GENETIC AND MORPHOLOGICAL CHARACTERIZATION OF WILD AND CULTURED SPECIES OF *CLARIAS* SCOPOLI, 1777 AND *HETEROBRANCHUS* GEOFFROY SAINT-HILAIRE, 1808

BY

ESTHER YOGBABALIAT, YASHIM

DEPARTMENT OF BIOLOGICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

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ESTHER YOGBABALIAT, YASHIM, BSC. ZOOLOGY, A.B.U. (2009)

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) DEGREE IN BIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

FEBRUARY, 2014

DECLARATION

I declare that the work in this thesis entitled "GENETIC AND MORPHOLOGICAL CHARACTERIZATION OF WILD AND CULTURED SPECIES OF *CLARIAS* SCOPOLI, 1777 AND *HETEROBRANCHUS* GEOFFROY SAINT-HILAIRE, 1808" was carried out by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree at this or any other institution.

ESTHER YOGBABALIAT YASHIM _____ _____

Signature Date

CERTIFICATION

This thesis entitled "GENETIC AND MORPHOLOGICAL CHARACTERIZATION OF WILD AND CULTURED SPECIES OF *CLARIAS* SCOPOLI, 1777 AND *HETEROBRANCHUS* GEOFFROY SAINT-HILAIRE, 1808" by ESTHER YOGBABALIAT YASHIM meets the regulations governing the award of the degree of Master of Science in Biology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. T. O. L. Aken'Ova _____ _____

Chairman, Supervisory Committee Signature Date

Department of Biological Sciences,

Ahmadu Bello University, Zaria

Prof. J. Auta _____ _____

Member, Supervisory Committee Signature Date

Department of Biological Sciences,

Ahmadu Bello University, Zaria

Prof. A. K. Adamu _____ _____

Head, Signature Date

Department of Biological Sciences,

Ahmadu Bello University, Zaria

Prof. A.A. Joshua _____ _____

Dean, School of Postgraduate Studies Signature Date

Ahmadu Bello University, Zaria

DEDICATION

This thesis is dedicated to the Father of Life, Jesus, The Rock on which I stand and to the Holy Spirit my Teacher.

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I sincerely appreciate all the fishermen and fish vendors at River Galma and Bagoma reservoir for the supply of fish species that were rare.

To all my friends, loved ones and siblings, you are the best.

ABSTRACT

Genetic and morphological studies on wild and cultured species of *Clarias* and *Heterobranchus* were conducted from August 2012 to January 2013. A total of forty (40) specimens comprising *Clarias gariepinus* (10), *C. anguillaris* (5), *C. galmaensis* (5), *Heterobranchus bidorsalis* (5), *H. longifilis* (10) and *H. isopterus* (5) were obtained from River Galma, Zaria and Bagoma Reservoir Birnin Gwari (wild), Miracle Fish Farm, Zaria and National Institute for Freshwater Fisheries Research (NIFFR), New Bussa (cultured). Morphometric data for each fish sampled were taken following Teugels (1986a) and the physico-chemical parameters of the habitats from which the fishes were collected were also determined. A total of eighty (80) samples of muscle and fin tissue of fish were obtained and preserved at -20°C for molecular analysis. DNA was extracted from the tissues using genomic Quick-gDNA™ MiniPrep (50 Preps) w/ Zymo-Spin IIN Columns DNA extraction kits. The cytochrome b gene was amplified and a portion of mtDNA containing the gene was amplified as described by Briolay *et al*. (1998). Restriction digest of the PCR-products was conducted. All products were separated, purified and visualized under ultra violet light and documented. The morphometrics showed that *Clarias gariepinus* (22.8-43.8cm) and *Heterobranchus longifilis* (26.3-39.8cm) had the largest sizes range (total length) followed by *Clarias anguillaris* (17.8-39.0cm) and *Heterobranchus bidorsalis* (21.7-38.5cm). The smallest in size were *Heterobranchus isopterus* (18.4-25.6cm) and *Clarias galmaensis* (15.7-20.5cm) from all the habitats, the same trend was also observed in other morphometric characters although there were some slight variations. Electrical conductivity (230$\pm$16.97µS), Total Dissolved Solids (114.25$\pm$8.84ppm) and alkalinity (6.2$\pm$1.50) had

higher values in the culture environment while lower values (59±11.0 µS, 29±5.0 ppm and 2.05±0.65 respectively) were obtained in the wild. Higher values were observed in Biochemical Oxygen Demand (4.38±2.09mg/L), Dissolved Oxygen (7.7±1.41mg/L) and PO_4-P (0.38±0.14mg/L) in the wild habitat whereas lower values were observed in the culture environment. Little variations were observed in pH, temperature, hardness and NO_3-N for both wild and culture environments, which were within acceptable limits for fish production. The marked positive correlation($r<0.9$) in morphometrics and physico-chemical parameters showed interaction between them. Significant differences ($P<0.05$) in morphometrics and physico-chemical parameters were observed. Amplification of cytochrome b gene in both fin and muscle tissues of the fish species was successful. Although genetic variation was observed between wild and cultured species, there is a close relationship between them, confirming that they are members of the same family, Clariidae, with good prospects for culture.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Genetic diversity plays a very important role in the survival and adaptation of a species. When the environment of a species changes, slight gene variations are necessary to produce changes in the organism's anatomy, which enable it to adapt and survive in the changed environment. A species that has a large degree of genetic diversity within its population will have more variations from which to choose fit alleles. Increase in genetic diversity is essential for the evolution of species; species that have little gene variation are at great risk. With very little gene variation within the species, healthy reproduction becomes increasingly difficult and offspring often deal with problems similar to those associated with inbreeding. Genetic variation in alleles that occurs within a population is important because it provides the raw materials for natural selection (www.nbii.gov, 2011). Alterations to the physical habitat could threaten animal populations through a possible erosion of their genetic variability by genetic drift or inbreeding leading to extinction (Gaines *et al.*, 1997).

Genetic variation between and within populations can be affected by geographical distances, barrier to migration and time. Over time, changes in genetic variations between populations can lead to evolutionary differences between them. These changes can be identified using allele frequencies at protein loci. Both gene flow and genetic drift can cause significant changes in allele frequencies at protein loci. Gene flow is the movement of genes from one population to another; it requires that individuals migrate between populations and breed.

Genetic drift is the evolutionary force that causes the alleles to be eliminated over time by random chance (The Fish Grinders, 2009).

Studies investigating the genetic composition of fish populations have been conducted for a number of decades, initially using protein coding allozyme loci (Utter *et al*., 1987) and then, starting in the mid-1980's, using mitochondrial DNA (mtDNA) polymorphisms (Avise, 2004). More recently, tandemly repeated microsatellite DNA markers have become the molecular markers of choice for determining intraspecific population genetic relationships. (Koskinen *et al*., 2002). In general, markers that are used today utilize the Polymerase Chain Reaction (PCR) as it enables analysis of archive materials such as scales.

Clariid catfishes (order Siluriformes) occur in Africa, Asia Minor, South-east Asia and the Indian subcontinent (Teugels and Adriaens, 2003). Two genera of the family Clariidae, *Clarias* and *Heterobranchus,* along with the cichlids are the most utilized in African aquaculture (Agnese *et al*., 1995); this is due to their fast growth rate, resistance to diseases and capacity for high stocking density in clariids (FAO, 2000). Clariidae are known as air-breathing or labyrinth catfishes, possessing suprabranchial organs that enable them to respire (Teugels, 1996). Their extreme hardiness and ability to survive in poorly oxygenated water makes them popular for cultivation.

Animals and plants remain the source of human food, with fish being one of the most important. This is because fish is the major source of protein for many people and constitutes the main part of the diet of many cultures. Fish and fisheries not only provide a significant portion of protein available for human consumption, but they are also an

economically significant activity, providing jobs and investment opportunities and, for many countries, a means of improving a balance of international trade (Ciftci and Okumus, 2002).

1.2 Statement of the problem

Fishery management has largely been concerned with the abundance and size of fish available for harvesting, but in the long term this may cause extinction of the population. Concern with reduction in/of genetic resources of fish is part of a larger global concern for the genetic resource of the biosphere. Despite their importance, their biological diversity is being threatened by various anthropogenic activities such as urbanization, habitat fragmentation, over-exploitation and the expansion of aquaculture business. Hybridization between *Clarias* and *Heterobranchus* also raises concern on genetic introgression when the hybrid invades natural waters which may destroy the genetic integrity of these species.

There is generally limited information on genetic variation among *Clarias* and *Heterobranchus* species and the lack of reliable basic taxonomic information greatly hampers an efficient and sustainable exploitation of these resources (Turan *et al*., 2005). Fingerlings of unknown or poor genetic quality may be sold to farmers as a result of little information available on the current status of the genetic diversity of these species.

1.3 Justification

This study will enhance an understanding of the level of genetic variation that will provide management guidelines for commercial use and conservation of *Clarias* and *Heterobranchus* species. There is also a need to understand the genetic composition of natural population in order to evaluate the latent genetic effects induced by hatchery

operations. *Clarias* and *Hesterobranchus* are the most culturable fish species in Nigeria (Tukura *et al*., 2005) and the use of molecular markers in genetic studies have yielded tremendous success (Gao *et al*., 2001; Fujii, 2002). Species for aquaculture must be properly identified and classified to preserve their germplasm and monitor genetic changes (Legendre *et al*., 1992).

1.4 Aim and Objectives

The aim of this research is to investigate the genetic and morphological diversity among wild and cultured catfish species utilized in aquaculture using Restriction Fragment Length Polymorphism (RFLP).

The objectives are to determine the:

1. Morphometric similarity and diversity within and between the populations of wild and cultured species of *Clarias* and *Heterobranchus.*
2. Physico-chemical properties of the aquatic habitats of the fishes and their interaction with the fishes.
3. Genetic diversity of wild and cultured *Clarias* and *Heterobranchus* spp.

1.5 Hypotheses

H_1: The morphometrics of wild and cultured *Clarias* and *Heterobranchus* spp. do not vary significantly.

H_2: Changes in physico-chemical properties of the aquatic environment have no effect on *Clarias* and *Heterobranchus* species.

H_3: Wild and cultured *Clarias* and *Heterobranchus* species do not show genetic variation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Clariid catfishes

The family Clariidae Bonaparte, 1846, in the order Siluriformes Cuvier, 1817 contributes significantly to annual freshwater fish production in South and Southeast Asia and Africa (Na Nakorn, 1999). This family is naturally distributed all over these areas with the highest genetic diversity found in Africa. One fifth of all known catfish genera occur in Africa and South-East Asia. However, the highest diversity is found in Africa with 14 genera and 92 species (Teugels, 1986a). Only 2 genera with about 17 species are presently known from Asia (Teugels, 1996).

Generally, the Clariidae are elongated, with long dorsal and anal fins, and four pairs of barbels. A remarkable character in this family is the presence of a suprabranchial organ, formed by folds of the second and fourth branchial arches. This organ enables the fishes to breathe atmospheric air, implying that they can survive outside water for some time. They are also known for "walking" on land over distances of several hundred meters using their pectoral spines for support (Teugels and Gourène, 1997).

The genus *Clarias* is the most common and most popular of the family Clariidae containing 32 species in Africa (Teugels, 1986a). One of them, *Clarias gariepinus* (Bürchell, 1822) is of great economic importance as it is the most cultured catfish in Africa and the third most cultured catfish species in the world (Garibaldi, 1996). Another economically important

species of this genus is *Clarias anguillaris* (Linnaeus, 1758), which is also cultured in Nigeria (Garibaldi, 1996).

Aken'Ova (2007) described a new species *Clarias galmaensis* bringing the total number of known *Clarias* spp. in Nigeria to eleven. *Clarias galmaensis* is characterized by its small size, oval head in dorsal outline and tiny cream to dirty-white spots on an olive to brownish background. This species is quite distinct from the other two species (*C. gariepinus* and *C. anguillaris*) in River Galma, Zaria, Nigeria.

The genus *Heterobranchus* is recognized and differentiated from *Clarias* by the presence of a dorsal adipose fin, in addition to the rayed dorsal fin. The following four species of this genus are known: *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire 1809, *H. longifilis* Valenciennes, 1840, *H. isopterus* Bleeker, 1863 and *H. boulengeri* Pellegrin, 1922; the last is the smallest member of the genus (Reed *et al.*, 1967; Froese and Pauly, 2013).

According to Teugels *et al*. (1990), *Heterobranchus* Geoffroy Saint-Hilaire, 1809 is generally considered the most primitive genus, mainly because of the presence of a large adipose fin (22-35% standard length) supported by 19 to 26 elongated neural spines. *Clarias* Scopoli, 1777 is the more speciose genus; Teugels (1986a) recognized 32 valid species in Africa and arranged them in 6 subgenera. All subgenera except *Clarias (Dinotopteroides)* Fowler, 1930, lack an adipose fin; it has a small adipose fin (6-16 % SL), supported by 6 to 12 elongated neural spines.

2.2 Genetic diversity of fishes

The studies of diversity and variation have been widely carried out in different populations and species of fish and form the basis of many biological investigations such as evolution (Cesaroni *et al*., 1994), animal conservation and management (Sherley, 1996; Britten *et al*., 1997) using different approaches including the study of morphological characters (Vicario *et al*., 1988) and DNA markers (Carvalho & Pritcher, 1995). One of the most important criteria for any efficient conservation and management programme is the taxonomic clarification of species complexes as well as the assessment of genetic biodiversity within and among populations. According to Paul-Michael *et al*. (2004), species are the currency of biology. Genetic variation study is important for stock management in fisheries. The concept of 'stock' is essential for both fisheries and endangered species management (Begg and Waldman 1999). Begg *et al*. (1999) described stocks as random groups of fish large enough to self-reproduce, with members of each group having similar life-history characteristics. Genetic variation study is useful to understand the stock structure of a species and design an appropriate management guideline in fisheries where multiple stocks are exploited differentially (Ricker, 1981). The process of defining fish stock is essential for effective management. Fisheries management generally aims to achieve maximum sustainable production from fish stocks (Begg & Waldman, 1999). DNA tests and sequencing are the most popular methods for species identification and conservation studies of animal specimens at present (Tsai *et al*., 2007; Gorgan, 2009).

2.3 Molecular markers used in fisheries

Molecular markers are DNA sequences used to "mark" or track particular loci on chromosomes, i.e. marker genes. A marker is a gene with a known location or clear phenotypic expression that is detected by analytical methods or an identifiable DNA sequence that facilitates the study of inheritance of a trait or a gene. The markers must be readily identifiable in the phenotype, for instance, by controlling an easily observable feature or by being readily detectable by molecular means, e.g. microsatellite marker (Williamson *et al*., 2001). Today, many molecular methods are available for studying fish populations but they are basically categorized under two types of markers, protein and DNA. There are three general classes of genetic markers that are routinely used in population genetic and phylogenetic studies: (1) allozymes/isozymes, (2) mitochondrial DNA, and (3) nuclear DNA. They have been subjects of a number of recent reviews (O'Connell and Wright, 1997; O'Reilly and Wright, 1998; Parker *et al*., 1998; Sunnucks, 2000; Hallerman, 2003; Avise, 2004).

2.4 Mitochondrial DNA molecular maker (mtDNA)

By the early 1980's, examination of the gene itself became possible by determining directly or indirectly, differences in the nucleotide sequence of the DNA molecule. A small portion (<1%) of the DNA of eukaryotic cells is non-nuclear; it is located within mitochondria.

