MORPHOMETRIC AND DNA VARIATION OF THE AFRICAN CATFISH (*Clarius gariepinus*) IN SELECTED CULTURED AND WILD POPULATIONS IN KENYA

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DECLARATION

I declare that this is my original work and has not been presented for any award degree or examination at any other university or institution of tertiary education.

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DEDICATION

This thesis is dedicated to my family, thank you for support.

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To God be all the Glory.

TABLE OF CONTENTS

DECLARATION ii
DEDICATION iii
ACKNOWLEDGEMENTS iv
TABLE OF CONTENTS v
LIST OF FIGURES viii
LIST OF TABLES ix
LIST OF APPENDICES x
LIST OF ABBREVIATIONS AND ACRONYMS xi
ABSTRACT xii
CHAPTER ONE 1
INTRODUCTION 1
1.1 Background Information 1
1.2 Problem Statement 4
1.3 Justification 5
1.4 Objective 6
1.4.1 Specific objectives 6
CHAPTER TWO 7
LITERATURE REVIEW 7
2.1 African Catfish Biology 7
2.2 African catfish (*Clarius gariepinus)* in aquaculture 7
2.3 Catfish Genetics 9
2.3.1 Morphological markers 10
2.3.2 Isozymes 11
2.3.3 Microsatellites 11
2.3.4 Expressed Sequence Tags 12
2.3.5 Single Nucleotide Polymorphism 12
2.3.6 Randomly amplified polymorphic DNA 12

2.3.7 Amplified fragment length polymorphism ... 13
2.3.8 Mitochondrial DNA ... 14
CHAPTER THREE ... 16
MATERIALS AND METHODS ... 16
3.1 Study Areas ... 16
3.1.1 Cultured catfish-Hatcheries ... 17
3.1.2 Wild catfish - Lake Naivasha and Lake Baringo ... 17
3.2 Data Collection ... 18
3.2.1 Morphometric measurements ... 18
3.2.2 DNA Extraction and Polymerase Chain Reaction of D-loop (PCR) ... 19
3.3 Data Analysis ... 21
3.3.1 Morphological Analysis ... 21
3.3.2 Genetic Analysis ... 22
CHAPTER FOUR ... 24
RESULTS ... 24
4.1 Preliminary Results ... 24
4.2 Morphological Results ... 25
4.2.1 Morphological variance results ... 25
4.2.2 Morphological variations ... 27
4.2.3 Canonical variate analysis (CVA) ... 28
4.2.4 Inter-group distances between catfish populations ... 29
4.2.5 Principal component analysis (PCA) ... 30
4.3 Genetic diversity using Mitochondrial D-Loop Analysis ... 32
4.3.1 Evolutionary History ... 32
4.3.2 Genetic Diversity ... 34
4.3.3 Haplotype Distribution ... 35
4.3.4 Genetic differentiation and AMOVA ... 39
4.3.5 Neutrality test results ... 40
CHAPTER FIVE ... 41
DISCUSSION ... 41
5.1 Breeding and Management practices ... 41

5.3 Morphology variation and biogeography 42
5.2 Genetic differentiation and diversity 43
5.4 Phylogenetic structure 45
5.5 Population selection 46
CHAPTER SIX 47
CONCLUSION AND RECOMMENDATION 47
6.1 Conclusion 47
6.2 Recommendations 47
REFERENCES 49
APPENDICES 60

LIST OF FIGURES

Figure 1: African catfish-*Clarius gariepinus* (Munguti *et al.,* 2014) 2

Figure 2.Fishery production in Kenya from 1980 (FAO Fishery Statistic, 2015) 8

Figure 3. Aquaculture production by species in 2009 (adapted from Otieno, 2011)...................... 8

Figure 4. Map of Kenya showing location of the six study sites: Lake Baringo, Lake Naivasha, Athi River, Sagana, Kisii and Jewlet hatcheries. 16

Figure 5. Image of 500bp PCR products run on 1% agarose gel. Lane a is the molecular ladder lane, x is the 500bp marker of the 1kbplus molecular ladder.1-19 represents PCR samples from Athi River... 20

Figure 6. Screen shot of survey data in SPSS version 23 21

Figure 7.Canonical variate analysis of catfish from Athi River, Kisii, Naivasha. Jewlett and Sagana 29

Figure 8. Component plot loadings of the variables: HL (head length), IOW(interorbittal width), PDL(predorsal length), VMW(vomerine toothplate width), OPW(occipital process width), OPL(occipital process length), PPEL(prepectoral length), PAL(preanal length), PPL(prepelvic length), PMW(premaxillary width), SL(standard length), AFL(anal fin length), DFL(dorsal fin length) 30

