFL), Pectoral spine length (PSL); measurements taken on the head region were;Dorso-caudal length (DDCF), Dorso-occipital length (DODF), Head length (HL), Head width (HW), Snout length (SNL), Inter-orbital distance (ID), Eye diameter (ED), Length of occipital fontanelle (OFL), Width of occipital fontanelle (OFW) and Snout-occipital length (DSO). The meristic counts were; Dorsal fin ray count (DFRC), Pectoral fin ray count (PFRC), Anal fin ray count (AFRC) and Caudal fin ray count (CFRC).The total length (TL) and body weight (BW) of each fish sample was used to compute Length-Weight relationships using the formula: $W = \log a + b \log L$ and $K = 100W/L^3$ was used to compute Condition Factor. Blood sample was taken from each fishsampleby severing the caudal peduncle and drained into FTA cards for DNA extraction, Polymerase Chain Reaction and electrophoresis to determine genetic variationbetween the Clariid fish populations. Data gotten from the morphometric measurements were analysed appropriately using GLM procedures of SAS 9.4 to show the influence of river location, strain, and sex, Duncan multiple range test was used for mean separation, Principal component analysis of SPSS was used for possible data reduction, and Genealex 6.4 software package was used to analyse the resolve bands from DNA extraction to determine their base pair and genetic variation. Body weight, morphometric measurements and meristic countswere significantly affected ($P<0.01,0.05$) by location

and strain while sex had effect (P<0.01, 0.05) only on total length, standard length, dorsal fin length, dorso-caudal length, caudal peduncle depth, anal fin length, head length, inter-orbital distance, eye diameter and length of occipital fontanelle. The equation for the length-weight relationship for the three strains were: *C. gariepinus* = -329.86+17.56TL and*H. longifilis*= -241.49+14.28TL.The condition factors showed varying degree of wellbeing of fish samples in their habitat (K = 0.37 to 0.89). Tiga dam had the best condition factor (0.81-0.89) followed by fishes caught in River Ghari (0.74-0.88). Pearson correlation analysis for all the variables measured showed that relationship between Body Weight and all the morphometric measurements were positive and significant. The 'r' values ranged from low (0.23) to high (0.80) for BW/PDD and BW/DDCF. The other measurements had positive and significant relationships with values ranging from 0.30 for ED/SL to 0.92 for TL/SL. Principal component analysis indicated that most of the variables could be used for discrimination with regard to the species with a total variance of 82.52% shared as 47.66%, 19.16%, 8.67% and 7.03% for PC1, PC2, PC3 and PC4, respectively. Among the populations sampled, the genetic similarity ranged from 0.018 to 0.079 whilethe genetic distance ranged from 0.112 to 0.998. The Fst values ranged from 0.000 to 0.663, Fit ranged from -0.041 to 0.115, Fis ranged from -0.350 to -0.262. The result indicated a large number of gene flow (exchange) among the populations with a range of 0.455 to 0.866. The populations were not genetically pure but heterogeneous with varying degrees of genetic similarity and distance. Since there was no inbreeding as shown in the study, none of the population exhibited genetic uniqueness. The populations had a high genetic differentiation between populations but moderate differentiation within populations. The populations were outbred populations; an indication that relatives avoided mating in the population. There was an established magnitude of genetic divergence (91.86%) among the populations as shown by the result of the percentage polymorphism which depends on the number of alleles detected per locus and their frequencies. The study indicated that river location and species of fish had a significant influence on Clariid fish morphometric measurements and meristic counts. The study also gave an indication that the growth pattern of Clariid species in Kano State Rivers was positive allometric growth pattern (b>3) and the Clariid fishes are in good condition of wellbeing as indicated in the condition factor.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Animal Variation

Variability is the fundamental and basic characteristics of life. Every level of organization of life displays variation in some parameters, in space or time, within and between cells, tissues, organisms, populations and communities. The existence of variations in natural populations of organisms is a necessary condition for evolution. While variability is both a product and foundation of the evolutionary process, biologists are still confronted with the basic problems of explaining the nature, extent and causes of this web of complexity (Reynaldo and Cesar, 2014). Genetic variation is one key factor in the survival of species. Natural populations are perhaps the best gene banks which are critical resources for genetic variation for current and future application in improvement of farmed species of fish (Dunham, 2004). Morphological differentiation is one of the several approaches which have proved useful in studying variability. Morphological data alone, however, is insufficient to explain variability. Molecular biology, biochemical analysis and other methods coupled with morphology are powerful means in understanding variability and evolutionary relationships among and within populations of organisms (Reynaldo and Cesar, 2014).

Among populations, genetic diversity can also be gained when populations that are not normally in contact with another hybridize that is when isolated population experienced migration, gene flow and genetic drift. This can occur when physical barriers are removed such as when fishes are introduced to an area or escape, or when migration patterns changes due to environmental condition. Populations of many species of organisms may

respond differently, both morphologically and genetically, to a changed environment. Individuals tend to express different phenotypes (morphological, physiological or behavioural) when surviving in varied environments (Freeman and Herron, 1998). To this end, genetic studies of fish populations play an important role in the sustenance of genetic diversity (Seeb *et al.,* 2007). Genetic markers can provide valuable information about geographic structuring, gene flow and demographic history of populations that can be highly relevant for conservation and management purposes (Maes and Volckaert, 2007).

Of all the animals and plants in the aquatic environment, fish is the most important source of human food (Yilmaz *et al.*, 2000). Fish plays an important role in the development of a nation. Apart from being a cheap source of highly nutritive protein, it also contains other essential nutrients required by the body (Sikoki and Otobotekere, 1999). Fishes are highly important in the development of Nigeria both economically and health-wise as source of protein with low cholesterol level in the diets of many populace.Fish and fish products are economically significant as they provide jobs and investment opportunities and, for many countries, a means of improving the balance of international trade (Yilmaz *et al.*, 2000). Fish is a high quality food and apart from its protein contents, it is also rich in vitamins and contains variable quantities of fat and minerals for human health (Adeniyi*et al.,* 2010). Fish oil contains vitamins A, D, E and K which have been successfully used in controlling coronary heart diseases, arthritis, atherosclerosis, asthma, auto-immune deficiency diseases and cancer (Bhuiyan *et al.,* 1993). Fish is often recommended for cardio-vascular disease patients because of its unique fat, which is composed mainly of Omega- 3 polyunsaturated fatty acid. In addition to its nutritious flesh, vitamins A and D present in fish oil are important especially for infants and children (Fasakin, 2006). Fish also supplies to the body, a range of inorganic minerals such as Phosphorus, Fluorine,

Potassium, Iron, Zinc, Magnesium, and Copper and in marine species Iodine as well as vitamins A and B complex (Adeniyi *et al.,* 2010). The proximate composition, nutritive values and mineral composition of fishes in Nigeria have been documented (Olatunde, 1980; Abdullahi and Abolude, 2006; Dankishiya and Kabir, 2006; Abdulkarim and Abdullahi, 2009). Most of the fish used for human consumption is obtained through exploitation of wild populations.

Water quality tolerance of catfish is diverse due to environmental changes. The warmer the water, the less the dissolved oxygen likewise, the greater the altitude, the less the dissolves oxygen, causing severe cases and death aquatic organisms including catfish. According to F.A.O., (2003), water quality requirement for catfish are as follows; temperature – 26 to 32^{o}C, dissolved oxygen – 3 to 10 mg/l or > 3ppm, pH – 6 to 8, Alkalinity – 50 to 250 mg/l, Ammonia – 0 to 0.03% and Nitrite – 0 to 0.6mg/l. It also reported that for advanced fry, the requirement are as follows; dissolved oxygen – 3-5ppm, temperature – 30^{o}C, ammonia – 0.1 to 1.0ppm, nitrite – 0.5ppm, nitrate – 100ppm, pH – 6 to 9, carbon dioxide – 6 to 15ppm and salinity – 10 to 16ppt.

1.2 Statement of Research Problem

Reduction in the genetic resources of natural fish populations is an important management problem. Not only has the genetic diversity of many fish populations been altered, but many populations and species have been extirpated by pollution, overfishing, destruction of habitat, blockage of migration routes and other human developments (Ferguson, 1995). Loss of genetic diversity and locally adapted populations (and species) can compromise stability and recovery potential of marine ecosystems as well as impair their ability to adapt to changing environmental condition.There is generally limited information on

genetic variation amongand within Clarias and Heterobranchus species and this greatly hampers an efficient and sustainable exploitation of these resources (Worm *et al.*, 2009).

1.3Justification of the Study

The quantification of specific characteristics of an organism or group of organisms is a demonstration of the degree of speciation induced by both biotic and abiotic conditions, thereby contributing to the definition of different stock of species (Bailey, 1997). The African catfish *Clarias gariepinus* and *Heterobranchus longifilis* are economically important species, but little is known about the genetic background of the natural populations of these species. Also, genetic study is needed for proper identification of the two species and determination of the genetic connection between them. Although, morphometric parameters have been used in the past to identify these too species but more specific tool is needed for a more concise differentiation.

Genetic improvement of aquaculture species offers a substantial opportunity for increasing production efficiency, health, product quality and ultimate profitability. It entails a lot of parameters including the description of their population and differences. When different populations arise, with little or no connection between them, they become genetically different from one another. Loss of such populations thus results in loss of genetic diversity within the species. The existence of multiple spawning units is an indicator that populations may be reproductively isolated, particularly if the species shows spawning site fidelity or homing ability. The organization of these populations in time and space, along with the ratio of within and among population variation are important to maintain in order to avoid negative genetic effects (Altukhov and Salmnekova, 1994). Scientifically, sound management of fish resources relies on the information on the biology of the species which include information on population structure.

There is little information on the genetic characteristics of clariid species of fish in natural waters in Northern Nigeria, especially Kano State. The study is expected to made known some useful inferences about the magnitude of stock delimitation may be possible with the notion that genetics-cum-environment influences phenotypic expression.

Microsatellite DNA marker has been the most widely used for genetic studies, due to its easy use by simple Polymerase Chain Reaction, followed by a denaturing gel electrophoresis for allele size determination, and to the high degree of information provided by its large number of alleles per locus(Vignal *et al.*,2002).

1.4 Objectives of the Study

The aim of the study is to characterize morphologically and genetically the clariid species in Kano State using microsatellite markers. The specific objectives are as follows:

i) To determine morphometric characters and meristic counts of Clariid species in Kano state
ii) To determine the influence of location, strain and sex on the morphometric and meristic attributes of Clariid species.
iii) To determine length-weight relationships and condition factor (ponderal index) in Clariid species in Kano State
iv) To evaluate genetic variation within and among Clariid species populations in Kano state
v) To determine relationships between morphometric measurements from the studied fish populations.

1.5 Research Hypotheses (Null and Alternative)

i **Ho:** Location, strain and sex does not have any influence on the morphometric and meristic count in Clariid species.

Ha: Location, strain and sex does have any influence on the morphometric and meristic count in Clariid species.

ii. **Ho:** The length-weight relationship and condition factor of Clariid species do not differ.

Ha: The length-weight relationship and condition factor between Clariid species differ.

iii. **Ho:** There is no genetic variation within and among Clariid populations.

Ha: There is genetic variation within and among Clariid populations.

iv. **Ho:** There is no relationship between morphometric measurements in the studied *clariid*

populations.

Ha: There is relationship between morphometric measurements in the studied *clariid* populations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The African Catfish *(Clarias gariepinus)*

Catfish (*Clarias gariepinus and Heterobranchus longifilis*) are commercially important warm water fishes which are distributed all over the world. The family of air-breathing or labyrinth catfishes (Clariidae) has over one hundred representative species native to most of Africa and Southeast Asia (Burgess, 1989). This specie has drawn attention because of its biological attributes that includes faster growth rate, resistance to diseases and possibility of being highly prolific. Its natural distribution extends all over Africa and Asia Minors; it is cultured intensively and extensively in Africa, Europe and Asia (Huisman and Richter, 1987).

Catfishes, especially members of the family Clariidae, are very important in Africa and are very abundant in commercial catches in Nigeria especially in the dry season (Okaeme, 2005). Members of this family that are very common include Heterobranchus species (*Heterobranchus bidorsalis and Heterobranchus longifilis*) and Clarias species (*Clarias gariepinus and Clarias anguillaris*) and their cultured hybrids (*Heteroclarias*). These species are very important because they not only keep well in both fresh and dried conditions, but are highly valued and enjoyed a greater level of popularity among consumers (Holden and Reed, 1978). The economic importance of Heterobranchus and Clarias catfishes and their hybrids is enhanced by their hardiness and adaptability to adverse environmental conditions, tolerance of high density culture, resistance to diseases, fast growth rate, high consumer ranking and their ability to accept or thrive on cheap feed (Olatunde, 1989). The growth of aquaculture animal protein has been increasing at a rate

of over 10% annually compared to 0.7% and 5.2% in beef and poultry respectively (Dunham, 2004). Even as aquaculture closes the gap or surpasses the value of commercial fisheries, the genetic management and conservation of natural fish stocks and gene pools is of great importance (Dunham, 2004). There is an increase in research interest in the maintenance and preservation of genetic diversity of fish as an essential ecological resource and life support system (Ekelemu and Zelibe, 2006). African catfish is an economically important species, apart from being a model organism in research (Volckaert *et al.,* 1994). The desire of fish farmers is to produce table sized fish within the shortest possible time. Long term success in meeting this goal and having an all-year round supply of fish, depends on the ability of the farmer to control the entire life cycle of the fish (Ezenwaji, 1989; Ekelemu and Ekokotu, 1999).This desire is met by two popular catfishes of the genera Clarias and Heterobranchus. *Clarias gariepinus* occupies a unique and prominent position in the commercial fisheries in Nigeria because it is tasty, hardy and can tolerate poor water quality conditions (Idodo-Umeh, 2003). It is also capable of reproducing in captivity and growing to a size of 7.0 kg (Holden and Reed, 1978; Idodo-Umeh, 2003).It has an efficient feed conversion especially in the males (Nweke and Ugwumba, 2005) and so attracts high market price. In a similar vein, Heterobranchus spp are also important and can grow to a size of about 14.0 kg but not as hardy as the Clarias spp (Idodo-Umeh, 2003). Aquaculturists have been able to harness the qualities of these two species by cross breeding them to produce a hybrid (Heteroclarias or Clariobranchus) which is hardy and grows to a large size (Miller, 2003; Keremah and Green, 2005) and they often display unequal growth patternwhen used to stock ponds.The hybrids of Clariasand Heterobranchus exhibit fast growing quality of Heterobranchusreaching up to 1.0 kg under eight months in ponds.On the other hand, observations using pure Clarias and Heterobranchus species to stock ponds shows that they grow uniformly (Ekelemu and

Ogba, 2005). These observations on the growth patterns of Heteroclarias, Clarias and Heterobranchus species notwithstanding, farmers are divergent in their opinion as to which set of fish to use in stocking their ponds

Studies on Heterobranchus and Clarias spp. have shown them to be quality animal protein sources (Afolabi, 1984). Essential amino acids in Heterobranchus and Clarias spp. have been reported to include lysine, leucine, isoleucine, arginine, histidine, methionine, threonine, phenylalanine, valine and tryptophan (Effiong and Tafa, 2005). Other essential amino acids like alanine, asparagine, glycine and glutamic acid were similarly reported in Heteroclarias hybrids (Effiong and Tafa, 2005). The presence of all these essential amino acids necessary for healthy human life in these catfishes underscores their nutritive value and hence the importance of their consumption. (Esobhawan, 2010).

The African catfish is indigenous to the Northern parts of the continent which has been trans- located into many rivers outside its natural range. Its common names include African catfish, African magur, sharptoothed catfish, barbell and skerptand barber (Sheasby, 2009). It is a dominant freshwater fish. Its body colouration varies from olive green to brown and black with the flanks often uniform grey to olive-yellow with dark slate or greenish brown black. Underparts are pale olive to white and are mottled irregularly with dark brownish green or uniform silvery olive (Sheasby, 2009)

The catfish genus can be defined as displaying an eel shape, having an elongated cylindrical body with dorsal and anal fins being extremely long (nearly reaching or reaching the caudal fin) with both fins containing only soft fin rays. The outer pectoral ray is in the form of a spine and the pelvic fin normally has six soft rays. The head is flattened, highly ossified, the skull bones (above and on the sides) forming a casque and the body is covered with a smooth, scaleless skin. The skin is generally darkly pigmented on the

dorsal and lateral parts of the body. The colour is uniformly marbled and changes from greyish olive to blackish according to the substrate. On exposure to light the skin colour generally becomes lighter (F.A.O., 2003). They have four pairs of unbranched barbels, one nasal, one maxillar (longest and most mobile) on the vomer and two mandibulars (inner and outer) on the jaw. Tooth plates are present on the jaws as well as on the vomer. The major function of the barbels is for prey detection (F.A.O., 2003).

Catfish is a heavy boned, flat headed fish with premaxilla and lower jaw pointed teeth arranged in several rows. It has a high number of gill rakers varying from 24 to 110, the number increasing with the size of the fish (Sheasby, 2009). The body is elongated with long, low dorsal and anal fins and a smoothly rounded tail fin. The skin is leathery and has no scales. It has a small but powerful pectoral fin set immediately in front of the anal fin which has a serrated spine. The eyes are small and set far forward in a flat and bony head. At the back of the head there is a subsidiary breathing organ above the gills which enables this animal to breathe air directly (Sheasby, 2009)

Having a bi-loded swim bladder which is connected to the oesophagus via a narrow pneumatic duct all making the catfish buoyant. The swim bladder is reduced to compensate for this buoyancy. Air is retained in the suprabranchial chamber when a vertical stationary position is required but the air is expelled when the fish need to plunge down suddenly to avoid predation (De Moor and Bruton, 1988). Clarias spp. inhabits calm water from lakes, streams, rivers, swamps to floodplains, many of which are subject to seasonal drying. The most common habitats frequented are floodplain swamps and pools in which the catfish can survive during the dry season due to the presence of their accessory air breathing organs (F.A.O., 2003).Although numerous studies on the food composition of *Clarias gariepinus* have been carried out, a consistent pattern has not

emerged and they are generally classified as omnivores or predators. They have been found to feed on terrestrial insects, molluscs and fruits (F.A.O., 2003).

With increasing demand for aquaculture food, application of molecular genetics to production traits is desirable through multiple trait selection programmes with the aid of marker assisted selection (MAS). This will greatly contribute to production efficiency; thus, enhancing production and increasing sustainability. Genetically improved fish removes the impediments to sustainability like slow growth rate, inefficient feed conversion, and heavy mortality from disease and associated use of chemicals, loss of fish from low oxygen levels, inefficient harvest, poor reproduction and processing loss. African catfish,*Clarias gariepinus* and *Heterobranchus longifilis,*are important species, but little is known about the genetic background of their natural populations. Also, genetic study is needed for proper identification of the two species for more concise differentiation and determination of the genetic connection between them. Also, specific highly polymorphic markers are required to assess the inbreeding level associated with the aquaculture of this species (Van Der Bank *et al.,* 1992).

Being a freshwater fish it is often found in rivers, dams, weirs, lakes, swamps, muddy waters, floodplains and other water bodies. They can be found at depths between 4 and 80m. It is able to bury itself in the river bed when there is a decrease in water or drought is occurring. They have been known to stay in muddy ground of ponds gulping air directly using their accessory breathing organ instead of their gills. Although they are unlikely to survive in ground that has dried completely, they have been known to 'walk' over land when there are damp conditions or to look for food and can survive extreme conditions and harsh environments. They can survive low oxygen concentrations in water of

temperature extremes from 8 - 35°C with salinity levels between 0 and 10% as well as a wide tolerance of pH range (De Moor and Bruton, 1988).

Clarias gariepinus is a non-guarding, substrate spawner, which awaits optimal conditions before spawning commences. The average number of eggs produced is 45000 eggs for a 2kg fish (Sheasby, 2009).Breeding of this species occurs in very shallow, weedy waters normally after heavy rains and usually once the fish have migrated upstream. Breeding normally takes place after heavy rains, often within 2 to 3 days of the new moon of last quarter. Courtship, spawning and egg deposition take place at night with the peak spawning times between 8pm and 2.30am. Tiny fertilized eggs hatch out within 24 to 36 hours of being attached to plants and debris in the water. The larvae swim after 50 hours and begin to feed by 80 hours. The fry live in inshore vegetated zones. *Clarias gariepinus* shows a seasonal gonadal maturation which is usually associated with the rainy season. The maturation processes of *Clarias gariepinus* are influenced by annual changes in water temperature and photoperiodicity and the final triggering of spawning is caused by a rise in water level due to rainfall (De Graaf *et al*., 1995). There is no parental care for ensuring the survival of the catfish offspring except by the careful choice of a suitable site. Development of eggs and larvae is rapid and the larvae are usually capable of swimming within 48-72 hours after fertilization at 23-28^{o}C (De Graaf *et al*., 1995).

Clarias is a voracious predator and eats almost anything including insects, crabs, plankton, snails, fish, young birds, amphibians, reptiles, rotting flesh, plants and fruits. It is normally an individual bottom feeder, but are known to be extremely adaptable to conditions and feed in groups at the water surface. They hunt socially, swimming in formations on the water surface or in a claw like formation to the shore (Sheasby, 2009).

The mouth is wide, subterminal, traverse and capable of opening extremely wide for engulfing prey items or sucking in large amounts of water which is flushed through the gills for filter feeding. Once the prey is in the mouth, the jaws snap closed and the broad bands of sharp teeth on both the upper and lower jaws prevent the prey from wriggling free. The prey is swallowed whole. The oesophagus is short, muscular and dilatable. It opens into a distended stomach typical of creatures capable of carnivory. The four types of feeding as described by De Moor and Bruton(1988) are:

a) Individual foraging (general solitary searching for food through the water column)

b) Individual shovelling (moving detritus and debris on the river floor and eating hidden organisms)

c) Surface feeding (moving water through the mouth and gills to capture organisms on the surface)

d) Formation feeding (includes social hunting, pack formation and feeding frenzies)

Catfish are opportunistic feeders and will take any fish species which is abundant. They respond quickly to newly available food sources and will change their feeding patterns to match organisms freely available. The youngs feed mostly on small invertebrates in shallow inshore areas.Factors which restrict the food niche of these fish are interspecific competition; predation pressure, constant low supply levels of food and harsh physic-chemical conditions. Interspecific competition and predation pressure are buffered by the large body size of the catfish, the shape and protection of the head, pectoral spines and piscivorous habits. The versatility of their physical adaptations enable them to survive almost all conditions (De Moor and Bruton, 1988).Clarias species is a nutritious food source in Africa and there is contention between various sources on its viability as an

aquaculture species. It can be housed in higher densities than most fish species and is a hardy, quick growing and tasty fish.

2.2*Heterobranchus longifilis*

Genus *Hetrobranchus longifilis* are mainly recognized and differentiated from *Clarias* by the presence of an arrayed dorsal fin. Four species of this genus are known, they are *Heterobranchus longifilis* (Valenciennes, 1840), *Heterobranchus bidorsalis* (Geoffroy, 1808), *Heterobranchus boulengen* (Pallegrin, 1912) and *Heterobranchus isopterus* (Bleeker, 1863) which is the smallest member of the genus (Reed *et al.*, 1967), but only two species are available in Nigeria, *Heterobranchus longifilis* and *Hetrobranchus bidorsalis*.

This is a very wide-ranging species with an almost pan-African distribution.

Central Africa:*Heterobranchus longifilis* are known throughout the Congo River basin. In Lower Guinea, it occurs in the Cross Sanaga in Cameroon, and the Ogowe in Gabon (Azeroual *et al.,* 2010).

Eastern Africa:*Heterobranchus longifilis*are present in Lake Tanganyika, Malagarasi River, Lake Rukwa drainage, Rufiji and Wami, Lower Shire River, Lake Rukwa system, Lake Edward, Murchision Nile, and Lake Turkana (Azeroual *et al.,* 2010).

Northern Africa: They are present but rare in Lower Egyptian Nile (Cairo) and sometimes found in Upper Egyptian Nile (Luxor and Aswan), as well as Lake Nasser (Lake Nubia) (Azeroual *et al.,* 2010).

Northeast Africa: They are found in Ghazal and Jebel systems, White Nile, Nile and Lake Nasser in Sudan. They are also found in Baro and Omo Rivers in Ethiopia (Azeroual *et al.,* 2010).

Southern Africa: They are known from middle and lower Zambezi and North into Central Africa. There are also new records of their existence in the Rovuna River (Azeroual *et al.,* 2010).

Western Africa: They are known from Gambia, upper Senagal, Niger, Benue, Lake Chad, Volta and coastal basins from Guinea to Nigeria, including the delta of Niger and the Cross River (Azeroual *et al.,* 2010).

Heterobranchus longifilis are demersal, potamodromous specie. They are uncommon and inhabits large rivers (Eccles, 1992) and found in quite waters with deep pools and stretches, not necessarily associated with vegetation, in large waterways and main river channels (Teugels *et al.,* 1990). They are omnivorous scavengers, most active at night, feeding on any available food, including invertebrates and insects when small, and fish and other vertebrates when large. They scavenges off large carcasses and offals from riverside villages (Skelton, 1993). THey undergoes a spawning migration from the normal dry season habitat into the tributary rivers and floodplains during the rainy season (Teugels *et al.,* 1990). Eggs and juveniles are found among plant roots in shallow water. It lives for 12 or more years and can be caught with trammel or sinking gill-nets.

Aquaculture production of the African catfishes,*Clarias gariepinus* and *Heterobranchus longifilis,*has been practiced for a long time in Africa. Increased productivity of fry and fingerlings with attributes of faster growth rates and better environmental tolerance is *sine qua non* to ensuring fish food security in Africa. Genetic techniques are therefore needed

to ensure that a faster growth rate leading to a shorter production cycle as well as a greater tolerance for poor water condition is achieved. The USDA (1988) pointed out that the lack of reliable estimates of genetic parameters such as genetic and phenotypic variances, covariance, and genetic and phenotypic correlations for commercially important traits and the lack of designed selection programs to test their validity as the major constraints to rapid development of stocks for commercial production of aquaculture species.

2.3 Fish Farming in Nigeria

Food is a basic necessity of life, third only to air and water. The global experience of energy crisis as a result of ever increasing human population has shifted survival pressure from land to water. The global food equation recognizes two major components, namely: food crop component and animal protein component (Balogun, 1998). Animal protein sources include fish, poultry and livestock. Fish consumption in Nigeria is higher due to its availability and comparatively cheaper pricesthan livestock production. The biomass of fish that can be produced per surface area is much greater than that for terrestrial animals indicating that fish production or aquaculture could be the key for providing global food security (Dunham, 2004).

Fish farming is the growing and cultivation of different species of fish including other aquatic animals for various purposes such as feeding, decoration, ornamental and for advanced research. This branch of agriculture has become very important being that they are good sources of protein, vitamins, oils, etc (Solomon and Ezigbo, 2010). The demand for protein normally rises as the population increases hence, the need to invest on animal and fish species that can reproduce and grow rapidly.

Fish farming, also called aquaculture, plays a major role in agriculture in Africa and especially Nigeria. This could be practiced in either their natural environment or by

artificial methods most especially to boost productivity. Artificial fish farming can be carried out with the use of ponds, tanks and aquariums, making available facilities which will enhance fish growth. Researches and studies are enhanced or encouraged with artificial fish farming with the aim of improving growth yield, prevention of diseases, improved quantities and other areas of study (Solomon and Ezigbo, 2010). In the review by Ita (1998), it was stated that early fish farmers in Nigeria raised their fish in burrow pits, abandoned minefields and in earthen ponds in extensive production system. The introduction of concrete tanks allows for manageable pond size and modification of the environment through a water flow-through system and supplementary feeding thus allowing for higher fish yield. The story of aquaculture in Nigeria is essentially the story of catfish culture and the hope of fish supply in Nigeria hangs on its development and culture.According to Olatunde (1989), Nigerian fish constitute almost 40% of animal protein intake and the trend has increased into the 21st century.

With the effort of FAO, modern aquaculture and aquaculture technology has been invented into the system, most especially with catfish and others which are facilitated with the acquisition of induced breeding technology (FAO, 2003). This effort seeks to improve fish yield and productivity. Its benefits range from rural development income generation, farm sustainability as well as reduction in vulnerability. This effort also makes use of land which are considered unsuitable for agriculture such as swamps or saline areas. Ayinla (1991) stated that over 9570MT of all the fish consumed in Nigeria are from the wild. Eyo *et al.* (2003) reported that since aquatic resources are finite although renewable, every effort should be made towards increased fish production through improved resource management and conservation and also intensive aquaculture practices.

Nigeria has high potentials in aquaculture which are hardly tapped. Ayinla (1991) stated that aquaculture has provided food of high animal protein, generated income and

employment thereby promoting the socio-economic development of Nigerians. Fish production when combined with improved inland fisheries management will eliminate fish importation and earn substantial foreign exchange.

To solve the problem of high demand for fish and fish products, Nigerians must turn to their underutilized inland waters for improved fish production and aquaculture. Fish farming in the country is already a very lucrative business and it is mainly boosted by the continuous rise in the demand for the African catfish. This trend thus makes catfish culture the most popular form of the fish farming in Nigeria with *Clarias gariepinus,* Heterobranchus spp and Heteroclarias spp being the most desirable culture and they have remained an important species for research in agriculture (Adekoya *et al.,* 2006). Catfish family,Clariidae is very popular in Nigeria due to its culture characteristics which has endeared it to many fish farmers. Ninety percent of the catfish supply in Sub-Sahara Africa in 2000 occurred in Nigeria (F.A.O., 2004).

Clarias gariepinus and Heterobranchus spp are species of high aquaculture importance in Nigeria. They are widely cultured owing to their high market price, fast growth rate and ability to withstand adverse pond conditions especially low oxygen content. Heterobranchus grows faster and attain bigger size than Clarias which matures earlier, more adaptable and has higher fecundity (Bartley *et al.,* 2000). They reported that interspecific hybrid fishes transfer desirable traits between species, combine desirable traits of two species into a single group of fishes. The hybrids of Heterobranchus and Clarias exhibit the fast growing quality of Heterobranchus reaching up to 1kg under eight month in pond and resistant to diseases (Bartley *et al.,* 2000).

Hybridization is one of the genetic improvements in aquaculture industry which has been recognized as a tool for stock improvement and management purposes. Several studies have demonstrated that *Clarias gariepinus* X *Heterobranchus bidorsalis* hybrid exhibits

superior growth, improved survival and general hardiness than true breed of either *Clarias gariepinus* or *Heterobranchus bidorsalis* (Nwadukwe, 1995b). The technology has been widely accepted as it gives 58% internal rate of return (IRR) on investment (Adeogun *et al.,* 1999).

2.4 Morphological and Meristic Characteristics

One of the most ignored areas in aquatic genetics and biotechnological research is the effect of the environment and experimental procedures on genetic expression, the phenotype and phenotypic variation (Dunham, 2004). Determination of population structure of exploited species is an essential component in successful management of fisheries. Specifically, this information can be used for applications ranging from the determination of appropriate conservation units to estimation of stock composition in mixed stock fisheries. Fish stocks are identified on the basis of differences in stock characteristics. The quantification of specific characteristics of an individual or group of individuals can demonstrate the degree of speciation induced by both biotic and abiotic conditions, and contribute to the definition of different stock species (Bailey, 1997). Characters used to identify fish stocks can be purely genetics, purely environmental or those that may reflect both genetic and environmental variation (Swain *et al.,* 1991). Morphometrics and meristics are the two types of morphological characters that have been most frequently used to delineate stocks of a variety of exploited fish species (Silva, 1985; Turan, 2004).

The morphology of fishes historically has been the primary source of information for taxonomical and evolutionary studies. Despite the value and availability of genetic, physiological, behavioural and ecological data for such studies, systematic ichthyologists

continue to depend heavily on morphology for taxonomic characters (Moyle and Cech, 1981). Species have characteristic shapes, sizes, pigmentation patterns, disposition of fins and other internal features that aid in recognition, identification and classification. In addition, there are important characters that can be examined by dissection or other means of internal examination (Bond, 1979). Morphological terms and conventional measurements include body sections, features of the head, fins, lateral line, scales and other dermal structures, pigmentation and colour patterns. Meristic characteristics include the vertebrae column, fin rays, scales, gill rakers and other rudiments (Strauss and Fuiman, 1985). Morphometric and meristic studies have provided useful results for identifying marine fish stocks and describing their spatial distributions (Ihssen *et al.,* 1981).

Morphomerics is the empirical fusion of geometry with biology (Bookstein, 1997). Patterns of morphometric variation in fishes indicate differences in growth and maturation rates because body form is a product of ontogeny. Phenotypic plasticity of fish allows them to respond adaptively to environmental changes by modification in their physiology and behaviour which leads to changes in their morphology, reproduction or survival that mitigate the effects of environmental variation (Meyer, 1987).

Morphological variation is a central theme in animal production because it provides inference into evolution, ontogeny, ethology, life history, and ecology. Shape variation is expected in freshwater fishes due to adaptation and occurs with development (Cadrin and Silva, 2005), variation in body size (Kimmel *et al.*, 2008), sexual maturity (Pyron, 1996), species interactions (Shine, 1989), ecology (Carlson and Wainwright, 2010), dispersal (Walker and Bell, 2000) and environmental variation (Haas *et al.,* 2010).

Morphology is primarily constrained by phylogeny, allometry and physiology (Norton *et al.,* 1995). Phylogeny is expected to be most indicative of interspecific morphological

variation. Environmental factors such as prey availability, habitat variation, and geography and assemblage composition likely contribute to intraspecific morphologic variation. Environmental influences on intraspecific morphology act through phenotypic plasticity (West-Eberhard, 1989). However, phenotypic plasticity is not necessarily independent of phylogeny, allometry and physiology. Ontogeny associated with maturation and body size increase (allometry) is a primary source of morphological variation (Hood and Heins, 2000).

Ontogenetic variation in morphology is frequently attributed to variation in feeding and habitat adaptations. For example, Haas*et al* (2010) attributed morphological allometry of feeding functions to changes in diet with increased body size. Similarly, Hugueny and Pouilly (1999) identified persistent and convergent morphological patterns across taxa with similar life history strategies for diet, anatomy, and physiology in a phylogenetic study of a West African freshwater fish assemblage. One caveat is that the ontogenic trends in shape for some proportion of taxa may only represent a covariate of size and not be adaptive (Cadrin and Silva, 2005). Physiological differences between males and females are an additional major source of morphological variation. Sexual dimorphism is expected in fishes as a result of selection for female fecundity, male–male competition and sperm competition (Parker, 1992).

Sexual dimorphism in shape differs with sex-specific ontogeny and differences in timing of sexual maturity and investments of energy into egg and sperm production. For example, Pyron *et al* (2007) identified sex-specific shape responses to stream discharge variation, suggesting a trade -off between sexual selection and natural selection in a North American cyprinid. However, identification that sexual selection is a cause of observed

morphological variation requires additional evidence of an association with intrasexual competition and/or mate preferences.

Phenotypic plasticity that is revealed by environmental variation is an additional component of morphological variation (West-Eberhard, 1989). Given the relationships of form and function (Wainwright and Richard, 1995), the ability of a single genotype to produce multiple phenotypes likely contributes to the persistence, distribution, and ecology of organisms. For example, Langerhans *et al* (2003) identified morphological shifts within populations of several Neotropical fishes that occurred in habitats with different flow variation. Similarly, Haas *et al.* (2010) identified morphological divergence associated with habitat change from lotic to lentic conditions that coincided with deeper and more robust body shapes in a species of Cyprinidae

Analyses of enumerable body features (meristics) have been widely used for studying stock structure of fishes. The most commonly enumerated features have been external, including number of fins spines and fin rays, gill rakers and scales. There is a long history of stock identification of fishes through meristic analysis; most fish species that occur as multiple stocks and that have been subject of fishery management, also have received at least some meristic analysis (Waldman, 2005). Ecophenotypic variation of meristic expression has been clearly demonstrated in many species of fishes. There are instances in which ecophenotypic variation of meristic traits were effective towards stock identification than are genetic approaches (Waldman, 2005). The morphometric and meristic characteristics are often analysed together for the purpose of population structure analysis.

Fish morphology refers to the variety of anatomical design among species. Body architecture can be discussed in terms of the characteristics depth, predation style and

other swimming specialization required for the survival success of a given species. For example, the type, size and arrangement of a fish fins are inextricably related to their ecological niche. All fins (except the adipose fin) are supported internally by fin ray (Farag *et al.*, 2014). Catfishes are bottom rovers and have a shape similar to the rover-predator but have a flattened head, humped back and enlarged pectoral fins. These fishes possess barbels or whiskers with taste buds to locate prey in muddy water. The mouth shapes vary to exploit different food sources found on the bottom (Farag *et al.*, 2014).

Describing the general body shape of fishes, Bookstein *et al* (1997), posited that most of them possess a fusiform body form in which head, trunk and tail merge smoothly into one another.

Body formation: Fish have a variety of different body plans. The fusiform body has a streamlined plan to aid fast movement. According to Frooze *et al* (2014), they may also possess filiform (eel-shaped) or vermiform (worm-shaped). They are often either compressed (laterally thin) or depressed (dorso-ventrally flat). The lateral line is a sense organ used to detect movement and vibration in the surrounding water. The lateral line system is designed to follow the vortices produced by fleeing prey. In most species, it consists of line receptors running along each side of the fish (Frooze *et al.*, 2014).

However, the shapes of fishes are diverse. Even closely related species may differ markedly in relative body length, depth and width and in the relative size, shape, and placement of fins and other external structures. For this reason, body proportions are often used to describe taxa and discriminate closely related species. Interspecific shape comparisons are best done after an analysis of withinspeciesvariation has been completed. Variation within species has two basic components; geographic variation among populations due to genetic divergence of phenotypic response to environmental factors and within population variation (Barlow, 1961). This second component can be partitioned

into body-size variation, sexual dimorphism and functional effects correlated with age, seasonality, nutrition, etc. Body size is an important source of shape variation because most fishes have continuous, indeterminate growth and change shape allometrically as a function of size (Bookstein *et al.,* 1997). Allometricgrowth results in systematically changing proportions among morphological structures during ontogeny and it can sometimes result in substantial differences in body form between juveniles and adults (Strauss, 1984). Morphometric traits are majorly used for discrimination among sexes or species, categorization of uncertain specimens like putative hybrids, description of patterns of morphological variation among populations or species; and classification and assessment of phylogenetic relationships (Winans, 1985; Tailor *et al.,* 1986).

Morphometric variation between stocks can provide a basis for stock structure, and may be applicable for studying short-term, environmentally induced variation geared towards successful fisheries management (Pinheiro *et al.,* 2005). It is widely used to identify differences between fish populations (Torres *et al.,* 2010).Grouping species according to traits, such as size, morphology, or behaviour, is a means of simplifying specie-rich communities, and sometimes provides better predictive capabilities than analyses performed at higher levels of taxonomic resolution (Layman *et al.,* 2005).

2.5 Fish Body Shape and Skin Pigmentation

Over the past few decades, body shape and skin pigmentation have become valuable appearance traits in commercial fish. Due to increasing market sophistication, fish size, meat quality, and other traditional traits are not the only attributes that influence consumer choice at point of sale, especially when fish are sold whole (Nelson and Christian, 2014) but according to Nelson and Christian (2014), shape and skin color in fish are complex traits, involving numerous genetic and environmental factors. Thus, progress in this field

will depend in part on dissecting the underlying genetics of these traits for future implementation of modern selection strategies, such as marker-assisted selection based on molecular data.

The domesticated phenotype phenomenon arises because the body shape of an organism results from the integration of morphological, behavioral, and physiological traits (Reid and Peichel, 2010), where different genetic and environmental pressures can lead to functional trade-offs (Walker, 2010). This creates functional constraints, where those changes with the greatest positive and fewest negative effects on fitness will be selected (Reid and Peichel, 2010). For example, in natural populations, there is a relationship between body shape and swimming performance, but body shape is also influenced by foraging behavior, the risk of predation, and stream velocity (Walker, 1997). The trade-off for body shape also operates in captive populations. For instance, cultured populations of rainbow trout selected for rapid growth result in more rotund fish, given the existence of a positive genetic correlation of body mass with body shape and condition factor (Kause *et al.,* 2003) that is, mass gain in fish achieved by increasing body width and height rather than by increasing body length.It has been shown that other factors, such as water velocity (Pakkasmaa and Piironen, 2001), rearing environment (Swain *et al.,* 1991), fish density, and diet (Jenkins *et al.,* 1999) may also modify body shape in fish. This phenomenon occurs given that many morphological growth-related traits show phenotypic and genetic correlations in fish (Martyniuk *et al.,* 2003),

2.6Length-Weight Relationship and Condition Factor in Aquatic Organisms

Fish found in tropical and sub-tropical water system experience frequent growth fluctuation due to factors such as food composition changes, environmental changes, rate

of spawning, etc. Length-weight and length-length relationships can be used to assess the influence of these factors (Adeyemi, 2011). In order to estimate the biomass, it is necessary to know the length-weight relationships of the species under study. Relative weight is considered as management goal by fishery manager for monitoring status of fishes and for comparative growth studies. Relative weight is a suitable index for comparing condition across populations and species (Froese, 2006). Knowledge of quantitative aspects such as length-weight relationship and condition factor (K) or Ponderal index is an important tool for the study of aquatic organisms. The knowledge can be used to predict weight from length measurements made in yield assessment (Pauly, 1993). Fish can attain either isometric growth, negative allometric growth or positive allometric growth. Isometric growth is associated with no change in body shape as an organism grows. Negative allometric growth implies the fish becomes more slender as it increases in weight while positive allometric growth implies the fish becomes relatively stouter or deeper-bodied as it increases in length (Riedel *et al.*, 2007). The condition factor (K) serves as an indicator of physiological state of the fish in relation to its welfare. It also provides information when comparing two populations living under different feeding densities and climatic conditions (Weatherly and Gills, 1987). Thus, condition factor is important in understanding the life cycle of fish species and it contributes to adequate management of these species, thereby maintaining equilibrium in the ecosystem.