The following are the major features of mitochondrial DNA (mtDNA):

a. Maternally inherited, a haploid single molecule;

b. The entire genome is transcribed as a unit;

c. Not subject to recombination and provides homologous markers;

d. Mainly selectively neutral and occurs in multiple copies in each cell;

e. Replication is continuous, unidirectional and symmetrical without any apparent editing or repair mechanism; and

f. Optimal size, with no introns present (Billington, 2003).

Mitochondrial DNA is physically separate from the rest of the cell's DNA and it is relatively easy to isolate. Any tissue or blood can be used for isolation of mtDNA. The rapid rate of evolution, the maternal mode of inheritance and the relatively small size of mtDNA make the Restriction Fragment Length Polymorphism (RFLP) analysis of this molecule one of the methods of choice for many population studies (Ferguson *et al*., 1995). RFLP is a polymorphism in an individual defined by restriction fragment sizes of distinctive lengths produced by a specific restriction endonuclease. Variation in the mtDNA may be analysed mainly using two approaches:

i) RFLP analysis of whole purified mtDNA obtained from fresh tissue (usually liver or gonad) by digesting it with restriction endonucleases;

ii) RFLP analysis or DNA sequencing of small segments of the mtDNA molecule obtained by means of PCR amplification (Billington, 2003).

2.5 Morphometrics of clariid fishes

The morphometric characteristics of these species are important, because hybrids of *C. gariepinus* and other species have been cultured. Moreover, the closely related species like *C. anguillaris* and *C. macrocephalus* are cultured on a large scale, thus, the detailed characteristics of the morphometric features are needed to distinguish between the different

species and hybrids within the genus *Clarias*. *Heterobranchus* differs from *Clarias* mainly by the presence of a large adipose fin for example, *Heterobranchus longifilis* has an adipose fin that is 24.3-32.8% standard.length, supported by 21 to 27 extended neural spines; it has a shorter dorsal fin than *C. gariepinus* 26.9-34.1% standard length versus 56.6-67.4% standard length; fewer dorsal fin rays 26-35 versus 61-79 and fewer gill rakers on the first branchial arch (16-29 versus 24-110) (Legendre, *et al*., 1992). Within the genus *Heterobranchus*, two subunits are recognized. In the first, *H. bidorsalis* appears to be the most primitive species: it has the smallest adipose fin, with the fewest extended neural spines; the other two species, *Heterobranchus longifilis* and *H. isopterus*, form a monophyletic lineage recognized by the adipose fin length, exceeding 25% standard length; pectoral fin spines are smooth in both *H. bidorsalis* and *H. boulengeri*, but in *H. isopterus* and *H. longijilis* they are serrated along the outer margin. Serrated pectoral spines are commonly distributed in siluroids and it might appear that loss of serrations is a derived feature. Both *H. isopterus* and *H. longijilis* show an increased number of neural spines with a corresponding enlargement of the adipose fin and reduction of the rayed dorsal fin. *Heterobranchus isopterus* is distinguished from its congeners by its smallest toothplates. The width of toothplates is variable amongst clariids, but can none-the-less be used to distinguish sub-groups. The second subunit is monospecific, containing only *Heterobranchus boulengeri* which is characterized by its extended lower jaw, the extended fourth infraorbital bone, the numerous gill rakers and the short barbels (Teugels, 1986b).

Morphological features are used in many genetic and breeding studies to identify both parental and hybrid stocks and to eliminate undesirable feral species (Omoniyi and Agbon,

2004). This morphometric approach, in spite of different modifications might not be always conclusive due to environmental influence on their morphology (Anyanwu *et al*., 2002).

2.6 Physico-chemical properties of the aquatic habitat

Water quality is the suitability of water for the survival and growth of aquatic organisms such as fish (Boyd, 1982). It is defined by the physical, chemical and biological characteristics of water, which include: temperature, turbidity, dissolved oxygen (DO) content, biochemical oxygen demand (BOD), pH, alkalinity, hardness, nutrient (nitrate and phosphate) content, ammonia and nitrite content, faecal coliform content etc. (Nnaji *et al*., 2011).

Water is a vital resource for fish in that it is the medium in which the fish lives; the growth of any fish is therefore directly related to water quality (Jain, 2006). Okram *et al*. (2003) noted that physico-chemical features of water and sediment play important roles in the structure and functioning of lake and reservoir ecosystems. The increasing emphasis on the improvement of the quality of aquatic systems and the monitoring of surface water has highlighted the need to know what factors cause environmental deterioration (Ozean *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

Fish specimens and water samples were collected from Bagoma Reservoir, Birnin-Gwari (Latitude 11° 1' 34" N, Longitude 6° 47' 23" E) and River Galma, Zaria (Latitude 10° 27'N, 11° 24'N Longitude 7°23'E and 8° 45'E). These two localities represented the wild habitat. The National Institute for Freshwater Fisheries Research, New Bussa (Latitude 9° 52' 19" N, Longitude 4° 30' 53" E) and Miracle fish farm Area BZ residential quarters, Ahmadu Bello University, Zaria (Latitude 11º3' N and Longitude 7º40' E) represented the culture habitat (Figure 1). The localities were selected based on the availability of fish species. Data were collected between August 2012 and January 2013.

Physico-chemical parameters of the water samples collected were determined in the Hydrobiology Laboratory and morphometric data of the fish species were taken in the Fisheries Laboratory, both in the Department of Biological Sciences, Ahmadu Bello University, Zaria. The molecular studies were conducted in the Centre for Biotechnology, Ahmadu Bello University, Zaria.

3.2 Collection of fish specimens (from wild and culture environments)

Fin (rayed dorsal and adipose) and muscle tissues were obtained from 40 specimens of cultured and wild fish (Table 1) using a pair of forceps and dissecting scissors, then frozen at -20°C.

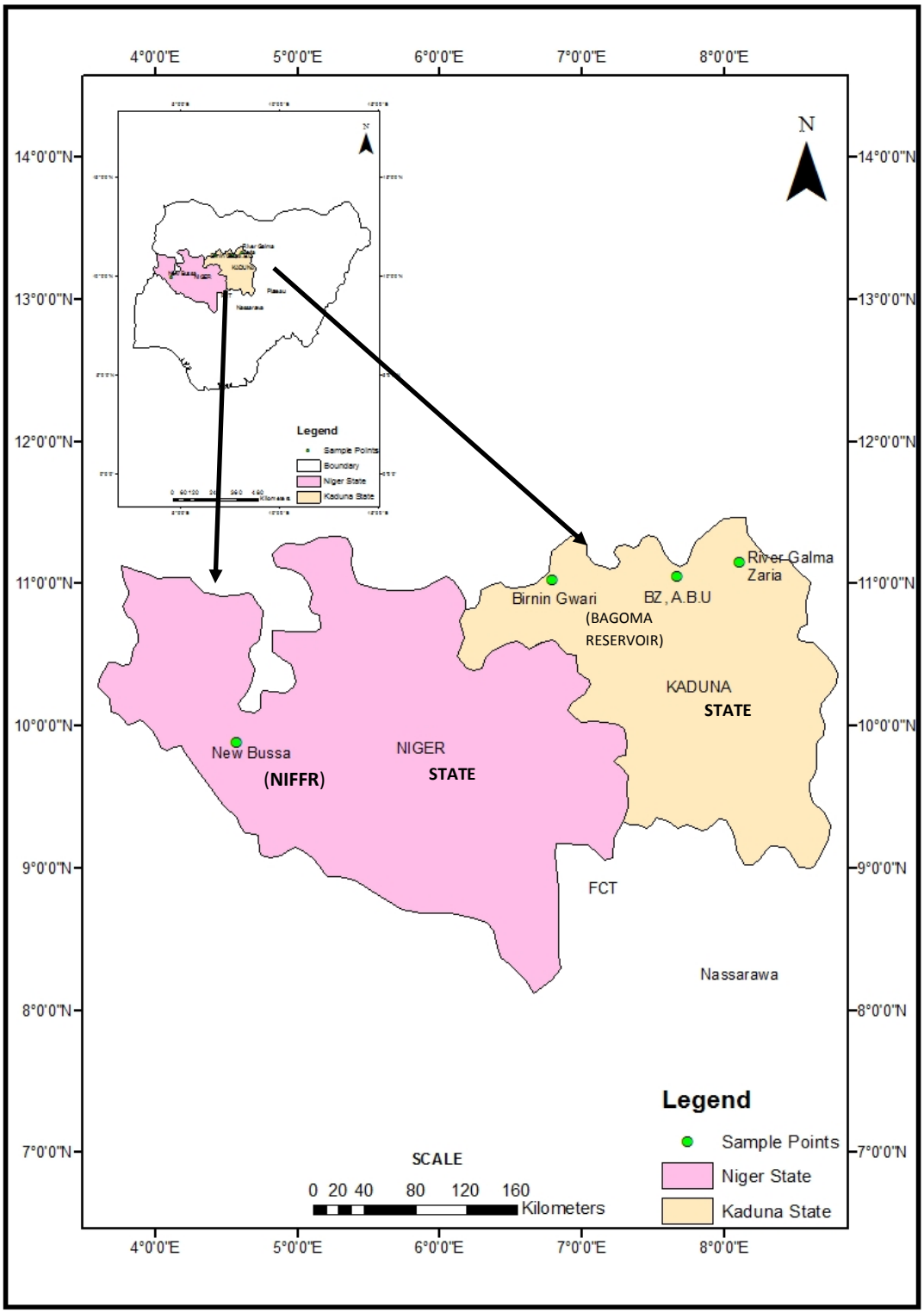


Figure 1: Map of Nigeria showing the study area

Source: Adapted and Modified from Administrative Map of Nigeria

Fish species collected and used in the study include:

A. *CLARIAS*

1. *Clarias gariepinus* (Bürchell, 1822)
2. *Clarias anguillaris* (Linnaeus, 1758)
3. *Clarias galmaensis* Aken'Ova, 2007

B. HETEROBRANCHUS

1. *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809
2. *Heterobranchus longifilis* Valenciennes, 1840
3. *Heterobranchus isopterus* Bleeker, 1863

IDENTIFICATION: Keys/descriptions of Teugels (2003); Aken'Ova (2007) and Froese and Pauly (2013) were used to identify the different species.

A total of eighty (80) pieces of tissue i.e. forty (40) fin and forty (40) muscle were collected from the fishes in the study areas. Five (5) adults of each of the representative species were sampled during the rainy season. Thirty (30) specimens were obtained from the wild including: *Clarias gariepinus* (5), *Clarias anguillaris* (5), *Clarias galmaensis* (5), *Heterobranchus bidorsalis* (5), *Heterobranchus longifilis* (5) and *Heterobranchus isopterus* (5). Ten (10) specimens were obtained from culture environment as follows: *Heterobranchus longifilis* (5) and *Clarias gariepinus* (5); only *Heterobranchus longifilis* and *Clarias gariepinus* are usually cultured. The greatest numbers of *Clarias* and *Heterobranchus* species were obtained from River Galma. *Heterobranchus* species and *Clarias galmaensis* were not available from Bagoma reservoir. Only two cultured species, *Clarias gariepinus* and *Heterobranchus longifilis* were available at Miracle Fish Farm, Zaria

and NIFFR, New Bussa respectively at the time of collection (Table 1). Plates I-VI are photographs of the clariids used in the study.

Table 1: List of the clariids used in the study and their sources

S/N	Species	Autopsy No.	Source	Specimens (n)	Habitat
1	*Clarias gariepinus*	CgaGAL	River Galma	3	Wild
		CgaBAG	Bagoma Reservior	2	Wild
		CgaMIR	Miracle Fish Farm	5	Culture
2	*Clarias anguillaris*	CanGAL	River Galma	3	Wild
		CanBAG	Bagoma Reservior	2	Wild
3	*Clarias galmaensis*	CgmGAL	River Galma	5	Wild
4	*Heterobranchus bidorsalis*	HbiGAL	River Galma	5	Wild
5	*Heterobranchus longifilis*	HloGAL	River Galma	5	Wild
		HloNFR	NIFFR	5	Culture
6	*Heterobranchus isopterus*	HisGAL	River Galma	5	Wild

Key: n= Number of specimens collected; CgaGAL= *Clarias gariepinus* from River Galma; CgaBAG *Clarias gariepinus* from Bagoma Reservior; CgaMIR= *Clarias gariepinus* from Miracle Fish Farm; CanGAL= *Clarias anguillaris* from River Galma; CanBAG= *Clarias anguillaris* from Bagoma Reservior; CgmGAL= *Clarias galmaensis* from River Galma; HbiGAL= *Heterobranchus bidorsalis* from River Galma; HloGAL= *Heterobranchus longifilis* from River Galma; HloNFR= *Heterobranchus longifilis* from NIFFR; HisGAL= *Heterobranchus isopterus* from River Galma

Plate I: *Clarias gariepinus* (Bürchell, 1822) from Miracle Fish Farm, Zaria. Note the marbled colour distribution without black spots on caudal fin or any other part of the body.

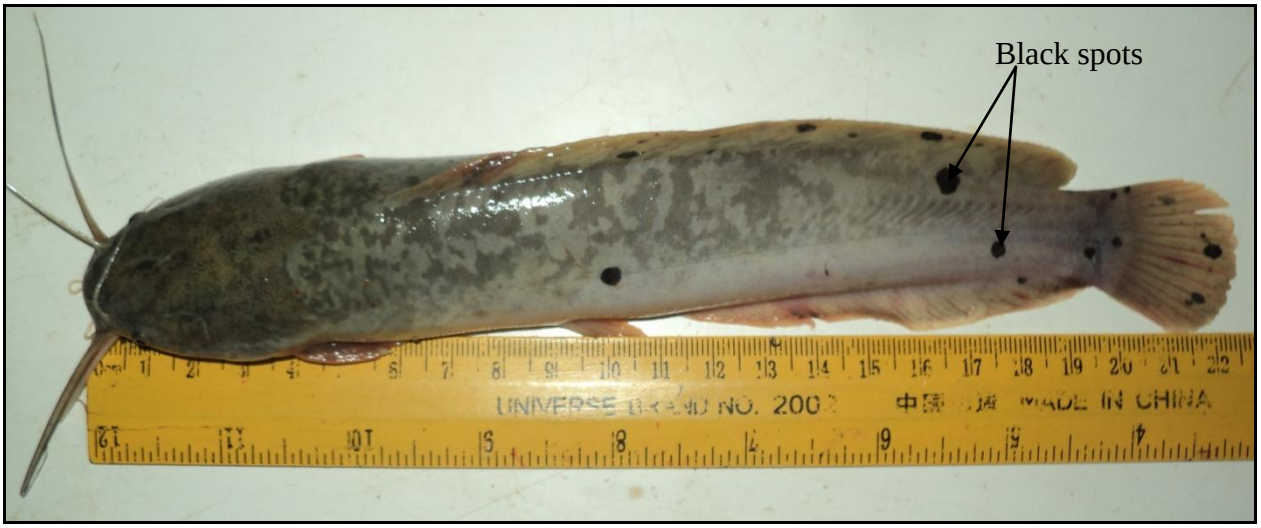


Plate II: *Clarias anguillaris* (Linnaeus, 1758) from River Galma. Note the marbled colour distribution with black spots (arrows) on the caudal fin that even extend into the dorsal fin and flanks. The black spots serve to distinguish this species from *C. gariepinus* in the field.

Plate III: *Clarias galmaensis* Aken'Ova, 2007 from River Galma. Note the small creamy spots on the dark background.