Figure 9. Binned Samples labeled by source (1.Athi River, 2. Kisii,, 3. Naivasha,4. Jewlett,5. Sagana and 6.Baringo) 31

Figure 10. Combined variables and object points by sources of fish 31

Figure 11. Sample sequences aligned and trimmed in Mega 7.. 32

Figure 12.Evolutionary relationships of the African catfish from Athi River (AR), Jewlett(J), Kisii(K) ,Sagana(S), Baringo (B) and Naivasha. 33

Figure 13. Median Network tree of African catfish mitochondrial DNA haplotypes(H) Athi River, Jewlett, Sagana, Kisii, Baringo and Naivasha populations... 38

LIST OF TABLES

Table 1. Challenges faced in catfish culture production 25

Table 2.Source of morphological variation and interaction among the catfish populations 26

Table 3. Analysis of variance showing differences in variables among populations (p<0.01). ... 26

Table 4.The standardized variables by ratios grouped by source of the catfish. 27

Table 5. Distance between populations Athi River, Kisii, Naivasha, Jewlett and Sagana 29

Table 6. Table showing haplotype diversity of the catfish populations 34

Table 7. Haplotype distribution among the sampled catfish populations 36

Table 8. Pairwise genetic differences of the six sampled populations 39

Table 9. Hierarchical analyses of molecular variance showing amount of population genetic structure.. 40

Table 10. Neutrality indices of sampled catfish populations 40

LIST OF APPENDICES

Appendix 1: Questionnaire for Hatcheries 60

Appendix 2. Catfish measurements 67

Appendix 3: Laboratory reagent preparation procedure: Agarose gel preparation 68

Appendix 4: DNA extraction: Qiagen Kit protocol 68

Appendix 5: Table of means and significance levels.. 70

LIST OF ABBREVIATIONS AND ACRONYMS

Hd haplotype diversity

π Nucleotide diversity

df degree of freedom

ANOVA analysis of variance

MS Means of squares

SS sum of squares

VR variance ratios

DNA deoxyribonucleic acid

PCR Polymerase Chain Reaction

MtDNA mitochondrial DNA

ABSTRACT

The study seeks to define and understand the population diversity, morphological and genetic differences of these fish in their natural habitats from which brood stock is obtained and in hatcheries in Kenya. Catfish samples were obtained from six sites in the country namely Athi River hatchery, Kisii Fingerling Production Centre (FPC), Jewlett hatchery, Sagana Station, Lake Baringo and Lake Naivasha. The samples were characterized using morphometric and genetic markers.

For morphometric study, canonical variate and principal component analyses were used to cluster the populations in Genstat and SPSS so as to determine population variation. Thirteen variables of one hundred forty-five African catfish were subjected to the analyses for morphological characterization. For molecular study, DNA was extracted from the muscle tissue samples, followed by amplification and sequencing of the D-loop region of mitochondrial DNA. The sequences were subjected to diversity analyses in MEGA 7, DNASP V5 and Arlequin V3.5.

Morphometric clustering of fish was evident with four populations having an overlap in clusters i.e. Kisii, Jewlett, Sagana and Baringo. The Naivasha and Athi River population did not overlap. The two clustered distantly from each other and from the overlapped three populations. Diversity as derived from mitochondrial DNA markers for the populations as a whole was Hd: 0.884 ±0.017 and the nucleotide diversity (π) was 0.09018± 0.10414. The diversity in each site population varied with Baringo having the highest at 0.913±0.035 while Naivasha had no diversity at 0.00±0.00. The Naivasha population had only one haplotype while the others had more than one haplotype.

The genetic and morphometric results were congruent confirming the results. The Sagana, Kisii FPC, Jewlett and Baringo population overlapped when assessed using both morphology and genetic methods indicating possibly shared source of broodstock. The Naivasha populations being distinct is not likely to have originated from the catfish in the other sampled sites or could have undergone mutations in the region resulting in the high differentiation. The Athi River population was distant and distinctiveness is attributed to imported broodstock. The Athi River hatchery population and Lake Baringo population have the highest diversities hence should be targeted as source of fingerlings.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Open access fisheries are an important source of income and food locally in Kenya and for export. A variety of fish species from natural water bodies are consumed in the Kenya. However, the diversity of fish in major water bodies and rivers has been decreasing. A major example is the Lake Victoria that had up to 14 groups of fish (Oguto Ohwayo, 1990) in the early 19th century including the catfish. Currently, the lake has three major species namely; -the Nile perch (*Lates niloticas)*, Nile tilapia (*Oreochromis niloticus*) which were introduced species and the third a native cyprinid locally known as dagaa (*Rastrineobola argentea*). However, the African catfish still occurs in the Lake Victoria among many other fresh water fish species. Another case Lake Naivasha has no native species, and the only living native species *Aplocheilichthys antinori* reported to having been eliminated as was last recorded in 1962 (Elder *et al.,* 1971). With minimal management interventions more fish species continue to face extinction threats.