Length and weight are two basic components in the study of the biology of fish species at individual and population levels (Ekelemu and Zelibe, 2006). Length – weight relationship (LWR) is an important factor in the biological and ecological study of fish. It is of prime importance in fish yield equations and stock assessments, estimation of biomass and in the prediction of the weight of fish from a given length in yield assessment. It is further employed in the studies of gonadal development, condition factor (the general well-being

or relative fitness of the individual) as well as providing information on growth patterns (Ekelemu and Zelibe, 2006). Effective management of any fishery requires considerable knowledge regarding population parameters such as length-weight, age and growth, mortality and recruitment of the exploited stock. Thus length-weight relationship (LWR) and population dynamics are studied with the major objective being the rational management and conservation of the resources. Fish, like other animals, have a requirement for essential nutrients in order to grow properly. In the wild, such essential foods are available for the fish to forage. In doing this, they are able to meet their body needs by feeding extensively on these foods. To successfully meet these needs, there are some variable factors such as the type of environment, season of the year, location which determines the abundance of the food and the distance to which the fish migrates when foraging. Such natural environments are comprised of lakes, streams, rivers, seas, oceans and other water bodies. Fishes in these water bodies, subsist essentially by feeding on a variety of foods such as the small microscopic organisms known as plankton, aquatic plants and animals including insects, snails, worms and decaying organic matter. In this process, some fish species even feed on others. When fish are removed from their natural environment, to an artificial environment, enough food must be supplied to enable them grow.

Weight-length relationships (WLR) are used for estimating the weight corresponding to a given length and condition factors are used for comparing the condition, fatness or wellbeing of fish based on the assumption that heavier fish of a given length are in better condition and growth quality (Tesch, 1968). The concept helps scientist in converting growth-in-length equation to growth in weight in stock assessment models (Bobori *et al.*, 2010). Length-weight equation has been used in yield per recruit model analyses and age

structured analyses (Lee and Liu, 1996). LWR allows life history and morphological comparison between different fish species or between fish population from different habitats or regions (Santos *et al.,* 2002). Condition factor on the other hand gives information on how a population lives in certain feeding, density, climate and other conditions; when determining the period of gonadal maturation; and when following up the degree of feeding activity of a species to verify whether it is making good use of its feeding source (Lazima *et al.*, 2002).

Length-weight relationship gives the condition and growth pattern of fish. It provides important information concerning the structure and function of fish populations (Hirpo, 2013), and allows for interchangeable calculations during hatchery and production operation. Fish is said to be growing isometrically if the length increases with equal proportion to the body weight for constant specific gravity (Taylor *et al.,* 2010) while allometric growth is assumed when the increase in any of the parameters is unproportional to the other. It is therefore possible to estimate the weight or length of fish from either of each parameter that is available from a formula that takes into account the growth pattern (whether isometric or allometric). Condition factor refers to the wellbeing of the fish in question and by extension its health status and fishing pressure (Blackwell *et al.,* 2000). It is therefore an index reflecting interactions between biotic and abiotic factors to the physiological condition of fish. It is estimated by comparing individual fish weight of a given length to a standard weight. It is assumed that heavier fish reflects a heavier physiological status. Condition factor reflects the fluctuations by interaction among feeding condition, parasitic infection, physiological factors and recent physical and biological circumstances (Shahlina and Biswas, 2014). When a condition factor value is higher, it means that the fish has attained a better condition. The condition factor can be

affected by a number of factors such as stress, sex, season, availability of feeds, and other water quality parameters (Khallaf *et al.*, 2003).

The length-weight relationship is a very important tool in proper exploitation and management of fish population (Anene, 2005) and is very essential for stabilizing the taxonomic characters of the specie (Pervin and Mortuza, 2008). Data from L-W equations are used for sampling programme, estimation of growth rate, length and age structure and other components of fish population dynamics (Kolher *et al.,* 1995), that could be used to compare life history and morphological aspects of populations inhabiting different regions (Stergiou and Moutopoulos, 2001).

The length-weight relationship and condition factor of five fish species from Nkoro River in the Niger delta region of Nigeria was studied by Abowei *et al.* (2009). The respective exponential equations for the length weight relationship are: *Ethmalosa fimbrata* (Wt = 0.0162 (TL)3.199); *Ilishia africana* (Wt = 0.5998(TL)2.719) *Sardinella maderensis* (Wt = 0.0478(TL)3.580) and *Cynoglossus senegalensis* (Wt = 0.0326(TL)3.508). All species studied exhibited isometric growth (b.3) except *Sardinellamaderensis* and *C. senegalensis*that exhibited positive allometric growth with b.3.6 and 3.5 respectively. The condition factor ranged from 0.917(*I. africana*) to 0.985 (*C. senegalensis*). There was difference in the condition factors for the combined fish species and the monthly factor for each fish species studied: *E. fimbrata* (0.85±0.015), *I. africana* (0.96±0.061), *S. maderensis* (0.87±0.072) and *E. senegalensis* (0.62±0.011), while*C. senegalensis* was 1.10±0.042

The length-weight, length-length relationship and condition factor of Synodontis robbianus in the lower Niger River (Idah), Nigeria was carried out Adeyemi, (2011). Results showed allometric growth for the sampled fish with regression coefficient (b-

value) of 2.9926, 2.7620 and 2.8355 for males, females and combined sex respectively. The regression coefficients (b-value) for the length-length relationship were 0.9418, 0.9602 and 0.9510 for males, females and combined sex while the condition factor of all the sampled population varied from 1.57-3.83 indicating that the sampled site is a good environment for growth, reproduction and survival of the fish species.

The length-weight relationships (LWR) and condition factors of 21 fish species from 15 families of ecological and economic importance, found in Ologe Lagoon, Lagos, Nigeria were studied by Kumolu-Johnson and Ndimele (2010). The slope (b) values obtained for the 21 fish species ranged from 2.012 to 2.991, and differed significantly ($p<0.005$) from 3, which indicates that most of the fish species have negative allometric growth. *Liza falcipinnis* was the only species with isometric growth (b = 2.99). The condition factors (K) of the fish species ranged from 0.12 in Polydactylus quadrifilis to 16.29 in *Eutropius niloticus* and about 86% of these condition factors fall outside the range recommended as suitable for matured fresh water species in the tropics. This indicates that Ologe Lagoon may be unfavourable to fishes in the lagoon.

2.7 Identification of *Clariid* Catfishes as Important Tools in Breeding and Genetics

The family Clariidae is divided into two species, namely: - Clarias and Heterobranchus. Clariid catfishes occur both in South-east Asia and in Africa. The highest generic diversity is found on the African continent where some 14 genera have been reported (Legendre *et al.,* 1992) against two in South East Asia. In both continents,Clariidae are of great economic importance as food fish. Aluko and Shaba (1999) stated that African catfish, Clarias and Heterobranchus, are widely cultured in Africa and Europe and of late, African catfish is being cultured in Asia. Teugels (1982) while systematically revising the genus

Clarias in Africa treated four species as *Clarias anguillaris,Clarias gariepinus*, *Clarias lazera* and *Clarias mossambicus*. However, since the author could not find significant differences between *Clarias mossambicus*, *Clarias gariepinus* and *Clarias lazera*. The author concluded that all three are synonymous and that *Clarias gariepinus* should be used in the future. Another African *Clariid* of the genusHeterobranchus has four species, these are *Heterobranchus longifilis* (Valenciennes, 1840). *Heterobranchus bidorsalis* (Geoffroy. 1809), *Heterobranchus boulengen* (Pallegrin, 1912) and *Heterobranchus isopterus* (Bleeker, 1863) which is the smallest member of the genus (Reed *et al.*, 1967). The fish species Clarias and Heterobranchusare very common and widely distributed throughout Africa. They go by different names in various localities and in Nigeria are collectively referred to as the mudfishes. The hybrid mudfish is the crossbreed between the Heterobranchus and Clarias species (Madu*et al.*, 1999). They also reported that two species of Clarias, *Clarias anguillaris* and *Clarias gariepinus*, are most popular for fish farming in Nigeria. Similarly, only two species of Heterobranchus (*Heterobranchus bidorsalis* and *Heterobranchus longifilis*) are commonly available in Nigeria (Madu*et al.*, 1999).

2.7.1 Parameters necessary for fish identification

Fish identification and classification sometimes allow managers to determine if samples of fish are from different populations and to determine the relative contribution of stocks to a mixed stock fishery (Sea Grant Research, 1987). Among the wide array of fish species, proper identification of each is a problem without reference to certain parameters. In identifying fish, the parameters are drawn around the external and internal features. Occasionally, the breeding pattern according to Ogueri(2001) is used but that requires close examination of the fish in its habitat. According to him, equally used in

identification of fish is information from genetic studies and haematological studies (of the blood of the specimens, through electrophoresis). He observed that the most common features used in identifying fish are the number of dorsal and anal fin spines and rays, position of mouth, number and location of teeth, type and number of scales in the lateral line, shape of caudal fin, number of gill rakers and colour.

Use of fins: Reed *et al* (1967) explained that when identifying a fish, the fins are the first thing which should be examined. The number of fins, their types, sizes, situation on the body and positions in relation to each other are most important. Some species of fish (e g. Heterobranchus) have two dorsal fins, the second of which is often an adipose fin, composed only of soft, fleshy tissue and usually without rays of any kind. The size and shape of this fin is often used as a clue to the identity of the fish.

Use of Teeth: The character and position of teeth are sometimes important in the classification of fish. Teeth may be pointed and with a varying number of cusps, in which case they are termed unicuspid, bicuspid, etc, or they are said to be granulated (e.g. Heterobranchus and Clarias spp.) when they are numerous and flat, forming a surface like rough sand paper. Some fish, like Tetraodon and Protoptertis, have fusiform teeth coalesced into massive ridges or beak-like structures; those are generally used for cracking shells and seeds (Reed *et al.,* 1967). They also explained that, the terms used to describe the positions of teeth are Pre-maxillary, when the teeth are situated in the front margin of the upper jaw; Maxillary, when on the sides of the upper jaw on a separate bone; Mandibular, when on the margin of the lower jaw; Voinerine, when on the front part of the roof of the buccal cavity; Palatine, when further back of the palate and Pharyngeal, when they are situated in the throat

2.8 Genetic Diversity or Variability

Populations of many species of organisms respond both morphologically and genetically to a changed environment. Geographically structured variation in phenotypic traits can result from genetic and environmental factors. Divergence may be primarily caused by environmental effects, which are mediated by phenotypic plasticity, the differential expression of traits under different environmental conditions. Genetic differentiation of quantitative traits among populations has been shown in many species, yet the genetic changes that can accompany divergence have received less attention. These can include changes in phenotypic plasticity, i.e. the ability of an organism to respond to environmental variation, and in genetic architecture, i.e. the genetic factors underlying trait variation (Musick, 1998). Within species, genetic diversity is partitioned among and within populations. Long before species declines into extinction, it will suffer a reduction in the level of genetic diversity within and among its populations. Often, range contraction and fragmentation of former distribution occur (Musick *et al.*, 2000). Fragmentation causes the formation of small isolated populations which are more vulnerable to genetic degradation. While documented extinctions of marine species are rare, the extinction of population (extirpation) is more common (Musick, 1998; Musick *et al.*, 2000).

Genetic variation is important for the long-term survival of species; it ensures the fitness of species or populations by giving the species or populations the ability to adapt to changing environment; and lack of genetic variation or too much of homozygosity are detrimental to survival and fitness as a result of inbreeding depression (Dunham, 2004),when a population of an organism contains a large gene pool—that is, if the genetic blueprints of individuals in the population vary significantly—the group has a greater chance of surviving and flourishing than a population with limited genetic variability. Homozygosity has been correlated with bilateral asymmetry (fluctuating asymmetry)

which is the unbalanced meristic counts of the right and left halves of the body in fishes (Dunham, 2004).

Genetic improvement of aquaculture species offers a substantial opportunity for increased production efficiency, health, product quality and profitability (Davis and Hetzel, 2000). Genetic improvement techniques for delivering genetic gains include definition of breeding objectives, estimation of genetic parameters that describe populations and their differences, and evaluation of additive and non-additive genetic merit of individuals or families (Davis and Hetzel, 2000).

There is a number of different molecular tagging methods which have been designed to study fish populations. The use of biochemical methods such as isozymes and protein electrophoretic techniques for species identification have been widely applied in fish (Burgess, 1989; Manohar *et al.,* 2005; Na-Nacron *et al.,* 2004; Saad *et al.,* 2002; Wei and Musa, 2008; Yilmaz *et al.,* 2007). Also, the use of molecular methods such as RAPD techniques (Almeida and Sodre, 2002; Quibai *et al.,* 2006), AFLP techniques (Mickett *et al.,* 2003; Simmones *et al.,* 2006) and microsatellite DNA (Perales *et al.,* 2007; Wachirachaikam and Na-Nakron, 2007) have been widely applied in fish characterization. The greatest advantages for RAPD techniques are that they can potentially sample a large number of loci and that no prior DNA sequence information is needed to perform the assay (Christopher *et al.,* 2004).

2.9 Fish Genetics

2.9.1 Emergence of fish genetics

According to Dunham (2004), fish genetics programmes first emerged in the 1900s after the basic principles of genetics and quantitative genetics had been established. However,

there was not a substantial effort in fish genetics research and the application of genetic enhancement programmes until the 1960s because of the infancy and small scale of aquaculture, a lack of knowledge of fish genetics and a lack of appreciation of genetic principles by natural resource managers regarding genetic enhancement, population genetics and conservation genetics. Slightly earlier, Ellis Prather conducted selection of largemouth bass, *Micropterus salmoides*, for improved feed conversion efficiency in the 1940s and Donaldson selected rainbow trout for increased growth in the 1950s, but neither utilized genetic controls, making any genetic progress unverifiable (Dunham, 2004). However, since the 1960s, fish genetics research and application of genetically improved fish and genetics principles have been gaining momentum with each passing decade. In 1959, *H. Swarup* was one of the first to induce triploidy in fish – the three-spined stickleback, *Gasterosteus aculeatus*. Giora Wohlfarth and Rom Moav also initiated a considerable amount of research on traditional selective breeding of common carp in the 1960s in Israel (Dunham, 2004). This led to the development of traditional catfish selective breeding efforts of Rex Dunham and R. Oneal Smitherman in the 1970s and 1980s in the USA after initial collaboration between the Israelis and Smitherman. Also in the 1970s, Trgve Gjedrem, Harold Kincaid and, later, William Hershberger initiated long-term selection programmes for various salmonids (Dunham, 2004). This early work on selective breeding was the predecessor to later research on molecular genetics of aquaculture species. Also in the 1970s, Rafael Guerrero III and William Shelton developed sex-reversal technology for tilapia, which would later lead to the development and worldwide application of genetically male tilapia (Dunham, 2004).

In 1970, Werner Arber, Hamilton O. Smith and Daniel Nathans isolated restriction endonucleases which was the key discovery allowing the development of gene cloning

(1978), genetic engineering (1978) and various restriction- fragment technologies (Glick and Pasternak, 1998). The discovery of reverse transcriptase by Howard Temin and David Baltimore was, of course, also key for the development of modern recombinant DNA technology. Then in 1973, Stanley Cohen and Herbert Boyer devised recombinant DNA technology (Cohen *et al.*, 1973). This type of research was further enhanced in 1975 with the development of procedures to rapidly obtain DNA sequences (Sanger *et al.*, 1977) and to visualize specific DNAfragments (Southern, 1975). The 1980s saw more quantum leaps in molecular genetic biotechnology. Around 1980, Palmiter, Brinster and Wagner produced the first transgenic animals, mice, and Palmiter and Brinster demonstrated that the transgenesis could lead to greatly accelerated growth in the mice. They also demonstrated the dramatic phenotypic alterations that could be realized through gene transfer. This provided the motivation and impetus for the development of technology for the generation of the first transgenic fish. In a year-and-a-half span from 1985 to 1987, Zhou first transferred genes into goldfish in China, followed by Ozato in Japan with medaka, Daniel Chourrout in France with rainbow trout and Rex Dunham in the USA with channel catfish. In 1985, Jeffreys developed DNA finger- printing technology (Jeffreys *et al.*, 1985), revolutionizing not only population genetic analysis and gene-mapping technology, but also forensic and criminal science. The current state of modern molecular genetics and genomics research would not have been possible without the revolutionary invention of the polymerase chain reaction (PCR) by Kary Mullis in 1985 (Dunham, 2004). The new biotechnologies, such as sex reversal and breeding and polyploidy, began to have a major impact on aquaculture production in the late 1980s and early 1990s by not only improving growth rates but allowing major improvement of flesh quality in species that exhibit sexual dimorphic and sexual maturation effects. Chourrout (1982) induced the first viable tetraploid fish, rainbow trout, Standish Allen developed triploid technology for

shellfish during the late 1980s and Gary Thorgaard developed clonal lines of rainbow trout via androgenesis. The pioneering research on sex reversal and breeding technology by Shelton and Guerrero led to worldwide production of monosex Nile tilapia in the 1980s and 1990s, and Graham Mair took this technology one step further in the 1990s, leading to the development of YY populations of Nile tilapia and the production of genetically male tilapia (GMT) populations in many countries. Traditional breeding is being utilized in concert with these new biotechnologies. The 1990s brought continued rapid progress in molecular genetics and biotechnology. DNA marker and gene-mapping technology has exploded in the last decade. Microsatellites were developed in 1989, radiation hybridization in 1990, random amplified polymorphic DNA (RAPD) and expressed sequence tag (EST) technologies in 1991, the amplified fragment length polymorphism (AFLP) technique in 1995 and single nucleotide polymorphism (SNP) procedures in 1998. Another major advance was the first nuclear cloning of a mammal, sheep, in 1997 (Dunham, 2004).

Since the early 1980s, research in aquaculture and fisheries genetic biotechnology has steadily grown, and now research in this area is extremely active. Currently, efforts are well established in the areas of traditional selective breeding, biotechnology and molecular genetics of aquatic organisms. Cultured fish are being improved for a multitude of traits, including growth rate, feed conversion efficiency, disease resistance, tolerance of low water quality, cold tolerance, body shape, dress-out percentage, carcass quality, fish quality, fertility and reproduction, and harvestability (Dunham, 2004).

2.9.2Application of biotechnology in fish population genetics

Traditionally, morphological characterization of fish has been useful in determining fish species, sex and larva stages, but it does not offer a definite or reliable method for specie

identification. Therefore, genetic or DNA-based techniques are being used for such purposes (Wong, 2011). The use of molecular genetic techniques in fisheries research has increased dramatically over the past years, largely due to the increased availability of techniques and an increased awareness of the value of genetic data (Park and Moran, 1994). At present, the boundaries of fisheries related molecular genetics research encompass topics from the identification of markers for stock discrimination (Park *et al.,* 1993) to the genetics of pathogenic organisms of commercially important species (Meyers *et al.,* 1992) and to the expression of growth factors during maturation (Duguay *et al.,* 1992). Population genetics is the study of genetic variation within populations, and involves the examination and modelling of changes in the frequencies of genes and alleles in populations over space and time. Population genetics is the study of genetic variation among species, individuals and populations; fundamentally, it shows that distribution of genetic variability is affected by evolutionary forces of mutation, migration, selection and random genetic drift (Hansen, 2003). As relative allele frequencies change, relative genotype frequencies may also change. Each genotype in the population usually has a different fitness for that particular environment. In other words, some genotypes will be favored, and individuals with those genotypes will continue to reproduce. Other genotypes will not be favored: individuals with those genotypes will be less likely to reproduce. Unfavorable genotypes take many forms, such as increased risk of predation, decreased access to mates, or decreased access to resources that maintain health. Overall, the forces that cause relative allele frequencies to change at the population level can also influence the selection forces that shape them over successive generations (Hansen, 2003).

Specific highly polymorphic markers are required to assess the inbreeding level associated with these aquacultured species (Bentzen *et al.,* 1991; Van Der Bank *et al.,* 1992). The

study of parentage in population genetics and quantitative genetics utilize microsatellite markers due to its relatively high polymorphism (Tautz, 1989). Queller *et al.* (1993) developed specific microsatellite primers for *Clarias gariepinus*in order to perform paternity tests and to characterize wild and domesticated populations. They reported the isolation and characterization of seven (GT)n markers and their potential use in other catfish species. The microsatellite primers were isolated through the production and screening of a library of short fragments of genomic DNA of *Clarias gariepinus.*

Microsatellite or Simple Sequence Repeats (SSRs) represent an abundant source of genetic markers which are highly abundant and dispersed evenly throughout eukaryotic genomes. They have become the markers of choice for a wide range of applications in population genetics, conservation and evolutionary biology. Microsatellites represent ideal molecular markers because they have multiple alleles which are highly polymorphic among individuals. Polymorphism is achieved by having variable numbers of tandem repeat motifs resulting in size variation which can then be visualized by PCR with pairs of locus-specific flanking primers, followed by electrophoresis of the amplification product. Microsatellite motifs occur once every 10kb in fishes. They are inherited in a co-dominant fashion, and are fast and easy to assay. They are co-dominant in nature with high level of polymorphism and can reproduce very well. This gives microsatellite an edge over others for estimating population structure and genetic diversity (Mojekwu and Anumudu, 2013).

2.9.3 Application of molecular markers

In genetics, a molecular markeris a fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA. There

are many types of genetic markers, each with particular limitations and strengths. Within genetic markers there are three different categories: "First Generation Markers", "Second Generation Markers", and "New Generation Markers (Maheswaran, 2014).These types of markers may also identify dominance and co-dominance within the genome (Maheswaran, 2014).Identifying dominance and co-dominance with a marker may help identify heterozygotes from homozygotes within the organism. Co-dominant markers are more beneficial because they identify more than one allele thus enabling someone to follow a particular trait through mapping techniques. These markers allow for the amplification of particular sequence within the genome for comparison and analysis. Molecular markers are effective because they identify an abundance of genetic linkage between identifiable locations within a chromosome and are able to be repeated for verification. They can identify small changes within the mapping population enabling distinction between a mapping species, allowing for segregation of traits and identity. They identify particular locations on a chromosome, allowing for physical maps to be created. Lastly they can identify how many alleles an organism has for a particular trait (bi allelic or poly allelic) (Maheswaran, 2014).

i) Isozymes and enzymes: Isozymes are multiple molecular forms of individual enzymes. These multiple forms can be alleles of one another at a single locus – allozymes – or can be products of different loci where there are multiple copies of genes making the same enzyme or enzyme sub- units. Temporal differences in isozyme expression exist, which

can be utilized in the study of developmental genetics – spatial or tissue-specific expression as well as allelic variation. Isozyme and enzyme analyses are technically easy, but are limited in both the numbers of loci available and polymorphism. For example, isozyme variation is low in Nile tilapia (Agnese *et al.*, 1997a). However, one major advantage is that genetic variation is being measured, which is directly related to protein products that actually affect performance. For example, Hallerman *et al* (1986) demonstrated that isozyme variation is associated with growth rate in channel catfish. Isozyme variation has also been linked with disease resistance, temperature tolerance, developmental speed and salinity tolerance in fish (Dunham, 1996). Another major advantage is that isozymes are inherited in a codominant fashion. This makes heterozygotes and homozygotes readily distinguishable, thus strengthening applications for gene mapping, population-genetic studies and determining parentage.

ii) Restriction fragment length polymorphism: RFLP was once widely used and is still very useful, and has been used to construct genetic maps of many species (Yang and Womack, 1997). Restriction endonuclease enzymes are used in this method to directly cut the DNA at restriction sites. Base substitution at the restriction sites, insertions, deletions or DNA fragment rearrangements at or between the restriction sites cause the polymorphism. The resulting products are then separated on agarose gel, transferred to a membrane and hybridized with labelled probes to produce DNA fingerprints. The advantages of RFLP include codominant inheritance and easy interpretation and scoring. This technique is now less frequently used because it is time- consuming and requires tedious Southern blotting. Additionally, probe development is required for RFLP analysis, polymorphism is generally low and sequence information is needed if using polymerase

chain reaction. This technique is too slow and tedious to generate large numbers of markers.

iii) Mitochondrial DNA: The analysis of mtDNA variation is an alternative for studying population genetics in fish (Agnese *et al.,* 1997b). For species such as striped bass where isozyme variation was minimal, significant mtDNA variation was observed (Wirgin *et al.,* 1989). The mutation rate of mtDNA is about an order of magnitude higher than that of the nuclear genome, and the control region is particularly hypervariable, thus allowing studies on recent evolution. Since the mitochondrion is the major site of cellular respiratory metabolism and a possible source of maternal effect, genetic improvement programmes should be concerned with mtDNA as well as nuclear DNA. MtDNA analysis often reveal genetic differences among populations of fish that are homogeneous for isozyme variability. Three types of polymorphisms can be detected for mtDNA in fish: length polymorphisms, restriction-site polymorphisms caused by base-pair additions, deletions or both, and heteroplasmy. MtDNA heteroplasmy is the existence of more than one form (genotype or haplotype) of mtDNAin an individual.

iv) Random amplified polymorphic DNA:RAPD markers are polymorphic DNA sequences separated by gel electrophoresis after PCR, using one or a pair of short (8–12 base pairs (bp)) random oligonucleotide primers (Liu *et al.,*1999b). Polymorphisms are a result of base changes in the primer-binding sites or of sequence-length changes caused by insertions, deletions or rearrangements. RAPD is very powerful in detecting large numbers of polymorphisms because oligonucleotide primers scan the whole genome for perfect and subperfect binding sites in a PCR reaction. When two binding sites are close enough (3000 bp or less), a RAPD band is produced on the gel. Each RAPD primer usually amplifies

several bands, some of which are polymorphic in even closely related populations, which can be either tremendously advantageous or disadvantageous.

v) Amplified fragment length polymorphism:AFLP combines the strengths of RFLP and RAPDLiu and Dunham, (1998). Genomic DNA is digested with two restriction enzymes EcoRI and MseI, suitable adaptors are ligated to the fragments and the ligated DNAfragments are selectively amplified with different primer combinations (Liu*et al.*, 1998); then the products are resolved by gel electrophoresis. AFLPs are highly polymorphic and the technique is simple and fast. The molecular bases of AFLP are base substitutions at the restriction sites, insertion or deletion between the two restriction sites, base substitution at the pre-selection and selection bases and chromosomal rearrangements. The advantages of AFLP include its PCR-based approach, requiring a small amount of DNA; no requirement for any known sequence information or probes; and the specific amplification of a subpopulation of the restriction fragments because the long PCR primers in the procedure allow high annealing temperature and high repeatability. Perhaps the greatest advantage of AFLP analysis is that it is capable of producing large numbers of polymorphic bands in a single analysis at a relatively low cost per marker (Liu and Dunham, 1998). The generation of hundreds or up to thousands of bands with limited numbers of primer combinations makes AFLP a very efficient and economical system for genetic analysis. There can be more than 100 loci per primer combination and over 4000 primer combinations to evaluate, and more than one restriction enzyme can be used, making it possible to generate tremendous numbers of markers. AFLPbands are widespread and evenly distributed, giving near genome-wide coverage. AFLP is highly reliable because it combines the advantage of RFLP and RAPD and is devoid of the disadvantages of the slow speed and low levels of polymorphism of RFLP

and the low reproducibility of RAPD. The AFLP procedure allows the genetic analysis of closely related populations. The disadvantages of AFLP are that they are dominantly inherited and that more technically demanding and specialized and expensive equipment, such as DNAsequencers, is required.

vi) Expressed sequence tags:ESTs are short, single-pass complementary DNA (cDNA) sequences reverse-transcribed from mRNAs and generated from randomly selected cDNA-library clones (Liu *et al.,* 1999a). The single-pass sequencing is at both the upstream and the downstream segments of cDNAs. The basis of EST analysis is that specific mRNAs and transcript quantities vary in different tissues, in different developmental stages or when the organism faces different environmental conditions. Characterization of ESTs is a relatively easy and rapid way for identification of new genes in various organisms (Tilghman, 1996). Because of the relative ease of EST analysis, the EST database is the fastest-growing division of GenBank (Karsi, 2001) and than 415,000 human ESTs have been characterized (Wolfsberg and Landsman, 1997). Extensive EST analysis is not only an efficient way to identify genes, but is also powerful for the analysis of their expression (Karsi, 2001). ESTs indicate when, where and how strongly genes are expressed, and each EST represents a gene, so they can be used in functional genomics analysis. ESTs are particularly useful for the development of cDNA microarrays, which allows the study of differentially expressed genes in a systematic way. EST analysis is useful for comparative genomics by determining orthologous counterparts of genes through evolution. Profiling of expression provides a rapid means of examining gene expression or differential gene expression in specific tissue types, in biological pathways, under specific physiological conditions, during specific developmental stages or in response to various environmental challenges. ESTs are also efficient molecular markers

for genomic mapping (Schuler*et al.,* 1996), and microsatellites can be found within ESTs, making ESTs even more useful for gene-mapping research. However, only 4.6% of ESTs characterized from the skin of channel catfish contained microsatellites (Karsi *et al.,* 2002). Disadvantages of EST analysis are that a large amount of preparatory work is required, the cDNA library may not contain transcripts of low abundance and normalization of the cDNAlibrary may be necessary.

vii) Single nucleotide polymorphisms:SNP is caused by base variation among individuals at any site of the genome (Kocabas, 2001). This single base variation can be determined by DNA sequencing, primer extension typing, the designing of allele-specific oligo and gene-chip technology. The advantages of SNP are that SNP sites are abundant throughout the entire genome (3×10^7 different sites have SNP in humans), they are highly polymorphic and the SNP analysis is the only system that identifies every single difference or polymorphism among individuals. SNP analysis has several disadvantages, including the need for sequence information, the necessity of probes and hybridization, high expense and difficult genotyping.The channel catfish myostatin gene not only contained microsatellites, but also had many SNPs (Kocabas *et al.,* 2002). Many of these SNPs were neutral and did not change amino acid sequences, but five SNPs caused changes in amino acid sequences.

viii) Microsatellites: Microsatellites are simple-sequence, tandem repeats of 1–6 bp. The molecular basis of microsatellites is the difference in number of repeats (Tan *et al*., 1999). Microsatellite markers are ideal molecular markers because they are highly polymorphic, evenly distributed in genomes and codominantly inherited. They are highly useful among various types of DNA markers because their high rate of polymorphism and codominant inheritance allow precise genetic analyses, increase mapping accuracy, maximize the

genetic information generated and allow lineages of individuals or families (individual spawns) to be accurately traced (Waldbieser and Wolters, 1999). High levels of polymorphism also indicate that microsatellite markers may be highly useful for population genetic analysis and strain identification. Microsatellite loci are short in size, facilitating genotyping via PCR. Their disadvantage is that microsatellite analysis requires great effort, time and expense in library construction, screening, sequencing and PCR primer analysis, and they may have non-specific bands (Liu and Dunham, 1998). Characterization of large numbers of microsatellites for the construction of genetic maps with high resolution is a tedious and strenuous task. The majority of microsatellite loci can be amplified in both channel catfish and blue catfish, suggesting evolutionary conservation between these two catfish species (Liu *et al.,* 1999c), and microsatellite markers can be used as codominant markers in the interspecific hybrid system for genetic linkage analysis. Liu *et al.* (1999b) confirmed that microsatellite markers are inherited as codominant markers in catfish and are reported to be highly polymorphic in both channel catfish and blue catfish. Various classes of microsatellites exist and can be found at varying frequencies. In Eastern Oysters, dinucleotide motifs within microsatellites were dominated by AG, tri- nucleotide microsatellites had all possible motifs in equal frequencies and tetranucleotides were more prevalent than trinucleotides and were strongly associated with specific repetitive sequences, which was not the case for other classes of microsatellites (Gaffney, 2002).

Microsatellite markers have been developed for studies in various organisms, ranging from plants to animals. The use of microsatellites has become a standard method to estimate natural genetic diversity in livestock (Erhardt and Weimann, 2007). It was found that highly polymorphic microsatellites are more powerful for parentage control than the

conventional use of biochemical markers like blood groups or biochemical marker systems (Glowatzki-Mullis *et al.,* 1995). Due to the high genetic information content, microsatellites are one of the most useful molecular markers to estimate population genetic parameters, and also for their co-dominant allelic patterns, critical for the investigation of gene flow patterns and parentage composition (Morgante and Olivieri, 1993).

Microsatellite loci characteristically exhibit high levels of length mutation, resulting in extensive allelic variation and levels of heterozygousity in fish ranging from 24% to 90% (O'Connell and Wright, 1997). Such levels of variation make them especially suitable for stock identification in species previously exhibiting low levels of detectable variation using allozymes or mtDNA (Bentzen *et al.,* 1991); for example, Ruzzante *et al (*1998) found significant heterogeneity at small geographical scales among Atlantic cod populations using microsatellites, in contrast to previous surveys using allozymes (Mork *et al.,* 1985) or mtDNA (Carr and Marshall, 1991). Similarly, O'Connell *et al.,* (1998) used microsatellite markers to demonstrate significant genetic heterogeneity among Pacific herring populations from the Gulf of Alaska and the Bering Sea previously thought to be genetically homogeneous (Grant and Utter, 1984).

Microsatellite markers have been extensively characterized in various fish species to show variation. For example, variations at three microsatellite loci were examined in four populations of *Chrysichthys nigrodigitatus* using microsatellite markers and the result showed high number of alleles in all the three loci (29 to 30) with average number of allele per population varying from 6.67 to 22.0. The observed heterozygousity was also high. These results showed that microsatellite is the tool of desire for population

monitoring, preservation of genetic diversity and breeding in aquaculture (Kotoulas *et al.,* 1997).

Clarias gariepinus primers have been used to amplify microsatellite loci in other species including *Clarias anguillaris, Heterobranchus longifilis* and *Clarias alluaudii*. The possibility has also been demonstrated in Cyprinids (Zhen *et al.,* 1995). Agnèse *et al* (1997b) used seven primer sets for *Clarias gariepinus* described by Galbusera *et al* (1996) Cga 01, 02, 03,05,06,09 and 10 including a newly developed marker Cga 07 (Gene bank submission number: U30868) to characterize sympatric populations of *Clarias gariepinus* and *Clarias anguillaris* from Senegal. The microsatellites confirmed the presence of two species and allowed a precise characterization of both, but the two species were closely related genetically and no diagnostic locus was found in the study.

Preliminary works have shown that microsatellites are for estimating heterozygousity of stocks, and will be very useful for tracking parentage in selection experiments (Kocher, 1997). Expected heterozygousity from characterization of eleven polymorphic tetranucleotide microsatellites in 37 Banggai Cardinal fish, *Pterapogon Kauderni* from a natural population using the phenol–chloroform procedure (Sambrook *et al.,* 1989) gave values ranging from 0.107 to 0.928, while polymorphism at the characterized loci ranged from 2 to 10 alleles. The result obtained from this study enabled high resolution genetic studies of the species (Hoffman *et al.,* 2004). Magoulas *et al.*(1997) reported the use of microsatellites to identify the parents of offspring from mass mating in cultured species of gilthead sea bream and Japanese oyster. This method was used for the estimation of heritability and evaluation of genetic quality of parents through the predominance of their offspring.

Microsatellites have been used as ideal tools to show variation within and between populations. Variation at three microsatellite loci was examined in four natural populations of the West African catfish *Chrysichthys nigrodigitatus*: Senegal, Selingue Dam, Niger, Ivory Coast, Volta Lake and one farmed population, Société ivoirienne d'aquaculture lagunaire (Sial) in Ebrie Lagoon, created with 700 founders and farmed for five generations. The number of alleles was high in al1 three loci (29 to 30) and the average number of alleles per population varied from 6.67 to 22.0. Heterozygousity was also high, but the higher mean heterozygousity was observed to be exhibited by the locus with the smaller number of alleles, showing high polymorphism. (Kotoulas *et al.*, 1997).

Microsatellites have also been used as a tool to investigate genetic diversity. Chiang *et al* (2008) characterized microsatellite loci in *Varicorhinus alticorpus* (Cyprinidae), an endangered fresh water fish in Taiwan and tested nine newly developed polymorphic microsatellites on 20 species of *Varicorhinus alticorpus*. The number of alleles ranged from 4 to 13. Expected (He) and observed (Ho) heterozygousities ranged from 0.692 to 0.892 (averaged at 0.821) and from 0.000 to 0.350 (averaged at 0.088), respectively. This suite of highly polymorphic microsatellites provided the chance to undertake a conservation program for this species in Taiwan.

Islam *et al* (2010) used microsatellites to detect genetic bottleneck in three wild populations (*Kella beel*, *Hakaluki haor*, and *Shobornokhali beel*) and one hatchery population of the freshwater walking catfish, *Clarias batrachus*, in Bangladesh. Analysis of seven microsatellite loci revealed genetic variability between the two populations. The observed heterozygousity was significantly higher than the expected in the *Hakaluki haor* and *Shobornokhali beel* populations, indicating a recent genetic bottleneck. The results reflected some degree of genetic variability in *Clarias batrachus* populations and a recent

bottleneck in some wild populations of *Clarias batrachus* indicating potentialities for improving this species through a selective breeding program. It was also used to detect loss of genetic diversity as a result of genetic bottleneck in Hippocampus capensis, the Knysna seahorse (Galbusera *et al.*, 2007).

Genetic linkage maps have been constructed with microsatellites in intercrossed species of *Astatotilapiaburtoni* from Tanzania and Zambia regions of Lake Tanganyika by Sanetra *et al.* (2008). About 97% of the 10 microsatellites genotyped could be linked and the map revealed 25 linkage groups which can be used to facilitate efficient genome scans and comparisons of Cichlid genomes thus providing useful future tool for studying the genetic basis of adaptive traits that play a major role in the rapid diversification of cichlid fishes. Kocher (1997) also characterized 62 microsatellites in Tilapia species for the construction of genetic linkage maps. About 93% of the markers tested showed detectable linkage to another marker.

Microsatellites have also been observed to be an important tool for wild and aquaculture population studies. They were isolated and characterized by Sanches and Galetti (2006) for analysis of genetic variability in wild and cultured populations of *Brycon hilarii*, a migratory fish species inhabiting the Paraguay River basin of Brazil. Genetic variability in both domestic and wild populations of African catfish has also been assessed using microsatellite DNA as markers. Analysis of African catfish using seven microsatellites in natural populations from eleven localities within the native range indicated a generally high genetic variability (mean expected heterozygousity He=78%), particularly when comparing populations from difference catchments. Variability in African catfish is generally higher than in other riverine catfish species such as *Eutropius niloticus, Schilbe mystus, Chrysichthys nigrodigitatus* and *Clarias anguillaris*. There are however some

significant departures from this finding, particularly in South Africa where the population from the van der Kloof Dam (Orange River) has a much lower degree of variability. Domesticated populations have generally been found to have a low genetic variability in comparison with wild populations. (Galbusera *et al.*,1996; Teugels *et al.*, 1992; Volckaert and Agnèse, 1996).

Sabina De Innocentiis *et al* (2002) used microsatellites for determination of seed origin and to infer geographical origin of individual breeders from an Italian commercial hatchery. An aquaculture broodstock of gilthead sea bream (*Sparus aurata*) from an Italian commercial hatchery was genetically characterized by means of microsatellite markers. At the same time, with the same technique, six natural populations from the Tyrrhenian, Adriatic and Atlantic coasts were assayed, in order to investigate the genetic relationships between domestic and wild stocks. Individual profiles at four loci were used to infer the most likely geographical origin of each single commercial breeder. The genetic variation of *Clarias gariepinus* collected from four hatchery stocks (Supanburi, Angthong, Nakornpathom and Nakornsawan) in Thailand were also assessed using six microsatellite loci. Relatively moderate high levels of polymorphism were observed across all populations with an average of 4.63-12.33 alleles per locus, 5.19-9.88 allelic richness per locus and average heterozygousity at all loci from 0.50-0.69. Genotype frequencies of all populations except Nakornsawan hatchery stock deviated from Hardy-Weinberg equilibrium. An FST value of 0.1453 indicated high genetic diversity among populations. The most prominent genetic variation was observed in the Angthong population, followed by Nakornsawan, Nakornpathom and Supanburi. A phylogenetic dendogram clearly divided the populations into two groups: Angthong-Supanburi and Nakornpathom-Nakornsawan. (Wachirachaikam and Na-Nacron, 2007)

2.9.4Marker-assisted selection (MAS) in fish

Marker assisted selection or marker aided selection(MAS**)** is an indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical or DNA/RNA variation) linked to a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality), rather than on the trait itself (Ribaut et al, 2001). For example, using MAS to select individuals with disease resistance involves identifying a marker allele that is linked with disease resistance rather than the level of disease resistance. The assumption is that the marker associates at high frequency with the gene or quantitative trait locus (QTL) of interest, due to genetic linkage (close proximity, on the chromosome, of the marker locus and the disease resistance-determining locus). MAS can be useful to select for traits that are difficult or expensive to measure, exhibit low heritability and/or are expressed late in development. At certain points in the breeding process the specimens are examined to ensure that they express the desired trait. The majority of MAS work in the present era uses DNA-based markers. However, the first markers that allowed indirect selection of a trait of interest were morphological markers. Markers may be: Morphological - These markers are often detectable by eye, by simple visual inspection. Biochemical- A protein that can be extracted and observed; for example, isozyme and storage proteins. Cytological- The chromosomal banding produced by different stains; for example, G banding.

Only a few examples of actual MAS exist for fish. Using mtDNA marker for selection, the growth rate of rainbow trout was increased by 26% but this method was strain- specific because the relative performance of fish with the specific haplotype was consistent across males within strains but not across strains (Ferguson and Danzmann, 1999). In contrast, six generations of traditional selection increased body weight by 30% in rainbow trout

(Kincaid, 1983). MAS has also been used to improve feed-conversion efficiency by 11% for aquaculture species, while traditional selection was able to improve feed-conversion efficiency by only 4.3% (Davis and Hetzel, 2000). In rainbow trout, 25% of progeny exhibited a high degree of upper-temperature tolerance after MAS for heat tolerance (Danzmann *et al.,* 1999). It therefore appears that MAS has the potential to accelerate genetic improvement of aquaculture species.