Plate IV: *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809 from River Galma. Note the conspicuous adipose fin (Ad.) on the dorsal surface which distinguishes *Heterobranchus* species from *Clarias* species.

Plate V: *Heterobranchus longifilis* Valenciennes, 1840 from the National Institute For Freshwater Fisheries Research, New Bussa. Note the terminal blue-black spot on the adipose fin (Ad.) that distinguishes this species from its congeners.

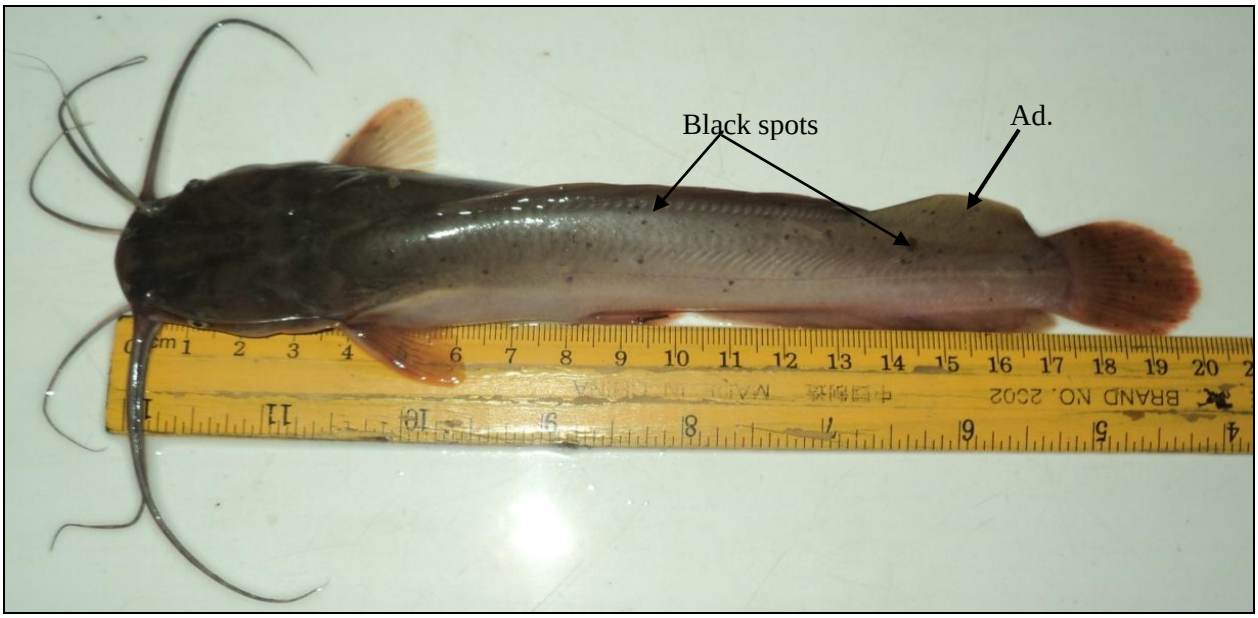


Plate VI: *Heterobranchus isopterus* Bleeker, 1863 from River Galma. Note the adipose fin (Ad.) and tiny black spots on the body surface used as a distinguishing feature of this species.

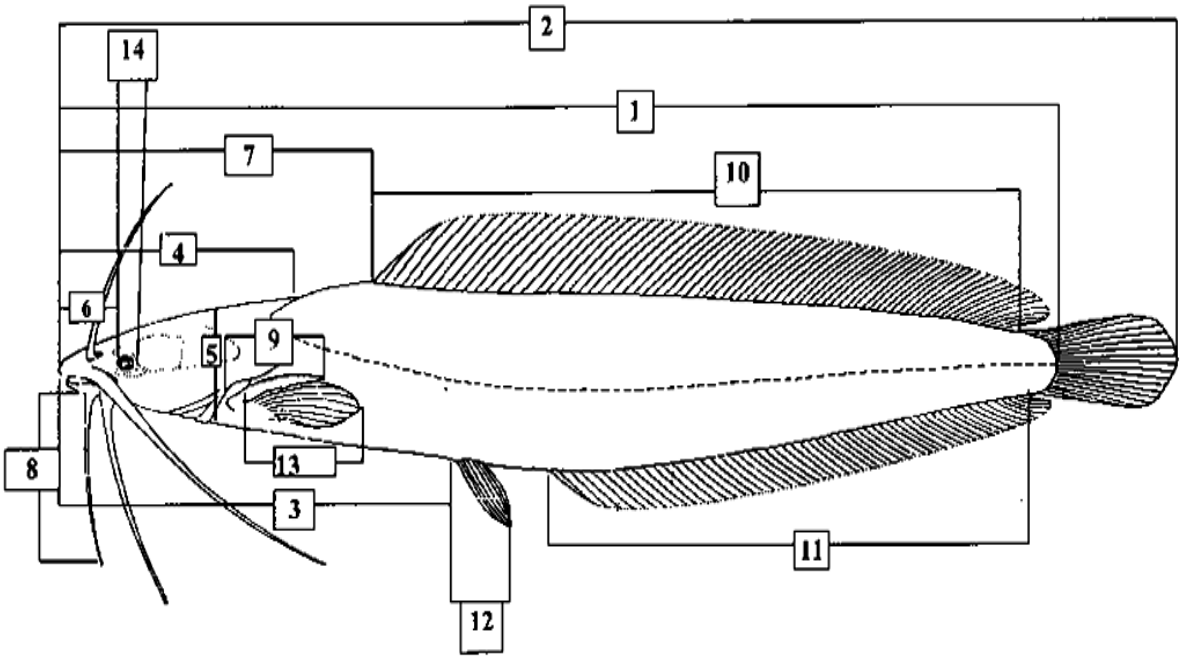
From the keys and description of Teugels (2003); Aken'Ova (2007) and Froese and Pauly, 2013; *Clarias gariepinus* (Plate I) was distinguished from other *Clarias* species by a high number of gill rakers on the first branchial arch, varying from 24 to 110; the number increases with size. *Clarias anguillaris* (Plate II) has 16-40 gill rakers rarely exceeding 40, irrespective of fish size, irregular black spots occur on the caudal fin and on the caudal peduncle which may also spread into the body surface. *Clarias galmaensis* (Plate III) on the other hand, is characterized by its small size, oval head in dorsal outline and olive to brownish colour with tiny cream to dirty-white spots. *Heterobranchus* species are distinguished from *Clarias* species by their adipose fin which is posterior to the rayed dorsal fin, close to the caudal fin; *H. bidorsalis* has a shorter adipose fin than *Heterobranchus longifilis* and *Heterobranchus isoperus* (Plates IV, V and VI), whereas the posterior extremity of the adipose fin of *H. longifilis* (Plate V) is coloured blue-black, *Heterobranchus isopterus*has black spots scattered all over the body *(*Plate VI).

3.3 Physico-chemical analysis

The following physico-chemical parameters: Temperature, pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Hardness, Total Dissolved Solids (TDS), Phosphate-phosphorus (PO_4-P), Nitrate-nitrogen (NO_3-N) and alkalinity were determined in the field using a pre-calibrated HANNA pH/temperature/conductivity/ Total dissolved solids meter (Model 27397). Standard methods described by Boyd (1979) and APHA (1991) were also used in the laboratory. Water samples were collected once in sample bottles in the morning during the period of the study.

3.4 Morphometrics

The morphometric data of the fish species samples were taken following the measurement outlines represented in Figure 1. A pair of dividers with a meter rule was used for measurement.



Source of Figure: Teugels (1986a).

Figure 2: Diagram of *Clarias* showing how measurements were taken; *Heterobranchus* has an adipose fin, whose length and distance from rayed dorsal fin were measured using a pair of dividers.

KEY: **1** = Standard Length (SL); **2** = Total Length (TL); **3** = Snout to Pelvic Fin Origin (STPFO); **4** = Head Length (HL); **5** = Head Width (HW); **6** = Snout Length (SnL); **7** = Snout to Dorsal Fin Origin (STDFO); **8** = Barbel Length (BL); **9** = Pectoral Spine Length (PSL); **10** = Dorsal Fin Length (DFL); **11** = Anal Fin Length (AFL); **12** = Pectoral Fin Length (PFL); **13** = Pelvic Fin Length (PFL); **14** = Eye Diameter (ED)

3.5. Molecular analyses:

Genetic variability in species of the two genera (*Clarias* and *Heterobranchus*) was investigated by Polymerase Chain Reaction (PCR) amplification of cytochrome b gene and Restriction Fragment Length Polymorphism (RFLP) as follows:

3.5.1 Extraction of Deoxyribonucleic acid (DNA)

DNA was extracted from fish fins and muscle using genomic Quick-gDNA™ MiniPrep (50 Preps) w/ Zymo-Spin IIN Columns DNA extraction kits (Inqaba Biotech Laboratory, South Africa) following the manufacturer's guide.

APPARATUS: Genomic Quick-gDNA™ MiniPrep (50 Preps) w/ Zymo-Spin IIN Columns DNA extraction kits, micropipette, pipette tips, Eppendorf Micro-Centrifuge, Eppendorf tubes, hand gloves, electrophoresis set, Agarose powder, Mini Sensitive Scale, Conical Flask, Microwave oven, Ethidium Bromide Solution, 50 × TAE (Tris-Acetate- EDTA) Solution, Distilled Water, UV Trans-illuminator.

PROCEDURE

1. 25mg of frozen tissue (muscle and fin) was mechanically homogenized in 500µl of genomic lysis buffer.
2. The lysate was centrifuged at top speed (10,000 rpm) for 5 minutes. Making sure not to disturb the pelleted debris, the supernatant was transferred to a Zymo-spin ^{Tm} column in a collection tube and centrifuged at 10,000 rpm for one minute. The collection tube was discarded with the flow through.

3. The Zymo-spin [Tm] column was transferred to a new collection tube and 200µl of DNA Pre-Wash Buffer was added to the spin column and centrifuged at 10,000 rpm for one minute.
4. 500µl of g-DNA Wash Buffer was added to the spin column and centrifuged at 10,000rpm for one minute.
5. The spin column was transferred to a clean micro-centrifuge tube and ≥50µl of DNA Elution Buffer (or distilled water) was added to the spin column and incubated for 2-5 minutes at room temperature, then centrifuged at top speed for 30 seconds to elute the DNA. The eluted DNA was stored at ≤-20°C.
6. Eluted DNA was separated and purified on 0.8% agarose gel in 50 × Tris-Acetate EDTA (TAE) buffer solutions and visualized under ultra violet (UV) light and documented.

3.5.2 Polymerase Chain Reaction

APPARATUS: PCR Thermocycler, Extracted DNA, DreamTaq™ Green PCR Master Mix (2×), Forward and Reverse Primer Sets, Nuclease-free water, Eppendorf tubes, micropipette, pipette tips, hand gloves, electrophoresis set-up, Agarose powder, Mini Sensitive Scale, Conical Flask, Microwave oven, Ethidium Bromide Solution, 50 × TAE (Tris-Acetate-EDTA) Solution, Distilled Water, UV Trans-illuminator.

PROCEDURE

The PCR was performed using DreamTaq™ Green PCR Master Mix (2×), 200 reactions (Inqaba Biotech lab, South Africa) in appropriate volumes (Table1). Cytochrome *b* gene

(585 bp) was amplified using primers L15267, 5'-AAT GAC TTG AAG AAC CAC CGT-3' and H15891, 5'-GTT TGA TCC CGT TTC GTG TA-3' (Briolay *et al*., 1998 of 7min at 72°C (Nwafili and Gao, 2007). Products of PCR amplification were run with PCR cycling conditions: 2min initial denaturation at 95°C and 35 cycles of 30s at 95°C for denaturation, 30s at 58.3°C for annealing, 1min at 72°C for extension, and a final extension at 75°C for 10min, amplicons were loaded on 0.8-1.5% agarose gel and photographed under UV light.

Table 2: Optimized volumes for PCR

Component	**Volume** (µl)
PCR Master mix	25
Primer L	1
Primer H	1
Nuclease free H_20	18
DNA template	5
Total volume	50

3.5.3 Restriction Fragment Length Polymorphism (RFLP)

Restriction digestion was carried out using Eco RI ('5...GAATTC...3'; 3'...CTTAAG...5'), Hae III (5'...GGCC ...3'; 3'...CCGG...5') and Hind III (5'...AAGCTT...3'; 3'...TTCGAA...5') restriction enzyme (Inqaba Biotech Lab, South Africa) following the manufacturer's guide.

APPARATUS: PCR products, restriction enzymes, heat block, micropipette, pipette tips, Eppendorf tubes, gloves, electrophoresis set-up, Agarose powder, Mini Sensitive Scale,

Conical Flask, Microwave oven, Ethidium Bromide Solution, 50 × TAE (Tris-Acetate-EDTA) Solution, Distilled Water and UV Trans-illuminator.

PROCEDURE

1. The reaction components for PCR product include: 17µl of nuclease-free water, 2µl of 10× FastDigest Green Buffer, DNA (PCR product) 10µl and FastDigest enzyme 1µl.
2. The components were gently mixed and spinned down for 30sec at 10000 rpm.
3. The reaction mixture was incubated at 37°C in a heat block for 20min
4. An aliquot of the mixture was loaded directly on 1% agarose gel electrophoresis tank; the gel was visualized for possible restriction and photographed.

3.6 Statistical Analysis

Data collected were recorded in tables and analysed. Factorial ANOVA was used to compare the whole morphometrics of the fish species and physico-chemical parameters of wild and culture environment while means were separated by Fisher Least Significant Difference (LSD) using Statistica version 8.0. Level of significance was set at $P<0.05$. Principal Component Analysis (PCA) was also used to correlate ($r<1$) the morphometrics of the two genera as well as the physico-chemical properties of both wild and culture habitat using PAST for windows version 2.09. Canonical Correspondence Analysis (CCA) was also used to relate the morphometrics of the two genera with the wild and culture habitats using CANOCO for windows version 4.5.

DARwin5 (version 5.0.157) software was used to illustrate the genetic variations observed.

CHAPTER FOUR

4.0 RESULTS

4.1 Morphometrics of fish species

Table 3 shows the morphometrics of wild and cultured species of *Clarias* and *Heterobranchus*, from two sources each, between August 2012 and January 2013. The morphometrics taken for each species shows no values for the adipose fin and the distance between adipose and dorsal fin in all *Clarias* species which are unique to and distinguishing feature of *Heterobranchus* species.

Relative to the standard length, snout to pelvic fin origin and snout to dorsal fin origin had higher values in *Heterobranchus species* than *Clarias species* with the highest values found in *Heterobranchus bordosalis* (46.29% SL) and *Heterobranchus longifilis* (35.97% SL). Higher values were also recorded in *Heterobranchus* spp. for pectoral spine length (11.94% SL). On the other hand dorsal and anal fin lengths were observed to be higher in *Clarias* spp. than *Heterobranchus* spp. with the highest values in *Clarias galmaensis* (66.84% SL and 51.87% SL respectively). Pelvic and pectoral fin length were greater relative to the standard length in *Heterobranchus isopterus* (11.60% SL and 14.29% SL respectively).

The adipose fin in *Heterobranchus* species had the highest value in *Heterobranchus isopterus* (25.45%) while the distance between the adipose and dorsal fin was highest in *Heterobranchus longifilis* (4.63%) relative to the standard length.

The head width, snout length and barbel length were higher in *Heterobranchus bidorsalis* (81.52%, 50.00% and 176.09% respectively) while eye diameter was higher in *Heterobranchus longifilis* (10.37%) relative to the head lenght. Overall, the head length as percentage of standard length was greater in *Heterobranchus* species than in *Clarias* species (Table 3).