2.10DNA Extraction, Polymerase Chain Reaction and Elctrophoresis

In recent years, molecular biology-based methods have become widely used in laboratory studies primarily because of the great versatility of the PCR-based methods (Neumaier *et al.,* 1998). The high sensitivity of amplification techniques and the complexity of the correlated molecular methods have produced an obvious demand for assay standardization and quality assurance, thereby emphasizing the importance of proficiency testing in molecular genetics (Neumaier *et al.,* 1998). The techniques for evaluating DNA extraction and amplification/scoring and the analysis of product after PCR was developed. These involve three levels; DNA extraction (quality and quantity), PCR performance (specificity and efficiency) and interpretation of result after electrophoresis (Orlando *et al.,* 2000). Reliability, feasibility and reproducibility of molecular genetics studies are often limited by the preliminary step of DNA isolation. Obtaining great amounts of high quality DNA from small quantities of tissue is often a laborious task.

DNA extraction methods should ideally be straightforward, quick, efficient, and reproducible while minimizing the potential for cross-contamination. It should also be suitable for extracting multiple samples and generate minimal risk for the operator. Safety,

time and costs are also main considerations. DNA quality is a critical issue for most amplification-based analysis, since the DNA amplification is influenced by the presence of co-purifying inhibitors from matrix or extraction reagents, which can reduce subsequent PCR efficiency. DNA damage may also occur during the extraction procedure due to oxidation and enzymatic hydrolysis problems, associated with extraction buffers formulation (Smith *et al.,* 2005) and excessive mechanical shearing (Marmiroli *et al.,* 2003).

The great majority of methods for DNA extraction were generated for human (especially blood samples) and for other mammalian or plant species (Yue and Orban, 2001). The traditional methods for DNA extraction are time-consuming (Blin and Stafford, 1976) and required the use of health hazard reagents and possible contaminants of the extracted genomic DNA. Phenol-chloroform extraction (Köchl *et al.,* 2005), salting out procedure (Miller *et al.,* 1988), silica-guanidinium thiocyanate method (Carter and Milton, 1993), CTAB procedure (Tel-Zur *et al.,* 1999) and Chelex-based extraction (Walsh *et al.,* 1991) are the most used protocols. Nowadays, commercial DNA extraction kits are available, employing a variety of solvents and/or specialized columns containing DNA-binding substances, procedures are shorter and easier to handle and does not require using toxic products, such as phenol (Pepinski *et al.,* 2002).

Polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. It is an easy, cheap, and reliable way to repeatedly replicate a focused segment of DNA, a concept which is applicable to numerous fields in modern biology and related sciences(Bartlett and Stirling, 2003).Typically, PCR consists of a series of 20–40 repeated temperature changes,

called cycles, with each cycle commonly consisting of two or three discrete temperature steps. The cycling is often preceded by a single temperature step at a very high temperature (>90 °C (194 °F)), and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters, including the enzyme used for DNA synthesis, the concentration of bivalent ions and dNTPs in the reaction, and the melting temperature(*Tm*) of the primers (Bartlett and Stirling, 2003). The individual steps common to most PCR methods are as follows:

Initialization: This step is only required for DNA polymerases that require heat activation by hotstars PCR. It consists of heating the reaction chamber to a temperature of 94–96 °C (201–205 °F), or 98 °C (208 °F) if extremely thermostable polymerases are used, which is then held for 1–10 minutes. Denaturation: This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C (201–208 °F) for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules. Annealing: In this step, the reaction temperature is lowered to 50–65 °C (122–149 °F) for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand (Bartlett and Stirling, 2003).

In practice, PCR can fail for various reasons, in part due to its sensitivity to contamination causing amplification of spurious DNA products. Because of this, a number of techniques

and procedures have been developed for optimizing PCR conditions. Contamination with extraneous DNA is addressed with lab protocols and procedures that separate pre-PCR mixtures from potential DNA contaminants. This usually involves spatial separation of PCR-setup areas from areas for analysis or purification of PCR products, use of disposable plasticware, and thoroughly cleaning the work surface between reaction setups. Primer-design techniques are important in improving PCR product yield and in avoiding the formation of spurious products, and the usage of alternate buffer components or polymerase enzymes can help with amplification of long or otherwise problematic regions of DNA. Addition of reagents, such as formamide, in buffer systems may increase the specificity and yield of PCR. Computer simulations of theoretical PCR results (electronic PCR) may be performed to assist in primer design(Bartlett and Stirling, 2003).

Electrophoresis is a process which enables the sorting of molecules based on size. Using an electric field, molecules (such as DNA) can be made to move through a gel made of agar or polyacrylamide. The electric field consists of a negative charge at one end which pushes the molecules through the gel, and a positive charge at the other end that pulls the molecules through the gel. The molecules being sorted are dispensed into a well in the gel material. The gel is placed in an electrophoresis chamber, which is then connected to a power source. When the electric current is applied, the larger molecules move more slowly through the gel while the smaller molecules move faster. The different sized molecules form distinct bands on the gel (Sambrook and Russel, 2001).

Gel in this instance refers to the matrix used to contain, then separate the target molecules. In most cases, the gel is a crosslinked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed. When separating proteins or small nucleic acid (DNA, RNA or oligonucleotides) the gel is

usually composed of different concentrations of acrylamide and a cross-linker, producing different sized mesh networks of polyacrylamide. When separating larger nucleic acids (greater than a few hundred bases), the preferred matrix is purified agarose. In both cases, the gel forms a solid, yet porous matrix. Acrylamide, in contrast to polyacrylamide, is a neurotoxins and must be handled using appropriate safety precautions to avoid poisoning. Agarose is composed of long unbranched chains of uncharged carbohydrate without cross links resulting in a gel with large pores allowing for the separation of macromolecules and macromolecular complexes (Sambrook and Russel, 2001).

If several samples have been loaded into adjacent wells in the gel, they will run parallel in individual lanes. Depending on the number of different molecules, each lane shows separation of the components from the original mixture as one or more distinct bands, one band per component. Incomplete separation of the components can lead to overlapping bands, or to indistinguishable smears representing multiple unresolved components (Sambrook and Russel, 2001).

Bands in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size. There are molecular weight size markers available that contain a mixture of molecules of known sizes. If such a marker was run on one lane in the gel parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size. The distance a band travels is approximately inversely proportional to the logarithm of the size of the molecule (Sambrook and Russel, 2001).

2.11 Studies on Population Genetics in Aquaculture

The pattern of morphometric differentiation among six populations of *Clarias gariepinus* sited in the Asi, Seyhan, Ceyhan, Göksu, Aksu, and Sakarya river systems in Turkey was examined by Cemal *et al* (2005). Univariate analysis of variance revealed significant differences between means of the six samples for 18 out of 20 standardized morphometric measurements. The first canonical function accounted for 39 % and the second for 29 % of between–group variability. In principal component analysis, the first component accounted for 20 % and the second for 12 % of the shape variations among the samples. Plotting the first and second principal components showed that the observed differences were mainly from measurements taken from the head of fish, indicating this region to be important in the description of population characteristics. Visual examination of the samples along the canonical functions revealed a clear between-sample differentiation. All the samples except the Seyhan and Aksu samples were clearly distinct from each other. Sakarya and Göksu samples were mostly isolated from each other and from all other samples. The overall random assignment of individuals into their original groups was high (78%). The proportion of correctly classified individuals into their original group was highest in the Sakarya sample (93%) and high in the Göksu (88 %) and the Ceyhan (86 %) samples, indicating that these samples were highly divergent from each other.

Intra-specific morphological variation in the indigenous catfish,*Arius jella* (Ariidae), was studied in five estuarine localities in Sri Lanka (Koggala lagoon, Walawe estuary, Garanduwa lagoon, Nilwala estuary and Chilaw lagoon) using morphometric analysis by Suneetha (2007). Five morphometric characteristics describing the shape of the fish were significantly different among some of the locations. Significantly shorter pre-orbital length (8.3% SL vs 9.2-10.7% SL in the other populations) in specimens from Walawe estuary is

a populationspecific character. Significant differences in other characteristics were found indicating heterogeneity in morphology. The first two canonical variates (CV) explained 82.7% of the total variation in the data, yet the plot of the CV failed to display significant separation of the sample populations. Derived classification functions could correctly classify an average of less than 50% individuals into their a priori groups.The results indicate a small degree of spatial separation in morphology in A. jella among the studied estuarine localities.

Patterns of morphometric and genetic variation using RAPD-PCR techniques were studied on three species of Garra, viz. G. mullya, G. kalakadensis and G. gotyla stenorhynchus, collected from various river basins of South-India by Arulraj*et al.* (2011). The results of morphological analysis revealed that G. mullya and G. kalakadensis hold many similar characters compared to the other congener, G. gotyla stenorhynchus. However, the G. gotyla stenorhynchus fish species exhibited distinct variation in the morphological characters such as snout length, pre-nasal length, inter-nasal width, gap width, lower jaw to isthmus, head depth at pupil, dorsal fin length and disc width from the other two species of Garra. However, certain morphometric characters overlapped hence the RAPD finger printing was used to assess the levels of genetic variation in Garra spp. using RAPD-PCR technique. A total of 72 reliable fragments were detected using 10 Operon primers, ranging from 2600 molecular weight to 3100. The shared RAPD fragments found in both G. mullya and G. kalakadensis with fixed frequencies were observed with all the investigated primers, implying their genetically closer relationship. However, the similarity index observed for G. gotyla stenorhynchus was less with the other two species specifying a genetically distant link.

Two Clariid species, *Clarias gariepinus* and *Heterobranchus bidorsalis* were compared at four loci using *Clarias gariepinus* microsatellite markers (Cga01, Cga02, Cga03, and Cga05) by Agbebi *et al* (2013). The heterozygosities observed were found to be 0.450 ± 0.050 and 0.442 ± 0.127, while 100% polymorphism was observed in both species. Overall, 95% of the samples amplified upon polymerase chain reaction (PCR) amplification and 44.3% of the total alleles observed for all the loci were heterozygotes. Conformity to Hardy-Weinberg equilibrium using the Chi-Square test showed that 81.25% of locus-population relationship conformed to Hardy-Weinberg equilibrium. The neighbor-joining phylogenetic dendrogram obtained gave a bootstrap value of 72, indicating that the genetic distance between the two species is distinct.

Morphology frequently varies with phylogeny, body size, sex, and phenotypic plasticity. However, the relative influence of these variables is unknown for most taxa. Morphological variation of Freshwater Drum,*Aplodinotus grunniens,* in the Wabash River, USA was described using geometric morphometrics by Jacquemin and Pyron (2013). A MANCOVA model of shape indicated that morphological variation was primarily influenced by allometry (body size), sex and river location. Among all individuals, at least 50% of the variability in morphology was a product of body size while sex and river location (collection locale) accounted for 10% and 5% of the overall variability in shape, respectively. However, when mature and immature individuals were analyzed separately, mature individuals demonstrated no morphological signal concurrent with river location, while at least 45% of the variability in immature shape was attributed to river locale. The contributions of allometry, sex and river gradient on Freshwater Drum morphology suggest that morphological variation is largely a result of a combination of developmental, sexual, and environmental influences.

A study to evaluate the genetic similarities and differences among 11 specimens of cichlids and four specimens of Mud catfishes obtained from Warri River was carried out through DNA fingerprinting analysis using random amplified polymorphic DNA (RAPD) by Asagbra *et al* (2014). PCR amplification with seven decamer primers and dendrograms through UPGMA cluster analysis. The total number of bands generated by the seven RAPD primers ranged between 2 to 33 for the Cichlids and 8 to 28 for the Catfish family with band sizes 100 to 800 bp. The primers produced 28 228 bands in total for the Cichlids and 109 for the catfishes, with 24% polymorphism.899 Considerable genetic variation was observed within species, between species of the same genera and among Cichlids and Catfishes.

Two species of fishes, *Acanthocobitis botia* and *Barilius bendelisis*, belonging to the families *Cobitidae* and *Cyprinidae*, respectively, were studied using karyotype and the cytochrome b gene by Ashoktaru*et al* (2012). Both species were collected from Kosi River, near Almora, Uttarakhand, India. The somatic karyotypes of A. botia (2n=50; 12m + 14sm + 10st + 14T; NF = 80) and B. bendelisis (2n=50; 16m + 14sm + 10st + 10T; NF = 80) were described. A fragment of approximately 307 bp that corresponds to the cytochrome b region of mtDNA was also amplified by polymerase chain reaction (PCR). The mtDNA sequencing indicated high per cent sequence divergences between the two species. Overall average percentage of nucleotide composition in A. botia was A(0.303), T(0.329), G(0.136) and C(0.232) and in B. bendelisis A(0.216), T(0.293), G(0.207) and C(0.284). Phylogenetic analysis based on the neighbor-joining (NJ) method using p-distance revealed that A. botia and B. bendelisis belonged to two different clades; this was also supported by species-specific karyotypes in the two species.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

The study was conducted in six selected water bodies in Kano State. These are Rivers Thomas and Ghari to the North, River Duddurun Gaya to the east,River Kano (Tiga dam) to the South, Rivers Karaye and Bagwai to the western part of the state. The first three locations were selected due to their independent flow and their disconnected tributaries. The remaining three locations have the same origin as the Kano River and two flow towards the western part of the State while Tiga dam flows toward the Southern part (Figure 3.1).

Kano State is located in the semi-arid area of North-western Nigeria. It has a population of 9,383,682 comprising of 4,844,128 males and 4,539,534 females (NPC, 2006). Kano State is the commercial nerve centre of Northern Nigeria. It is located between latitude $10^{o}33^{'}$ and $12^{o}27^{'}$North of the equator and longitude $7^{o}34^{'}$ and $9^{o}29^{'}$ East of the Greenwich meridian and as such it is part of Sudano-Sahelian vegetation zone of Nigeria. Kano has a hot semi-arid steppe climate which exhibits a tropical wet-dry season with a mono-modal rainfall distribution. The city sees on average about 980 mm (38.6 in) of precipitation per year, the bulk of which falls from June through September. Kano is typically very hot throughout the year, though from December through February, the city is noticeably cooler. Nighttime temperatures are cool during the months of December, January and February, with average low temperatures of 11 to 15 °C (52 to 59 °F).Ambient temperature varies sharply depending on the season, ranging from19.6^{o}C in December-January to 40^{o}C in March to April (KNARDA, 2001). The mean monthly sunshine hours

measured with Campbell Stoke recorder varies from 220.1 to 266.6 W/m^2 (KNSG, 2004). The average annual relative humidity is 31.1% and average monthly relative humidity ranges from 11% in Marchto 68% in August (Weatherspark, 2013). Kano State occupies a total land area of about 42,582.8 km^2out of which 754,200 ha are being used for agricultural production while forest and grazing land occupy 75,000 ha. The state is located at an elevation of 481m above sea level. The metropolitan area covers an estimated 499 km^2. Major occupation of the populace include cropping, fishing and livestock husbandry, and merchandizing. Kano rivers are the River Kano, Chalawa, Iggi and Gaya in the south while River Gari, Thomas and Jakara are situated in the north. These rivers are dammed to store water in surface reservoirs for multipurpose use including irrigation, domestic supply, fisheries and recreation (Ahmad, 1998).

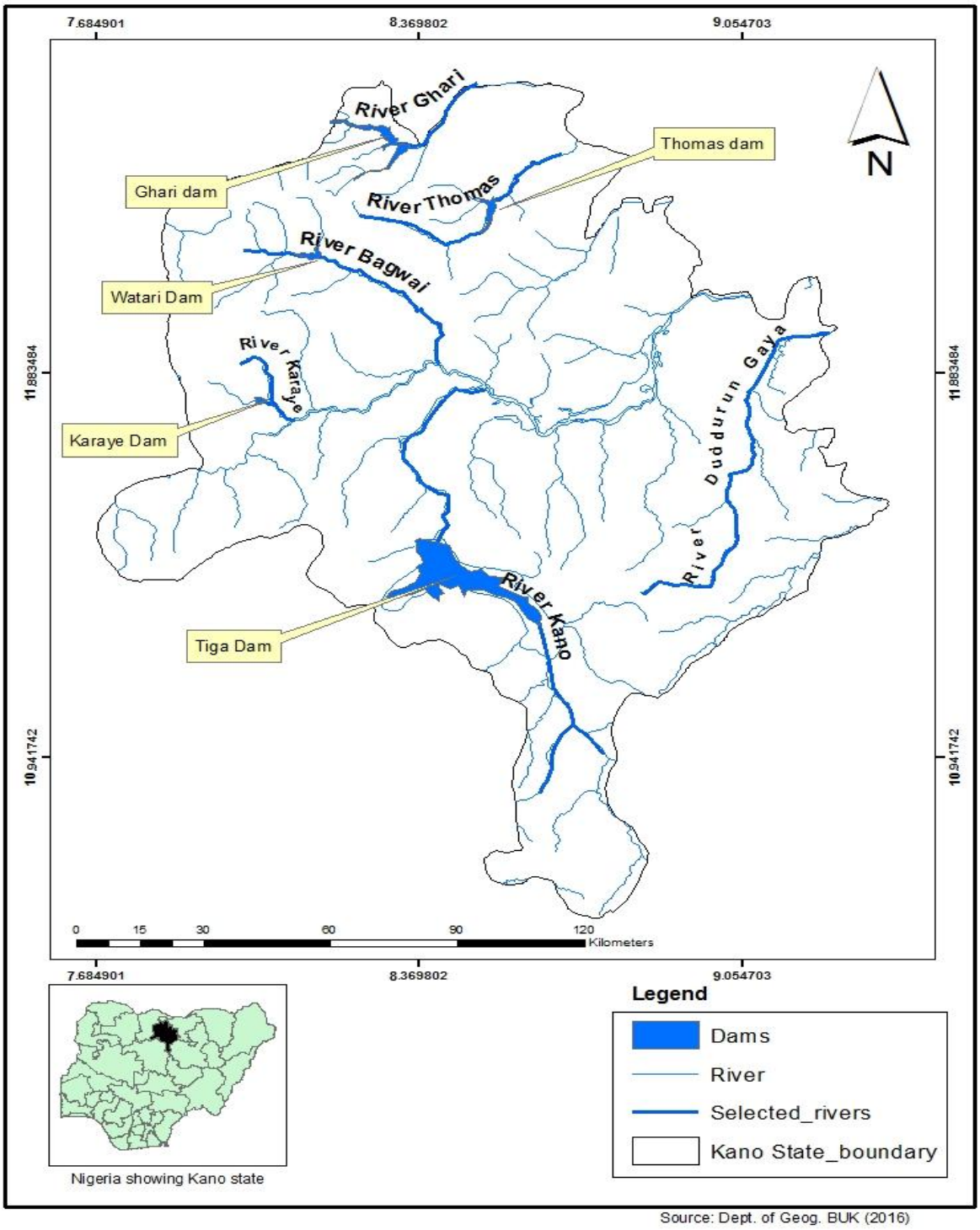


Figure 3.1: The map of Kano state showing the sampling locations (Rivers)

3.2 Study I: Morphometric Characters, Meristic Count, Length-Weight Relationship and Condition Factor in *Clarias gariepinus and Heterobranchus longifilis* in Kano State.

The fish samples were identified using an exposition for identification by Moses and Olufeagba, (2009), and confirmed using local names provided by the fishermen. Live fish samples of *Clarias gariepinus* (143) and*Heterobranchus longifilis* (29) from the six locations described above were purchased from commercial catches of the fishermen. *Clarias gariepinus* were collected from all locations except Tiga dam. Likewise, *Heterobranchus longifilis* were collected in all locations except Rivers Duddurun Gaya and Thomas. The samples were adults and were transported in large bowls to the Laboratory of the Department of Animal Science, Bayero University Kano, where measurements and counts were taken immediately. Sex was determined by identifying the presence of papillae in male and absent in female. Each fish sample, after draining off using filter paper, was subsequently given a serial identity number.

Table 3.1: Distribution of fish sample collected based on location

Location	Cg	Hl
River Thomas	4	-
River Ghari	26	4
River DudusinGaya	50	-
River Karaye	25	8
River Bagwai	38	4
River Tiga	-	13

Cg = Clarias gariepinus

Hl = Heterobranchus longifilis

Twenty-two (22) morphometric characters (using flexible tape), body weight and four (4) meristic counts were taken on each specimen. The morphometric measurements were shown in figure 3.2 below and they were as follows:

3.2.1 Morphometric measurements (BW was in gramm while others were in centimeters)

1. Body weight (BW):- This was taken in grams using a sensory weighing electronic balance (GT4100 model).
2. Total length (TL):- The distance between the snout and the end of the caudal fin.
3. Standard length (SL):- The distance between the snout and the origin of caudal fin.
4. Predorsal distance (PDD):- The distance between the snout and the origin of dorsal fin.
5. Preanal distance (PAD):- The distance between the snout and origin of anal fin.
6. Preventral distance (PVD):- The distance between the snout and origin of pectoral spine.
7. Prepectoral distance (PPD):- The distance between the snout and the origin of pectoral fin.
8. Dorsal fin length (DFL):- The distance of the dorsal fin from origin to the end.
9. Anal fin length (AFL):- The distance of the anal fin from origin to the end.
10. Pectoral fin length (PFL):- The length of the pectoral fin from origin to the end.
11. Pectoral spine length (PSL):- The length of the pectoral spine from origin to the end.
12. Dorso-caudal length (DDCF):- The distance between the dorsal and the caudal fin.
13. Dorso-occipital length (DODF):- The distance between the occipital process and dorsal fin.

14. Caudal peduncle depth (CPD):- The thickness of the body from the end of the dorsal fin to the end of the anal fin.
15. Body depth at anus (BDA):-Thickness of the body from the spine to the anus.
16. Head length (HL):- Longitudinal distance from the snout to the base of the head.
17. Head width (HW):- Lateral distance across the head.
18. Snout length (SNL):- The distance between frontal position of the eye to the tip of the mouth.
19. Inter-orbital distance (ID):- The distance between the eyes across the head.
20. Eye diameter (ED):- The diameter of the spherical eye.
21. Length of occipital fontanelle (OFL):- The longitudinal length of the knob at the base of the head.
22. Width of occipital fontanelle (OFW):- The lateral length of the knob at the base of the head.
23. Snout-occipital length (DSO):- Distance between snout and origin of occipital frontanelle.

The entire measurements were divided into three segments;

1. Measurements taken on the body comprising total length, standard length, pre-dorsal distance, pre-anal distance, pre-pectoral distance, pre-ventral distance, caudal peduncle depth and body depth at anus.
2. Measurements taken on the fins which include dorsal fin length, anal fin length, pectoral fin length and pectoral spine length.
3. Measurements taken on the head region are head length, head width, Dorso-caudal length, Dorso-Occipital length, Snout Length, Inter-orbital Distance, Eye Distance,

Length of Occipital Fontanelle, Width of Occipital Fontalle and Snout Occipital length

3.2.2 Meristic counts (All counts were done visually)

1. DFRC :- Dorsal fin ray count
2. PFRC :- Pectoral fin ray count
3. AFRC :- Anal fin ray count
4. CFRC :- Caudal fin ray count

The following statistical model was used for the analysis of morphometric characters and meristic counts:

$Y_{ijk} = \mu + C_i + X_j + S_k + e_{ijk}$

Y_{ijk}= morphometric charcteristics or meristic counts

μ = Population mean

C_i= Fixed effect of i^{th} strain ($i_{1,2}$ = 1. *Clarias gariepinus and*2. *Heterobranchus longifilis*)

X_j= Fixed effect of j^{th} sex (j = 1, male and 2, female)

S_k= Fixed effect of k^{th} location (k = 1, 2, 3, 4, 5,6)

e_{ijk} = Error term (random error)

3.2.3 Length-weight relationship and condition factor (Ponderal index):- The determined total length of fish samples were used alongside the determined weight to estimate length-weight relationship of the samples according to Ricker (1978).

The formula $W = a \times L^b$ or $\log W = \log a + b \log L$ was used,

where:

W = weight of fish samples,

L = total length of fish samples,

a = intercept

b = regression coefficient.

The condition factor (K) was computed according to Pauly (1983) using the formula:

$K = 100W/L^3$

*where*W = weight of fish samples

L = total length of fish samples.

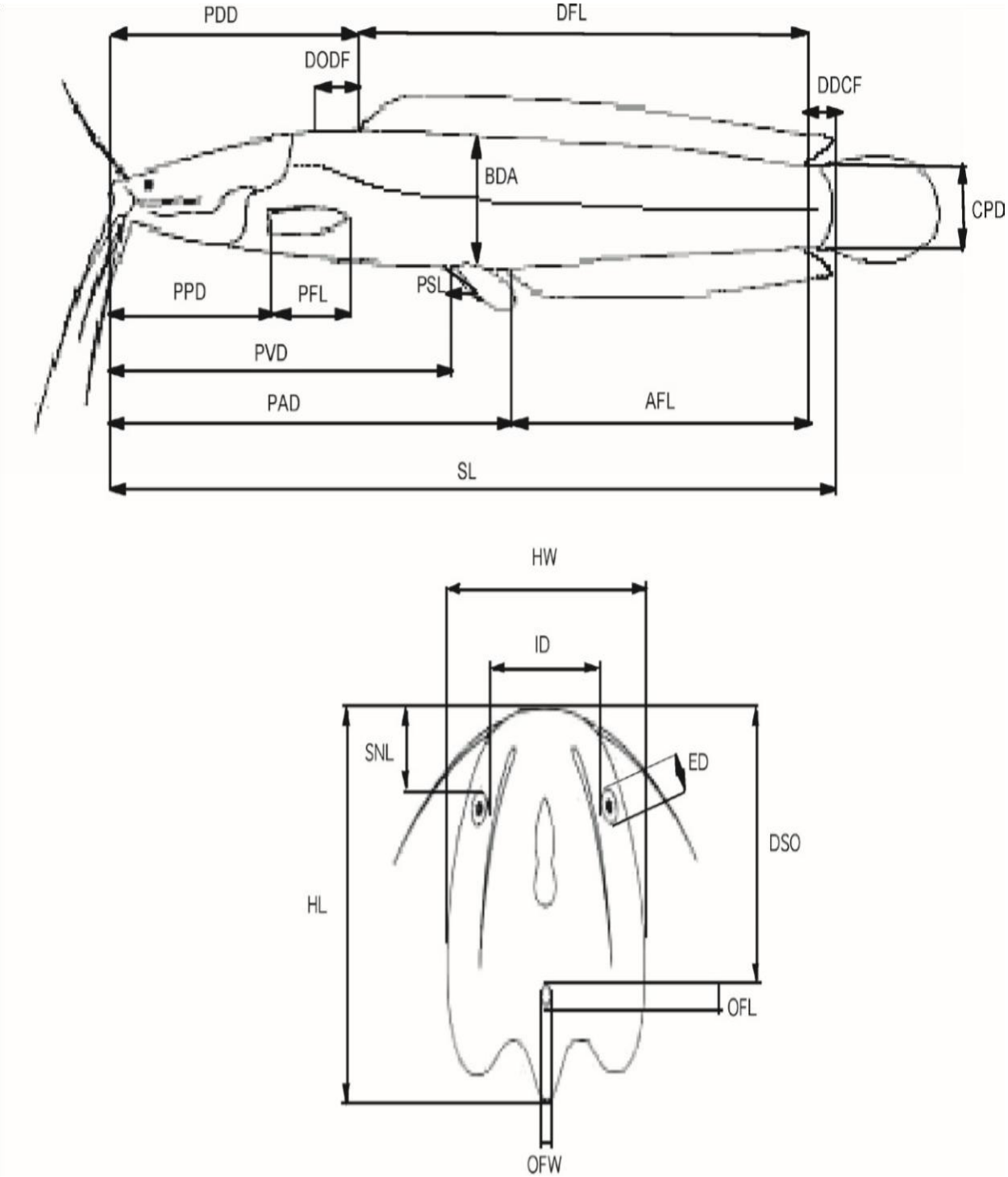


Figure 3.2: Image of a clariid species indicating the morphometric measurements. (Source: Cemal *et al.*2005)

3.3 Study II:Genetic Variation within and among *Clarias gariepinus and Heterobranchus longifilis* Populations in Kano State.

3.3.1 Sample collection and DNA extraction: The study was carried out using specimens in Study I above. Thirty-five blood samples (25 – *Clarias gariepinus* and 10 – *Heterobranchus longifilis*) were transferred to FTA® Classic Cards (Whatman Bioscience, Maidestone, UK) and left to air dry and subsequently used for DNA extraction. The caudal peduncle of each fish sample was severed to drain out blood into the FTA cards. The samples were taken to a commercial laboratory (DNA Lab) in Angwa Seriki, Kaduna Metropolis, Kaduna State.

Genomic DNA was extracted individually following the procedure of Agbebi *et al.* (2013) as follows:

1. Five 1.2mm disc of FTA® Classic Cards were punched into 1.5ml Eppendorf tubes.
2. 1000µl of 100mM Tris-base and 0.1% SDS buffer was added. Tubes were vortexed gently for 30minutes.
3. The supernatant was decanted, thereafter, 500µl of 5M guanidine thiocyanate was added and vortexed for 10minutes.
4. The supernatant was decanted again; thereafter, 500µl nuclease-free water was added and vortexed for 10 minutes and then the supernatant was decanted.
5. The same volume of nuclease-free water was added and left to stand for 10 minutes.
6. The supernatant was again decanted and finally, 50µlnuclease-free water was added to the discs.

7. The tubes were heated at 90^{o}C for 10minutes in a thermocycler to complete extraction.
8. The DNA concentration was further diluted with nuclease free water in 1:20 ratio to remove impurities and prevent smearing.
9. DNA concentration was determined by measuring the intensity of absorbance of the solution at the 600 nm with a spectrophotometer and comparing to a standard curve of known DNA concentrations.
10. Measuring the intensity of absorbance of the DNA solution at wavelengths 260 nm and 280 nmwas used as a measure of DNA purity. DNA absorbedUV light at 260 and 280 nanometres, and aromatic proteins absorbed UV light at 280 nm; a pure sample of DNA hada ratio of 1.8 at 260/280 and was relatively free from protein contamination. DNA was quantified by cutting the DNA with a restriction enzyme, running it on an agarose gel, staining with ethidium bromide and comparing the intensity of the DNA with a DNA marker.
11. DNA samples were stored at -20^{o}C in Tris EDTA buffer pH7.8/8.0.

3.3.2 Polymerase chain reaction

i) DNA Amplification and scoring: TemplateDNA was amplified using polymerase chain reaction (PCR). PCR was carried out in 10µl reaction volumes containing 20 to 40ng(50-120 ng/µl)genomic DNA, 2µl5x Taq mastermix of Taq DNA polymerase, dATP, dCTP, dGTP, dTTP, $(NH_4)_2SO_4$, $MgCl_2$, Tween-20, Nonidet P-40, red dye, gel loading buffer, stabilizers, 0.3µl each of the forward and reverse primer sets and 5.9µl of PCR grade water.

Seven microsatellite markers isolated by Galbusera *et al*(1996) were used as primers. They were obtained from Jena Bioscience, Lobstedter, Germany. The primers are Cga01,

Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10. The list of primers showing their relevant information is presented in Table 3.2. Amplifications were carried out in a Real-time thermocycler as follows: 1 cycle at 94^{o}C (5min) for pre-denaturation, 35 cycles at 94^{o}C (30sec) for denaturation, 35 cycles at 55^{o}C (30sec) for annealing, 35 cycles at 72^{o}C (30sec) for extension and 1 cycle at 72^{o}C (5min) for final extension to complete amplification. PCR condition for each marker was optimized: 1cycle for 2minutes at 95^{0}C, 25 cycles for 15seconds at 95^{0}C, 15seconds at 55^{0}C, 45seconds at 68^{0}C, 1 cycle for 5minutes at 68^{0}C and finally, 1 cycle 2days at 4-10^{0}C.

ii) Electrophoresis: Electrophoresis was conducted on 2% Agarose gel containing a negative control to detect potential PCR contamination, and each gel contained a positive control using a sample that had been genotyped in order to standardize allele scoring among gels. Scoring was done by comparison to 8 bp (75, 154, 220, 298, 344, 396, 504 & 1632) standard DNA ladderto identify the approximate size of a molecule run on a gel during electrophoresis, with the aid of gel analyser (Jena Bioscience, Lobstedter, Germany).

Table 3.2: List of primers showing their relevant information

Locus	Repeat Array	Primer Sequence 5'-3'	Genebank Accession Number	$MgCl_2$(mM)	Annealing Temperature	Range of Base Pair	Expected Allele Number
Cga01	$(GT)_{15}$	GGCTAAAAGAACCCTGTCTG	U30862	1	59	92-104	5
		TACAGCGTCGATAAGCCAGG					
Cga02	$(GT)_{10}N_2(GT)_8$	GCTAGTGTGAACGCAAGGC	U30863	1	58	102-110	5
		ACCTCTGAGATAAAACACAGC					
Cga03	$(GT)_{21}$	CACTTCTTACATTTGTGCCC	U30864	1	56	142-168	13
		ACCTGTATTGATTTCTTGCC					
Cga05	$(GT)_{11}N_2(GT)_2$	TCCACATTAAGGACAACCACCG	U30866	1.5	60	204-212	5
		TTTGCAGTTCACGATTGCCG					
Cga06	$(GT)_5N_2(GT)_9$	CAGCTCGTGTTTAATTTGGC	U30867	1.5	60	134-1422	5
		TTGTACGAGAACCGTGCCAGG					
Cga09	$(GT)_3N_3(GT)_{11}$	CGTCCACTTCCCCTAGAGCG	U30871	1	65	180-196	7
	$N(GT)_6N_2(GT)_4$	CCAGCTGCATTACCATACATGG					
Cga10	$(GT)_2N_2(GT)_{15}$	GCTGTAGCAAAAATGCAGATGC	U30870	1	60	102-138	14
		TCTCCAGAGATCTAGGCTGTCC					

Source: Galbusera*et al.,* (1996)

3.4 Data Analysis

Study I: The data from the morphometric measurements and meristic counts were computed in excel and analysed using GLM procedures of SAS 9.4, (2000), and Duncan Multiple Range Test was used to separate the means. Simple linear Regression (Y = a+bx) was used to determine the relationship between length and weight. Pearson correlation was used to determine the relationships among variables (SAS, 2000).It has a value between +1 and −1, where 1 is total positive linear correlation, 0 is no linear correlation, and −1 is total negative linear correlation. Principal Component Analysis using the morphometric measurements for data reduction (SPSS, 2007). Dendrogram was constructed using morphometric measurements to determine the genetic distance among populations.

Study II: Allele frequencies for each locus within each sampled population were computed. This was calculated locus by locus with the expression:

$$FreqAllele_x = \frac{2\mathrm{Nxx} + \mathrm{Nxy}}{2\mathrm{N}}$$

Where Nxx is the number of homozygotes for allele X (XX), and Nxy is the number of heterozygotes containing the allele X (Y was any other allele to be tested). N = the number of samples. These were tested for deviation of observed genotype frequencies from those under Hardy-Weinberg equilibrium using the Markov chain exact tests provided in the Genepop software (Raymond and Rousset, 1995). Estimate of microsatellite diversity within populations such as total alleles TNA, mean number of alleles MNA, allelic richness Ar, observed and expected (H_o and H_e) heterozygote as well as nuclear pairwise F_t, values corrected for multiple testing was calculated using MS analyser 4.05 (Dieriener and Schloetterer, 2003).Genetix (Belkher *et al.*,2004)

wasused to infer genetic inbreeding coefficient $F_{is.}$To quantify the extent of molecular variation, locus-by-locus analysis of molecular variance (AMOVA) was performed using Genealex 6.4. In the current study, Fst was used to determine the potential differences between the two statistics. F-statistics were obtained using AMOVA approach and population pairwise based on microsatellite loci as implemented in Genealex 6.4. Genetic Distance were calculated by choosing the option *Distance* from the GenAlEx menu and then selected *Genetic* from the submenu. It was ensured that locus and sample parameters were correct in the Genetic Distance Options dialog box. Then the appropriate Distance Calculation was selected and output options required. Title and Worksheet Prefix was enteredand then click Ok. Genetic distance was output to sheet.

CHAPTER FOUR

4.0 RESULTS

4.1 Morphological and Meristic Characterization of Clariid Species in Kano State

Table 4.1 shows the summary statistics for the morphometric characteristics measured in *Clarias gariepinus* and *Heterobranchus longifilis*. In *Clarias gariepinus,* the average body weight was 190.54g and coefficient of variability was 72.73% while it was 67.39% in *Heterobrancus longifilis* with average body weight of 318.73g. The body weight of the two strains showed a significantly wide variation indicating a lot of differences within the strains when body weight is considered.

On the measurements taken on the body, *Heterobranchus longifilis* recorded higher values compared to *Clarias gariepinus,* these are total length, standard length, pre-dorsal distance, pre-anal distance, pre-pectoral distance, pre-ventral distamce caudal peduncle depth and body depth at anus. The coefficient of variation of *Claria gariepinus* ranged from 21.34% for pre-anal distance to 59.73 for pre-ventral distance. The coefficient of variation of *Heterobranchus longifilis* ranged from 13.71% for total length to 51.00% for pre-ventral distance. These showed that the variability observed in them were moderate to high and could be regarded as variable.

Dorsal fin length and anal fin length were higher in *Clarias gariepinus* compared to *Heterobranchus longifilis* due to the presence of adipose tissue on the dorsal part of *Heterobranchus longifilis* while *Heterobranchus longifilis* gave a higher measurements in pectoral fin length and pectoral spine length than *Clarias gariepinus*. The coefficient of variation for fin measurements in *Clarias gariepinu*s were 21.61% for dorsal fin length to 51.48% for pectoral fin length while in *Heterobranchus longifilis,* the

coefficient of variation ranged from 21.80% for dorsal fin length to 47.01% for anal fin length. The variability observed in them were high and they are optimally wide apart.

Concerning the head region measurements, *Clarias gariepinus* had higher measurement in head length and inter-orbital distance while *Heterobranchus longifilis* had higher measurement in head width, dors-caudal length, dorso-occipital length, snout length, eye distance, length of occipital fontenale, width of occipital fontenale and snout occipital length. The coefficient of variationfor *Clarias gariepinus* ranged from 20.59% for head length to 32.45% for eye distance while coefficient of variation for *Heterobranchus longifilis*ranged from 13.21% for snout occipital length to 62.08% for dorso-caudal length. These showed moderate to high variability and they are widely separated in terms of phenotypic plasticity.

Table 4.2 shows the summary statistics for the meristic counts taken on the samples. The ray counts are the number of webbed rays on the fins that aid locomotion. *Clarias gariepinus* recorded a higher count in dorsal fin ray count, anal fin ray count and pectoral fin ray count while *Heterobranchus longifilis* only gave a higher count in caudal fin ray count. The coefficient of variation for*Clarias gariepinus* on meristic count were 5.33% for caudal fin ray count, 6.87 for pectoral fin ray count, 22.17 for anal fin ray count and 24.50 for dorsal fin ray count.For *Heterobranchus longifilis*, the coefficient of variation were 5.26 for caudal fin ray count, 7.05 pectoral fin ray count, 47.06 for anal fin ray count and 58.31 for dorsal fin ray count. Pectoral and caudal fins ray count showed a very low percentage of variation while dorsal and anal fins ray count were moderate to high variability.

Table 4.1: Summary Statistics for the Morphometric Characteristics of the fish Populations in the study area

	Clarias gariepinus		*Heterobranchus longifilis*	
Characteristics (cm)	Mean±SE	CV%	Mean±SE	CV%
Body measurement				
Body Weight (g)	190.54±11.59	72.73	318.73±42.12	67.39
Total Length	29.63±0.55	22.11	39.23±1.06	13.71
Standard Length	26.23±0.50	22.63	29.05±1.04	18.24
Pre-Dorsal Distance	8.64±0.16	21.60	11.46±0.41	18.30
Pre-Anal Distance	13.96±0.25	21.34	20.52±0.68	16.99
Pre-Pectoral Distance	10.28±0.21	24.82	11.74±0.71	30.79
Pre-Ventral Distance	6.55±0.33	59.73	9.79±0.99	51.00
Caudal Peduncle Depth	2.10±0.05	25.82	2.29±0.11	24.98
Body Depth at Anus	5.18±0.10	22.84	6.60±0.29	22.53
Fin measurement				
Dorsal Fin Length	15.69±0.28	21.61	5.33±0.23	21.80
Anal Fin Length	12.88±0.39	35.99	3.67±0.35	47.01
Pectoral Fin Length	2.48±0.11	51.48	4.44±0.21	24.43
Pectoral Spine Length	2.98±0.07	28.29	4.79±0.21	22.80
Head region				
Head Length	7.34±0.13	20.59	7.10±0.25	17.99
Head Width	5.60±0.10	21.64	6.17±0.18	15.14
Dorso-caudal length	1.64±0.04	28.81	8.44±1.03	62.08
Dorso-Occipital length	6.14±0.14	26.30	9.37±0.35	18.21
Snout Length	1.73±0.04	28.08	2.59±0.13	24.52
Inter-orbital Distance	3.30±0.06	21.38	2.46±0.09	19.06
Eye Distance	0.34±0.01	32.45	0.65±0.03	22.16
Occipital Fontanelle Length	1.96±0.04	25.55	4.58±0.19	21.51
OccipitalFontanelleWidth	0.45±0.01	30.27	0.67±0.04	28.67
Snout Occipital length	1.77±0.04	24.35	2.11±0.06	13.21

CV = Coefficient of variation, SE = Standard error.

Table 4.2: Summary Statistics for the Meristic Characteristics of the Populations Investigated

	Clarias gariepinus		*Heterobranchus longifilis*	
Character	Mean±SE	CV%	Mean±SE	CV%
Dorsal Fin Ray Count	7.83±0.05	24.50	3.29±0.02	58.31
Pectoral Fin Ray Count	2.33±0.01	6.87	2.27±0.02	7.05
Anal Fin Ray Count	6.92±0.04	22.17	3.26±0.04	47.06
Caudal Fin Ray Count	4.28±0.02	5.33	4.34±0.03	5.26

CV = Coefficient of variability

SE = Standard error.