Table 3: Morphometrics of the fishes in ranges and as percentage of standard length

Morphometrics (cm)	*Clarias gariepinus*	*Clarias anguillaris*	*Clarias galmaensis*	*Heterobranchus bidorsalis*	*Heterobranchus longifilis*	*Heterobranchus isopterus*
			Min – Max (% of SL)			
SL	20.7-38.4	16.5-34.5	14.1-18.7	19.2-33.5	23.7-36.7	17.7-22.4
TL	22.8-43.8	17.8-39.0	15.7-20.5	21.7-38.5	26.3-39.8	18.4-25.6
STPFO	9.3-16.8 (43.75)	7.0-14.3 (41.45)	5.5-6.3 (33.69)	10.3-15.5(46.29)	11.1-16.7 (45.50)	8.2-10.0 (44.64)
STDFO	6.8-13.0 (33.85)	4.7-10.0 (28.99)	4.2-5.7 (30.48)	6.9-11.5 (34.33)	8.7-13.2 (35.97)	4.4-7.6 (33.93)
PSL	1.3-3.3 (8.59)	1.8-2.4 (6.96)	0.8-1.3 (6.95)	2.1-4.0 (11.94)	1.5-4.2 (11.44)	1.2-1.7 (7.59)
DFL	13.5-25.6 (66.66)	10.8-22.5 (65.22)	9.8-12.5 (66.84)	7.9-13.5 (40.30)	8.0-13.5 (36.78)	6.9-9.8 (43.75)
AFL	8.8-16.4 (42.70)	7.8-16.3 (47.25)	7.2-9.7 (51.87)	7.3-13.7 (40.90)	8.5-14.4 (39.24)	5.8-9.7 (43.30)
PCFL	2.1-4.8 (9.63)	2.1-4.4 (8.41)	1.1-1.8 (6.95)	2.6-4.6 (11.34)	3.1-4.5 (10.63)	2.2-3.2 (11.60)
PLFL	1.5-3.7 (12.50)	1.7-2.9 (12.75)	0.9-1.3 (9.63)	2.1-3.8 (13.73)	2.6-3.9 (12.26)	1.4-2.6 (14.29)
ADFL	-	-	-	4.0-8.0 (23.88)	5.0-8.5 (23.16)	3.4-5.7 (25.45)
ADFD	-	-	-	0.1-1.0 (2.99)	1.0-1.7 (4.63)	0.4-0.6 (5.71)
			Min – Max (% of HL)			
HL	5.2-10.6	4.2-8.9	3.1-4.1	5.4-9.2	7.1-10.6	4.5-7.0
HW	3.5-7.8 (73.58)	2.3-6.6 (74. 16)	2.3-3.0 (73.17)	4.2-7.5 (81.52)	4.7-8.5(80.19)	3.2-5.1 (72.86)
ED	0.3-0.6 (5.66)	0.2-0.6 (6.74)	0.2-0.3 (7.31)	0.4-0.7 (7.61)	0.4-1.1 (10.37)	0.3-0.4 (5.71)
BL	5.0-11.3 (106.60)	3.3-7.4 (83.15)	3.2-5.2 (126.83)	10.4-16.2 (176.09)	8.1-15.4 (145.28)	7.6-9.6 (134.14)
Sn L	1.9-4.1(38.68)	1.2-3.5 (39.33)	1.0-1.6 (39.02)	2.8-4.6 (50.00)	2.5-4.3 (40.57)	1.8-3.0 (42.86)

NB. Five specimens of each species were measured

Key: SL = Standard Length; **TL** = Total Length; **STPFO** = Snout To Pelvic Fin Origin; **HL**= Head Length; **HW** = Head Width; **SnL** = Snout Length; **STDFO** = Snout To Dorsal Fin Origin; **BL**= Barbel Length; **PSL** = Pectoral Spine Length; **DFL** = Dorsal Fin Length; **AFL** = Anal Fin Length; **PCFL** = Pectoral Fin Length; **PLFL** = Pelvic Fin Length; **ED** = Eye Diameter **ADFL** = Adipose Fin Length; **ADFD** = Distance Between Dorsal And Adipose Fin

Table 4: Comparison of statistical values of morphometrics

Measurements	Species	Culture type
	F-value	F-value
SL	6.30*	1.17
TL	6.19*	2.32
STPFO	10.29*	3.55
HL	8.45*	1.15
HW	8.69*	0.00
SL	8.80*	1.11
STDFO	8.97*	1.46
BL	24.19*	10.65*
PSL	5.93*	1.11
DFL	12.37*	4.69*
AFL	4.14*	6.06
PCFL	5.70*	0.77
PLFL	4.64*	8.19*
ED	7.48*	33.59*

*** Significant at P< 0.05.**

Table 4 shows significant differences in morphometrics for all species, whereas with respect to the environment; significant differences were observed only in snout to pelvic fin origin, barbel length, dorsal fin length, pelvic fin length and eye diameter between wild and culture environments ($P<0.05$).

Tthe mean morphometrics of cultured *Clarias* species were higher than those of the wild species; there were also significant differences between cultured and wild *Clarias* and *Heterobranchus* species. Some mean morphometric values for cultured *Heterobranchus* were higher than the wild species while other mean morphometric values of wild *Clarias* and *Heterobranchus* were higher than those of cultured species (Table 5).

Table 5: Morphometrics of wild (W) and cultured (C) species of *Clarias* and *Heterobranchus*

Species	SL	TL	STPFO	HL	HW	SL	STDFO	BL
Clarias gariepinus (W)	26.04±2.83^{b}	29.26±3.17^{b}	11.24±1.08^{b}	6.76±0.89^{b}	5.20±0.80^{b}	2.50±0.34^{b}	8.48±0.93^{b}	6.12±0.51$^{a\,b}$
Clarias anguillaris (W)	24.0±3.36^{b}	26.88±3.85^{b}	10.5±1.37^{b}	5.92± 0.84^{b}	4.30±0.74^{b}	2.22±0.53^{a}	7.64±1.04^{b}	5.52±0.80$^{a\,b}$
Clarias galmaensis (W)	16.26±0.90^{a}	17.76±0.98^{a}	6.1±0.31^{a}	3.58±0.17^{a}	2.54±0.12^{a}	1.34±0.11^{a}	4.86±0.31^{a}	4.22±0.32^{a}
Heterobranchus bidorsalis (W)	28.48±2.53^{b}	32.08±2.87^{b}	13.42±0.90^{b}	8.08±0.68^{b}	6.38±0.57^{b}	3.70±0.30$^{a\,b}$	9.76±0.80^{b}	11.78±1.11$^{a\,b}$
Heterobranchus longifilis (W)	31.56±2.14^{b}	34.44±2.29^{b}	14.22±0.94^{b}	9.24±0.63^{b}	7.02±0.65$^{a\,b}$	3.50±0.29$^{a\,b}$	11.36±0.84$^{a\,b}$	11.64±1.04$^{a\,b}$
Heterobranchus isopterus (W)	18.88±1.13^{ab}	21.66±1.25$^{a\,b}$	8.92±0.33$^{a\,b}$	5.34±0.47^{ab}	3.96±0.33$^{a\,b}$	2.40±0.19^{b}	6.22±0.57$^{a\,b}$	8.76±0.34^{b}
Clarias gariepinus (C)	30.64±2.58^{c}	35.5±2.70^{c}	14.12±1.06^{c}	8.60±0.81^{c}	5.76±0.55^{c}	2.88±0.35^{c}	10.40±0.94^{c}	10.06±0.39^{c}
Heterobranchus longifilis (C)	31.88±1.60^{c}	35.84±1.49^{c}	14.8±0.72^{c}	8.84±0.40^{c}	6.44±0.36^{c}	3.74±0.19^{c}	11.32±0.44^{c}	12.74±0.92^{c}

Key: SL = Standard Length; **TL** = Total Length; **STPFO** = Snout To Pelvic Fin Origin; **HL**= Head Length; **HW** = Head Width; **SLN** = Snout Length; **STDFO** = Snout To Dorsal Fin Origin; **BL**= Barbel Length.

All measurements are in Centimeters

NB: Along columns, superscripts with the same alphabets are not significantly different (P≥0.05).

Table 5 Continued: Morphometrics of *Clarias* and *Heterobranchus* species in wild (W) and culture (C) habitats

Species	PSL	DFL	AFL	PCFL	PLFL	ED
Clarias gariepinus (W)	2.1±0.38[b]	17.06±1.76[b]	11.68±1.24[b]	2.82±0.30[b]	2.32±0.32[b]	0.38±0.04[b]
Clarias anguillaris (W)	2.10±0.13[b]	15.62±2.21[b]	10.52±1.71[b]	3.0±0.45[b]	2.12±0.21[b]	0.36±0.07[b]
Clarias galmaensis (W)	1.16±0.10[a]	11.0±0.5[a]	8.29±0.55[a]	1.54±0.13[a]	1.10±0.08[a]	0.28±0.02[b]
Heterobranchus bidorsalis (W)	2.82±0.36[b]	11.14±0.96[a b]	11.02±1.11[b]	3.76±0.35[b]	3.02±0.29[b]	0.52±0.05[b]
Heterobranchus longifilis (W)	2.64±0.32[b]	10.34±0.90[a b]	12.06±0.99[a b]	3.78±0.27[b]	3.34±0.18[a b]	0.66±0.11[a]
Heterobranchus isopterus (W)	1.36±0.09[a b]	8.22±0.48[a b]	7.80±0.66[b]	2.5±0.18[a b]	1.92±0.20[b]	0.38±0.02[b]
Clarias gariepinus (C)	2.50±0.28[c]	20.72±1.37[c]	13.38±1.04[c]	4.0±0.34[c]	3.04±0.24[c]	0.46±0.05[c]
Heterobranchus longifilis (C)	2.82±0.36[c]	11.08±0.53[c]	12.02±0.50[c]	3.76±0.15[c]	3.18±0.22[c]	0.46±0.04[c]

Key: PSL = Pectoral Spine Length; **DFL** = Dorsal Fin Length; **AFL** = Anal Fin Length; **PCFL** = Pectoral Fin Length; **PLFL** = Pelvic Fin Length; **ED** = Eye Diameter **ADFL** = Adipose Fin Length; **DAFL** = Distance Between Dorsal and Adipose Fin

All measurements are in centimeters

NB: Along columns, superscripts with the same alphabets are not significantly different ($P \geq 0.05$).

Table 6: Physico-chemical properties of the water bodies sampled

HABITAT	C.D.	pH	TEMP.(°C)	EC (µS/cm)	TDS (ppm)	BOD (mg/L)	DO. (mg/L)	ALK. (mg/ L. $CaCO_3$)	HARD. (mg/L)	PO_4-P (mg/L)	NO_3-N (mg/L)
River Galma	05/09/12	6.42	27.70	48.00	24.00	5.85	6.70	1.40	1.40	0.48	0.12
Bagoma Reservoir	24/10/12	7.89	31.70	70.00	34.00	2.90	8.70	2.70	1.40	0.28	0.32
Mean±SE		7.16 ± 0.73^{a}	29.7 ± 2.0^{a}	59 ± 11.0^{a}	29 ± 5.0^{a}	4.38 ± 1.48^{a}	7.7 ± 1.0^{a}	2.05 ± 0.65^{a}	1.4 ± 0.0^{a}	0.38 ± 0.10^{a}	0.22 ± 0.10^{a}
Miracle Fish Farm	23/09/12	7.25	27.50	242.	120.50	1.30	1.10	4.70	1.3	0.22	0.18
NIFFR	30/10/12	7.82	28.9	218.00	108.00	1.90	3.95	7.7	1.5	0.25	0.42
Mean±SE		7.34 ± 0.2^{b}	28.2 ± 0.70^{b}	230 ± 12.0^{b}	114.25 ± 6.25^{b}	1.60 ± 0.30^{b}	2.53 ± 1.43^{b}	6.2 ± 1.50^{b}	1.4 ± 0.10^{b}	0.24 ± 0.02^{b}	0.3 ± 0.12^{b}

Key:
C.D., Collection Date; TEMP., Temperature; EC, Electrical Conductivity; TDS, Total Dissoved Solids; BOD, Biochemical Oxygen Demand; DO, Dissoved Oxygen; ALK., Alkalinity; HARD., Hardness

NB: Along columns, superscripts with the same alphabets are not significantly different ($P\geq0.05$).

4.2 Physico-chemical properties of the water bodies sampled

There were significant variations ($P< 0.05$) in some water quality parameters of the wild and culture habitats. Electrical conductivity (230±16.97µS), Total Dissolved Solids (114.25±8.84ppm) and alkalinity had higher values in the culture environment while lower values were observed in the wild. Higher values were observed in Biochemical Oxygen Demand (4.38±2.09mg/L), Dissolved Oxygen (7.7±1.41mg/L) and PO_4-P (0.38±0.14mg/L) in wild habitat whereas lower values were observed in culture environment. Little variations were observed in pH, temperature, water hardness and NO_3-N for both wild and culture environments (Table 6).

4.3 Correlation analysis for morphometrics of the fishes and physico-chemical properties of the habitat

There was a strong positive correlation between all the measurements taken on the vertical axis ($r>0.6$) however, a weak negative correlation was observed between eye diameter, barbel length and standard length as against the pelvic, anal and dorsal fin lengths ($r<0.6$) (Figure 3).

In wild and culture habitats, a positive correlation ($r>0.6$) was observed in electrical conductivity, total dissolved solids, alkalinity, NO_3-N and temperature on the vertical axis as well as in hardness, dissolved oxygen, Biochemical Oxygen Demand and PO_4-P. On the horizontal axis there was a negative correlation ($r<0.6$) between electrical conductivity, total dissolved solids, alkalinity, NO_3-N, Temperature, Hardness, and Dissolved Oxygen against Biochemical Oxygen Demand with PO_4-P (Figure 4)

There were significant differences in Electrical Conductivity, Total Dissolved Solids, Biochemical Oxygen Demand, Alkalinity, Hardness, and PO_4-N in the habitats (wild and culture) of all *Clarias* and *Heterobranchus* species (Table 7).