Tables 4.3a, 4.3b and 4.3c show the effect of location, strain and sex on the morphometric measurements taken on the fish samples respectively. All the parameters showed a significant difference (P<0.01) of location and also in strain except standard length, pre-dorsal distance and head length that showed significant difference at only 5% while sex only had significant difference (P<0.01) on total length, standard length, dorsal fin length, dorso-caudal length, caudal peduncle depth, anal fin length, head length, inter-orbital distance, eye diameter and length of occipital fontalle. On body weight, Clariid strains of River Thomas recorded the highest body weight of 460.33g while those of River Duddurun Gaya had the lowest body weight of 114.01g. Clariis strains of River Thomas also gave higher (P<0.01) measurements in all the morphometric measurements except in pre-ventral distance, dorso-cauda distance, snout length, eye diameter and snout-occipital length. On the effect of strain, *Heterobranchus longifilis*was higher (P<0.01) in body weight and some of the morphometric measurements except in pre-pectoral distance, dorso-caudal length, caudal peduncle depth, anal fin length, dorsal fin length, head width, head length, snout length, inter-orbital distance, eye distance and snout-occipital length. *Clarias gariepinus* was only

higher in dorsal fin length and anal fin length. On the effect of sex, there were significant difference between males and females whereby males had a higher measurement in total length, standard length, dorsal fin length, anal fin length, caudal peduncle depth, head length, inter-orbital distance, body weight, pre-dorsal distance, pre-anal distance, pre-ventral distance, pre-pectoral distance and body depth at anus. The females on the other hand, had a higher measurement in pectoral fin length, snout length, eye diameter, dorso-caudal length, snout-occipita length and length of occipital fontanelle. With respect to sex, there were no significant effect on WT, PDD, PAD, PVD, PPD, PFL, PSL, DODF, BDA, HW, SNL, OFL and DSO.

Table 4.3a: Morphometric Characteristics of strains of Clariid Species in relation to location, strains and sex

Factor	N	WT (g)	TL (cm)	SL (cm)	PDD (cm)	PAD (cm)	PVD (cm)	PPD (cm)	DFL (cm)
Location	177	****	**	**	**	**	**	**	
River Thomas	4	460.33^{a}±111.44	42.75^{a}±2.87	38.00^{a}±2.68	13.34^{a}±0.94	19.13^{a}±3.07	8.00^{b}±0.58	17.00^{a}±1.46	25.75^{a}±1.83
RiverGhari	27	260.70^{bc}±22.48	31.03^{c}±0.99	27.92^{bc}±0.86	8.87^{c}±0.31	14.30^{b}±0.51	5.21^{d}±0.25	11.69^{b}±0.40	16.36^{b}±0.52
River.D/Gaya	50	114.01^{d}±8.09	25.74^{d}±0.81	22.64^{d}±0.77	7.53^{d}±0.16	12.32^{b}±0.25	4.58^{d}±0.11	10.29^{c}±0.21	14.51^{bc}±0.46
RiverKaraye	33	323.47^{b}±35.87	37.46^{b}±1.29	31.30^{b}±1.06	11.15^{b}±0.43	18.58^{a}±0.78	15.04^{a}±0.47	6.59^{d}±0.24	12.16^{c}±0.75
RiverTiga	21	259.12^{bc}±43.11	33.28^{c}±0.94	24.73^{cd}±0.54	10.09^{b}±0.22	18.08^{a}±0.47	6.36^{c}±0.11	12.86^{b}±0.26	4.12^{d}±0.21
RiverBagwai	42	160.16^{cd}±8.75	30.03^{c}±0.76	26.22^{cd}±0.54	8.71^{c}±0.21	14.37^{b}±0.46	5.01^{d}±0.13	11.63^{b}±0.28	15.47^{b}±0.61
Strain	177	**	**	*	**	**	**	*	**
C. gariepinus	143	190.54^{b}±11.59	29.63^{b}±0.55	26.23^{b}±0.50	8.64^{b}±0.16	13.96^{b}±0.25	6.55^{b}±0.33	10.28^{b}±0.21	15.69^{a}±0.28
H. longifilis	29	318.73^{a}±42.12	39.23^{a}±1.06	29.05^{a}±1.04	11.46^{a}±0.41	20.52^{a}±0.68	9.79^{a}±0.98	11.74^{a}±0.71	5.33^{b}±0.23
Sex	177	NS	*	**	NS	NS	NS	NS	**
Male	83	233.93±18.06	32.40^{a}±0.78	28.36^{a}±0.69	9.28±0.22	15.12±0.39	7.05±0.44	10.94±0.34	15.99^{a}±0.49
Female	94	191.57±15.13	29.82^{b}±0.72	24.86^{b}±0.51	8.98±0.23	15.11±0.43	6.99±0.44	10.28±0.25	11.49^{b}±0.52

a,b,c,d means within the same column and factors with different superscripts differ significantl**=P<0.01;*=P<0.05
WT= weight, PAD = pre-anal distance, DFL = dorsal fin length,
SL=standard length, PPD = pre-pectoral distance, TL= Total length
PDD=pre-dorsal distance,NS- Not significant PVD = pre-ventral distance

Table 4.3b: Morphometric Characteristics of strains of Clariid Species in relation to location, strains and sex

Factor	N	PFL (cm)	PSL (cm)	DDCF (cm)	DODF (cm)	CPD (cm)	AFL (cm)	BDA (cm)
Location	177	**	**	**	**	**	**	**
River Thomas	4	4.03^{a}0±0.27	4.53^{a}±0.30	2.78^{a}±0.29	10.10^{a}±1.36	3.00^{a}±0.71	19.13^{a}±1.23	7.18^{a}±0.69
RiverGhari	27	2.55^{b}±0.14	3.18^{b}±0.17	1.79^{cd}±0.07	5.76^{c}±0.39	2.07^{cd}±0.10	12.63^{b}±0.70	5.33^{bc}±0.24
River D/Gaya	50	2.21^{b}±0.27	2.43^{c}±0.06	1.32^{d}±0.05	5.96^{c}±0.11	1.82^{d}±0.05	9.73^{b}±0.20	4.53^{c}±0.11
RiverKaraye	33	3.75^{a}±0.24	4.43^{a}±0.21	2.35^{bc}±0.18	8.17^{b}±0.37	2.67^{ab}±0.09	16.71^{a}±1.37	6.79^{a}±0.26
RiverTiga	21	3.58^{a}±0.09	3.98^{a}±0.08	3.12^{a}±0.34	8.18^{b}±0.28	2.39^{bc}±0.16	2.52^{c}±0.14	5.88^{b}±0.20
River Bagwai	42	2.49^{b}±0.14	3.05^{b}±0.14	1.79^{cd}±0.07	5.85^{c}±0.25	2.07^{cd}±0.06	10.88^{b}±0.45	5.16^{bc}±0.14
Strain	177	**	**	**	**	NS**	**	
C. gariepinus	143	2.48^{b}±0.11	2.48^{b}±0.07	1.64^{b}±0.04	6.14^{b}±0.14	2.10±0.05	12.88^{a}±0.39	5.18^{b}±0.10
H. longifilis	29	4.44^{a}±0.21	4.79^{a}±0.21	8.44^{b}±1.03	9.37^{a}±0.34	2.29±0.11	3.67^{b}±0.35	6.60^{a}±0.29
Sex	177	NS	NS **	NS	*	**	NS	
Male	83	2.68±0.10	3.30±0.11	2.02^{b}±0.19	6.47±0.21	2.27^{a}±0.07	13.48^{a}±0.59	5.56±0.15
Female	94	2.94±0.18	3.30±0.12	4.11^{a}±0.49	6.85±0.21	2.09^{b}±0.06	8.92^{b}±0.32	5.34±0.14

a,b,c,d means within the same column and factors with different superscripts differ significantly. **=P<0.01;*=P<0.05

AFL=anal fin length PFL=pectoral fin length, PSL=pectoral spine length, DDCF=dorso-caudal length, DODF=dorso-occipital length, CPD=caudal peduncle depth BDA=body depth at anus. NS – Not significant

Table 4.3c: Morphometric Characteristics of strains of Clariid Species in relation to location, strains and sex

Factor	N	HL (cm)	HW (cm)	SNL (cm)	ID (cm)	ED (cm)	OFL (cm)	OFW (cm)	DSO (cm)
Location	177	**	**	**	**	**	**	**	**
River Thomas	4	$10.48^{a}\pm0.88$	$8.00^{a}\pm0.61$	$2.25^{b}\pm0.20$	$4.55^{a}\pm0.40$	$0.53^{b}\pm0.08$	$2.63^{bc}\pm0.24$	$0.65^{a}\pm0.12$	$1.70^{c}\pm0.15$
RiverGhari	27	$7.88b^{c}\pm0.21$	$5.84^{c}\pm0.21$	$1.72^{c}\pm0.08$	$3.30^{bc}\pm0.09$	$0.42^{b}\pm0.02$	$1.99^{cd}\pm0.14$	$0.50^{bc}\pm0.02$	$1.80^{c}\pm0.05$
River D/Gaya	50	$6.24^{d}\pm0.12$	$4.86^{d}\pm0.12$	$1.51^{c}\pm0.05$	$2.93^{c}\pm0.07$	$0.26^{c}\pm0.01$	$1.78^{d}\pm0.05$	$0.39^{c}\pm0.02$	$1.57^{c}\pm0.06$
RiverKaraye	33	$8.40^{b}\pm0.28$	$6.42^{bc}\pm0.22$	$2.53^{b}\pm0.12$	$3.60^{b}\pm0.16$	$0.40^{bc}\pm0.03$	$3.21^{ab}\pm0.25$	$0.52^{b}\pm0.05$	$2.18^{b}\pm0.09$
RiverTiga	21	$7.21^{c}\pm0.34$	$6.81^{b}\pm0.38$	$3.04^{a}\pm0.26$	$2.32^{d}\pm0.08$	$0.86^{a}\pm0.09$	$3.51^{a}\pm0.28$	$0.51^{bc}\pm0.03$	$2.65^{a}\pm0.20$
RiverBagwai	42	$7.41^{c}\pm0.15$	$5.76^{c}\pm0.15$	$1.68^{c}\pm0.06$	$3.21^{bc}\pm0.10$	$0.44^{b}\pm0.02$	$1.99^{cd}\pm0.09$	$0.50^{bc}\pm0.02$	$1.80^{c}\pm0.05$
Strain	177	NS	NS	NS	**	**	**	**	**
C. gariepinus	143	7.34±0.13	5.60±0.10	5.60±0.04	$3.30^{a}\pm0.06$	$0.34^{b}\pm0.01$	$1.96^{b}\pm0.04$	$0.45^{b}\pm0.01$	$1.77^{b}\pm0.04$
H. longifilis	29	7.10±0.25	6.17±0.18	6.17±0.13	$2.46^{b}\pm0.09$	$0.65^{a}\pm0.03$	$4.58^{a}\pm0.19$	$0.67^{a}\pm0.04$	$2.11^{a}\pm0.05$
Sex	177	**	NS	NS	**	*	*	NS	NS
Male	83	$7.80^{a}\pm0.17$	5.95±0.15	1.92±0.06	$3.41^{a}\pm0.08$	$0.39^{b}\pm0.02$	$2.15^{b}\pm0.08$	0.46±0.02	1.90±0.05
Female	94	$7.00^{b}\pm0.14$	5.70±0.14	2.02±0.10	$2.91^{b}\pm0.07$	$0.47^{a}\pm0.03$	$2.53^{a}\pm0.14$	0.49±0.02	1.91±0.07

a,b,c,d means within the same column and factors with different superscripts differ significantly .**=P<0.01;*=P<0.05

HL=head length, HW=head width, SNL=snout length, ID=inter-orbital distance,

OFL=length of occipital fontanelle, ED= eye diameter ID=inter-orbital distance NS=not significant

OFW=width of occipital fontanelle, DSO=snout-occipital length.

Tables 4.4a, 4.4b and 4.4c show the interaction effect between river location and sex on the morphometric measurements. River Thomas had a significant effect ($P<0.01$) on sex with higher measurements in males than females in body weight, total length, standard length, pre-dorsal distance, pre-anal distance, pre-ventral distance, pre-pectoral distance, dorsal fin length, caudal peduncle depth, anal fin length, body depth at anus, head length, head width, snout length and inter-orbital distance. Females had a higher measurements in dorso-occiptal length. In River Ghari, location effect ($P<0.01$) was more on males then females in body weight, total length, standard length, pectoral spine length, anal fin length and body depth at anus.The same effect ($P<0.01$) was recorded in River Duddurun Gaya as the effect were more on meales than females in body weight, total length, standard length, pre-anal distance, pre-pectoral distance, dorsal fin length, dorso-occipital length, caudal peduncle depth and anal fin length. In River Karaye, the effect of location ($P<0.01$) was seen on males in dorsal fin length and anal fin length while the effect was more on felames than males in pre-dorsal distance, pre-anal distance, pectoral fin length, pectoral spine length, dorso-caudal length and dorso-occipital length. In Tiga dam, effect of location ($P<0.01$) was significant on females than males in body weight, standard length, pre-dorsal distance, pre-pectoral distance and dorso-occipital length. In River Bagwai, effect of location was significant ($P<0.01$) in males than females on body weight, total length, standard length, dorsal fin length, dorso-occipital length and anal fin length.

Table 4.4a: Morphometric Characteristics of strains of Clariid Species in relation to interaction between location and sex

Factor	WT	TL	SL	PDD	PAD	PVD	PPD	DFL
		(g)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)
Location	sex							
River Thomas	F	274 80^{b}±47.90	38.00^{b}±2.00	33.50^{b}±1.50	11.754^{b}±0.25	14.75^{b}±4.25	7.00^{b}±0.00	14.50^{b}±0.50 22.75^{b}±1.25
River Thomas	M	635.85^{a}±58.15	47.50^{a}±0.50	42.50^{a}±0.50	15.00^{a}±0.00	23.50^{a}±0.50	9.00^{a}±0.00	19.50^{a}±0.00 28.75^{a}±0.75
River Ghari	F	193.00^{b}±11.95	28.12^{b}±0.68	25.44^{b}±0.62	8.43±0.31	13.69±0.62	4.64±0.14	11.00±0.37 16.04±0.72
River Ghari	M	294.56^{a}±30.74	32.39^{a}±1.33	29.16^{a}±1.15	9.09±0.43	14.61±0.70	5.49±0.35	12.04±0.55 16.50±0.70
River.D/Gaya	F	96.70^{b}±6.04	23.84^{b}±0.49	21.00^{b}±0.42	7.19±0.15	11.86^{b}±0.25	4.41±0.11	9.86^{b}±0.20 13.42^{b}±0.29
River D/Gaya	M	147.62^{a}±18.48	29.43^{a}±1.93	25.80^{a}±1.94	8.19±0.33	13.21^{a}±0.50	4.92±0.22	11.13^{a}±0.41 16.62^{a}±1.06
River Karaye	F	321.71±39.35	38.62±1.72	31.38±1.26	11.82^{a}±0.59	19.94^{a}±1.15	15.60±0.58	6.56±0.31 9.91^{b}±1.02
River Karaye	M	325.24±62.49	36.23±1.92	31.22±1.77	10.42^{b}±0.61	17.12^{b}±0.94	14.44±0.75	6.62±0.38 14.54^{a}±0.76
Tiga Dam	F	284.10^{a}±72.85	35.74±0.69	25.81^{a}±0.43	10.17^{a}±0.20	18.13±0.63	6.40±0.11	13.23^{a}±0.21 4.73±0.20
Tiga Dam	M	143.00^{b}±32.99	35.00±0.88	24.00^{b}±0.66	9.00^{b}±0.19	17.50±0.22	6.00±0.15	12.00^{b}±0.47 4.00±-.11
River Bagwai	F	146.24^{b}±13.84	28.41^{b}±0.96	24.58^{b}±0.75	8.15±0.32	13.86±0.65	4.96±0.32	11.17±0.41 14.25^{b}±1.15
River Bagwai	M	167.13^{a}±11.09	30.84^{a}±1.02	27.04^{a}±0.68	9.04±0.26	14.62±0.62	5.04±0.11	11.86±0.36 16.08^{a}±0.70

a,b, means within the same column and factors with different superscripts differ significantly. P<0.01

WT= weight, PAD = pre-anal distance, DFL = dorsal fin length, PVD = pre-ventral distance F=Female

SL=standard length, PPD = pre-pectoral distance, TL= Total length PDD=pre-dorsal distance, M=Male

Table 4.4b: Morphometric Characteristics of strains of Clariid Species in relation to interaction between location and sex

Factor		PFL (cm)	PSL (cm)	DDCF (cm)	DODF (cm)	CPD (cm)	AFL (cm)	BDA (cm)
Location	sex							
River Thomas	F	3.65^{b}±0.05	4.10±0.40	2.65±0.65	9.05^{a}±0.05	2.00^{b}±0.00	17.00^{b}±0.00	6.10^{b}±0.10
River Thomas	M	4.40^{a}±0.40	4.95±0.05	2.90±0.20	8.15^{b}±1.85	4.00^{a}±1.00	21.25^{a}±0.25	8.25^{a}±0.75
River Ghari	F	2.22±0.09	2.77^{b}±0.15	1.73±0.10	5.74±0.38	2.02±0.02	11.57^{b}±0.42	4.89^{b}±0.20
River Ghari	M	2.71±0.20	3.39^{a}±0.23	1.81±0.10	5.77±0.56	2.10±0.14	13.16^{a}±1.01	5.56^{a}±0.33
River.D/Gaya	F	2.27±0.40	2.34±0.06	1.26±0.04	5.79^{b}±0.12	1.74^{b}±0.04	9.29^{b}±0.20	4.34±0.12
River D/Gaya	M	2.10±0.08	2.62±0.11	1.42±0.10	6.28^{a}±0.23	2.01^{a}±0.09	10.57^{a}±0.38	4.91±-.22
River Karaye	F	4.19^{a}±0.38	4.75^{a}±0.31	2.81^{a}±0.27	9.10^{a}±0.53	2.59±0.11	12.44^{b}±1.99	7.16±0.35
River Karaye	M	3.28^{b}±0.22	4.09^{b}±0.26	1.86^{b}±0.14	7.19^{b}±0.37	2.76±0.14	20.98^{a}±1.16	6.41±0.36
Tiga Dam	F	3.56±0.09	3.95±0.11	3.55±0.28	8.78^{a}±0.33	1.98±0.06	2.89±0.13	5.60±0.11
Tiga Dam	M	3.10±0.21	3.50±0.16	3.00±0.19	7.50^{b}±0.28	1.60±0.03	2.90±0.18	5.00±0.14
River Bagwai	F	2.52±0.31	3.08±0.33	1.81±0.09	5.20^{b}±0.41	1.97±0.07	9.88^{b}±0.79	5.05±0.21
River Bagwai	M	2.48±0.14	3.04±0.14	1.77±0.09	6.18^{a}±0.20	2.12±0.08	11.38^{a}±0.52	5.21±0.18

a,b, means within the same column and factors with different superscripts differ significantly. P<0.01

PFL=pectoral fin length, DODF=Dorso-occipital length, BDA=Body depth at anus, F=Female M=Male

PSL=pectoral spine length, CPD=Caudal peduncle deepth, DDCF=Dorso-caudal length, AFL=Anal fin length

Table 4.4c: Morphometric Characteristics of strains of Clariid Species in relation to interaction between location and sex

Factor	HL (cm)	HW (cm)	SNL (cm)	ID (cm)	ED (cm)	OFL (cm)	OFW	DSO
Location	sex							
River Thomas	F	9.10^{b}±0.90	7.00^{b}±0.50	1.95^{b}±0.05	4.00^{b}±0.00	0.45±0.03	2.75±0.25	0.80±0.20
River Thomas	M	11.85^{a}±0.15	9.00^{a}±0.00	2.55^{a}±0.25	5.10^{a}±0.06	0.60±0.00	2.50±0.50	0.50±0.001.50±0.20
River Ghari	F	7.37±0.34	5.93±0.31	1.57±0.09	3.37±0.18	0.36±0.02	1.87±0.09	0.47±0.041.68± 0.09
River Ghari	M	8.13±0.25	5.79±0.28	1.79±0.09	3.27±0.11	0.44±0.02	2.06±0.07	0.51±0.031.86±0.05
River.D/Gaya	F	6.03±0.11	4.71±0.11	1.42±0.06	2.82±0.06	0.26±0.01	1.72±0.05	0.36±0.111.49±0.06
River D/Gaya	M	6.64±0.25	5.16±0.28	1.71±0.06	3.14±0.15	0.28±0.02	1.91±0.09	0.45±0.041.74±0.12
River Karaye	F	8.17±0.30	6.35±0.24	2.62±0.19	3.36±0.18	0.50±0.05	3.76±0.41	0.68±0.062.18±0.12
River Karaye	M	8.64±0.49	6.50±0.38	2.44±0.14	3.86±0.26	0.29±0.02	2.63±0.18	0.36±0.042.19±0.12
Tiga Dam	F	6.18±0.14	5.75±0.15	2.28±0.11	2.17±0.07	0.61±0.03	4.38±0.26	0.58±0.031.99±0.06
Tiga Dam	M	6.00±0.17	5.00±0.18	2.00±0.17	2.30±0.09	0.60±0.02	4.00±0.22	0.50±0.042.00±0.05
River Bagwai	F	7.11±0.27	5.41±0.19	1.66±0.08	2.93±0.17	0.46±0.04	1.99±0.19	0.49±0.031.75±0.08
River Bagwai	M	7.56±0.18	5.94±0.11	1.69±0.09	3.35±0.11	0.43±0.03	1.99±0.11	0.50±0.021.82±0.07

a,b, means within the same column and factors with different superscripts differ significantly$P<0.01$

HL=Head length, SNL=Snout length, ED=Eye diameter, OFW=width of occipital fontenele F=Male

HW=Head width, ID=interorbital distance, OFL=length of occipital fontenele, DSO=Snout-occipital length M=Male

Tables 4.5a, 4.5b and 4.5c show the interaction effect between river location and strain on morphometric measurements. River Ghari had more significant effect (P<0.01) on *Heterobranchus longifilis* than *Clarias gariepinus* in body weight, total length, standard length, pre-dorsal distance, pre-anal distance, pre-pectoral distance, pre-ventral distance, pectoral fin length, pectoral spine length, dorso-occipiatal length, caudal peduncle depth, body depth at anus, head length, head width, length of occipital fontenale and width of occipital fontenale while the significance was more on *Clarias gariepinus* in dorsal fin length and anal fin length.In River Karaye, a significant effect (P<0.01) was equally more on *Heterobranchus longifilis* than *Clarias gariepinus* in body weight, total length, standard length, pre-dorsal distance, pre-anal distance, pre-ventral distance, pectoral fin length, pectoral spine length, dorso-caudal length, dorso-occipital length, body depth at anus, eye distance, length of occipital fontenale and width of occipital fontenale while the significance was more on Clarias gariepinus in dorsal fin length, anal fin length and inter-orbital distance.In River Bagwai, a significant effect (P<0.01) was observed on *Heterobranchus longifilis* than *Clarias gariepinus* in body weight, total length, standard length, pre-dorsal distance, pre-anal distance, pre-pectoral distance, pre-ventral distance, pectoral fin length, pectoral spine length, dorso-occipiatal length, caudal peduncle depth, body depth at anus, head width, snout length, eye distance, length of occipital fontenale and snout occipital length while the significance was more on *Clarias gariepinus* in dorsal fin length, anal fin length and inter-orbital distance.

Table 4.5a: Morphometric Characteristics of strains of Clariid Species in relation to interaction between location and strain

Factor	WT	TL	SL	PDD	PAD	PVD	PPD	DFL
		(g)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)
Location	strain							
RiverGhari	Cg	243.06^{b}±14.82	30.46^{b}±0.84	27.45^{b}±0.75	8.64^{b}±0.21	14.00^{b}±0.43	5.07^{b}±0.21	11.41^{b}±0.29	16.64^{a}±0.44
River Ghari	Hl	719.40^{a}±52.17	46.00^{a}±0.80	40.00^{a}±1.41	15.00^{a}±0.98	22.00^{a}±1.52	9.00^{a}±0.28	19.00^{a}±0.55	8.70^{b}±0.36
River Karaye	Cg	298.79^{b}±43.94	35.36^{b}±1.40	30.54^{b}±1.31	10.35^{b}±0.44	16.86^{b}±0.69	14.43^{b}±0.55	6.48±0.30	14.12^{a}±0.57
River Karaye	Hl	400.60^{a}±49.48	44.00^{a}±1.41	33.69^{a}±1.37	13.61^{a}±0.60	23.94^{a}±0.89	16.94^{a}±0.62	6.94±0.28	6.01^{b}±0.28
River Bagwai	Cg	155.69^{b}±9.29	29.03^{b}±0.57	26.03^{b}±0.51	8.52^{b}±0.19	13.64^{b}±0.30	4.83^{b}±0.09	11.27^{b}±0.23	16.55^{a}±0.36
River Bagwai	Hl	202.65^{a}±14.24	39.50^{a}±3.62	28.00^{a}±3.24	10.75^{a}±0.75	21.25^{a}±1.65	6.75^{a}±0.48	15.00^{a}±0.91	5.25^{b}±0.48

a,b, means within the same column and factors with different superscripts differ significantly. P<0.01

WT= weight, PAD = pre-anal distance, DFL = dorsal fin length, PVD = pre-ventral distance Cg=*Clariasgariepinus*

SL=standard length, PPD = pre-pectoral distance, TL= Total length PDD=pre-dorsal distance, Hl=*Heterobranchuslongifilis*

Table 4.5b: Morphometric Characteristics of strains of Clariid Species in relation to interaction between location and strain

Factor		PFL (cm)	PSL (cm)	DDCF (cm)	DODF (cm)	CPD (cm)	AFL (cm)	BDA (cm)
Location	strain							
RiverGhari	Cg	2.45^{b}±0.11	3.07^{b}±0.13	1.77±0.07	5.52^{b}±0.32	1.99^{b}±0.06	12.69^{a}±0.72	5.15^{b}±0.16
River Ghari	Hl	5.00^{a}±0.16	6.00^{a}±0.19	2.20±0.17	11.90^{a}±0.92	4.20^{a}±0.11	5.00^{b}±0.33	10.00^{a}±0.35
River Karaye	Cg	3.14^{b}±0.17	3.97^{b}±0.19	1.86^{b}±0.11	7.35^{b}±0.30	2.68±0.10	20.21^{a}±0.87	6.46^{b}±0.28
River Karaye	Hl	5.64^{a}±0.21	5.86^{a}±0.19	3.88^{a}±0.13	10.75^{a}±0.53	2.63±0.14	4.21^{b}±0.27	7.84^{a}±0.29
River Bagwai	Cg	2.25^{b}±0.06	2.83^{b}±0.08	1.70±0.05	5.60^{b}±0.23	2.05±0.06	11.62^{a}±0.26	5.00^{b}±0.11
River Bagwai	Hl	4.85^{a}±0.43	5.20^{a}±0.64	2.65±0.24	8.25^{a}±0.85	2.25±0.14	3.40^{b}±0.43	6.68^{a}±0.71

a,b, means within the same column and factors with different superscripts differ significantly. P<0.01

PFL=pectoral fin length, DODF=Dorso-occipital length, BDA=Body depth at anus, Cg=*Clariasgariepinus*

PSL=pectoral spine length, CPD=Caudal peduncle deepth, DDCF=Dorso-caudal length, AFL=Anal fin length

Hl=*Heterobranchuslongifilis*

Table 4.5c: Morphometric Characteristics of strains of Clariid Species in relation to interaction between location and strain

Factor		HL (cm)	HW (cm)	SNL (cm)	ID (cm)	ED (cm)	OFL (cm)	OFW	DSO
Location	strain								
RiverGhari	Cg	7.84^{b}±0.22	5.72^{b}±0.18	1.70±0.08	3.30±0.09	0.42±0.02	1.88^{b}±0.08	0.49^{b}±0.02	1.80±0.05
River Ghari	Hl	9.00^{a}±0.59	9.00^{a}±0.43	2.20±0.21	3.50±0.16	0.30±0.01	5.00^{a}±0.36	0.70^{a}±0.05	1.90±0.06
River Karaye	Cg	8.39±0.36	6.43±0.27	2.32±0.11	3.83^{a}±0.18	0.31^{b}±0.02	2.50^{b}±0.13	0.41^{b}±0.03	2.18±0.11
River Karaye	Hl	8.41±0.33	6.40±0.32	3.19±0.24	2.89^{b}±0.11	0.69^{a}±0.03	5.44^{a}±0.22	0.89^{a}±0.06	2.19±0.08
River Bagwai	Cg	7.45±0.16	5.68^{b}±0.16	1.59^{b}±0.04	3.31^{a}±0.09	0.40^{b}±0.01	1.83^{b}±0.05	0.49±0.02	1.74^{b}±0.04
River Bagwai	Hl	7.05±0.55	6.53^{a}±0.28	2.55^{a}±0.26	2.25^{b}±0.18	0.83^{a}±0.03	3.50^{a}±0.29	0.58±0.03	2.37^{a}±0.21

a,b, means within the same column and factors with different superscripts differ significantly. P<0.01

HL=Head length, SNL=Snout length, ED=Eye diameter, OFW=width of occipital fontenele Cg=*Clariasgariepinus*

HW=Head width, ID=interorbital distance, OFL=length of occipital fontenele, DSO=Snout-occipital length

Hl=*Heterobranchuslongifilis*

Tables 4.6a, 4.6b and 4.6c show the interaction effect between sex and strain on morphometric measurements.There was a significant effect of strain ($P<0.01$) on male *Clarias gariepinus* than female *Clarias gariepinus* on body weight, total length, standard length, pre-dorsal distance, pre-anal distance, pre-ventral distance, anal fin length and head length. In *Heterobranchus longifilis*, a significant effect ($P<0.01$) was observed in males than females in total length, standard length, pre-anal distance, pre-ventral distance, pre-pectoral distance, anal fin length, body depth at anus, head length and head width while significat effect ($P<0.01$) was seen in females on pre-ventral distance and dorso-caudal distance.

Table 4.7 shows the effect of location, strain and sex on the meristic counts taken on the fish samples. Location had significant effect ($P<0.01$) on dorsal fin ray count, pectoral fin ray count and anal fin ray count while having no significant effect on caudal fin ray count ($P> 0.05$). Strain and sex on the other hand had significant effect ($P<0.01$) on dorsal fin ray count and anal fin ray count but not on pectoral fin ray count and caudal fin ray count ($P>0.05$). River Thomas had higher counts compared to the remaining rivers followed by River DudusinGaya and River Ghari, due to the predominance of *Clarias gariepinus* in those water bodies. *Clarias gariepinus* gave higher counts, due to the presence of dorsal adipose tissue on the *Heterobranchus longifilis* and *Synodontis clarias* that halved the dorsal parts of the latter fish strains mentioned. On the effect of sex, dorsal fin ray count and anal fin ray count were more ($P<0.05$) in the males than the females; but pectoral fin ray count and caudal fin ray count were similar for the sexes. The interaction effect between river location and sex, between location and strain, and between sex and strain were not significant hence they were dropped from further analysis.

Table 4.6a: Morphometric Characteristics of strains of Clariid Species in relation to interaction between sex and strain

Factor		WT	TL	SL	PDD	PAD	PVD	PPD	DFL
		(g)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)	(cm)
Strain	sex								
Cg	F	145.10^{b}±11.07	26.94^{b}±0.63	23.88^{b}±0.56	8.06^{b}±0.20	13.05^{b}±0.30	5.92^{b}±0.45	9.83±0.26	14.50±0.33
Cg	M	228.41^{a}±18.12	31.88^{a}±0.77	28.18^{a}±0.71	9.15^{a}±0.22	14.72^{a}±0.36	7.07^{a}±0.47	10.65±0.32	16.67±0.41
Hl	F	316.90±45.22	38.54^{b}±1.11	28.38^{b}±1.05	11.36±0.44	20.28^{b}±0.76	10.34^{a}±1.11	10.97^{b}±0.68	5.22±0.21
Hl	M	328.80±131.73	43.00^{a}±2.68	32.75^{a}±3.30	12.00±1.22	21.88^{a}±1.59	6.75^{b}±0.75	16.00^{a}±1.47	5.93±1.01

a,b, means within the same column and factors with different superscripts differ significantly

WT= weight, PAD = pre-anal distance, DFL = dorsal fin length, PVD = pre-ventral distance Cg=*Clariasgariepinus*

SL=standard length, PPD = pre-pectoral distance, TL= Total length PDD=pre-dorsal distance, Hl=*Heterobranchuslongifilis*

F=Female M=Male

Table 4.6b: Morphometric Characteristics of strains of Clariid Species in relation to interaction between sex and strain

Factor		PFL (cm)	PSL (cm)	DDCF (cm)	DODF (cm)	CPD (cm)	AFL (cm)	BDA (cm)
Strain	sex							
Cg	F	2.36±0.21	2.71±0.09	1.54±0.06	5.95±0.17	1.94±0.05	11.47^{b}±0.46	4.89±0.14
Cg	M	2.57±0.09	3.21±0.10	1.72±0.05	6.30±0.20	2.23±0.07	14.04^{a}±0.57	5.42±0.13
Hl	F	4.45±0.24	4.79±0.24	9.00^{a}±1.10	9.33±0.37	2.22±0.09	3.39^{b}±0.19	5.41^{b}±0.28
Hl	M	4.38±0.44	4.83±0.51	5.30^{b}±2.57	9.60±0.92	2.70±0.54	5.15^{a}±1.95	7.63^{a}±1.07

a,b, means within the same column and factors with different superscripts differ significantly

PFL=pectoral fin length, DODF=Dorso-occipital length, BDA=Body depth at anus, Cg=*Clariasgariepinus*

PSL=pectoral spine length, CPD=Caudal peduncle deepth, DDCF=Dorso-caudal length, AFL=Anal fin length

Hl=*Heterobranchuslongifilis*

F=Female M=Male

Table 4.6c: Morphometric Characteristics of strains of Clariid Species in relation to interaction between sex and strain

Factor (cm)	(cm)	(cm)	HL (cm)	HW (cm)	SNL (cm)	ID (cm)	ED	OFL	OFW	DSO	(cm)
Strain	sex										
Cg	F		6.81[b]±0.16	5.28±0.13	1.58±0.05	3.11±0.07	0.31±0.01	1.85±0.05	0.43±0.02	1.66±0.05	
Cg	M		7.79[a]±0.18	5.86±0.15	1.85±0.06	3.45±0.09	0.37±0.01	2.05±0.06	0.46±0.02	1.86±0.05	
Hl	F		6.89[b]±0.27	6.01[b]±0.15	2.60±0.14	2.41±0.09	0.66±0.03	4.68±0.21	0.69±0.04	2.08±0.05	
Hl	M		7.25[a]±0.73	7.00[a]±0.82	2.55±0.26	2.72±0.27	0.65±0.13	4.00±0.41	0.60±0.04	2.28±0.25	

a,b, means within the same column and factors with different superscripts differ significantly

HL=Head length, SNL=Snout length, ED=Eye diameter, OFW=width of occipital fontenele Cg=*Clariasgariepinus*

HW=Head width, ID=interorbital distance, OFL=length of occipital fontenele, DSO=Snout-occipital length

Hl=*Heterobranchuslongifilis*

F=Female M=Male

Table 4.7: Meristic Counts of Strains of Clariid Species in relation to location, strains and sex

Factor	N	DFRC	PFRC	AFRC	CFRC
Location	177	**	**	*	NS
River Thomas	4	8.15^{a}±0.11	2.64^{a}±0.19	6.83^{ab}±0.35	4.41±0.12
River Ghari	27	7.01^{c}±0.15	2.26^{c}±0.02	6.46^{c}±0.14	4.29±0.04
River D/Gaya	50	7.97^{ab}±0.07	2.39^{b}±0.03	7.03^{a}±0.06	4.24±0.03
River Karaye	33	6.56^{d}±0.33	2.31^{bc}±0.02	5.99^{d}±0.28	4.30±0.06
River Tiga	21	3.11^{e}±0.06	2.31^{bc}±0.02	3.17^{e}±0.04	4.31±0.02
River Bagwai	42	7.74^{ab}±0.24	2.26^{c}±0.01	6.69^{bc}±0.20	4.32±0.03
Strain	177	**	NS	**	NS
C. gariepinus	143	7.83^{a}±0.05	2.33±0.01	6.92^{a}±0.04	4.28±0.02
H. longifilis	26	3.29^{b}±0.02	2.27±0.02	3.26^{b}±0.04	4.34±0.03
Sex	177	**	NS	**	NS
Male	83	7.54^{a}±0.25	2.30±0.02	6.75^{a}±0.06	4.30±0.12
Female	94	6.40^{b}±0.33	2.34±0.03	5.72^{b}±0.04	4.28±0.03

a,b,c,d means within the same column and factors with different superscripts differ significantly .**=P<0.01;*=P<0.05.

DFRC=dorsal fin ray count, PFRC=pectoral fin ray count,AFRC=anal fin ray count,CFRC=caudal fin ray count. NS – Not siginificant

4.1.1 Condition factor of Clariid species in Kano state

Table 4.8 shows the Condition factor (Ponderal index) for the twostrains of Clariid specie studied. The condition factor which showed the degree of wellbeing of the fish in their habitat is expressed by ‘coefficient of condition’. The condition factor of the Clariid species vary in all the rivers sampled. The population of *Clarias gariepinus* in Rivers Thomas, Ghari and Duddurun Gaya had a better condition of wellbeing with ‘K’ values of 0.64, 0.74 and 0.67 respectively than those in Rivers Karaye (0.46) and Bagwai (0.37). The population

of*Heterobarnchus longifilis* were all in good condition of wellbeing in all the rivers sampled with 'K' values of 0.61 in River Karaye, 0.88 in River Ghari and 0.81 in Tiga dam.

4.1.2 Length-weight relationship in Clariid species in Kano state

Table 4.9 shows the length-weight relationship of the two strains of Clariid species found in Kano State. The equations indicate the pattern of growth, either isometric or allometric. Isometric growth is associated with no change in body shape as an organism grows. Negative allometric growth implies the fish becomes more slender as it increases in weight while positive allometric growth implies the fish becomes relatively stouter or deeper-bodied as it increases in length (Riedel *et al.,* 2007). From the equation, the present result showed that b (regression coefficient) were 17.56 for *Clarias gariepinus* and 14.28 for *Heterobranchus longifilis*, indicating positive allometric pattern of growth for Clariid species. When the regression coefficient is 3, the growth pattern is isometric. Negative allometric growth pattern is when the regression coefficient is less than 3.The R^2 for *Clarias gariepinus* (0.69) indicates that the prediction equation explains mostof the variability of the response data around its mean and the relationship is significant while 0.13 of *Heterobranchus longifilis* shows that the prediction equation explains few of the variability of the response data around its mean.

Table 4.8: Condition Factor of Clariid Species in the Six Location Sampled

Location	Cg	Hl
River Thomas	0.64(4)	-
River Ghari	0.74(26)	0.88(4)
River DudusinGaya	0.67(50)	-
River Karaye	0.46(25)	0.61(8)
River Bagwai	0.37(38)	0.62(4)

River Tiga	-	0.81(13)

Cg = Clarias gariepinus

Hl = Heterobranchus longifilis

Sc = Synodontis clarias

Table 4.9: Regression equation for the length-weight relationship in the strains sampled

Strains	Prediction equation	±SE	R^2	LS
C. gariepinus	-329.86+17.56TL	30.11	0.69	**
H. longifilis	-241.49+14.28TL	30.14	0.13	NS

LS=level of significance

R^2= coefficient of determination

±SE=standard error

4.1.3 Pearson correlation coefficient among morphometric masurements

Table 4.10a, 4.10b and 4.10c show the Pearson correlation analysis for all the variables measured. The relationship between Body Weight and all the morphometric measurements were positive and significant except PDD, DDCF, ID and ED. These 'r' values were; BW/TL (0.75), BW/SL (0.73), BW/PDD (0.80), BW/PAD (0.74), BW/PVD (0.58), BW/DFL (0.65), BW/AFL (0.51), BW/PFL (0.47), BW/PSL (0.66), BW/DODF (0.68), BW/CPD (0.65), BW/BDA (0.76), BW/HL (0.69), BW/HW (0.67), BW/SNL (0.55), BW/ID (0.50), BW/OFL (0.56), BW/OFW (0.38) and BW/DSO (0.42).The morphometric measurements with negative and significant correlations were PPD/PVD (r= -0.45), DDCF/AFL (r= -0.67), DDCF/PFL (r= -0.54), SNL/DFL (r= -0.41), ID/DDCF (r= -0.36), ED/DFL (r= -0.50), ED/AFL (r= -0.45), OFL/DFL (r= -0.48) and DSO/DFL (r= -0.34). The remaining measurements had positive and significant relationships with 'r' values ranging from 0.30 for ED/TL, PAD/ID, ID/OFL and PDD/DDCF to 0.92 for TL/SL.