Table 7: Comparison of statistical values of physico-chemical parameters

Parameters	Species F-value	Culture type F-value
pH	1.38	16.21*
Temperature	1.42	0.30
Electrical conductivity	7.58*	3588.25*
Total dissolved solids	8.13	3890.35*
Biochemical oxygen demand	3.02*	103.73*
Dissolved oxygen	1.99	131.55*
Alkalinity	2.97*	174.01*
Hardness	6.60*	0.00
$P0_4$-N	2.78*	69.95*
$N0_3$-N	1.72	14.44*

* = significant at $P<0.05$

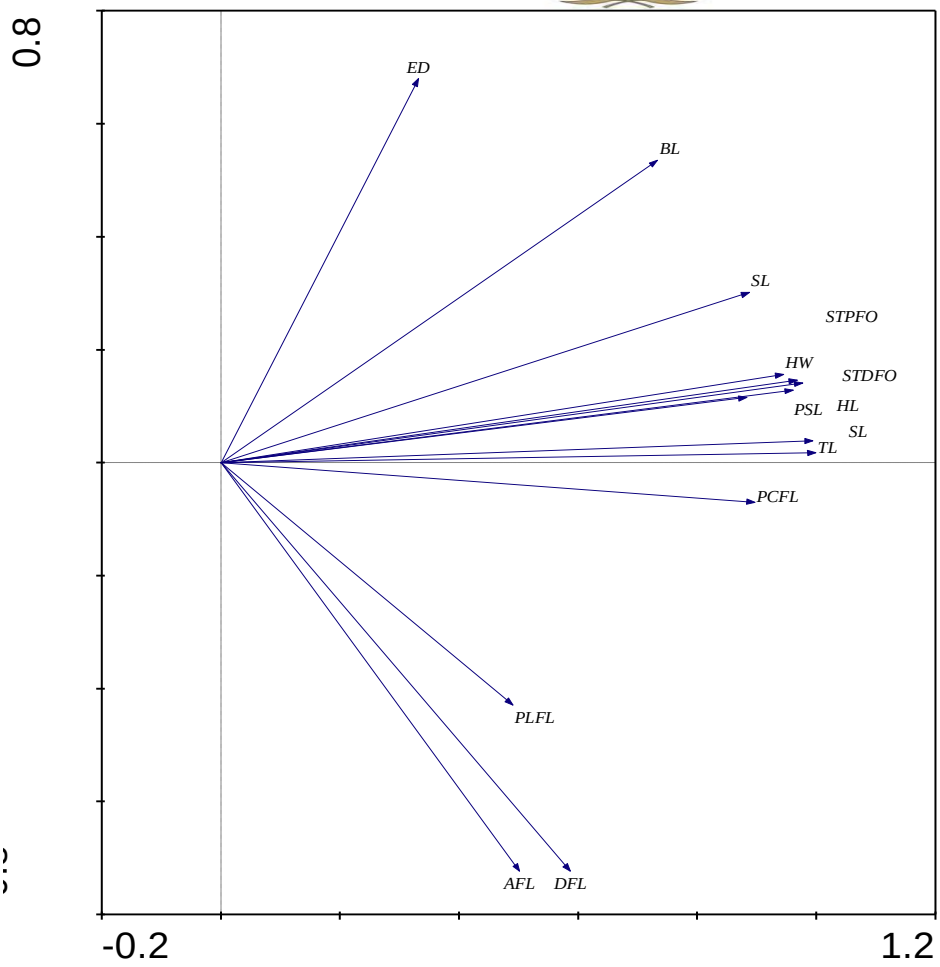


Figure 3: Principal Component Analysis showing the correlation of all the morphometrics of *Clarias* and *Heterobranchus* species from wild and culture habitats.

Key: SL = Standard Length; **TL** = Total Length; **STPFO** = Snout To Pelvic Fin Origin; **HL**= Head Length; **HW** = Head Width; **SnL** = Snout Length; **STDFO** = Snout To Dorsal Fin Origin; **BL**= Barbel Length; **PSL** = Pectoral Spine Length; **DFL** = Dorsal Fin Length; **AFL** = Anal Fin Length; **PCFL** = Pectoral Fin Length; **PLFL** = Pelvic Fin Length; **ED** = Eye Diameter **ADFL** = Adipose Fin Length; **DAFL** = Distance Between Dorsal And Adipose Fin

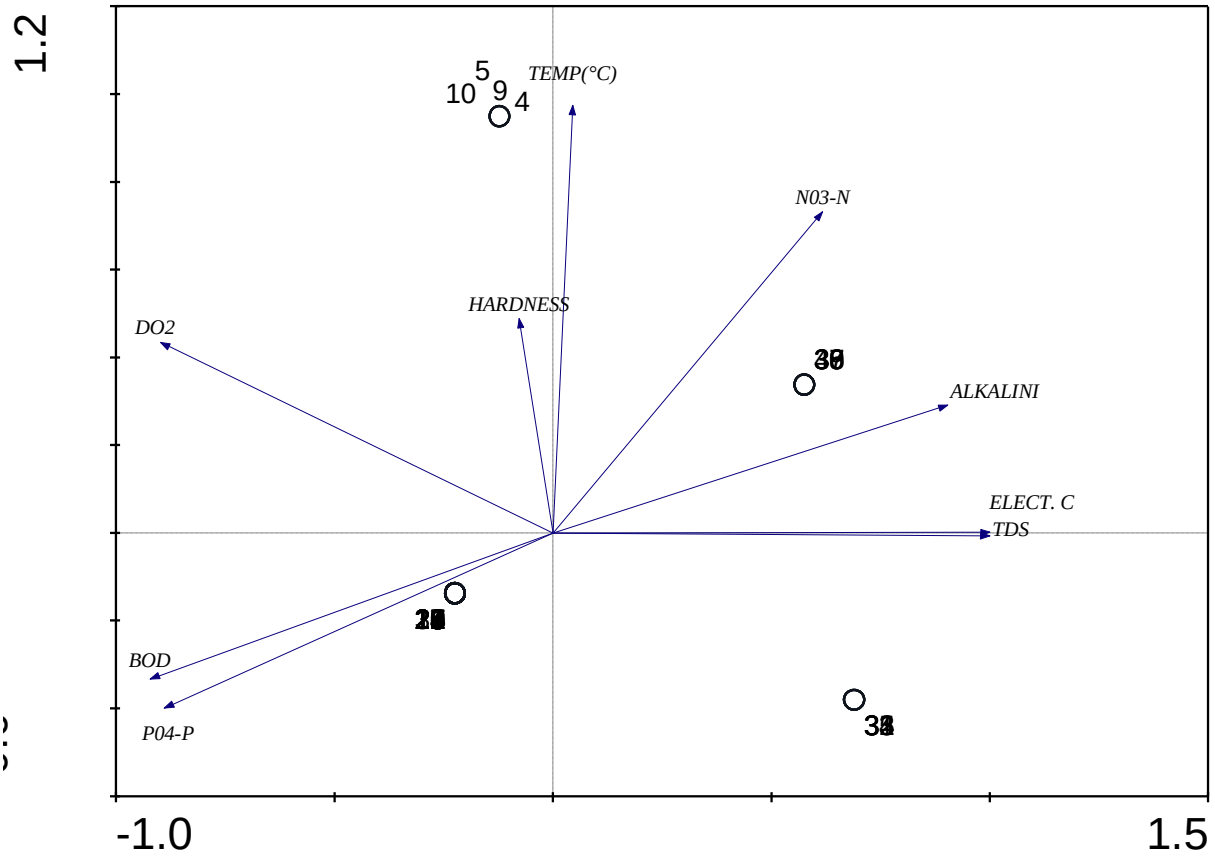


Figure 4: Principal Component Analysis showing the correlation of total physico-chemical parameters of wild and culture environment.

Key: Temp = Temperature; DO = Dissolved Oxygen; NO_3-N = Nitrate Nitrogen; Alkalini = Alkalinity; Elect. C = Electrical conductivity, TDS = Total Dissolved Solids; BOD = Biochemical Oxygen Demand and PO_4-P = Phosphate Phosphorus. (r>0.6 is positively correlated; r<0.6 negatively correlated)

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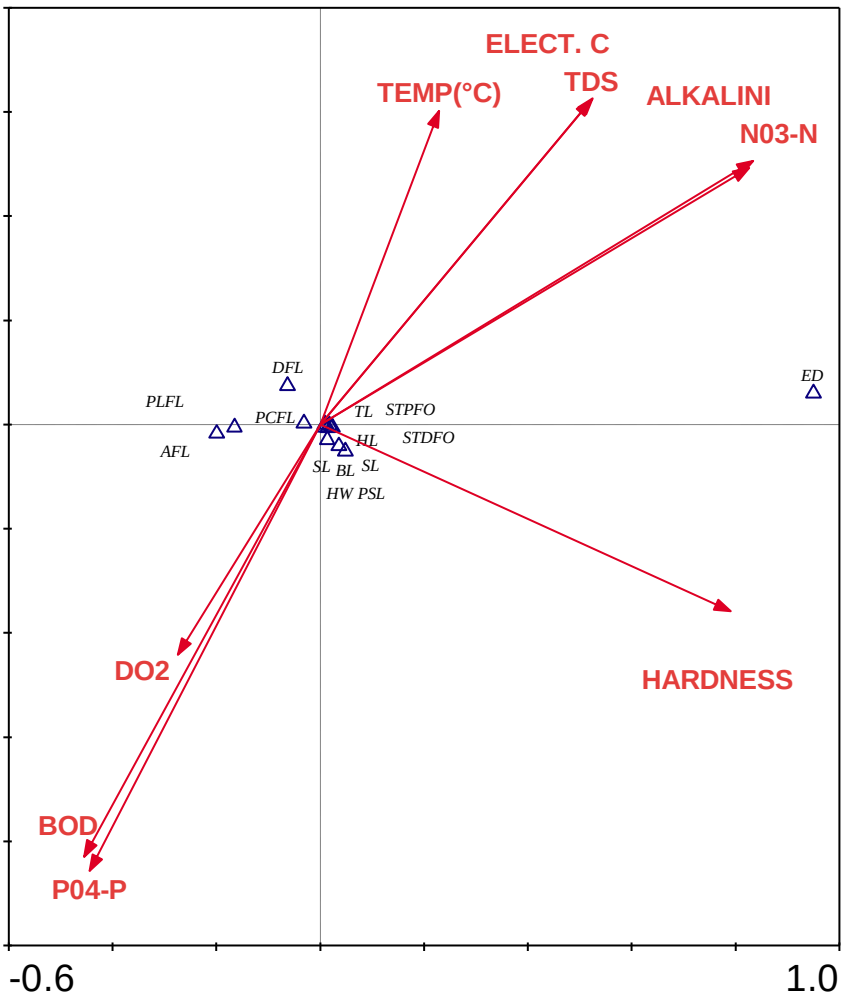

Figure 5: Canonical Correspondence Analysis showing interactions between morphometrics of *Clarias* and *Heterobranchus* species and the physico-chemical parameters of their habitat.

Key: SL = Standard Length; **TL** = Total Length; **STPFO** = Snout To Pelvic Fin Origin; **HL**= Head Length; **HW** = Head Width; **SnL** = Snout Length; **STDFO** = Snout To Dorsal Fin Origin; **BL**= Barbel Length; **PSL** = Pectoral Spine Length; **DFL** = Dorsal Fin Length; **AFL** = Anal Fin Length; **PCFL** = Pectoral Fin Length; **PLFL** = Pelvic Fin Length; **ED** = Eye Diameter **ADFL** = Adipose Fin Length; **DAFL** = Distance Between Dorsal And Adipose Fin

With respect to the interaction between all the physico-chemical properties and morphometrics, there was a positive correlation between Electrical Conductivity, Total Dissolved Solids, Alkalinity, NO_3-N, Temperature, Hardness (physico-chemical properties) and eye diameter, total length, snout to pelvic fin origin, snout to dorsal fin origin, eye diameter, head length, snout

length, barbel length, head width, pectoral spine length (morphometrics) on the vertical axis, as well as between dissolved oxygen, biochemical oxygen demand, PO_4-P (physico-chemical properties) and the lengths of the pelvic fin, anal fin, pectoral fin and dorsal fin (morphometrics) while a negative correlation exist between them (r<0.6) in the opposite direction (Figure 5).

Interactions of the morphometrics of wild *Clarias gariepinus* and *Heterobranchus longifilis* with Electrical Conductivity, Total Dissolved Solids, Alkalinity, NO_3-N, Temperature, pH, Dissolved Oxygen (physico-chemical properties) showed a positive correlation except in the dorsal fin length. There was also a positive correlation between Biochemical Oxygen Demand, PO4-P, Hardness and eye diameter, total length, snout to pelvic fin origin, snout to dorsal fin origin, head length, snout length, barbel length, head width, pectoral spine length, pelvic fin length, anal fin length, pectoral fin length, standard length (morphometrics), whereas, there was a negative correlation between them (r<0.6) in the opposite direction (Figure 6).

On the other hand, interactions between the morphometrics of cultured *Clarias gariepinus* and *Heterobranchus longifilis* showed positive correlation with Electrical Conductivity, Total Dissolved Solids (physico-chemical properties) and dorsal fin length, pelvic fin length, anal fin length, pectoral fin length (morphometrics). Also there is a positive correlation between Alkalinity, NO_3-N, temperature, pH, Dissolved Oxygen, Biochemical Oxygen Demand, PO_4-P, Hardness (physico-chemical properties) and eye diameter, total length, snout to pelvic fin origin, snout to dorsal fin origin, head length, snout length, barbel length, head width, pectoral spine length, standard length (morphometrics) while there was a negative correlation between them (r<0.6) (Figure 7).

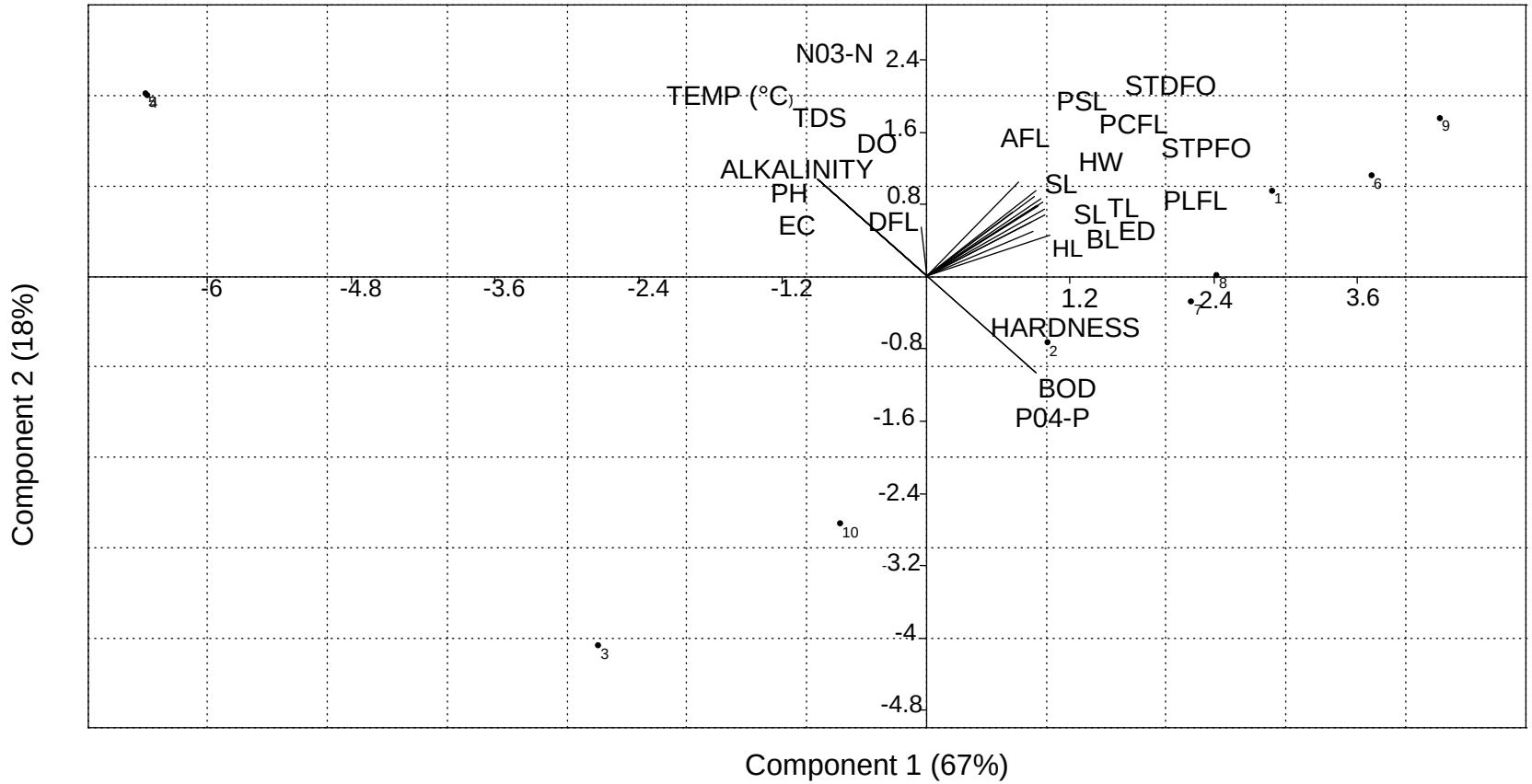

Figure 6: Principal Component Analysis biplot for wild *Clarias gariepinus* and *Heterobranchus longifilis*