Table 4.10a: Pearson correlation analysis of the measured morphometric traits

	WT	TL	SL	PDD	PAD	PVD	PPD	DFL	AFL	PFL	PSL	DDCF
WT	-											
TL	0.75**	-										
SL	0.73**	0.92**	-									
PDD	0.80**	0.86**	0.77**	-								
PAD	0.74**	0.83**	0.67**	0.91**	-							
PVD	0.58**	0.66**	0.59**	0.71**	0.68**	-						
PPD	0.21	0.20	0.17	0.23	0.23	-0.45**	-					
DFL	0.65**	-0.04	0.24	-0.06	-0.24	-0.14	0.14	-				
AFL	0.51**	0.26	0.48**	0.20	0.04	0.44**	-0.24	0.68**	-			
PFL	0.47**	0.56**	0.45**	0.62**	0.64**	0.49**	0.09	-0.26	-0.04	-		
PSL	0.66**	0.78**	0.63**	0.81**	0.89**	0.70**	0.09	-0.28	0.09	0.66**	-	
DDCF	0.20	0.25	-0.00	0.30**	0.42**	0.04	0.32**	-0.67**	-0.54**	0.29	0.35**	-

WT-Body weight
TL-Total length
SL-Standard length
PDD-Pre-dorsal distance
PAD-Pre-anal distance
PVD-Pre-ventral distance
PPD-Pre-pectoral distance
DFL-Dorsalfin length
AFL-Anal fin length
PFL-Pectoral fin length
PSL-Pectoral spine length
DDCF-Dorso-caudal length

Table 4.10b: Pearson correlation analysis of the measured morphometric traits

	DODF	CPD	BDA	HL	HW	SNL	ID	ED	OFL	OFW	DSO
DODF	-										
CPD	0.62**	-									
BDA	0.75**	0.75**	-								
HL	0.45**	0.71**	0.75**	-							
HW	0.63**	0.84**	0.77**	0.80**	-						
SNL	0.58**	0.66**	0.67**	0.58**	0.73**	-					
ID	0.23	0.54**	0.49**	0.72**	0.53**	0.13	-				
ED	0.31**	0.35**	0.36**	0.31**	0.47**	0.69**	-0.22	-			
OFL	0.74**	0.37**	0.60**	0.24	0.36**	0.50**	-0.05	0.23	-		
OFW	0.47**	0.23	0.46**	0.32**	0.31**	0.30**	0.12	0.29	0.58**	-	
DSO	0.42**	0.58**	0.57**	0.56**	0.70**	0.81	0.18	0.66**	0.28	0.17	-

DODF-Dorso-occipital length
CPD-Caudal peduncle depth
BDA-Body depth at anus
HL-Head length
HW-Head width
SNL-Snout length
ID-inter-orbital distance
ED-Eye diameter
OFL length of occipital fontenale
OFW-width of occipital fontenale
DSO-Snout-occipital length

Table 4.10c: Pearson correlation analysis of the measured morphometric traits

	WT	TL	SL	PDD	PAD	PVD	PPD	DFL	AFL	PFL	PSL	DDCF
DODF	0.68**	0.73**	0.59**	0.82**	0.83**	0.58**	0.21	-0.21	0.02	0.57**	0.72**	0.40**
CPD	0.65**	0.60**	0.60**	0.74**	0.68**	0.57**	0.18	0.02	0.31**	0.44**	0.63**	0.22
BDA	0.76**	0.80**	0.74**	0.88**	0.86**	0.70**	0.16	-0.06	0.25	0.59**	0.80**	0.24
HL	0.69**	0.65**	0.71**	0.77**	0.67**	0.53**	0.24	0.32**	0.48**	0.37**	0.57**	0.05
HW	0.67**	0.64**	0.61**	0.77**	0.73**	0.47**	0.48**	0.01	0.22	0.45**	0.62**	0.35**
SNL	0.55**	0.53**	0.39**	0.67**	0.73**	0.52**	0.10	-0.41**	-0.11	0.50**	0.68**	0.57**
ID	0.50**	0.41**	0.60**	0.48**	0.30**	0.41**	0.07	0.66**	0.77**	0.11	0.21	-0.36**
ED	0.23	0.30**	0.11	0.39**	0.47**	0.10	0.29	-0.50**	-0.45**	0.36**	0.48**	0.64**
OFL	0.56**	0.65**	0.42**	0.70**	0.77**	0.56**	0.09	-0.48**	-0.23	0.57**	0.73**	0.52**
OFW	0.38**	0.47**	0.39**	0.56**	0.56**	0.29	0.19	-0.09	-0.18	0.35**	0.48**	0.19
DSO	0.42**	0.42**	0.36**	0.55**	0.60**	0.40**	0.15	-0.34**	-0.03	0.38**	0.54**	0.50**

WT-Body weight
CPD-Caudal peduncle depth
BDA-Body depth at anus
HL-Head length
HW-Head width
SNL-Snout length
ID-inter-orbital distance
ED-Eye diameter

DODF-Dorso-occipital length
TL-Total length
SL-Standard length
PDD-Pre-dorsal distance
PAD-Pre-anal distance
PVD-Pre-ventral distance
PPD-Pre-pectoral distance
AFL-Anal fin length

DFL-Dorsalfin length
OFL length of occipital fontenale
OFW-width of occipital fontenale
DSO-Snout-occipital length

PFL-Pectoral fin length
PSL-Pectoral spine length
DDCF-Dorso-caudal length

4.1.4 Pearson correlation coefficient among morphometric masurements for *Clarias gariepinus*

Table 4.11a, 4.11b and 4.11c show the Pearson correlation analysis for all the variables measured for *Clarias gariepinus*. The relationship between Body Weight and all the morphometric measurements were positive and significant except PDD, ED, OFL and OFW. These 'r' values were; BW/TL (0.83), BW/SL (0.81), BW/PDD (0.86), BW/PAD (0.86), BW/PVD (0.58), BW/DFL (0.55), BW/AFL (0.79), BW/PFL (0.42), BW/PSL (0.74), BW/DODF (0.66), BW/DDCF (0.47), BW/CPD (0.73), BW/BDA (0.82), BW/HL (0.84), BW/HW (0.80), BW/SNL (0.69), BW/ID (0.79), BW/OFL (0.64), BW/ED (0.34) and BW/DSO (0.61). The remaining morphometric measurements had positive and significant relationships with 'r' values ranging from 0.30 for ED/PAD to 0.98 for SL/TL.

4.1.5 Pearson correlation coefficient among morphometric masurements for *Heterobranchus longifilis*

Table 4.12a, 4.12b and 4.12c show the Pearson correlation analysis for all the variables measured for *Heterobranchus longifilis*. The relationship between Body Weight and all the morphometric measurements were positive and significant except PVD, PFL, DDCF, ED and DSO. These 'r' values were; BW/TL (0.36), BW/SL (0.48), BW/PDD (0.55), BW/PAD (0.43), BW/PPD (0.34), BW/DFL (0.51), BW/AFL (0.45), BW/PSL (0.34), BW/DODF (0.68), BW/CPD (0.51), BW/BDA (0.53), BW/HL (0.45), BW/HW (0.55), BW/SNL (0.58), BW/ID (0.52), BW/OFL (0.57) and BW/DSO (0.44). The remaining morphometric measurements had positive and significant relationships with 'r' values ranging from 0.32 for PDD/HW to 0.95 for SL/TL and SL/PDD.

Table 4.11a: Pearson correlation analysis of the measured morphometric traits for *Clarias gariepinus*

	WT	TL	SL	PDD	PAD	PVD	PPD	DFL	AFL	PFL	PSL	DDCF
WT	-											
TL	0.83**	-										
SL	0.81**	0.98**	-									
PDD	0.86**	0.83**	0.79**	-								
PAD	0.86**	0.80**	0.76**	0.91**	-							
PVD	0.58**	0.62**	0.55**	0.68**	0.69**	-						
PPD	0.23	0.20	0.24	0.28	0.24	0.03	-					
DFL	0.55**	0.52**	0.54**	0.62**	0.57**	0.88**	0.45**	-				
AFL	0.79**	0.79**	0.74**	0.80**	0.81**	0.32**	0.70**	0.31*	-			
PFL	0.42**	0.40**	0.41**	0.45**	0.42**	0.21	-0.14	0.23	0.44**	-		
PSL	0.74**	0.76**	0.73**	0.77**	0.76**	0.70**	0.07	0.41**	0.87**	0.43**	-	
DDCF	0.47**	0.53**	0.51**	0.59**	0.52**	0.04	0.25	0.44**	0.53**	0.20	0.53**	-

WT-Body weight

TL-Total length

SL-Standard length

PDD-Pre-dorsal distance

PAD-Pre-anal distance

PVD-Pre-ventral distance

PPD-Pre-pectoral distance

DFL-Dorsalfin length

AFL-Anal fin length

PFL-Pectoral fin length

PSL-Pectoral spine length

DDCF-Dorso-caudal length

Table 4.11b: Pearson correlation analysis of the measured morphometric traits for *Clarias gariepinus*

	DODF	CPD	BDA	HL	HW	SNL	ID	ED	OFL	OFW	DSO
DODF	-										
CPD	0.68**	-									
BDA	0.69**	0.75**	-								
HL	0.56**	0.68**	0.82**	-							
HW	0.72**	0.81**	0.81**	0.84**	-						
SNL	0.55**	0.67**	0.71**	0.72**	0.68**	-					
ID	0.65**	0.78**	0.82**	0.83**	0.87**	0.69*	-				
ED	0.08	0.8	0.34**	0.48**	0.28	0.23	0.25	-			
OFL	0.59**	0.66**	0.67**	0.64**	0.65**	0.62**	0.68**	0.02	-		
OFW	0.16	0.15	0.29	0.40**	0.37**	0.14	0.36**	0.40**	0.24	-	
DSO	0.48**	0.52**	0.63**	0.64**	0.60**	0.70**	0.61**	0.30*	0.48**	0.23	-

DODF-Dorso-occipital length

CPD-Caudal peduncle depth

BDA-Body depth at anus

HL-Head length

HW-Head width

SNL-Snout length

ID-inter-orbital distance

ED-Eye diameter

OFL length of occipital fontenale

OFW-width of occipital fontenale

DSO-Snout-occipital length

Table 4.11c: Pearson correlation analysis of the measured morphometric traits for *Clarias gariepinus*

	WT	TL	SL	PDD	PAD	PVD	PPD	DFL	AFL	PFL	PSL	DDCF
DODF	0.66**	0.63**	0.60**	0.75**	0.75**	0.21	0.53**	0.48**	0.65**	0.37**	0.60**	0.42**
CPD	0.73**	0.68**	0.65**	0.79**	0.78**	0.13	0.66**	0.50**	0.76**	0.38**	0.70**	0.51**
BDA	0.82**	0.78**	0.76**	0.85**	0.86**	0.14	0.70**	0.52**	0.81**	0.44**	0.79**	0.51**
HL	0.84**	0.79**	0.77**	0.91**	0.89**	0.35*	0.57**	0.67**	0.75**	0.39**	0.75**	0.61**
HW	0.80**	0.77**	0.75**	0.88**	0.87**	0.35*	0.57**	0.61**	0.73**	0.40**	0.70**	0.58**
SNL	0.69**	0.67**	0.63**	0.74**	0.75**	-0.03	0.73**	0.37*	0.81**	0.36**	0.74**	0.42**
ID	0.79**	0.73**	0.72**	0.87**	0.81**	0.28	0.60**	0.58**	0.73**	0.40**	0.68**	0.50**
ED	0.34**	0.36**	0.40**	0.35**	0.30**	0.40**	-0.01	0.48**	0.19	0.10	0.35**	0.36**
OFL	0.64**	0.61**	0.55**	0.71**	0.74**	0.05	0.66**	0.32**	0.72**	0.34**	0.66**	0.46**
OFW	0.24	0.26	0.29	0.37**	0.32**	0.40**	-0.01	0.45**	0.10	0.05	0.17	0.36**
DSO	0.61**	0.64**	0.61**	0.67**	0.66**	-0.01	0.61**	0.25	0.65**	0.30**	0.61**	0.36**

WT-Body weight
CPD-Caudal peduncle depth
BDA-Body depth at anus
HL-Head length
HW-Head width
SNL-Snout length
ID-inter-orbital distance
ED-Eye diameter

DODF-Dorso-occipital length
TL-Total length
SL-Standard length
PDD-Pre-dorsal distance
PAD-Pre-anal distance
PVD-Pre-ventral distance
PPD-Pre-pectoral distance
AFL-Anal fin length

DFL-Dorsalfin length
OFL length of occipital fontenale
OFW-width of occipital fontenale
DSO-Snout-occipital length

PFL-Pectoral fin length
PSL-Pectoral spine length
DDCF-Dorso-caudal length

Table 4.12a: Pearson correlation analysis of the measured morphometric traits for *Heterobranchus longifilis*

	WT	TL	SL	PDD	PAD	PVD	PPD	DFL	AFL	PFL	PSL	DDCF
WT	-											
TL	0.36*	-										
SL	0.48*	0.95**	-									
PDD	0.55*	0.89**	0.95**	-								
PAD	0.43*	0.82**	0.82**	0.80**	-							
PVD	-0.08	-0.26	-0.23	-0.37*	-0.39*	-						
PPD	0.34*	0.67*	0.69*	0.80**	0.72**	-0.83*	-					
DFL	0.51*	0.70**	0.81**	0.79**	0.62*	-0.07	0.51*	-				
AFL	0.45*	0.53*	0.66*	0.63*	0.37*	0.13	0.32	0.82**	-			
PFL	0.29	0.70**	0.71**	0.76**	0.77**	-0.52*	0.82**	0.71**	0.49*	-		
PSL	0.34*	0.59*	0.64*	0.69**	0.79**	-0.43*	0.72**	0.77**	0.58*	0.95**	-	
DDCF	-0.22	-0.62*	-0.60	-0.61*	-0.65*	0.32	-0.63*	-0.54*	-0.50*	-0.81**	-0.78**	-

WT-Body weight

TL-Total length

SL-Standard length

PDD-Pre-dorsal distance

PAD-Pre-anal distance

PVD-Pre-ventral distance

PPD-Pre-pectoral distance

DFL-Dorsalfin length

AFL-Anal fin length

PFL-Pectoral fin length

PSL-Pectoral spine length

DDCF-Dorso-caudal length

Table 4.12b: Pearson correlation analysis of the measured morphometric traits for *Heterobranchus longifilis*

	DODF	CPD	BDA	HL	HW	SNL	ID	ED	OFL	OFW	DSO
DODF	-										
CPD	0.73**	-									
BDA	0.80**	0.83**	-								
HL	0.79**	0.77**	0.93**	-							
HW	0.68**	0.85**	0.81**	0.72**	-						
SNL	0.76**	0.49*	0.69**	0.72**	0.48*	-					
ID	0.79**	0.79**	0.90**	0.90**	0.74**	0.62*	-				
ED	-0.14	-0.08	0.06	0.10	0.01	0.31	-0.05	-			
OFL	0.72**	0.47*	0.50*	0.55*	0.40*	0.58*	0.53*	-0.20	-		
OFW	0.70**	0.63*	0.62*	0.68**	0.39*	0.77**	0.67*	0.23	0.59*	-	
DSO	0.17	0.17	0.44*	0.42*	0.33*	0.39*	0.41*	0.50*	0.05	0.18	-

DODF-Dorso-occipital length

CPD-Caudal peduncle depth

BDA-Body depth at anus

HL-Head length

HW-Head width

SNL-Snout length

ID-inter-orbital distance

ED-Eye diameter

OFL length of occipital fontenale

OFW-width of occipital fontenale

DSO-Snout-occipital length

Table 4.12c: Pearson correlation analysis of the measured morphometric traits for *Heterobranchus longifilis*

	WT	TL	SL	PDD	PAD	PVD	PPD	DFL	AFL	PFL	PSL	DDCF
DODF	0.68**	0.74**	0.82**	0.85**	0.71**	-0.30	0.63*	0.63*	0.51*	0.55*	0.46*	-0.34*
CPD	0.51*	0.70**	0.83**	0.81**	0.61*	-0.05	0.50*	0.84**	0.81**	0.62*	0.66*	-0.56*
BDA	0.53*	0.90**	0.94**	0.91**	0.82**	-0.19	0.67*	0.78**	0.72**	0.75**	0.69**	-0.69**
HL	0.45*	0.94**	0.95**	0.94**	0.83**	-0.36*	0.78**	0.75**	0.60*	0.81**	0.72**	-0.69**
HW	0.55*	0.74**	0.79**	0.74**	0.59*	0.21	0.32*	0.82**	0.75**	0.56*	0.56*	-0.49*
SNL	0.58*	0.67*	0.68**	0.68**	0.80**	-0.47*	0.68**	0.44*	0.17	0.66*	0.57*	-0.47*
ID	0.52*	0.87**	0.92**	0.92**	0.82**	-0.20	0.71**	0.79**	0.70**	0.68*	0.64*	-0.56*
ED	-0.23	0.19	0.05	0.07	0.31	-0.13	0.17	-0.02	-0.33	0.34*	0.30	-0.32
OFL	0.57*	0.46*	0.52*	0.61*	0.50*	-0.49*	0.65*	0.38*	0.24	0.46*	0.37*	-0.14
OFW	0.44*	0.58*	0.63*	0.70**	0.73**	-0.62*	0.78**	0.57*	0.30	0.71**	0.68**	-0.47*
DSO	0.00	0.48*	0.37*	0.35*	0.56*	0.01	0.24	0.09	0.01	0.38*	0.28	-0.40*

WT-Body weight

CPD-Caudal peduncle depth

BDA-Body depth at anus

HL-Head length

HW-Head width

SNL-Snout length

ID-inter-orbital distance

ED-Eye diameter

DFL-Dorsalfin length

OFL length of occipital fontenale

OFW-width of occipital fontenale

DSO-Snout-occipital length

DODF-Dorso-occipital length

TL-Total length

SL-Standard length

PDD-Pre-dorsal distance

PAD-Pre-anal distance

PVD-Pre-ventral distance

PPD-Pre-pectoral distance

AFL-Anal fin length

PFL-Pectoral fin length

PSL-Pectoral spine length

DDCF-Dorso-caudal length

4.1.6 Principal component analysis for the variables

Table 4.13a shows the principal component analysis for pooled data where four components were extracted. The morphometric measurements had a total variance of 82.52% shared as 47.66%, 19.16%, 8.67% and 7.03% for PC1, PC2, PC3 and PC4, respectively. The factor loading accounted for by PC1 were thirteen of the morphometric characteristics (in bold) and PC2 accounted for only three (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high ranging from 0.52 to 0.96.

Table 4.13b shows the Eigenvalues and share of total variance along with factor loadings and communalities of morphometric traits for River Bagwai.Four components were also extracted. The morphometric measurements had a total variance of 89.46% shared as 62.46%, 12.15%, 8.91% and 5.94% for PC1, PC2, PC3 and PC4, respectively. The factor loading accounted for by PC1 were sixteen of the morphometric characteristics (in bold), PC2 accounted for only one (in bold) and PC4 for one of the traits (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high ranging from 0.77 to 0.95.

Table 4.13c shows the principal component analysis for River Dudusingaya data where four components were extracted. The morphometric measurements had a total variance of 74.73% shared as 56.31%, 8.07%, 5.69% and 4.66% for PC1, PC2, PC3 and PC4, respectively. The factor loading accounted for by PC1 were thirteen of the morphometric characteristics (in bold) and PC3 for two traits (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high ranging from 0.53 to 0.99.

Table 4.13a:Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of two Clariid species.

Variables	PC1	PC2	PC3	PC4	Communality
Total Length	**0.87**	0.16	-0.15	0.21	0.84
Standard Length	**0.76**	0.43	-0.07	0.20	0.81
Pre-Dorsal Distance	**0.85**	0.39	-0.48	0.63	0.87
Pre-Anal Distance	**0.94**	-0.09	-0.09	0.11	0.91
Pre-Pectoral Distance	0.22	-0.15	**0.67**	0.63	0.92
Pre-Ventral Distance	**0.71**	0.25	-0.52	-0.35	0.96
Caudal Peduncle Depth	**0.81**	0.21	0.20	-0.19	0.76
Body Depth at Anus	**0.92**	0.15	-0.04	0.02	0.87
Dorsal Fin Length	-0.17	**0.86**	0.24	0.26	0.91
Anal Fin Length	0.15	**0.91**	-0.07	-0.21	0.90
Pectoral Fin Length	**0.67**	-0.15	-0.21	0.06	0.52
Pectoral Spine Length	**0.88**	-0.10	-0.21	-0.01	0.82
Head Length	**0.77**	0.43	0.30	0.06	0.87
Head Width	**0.85**	0.12	0.40	0.08	0.89
Dorso-caudal length	**0.42**	-0.74	0.19	-0.03	0.76
Dorso-Occipital length	**0.82**	-0.09	-0.19	0.20	0.75
Snout Length	**0.81**	-0.29	0.17	-0.34	0.88
Inter-orbital Distance	0.42	**0.82**	0.17	-0.02	0.87
Eye Distance	**0.52**	-0.59	0.37	-0.15	0.77
Length Occipital Fontalle	**0.70**	-0.36	-0.43	0.27	0.88
Width Occipital Fontalle	**0.52**	-0.14	-0.21	0.51	0.59
Snout Occipital length	**0.69**	-0.22	0.37	-0.43	0.84
Eigenvalue	10.01	4.02	1.82	1.48	
% Variance	47.66	19.16	8.67	7.03	

PC1- Principal Component 1, PC2- Principal Component 2,
PC3- Principal Component 3, PC4- Principal Component 4

Table 4.13b: Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of River Bagwai.

Variables	PC1	PC2	PC3	PC4	Communality
Total Length	**0.90**	0.05	0.15	0.23	0.89

Standard Length	**0.94**	0.17	0.20	0.15	0.94
Pre-Dorsal Distance	**0.96**	0.60	-0.70	**0.81**	0.93
Pre-Anal Distance	**0.89**	-0.22	0.14	0.18	0.87
Pre-Pectoral Distance	-0.39	**0.74**	0.45	0.21	0.95
Pre-Ventral Distance	**0.83**	-0.44	-0.25	-0.14	0.95
Caudal Peduncle Depth	**0.85**	0.39	-0.04	-0.11	0.88
Body Depth at Anus	**0.94**	0.18	0.10	0.10	0.94
Dorsal Fin Length	**0.83**	0.36	0.02	-0.28	0.89
Anal Fin Length	**0.65**	0.64	-0.01	-0.32	0.93
Pectoral Fin Length	**0.86**	-0.26	0.14	-0.32	0.93
Pectoral Spine Length	**0.81**	-0.15	0.15	-0.48	0.93
Head Length	**0.96**	0.02	0.03	0.08	0.93
Head Width	**0.77**	0.49	0.19	0.10	0.89
Dorso-caudal length	-0.72	0.14	-0.39	0.38	0.83
Dorso-Occipital length	**0.83**	0.11	-0.35	0.30	0.92
Snout Length	**0.80**	-0.38	-0.08	0.28	0.86
Inter-orbital Distance	**0.93**	0.17	-0.04	0.13	0.90
Eye Distance	0.16	-0.62	**0.66**	-0.01	0.84
Length Occipital Fontalle	**0.60**	-0.11	-0.59	0.23	0.77
Width Occipital Fontalle	**0.81**	-0.36	-0.26	-0.08	0.86
Snout Occipital length	0.39	-0.23	**0.66**	0.45	0.84
Eigenvalue	13.74	2.76	1.96	1.31	
% Variance	62.46	12.15	8.91	5.94	

PC1- Principal Component 1, PC2- Principal Component 2,
PC3- Principal Component 3, PC4- Principal Component 4

Table 4.13c: Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of River Duddurun Gaya.

Variables	PC1	PC2	PC3	PC4	Communality
Total Length	**0.64**	0.62	0.15	0.28	0.89
Standard Length	0.58	**0.68**	0.18	0.28	0.91
Pre-Dorsal Distance	**0.93**	-0.02	0.08	-0.05	0.99
Pre-Anal Distance	**0.95**	-0.06	0.50	-0.02	0.91
Pre-Pectoral Distance	**0.91**	-0.13	0.08	0.03	0.85
Pre-Ventral Distance	**0.86**	-0.19	0.06	0.05	0.78
Caudal Peduncle Depth	**0.78**	-0.28	0.07	-0.01	0.69
Body Depth at Anus	**0.81**	0.08	0.15	0.01	0.69
Dorsal Fin Length	**0.56**	0.14	0.13	-0.56	0.66
Anal Fin Length	**0.92**	0.03	-0.03	-0.06	0.85
Pectoral Fin Length	0.12	-0.11	**0.74**	0.18	0.60
Pectoral Spine Length	**0.80**	0.06	0.04	-0.12	0.66
Head Length	**0.92**	-0.03	0.06	-0.15	0.88
Head Width	**0.81**	-0.26	0.08	0.20	0.77
Dorso-caudal length	**0.73**	-0.19	-0.24	0.06	0.63
Dorso-Occipital length	**0.88**	-0.01	0.13	-0.10	0.80
Snout Length	**0.66**	0.04	-0.37	-0.31	0.67
Inter-orbital Distance	**0.91**	-0.23	0.04	0.01	0.87
Eye Distance	0.32	**0.68**	-0.28	-0.16	0.67
Length Occipital Fontalle	**0.62**	-0.30	-0.24	0.27	0.60
Width Occipital Fontalle	**0.47**	-0.07	-0.46	0.47	0.65
Snout Occipital length	**0.67**	0.21	-0.17	0.09	0.53
Eigenvalue	12.39	1.78	1.25	1.03	
% Variance	56.31	8.07	5.69	4.66	

Table 4.13d shows the Eigen values and share of total variance along with factor loadings and communalities of morphometric traits for River Ghari. Four components were also extracted. The morphometric measurements had a total variance of 82.94% shared as 55.18%, 13.50%, 8.33% and 5.93% for PC1, PC2, PC3 and PC4, respectively. The factor loading accounted for by PC1 were thirteen of the morphometric characteristics (in bold), PC2 accounted for only one (in bold) and PC3 for one traits (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high ranging from 0.73 to 0.98.

Table 4.13e shows the principal component analysis for River Karaye data where four components were extracted. The morphometric measurements had a total variance of 82.52% shared as 47.66%, 19.16%, 8.67% and 7.03% for PC1, PC2, PC3 and PC4, respectively. The factor loading accounted for by PC1 were thirteen of the morphometric characteristics (in bold) and PC2 accounted for only rhree (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high ranging from 0.52 to 0.96.

Table 4.13f shows the Eigenvalues and share of total variance along with factor loadings and communalities of morphometric traits for River Tiga.Three components were also extracted. The morphometric measurements had a total variance of 84.06% shared as 39.89%, 38.75% and 5.42% for PC1, PC2 and PC3, respectively. The factor loading accounted for by PC1 were ten of the morphometric characteristics (in bold) and PC2 accounted for six (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high ranging from 0.63 to 0.97.

Table 4.13d: Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of River Ghari.

Variables	PC1	PC2	PC3	PC4	Communality
Total Length	**0.71**	-0.39	0.39	0.18	0.83
Standard Length	**0.72**	-0.38	0.38	0.10	0.83
Pre-Dorsal Distance	**0.94**	0.05	-0.22	0.19	0.98
Pre-Anal Distance	**0.91**	0.16	-0.24	-0.01	0.90
Pre-Pectoral Distance	**0.95**	-0.02	-0.19	-0.02	0.93
Pre-Ventral Distance	**0.88**	-0.05	0.22	0.06	0.82
Caudal Peduncle Depth	**0.89**	-0.22	-0.29	-0.04	0.92
Body Depth at Anus	**0.93**	-0.03	-0.22	0.12	0.92
Dorsal Fin Length	0.02	**0.90**	0.08	-0.15	0.84
Anal Fin Length	0.51	0.09	**0.71**	-0.32	0.87
Pectoral Fin Length	**0.86**	-0.33	0.31	0.05	0.94
Pectoral Spine Length	**0.85**	-0.29	0.18	-0.05	0.85
Head Length	**0.68**	0.61	-0.02	0.06	0.84
Head Width	**0.88**	0.01	-0.27	-0.26	0.91
Dorso-caudal length	**0.58**	0.28	0.40	0.41	0.74
Dorso-Occipital length	**0.84**	-0.08	-0.15	0.06	0.73
Snout Length	**0.69**	0.21	0.10	-0.46	0.74
Inter-orbital Distance	**0.56**	0.37	-0.11	-0.46	0.67
Eye Distance	-0.11	**0.64**	0.32	0.41	0.69
Length Occipital Fontalle	**0.89**	-0.23	-0.30	0.12	0.94
Width Occipital Fontalle	0.48	**0.54**	-0.27	0.44	0.80
Snout Occipital length	**0.55**	0.45	0.17	-0.20	0.57
Eigenvalue	12.14	2.97	1.83	1.30	
% Variance	55.18	13.50	8.33	5.93	

PC1- Principal Component 1, PC2- Principal Component 2,

PC3- Principal Component 3, PC4- Principal Component 4

Table 4.13e: Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of River Karaye.

Variables	PC1	PC2	PC3	PC4	Communality
Total Length	**0.87**	0.16	-0.15	0.21	0.84
Standard Length	**0.76**	0.43	-0.07	0.20	0.81
Pre-Dorsal Distance	**0.92**	0.08	-0.29	0.17	0.88
Pre-Anal Distance	**0.94**	-0.09	-0.09	0.11	0.92
Pre-Pectoral Distance	0.22	-0.15	**0.66**	0.63	0.92
Pre-Ventral Distance	**0.71**	0.25	-0.52	-0.35	0.96
Caudal Peduncle Depth	**0.81**	0.21	0.20	-0.19	0.76
Body Depth at Anus	**0.92**	0.15	-0.04	0.02	0.87
Dorsal Fin Length	-0.17	**0.86**	0.24	0.26	0.91
Anal Fin Length	0.15	**0.91**	-0.07	-0.21	0.90
Pectoral Fin Length	**0.67**	-0.15	-0.21	0.06	0.52
Pectoral Spine Length	**0.88**	-0.10	-0.21	-0.01	0.82
Head Length	**0.77**	0.43	0.30	-0.06	0.89
Head Width	**0.85**	0.12	0.40	-0.08	0.88
Dorso-caudal length	**0.42**	-0.74	0.19	-0.03	0.76
Dorso-Occipital length	**0.82**	-0.09	-0.19	0.20	0.75
Snout Length	**0.81**	-0-29	0.17	-0.34	0.88
Inter-orbital Distance	0.42	**0.82**	0.17	-0.02	0.87
Eye Distance	**0.52**	-0.59	0.36	-0.15	0.77
Length Occipital Fontalle	**0.70**	-0.36	-0.43	0.27	0.88
Width Occipital Fontalle	**0.52**	-0.14	-0.21	0.51	0.59
Snout Occipital length	**0.69**	-0.22	0.36	-0.43	0.84
Eigenvalue	10.01	4.02	1.82	1.48	
% Variance	47.66	19.16	8.67	7.03	

PC1- Principal Component 1, PC2- Principal Component 2,

PC3- Principal Component 3, PC4- Principal Component 4

Table 4.13f: Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of River Tiga.

Variables	PC1	PC2	PC3	Communality
Total Length	0.22	**0.92**	-0.22	0.96
Standard Length	0.51	**0.82**	-0.20	0.97
Pre-Dorsal Distance	**0.82**	0.42	-0.02	0.86
Pre-Anal Distance	**0.63**	0.32	0.40	0.66
Pre-Pectoral Distance	**0.64**	0.66	-0.16	0.87
Pre-Ventral Distance	**0.71**	0.46	-0.36	0.84
Caudal Peduncle Depth	**0.84**	-0.46	0.12	0.93
Body Depth at Anus	**0.87**	-0.08	-0.09	0.76
Dorsal Fin Length	0.10	**0.93**	-0.04	0.87
Anal Fin Length	0.04	**0.85**	-0.33	0.84
Pectoral Fin Length	**0.85**	0.27	-0.01	0.79
Pectoral Spine Length	**0.77**	0.20	0.04	0.63
Head Length	**0.81**	-0.56	-0.02	0.97
Head Width	**0.83**	-0.51	0.08	0.96
Dorso-caudal length	0.61	**0.67**	0.13	0.84
Dorso-Occipital length	0.16	**0.70**	0.51	0.77
Snout Length	**0.75**	-0.59	0.14	0.93
Inter-orbital Distance	**0.90**	-0.13	0.06	0.83
Eye Distance	0.32	-0.75	-0.04	0.67
Length Occipital Fontalle	-0.31	**0.82**	0.19	0.81
Width Occipital Fontalle	-0.11	**0.69**	0.56	0.80
Snout Occipital length	**0.64**	-0.74	0.01	0.96
Eigenvalue	8.78	8.53	1.19	
% Variance	39.89	38.75	5.42	

PC1- Principal Component 1, PC2- Principal Component 2, PC3- Principal Component 3

Table 4.13g shows the principal component analysis for River Thomas data where three components were extracted. The morphometric measurements had a total variance of 100% shared as 74.52%, 14.83% and 10.65% for PC1, PC2 and PC3, respectively. The factor loading accounted for by PC1 were sixteen of the morphometric characteristics (in bold), PC2 accounted for only two (in bold) and PC3 for one traits (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high (1.00).

Table 4.13h shows the Eigenvalues and share of total variance along with factor loadings and communalities of morphometric traits for *Clarias gariepinus*. Three components were also extracted. The morphometric measurements had a total variance of 74.83% shared as 57.30%, 12.43% and 5.10% for PC1, PC2 and PC3, respectively. The factor loading accounted for by PC1 were fifteen of the morphometric characteristics (in bold) and PC2 accounted for one (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high ranging from 0.46 to 0.93.

Table 4.13i shows the principal component analysis for *Heterobranchus longifilis* where four components were extracted. The morphometric measurements had a total variance of 89.46% shared as 62.46%, 12.15%, 8.91% and 5.94% for PC1, PC2, PC3 and PC4, respectively. The factor loading accounted for by PC1 were sixteen of the morphometric characteristics (in bold) and PC2 accounted for only one (in bold). The communality values, which represent the proportion of the variance in the original variables that is accounted for by the factor solution are very high ranging from 0.77 to 0.95.

Table 4.13g: Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of River Thomas.

Variables	PC1	PC2	PC3	Communality
Total Length	**0.99**	0.08	-0.14	1.00
Standard Length	**0.99**	0.02	-0.17	1.00
Pre-Dorsal Distance	**0.98**	-0.12	-0.15	1.00
Pre-Anal Distance	**0.95**	0.30	0.10	1.00
Pre-Pectoral Distance	**0.99**	-0.01	-0.14	1.00
Body Depth at Anus	**0.91**	-0.31	0.26	1.00
Dorsal Fin Length	**0.99**	-0.02	0.08	1.00
Anal Fin Length	**0.96**	-0.25	-0.11	1.00
Pectoral Fin Length	**0.84**	-0.33	0.43	1.00
Pectoral Spine Length	**0.95**	0.29	0.10	1.00
Head Length	**0.97**	0.21	-0.09	1.00
Head Width	**0.99**	0.10	0.06	1.00
Dorso-caudal length	0.47	**0.89**	0.01	1.00
Dorso-Occipital length	**0.95**	0.32	0.05	1.00
Snout Length	**0.80**	0.10	-0.59	1.00
Inter-orbital Distance	**0.82**	-0.40	0.42	1.00
Eye Distance	**0.77**	0.61	0.18	1.00
Length Occipital Fontalle	-0.10	0.12	**0.99**	1.00
Width Occipital Fontalle	-0.51	**0.77**	0.37	1.00
Snout Occipital length	-0.73	**0.63**	-0.27	1.00
Eigenvalue	14.90	2.97	2.13	
% Variance	74.52	14.83	10.65	

PC1- Principal Component 1, PC2- Principal Component 2, PC3- Principal Component 3

Table 4.13h: Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of *Clarias gariepinus*.

Variables	PC1	PC2	PC3	Communality

Total Length	**0.89**		0.02		0.10		0.80	
Standard Length		**0.86**		0.09		0.12		0.76
Pre-Anal Distance		**0.94**		-0.01		0.09		0.89
Pre-Pectoral Distance		0.22		**0.87**		-0.29		0.89
Pre-Ventral Distance		**0.73**		-0.62		0.11		0.93
Caudal Peduncle Depth		**0.84**		-0.11		-0.21		0.76
Body Depth at Anus		**0.91**		-0.04		0.01		0.83
Dorsal Fin Length		0.60		**0.65**		-0.18		0.81
Anal Fin Length	**0.89**		-0.36		0.08		0.92	
Pectoral Fin Length		**0.48**		-0.11		-0.21		0.69
Pectoral Spine Length		**0.86**		-0.15		0.14		0.78
Head Length	**0.91**		0.19		0.10		0.88	
Head Width	**0.91**		0.13		-0.17		0.86	
Dorso-caudal length		**0.63**		0.22		0.11		0.46
Dorso-Occipital length		**0.75**		-0.04		-0.40		0.72
Snout Length	**0.82**		-0.24		0.13		0.74	
Inter-orbital Distance		**0.89**		0.07		-0.14		0.81
Eye Distance	0.37		**0.57**		0.60		0.80	
Length Occipital Fontalle		**0.76**		-0.20		-0.22		0.66
Width Occipital Fontalle		0.34		**0.57**		0.19		0.47
Snout Occipital length		**0.72**		-0.16		0.34		0.65
Eigenvalue		12.03		2.61		1.07		
% Variance		57.30		12.43		5.10		

PC1- Principal Component 1, PC2- Principal Component 2, PC3- Principal Component 3

Table 4.13i: Variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of *Heterobranchus longifilis*.

Variables	PC1	PC2	PC3	PC4	Communality
Total Length	**0.90**	0.05	0.15	0.23	0.89

Standard Length	**0.94**	0.17	0.02	0.15	0.94
Pre-Dorsal Distance	**0.96**	0.06	-0.07	0.08	0.93
Pre-Anal Distance	**0.88**	-0.22	0.14	0.18	0.87
Pre-Pectoral Distance	-0.39	**0.74**	0.45	0.21	0.95
Pre-Ventral Distance	**0.83**	-0.44	-0.25	-0.14	0.95
Caudal Peduncle Depth	**0.85**	0.39	-0.04	-0.11	0.88
Body Depth at Anus	**0.94**	0.18	0.10	0.10	0.94
Dorsal Fin Length	**0.83**	0.36	0.02	-0.28	0.89
Anal Fin Length	**0.65**	0.64	-0.04	-0.32	0.93
Pectoral Fin Length	**0.86**	-0.25	0.14	-0.32	0.93
Pectoral Spine Length	**0.81**	-0.15	0.14	-0.48	0.93
Head Length	**0.96**	0.02	0.03	0.08	0.93
Head Width	**0.77**	0.49	0.19	0.10	0.89
Dorso-caudal length	-0.72	0.14	-0.39	0.38	0.83
Dorso-Occipital length	**0.83**	0.11	-0.35	0.30	0.92
Snout Length	**0.80**	-0.38	-0.08	0.28	0.86
Inter-orbital Distance	**0.93**	0.17	-0.04	0.13	0.90
Eye Distance	0.16	-0.62	**0.66**	-0.01	0.84
Length Occipital Fontalle	**0.60**	-0.11	-0.59	0.23	0.77
Width Occipital Fontalle	**0.81**	-0.36	-0.26	-0.08	0.86
Snout Occipital length	0.39	-0.23	**0.66**	0.45	0.84
Eigenvalue	13.74	2.67	1.96	1.31	
% Variance	62.46	12.15	8.91	5.94	

PC1- Principal Component 1, PC2- Principal Component 2,

Genetic Characterization of Clariid (*Clarias gariepinus* and *Heterobranchus longifilis*) species in Kano state

Thirty-five blood samples (25 from *Clarias gariepinus* and 10 from *Heterobranchus longifilis*) were subjected to DNA extraction, polymerase chain reaction (PCR) and electrophoresis. Resolve bands by location from the laboratory analysis are presented in plates 4.1-4.7.

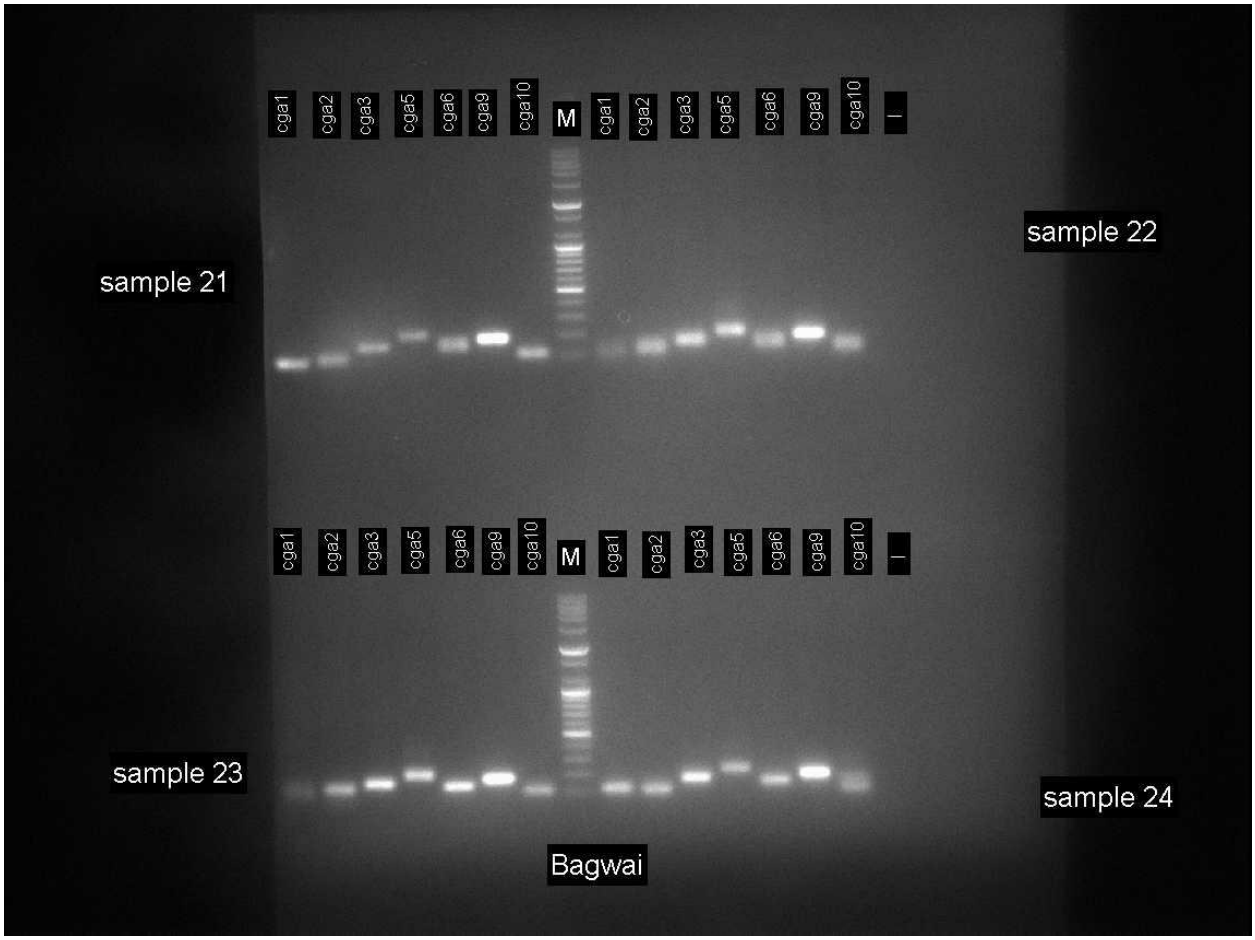


Plate 4.1: Gel images for *Clarias gariepinus* from River Bagwai showing the bands of the used microsatellite marker

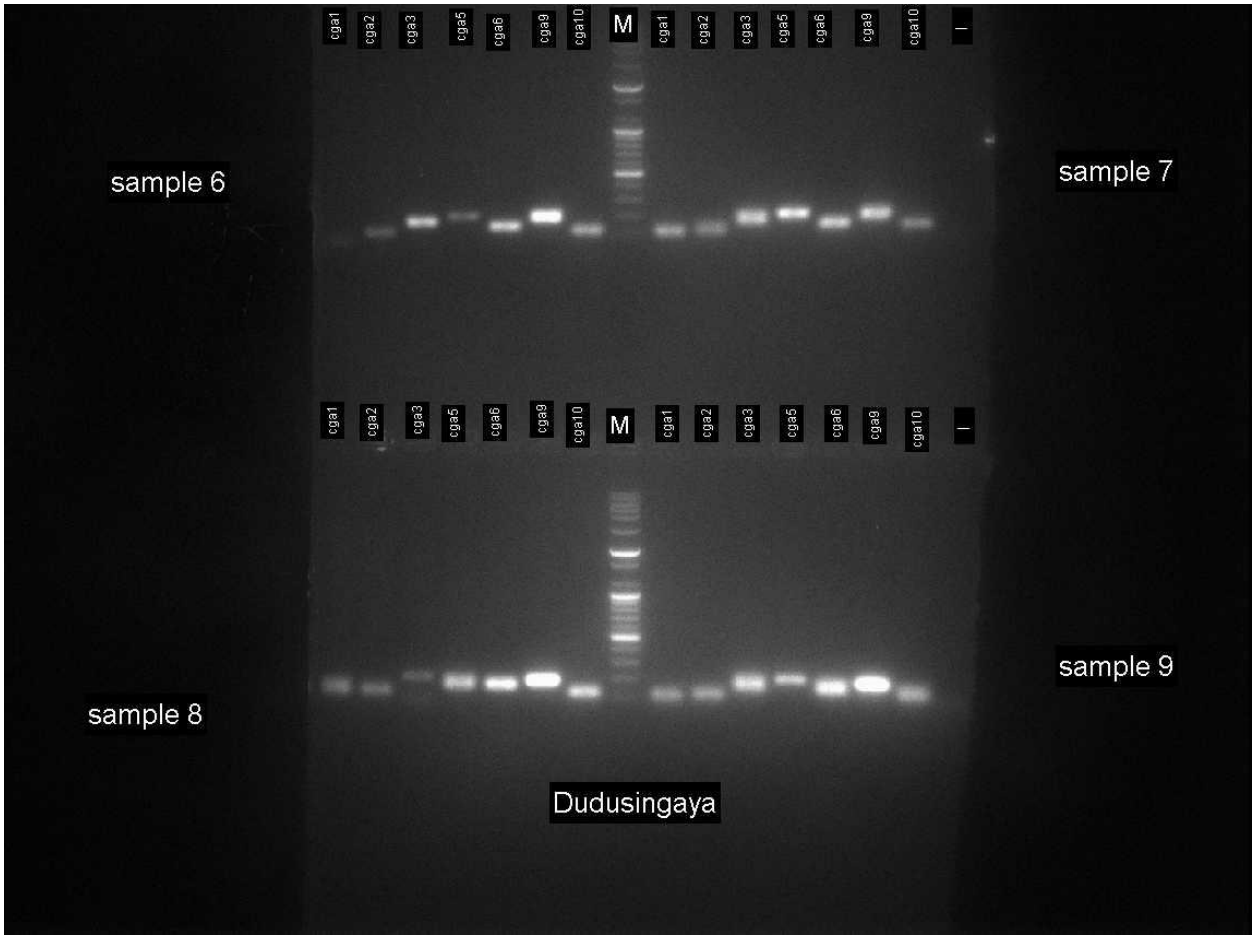


Plate 4.2: Gel images for *Clarias gariepinus* from River Dudusingaya showing the bands of the used microsatellite marker

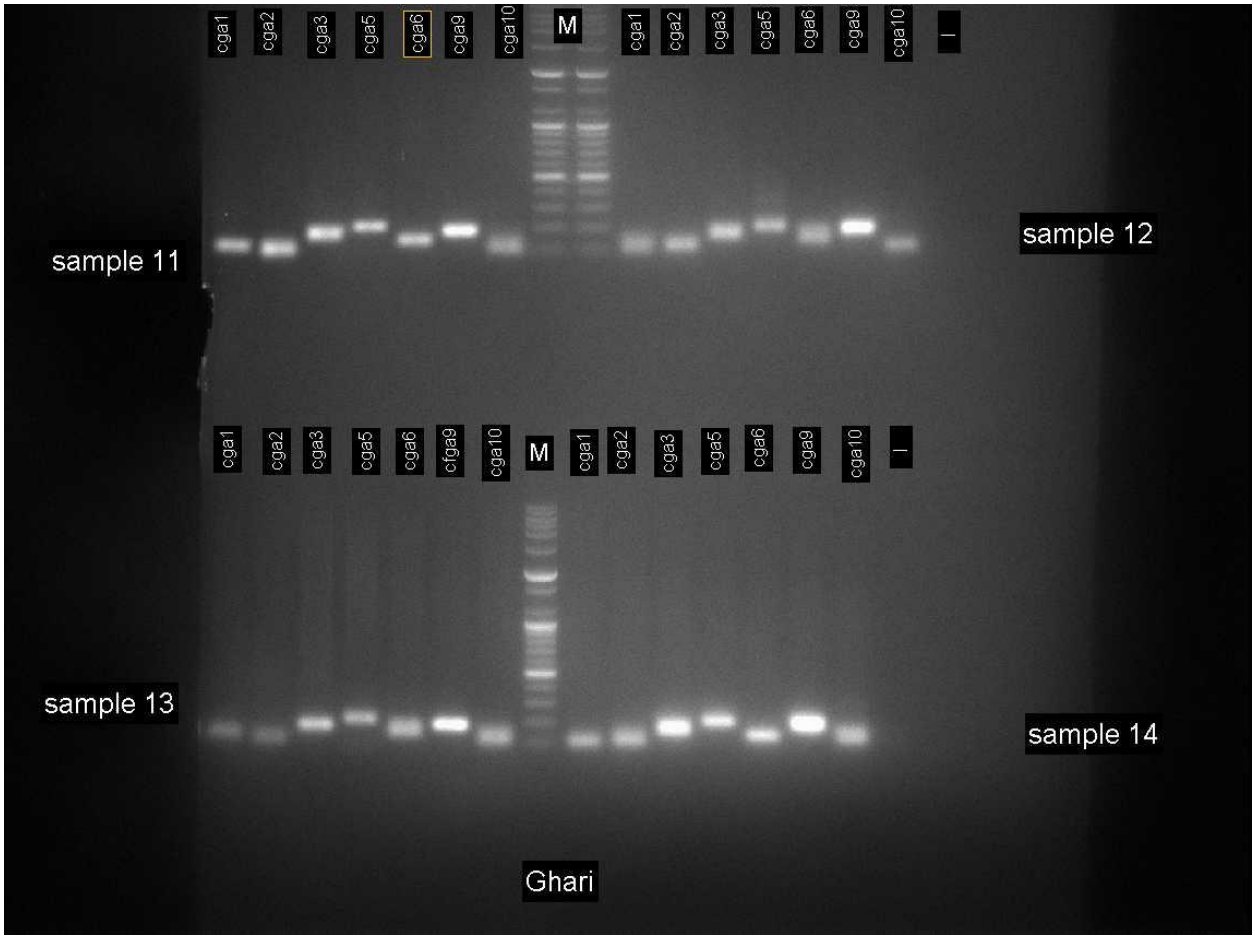


Plate 4.3: Gel images for *Clarias gariepinus* from River Ghari showing the bands of the used microsatellite marker

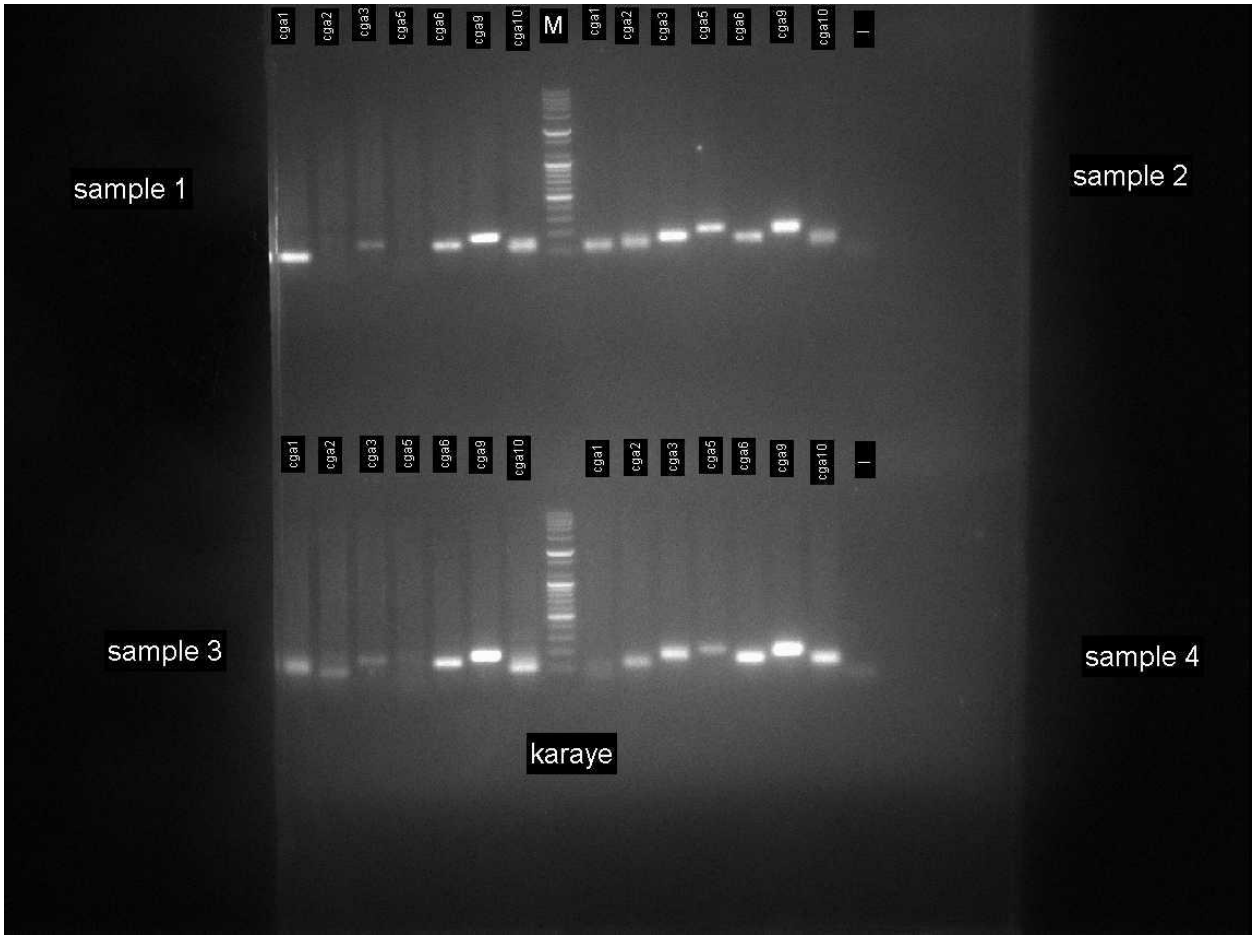


Plate 4.4: Gel images for *Clarias gariepinus* from River Karaye showing the bands of the used microsatellite marker

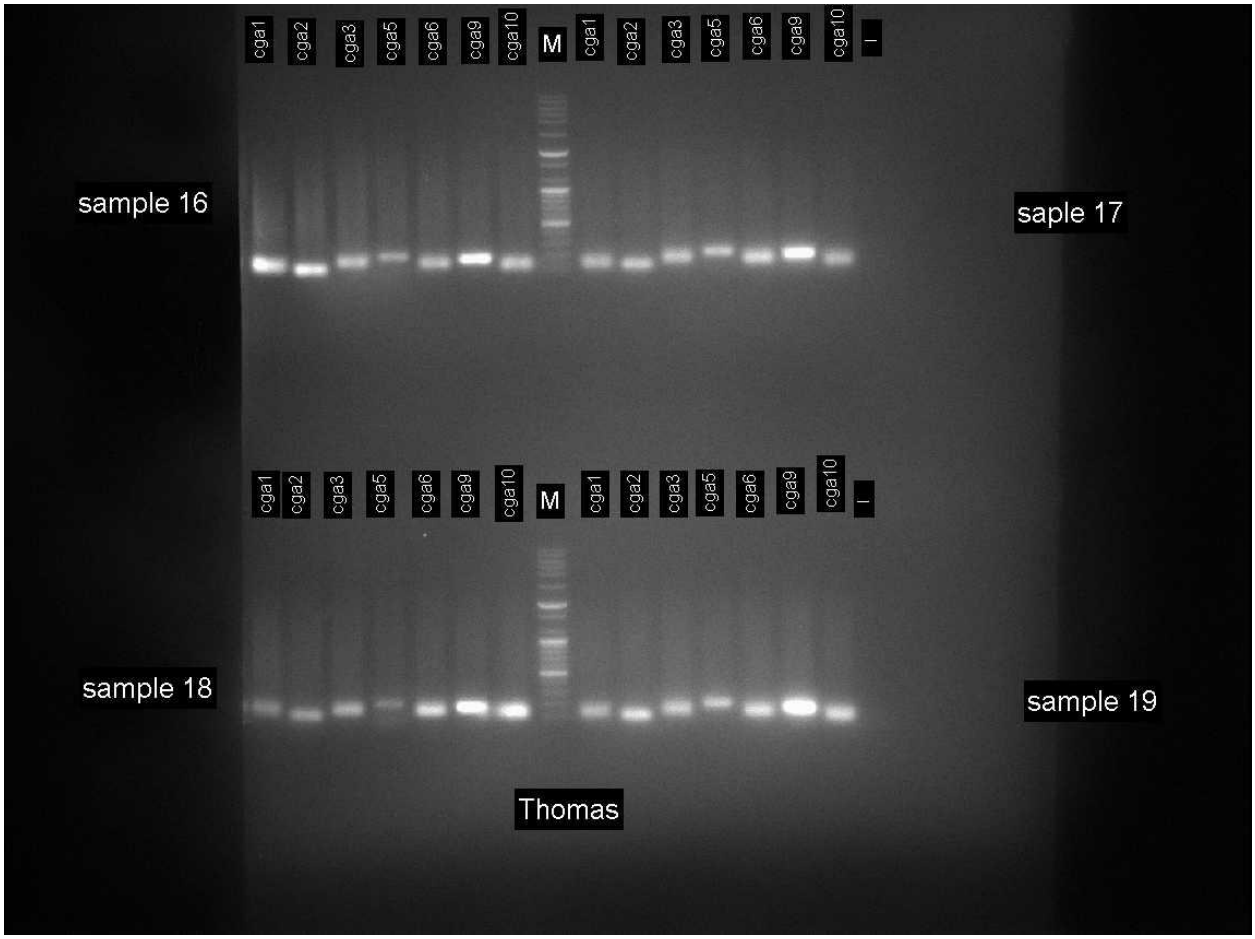


Plate 4.5: Gel images for *Clarias gariepinus* from River Thomas showing the bands of the used microsatellite marker

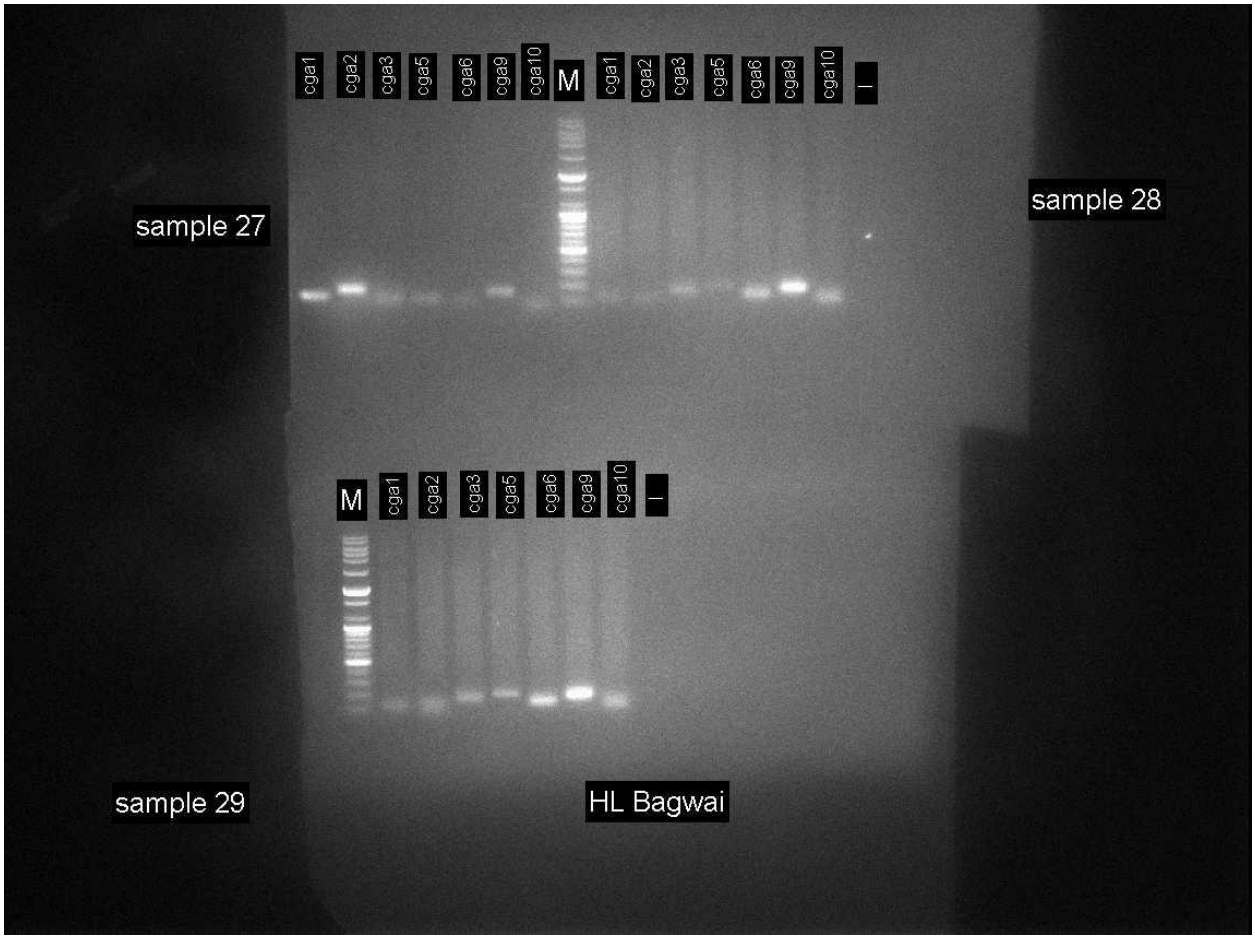


Plate 4.6: Gel images for *Heterrobranchus longifilis* from River Bagwai showing the bands of the used microsatellite marker

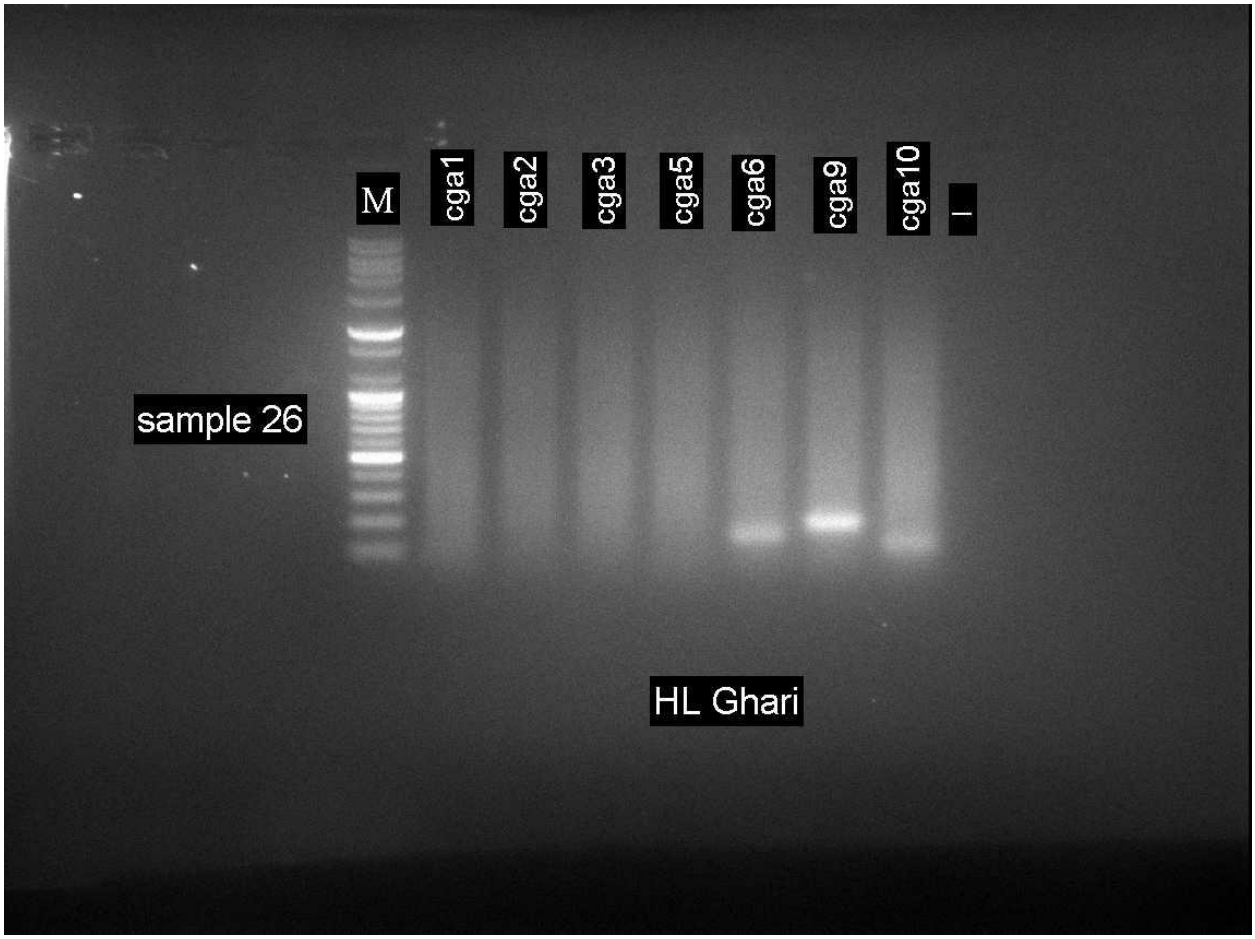


Plate 4.7: Gel images for *Heterrobranchus longifilis* from River Ghari showing the bands of the used microsatellite marker

The similarity values of the population of Clariid specie studied is presented in Table 4.14. The table showed that *Clarias gariepinus* from River Duddurun Gaya was related to that of River Ghari with a similarity value of 0.040, River Thomas with 0.018 and River Bagwai with 0.060. The *Clarias gariepinus* from River Ghari was related to that of River Karaye with a value of 0.040 and River Bagwai with 0.044. *Clarias gariepinus* from river Ghari was also related to *Heterobranchus longifilis* of River Bagwai with a similarity value of 0.026. *Heterobranchus longifilis*from river Bagwai had a relationship with *Clarias gariepinus* of River Thomas and River Karaye with values 0.070 and 0.066, respectively. The *Clarias gariepinus* of River Thomas was related to that of River Karaye with 0.018 and *Hetrobranchus longifilis* of River Ghari with 0.079. Others with values 0.000 are not related meaning that there are no tributaries connecting them.

The unbiased genetic similarity values give the relatedness when all sources of error are removed from analysis. Table 4.15 gives the similarity values and confirmed the values in Table 4.14 except that the values differed in strength.

Table 4.14: Nei's Genetic Similarity values among the investigated populations

	CgD	CgG	CgB	CgT	CgK	HlG	HlB
CgD	-						
CgG	0.040	-					
CgB	0.060	0.044	-				
CgT	0.018	0.000	0.020	-			
CgK	0.000	0.040	0.000	0.018	-		
HlG	0.000	0.000	0.000	0.079	0.000	-	
HlB	0.000	0.026	0.000	0.070	0.066	0.000	-

Cg=Clarias gariepinus

Hl=Heterobranchus longifilis.

D-River Dudusingaya,

G-River Ghari,

B-River Bagwai,

T-River Thomas

K- River Karaye

Table 4.15: Nei's Unbiased Similarity values among the investigated populations

	CgD	CgG	CgB	CgT	CgK	HlG	HlB
CgD	-						
CgG	0.080	-					
CgB	0.125	0.107	-				
CgT	0.032	0.000	0.043	-			
CgK	0.000	0.091	0.000	0.037	-		
HlG	0.000	0.000	0.000	0.065	0.000	-	
HlB	0.000	0.057	0.000	0.137	0.143	0.000	-

Cg=Clarias gariepinus

Hl=Heterobranchus longifilis.

D-River Dudusingaya,

G-River Ghari,

B-River Bagwai,

T-River Thomas

K- River Karaye

The genetic distance of the populations studied is presented in table 4.16. Nei's genetic distances among all populations ranged from 0.112 (CgG vs CgB) to 0.998 (CgT vs CgK). The Table shows that *Clarias gariepinus* of River Dudusingaya were genetically closer to *Clarias gariepinus* of River Ghari with value 0.223 while they were much more genetically distanced from those of River Thomas with 0.907 and River Bagwai with 0.816. *Clarias gariepinus* of River Ghari had genetic closeness to those of River Karaye (0.214), River Bagwai (0.112) and *Heterobranchus longifilis* of River Bagwai (0.667). *Heterobranchus longifilis* of River Bagwai shared distant genetic attributes with *Clarias gariepinus* of Rivers Thomas (0.659) and Karaye (0.725). *Clarias gariepinus* of River Thomasis genetically distanced to those of Rivers Karaye (0.998) and Bagwai (0.897), but closer to *Heterobrancus longifilis* of River Ghari (0.544).

The unbiased genetic distance values give the genetic relatedness when all sources of error are removed from analysis. Table 4.17 gives the unbiased genetic distance values and confirmed the values in Table 4.16 except that the values differed in strength.

The pairwise genetic differentiation values (F_{st}) shown in Table 4.18 were calculated for the seven populations showed genetic differentiation between each of the populations. Populations from different genetic clusters appeared to be more differentiated from each other, corresponding well to the classification of the genetic cluster. The values obtained showed that there was free interbreeding among the population in different magnitude. The values ranged from 0.000 for complete panmixis, to 0.663 indicating that some of the populations still share some amount of genetic diversity and the populations were highly differentiated.

Table 4.16: Nei's Genetic Distance among populations of two clariid strains

	CgD	CgG	CgB	CgT	CgK	HlG	HlB
CgD	-						
CgG	0.223	-					
CgB	0.816	0.112	-				
CgT	0.907	*	0.897	-			
CgK	*	0.214	*	0.998	-		
HlG	*	*	*	0.544	*	-	
HlB	*	0.667	*	0.659	0.725	*	-

* = undefined

Cg=Clarias gariepinus

Hl=Heterobranchus longifilis.

D-River Dudusingaya,

G-River Ghari,

B-River Bagwai,

T-River Thomas

K- River Karaye

Table 4.17: Nei's Unbiased Genetic Distance among populations of two clariid strains

	CgD	CgG	CgB	CgT	CgK	HlG	HlB
CgD	-						
CgG	0.523	-					
CgB	0.790	0.235	-				
CgT	0.429	*	0.140	-			
CgK	*	0.401	*	0.307	-		
HlG	*	*	*	0.736	*	-	
HlB	*	0.857	*	0.971	0.943	*	-

* = undefined

Cg=Clarias gariepinus

Hl=Heterobranchus longifilis.

D-River Dudusingaya,

G-River Ghari,

B-River Bagwai,

T-River Thomas

K- River Karaye

Table 4.18: Population pairwise genetic differentiation values based on microsatellite loci

(Fst) (gene differentiation)

	CgD	CgG	CgB	CgT	CgK	HlG	HlB
CgD	-						
CgG	0.003	-					
CgB	0.000	0.003	-				
CgT	0.006	0.009	0.003	-			
CgK	0.012	0.015	0.003	0.018	-		
HlG	0.647	0.655	0.588	0.663	0.440	-	
HlB	0.000	0.003	0.000	0.006	0.000	0.279	-

Cg=Clarias gariepinus

Hl=Heterobranchus longifilis.

D-River Dudusingaya,

G-River Ghari,

B-River Bagwai,

T-River Thomas

K- River Karaye

Tables 4.19a, 4.19b and 4.19c show the sample size, number of alleles, number of effective alleles, Shanon information index, observed heterozygosity, expected heterozygosity and fixation index for each of the populations studied locus by locus measuring the amount of genetic diversity among the populations studied. Their grand means and standard errors over loci are shown in Table 4.20. The highest genetic diversity occurred in Cga03 (Ne = 8.000, Ho = 1.000 and He = 0.875) for CgG (Table 4.19a) and Cga09 (Ne = 8.000, Ho = 1.000 and He = 0.875) for CgK (Table 4.19b). The lowest genetic diversity was observed in Cga06, Cga09 and Cga10 (Ne = 2.000, Ho = 1.000 and He = 0.5000) for HlG (Table 4.19b). In Table 4.20, total number of sample size was 3.245 with a range of 2 to 4 across loci (Tables 4.19a, b and c). Mean number of alleles were 4.388 and detected across all populations for the seven microsatellites examined. Number of alleles per locus overall populations ranged between 3 and 8 with grand mean of 4.388 for all alleles in the entire population. The number of effective alleles were totalled 4.047 while the total information index was 1.351. Total observed heterozygosity was 0.918 indicating that the markers were sufficiently polymorphic to determine genetic diversity in the fish populations studied. Expected and unbiased heterozygosity were 0.687 and 0.821 respectively. Mean Ho was higher than He indicating efficiency of heterozygotes at these loci. The mean fixation index for the population was -0.364 indicating that the population had more heterozygotes than expected in contrast to positive values that indicate fewer heterozygotes.

Table 4.19a:Sample Size, No of Alleles, No of Effective Alleles, Information Index, Observed Heterozygosity, Expected and unbiased expected Heterozygosity, and Fixation Index for CgD, CgG and HlB

Locus	N	Na	Ne	IH_OH_e	uHe	F		
CgD								
Cga01	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Cga02	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Cga03	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Cga05	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Cga06	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Cga09	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Cga10	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Mean	4.000	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
SE	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CgG								
Cga01	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Cga02	4	4.000	4.000	1.386	1.000	0.750	0.857	-0.333
Cga03	4	8.000	8.000	2.079	1.000	0.875	1.000	-0.143
Cga05	4	5.000	4.000	1.494	1.000	0.750	0.857	-0.333
Cga06	4	7.000	6.400	1.906	1.000	0.844	0.964	-0.185
Cga09	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Cga10	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Mean	4.000	5.714	5.295	1.674	1.000	0.799	0.913	-0.256
SE	0.000	0.565	0.568	0.100	0.000	0.019	0.022	0.030
HlB								
Cga01	3	4.000	4.000	3.600	1.000	0.722	0.867	-0.385
Cga02	3	3.000	4.000	2.571	1.000	0.611	0.733	-0.639
Cga03	3	4.000	8.000	3.600	1.000	0.722	0.867	-0.385
Cga05	3	5.000	4.000	4.500	1.000	0.778	0.933	-0.286
Cga06	3	5.000	6.400	4.500	1.000	0.778	0.933	-0.286
Cga09	3	5.000	5.333	4.500	1.000	0.778	0.933	-0.286
Cga10	3	4.000	5.333	3.600	1.000	0.722	0.867	-0.385
Mean	3.000	4.286	3.839	1.383	1.000	0.730	0.876	-0.378
SE	0.000	0.286	0.271	0.076	0.000	0.022	0.027	0.047

N-Sample Size, Na-No of Alleles, Ne-No of Effective Alleles, I- Information Index, Ho-Observed Heterozygosity, He-Expected, uHe-unbiased expected Heterozygosity, F-Fixation Index

Table 4.19b:Sample Size, No of Alleles, No of Effective Alleles, Information Index, Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity, and Fixation Index for CgT, CgK and HlG

Locus	N	Na	Ne	IH_OH_e	uHe	F		
CgT								
Cga01	4	5.000	4.671	1.560	1.000	0.781	0.893	-0.280
Cga02	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Cga03	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Cga05	4	4.000	3.556	1.321	1.000	0.719	0.821	-0.391
Cga06	4	5.000	4.000	1.494	1.000	0.750	0.857	-0.333
Cga09	4	3.000	2.667	1.040	1.000	0.625	0.714	-0.600
Cga10	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Mean	4.000	5.000	4.399	1.516	1.000	0.759	0.867	-0.378
SE	0.000	0.436	0.394	0.099	0.000	0.026	0.030	0.047
CgK								
Cga01	3	5.000	4.500	1.561	1.000	0.778	0.933	-0.286
Cga02	3	4.000	3.600	1.330	1.000	0.722	0.867	-0.385
Cga03	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Cga05	2	2.000	2.000	0.693	1.000	0.500	0.667	-1.000
Cga06	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Cga09	4	8.000	8.000	2.079	1.000	0.875	1.000	-0.143
Cga10	4	5.000	4.571	1.560	1.000	0.781	0.893	-0.280
Mean	3.429	5.143	4.399	1.516	1.000	0.759	0.867	-0.378
SE	0.297	0.705	0.394	0.099	0.000	0.026	0.030	0.047
HlG								
Cga01	0	0	0	0	0	0	0	0
Cga02	0	0	0	0	0	0	0	0
Cga03	0	0	0	0	0	0	0	0
Cga05	0	0	0	0	0	0	0	0
Cga06	1	2.000	2.000	0.693	1.000	0.500	1.000	-1.000
Cga09	1	2.000	2.000	0.693	1.000	0.500	1.000	-1.000
Cga10	1	2.000	2.000	0.693	1.000	0.500	1.000	-1.000
Mean	0.429	0.857	0.857	0.297	0.429	0.214	0.429	-1.000
SE	0.297	0.404	0.404	0.140	0.202	0.101	0.202	0.000

N-Sample Size, Na-No of Alleles, Ne-No of Effective Alleles, I- Information Index, Ho-Observed Heterozygosity, He-Expected, uHe-unbiased expected Heterozygosity, F-Fixation Index

Table 4.19c:Sample Size, No of Alleles, No of Effective Alleles, Information Index, Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity, and Fixation Index for CgB

Locus	N	Na	Ne	IH_OH_e	uHe	F		
CgB								
Cga01	3	4.000	3.600	1.330	1.000	0.722	0.867	-0.385
Cga02	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Cga03	4	5.000	4.571	1.560	1.000	0.781	0.893	-0.280
Cga05	4	7.000	6.400	1.906	1.000	0.844	0.964	-0.185
Cga06	4	5.000	4.571	1.560	1.000	0.781	0.893	-0.280
Cga09	4	7.000	6.400	1.906	1.000	0.844	0.964	-0.185
Cga10	4	6.000	5.333	1.733	1.000	0.813	0.929	-0.231
Mean	3.857	5.714	5.173	1.675	1.000	0.800	0.920	-0.254
SE	0.143	0.421	0.386	0.079	0.000	0.016	0.014	0.026

N-Sample Size, Na-No of Alleles, Ne-No of Effective Alleles, I- Information Index, Ho-Observed Heterozygosity, He-Expected, uHe-unbiased expected Heterozygosity, F-Fixation Index

Table 4.20:Grand mean for Sample Size, No. Alleles, No. Effective Alleles, Information Index, Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity, and Fixation Index

	N	Na	Ne	IH_OH_e	uHe	F		
Mean	3.245	4.388	4.047	1.351	0.918	0.687	0.821	-0.364
SE	0.181	0.276	0.254	0.074	0.040	0.032	0.037	0.032

N-Sample Size, Na-No of Alleles, Ne-No of Effective Alleles, I- Information Index, Ho-Observed Heterozygosity, He-Expected, uHe-unbiased expected Heterozygosity, F-Fixation Index

Table 4.21 shows the levels of intra and inter-population variation of Fis, Fst and Fit. These are inbreeding coefficients: within population (Fis), between population (Fst) and overall population (Fit) for the seven microsatellite loci investigated. Inbreeding within population (Fis) was negative ranging from -0.262 to -0.382. The inbreeding between populations (Fst) ranged from 0.224 to 0.342. Inbreeding in the overall population (Fit) ranged from negative to positive (-0.041 to 0.115). The estimate of gene flow ranged from 0.482 to 0.866 indicating effective gene flow in the population. The degree of genetic differentiation within population was very low, that of between populations was very high while overall showed a moderate degree of genetic differentiation. The percentage polymorphism was 100% except for the sixth locus (Cga09) where it was 48.86% indicating that Cga09 had a lower genetic polymorphic loci in the populations investigation.

Table 4.22 shows the Analysis of molecular variance (AMOVA) of hierarchical gene diversity. The result indicated that 83% of the genetic variation was explained by within-individual, whereas 7% of the variation was explained by among-individual and 10% of the variation was explained by among population with estimated variability ranging from 0.247 to 2.839. The analysis of molecular variation indicated that majority of the variation were partitioned within individual in the populations.

Figure 3 shows the hierarchical clustering dendrogram obtained by different distances among river bodies using fish morphometric measurements. Genetic distance of the fish population in the river bodies were grouped into two clusters. The horizonal dimension gives the amount of genetic changeand distance in populations. The horizonal lines are branches and represent evolutionary lineages changing over time. The longer the branch in the horizonal dimension, the larger the amount of genetic change and disrance. From the dendrogram, it indicates that population of Clariid in River Thomas is substancially and quite different from the remaining

populations with value 1.80 thereby forming a separate cluster (Cluster 1). Clariid populations in River Ghari and Tiga dam are the most similar populations as indicated in dendrogram with value 0.01, they are similar to each other than the population of Clariid in River Karaye with value 0.49. In addition, Clariid populations in Rivers Duddurun Gaya and Bagwai are more similar (0.29) to each other than populations in River Ghari and Tiga dam (0.49) are to Clariid population in River Karaye. According to the dendrogram, the Clariid populations share some amount of genetic diversity.

Table 4.21: Inbreeding coefficients and Estimate of gene flow (Nm) over all populations for each locus

Locus	Fis	Fit	Fst	Nm	%polymorphic loci
Cga01	-0.332	0.111	0.332	0.502	100%
Cga02	-0.346	0.114	0.342	0.482	100%
Cga03	-0.262	0.115	0.299	0.585	100%
Cga05	-0.382	0.108	0.355	0.455	100%
Cga06	-0.342	-0.041	0.224	0.866	100%
Cga09	-0.350	-0.045	0.226	0.857	42.86%
Cga10	-0.348	-0.043	0.226	0.855	100%
Mean	-0.338	0.045	0.286	0.657	91.86%
SE	0.014	0.031	0.022	0.073	8.16

Fis, Fit and Fst – Inbreeding coefficients

Nm – Estimate of gene flow

Table 4.22: Analysis of molecular variation (AMOVA) of hierarchical gene diversity

Source	df	SS	MS	Est. Var	%
Among Population	6	36.21	6.04	0.34	10
Among Individual	21	70.00	3.33	0.25	7
Within Individual	28	79.50	2.84	2.84	83
Total	55	185.72		3.42	100

df – degree of freedom,

SS – sum of square,

MS – mean square,

Est Var – estimated variance

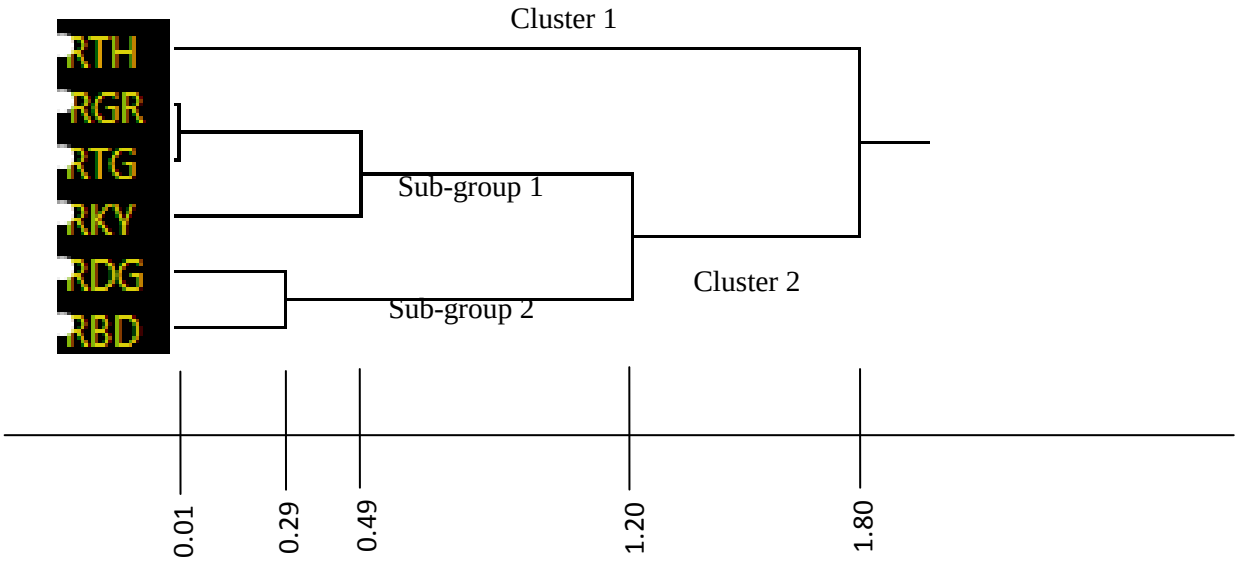


Figure 3: Hierarchical clustering dendrogram of Clariid populationsin genetic similarity and distance using morphometric measurements

RTH – River Thomas

RGR – River Ghari

RTG – Tiga dam

RKY – River Karaye

RDG – River Duddurun Gaya

RBD – River Bagwai

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of Location, Sex and Strain on Morphological and Meristic Counts in Clariid Species

Fish has been said to demonstrate greater variances in morphological traits both within and between populations of species than any other vertebrates (Wimberger, 1992). Morphometric and meristic studies provide useful results for identifying fish stocks.Morphometric and meristic characters have been used in this study as a step to analyze the potential differentiation of Clariid populations. Location and strain contributed strongly to morphological and meristic variations in Clariid species than variation from sex in the present study.