(r>0.6 is positively correlated; r<0.6 negatively correlated)

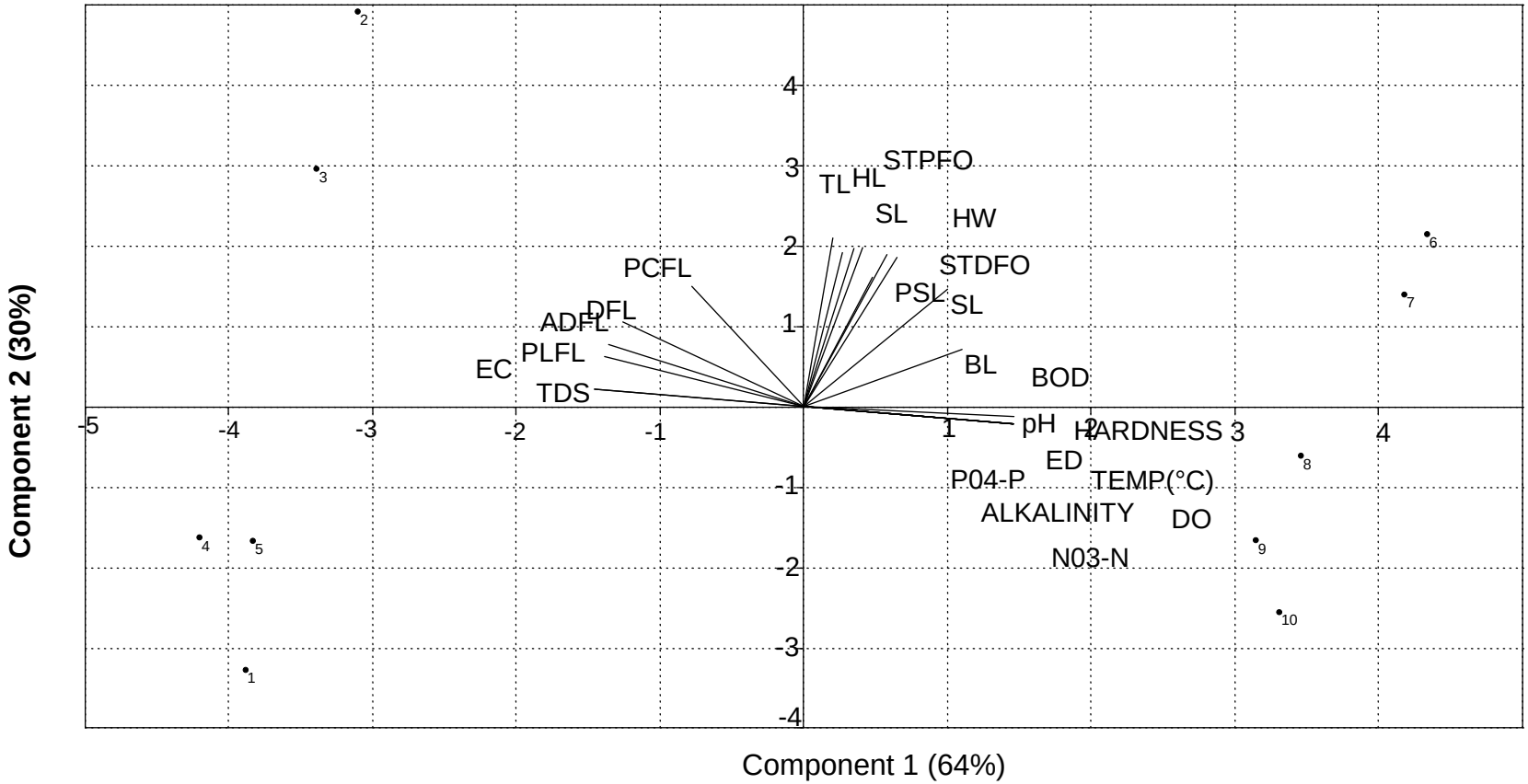

Figure 7: Principal Component Analysis biplot for cultured *Clarias gariepinus* and *Heterobranchus longifilis*

L A1 A2 A3 A4 A5 A6 A7 A8 A9 A10

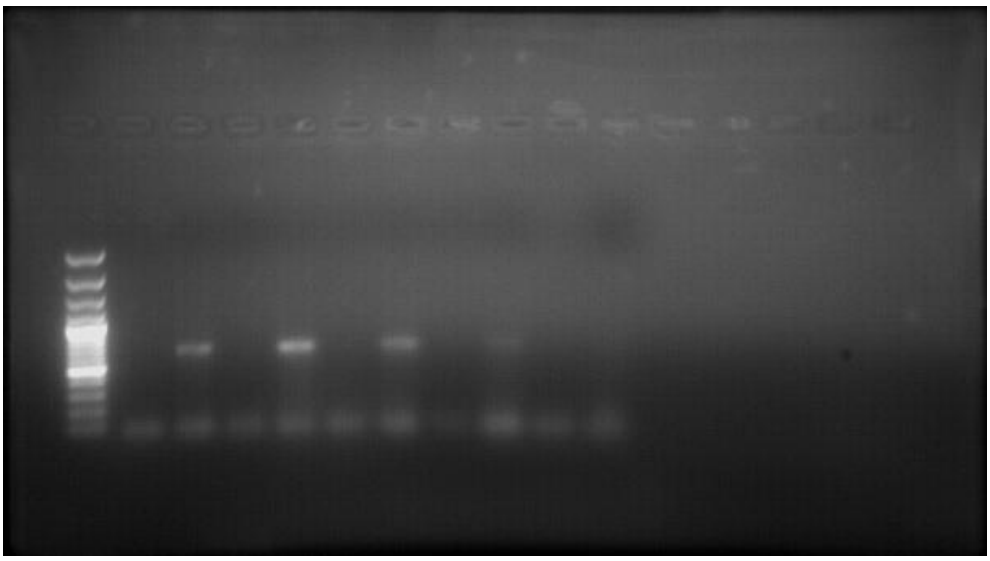

Plate VII: Agarose (0.8%) gel electrophoretic profile of PCR amplification of cytochrome b gene (585bp). L is ladder (1kb), (A1, A3, A5, A7, A9) are muscle samples of *Clarias anguillaris* (wild) while (A2, A4, A6, A8 & A10) are fin samples of *Clarias anguillaris* respectively (wild).

L B1 B2 C1 C2 D1 D2 E1 E2 F1 F2 G1 G2 H1 H2 L

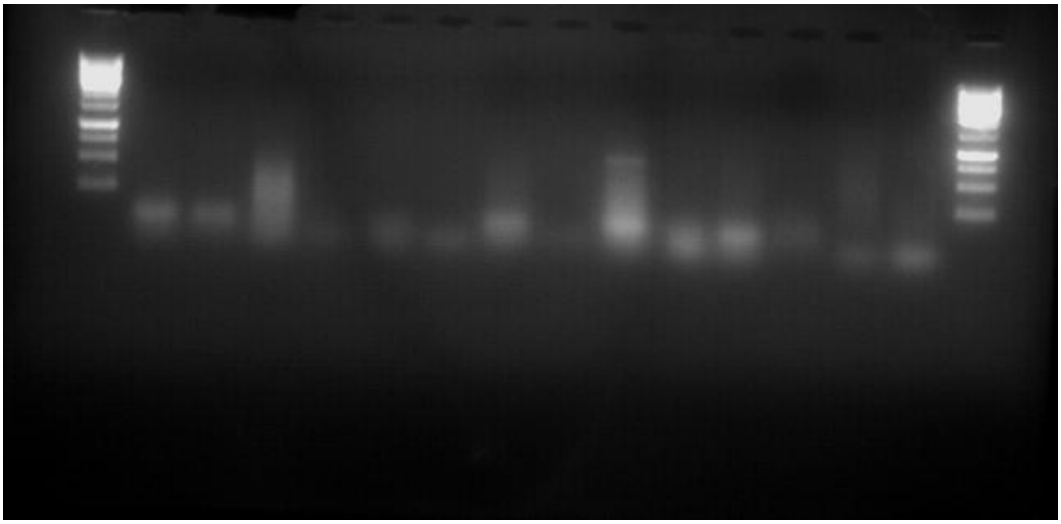

Plate VIII: Agarose (1.2 %) gel electrophoretic profile of PCR amplification of cytochrome b gene (585bp). L is ladder (1kb), (B1 & B2) are muscle and fin samples of *Clarias gariepinus* (wild), (C1& C2) are muscle and fin samples of *Clarias gariepinus* (cultured), (D1 & D2) are muscle and fin samples of *Heterobranchus longifilis* (wild), (E1 & E2) are muscle and fin samples of *Heterobranchus longifilis* (cultured), (F1 & F2) are muscle and fin samples of *Clarias galmaensis* (wild), (G1 & G2) are muscle and fin samples of *Heterobranchus bidorsalis* (wild), (H1 & H2) are muscle and fin samples of *Heterobranchus isopterus* (wild) respectively.

L B1 B2 C1 C2 D1 D2 E1 E2 F1 F2 G1 G2 H1 H2 L

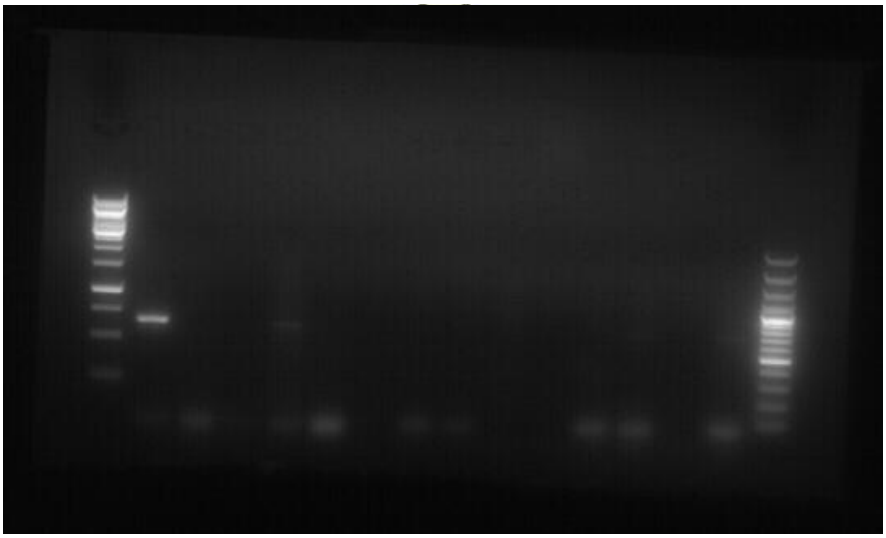

Plate IX: Agarose (1.2 %) gel electrophoretic profile of PCR amplification of cytochrome b gene (585bp). L is ladder (1kb), (B1 & B2) are muscle and fin samples of *Clarias gariepinus* (wild), (C1& C2) are muscle and fin samples of *Clarias gariepinus* (cultured), (D1 & D2) are muscle and fin samples of *Heterobranchus longifilis* (wild), (E1 & E2) are muscle and fin samples of *Heterobranchus longifilis* (cultured), (F1 & F2) are muscle and fin samples of *Clarias galmaensis* (wild), (G1 & G2) are muscle and fin samples of *Heterobranchus bidorsalis* (wild), (H1 & H2) are muscle and fin samples of *Heterobranchus isopterus* (wild) respectively.

L B1 C1 D1 E1 F1 G1 H1 B2 C2 D2 E2 F2 G2 H2 L

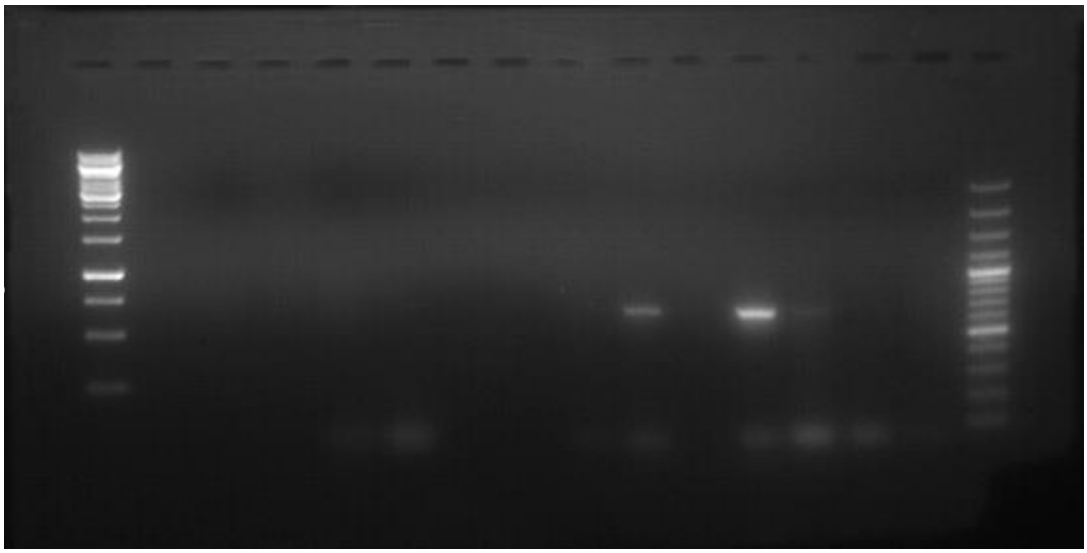

Plate X: Agarose (1.2 %) gel electrophoretic profile of PCR amplification of cytochrome b gene (585bp). L is ladder (1kb), (B1 & B2) are muscle and fin samples of *Clarias gariepinus* (wild), (C1& C2) are muscle and fin samples of *Clarias gariepinus* (cultured), (D1 & D2) are muscle and fin samples of *Heterobranchus longifilis* (wild), (E1 & E2) are muscle and fin samples of *Heterobranchus longifilis* (cultured), (F1 & F2) are muscle and fin samples of *Clarias galmaensis* (wild), (G1 & G2) are muscle and fin samples of *Heterobranchus bidorsalis* (wild), (H1 & H2) are muscle and fin samples of *Heterobranchus isopterus* (wild) respectively.

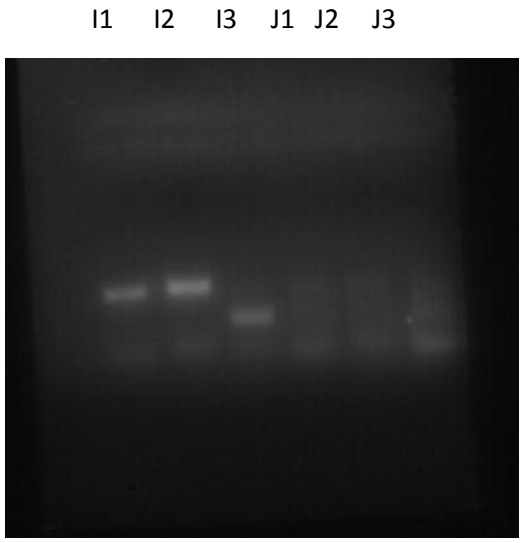

Plate XI: Agarose (1.0 %) gel electrophoretic pattern of restriction digest of PCR product for *Clarias gariepinus* (wild) and *Clarias gariepinus* (cultured) using Eco R1 (I1, J2); Hind 111 (I2, J2) and Hae 111(I3, J3).

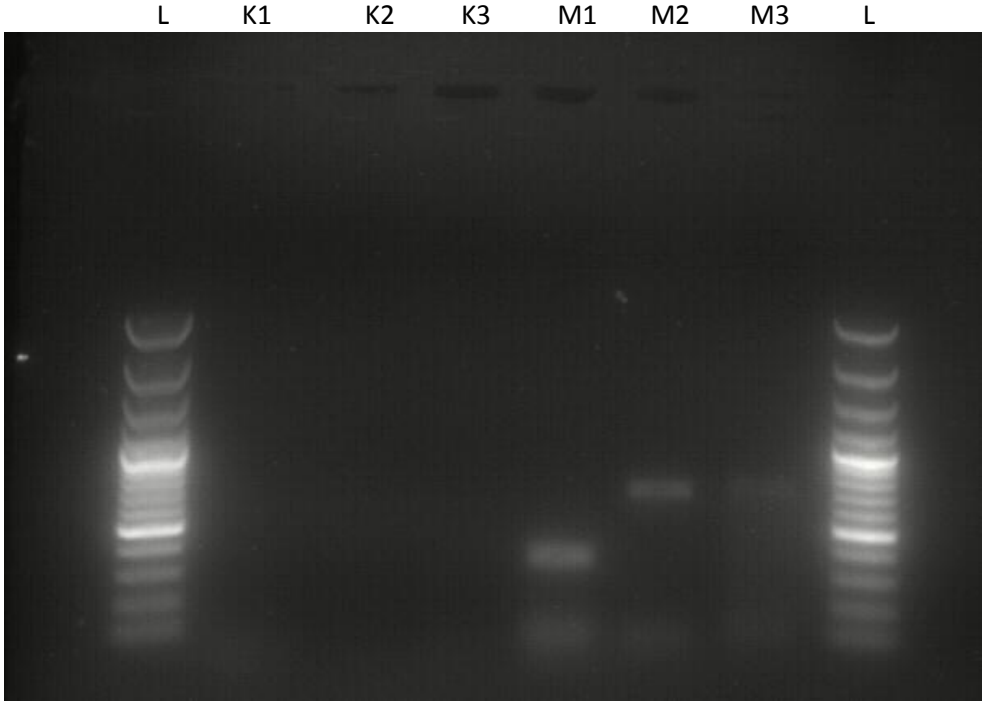

Plate XII: Agarose (1.0 %) gel electrophoretic pattern of restriction digest of PCR product for *Heterobranchus longifilis* (wild) and *Heterobranchus longifilis* (cultured) using Hae 111 (K1, M1); Eco R1 (K2, M2) and Hind 111 (K3, M3) respectively, L is the ladder(1kb).

L N1 N2 N3 O1 O2 O3 P1 P2 P3 Q1 Q2 Q3 L

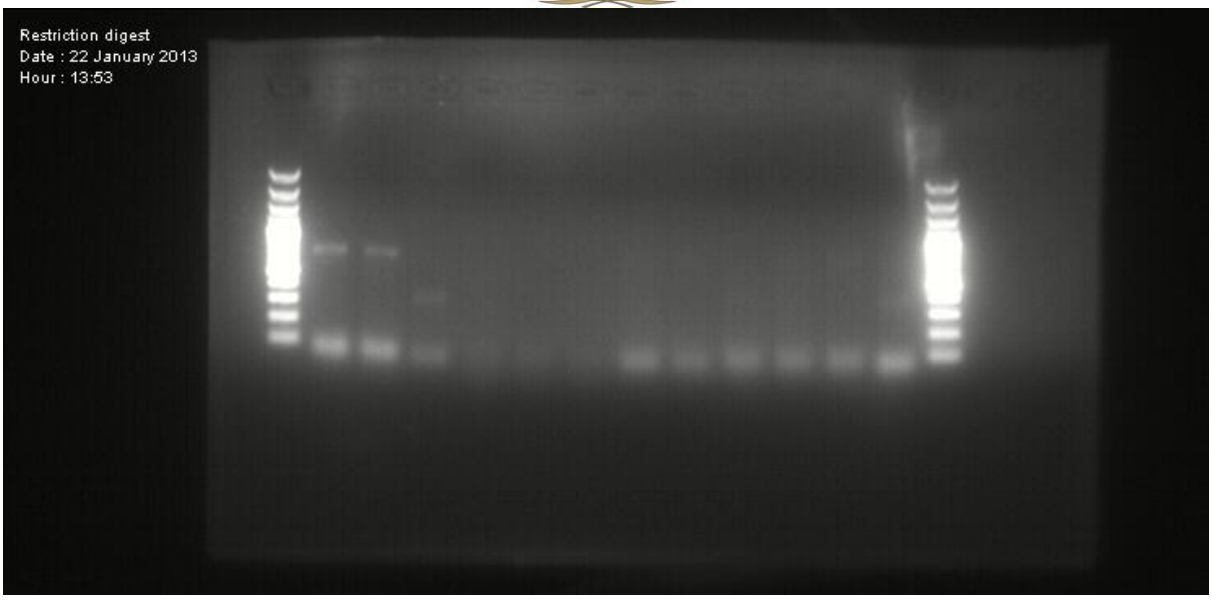

Plate XIII: Agarose (1.0 %) gel electrophoretic pattern of restriction digest of PCR product for *Clarias anguillaris* (wild), *Clarias galmaensis* (wild), *Heterobranchus bidorsalis* (wild) and *Heterobranchus isopterus* (wild) using Eco R1 (N1, O1, P1, Q1); Hind 111 (N2, O2, P2, Q2) and Hae111(N3, O3, P3, Q3) respectively, L is the ladder (1kb).

4.4 Polymerase Chain Reaction and Restriction Fragment Length Polymorphism

Generally, DNA was successfully extracted from preserved fin and muscle tissues. The amplification of cytochrome b gene (585bp) from a portion of mtDNA of wild and cultured *Clarias* and *Heterobranchus* species using primers L15267, 5'-AAT GAC TTG AAG AAC CAC CGT-3' and H15891, 5'-GTT TGA TCC CGT TTC GTG TA-3' was observed. Amplification was however more pronounced in fin than in muscle tissue. Depending on the intensity of the amplification restriction digest of the amplicons showed the same pattern for the digest using EcoR1, Hind 111 and Hae 111 (Plates VII-XIII). The restriction enzymes EcoR1 and Hind 111 cut the amplicons at the same level whereas Hae 111 cut at a slightly lower level.

4.5 Genetic Variation of *Clarias* and *Heterebranchus* species

A pictorial representation of the amplicons shows a level of diversity in the genotypes of wild and cultured *Clarias* and *Heterobranchus* species. Basically, the phylogeny showed two groups, the genotypes clustered together are closely related while their neighbours are joined closely.

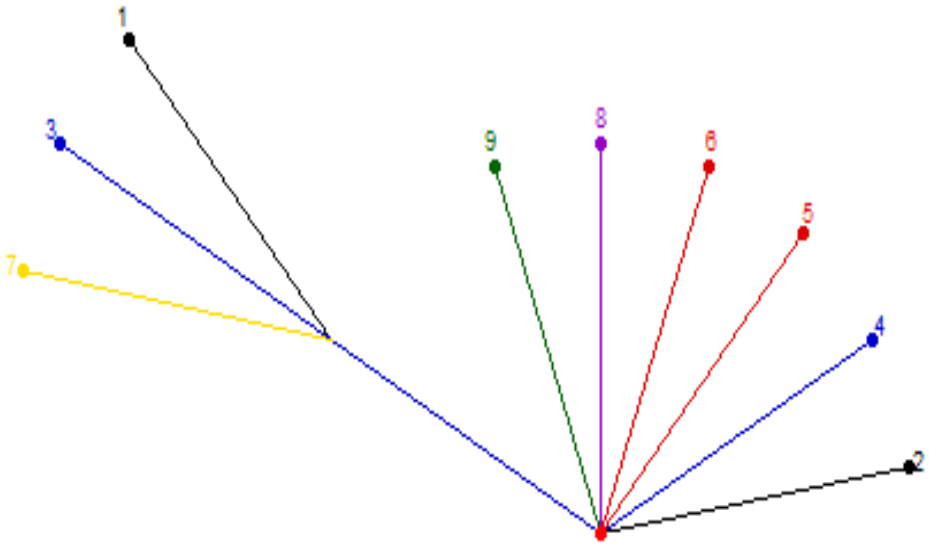

Figure 8: Radial phylogram (neighbour joining) representing genetic diversity of *Clarias* and *Heterobranchus* species

Key: Black = Indicator (1 & 2); Blue = *Clarias gariepinus* (3 & 4); Red = *Heterobranchus longifilis* (5 & 6); Yellow = *C. galmaensis* (7); Purple = *H. bidorsalis* (8); Green = *H. isopterus* (9)

Wild species of *Clarias gariepinus* (3) and *C. galmaensis* (7) respectively, were found to be closely related while cultured *Clarias gariepinus* (4), and wild and cultured *Heterobranchus longifilis* (5 and 6), wild *H. bidorsalis* (8) and *H. isopterus* (9) were also clustered together showing their close relatedness (Figure 8).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Interactions of fish species with their natural habitat

In this study the morphometrics of *Clarias* and *Heterobranchus* species as well as physico-chemical properties were used to assess genetic variability in relation to wild and culture environment. Measurements of morphological features have been widely used to differentiate various fish populations. Among the vertebrates, phenotypic (especially morphometrics) variability is considered to be greatest in fish, which have relatively higher intra-population coefficients of variation of phenotypes. This variability is likely to have arisen from the great phenotypic plasticity of fishes in response to changes in environmental factors (Gunawickrama, 2007).

5.1.1 Morphometrics of *Clarias* and *Heterobranchus* species

The morphometrics of *Clarias gariepinus* and *Heterobranchus longifilis* accounted for the highest measurements compared to the other species examined in this study. This large size of *Clarias gariepinus* and *Heterobranchus longifilis* can be attributed to their being very hardy omnivorous fish, whose potential growth rate is one of the fastest observed amongst African fish species tested in aquaculture; it has proved to be a fast growing protein source. Interspecific cross-breeding in these fish will lead to hybrids with valuable characteristics for culture such as sterility, monosex population, heterosis for disease resistance or growth rate (Legendre *et al*., 1992).

There were also significant differences in the morphometrics of all the species of fish examined, however, the mean morphometrics for cultured *Clarias* species were higher than wild species and some mean morphometric values for cultured *Heterobranchus* were higher than wild species probably due to seasonal variations and nature of the water bodies. The mean values of the morphometrics were within the range described by Teugels (2003) as standard ranges for *Clarias* and *Heterobranchus* species.

The only morphological distinguishing feature of *Clarias* and *Heterobranchus* species is the adipose fin in the later, which is absent from the former. The gap between the adipose fin and dorsal fin was very conspicuous in all *Heterobranchus* species. The relationship between the adipose fin and dorsal fin were not equidistant in any of the *Heterobranchus* species. The adipose fin lengths were shorter in length compared to the dorsal fin this could be due to intra-specific differenciations. In comparism with *Clarias* species, the anal fin is longer in *Heterobranchus* species whereas the dorsal fin is longer in *Clarias* than *Heterobranchus*, this could be attributable to the presence of an adipose fin and other physiological characters. This finding is similar to those of Hanssens (2009) and Agbebi *et al*. (2009). Aken'Ova (2007) described a new species *Clarias galmaensis* in River Galma, Zaria Nigeria; this species resembles other *Clarias* species found in Nigeria except for its consistently smaller size.

The positive correlation from the Principal Component Analysis (PCA) in all morphometrics, shows that the organism is able to survive as a living entity with indispensable body parts. The relationship observed between the morphometrics of the fishes and physico-chemical parameters of the water body is indicative of the interaction between the genes and the environment that

results in the phenotype of these fishes and any other organism. Principal Component Analysis (PCA) performed by Turan *et al*. (2005) on the catfishes from different regions of Turkey revealed that morphometric differentiation between samples from Turkish waters was largely located in the head of *C. gariepinus* (length of head, interorbital distance, the distance from the dorsal fin to the occipital process) as well as the placement of dorsal and ventral fins, indicating that these measurements are important in the description of population characteristics. Data obtained in this study showed also a high variability in the characters of to head, but a low variability in relation to the placement of all fins. I concur with the conclusion drawn by Turan *et al*. (2005) that such differences among populations may be related to different habitat characteristics such as temperature, turbidity, food availability, water depth and flow. It is also well known that morphometric characters can show high plasticity in response to differences in environmental conditions, such as food abundance and temperature; extreme differences in culture conditions may elucidate adaptive potential of a species and its morphological variability as an expression of ecological plasticity (Keszkae *et al*., 2009).

5.1.2 Physico-chemical properties of the aquatic environment

Athough there were significant differences in all physico-chemical properties between the wild and culture environments in this study, all the values were within the acceptable values for fish survival, especially for clariids (Heut, 1979; Adakole *et al.*, 1998). The little variations observed in pH, temperature, hardness and NO_3-3 in both wild and culture habitats imply that the impact of these values on the fishes is negligible.

Temperatures of between 27.7-31.7°C and 27.5-28.9°C recorded in wild and culture habitats respectively, during the study period fall within the range (25°C and 32°C) recommended by Afzal *et al*. (2007) for good performance of fishes. The pH ranges obtained during the study (6.42-7.89 and 7.25-7.82) fall within the range of 6.0 and 9.0, which is said to be productive in fish culture (Heut, 1979; Adakole *et al.*, 1998). Tukura *et al*. (2005) also obtained a mean pH of 6.59±0.10 for River Kubanni which is close to value obtained in this study for the wild habitat. Tukura *et al.* suggested that the value they obtained may be due to the fact that Kubanni reservoir water receives more pollutants since it is the main drainage channel for the Samaru-Ahmadu Bello University area; the same comment may apply here.

The nitrate values of <1mg/L obtained in River Galma and Bagoma Reservoir, as well as the culture habitats are within acceptable levels for fish survival. Nitrate is a form of nitrogen and a vital nutrient for growth, reproduction, and the survival of organisms but high nitrate levels (>1 mg/L) are not good for aquatic life (Johnson *et al.*, 2000). The phosphate content of artificial water bodies such as fish ponds (culture habitat) which were considered in this study had lower values of 0.24±0.02mg/L. River Galma and Bagoma reservoir also had lower values 0.38±0.10mg/L which could be attributed to intense farming and other anthropogenic activities carried out around them. Phosphate is considered to be the most significant among the nutrients responsible for eutrophication of lakes, as it is the primary initiating factor (Adeyemo, 2003). Fakayode (2005) found the mean PO_4^{3-} content in the downstream section of Alaro River, Ibadan to be 4.62±2.07 mg/L and while there are various sources of phosphate to rivers, such as firm rock deposit, runoff from surface catchments, and interaction between the water and sediment from plant and animal remains at the bottom of rivers; High Levels of both phosphate and

nitrate can lead to eutrophication, which increases algal growth, ultimately reducing dissolved oxygen levels in the water (Murdoch *et al.*, 2001).