Clariid population in River Thomas was distinctive in attributes measured and most divergent from others due to higher morphometric measurements recorded, indicating that the River is much more habitable for the Clariid species for good growth and development. This was closely followed by River Karaye. Morphometric characteristics can show high plasticity in response to differences in environmental condition, such as food abundance and temperature. The study has shown that morphometric parameters can be highly variable among and within conspecific populations, either correlating with geographical and habitat variation or having a genetic component, based on differences among groups in a common environment.

The mean values of the morphometric characteristics and meristic counts were within the range of value described by Eyo (2003) for Clariid strains with mean Total length of 24.02cm, Standard length of 20.61cm, Pre-dorsal distance of 7.21cm, Pre-pectoral of 4.64cm, Pre-anal distance of 11.68cm and other body measurements fall within the values in this study. However, the values were lower than the values described by Fagbuaro *et al*. (2015) with mean body weight and total length of 530.67±18.74g and 42.53±0.55cm and

507.02±26.26g and 42.40±0.58cm for controlled and uncontrolled fish populations respectively. This might due to the nature of the water bodies and difference in environment. The lack of ability to show significance for variation in mature male and female morphology from the Rivers investigated may be as a result of sex specific convergence on a common shape or increased movement and mixing of mature individuals(Jacquemin and Pyron, 2013).The result of this study concored with the report of Turan*et al*. (2005) who reported negligible sex variation in C. gariepinus from six wild populations in Turkey. Concerning strains, *Heterobranchus longifilis* revealed better morphometric measurements compared to *Clarias gariepinus.*The most important morphological difference between *Clarais guriepinus* and *Heterobranchus longifilis* is the presence in the latter species of a large adipose fin originating immediately behind the rayed dorsal fin and reaching the caudal fin base.This heterogeneity of morphometric measurments and meristic counts may be due to their strain variation.

Significant spatial heterogeneity in morphology of Clariid species among the river bodies was evident in this study. Spatial divergence brought a lot of significance in the strains studied as revealed by the influence of river location and strain in this study. These are in agreement with Elliot *et al* (1995), Uiblein (1995), Hurlbut and Clay (1998) and Cemal *et al* (2005) with the observation that different strains of fish species exhibit divergent morphological characteristics in various habitats in response to the condition factors prevailing in the water bodies.

Among the vertebrates, phenotypic variability is considered to be greatest in fish, which have relatively higher within-population coefficients of variation of phenotypes (Carvalho, 1993). This variability is likely to have arisen from the great phenotypic plasticity of fishes in response to changes in environmental factors (Wimberger 1991, 1992). It is well known that

the environment of the water bodies undergo great fluctuations in physico-chemical conditions as they are under the heavy influence of tidal fluctuations and fresh water influx mainly from rainfall. Under such variability in the environment, maintenance of distinctive phenotypes might be impractical (Suneetha, 2007).

Result obtained from this study showed evidence of large phenotypic differences between populations which indicates that any restriction on gene flow that may occur on these population units is sufficient to maintain them in complete isolation. The results also indicate that significant heterogeneity in morphology existed among all the studied populations ofClariid species. Morphological variation have occurred due to factors such as partial isolation of the populations and local adaptations. The populations of Clariid species as revealed by the present study are completely spatially isolated at present. This might be of evolutionary significance with respect to short-term or long-term divergence. These results on the influence of river location, sex and strain are in agreement with the study conducted by Hood and Heins (2000) and Wund *et al* (2012), where ontogenic shape patterns in other fish taxa were found to be associated with differences in sex, body size and environmental variation. Ecomorphology posits that a morphological trend with body size results from functional adaptations with variation in physiology, habitat, or dietary niche that covary with body size (Norton *et al.,* 1995).Since the identification of populations and their connectivity between each other is a major point for management, breeding and conservation of species, the use of the morphometric characteristics for these purposes under the present subtropical conditions appears promising.

5.2 Length-Weight Relationship and Condition Factor (Ponderal Index) for the Clariid Population Sampled and their Location

The study of Length-Weight relationship in Clariid populations is of paramount impotortance, as it assist in understanding the general well being and growth pattern in a fish population, which varies depending on the condition of life in aquatic environment. The regression coefficient obtained from this study indicated positive allometric growth pattern depicting that the clariid strains studied had stouter bodies as they increase in length. This was in agreement with the reports of Abowei *et al* (2009), Abowei and Hart, (2009), Olurin and Aderibigbe, (2006), Dankishiya (2013) and Suleiman *et al* (2015) who worked with *Clarias gariepinus* from cultured environment. Also, Ibrahim *et al* (2012) observed allometric pattern of growth in Kontagora Reservoir while Ude *et al* (2011) made similar findings in fish species of Ebonyi River.In fish, the condition factor (K) reflects, through its variation, information on the physiological atate of the fish in relation to its welfare. It gives information when comparing populations living in certain feeding intensity, density, climate and other conditions. The condition factor for all the Clariid populations in their water bodies ranged from 0.37 to 0.88 indicating the good condition and proper wellbeing of the fishes in their habitat.When condition factor value is higher it means that the fish has attained a better condition.The result obtained from this study agrees with the report of Ajayi (1982) with values 0.37-0.81 in Lake Oguta. Differences in Condition factor can be due to different reasons which includes; stress, sex, season, availability of feeds, andother water quality parameters (Khallaf et al., 2003).

5.3 Relationships among the Morphometric Measurements

The positive Pearson correlations for all morphometric measurements shows that Clariid species are able to survive as a living entity with indispensable body parts. The Pearson correlation analysis showed that body weight had a positive and significant relationship with all the morphometric characteristics indicating that selection for body weight might yield a correspondent positive result in other morphometric characteristics. The relationship between the variables measured showed a direct proportional relationship while some had negative relationship. This could be used in selection criteria for any breeding goal in fish breeding in which emphasis would be laid on those with positive and significant relationship against the variables with negative relationship. These findings were in agreement with reports of Umoh *et al* (2015) on studies carried out on hybrid of *Clarias gariepinus* and *Heterobranchus longifilis* in some selected farms in Southern Nigeria and Teugels *et al.,* (1990) on Clariid strains. The high positive correlations (r) exhibited by Clariid species in this study indicated that as length of the fish increased, its body weight also increased. This could be attributed to the availability of quality and quantity of food and plankton yield resulting from the water body within the ecological niches of the fish. Peepple and Ofor (2011) had made similar observation.

5.4Principal Component Analysis for Location and Strains using the Morphometric Characteristics

Result of the studies using principal component analysis revealed that most of the morphometric characteristics were discriminating attributes in the clariid specie studied as revealed in PC1 which loaded the highest percentages of variance as regard to location and strain. Factor pattern coefficients of the rotated factors showed the relative contribution of each trait to a particular component (PC1 and PC2). This is an indication that the differences

between the fish populations resulted from mainly all the morphometric characteristics depicting total diversity in the populations. These showed that morphometric characters enabled a clear separation among fish genotypes. These results conformed to the reports of Cemal *et al* (2005) on *Clarias gariepinus* in Turkey, Suneetha (2007) on catfish (*Arius jella)* in Sri Lanka and Umoh *et al* (2015) in Southern Nigeria.

5.5 Genetic Variability and Similarity of the Strains of Clariid Species Studied

i) Genetic similarity and relatedness

The result of the Nei's genetic similarity showed the population of *Clarias gariepinus* of river Duddurun Gaya were not related at all the populations of *Clarias gariepinus* of river Karaye and *Heterobranchus longifilis* of rivers Ghari and Bagwai, indicating that there was no gene exchange between them (0.000). The populations with values ranging from 0.018 to 0.079 had some level of genetic similarity among them similar to the findings of Agbebi *et al* (2013) with *Heterobranchus bidorsalis* and *Clarias gariepinus* strains. The close relationship between Clariid species had been emphasized by Teugels *et al* (1990) in a revisionary study using osteological features. Agnese *et al* (1997b) reported genetic variation at 25 protein and eight microsatellite loci, and two mitochondrial (mtDNA) segments in two sympatric Clariid strains from Senegal River. Results from this experiment were in congruent with those of Rognon *et al* (1998) in a morphometric and the allozyme study of nine populations of *Clarias gariepinus* and seven populations of *Clarias anguillaris* to quantify their intra and interspecific variation using *Clarias albopunctatus* and *Heterobranchus longifilis* as outgroups.

ii) Genetic distance

Genetic distance is a measure of the genetic divergence between species or between populations within a species as indicated by Nei's genetic distance values, Fst values, mean fixation index and hierarchical clustering dendrogram. The results of this study showed that some of the populations were genetically close while some were farther apart indicating that those that were close might have shared some tributaries and connection in terms of the movement or flow of the water bodies. Those that were farther apart might have some level of distinctiveness in terms of their habitat or there was little or no connection between the water bodies. The results were in agreement with the reports on aquatic organisms by Agnese (1989) who reported genetic distances varying from 0.271 to 0.916 in the genus *Chrysichthys* and Abban and Skibinski (1988) reported a distance range of 0.88 to 1.14 between *Eutropius niloticus* and *Schilbe mystus*. Agnèse (1991) alsoreported value for populations of *Chrysichthys auratus*to be 0.003 to 0.112 while Agbebi *et al* (2013) reported 0.84 to 0.89 in *Clarias gariepinus* and *heterobranchus bidorsalis*.

iii) Population diffrenctiation

The result obtained showed that the degree of genetic differentiation within population were very low, those of between populations were very high while overall population showed a moderate degree of genetic differentiation. This was an indicating that the populations are not experiencing panmixis (free interbreeding) but they still share some amount of genetic diversity and the populations were somehow differentiated. The mean inbreeding coefficient between (Fst = 0.286) observed in this study was lower than the values reported for *Clarias batrachus* (Fst = 0.545) and *Clarias nieuhofii* (Fst = 0.484) in Thailand by Na Nacron *et al*(2004). It was also lower than the Nei's among heterozygosity observed in *Clarias gariepinus* (Fst = 0.44) (Rognon *et al.,* 1998) and slightly greater than the one reported for *Clarias anguillaris* in Africa, (Fst = 0.15) by Rognon *et al*(1998). Values of inbreeding

coefficient within (Fis) in most natural populations are close to zero. The Fis value provides the non-random union of gametes in population, which is the mating among individuals in the population which is related more than average relationship. The higher the inbreeding coefficient (Fis) value, the more the degree of inbreeding. The negative values of inbreeding coefficient (Fis) point towards outbreeding and excess of heterozygotes in a population. For populations mating at random, genes are equally related-within or between individuals and in this case, value of inbreeding coefficient is zero (Fis = 0). Therefore, estimates of Fis that differ significantly indicate departures from random mating. Any avoidance of mating of relatives will cause Fis to exceed 0 and to be negative. More commonly, Fis is positive, which could be interpreted as evidence of inbreeding (Weir, 1996). In this study, Fis values were negative (-0.262 to -0.382, mean = -0.338) indicating a deviation from inbreeding and show an outbred populations meaning that they are less related. These results were in harmony with the reports of Cristianne and Alexandre (2009) who reported values of -0.25 to -0.59 and those of O'Reilly *et al* (2004) who obtained values in range of -0.17 to -0.46.

iv) Allelic variation

The seven loci assayed were polymorphic for all the populations, with each having at least four allele per population. The range of alleles observed per locus, three to eight was in perfect agreement with the three to eight range observed by Volckaert and Agnese (1996) in the analysis of five microsatellite loci in ten samples of *Clarias gariepinus*. However, Galbusera *et al* (1996) observed a range of five to 14 alleles in the characterization of seven microsatellite loci in *Clarias gariepinus*. The genetically heterogenous samples were observed to maintain the different allele frequencies for a microsatellite locus. This could be because those samples were under the differentselection pressures, hence they were forced to maintain the different allele frequencies or because of intensive exchange of migrants in the

populations. Allele frequencies in heterogeneous populations may be established as a result of random drift due to the long term isolation or because the populations are subjected to different selection pressures (Volckaert and Agnese, 1996). The result of this study were in agreement with those of Agbebi *et al* (2013) who used four microsatellite to characterize cultured *Clariid* fish strains in Abeokuta, Ogun State, Nigeria.

v) Genetic diversity and variability

The genotypic data obtained showed a good level of informativeness having a percentage polymorphism value of 100% for all loci exceptthe sixth locus, which was below the threshold for which genetic markers begin to be informative. The percentage of polymorphism value depends on the number of alleles detected per locus and their frequencies. The heterozygosity percentage observed for all the alleles (91.86%) fall within the observed levels of heterozygosity in fish, which range from 24 to 95% (O'Connell and Wright, 1997). The observed average heterozygosity (Ho = 0.918 ± 0.040) was higher than the values in*Clarias gariepinus* populations collected across Africa (0.06 to 0.15) by Teugels *et al*(1992 and Agnèse *et al*(1997b). The expected heterozygosity observed in this study (He = 0.687 ± 0.032) was also higher than the values observed from a previous study by Rognon *et al* (1998) (He = 0.05 to 0.15).However, the Ho and He values were within the range given by Shubina *et al*(2005); (Ho = 0.48 to 1.00, He = 0.70 to 0.95).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

These studies were carried out to examine the effect of location of water bodies, strains of the fish sampled and sex on morphometric characteristics and meristic counts in two strains of

Clariid species, to determine length-weight relationship and condition factors of Clariid species, to evaluate any existing genetic variation within and among Clariid species populations in Kano State and to dwtermine the relationships among morphometric characteristics measured. In achieving the objectives laid down for the studies, one hundred and seventy seven samples were gotten from six different water bodies in Kano state. On each fish sample, morphometric characteristics and meristic count were taken and recorded. Blood samples were taken from the fishes by severing the caudal peduncle on Watson FTA cards that were left to dry and later used for molecular and genetic characterization of the Clariid strains of fish. The result of the first study showed a significant effect of location and strain on the morphometric characteristic and meristic counts studied. The results equally showed that most of the morphometric characteristics could be used for the classification of the strains in their genus. The length and weight relationships were also examined with the result indicating a positive allometric pattern of growth. The condition factor (Ponderal indices) were calculated to determine the wellbeing of the fish sampled in their respective habitat. The result indicates a good wellbeing of these fishes.

The blood samples were subjected to molecular laboratory analysis. DNA were extracted, gel were run, PCR was performed for selected samples using seven microsatellite markers (Cga01, Cga02, Cga03, Cga05, Cga06, Cga09 and Cga10) to determine the existing genetic variation within and among the populations. The results of the genetic studies showed that the populations were heterogeneous with high heterozygosity percentage. It also showed some of the populations had some level of genetic variability while others were similar. The populations had a high genetic differentiation between populations but moderate differentiation within populations. The result indicated that populations were outbred meaning that relatives avoided mating in the population due to the negative values of

inbreeding coefficient within (Fis). There was an established magnitude of genetic divergence (91.86%) among the populations as shown by the result from the studies.

6.2 Conclusion

The following conclusions were drawn for this study:

i) Morphometric characteristics in fish alongside meristic count were determined and were found to be influenced by environmental factors and the genotype of the fish studied. Location of the water bodies and strains of the Clariid species had significant influence on morphometric characteristics and meristic counts in the Clariid species studied. The study of the influence of environmenton morphology continues to be developed as morphology is being found as an increasing useful predictor of population dynamics and response to environmental changes. Sex effect on morphometric characteristics and meristic counts were negligible in the studied populations contrary to sexual dimorphism in livestock animals. The secondhypothesis that location, strain and sex have influence on morphometric charcateristics and meristic counts in Clariid species is therefore accepted.

ii) Results of the length-weight relationships of the sampled Clariid species indicate positive allometric pattern of growth (Cube law of growth). The condition of living as revealed by Condition factor (Ponderal index) for the specie in their respective water bodies indicated that the physicochemical and biotic variables in the water bodies were within acceptable limits for fish production in freshwater ecosystem. The third hypothesis that length-weight relationship and condition factors of Clariid species differs is accepted.

iii) The study revealed a relatively high genetic diversity which is required for populations to be more adaptive with the environmental changes. The studied populations were not genetically pure but heterogeneous with varying degrees of genetic similarity (0.018-0.079) and distance (0.112-0.998). Since there was no inbreeding (-0.338) as shown in all the populations, none of the poplations exhibited genetic uniqueness, therefore, the populations were not isolated. Populations showing a great deal of variation will be able to adapt to changing circumstances whereas populations with less genetic variability will be less adaptable to sudden environmental changes. Therefore, the fouthhypothesis on within and among genetic variation of Clariid populations is accepted.

iv) Morphometric measurements could be used in breeding programme as a measure of direct selection for Clariid fish with better body weight traits (190.54±11.59 for *Clarias gariepinus* and 318.73±42.12 for *Heterobranchus longifilis*) and body configuration, therefore, the fifthhypothesis on existing relationships among morphometric measurements in Clariid species is accepted.

6.3 Recommendations

The following can be recommended from the findings of this study:

i) The findings of this research suggested that the morphological characteristics and meristic counts of Clariid species were determined by genetic, environment and the interaction between them, therefore it is recommended that the environment of the water bodies should be well managed interms of physico-chemical properties and water quality to enhance proper development of the Clariid fishes and better their morphological configuration.

ii) It is also recommended that morphometric characteristics and meristic counts should be used to generate reliable information for stock discrimination due to their morphological differences.

iii) Due to the positive and significant relationship between body weight and other morphometric measurements (r values ranges from 30 to 98), it is recommended that selection index method should be employed for better Clariid fish configuration.

iv)The Clariid strains were not available in all water bodies visited thus there is the need to stock all water bodies with Clariid strains since these are the major fish of economic importance to the artisan fishermen in the communities surrounding the area.

v) The wellbeing of *Clarias gariepinus* in River Karaye (0.46) and River Bagwai (0.37)are lower compared to other water bodies which could be as a result of their physico-chemical properties and water quality, hence, there is the need to look into the causes and proffer solution in subsequent research.

vi)Measures to conserve uniqueness and distinctness of Clariid fish populations should be sought through genetic improvement. Appropriate breeding policies and strategies should be developed and adopted to improve Clariid fish populations in Kano state.

vii) The genetic characterization revealed a well-managed and conserved population due to their genetic similarity (0.018-0.079), distance (0.112-0.998) and differentiation 0.003-0.663), thus there should be continuity in policy and decision making to retain and maintain the genetic resources available for breeding purposes in Kano state water bodies. This could be achieved through restocking programmes.

viii) Based on genetic distance and similarity, and high level of heterozygosity, it is better to employ all the loci as basis for selection through marker assisted selection (MAS) and cross breeding so as to ensure the highest genetic gain.

ix) More studies should be carried out on electrophoretic serum protein investigation using the Clariid fish species with hematological and biochemical profiles.

x) Research should also be carried out to show the genetic diversity between wild and cultured Clariid species.

CONTRIBUTIONS TO KNOWLEDGE

1) The study made known that the environment of the river location and the strain of Clariid fish have a significant impact on Clariid species's growth and development which were positive allometric growth pattern with a good condition of wellbeing as indicated in their condition factor.
2) The study discovered that populations of Clariid species in Kano state were outbred populations, were heterogenous and with varying degree of genetic similarity and distance.

REFERENCES

Abban, E.K. and Skibinski, D.O.F. (1988) Protein Variation in Schilbe mystus and Eutropius niloticus (Ruppel) (Pisces Siluriforms) in the Volta basin of Ghana, West-Africa. *Aquaculture and Fish Management,* 19:25-37

Abdulkarim, B. and Abdullahi, S. A. (2009). Studies on the proximate and mineral content analysis of three fresh water Fishes of the families Schilbeidae, Mormyridae and Mochokidae in Mairuwa Reservoir, Faskari, Kastina State Nigeria. *Kastina Journal of Pure and Applied Science*,1 (1): 17-22.

Abdullahi, S. A. and Abolude, D. S. (2006). Seasonal levels of some nutrients in the three Bagrids from two localities in Northern Nigeria. *Nigerian Journal of Scientific Research*, 5(2): 43-47.

Abowei, J.F.N., Davies, O.A. and Eli, A. A. (2009). Study of the Length–Weight Relationship and Condition Factor of Five Fish Species from Nkoro River, Niger Delta, Nigeria.*Current Research Journal of Biological Sciences*, 1(3): 94-98, 2009 ISSN: 2041-0778 © Maxwell Scientific Organization.

Abowei, F.N. and Hart, A.I. (2009). Some morphometric parameters of 10 finfish species from Nun River, Niger Delta, Nigeria. *Research Journal of Biological Sciences*, 4(3): 282-288.

Adekoya, B.B., Ayansawo, T.O., Idowu, A.A., Kudoro, O.A. and Salisu, A.A. eds (2006). In "Directory of fish hatcheries in Ogun State" Ogun State Agricultural Development Programme (OGADEP), Abeokuta, 18 pp.

Adeniyi, O. R., Omitoyin, S. A. and Aderibigbe, H. I. (2010). Profitability of Aquacultural apractices: Empirical experience from fish farmers in Epe Local Government Area of Lagos State. *Nigerian Journal of Fisheries*, 7(1and 2): 117-125.

Adeogun, O.A., Ayinla, O.A., Ajana, A.M. and Ajao, E.A. (1999). Economic Impact Assessmentof hybrid catfish *(Heteroclarias)* in Nigeria. Technical Report of National Agricultural Research Project (NARP). NIOMR, Victoria Island, Lagos. P27.

Adeyemi, S.O. (2011). Length-weight, Length-length relationships and Condition factor of *Synodontis robbianus* at Idah area of River Niger, Kogi State, Nigeria. *Pakistan Journal of Nutrition,* 10(6): 505-508. ISSN: 1680-5194.

Adikwu, I.A. and Haruna, A.B. (1999). Nutritional Evaluation of Hydrolysed Feather meal / plant protein blends as replacement for fishmeal protein in diets for juvenile *Clarias gariepinus (Burchell, 1882*). *African Journal of Applied Zoology*, 17: 17-22

Afolabi O.A. (1984). Quality changes of Nigeria traditionally processed freshwater species, Nutritive and Organoleptic changes, *Journal of Food Technology*, 1(19): 333 – 340.

Agbebi, O.T., Ilaboya, D.E. and Adebambo, A.O. (2013). Preliminary characterization of genetic strains in clariid species, *Clarias garepinus and Heterobranchus bidorsalis* using microsatellite markers. *African Journal of Biotechnology*,12(4): 364-369.

Agnèse, J.F.(1989). Différenciation génétique de plusieurs espèces de Silurifonnes Ouest-Africains ayantu n intérêt pour 'aquaculture. Ph.D. Thesis, University of Montpellier, France. Pp 87-89.

Agnèse, J.F. (1991). Taxonomic status and genetic differentiation among West African populations of the Chrysichthys auralus complex (Pisces, Siluriformes), based on protein electrophoresis. *Aquaculture and Fisheries Management,* 22: 229-237.

Agnese, J.F., Adepo-Gourne, B., Abban, E.K. and Fermon, Y. (1997a) Genetic differentiation among natural populations of Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae). *Heredity*, 79: 88–96.

Agnèse, J.F.; Teugels, G.G.; Galbusera, P.; Guyomard, R. and Volckaert, F., (1997b). Morphometric and genetic characterization of sympatric populations of *Clarias gariepinus and Clarias anguillaris*in Senegal. *Journal of Fish Biology*. 50: 1143-1157.

Ahmad, K. (1998). The Kano Physical Environment. Accessed online from www.kanoonline.com on 17th of November, 2014.

Alex N., Justin D. M. and Cyrus R. (2012) Length-Weight relationship and condition factor of tilapia species grown in marine and fresh water ponds. *Agriculture and Biology Journal of North America*, 3(3):117-124. ISSN Print: 2151-7517, ISSN Online: 2151-7525, doi:10.5251/abjna.2012 ScienceHuβ, http://www.scihub.org/ABJNA

Almeida, F.S. and Sodre, L.M.K. (2002). Comparative study by RAPD analysis of six species of the *Pimelodidea* family *(Osteichthyyes, Siluriformes)* from the Tibagi River, State of Parana, Brazil. *Maringa*, 24:513-517.

Altukhov, P. and Salmnekova, E.A. (1994). Straying intensity and genetic differentiation in Salmon populations. *Aquaculture and Fisheries Management,* 25(supplement 2):99-102.

Aluko, P.O. and Shaba, M. (1999). Intra-and Interspecific Hybridizatiòn studies between. Exotic *Clarias gariepinus* and two indigenous Clariid 'species. *Nigerian Journal of Genetics*, 14: 59- 63.

Aluko, P. O.(1995) Growth characteristics of first, second and backcross generations of the hybrids between *Heterobranchus longifilis* and *Clarias anguillaris*. National Institute for Freshwater Fisheries Research Annual Report. New Bussa, Nigeria. pp. 74 – 78.

Anene, A. (2005). Condition factors of four cichlid species of a man-made lake in Imo State, Southeast, and Nigeria. *Turkish Journal of Aquatic Science*, 5: 43-47.

Arulraj, D., Nabeel, M.A., Selvaraj, C., Ramaiya, S., Murugaiyan, K., Ramadoss, R., Subramanian, M. (2011). Assessment of Morphometric and Genetic variation in three freshwater fish species of the Genus *Garra (Osteichthyes: Cyprinidae). Notulac Scientia Biologicae,* 3(1): 12-16. ISSN: 2067-3205.

Asagbra, M.C., Adebayo, A.S., Ugwumba, O.A., Ugwumba, A.A.A. and Anumudu, C.I. (2014). Genetic characterization on fin fish species from the Warri River at Ubeji, Niger Delta, Nigeria. *African Journal of Biotechnology*, 13(27): 2689-2695. ISSN: 1684-5315.

Assan, N. (2013). Bioprediction of body weight and carcass parameters from morphometric measurements in livestock and poultry.*Scientific Journal of Review*. Vol 2:6

Ashoktaru B., Shahnawaz A., Surabhi K. and Prabhati K. S. (2012) Genetic characterization of two coldwater fishes from Kumaun Hills, Uttarakhand. *International Journal of Cytology, Cytosystematics and Cytogenetics* Vol. 65, No. 4, pp: 311–315.

Ayinla, O.A. (1991). Fish feed and Nutrition. Paper presented at the Fisheries Society of Nigeria (FISON) Symposium at Sokoto. Pp: 1-12.

Azeroual, A., Bills, R., Getahun, A., Hanssens, M., Kazembe, J.,Marshall, B. and Moelants, T. (2010). *Heterobranchus longifilis*. The IUCN Red List of Threatened Species (2010): e.T182390A7875559.http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T182390A7875559.en. Downloaded on 29 April 2016.

Bailey, K.M. (1997). Structural dynamics and ecology of flatfish populations. *Journal of Sea Resources*, 37(3-4): 269-280.

Balfour, H. and Yoel, P. (1981). Commercial fish farming. A Wiley-Interscience Publication. John Wiley and Sons, New York. Pp 259.

Balogun, C.Y. (1998). The sanctity of fish as food. Inaugural lecture series 14 on Fingerling from bigger size broodstocks has highermean weight gain as evidenced in the graph, Federal University of Technology, Akure, pp: 79.

Bard, J., De Kimpe, P. J., Lazard, J., Lemasson, J. and Lessent, P. (1976). Hand Book of Tropical Fish Culture. Centre Technique Forestier Tropical, France. Pp: 128.

Barlow, G.W. (1961). Causes and Significance of morphological variation in fishes. *Systematic Zoology*,10: 105-117.

Bartley, D.M., Rena, K. andImmink, A.J. (2000). The use of inter-specific hybrids in aquaculture and fisheries. *Reviews in Fish Biology and Fisheries*, 10: 325-337.

Belkher, K., Borsa, P., Chikhi, L., Ranfarte, N. and Bonhomme, F. (2004). Laboratoire Genome, Populations, Interactions CNRS UMR 5000. Universite de Montpellier II;

Montpellier: 2004. *GENETIX* 4.05, logiciel sous Windows pour la genetique des populations.

Bentzen, P.; Harris, A. and Wright, J.M. (1991). Cloning of hypervariable minisatellite and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species. In: Burke, T.; Dolf, G.A.; Jeffreys, A.J. and Wolf, R. (eds) *DNA fingerprinting: Approaches and Applications*, pp: 243-262. Birkhäuser, Basel.

Bhuiyan, A. K. M., Ratnayake, W. M. N. and Ackman, R. G. (1993). Nutritional composition of raw and smoke Atlantic mackerel (Scomber scombrus): oil-water soluble vitamins. *Journal Food Composition Analysis,* 6:172-184.

Billard, R. (2003). From polyculture to co-culture in fish-farming. In C.S. Lee (ed.). Aquaculture: retrospective and outlook – an aquaculture summit, pp 169-200. *Asian Fisheries Society*, Manila, Philippines and*World Aquaculture Society,* Baton Rouge, Louisiana, USA.

Bookstein, F.L., Chernoff, B., Elder, R.L., Humphries, J.M., Smith, G.R. and Straus, R.E. (1997). Morphometrics evolutionary biology: the geometry size and shape change, with examples from fishes. Special Publication Academy of Natural Sciences Philadelphia 15. *Biology of Fishes.* Saunders, Philadelphia.

Blackwell, B.G., Brown, M.L. and Willis, D.W. (2000). Relative weight: Status and current use in fisheries assessment and management. *Review in Fisheries Science*, 8:1-44.

Blin N., and Stafford D.W. (1976) A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Resources*, (3): 2303–2308.

Bobori, D.C., Moutopoulos, D.K., Bekri, M., Salvarina and A.P. (2010). Length-Weight Munoz,
Relationships of fresh water species caught in three Greek lakes. *Journal Biological Resources*, 14: 87-89.

Bond, C.E. (1979). Biology of Fishes. Saunders Publications Philadelphia, USA. Pp: 162-164.

Burgess, W.E. (1989). The Atlas of Fresh Water and Marine Catfishes(*Clarias gariepinus*). *Aquaculture*, 171: 49-64.

Cadrin, S. X., and Silva, V.M.(2005). Morphometric variation of yellowtail flounder. *ICESJournal of Marine Science,* 62:683–694.

Carr, S.M. and Marshall, H.D., (1991). Detection of intraspecific DNA-sequence variation in themitochondrial cytochrome-B dene of Atlantic cod (Gadus morhua) by the Polymerase Chain Reaction. *Canadian Journal of Fish Aquatic Science*, 48: 48-52.

Carter M.J. and Milton I.D. (1993). An inexpensive and simple method for DNA purifications on silica particles. *Nucleic Acids Resources*, 21 doi: 10.1093/nar/21.4.1044.

Carlson, R. L., and Wainwright, P.C.(2010). The ecological morphology of darter fishes (*Percidae*: *Etheostomatinae*). *Biological Journal of the Linnean Society,* 100:30–45.

Carvalho, G. R. (1993). Evolutionary aspects of fish distribution: Genetic variability and adaptation. *Journal of Fish Biology,* 43 (Supplement A): 53-73

Chiang, T.Y., Lee, T.W., Lin, F.J., Huang, K.H. and Lin, H.D., (2008). Isolation and Chartacterization of microsatellite loci in the endangered freshwater fish Varicorhinus alticorpus (Cyprinidae). *Conservation Genetics*, 9: 1399-1401.

.

Cemal, T., Sukran, Y., Funda, T., Emel, O. and Ihsan, A. (2005). Morphometric comparisons of African catfish, *Clarias gariepinus,* population in Turkey. *Folia Zoology*, 54(1-2): 165-172.

Cristianne, K. M. and Alexandre, W. S. H. (2009). Microsatellite variation and population genetic structure of a neotropical endangered Bryconinae species Brycon insignis Steindachner, 1877: implications for its conservation and sustainable management. *Neotropical Ichthyology*, 7(3):395-402. Sociedade Brasileira de Ictiologia

Christopher, W., Theodorakis, I. and John, W. (2004). Molecular characterization of contaminant-indicative RAPD markers. *Ecotoxicology,* 13: 303-309.

Cohen, S.A., Chang, C.Y., Boyer, H.W. and Helling, R.B. (1973). Construction of biologically functional bacterial plasmids in vitro. *Proceedings of the National Academy of Sciences*, USA 70, 3240–3244.

Chourrout, D. (1982). Tetraploidy induced by heat shocks in rainbow trout *(Salmo gairdneriR.)Reproduction, Nutrition and Development*, 22: pp 569-574.

Dankishiya, A. S. (2013). Length-Weight Relationship and Condition Factor of Five Fish Species from a Tropical WaterSupply Reservoir in Abuja, Nigeria. *American Journal of Research Communication*.www.usa-journals.com Pp: 175-187.

Dankishiya, A. S. and Kabir, H.M. (2006). The influence of Smoke-drying, Oven- drying and Sun-drying on the Nutritive value of *Oreochromis niloticus* in Gwagwalada. *Biological and Environmental Science Journal for the Tropics*, 3(2):132-134

Danzmann, R.G., Jackson, T.R. and Ferguson, M.M. (1999). Epistasis in allelic expression at upper temper- ature tolerance QTLin rainbow trout. *Aquaculture,* 173: 45–58.

Davis, G.P. and Hetzel, D.J.S. (2000). Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. *Aquaculture Research,* 31: 3–10.

De Graaf, G.J. and Janssen, H. (1996). Artificial reproduction and pond rearing of the African catfish *Clarias gariepinus* in Sub-Saharan Africa – a handbook. FAO Fisheries Technical Paper. No. 362. FAO, Rome, Italy. Pp 73.

De Graaf, G.J., Galemoni, F. and Banzoussi, B.(1995). The artificial reproduction and fingerling production of the African catfish *Clarias gariepinus* (Burchell 1822) in protected and unprotected ponds. *Aquaculture Research,* 26: 233-242.

De Moor, J.J. and Bruton, M.N. (1988). Atlas of alien and translocated indigenous aquatic animals in southern Africa. *South Africa National Scientific Programme Report,* 114, South Africa.

De Kimpe, P. and Micha, J.C. (1974). First guidelines for the culture of *Clarias lazera* in Central Africa. *Aquaculture,* 4:227-248.

De Silva S. and Davy, F.B. (2010). Success stories in Asian aquaculture. Springer. Pp 222.

Dieriener, D. and Schloetterer, C. (2003). Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes,* 3: 167-169.

Dorey, M., Mikolasek, O., Boureima, A. and Oswald, M. (2002). Savoir-faire paysan et exploitation piscicole de mares temporaires en zone sahélienne: cas du village de Tafouka (Niger). In D. Orange, R. Arfi, M. Kuper, P. Morand and Y. Poncet (éds), *Gestion Intégrée des Ressources Naturelles en Zone Inondables Tropicales.* Pp: 603–619. IRD, Paris, France.

Duguay, S.J., Park, L.K., Samadpour, L. and Dickoff, W.W. (1992). Nucleotide sequence and tissue distribution of three insulin like growth factor I prohormone in salmon. *Molecular endocrinology,* 6: 1202-1210.

Dunham, R.A. (1996). Contribution of genetically improved aquatic organisms to global food security. In: International Conference on Sustainable Contribution of Fisheries to Food Security. Government of Japan and FAO, Rome, Italy, pp: 50.

Dunham, R.A. (2004). Aquaculture and Fisheries Biotechnology: Genetic Approaches. CABI Publishing, CABI International, Wellingford, Oxfordshire. OX10 8DE UK. ISBN Number: 0851995069. www.cabi-publishing.org

Dunham, R.A., Majumdar, K., Hallerman, E., Bartley, D., Mair, G., Hulata, G., Liu, Z., Pongthana, N., Bakos, J., Penman, D., Gupta, M., Rothlisberg, P. and Hoerstgen-Schwark, G. (2001) Review of the status of aquaculture genetics. In: Subasinghe, R.P., Bueno, P., Phillips, M.J., Hough, C., McGladdery, S.E. and Arthur, J.R. (eds) *Technical Proceedings of the Conference on Aquaculture in the Third Millenium*, Bangkok, Thailand, 20–25 February 2000. NACA, Bangkok, and FAO, Rome, pp. 129–157

Eccles, D. H.(1992). FAO species identification sheets for fishery purposes. Field guide to the freshwater fishes of Tanzania, United Nations Development Programme, Rome, pp: 145.

Effiong, P.N. and Tafa, J. L. (2005). Proximate composition of nutrients in adult *Clarias gariepinus, Heterobranchus longifilis* and their hybrid *(Heteroclarias*), *Proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria* (FISON), Port Harcourt, 550 – 553.

Ekelemu, J.K. and Ekokotu, P.A. (1999): Principle and Practice of Fish seed production.In: Issues in Animal Science (ed. S.I.Omeje). Raykenedy Scientific Publishers Enugu Pp 182-196.

Ekelemu, J.K.and Ogba, O. (2005): Growth performance of *Clarias gariepinus* fed rations of maggot meal as replacement for fish meal. In *Proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria (FISON)* Port-Harcourt, 14th – 18th Nov, 2005. Pp 159 – 162.

Ekelemu, J.K. and Zelibe, S.A.A. (2006). Aspect of Hydrobiology of Lake Ona in Southern Nigeria. *Journal of Environmental Hydrology*, 14: 1-9.

Elliott, N. G., Haskard, K. and Koslow J. A. (1995). Morphometric analysis of Orange roughy *(Haplostethus atlanticus)* off the continental slope of southern Australia. *Journal of Fish Biology*, 46: 202-220.

Erhardt, G. and Weimann, C.(2007). Use of molecular markers for evaluation of genetic diversity and in animal production. *Arch. Latinoam Production Animal*, Vol.15(Supplement 1), 63-66.

Esobhawan, A.O. (2010). Resources use efficiency in fish smoking in Delta State, Nigeria: The case of women fish smokers in Udu and Uvwie Local Government Areas. *The Nigerian Journal of Agriculture and Forestry*, 2(2): 51 – 66.

Evanno, G., Regnant, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology Notes*, 14: 2611- 2620.

Eyo, J. E. (2003). Congeneric Discrimination of Morphometric Characters among members of the Pisces Genus Clarias (CLARIIDAE) in Anambra River, Nigeria. *The Zoologist,*2(1) 1- 17.

Eyo, A.A., Aluko, P.O., Okoye, F.C. and Mboko, H. (2003). Optimum Protein Requirements and Growth Performances of two sets of Genetically Improved Triploid Hybrid Fingerlings. *Nigerian Journal of Fisheries*. 1: 11-21.

Ezenwaji, O. (1989): Methods for Assement of Fish in Freshwater. IBP Handbook No. 3, Blackwell Scientific Publication, Oxford. Pp 136.

Fagbuaro, O., Oso, J.A., Olurotimi, M.B. and Akinyemi, O. (2015). Morphometric and Meristic Characteristics of Clarias gariepinus from Controlled and Uncontrolled Population from Southwestern Nigeria. *Journal of Agriculture and Ecology Research International* 2(1): 39-45, Article no.JAERI.2015.005

FAO, (2003). Review of the state of the world fishery resources: Inland fisheries. FAO fish circular No. 942 Rev. 1.

F.A.O. (2004). World Aquaculture supply of catfish and tilapia. FAO fisheries report No 733 FAO Rome, pp: 46.

Farag, F.M.M., Wally, Y.R., Daghash, S.M. and Ibrahim, A.M. (2014). Some gross morphological studies on the internal anatomy of the scaled common carp fish (*Cyprinus carpo*) and catfish (*Clariidea)* in Egypt. *Journal of veterinary anatomy*, 7(1) 15-29.

Fasakin, K. (2006). Fish farming (aquaculture) made easy. LUSIJ Publications Lagos State, Nigeria. 33

Ferguson, M. (1995). The role of molecular genetic markers in the management of cultured fish. G.R. Carvalho and T.J. Pitcher (eds). *Molecular Genetics in Fisheries*. London: Chapman and Hall, pp: 81-104.

Ferguson, M.M. and Danzmann, R.G. (1999) Inter-strain differences in the association between mitochon- drial DNA haplotype and growth in cultured Ontario rainbow trout (Oncorhynchus mykiss). *Aquaculture*, 178: 245–252.

Freeman, S. and Herron J.C. (1998). Evolutionary Analysis. Prentice Hall Publication, New Jersey.

Froese, R. (2006). Cube law, condition factor and length-weight relationships: history, meta-analysis and recommendations. *Applied Ichthyology*, 22: 241-253.

Frooze, W., Rainer, S.T. and Pauly, D. (2014). *"Clarias gariepinus and Heterobranchus longifilis"* in FishBase. March 2014 version. Retrieved from http://en.wikipedia.org/w/index.php?

Gaffney, P. (2002) Genomic approaches to marker development and mapping in the eastern oyster, *Crassostrea virginica. In*: Shimizu, N., Aoki, T., Hirono, I. and Takashima, F. (eds) Aquatic Genomics: Steps Toward a Great Future. Springer-Verlag, New York, pp. 84–91.

Galbusera, P., Volckaert, F.A., Hellemans, B. and Ollevier, F.(1996). Isolation and Characterization of microsatellite markers in the African catfish *Clarias gariepinus (Burchell, 1822*). *Molecular Ecology*, 5: 703-705.

Galbusera, P.,Volckaert, A.M and Ollevier, F.(2000). Gynogenesis in the African Catfish, *Clarias gariepinus (Burchell, 1822)* III. Induction of endomitosis and the presence of residual genetic variation. *Aquaculture*, 185: 25-42.

Galbusera P.H.; Gillemot, S.; Jouk, P.; Teske, P.R.; Hellemans, B. and Volckaert, M.J., (2007). Isolation of microsatellite markers for the endangered Knysna seahorse (*Hippocampus capensis)* and their use in the detection of a genetic bottleneck. *Molecular Ecology Notes*, 7: 638-640.

Glick, B.R. and Pasternak, J.J. (1998) Molecular Biotechnology: Principles and Applications of Recombinant DNA. ASM Press, Washington, DC.

Glowatzki-Mullis, M.L., Gaillard, C., Wigger, G. and Fries, R., (1995). Microsatellite-based parentage control in cattle. *Animal Genetics*, 26: 7-12

Grant, W. S. and Utter, F. M., (1984). Biochemical genetics of Pacific herring (Clupea pallasi). *Canadian Journal of Fish Aquatic Science*. 41: 856–864.

Hallerman, E.M., Dunham, R.A. and Smitherman, R.O. (1986) Selection or drift–isozyme allele frequency changes among channel catfish selected for rapid growth. *Transactions of theAmerican Fisheries Society*, 115: 60–68.

Haas, T. C., Blum, M.J. and Heins, D.C.(2010). Morphological responses of a stream fish to water impoundment. Biology Letters 6:803–806. Hjelm, J., L. Persson, and B. Christensen. (2000). Growth, morphological variation and ontogenetic niche shifts in perch *(Perca fluviatilis)* in relation to resource availability. *Oecologia*, 122:190–199.

Hansen, M.M. (2003). Application of molecular markers in population and conservation genetics, with special emphases on fishes. DSc Thesis, Faculty of Natural Sciences, University of Aarhus, 68 pp.

Hirpo, L.A. (2013). Reproductive Biology of *Oreochromis niloticus* in lake Beseka, Ethiopia. *Journal of Cell and Animal Biology*, 7: 116-120.

Hogendoorn, H. (1983). The African catfish *(Clarias lazera C. et V., 1840).* A new species for aquaculture. Dissertation, Wageningen Agriculture University, TheNetherlands. 135 pp.

Hoffman, E.A., Arguello, J.R., Kolm, N., Berglund, A. and Jones, A.G.(2004). Eleven polymorphic microsatellite loci in a Coral reef fish, *Pterapogon kauderni*. *Molecular Ecology Notes*, 4: 342-344.

Holden, M. and Reed, W. (1978): West African Freshwater Fishes. Nature Handbooks, Longman group Ltd., London. Pp 78.

Hood, C. S. and Heins, D.C.(2000). Ontogeny and allometry of body shape in the blacktail shiner, *Cyprinella venusta. Copeia,* pp: 270–275.

Höss, M. and Pääbo, S. (1993). DNA extraction from Pleistocene bones by a silica-based purification method. *Nucleic Acids Research*,21:3913–3914.

Hugueny, B., and Pouilly, M.(1999). Morphological correlates of diet in an assemblage of West African freshwater fishes. *Journal of Fish Biology*, 54:1310–1325.

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

Hurlbut, T. and Clay, D. (1998). Morphometric and meristic differences between shallow and deepwater populations of whitehake *(Urophycis tenuis)* in the southern Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences,* 55: 2274-2282.

Ibrahim, B.U., Auta, J., Balogun, J.K., Bolorunduro, P.I. and Dan-Kishiya, A.S. (2012). Length-weight relationship and condition factor of *Barilius niloticus*(Family: Cyprinidae) in Kontagora Reservoir, Niger State, Nigeria. *Biological and Environmental Sciences Journal for the Tropics*, 9(2): 155-158.

Idodo-Umeh, G. (2003): Freshwater Fishes of Nigeria (Taxonomy, Ecological Notes, Diets and utilization) Idodo Umeh Publishers Ltd. Benin City. Pp 232.

Imam, T. S., Bala, U., Balarabe, M. L. and Oyeyi, T. I. (2010). Length-weight relationship and condition factor of four fish species from Wasai Reservoir in Kano, Nigeria. *African Journal of General Agriculture*, 6(3): 125-130

Ihssen, P.E., Booke, H.E., Casselman, J.M., McGlade, J.M., Payne, N.R. and Utter, F.M. (1981). Stock identification: materials and methods. *Canadian Journal of Fish Aquatic Science*, 38: 1838-1855.

Islam, M.N.; Islam, M.S. and Alam, M.S., (2010). Genetic structure of different populations of walking catfish *(Clarias batrachus L.)* in Bangladesh. *Biochemical Genetics*, 45: 647- 662

Isyagi, N.A., Veverica, K.L., Asiimwe, R. and Daniels, W.H. (2009). Manual for the commercial pond production of the African catfish in Uganda. USAID-FISH Project. Pp 251.

Ita, E.O. (1998). Statistical Fisheries Survey System in Nigeria. Paper presentation at a National fish frame and catch assessment training programme, FACU, Abuja. Pp 1-4.

Jacquemin, S.J. and Pyron, M. (2013).Effects of Allometry, Sex, and River Location on Morphological Variation of Freshwater Drum *Aplodinotus grunniens* in the Wabash River, USA. *Copeia* 2013, No. 4, 740–749.

Jeffreys, A.J., Wilson, V. and Thien, S.L. (1985). Individual-specific 'fingerprints' of human DNA. *Nature,* 316: 76–79.

Jenkins, S.R., Trevor, A.N. and Stephen, J.H. (1999). Settlement and post-settlement interaction between *Semibalanus lalanoides (L) (Crustacea: Cirripedia)* and three species of fucoid canopy algae. *Journal of Experimental Marine Biology and Ecology*, 236: 49-67.

Karsi, A. (2001). Development of genomic resources for genetic enhancement of channel catfish *(Ictalurus punctatus)*. Doctoral dissertation, Auburn University, Auburn, Alabama, USA.

Karsi, A., Cao, D., Li, P., Patterson, A., Kocabas, A., Feng, J., Ju, Z., Mickett, K. and Liu, Z.J. (2002). Transcriptome analysis of channel catfish *(Ictalurus punctatus)*: initial analysis of gene expression and microsatellite-containing cDNAs in the skin. *Gene*, 285: 157–168.

Kause, A., Ritola, O., Paananen, T., Eskelinen, U. and Mantysaari, E. (2003). Big and beautiful? Quantitative genetic parameters for appearance of large rainbow trout. *Journal of Fish Biology*, (62) 610–622.

Keremah, R.I. and Green, H.J. (2005): Effect of Replacing Fish meal with Graded levels of Fish Offal on Growth and Survival hybrid Catfish fingerlings. *In Proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria* (FISON) Port-Harcourt 14th - 18th November, 2005. Pp 144 – 149.

Khallaf, E., Galal, M. and Athuman, M. (2003). The Biology of *Oreochromis niloticus* in a polluted canal. *Ecotoxicology*, 12: 405-416.

Kimmel, C. B., Aguirre, W.E., Ullmann, B., Currey, M. and Cresko, W.A.(2008). Allometric change accompanies opercular shape evolution in Alaskan threespine sticklebacks.*Behaviour*, 145:669–691.

Kincaid, H.L. (1983). Results from six generations of selection for accelerated growth rate in a rainbow trout population [abstract]. In: The Future of Aquaculture in North America. Fish Culture Section of the American Fisheries Society, Bethesda, Maryland, pp. 26–27

KNARDA, (2001). Kano Agricultural Rural Development Authority. Meteorological Station Report. Temperature Record Book, Land Management Unit. Number 11: 1-3.

KNSG, (2004). Kano State Government Official Diary, Directorate of Information, Kano, Nigeria. Pp 1-3.

Kocabas, A. (2001). Development of genomic resources and cDNA microarray technology for functional genomics of catfish. Doctoral dissertation, Auburn University, Auburn, Alabama, USA.

Kocabas, A., Kucuktas, H., Dunham, R.A. and Liu, Z.J. (2002) Molecular characterization and differen- tial expression of the myostatin gene in channel catfish *(Ictalurus punctatus).Biochemical et Biophysical Acta*, 1575: 99–107.

Köchl, S., Niederstätter, H. and Parson, W. (2005) DNA extraction and quantification of forensic samples using the phenol-chloroform method and real-time PCR. Methods *Molecular Biology*, 297:13–29.

Kocher, T.D., (1997). Genetic mapping of Tilapiine fishes. Aquaculture and Genetics in Africa, pp: 59-66.

Kolher, N., Casey, J and Turner, P. (1995). Length-weight relationship for 13 species of sharks from the western North Atlantic. *Fish Bulletin,* 93: 412-418.

Kotoulas, G.; Agnèse, J.F. and Zouros, E., (1997). Microsatellite variation in the African catfish *Chrysichthys nigrodidtatus (Lacepede, 1803) (Siluroidei, Claroteidae). Aquaculture and Genetics in Africa*, 285-288.

Kumolu-Johnson, C.A and Ndimele, P.E. (2010) Length-Weight Relationships and Condition Factors of Twenty-One Fish Species in Ologe Lagoon, Lagos, Nigeria. *Asian Journal of Agricultural Sciences* 2(4): 174-179, ISSN: 2041-3890 © Maxwell Scientific Organization.

Langerhans, R. B., Layman, C.A., Langerhans, A.K. and DeWitt, T.J.(2003). Habitat-associated morphological divergence in two Neotropical fish species. *Biological Journal of the Linnean Society,* 80:689–698.

Layman, C.A., Langerhans, R.B. and Winemiller, K.O. (2005). Body size, not other morphological traits, characterizes cascading effects in fish assemblage composition following commercial netting. *Canadian Journal of Fish Aquatic Science,* 62: 2802-2810.

Lazima, M., De los, A.P. and Ambrosio, A.M. (2002). Condition Factor in nine species of fish of the *Characidae* family in the upper Parana River Floodplain, Brazil. *Brazil Journal of Biology*, 62(1): 113-124.

Lee, Y.C. and Liu, H.C. (1996). An updated virtual population analysis of the Indian Ocean albacore stock. *Proceedings of the Sixth Expert Consult on Indian Ocean Tunas*. Pp: 269-278. Indo-pacific Tuna Development and Management Programme, Colombo, Sri Lanka, pp: 373.

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), *Heterobranchus longifilus* Valenciennes, 1840 and their reciprocal hybrids *(Pisces, Clariidae). Journal of Fish Biology*, 40: 59-79.

Little, D.C. and Edwards, P. (1999). Alternative strategies for livestock-fish integration with emphasis on Asia. *Ambio*, 28:118-124.

Liu, Z.J. and Dunham, R. A. (1998). Genetic Linkage and QTL Mapping of *Ictalurid* Catfish. Circular Bulletin 321, Alabama Agricultural Experiment Station, 19 pp. Available at: www.intl-pag.org

Liu, Z.J., Nichols, A., Li, P. and Dunham, R.A. (1998). Inheritance and usefulness of AFLP markers in chan- nel catfish (*Ictalurus punctatus)*, blue catfish *(I. furcatus*) and their Fl, F2 and backcross hybrids. *Molecular and General Genetics,* 258: 260–268.

Liu, Z. J., Karsi, A. and Dunham, R.A. (1999a). Development of polymorphic EST markers suitable for genetic linkage mapping of catfish. *Marine Biotechnology*, 1: 437–447

Liu, Z.J., Li, P., Argue, B.J. and Dunham, R.A. (1999b). Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. *Aquaculture*, 174: 59–68.

Liu, Z.J., Tan, G., Kucuktas, H., Li, P., Karsi, A., Yant, D.R. and Dunham, R.A. (1999c). High levels of con- servation at microsatellite loci among ictalurid catfishes. *Journal of Heredity*, 90: 307–312.

Madu, C.T., Ita, E.O. and Mohammed, S. (1999). Fishy Business. In: African Farming January/February, 1999. pp. 11-14.

Maes, G.E. and Volckaert, F.A.M. (2007). Challenges for genetic research in Europeaneel management. *ICES Journal of Marine. Science*,64: 1463-1471.

Magoulas, A.; Kotoulas, G.; Batargias, K. and Zouros, E., (1997). Genetic markers in marine biology and aquaculture research: when to use what. *Genetics and Aquaculture in Africa*, 67 –78.

Manohar, D., Damodar, G., Sreenivasulu, G., Senthilkumaran, B. and Gupta, A. (2005). Purification of vitellogenin from the air breathing catfish, *Clarias gariepinusFish Physiology and Biochemistry*, 31: 235-239.

Marttín, F. and De Graaf, G. (2001). Poverty alleviation through fish culture: homestead fish culture in Bangladesh. FAO Newsletter No. 27:8-11.

Marmiroli, N., Peano, C. and Maestri, E. (2003). Advanced PCR Techniques in Identifying Food Components. In: Lees M., editor. Food Authenticity and Traceability. Woodhead Publishing; Cambridge, UK. pp. 3–33.

Martyniuk, C. J., Perry, G. M. L., Moghadam, H. K., Ferguson, M. M. and Danzmann, R. G. (2003). The genetic architecture of correlations among growth-related traits and male age at maturation in rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Biology*, 63: 746–764.

Meyer, A. (1987). Morphological measurements from specimens and their X-rays: test of a method for the study of allometry and phenotypic plasticity in fishes. *Netherlands Journal of Zoology*, 37:315-321.

Meyers, T.R., Sullivan, J., Emmenegger, E., Follet, J., Short, S., Batts, W.N. and Winton, J.R. (1992). Identification of viral hemorrhagic septicaemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince Williams Sound Alaska, USA. *Dis. Aquatic Organization*, 12: 167-175.

Mickett, K., Morton, C., Feng, J., Li, P., Simmones, M., Cao, D.A., Dunham, R.A. and Lui, Z. (2003). Assessing genetic diversity of domestic population of channel catfish (*Letalurus punctatus)* in Alabama using AFLP markers. *Aquaculture*, 228: 91-105.

Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Resources*16 doi: 10.1093/nar/16.3.1215.

Miller, J. (2003): Private Sector Aquaculture Feed Development in Nigeria. In Fish Feed Development and Feeding practices in Aquaculture (ed. A.A.Eyo) A National workshop, organised by FISON/NIFFR/FAO – NSPFS. September 2003. Pp 114

Mohammad, H. G., Darioush, A. and Rahman, P. (2013). Length-Weight, Length-Length Relationship and Condition Factor of *Garra rufa* in Cholvar River of Iran. *World Journal of Fish and Marine Sciences*, 5 (4): 358-361. ISSN 2078-4589 © IDOSI Publications.

Mojekwu, T.O and Anumudu, C.I. (2013).Microsatellite markers in Aquaculture: Application in Fish population genetics. *IOSR Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT) e-ISSN: 2319-2402,p- ISSN: 2319-2399. Volume 5, Issue 4, Pp 43-48 www.Iosrjournals.Org

Morgante, M. and Olivieri, A.M. (1993). PCR_amplified microsatellites as markers in plant genetics. *Plant Journal*, 3: 175-182.

Mork, J., Ryman, N., Ståhl, G., Utter, F. and Sundnes, G.(1985). Genetic variation in Atlantic cod *(Gadus morhua)* throughout its range. *Canadian Journal of Fish Aquatic Science*. 42: 1580-1587.

Moses, Y. and Olufeagba, S.O. (2009). An Exposition on Field Identification of *Clariid* Catfishes as an Important Tool in Fish Breeding and Genetics. Manual of the National Institute for Freshwater Fisheries Research, New Bussa, Niger state.

Moyle, P.B. and Cech, J.J. Jr. (1981). Fishes: An Introduction to ichthyology. Prentice-Hall, Englewood Cliff, New Jersey.

Musick, J.A. (1998) Endangered marine fishes:criteria and identification of North Americanstocks at risk. *Fisheries*,23: 28–30.

Musick, J., Burgess, G., Cailliet, G., Camhi, M. and Fordham, S. (2000). Management of sharks and their relatives (*Elasmobranchii). Fisheries*, 25: 9-13.

Na-Nacron, U. (1999). Genetic factors in fish production: a case study of the catfish *Clarias*. In: Mustafa, S. (eds.). Genetics in sustainable Fisheries Management. Fishing News Books, London, pp. 175-187.

Na-Nacron, U., Kamonart, W. and Ngmsiri, T. (2004). Genetic diversity of walking catfish, *Clarias microcephalus* in Thailand and evidence of genetic introgression from introduced farmed *Clarias gariepinus.Aquaculture*, 240: 145-163

Nelson, C. and Christian, A. (2014). Appearance traits in fish farming: progress from classical genetics to genomics, providing insight into current and potential genetic improvement. *Front Genetics,* 5: 251-260.

Neumaier, M, Braun, A and Wagener, C (1998). Fundamentals of quality assessment of molecular amplification methods in clinical diagnosis. International Federation of Clinical Chemistry Scientific Division Committee on Molecular Biology Techniques. *Clinical Chemistry,* 44:12-26.

Norton,S.F.,Luczkovich, J.L.andMotta, P.J. (1995).Therole of ecomorphological studies in the comparative biology of fishes. *Environmental Biology of Fishes,* 44:287–304.

NPC, (2006). National Population Commission: Provisional Census Figures for Kano State, Federal Republic of Nigeria.

Nwadukwe, F. O.(1995a) Analysis of Production, Early Growth and Survival of *Clarias gariepinus* (Burchell), *Heterobranchus longifilis* (Val.) *(Pisces: Clariidae)* and their F_1 Hybrids in Ponds. *Netherlands Journal of Aquatic Ecology*, 29(2): 177 – 182.

Nwadukwe, F. O.(1995b). Hatchery Propagation of five Hybrid Groups by Artificial hybridization of *Clarias gariepinus* (B) and *Heterobranchus longifilis* (Val.) *(Clariidae*) using dry Powdered Carp Pituitary Hormone. *Journal of Aquaculture in the Tropics*,10: 1 – 11.

Nweke, S.I. and Ugwumba, A.A. (2005): Effects of Replacement of Fishmeal with Duckweed *(Lemna paucicostata)* meal on the Growth of *Clarias gariepinus(Burchell, 1822*) fingerlings. *In Proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria (*FISON) Port-Harcourt, 14th -18th November, 2005. Pp 163 – 167.

O'Connell, M. and Wright, J.M. (1997). Microsatellite DNA in fishes. *Revised Fish Biology*, 7: 331-363.

O'Connell, M., Dillon, M. C., Wright, J. M., Bentzen, P., Merkouris, S. and Seeb, J. (1998). Genetic structuring among Alaskan Pacific herring *(Clupea pallasi)* populations identified using microsatellite variability. *Journal of Fish Biology*. 53: 150–163

Ogueri, C. (2001). Fish Biology Simplified. Nimsay Printing and Publishing Company Nigeria Limited. Pp: 90.

Okaeme, A.N. (2005). Quality Control and Potential pathogens contamination in catfish production in Nigeria: Issues for Discussion, In: *Proceedings of the Annual Conference of Fisheries Society of Nigeria* (FISON), Port Harcourt, 561 – 566.

Olatunde, A. A. (1980). The Biochemical Composition and Nutritional Value of *Eutropius niloticus, Schilbe mystus* and *Physailia pellucida*. Family *Schilbeidae(Osteichthyes: Siluriformes)* from Lake Kainji, Nigeria. *Archituctural Hydrobiology*, 88: 500 – 504.

Olatunde, A.A. (1989). Approaches to the study of fisheries Biology in Nigerian Inland Waters. *Proceeding of the National Conference of Two Decades*.

Olurin,K.B. and Aderibigbe, O.A. (2006). Length-Weight Relationship and Condition Factor of Pond Reared Juvenile *Oreochromis niloticus*. *World Journal of Zoology*, 1 (2): 82-85. ISSN 1817-3098 © IDOSI Publications.

Orlando, C, Casini, R.C. and Pazzagli, M (2000). Molecular diagnosis and quality control: a program for DNA analysis with PCR amplification. *Clinical Chemistry* Lab: 7-A10.

O'Reilly, P.T., Canino, M.F., Bailey, K.M. and Bentzen, P. (2004). Inverse relationship between Fst and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Molecular Ecology,* 13: 1799–1814.

Outa, N. O., Kitaka, N.and Njiru, J.M. (2014). Length-weight relationship, condition factor, length at first maturity and sex ratio of Nile tilapia, Oreochromis niloticus in Lake Naivasha, Kenya. *International Journal of Fisheries and Aquatic Studies*; 2(2): 67-72

Pakkasmaa, S. and Piironen, J. (2001). Water velocity shapes juvenile salmonids. *Evolution Ecology,* 14: 721–730.

Park, L.K. and Moran, P. (1994). Development of Molecular genetics techniques in fisheries. Chapman and Halls, London.

Park, L.K, Brainard, M.A. and Dightman, D.A. (1993). Low levels of intraspecific variation in the mitochondrial DNA of *chum salmon*. *Molecular marine Biology Biotechnology*,2: 362-370.

Parker, G. A. (1992). The evolution of sexual size dimorphism in fish. *Journal of Fish Biology*, 41(Supplement B):1–20.

Pauly, D. (1983). Linear regressions in fisheries research. *Journal of the Fisheries Research Board of Canada*, 30: 409-434.

Pellegrin, J. (1912). The fresh water fish in Africa and their geographical distribution. *Mem Society of Zoology*, 25: 63-83 (in French)

Pepple, P.C.G. and Ofor, C.O. (2011). Length-weight relationship of *Heterobranchus longifilis* reared in earthen ponds. *Nigerian journal of Fisheries*. 8(2), Pp 315-321.

Pepinski, W., Soltyszewski, I., Janica, J., Skawronska, M. and Koc-Zorawska, E. (2002). Comparison of five commercial kits for DNA extraction from human blood, saliva and muscle samples. *Rocz. Akad. Med. Bialymst*, 47:270–275.

Perales, E., Sifuentes, A. and Garcia, J. (2007). Microsatallite variability analysis in farmed catfish (*Letalurus punctatus*) from Tamau lipas, Mexico. *Genetics and Molecular Biology*, 30: 570-574.

Pervin, M.R. and Mortuza, M.G. (2008). Notes on length-weight relationship and condition factor of fresh water fish, *Labeo boga* (Hamilton) (*Cpriniformes: Cyprinidae).* University *Journal of Zoology*, Rajshahi University, 27:97-98.

Pinheiro, A., Teixeira, C.M., Rego, A.L., Marques, J.F. and Cabral, H.N. (2005). Genetic and morphological variation of *Solea lascaris* along the Portuguese coast. *Fish Resources,* 73(1-2): 67-78.

Ponzoni, R.W and Nguyen, N.H. (2008). Program for African catfish *Clarias gariepinus* Proceedings of a Workshop on the Development of a Genetic Improvement Center, 130 pp. *World Fish Conference Proceedings* No. 1889. The WorldFish Center, Penang, Malaysia.

Pouomogne, V. (2008.) Capture-based aquaculture of *Clarias* catfish: case study of the Santchou fishers in western Cameroon. In A. Lovatelli & P.F. Holthus (eds.). Capture based aquaculture. Global overview. *FAO Fisheries Technical Paper*. No. 508:3-108. FAO, Rome, Italy.

Powles, H., Bradford, M.J., Bradford, R.G.,Doubleday, W.G., Innes, S. and Levings, C.D.(2000). Assessing and protecting endangeredmarine species. *ICES Journal of Marine Science*,57: 669–676.

Pritchirt, J.K., Stephens, M. and Donelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*,155: 945-959.

Pyron, M. (1996). Sexual size dimorphism and phylogeny in North American minnows. *Biological Journal of the Linnean Society*, 57:327–341.

Pyron, M., Fincel, M. and Dang, M(2007). Sexual size dimorphism and ecomorphology of spotfin shiner *(Cyprinella spiloptera)* from the Wabash River watershed. *Journal of Freshwater Ecology*, 22:687–696.

Quibai, Z., Fengbo, I., Li, Z. and Jianfang, G. (2006). RAPD markers between yellow catfish (*Pelteobagrus fulvidraco*) and long whiskers yellow catfish (*Pelteobagrus enpogen*) *Aeta-Hydrobiologica-sinica*, 30:482-485.

Queller, D.C., Strassmann, J.E. and Hughes, C.R. (1993) Microsatellites and kinship. Trends *in Ecology and Evolution*, 8: 285–288.

Raymond, M.L. and Rousset, F. (1995). An exact test for population differentiation. *Evolution* 49, 1280-1283.

Reynaldo, A.L. and Cesar, G.D. (2014). Enzyme polymorphism among natural population of damselfish collected in selected reefs of Iligan Bay and Camiguin Island, Philippines. *European Journal of Zoological Research,* 3(2): 18-22.

Reed, W., John, B.; Hopson, A.J. Jonathan, Y.I. and Yaro, J. (1967). Fish and fisheries of Northern Nigeria (First Edition). Published by Ministry of Agriculture Northern Nigeria. Pp 226.

Reid, D. T. and Peichel, C. L. (2010). Perspectives on the genetic architecture of divergence in body shape in sticklebacks. *Integration Computation Biology,* 50: 1057–1066.

Riedel, R., Caskey, L.M., and Hurlbert, S.H. (2007). Length-weight relations and growth rates of dominant fishes of the Salton Sea: implications for prediction by fish-eating birds. *Lake and Reservoir Management,* 23: 528-535.

Ricker, G.O. (1978). Computation and Interpretation of Biological Statistics of Fish Populations. Fish Research Biology. Canada Bulletin, 191.

Rognon, X., Teugels, G.G., Guyomard, R., Galbusera, P, Andriamanga, M, Volckaert, F. and Agnese J.F. (1998). Morphometric and allozyme variation in the African catfishes *Clarias gariepinus* and *Clarias anguillaris*. *Journal of Fish Biology*,53:192-207.

Rosenstein, S. and Hulata, G.(1993) Sex Reversal in the genus *Oreochromis* Immersion of eggs and embryos in oestrogen solutions is ineffective. *Aquaculture and Fisheries Management.* 23: 669 – 678.

Ruzzante, D.E.; Taggart, C.T. and Cook, D.(1998). A nuclear DNA basis for shelf and bank-scale population structure in NW Atlantic cod *(Gadus morhua):* Labrador to Georges Bank. *Molecular Ecology*, 7: 1663-1680.

Saad, Y.M., Rashed, M.A., El-deep, S.I., El-Gamal, A.A. and Saiid, M.M. (2002). Molecular genetic marker and phylogenetic relations for some Tilapia species. ISSN 1110-5372, 9th International conference, Aleppo University, Syria. *Journal of Union Arab Biologists* Cairo, 18: 27-44

Sabina De Innocentiis, Miggiano, E; Ungaro, A.; Lesti, A.; Andreoli, A.C.; Livi, S. Sola, L. and Crosetti, D.(2002). Use of microsatellites to infer the geographical origin of individual breeders from an Italian commercial hatchery. ICES CM.

Sahoo, S. K., Giri S. S., Sahu, A. K. and Ayyappan, S.(2003) Experimental Hybridization between Catfish *Clarias batrachus* (Linn.) x *Clarias gariepinus* (Bur.) and Performance of the Offspring in Rearing Operations. *Asian Fisheries Science,* 16: 157 – 166.

Sambrook, J.; Fritsch, E.F. and Maniatis, T., (1989). Molecular Cloning a Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Sanches, A. and Galetti, P.M., (2006). Microsatellites loci isolated in the freshwater fish *Brycon hilarii*. *Molecular Ecology Notes*, 6: 1045-1046.

Sanetra, M.; Henning, F.; Fukamachi, S. and Meyer, A., (2008). A microsatellite-based genetic linkage map of the Cichlid fish, *Astatotilapia burtoni (*Teleostei): A

comparison of genomic architectures among rapidly speciating *Cichlids*. *Genetics*, 182: 387-397.

Sanger, F., Nicklen, S. and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*, USA 74, 5463–5467.

Santos, M.S., Gasper, M.B., vasconselus, P. and Monterio, C.C. (2002). Weight-Length relationships for 50 selected fish species of the Algarve coast (southern Portugal). *Fisheries Research*, 59: 289-295.

SAS, (2000). The statistical analysis system for windows. SAS^{R} Software, version 9.0. Cary, NC. USA.

Sea Grant Research (1987). Genetics Guidelines for Fisheries Management. Report Number, 17

Seeb, L.W., Antonovich, A., Banks, A.A., Beacham, T.D., Campbell, R., Decovich, N.A., Garza, J.C., Guthrie, C.M., Kundrigan, T.A., Moran, P., Narum, S.R., Stephenson, J.J., Supernault, K.J., Teel, D.J., Templin D.W., Wenburg, J.K., Young S.E. and Smith, C.T. (2007). Development of Standardized DNA database for *Chinook salmon*, *Fisheries*,32: 540-552.

Schuler, G.D., Boguski, M.S., Hudson, T.J., Hui, L., Ma, J., Castle, A.B., Wu, X., Silva, J., Nusbaum, H.C., Birren, B.B., Slonim, D.K., Rozen, S., Stein, L.D., Page, D., Lander, E.S., Stewart, E.A., Aggarwal, A., Bajorek, E., Brady, S., Chu, S., Fang, N., Hadley, D., Harris, M., Hussain, S., Hudson, J.R. Jr (1996). Genome maps 7. The human transcript map. *Wall chart Science*, 274: 547–562.

Shahlina, H. and Biswas, S.P. (2014). Length-Weight relationship and condition factor of *Botia dario* (Hamilton-Buchanan). *International Journal of Fisheries and Aquatic Studies*, 2(1): 244-247.

Shaheena, S. and Yousuf, A.R. (2012). Length-weight relationship and condition factor in *Puntius conchonius* (Hamilton, 1822) from Dal Lake, Kashmir. *International Journal of Scientific and Research Publications*, 2(3) ISSN 2250-3153

Sheasby, C. (2009) African Catfish *(Clarias gariepinus)* Zandvlei Trust, accessed from http//www.scienceinafrica.co.za on 17/09/2015.

Shine, R. (1989). Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *The Quarterly Review of Biology*, 64:419–461.

Shubina, E.A., Ponomareva, E.V. and Glubokov, A.I. (2005). Microsatellite analysis of the population structure of the Bering Sea Pollock. CBS Pollock workshop June 2005 WP-10.

Sikoki, F.D. and Otobotekere, A.J.T.(1999). Fisheries. In: The Land People of Bayelsa State Central Niger Delta. E.C. Alagoa, (Ed.). Port Harcourt, pp: 301-319.

Silva, S.S. (1985). Body condition and nutritional ecology of *Oreochromis mossambicus* (Pisces, *Cichlidae)* population of man-made lakes in Sri-Lanka. *Journal of Fish Biology*, 27: 621-633.

Simmones, M., Mickett, K., Kucuktas, H., Li, P., Dunham, R.A. and Lui, J. (2006). Comparison of domestic and wild channel catfish *(Letalurus punctatus*) populations provides no evidence for genetic impact. *Aquaculture*, 252: 133-146.

Skelton, P. H. (2001) A complete guide to the freshwater fishes of Southern Afraca. Struik Publishers, Cape Town.

Skelton, P. H. (1993) A complete guide to the freshwater fishes of Southern Afraca. Southern Book Publishers, pp: 388 Cape Town.

Smith D.S., Maxwell P.W., and de Boer S.H. (2005). Comparison of several methods for the extraction of DNA from potatoes and potato-derived products. *Journal of Agriculture and Food Chemistry*53:9848–9859.

Solomon, J.R. and Ezigbo, M.N. (2010). Polyculture of Heteroclarias/Tilapia under different feeding regimes. *New York Science Journal,* 3(10): 42-57. http://www.sciencepub.net/newyork

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology*, 98: 503-507.

SPSS, (2007). Statistical Package for the Social Science. An analytical software for data analysis version 2015. IBM SPSS Modeler Solution Publishers

Stergiou, K.I. and Moutopoulos, D.K. (2001). A review of length-weight relationships of fishes from Greek marine waters. Naga, *ICLARMQuart*, 24(1-2): 23-39.

Stiassny, M.L.J., Teugels, G.G. and Hopkins, C.D. (2007). Poissons d'eaux douces et saumâtres de basse Guinée, oust de l'Afrique centrale / The Fresh Water Fishes of Lower Guinea West-Central Africa. Volume 1. IRD Editions, Paris, France. 800 pp.

Strauss, R.E. and Fuiman, L.A. (1985). Quantitative comparisons of body form and allometry in larval and adult pacific sculpins (*Teleoste: Cottidae*). *Canadian Journal of Zoology*,63: 1582-1589.

Strauss, R.E. (1984). Allometry and Functional feeding morphology in *haplochromine cichlids*. Pp 217-219 in A.A. Echelle and I. Kornfield, editors. Evolution of fish spp flocks. University of Marine at Orono press, Orono.

Suleiman**,** I.O.,Akpa, G.N. and Kabir, M. (2015). The Length-Weight Relationship and ConditionFactor (Ponderal Index) of Cultured African Catfish (*Clarias gariepinus). Proceeding of the 39th Annual Conference of the Genetic Society of Nigeria, 27th to 31st October, 2015 in Bauchi. Pp: 507-510.*

Suneetha, K.B.G. (2007) Morphological Heterogeneity in some estuaries populations of the Catfish *(Arius jella)* in Sri Lanka. *Cey, Journal.of Science (Biological Science)*, 36(2): 100-107

Swain, D.P., Ridell, B.E. and Murray, C.B. (1991). Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin. *Canadian Journal of Fish and Aquacultural Science*, 48(9): 1783–1791.

Tailor, J.N., Snyder, D.B. and Courtenay, W.R. Jr. (1986). Hybridization betweentwo introduced, substrate- spawning tilapias (*Pisces: Cichlidae*) in Florida. *Copeia*,1986: 903-909.

Tan, G., Karsi, A., Li, P., Kim, S., Zheng, X., Kucuktas, H., Argue, B.J., Dunham, R.A. and Liu, Z.J. (1999). Polymorphic microsatellite markers in *Ictalurus punctatus* and related catfish species. *Molecular Ecology*, 59: 190–194.

Tautz, D., (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, 17: 6463-6471.

Taylor, P., Blackwell, H.G., Brown, M.L. and Willis, D.W. (2010). Status and current use in fisheries assessment and management. *Review in Fisheries Science*, 8(1): 37-41.

Tel-Zur, N.A.S., Myslabodski, D. and Mizrahi, Y. (1999). Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera *hylocereus* and *selenicereus* (cactaceae).*PlantMolecular Biology. Rep*.,17:249–254.

Tesch, F.W. (1968). Age and Growth In: Methods for assessment of fish production in fresh waters. W.E. Rickers (Eds). Blackwell Scientific Publication, Oxford, pp:93-123.

Teske, P.R., Cowley, P.D.,Forget, R.G. and Beheregaray, L.B.(2009). Microsatellite markers for the roman, Chrysoblephus laticeps (Teleostei: Sparidae), an overexploited seabream from South Africa. *Permanent Genetic Resources Note*, pp:1162-1164.

Teugels, G.G. (1982). Preliminary results "of- morphological study or fine African Species of the subgenus *Clarias* (Pisces. Claridge). *Journal of Natural History*, 16: 439-4.64.

Teugels, G. G., Denayer, T. and Legendre U. (1990). Systematic revision of the African catfish genus *Heterobranchus* Geohrey saint Hilaire 1809 (Pisces *lasiidae)Zoological Journal of the Linnean Society*, 92: 237-257

Teugels, G.G., Ozouf-Costaz, C., Legendre, M. and Parrent, M., (1992). A karyological analysis of the artificial hybridization between *Clarias gariepinus* (Burchell 1822) and *Heterobranchus longifilis* (Valenciennes, 1840)(Pisces: Clariidae). *Journal of Fish Biology*, 40: 81-86.

Tilghman, S.M. (1996) Lessons learned, promises kept a biologist's eye view of the genome project. *Genome Research*, 6: 773–780.

Torres, R.G.A., Gonzales, P.S. and Pena, S.E. (2010). Anatomical, histological and ultrastructural description of the gills and liver of the Tilapia. *International Journal of Morphology*, 28(3): 703-712.

Turan, C. (2004). Stock IdentificationofMediterraneanHorseMackerel*(Trachurus mediterraneus*) using Morphometric and Meristic Characters. *ICES Journal of Marine Science,* 61: 774–781.

Ude, E.F., Ugwu, L.L.C., Mgbenka, B.O and Nwani, C.D. (2011). Evaluation of Length-weight relarionship of fish species of Ebonyi River, Nigeria. *Nigerian Journal of Fisheries,* 8(1):136-144.

Uiblein, F. (1995). Morphological variability between populations of *Neobythites (Pisces: Ophididae*) from the deep Red Sea and the Gulf of Aden. *Marine Ecology Progress Series,* 124: 23-29.

Umoh, I. A., Nlewadim, A., Obuba, L. E, and Oguntade, O. R. (2015). Morphometric and Meristic Characteristics of Hybrid Catfish from Selected Fish Farms in Southern Nigeria. *International Journal of Biotech Trends and Technology (IJBTT*) 10(2) 7-11.

USDA (United State*s* Department of Agriculture) (1988) Aquacultural Genetics and Breeding. National Research Priorities. USDA, Cooperative State Research Service, Washington, D.C. 61 pp.

Usman, B.A., Agbebi, O.T., Bankole, M.O., Oguntade, O.R. and Popoola, M.O. (2013) Molecular characterisation of two cichlids populations (Tilapia *guineensis* and *Sarotherodon melanotheron*) from different water bodies in Lagos State, Nigeria. *International Journal for Biotechnology and Molecular Biology Research*, 4(5): 71-77 ISSN 2141-2154. Academic Journals http:// www.academicjournals.org/IJBMBR

Van Der Bank, F.P., Grobler, J.P. and Preez, H.H. (1992). A comparative biochemical genetic study of three populations of domesticated and wild African catfish. *Comparative Biochemistry and Physiology*, 101B: 387-390.

Vignal, A.; Milan, .D, ; SanCristobal, M.; and Eggen, A., (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Sel. Evol*., 34: 275-305

Volckaert, F.A., Hellemans, B.A., Galbusera, P., Ollevier, F., Sekkali, B. and Belayew, A. (1994). Replication, expression and fate of foreign DNA during embryonic and larval development of the African catfish. *Molecular Marine Biology and Biotechnology,* 3(2): 57-69.

Volckaert, F. and Agnese, J.F.(1996). Evolutionary and population genetics of *Siluroidei*. In M. Legendre and J.P. Proteau (ed.), The biology and culture of catfishes. *Aquatic Living Resources*, 9, (Suppl.) 1:81-92.

Wachirachaikam, A. and Na-Nakorn, U.(2007). Subject: Fisheries.*Proceedings of the 45th Kasetsart University Annual Confernce,* Kasetsart, pp: 82-89.

Wainwright, P. C., and Richard. B.A.(1995). Predicting patterns of prey use from morphology of fishes. *Environmental Biology of Fishes,* 44:97–113.

Waldbieser, G.C. and Wolters, W.R. (1999) Application of polymorphic microsatellite loci in a channel catfish *Ictalurus punctatus* breeding program. *Journal of the World Aquaculture Society,* 30: 256–262.

Waldman, J.R. (2005). Definition of stocks: an evolving concept. In: Cadrin, S.X., Friedland, K.D., Waldman J.R. (eds) Stock identification methods. Applications in fisheries science. *Elsevier Academic Press,* San Francisco, pp 7–16.

Walker, J. A. (1997). Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biological. Journal of Linnean Society,* (61) 3–50.

Walker, J. A., and Bell. M.A.(2000). Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks *(Gasterosteus aculeatus*). *Journal of the Zoological Society of London,* 252:293–302.

Walker, J. A. (2010). An integrative model of evolutionary covariance: a symposium on body shape in fishes. *Integrated. Comparative Biology,* (50): 1051–1056.

Walsh, P.S., Metzger, D.A. and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, 10:506–513.

Weatherly, A.H. and Gills, H.S. (1987). The biology of fish growth. London academic Press. 433443.

Weatherspark, (2013). Historical weather for 2013 in Kano, Nigeria. Mallam Aminu Kano International Airport (Kano, Nigeria). Weatherspark.htm Retrieved 10th November, 2015.

Webster, C.D., Tiu, L.G. and Morgan, A.M.(1999): Effect of partial and total replacement of Fish meal on Growth and Body composition of Sunshine Bass *(Morone chrysops x M saxatilis)* Fed Practical Diets. *Journal of the world Aquaculture Society,*30(4): 443-453

Wei, L. and Musa, N. (2008). Phenotyping, genotyping and whole cell profiling of *Edwardstella tarda* isolated from cultivated and natural habitat fresh water fish. *American/EurAsian Journal of Agricultural and Environmental Science,* 3: 681-691

Weir, B. S. (1996). Genetic data analysis II. Sunderland (MA) Sinauer Associates, USA, pp. 56-88.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, 20:249–278.

Wimberger, P. H. (1991). Plasticity of jaw and skull morphology in the neotropical cichlids *Geophagus brasiliensis* and *G. steindachneri*. *Evolution*, 45: 1545-1561.

Wimberger, P. H. (1992). Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces: *Cichlidae*) *Biological Journal of the Linnaean Society*, 45: 197-218.

Winans, G.A. (1985). Geographical variation in the milkfish (*Chanos chanos*). II. Multivariate morphological evidence. *Copeia*,1985: 890-898.

Wirgin, I.I., Proenca, R. and Grossfield, J. (1989) Mitochondrial DNA diversity among populations of striped bass in the southeastern United States. *Canadian Journal of Zoology*, 67: 891–907.

Wolfsberg, T.G. and Landsman, D. (1997). A comparison of expressed sequence tags (ESTs) to human genomic sequences. *Nucleic Acids Research*, 25: 1626–1632.

Wong, L.L. (2011). DNA Barcoding and Related Molecular Markers for Fish Species: Authentication, Phylogenetic Assessment and Population Studies. Ph.D Thesis submitted to Graduate Faculty, Auburn University, Alabama, United States.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J. and Watson, R. (2009). Impacts of Biodiversity loss on ocean ecosystem services. *Science*, 314: 787-790.

Wund, M. A., Valena, S., Wood, S. and J. A. Baker. (2012). Ancestral plasticity and allometry in threespine sticklebacks reveal phenotypes associated with derived, freshwater ecotypes. *Biological Journal of the Linnean Society*, 105:573–583.

Yang, Y.P. and Womack, J.E. (1997) Construction of a bovine chromosome 19 linkage map with an inter-species hybrid backcross. *Mammalian Genome*, 8: 262–266.

Yilmaz, M., Cigremis, Y., Turkoz, Y. and Gaffaroglu, M. (2000). An electrophoretic taxonomic study on blood serum proteins of some fishes in Karakaya Dam Lake. *Eurasian Journal of Veterinary Sciences*, 16(1): 8992-8997.

Yilmaz, M., Yilmaz, H.R. and Alas, A. (2007). An electrophoresis taxonomic study on serum proteins of *Acanthobrama marmid, Leuciscus cephalus* and *Chondrostoma regium. Asia Journal of Bioscience*, I: 22-27.

Yue, G.H. and Orban, L. (2001). Rapid isolation of DNA from fresh and preserved fish scales for polymerase chain reaction. *Marine Biotechnology*,3:199–204.

Zhen, W.; Stacey, N.E.; Coffin, J. and Strobeck, C., (1995). Isolation and characterization of microsatellite in goldfish *Carassius auratus*. *Molecular Ecology*, 4: 791-792.