In this study a lower value of Dissolved Oxygen 1.10mg/L was obtained in culture habitat and this may be attributed to the smaller size of the pond and eutrophication due to over fertilization. According to Ufodike and Garba (1992) a minimum constant value of 4.0mg/L Dissolved Oxygen is adequate for most species and stages of aquatic life.

Higher values of Electrical conductivity, Biochemical Oxygen Demand, Total Dissolved Solids and alkalinity obtained in the culture environment were probably due to the concentration of solids and other ions in the ponds against what was observed in the wild, this is likely due to the continuous flow and mixing of water in rivers. In an analysis of upstream water from River Challawa Yisa, 2004 obtained similar ranges for physico-chemical parameters: EC 58-946S/cm; TDS, 34-520 mg/L; BOD, 10.0-9.35 mg/L etc. Values of physico-chemical parameters (DO, 1.4-4.8 mg/L and alkalinity, 24.2-25.4 $CaCO_3$mg/L) obtained by Fafioye *et al*. (2005) for water from Omi water body were generally higher than values obtained in this study.

The variations in some physico-chemical parameters of the wild (river) environments although within the acceptable limits in this study may be attributable to the fact that rivers flow and the effect of concentration of any pollutants would be mild compared to culture ponds in which the concentrations of pollutants from feeds and excretory products increase and accumulate, making the ponds prone to eutrophication.

5.2 Genetic Variation of the fishes

The PCR amplification of cytochrome b gene in *Clarias* and *Heterobranchus* species shows the presence of the gene in these species. The clustered genotype of wild *Clarias gariepinus* and *C. galmaensis* reveals their close relationship which could be attributed to the fact that they were both obtained from the wild. Although there were variations in the phenotype of wild and cultured *Heterobranchus* and *Clarias* species, the clustered groups of *Heterobranchus longifilis*, *H. isopterus*, *H. bidorsalis* and *Clarias gariepinus* shows a close relationship in their genotypes which is indicative of their phylogeny. Findings on serum protein pattern in interspecific and intergeneric hybrids of *Heterobranchus longiflis*, *Clarias gariepinus* and *Clarias anguillaris* in Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) by Akinwande *et al*. (2012) reported a high similarity coefficient between these species hinting at a very low genetic diversity. The close relationship between the *Heterobranchus* species and the species of the subgenus *Clarias* to which *Clarias gariepinus* and *C anguillaris* belong has been emphasized by Teugels *et al*. (1990) in a revisionary study using osteological features. Agnèse *et al*. (1997) reported genetic variation at 25 protein and eight microsatellite loci, and two mitochondrial (mtDNA) segments in two sympatric clariids from the Senegal River.

The results obtained here concur to some extent with those of Rognon *et al*. (1998) in a morphometric and the allozyme study of nine populations of *C. gariepinus* and seven populations of *C. anguillaris* to quantify their intra- and interspecific variation using *Clarias albopunctatus* and *Heterobranchus longifilis*, as outgroups. Morphometric and allozyme data were congruent for the Nilo-Sudanian populations of *C. gariepinus* and *C. anguillaris* in their

study however, allozyme data suggested that *C. gariepinus* is not a monophyletic group and that *C. albopunctatus* was more divergent from *C. gariepinus* and *C. anguillaris* than it was from *H. longifilis*. They stressed the need for a revision of clariid systematics.

There are several rationales for summarizing the cytochrome b gene literature which was found to be inherent in *Clarias* and *Heterobranchus* species studied: First, cyto b is the gene that is perhaps most extensively sequenced to date for vertebrates (Lydeard and Roe 1997; Moore and De-Filippis 1997). Second, the evolutionary dynamics of the cyt b gene and the biochemistry of the protein product are better characterized than most other molecular systems (Esposti *et al*., 1993). Third, levels of genetic divergence typically associated with sister species, congeners, and confamilial genera are usually in a range in which the cyt b gene is phylogenetically informative and unlikely to be severely compromised by superimposed nucleotide substitutions (Meyer 1994).

The nucleotide composition of cyt b sequences obtained in this study agree with these obtained similar studies i.e. low Guanine (G) content and almost equal amount of Adenosine (A), Thyamine (T) and Cytosine (C), the 585 bp sequence comprised 495 conserved and 58 parsimony informative sites (Briolay *et al*., 1998; Mwita & Nkwengulila, 2008).

The digestion of DNA with restriction enzymes which was also considered in this study cut DNA strands into specific nucleotide sequences. The resulting DNA fragments are called restriction fragment length polymorphisms (RFLP's) which for example, the restriction enzyme Hae111 cuts DNA or a particular gene at the four nucleotide sequence GGCC (it is called a four-

cutter). Variation at the GGCC site will permit the detection of DNA polymorphisms. Sequence variation of the maternally inherited mitochondrial DNA (cytochrome b gene) has been used for detecting genetic variation within and among populations, and among species. Sequence variation of coding nuclear genes, such as growth hormone and homeobox genes, are of phylogenetic interest.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Generally, the morphometrics of the six species studied showed variations in size irrespective of the environment. *Clarias gariepinus (*22.8-43.8) and *Heterobranchus longifilis* (26.3-39.8) had the largest sizes followed by *Clarias anguillaris* (17.8-39.0) and *Heterobranchus bidorsalis* (21.7-38.5). The smallest in size range of the samples collected were *Heterobranchus isopterus* (18.4-25.6) and *Clarias galmaensis* (15.7-20.5) from all the habitats. The differences in the morphometrics of the fish species were significant ($P<0.05$) and a correlation was observed between the morphometrics and the physico-chemical properties of their habitat. Variations were observed in some water quality parameters between the wild and culture habitats. Electrical conductivity ($230\pm16.97\mu S$), TDS (114.25 ± 8.84ppm) and alkalinity had higher values in the culture environment while lower values were observed in the wild. Higher values were observed in BOD (4.38 ± 2.09mg/L), DO (7.7 ± 1.41mg/L) and $P0_4$-P (0.38 ± 0.14mg/L) in wild habitat whereas lower values were observed in culture environment. Little variations were observed in pH, temperature, hardness and NO_3-N for both wild and culture environment. There were significant differences observed in all physico-chemical properties either with respect to the fish species and the environment ($P<0.05$).

Clusters of *Clarias gariepinus* and *C. galmaensis* (both wild species) showed a closer relationship than to the other groups; *Clarias gariepinus* (cultured), *Heterobranchus longifilis* (wild and cultured), *H. isopterus* and *H. bidorsalis* showed a close relationship.

6.2 Conclusions

There are significant differences between the morphometrics of *Clarias* and *Heterobranchus* species. The differences between the physico-chemical properties of the natural and artificial environments are significant. This is reflected in the relationship between the morphometrics and physico-chemical properties which are vital and can be useful in breeding programmes to produce more of these species with better quality (eg. fast growth) for market demand as well as for the conservation of the species; H_1 and H_2 are therefore rejected. The variation in genotypes of wild and cultured *Clarias* and *Heterobranchus* species is low, indicating a close relationship between the species. The low variation is evidence of the fact that they are members of the same family Clariidae; hypothesis H_3 is therefore rejected.

6.3 Recommendations

From this study it is recommended that:

- Fin and muscle tissues of fish are suitable for molecular analysis.
- Information on the morphometrics of *Clarias* and *Heterobranchus* species as well as the genetic variation obtained in this study can be used as baseline information and reference when assessing further changes caused by nature or humans on the environment as this will provide management guidelines for breeding and genetics of fishes.
- Studies like this should take into cognizance the morphometric and genetic variations that might have resulted from sexual physiological differences and dimorphism alongside other predisposing factors; these were not considered in this study.
- More research should be conducted on the morphometrics and genetics of *Clarias* and *Heterobranchus* species and all other economically important and culturable species of fish.

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APPENDICES

Appendix I: Morphometric data of wild and cultured species of *Clarias* and *Heterobranchus*

Morphometrics of wild fish

SAMPLES	SL	TL	STPFO	HL	HW	SL	STDFO	BL	PSL	DFL	AFL	PCFL	PLFL	ED	ADFL	DAFL
CgaGAL	35.4	39.0	14.8	9.3	7.8	3.8	11.6	7.7	3.3	22.6	15.0	3.7	3.2	0.5		
CgaGAL	29.8	34.5	12.7	8.5	6.3	2.4	9.6	6.9	2.7	19.7	14.3	3.3	2.9	0.4		
CgaGAL	20.7	22.8	9.3	5.6	3.5	1.9	7.0	5.0	1.3	13.5	8.8	2.1	1.5	0.3		
CgaBAG	22.3	25.0	9.8	5.2	4.1	2.4	7.4	5.4	1.7	14.2	10.1	2.3	2.0	0.3		
CgaBAG	22.0	25.0	9.6	5.2	4.3	2.0	6.8	5.6	1.5	15.3	10.2	2.7	2.0	0.4		
CanGAL	34.5	39.0	14.3	8.9	6.6	3.5	10.0	7.4	2.4	22.5	16.3	3.7	2.9	0.6		
CanGAL	20.4	22.7	9.3	4.5	3.6	1.2	6.5	5.6	2.0	12.3	7.8	2.3	1.9	0.2		
CnGAL	29.1	32.4	13.1	6.3	5.2	3.5	10.0	7.1	2.4	19.0	12.6	4.4	2.2	0.4		
CanBAG	19.5	22.5	8.8	5.7	3.8	1.7	7.0	4.2	1.9	13.5	8.1	2.5	1.7	0.3		
CanBAG	16.5	17.8	7.0	4.2	2.3	1.2	4.7	3.3	1.8	10.8	7.8	2.1	1.9	0.3		
CgmGAL	18.1	19.7	5.9	3.8	2.5	1.6	5.5	3.2	1.3	11.8	9.7	1.8	1.2	0.3		

CgmGAL	15.4	16.7	5.6	3.1	2.4	1.0	4.2	4.0	0.8	9.8	7.2	1.1	0.9	0.2		
CgmGAL	15.0	16.2	6.3	3.5	2.3	1.4	4.5	4.4	1.1	10.7	7.8	1.4	0.9	0.3		
CgmGAL	14.1	15.7	5.5	3.4	2.5	1.2	4.4	4.3	1.3	10.2	7.2	1.7	1.3	0.3		
CgmGAL	18.7	20.5	7.2	4.1	3.0	1.5	5.7	5.2	1.3	12.5	9.5	1.7	1.2	0.3		
HbiGAL	32.5	36.0	14.9	8.6	7.0	4.0	11.0	15.9	3.0	12.4	12.8	4.3	3.0	0.65	8.0	1.0
HbiGAL	28.8	31.9	13.3	8.7	6.4	3.5	9.9	14.2	3.0	10.4	10.5	3.8	3.4	0.5	6.0	0.5
HbiGAL	28.4	32.3	13.1	8.5	6.8	3.6	9,5	12.2	2.0	11.5	10.8	3.5	2.8	0.5	6.2	0.5
HbiGAL	33.5	38.5	15.5	9.2	7.5	4.6	11.5	16.2	4.0	13.5	13.7	4.6	3.8	0.6	8.0	0.2
HbiGAL	19.2	21.7	10.3	5.4	4.2	2.8	6.9	10.4	2.1	7.9	7.3	2.6	2.1	0.35	4.0	0.1
HloGAL	33.9	37.6	16.0	10.6	7.5	4.0	13.0	13.4	3.3	11.5	13.3	4.4	3.3	0.6	8.4	1.0
HloGAL	32.0	34.5	14.5	9.5	6.7	3.2	11.5	11.6	2.6	10.0	11.9	3.2	3.9	0.5	7.7	1.2
HloGAL	31.7	34.0	13.3	8.7	8.5	3.8	10.4	11.1	2.6	9.1	12.2	4.0	3.5	0.6	8.5	1.2
HloGAL	36.5	39.8	16.2	10.3	7.7	4.0	13.2	14.0	3.2	13.1	14.4	4.2	3.2	1.1	9.1	1.4
HloGAL	23.7	26.3	11.1	7.1	4.7	2.5	8.7	8.1	1.5	8.0	8.5	3.1	2.8	0.5	5.0	1.4
HisGAL	15.8	18.4	8.7	4.5	3.2	1.8	5.7	7.6	1.3	6.9	5.8	2.4	1.4	0.3	3.5	0.4
HisGAL	22.4	25.6	10.0	7.0	5.1	3.0	7.6	9.6	1.7	9.8	9.7	3.2	2.6	0.4	5.7	0.5

HisGAL	18.2	20.8	8.4	4.5	3.6	2.4	4.4	9.1	1.2	7.7	8.2	2.2	1.7	0.4	3.8	0.6
HisGAL	20.3	23.2	9.3	5.7	4.2	2.5	7.2	9.0	1.2	8.5	8.3	2.3	1.9	0.4	4.3	0.6
HisGAL	17.7	20.3	8.2	5.0	3.7	2.3	6.2	8.5	1.4	8.2	7.0	2.4	2.0	0.4	3.4	0.6

Morphometrics of cultured fish

SAMPLES	SL	TL	STPFO	HL	HW	SL	STDFO	BL	PSL	DFL	AFL	PCFL	PLFL	ED	ADFL	DAFL
CgaMIR	26.8	29.6	11.5	6.8	4.7	2.4	8.2	9.8	1.6	18.0	10.4	3.1	2.2	0.3		
CgaMIR	38.4	43.8	16.8	10.6	7.7	4.1	13.0	11.3	3.1	25.6	16.4	4.8	3.7	0.6		
CgaMIR	34.7	39.8	16.5	10.5	6.2	3.2	12.1	10.1	3.1	21.8	14.9	4.7	3.1	0.5		
CgaMIR	28.9	33.0	12.7	7.5	5.0	2.1	8.7	8.9	2.4	19.1	12.4	4.0	3.1	0.4		
CgaMIR	24.4	31.3	13.1	7.6	5.2	2.6	10.0	10.2	2.3	19.1	12.8	3.4	3.1	0.5		
HloNFR	36.7	39.8	16.0	9.3	7.5	4.3	12.6	13.4	4.2	12.6	12.8	4.5	3.1	0.6	8.4	1.6
HloNFR	34.0	38.5	16.7	10.1	7.0	4.0	12.0	15.4	2.7	11.0	13.5	3.4	3.4	0.5	8.5	1.5
HloNFR	31.5	35.6	14.5	7.8	6.3	3.7	11.2	13.0	2.4	11.7	11.5	4.2	3.9	0.4	8.1	1.7
HloNFR	29.6	33.5	14.2	8.6	5.8	3.5	10.3	9.7	2.6	9.4	11.6	3.4	2.9	0.4	7.1	1.3
HloNFR	27.6	31.8	12.6	8.4	5.6	3.2	10.5	12.2	2.2	10.7	10.7	3.3	2.6	0.4	8.1	1.4

Key: SL = Standard Length; **TL** = Total Length; **STPFO** = Snout To Pelvic Fin Origin; **HL**= Head Length; **HW** = Head Width; **SnL** = Snout Length; **STDFO** = Snout To Dorsal Fin Origin; **BL**= Barbel Length; **PSL** = Pectoral Spine Length; **DFL** = Dorsal Fin Length; **AFL** = Anal Fin Length; **PCFL** = Pectoral Fin Length; **PLFL** = Pelvic Fin Length; **ED** = Eye Diameter **ADFL** = Adipose Fin Length; **DAFL** = Distance Between Dorsal And Adipose Fin