As the pressure on open access fisheries increases, the need for culture fisheries to satisfy the demand also increases (Munguti *et al.,* 2014). Fish farming has been practiced in Kenya mostly in the central, nyanza, western, parts of Rift Valley and coastal regions (Musa *et al.,* 2012). The commonly cultured fish are African catfish, tilapia and ornamental fish species.

The African catfish *Clarias gariepinus* (Figure 1) is indigenous in Kenya and has a widespread distribution because of their ability to tolerate a wide range of environmental parameters. The African catfish is a dynamic freshwater fish that is generally classified as omnivores and are

cannibalistic. They feed on aquatic plants, invertebrates such as crayfish and small fish and animals like birds.

The African catfish spawns in response to the rise in water levels (Pillay, 1990). However, ovulation can be prompted by environmental manipulation and/or hormonal stimulation. *Clarias gariepinus* has a rapid growth rate dependent on ambient conditions and habitat (Bruton and Allanson, 1980) in the wild. The African catfish are non-guarders that scatter their eggs under suitable conditions before spawning (Brutton, 1996). *C. gariepinus* gonadal maturation is influenced by changes in temperature of water and photoperiodicity (Graaf *et al*., 1995).

C.gariepinus adapts well to artificial environments, and has rapidly gained status as an important aquaculture species (Hecht *et al.,* 1988). The species have a rapid growth and can grow up to one metre and beyond in length. Catfish have sturdy resistance to harsh environments.

Figure 1: African catfish-*Clarius gariepinus* (Munguti *et al.,* 2014)

There are many fish farms in Kenya that culture *Clarias gariepinus* due to the advantages over other species. Clarias species have the ability to utilize atmospheric air and survive in harsh conditions (Teugels, 1996). Examples of catfish farms in Kenya are Sagana and Athi River.

Culture fish populations from hatcheries in Kenya with brood stock obtained from different sources; including natural water bodies; are heavily relied on by farmers for production. Morphological and molecular markers were used in the study to characterize the catfish species. Morphological variables, diagnostic to the African catfish have been used for population characterization (Agnese *et al.,* 1997). The morphology has enabled identification of the fish from other fish species and broodstock for catfish hatcheries. Molecular tools such as mitochondrial DNA has been used to assess the phylogeny and haplotype variation of the African catfish in other studies. Mitochondrial variation assessments have enabled distinction of broodstock from wild populations for use by hatcheries in culturing populations.

1.2 Problem Statement

Fish harvested from natural fish populations is decreasing and there is increasing need for supplementing natural fish populations with aquaculture (fishpond) farming (Mwangi *et al.,* 2012). Fish culture in Kenya started with trout species in the1920s (Vernon and Someren, 1960). More aquaculture species were later introduced such as tilapia and catfish.

Aquaculture production has grown over the years and is continuing to grow especially catfish culture within the past five years. Amidst the increasing production there are a number of challenges such as poor breeding practices that affect quality and quantity of production. In order to get quality catfish, broodstock should be adequately characterized for use by farmers for good produce and more returns. The study seeks to define and understand the population structure, morphological and genetic differences of these fishes in their natural habitats from which brood stock is obtained and in hatcheries.

1.3 Justification

Genetic diversity loss may be detrimental to a population and it's sustainability. Genetic diversity indicators provide meaningful information about current status of populations, trends and their future survival. A reduced genetic pool may affect the population fitness due to reduced genetic diversity in the next generation.

Over fishing has affected the natural fish populations as well as other human activities that have contributed to separation of species and populations. A genetic study on the variations available in catfish will be useful to inform breeding practices. An understanding of the differences within wild and culture populations will provide information to inform breeding programs and practices.

The genetic diversity data will give fundamental information needed for aquaculture practices to locate stock with a higher degree of genetic diversity. Hatcheries lose some variability at microsatellite loci (Li *et al.,* 2004) once brought in from natural habitats hence the importance for genetic studies. This is because gene flow and interactions are prohibited. The study will provide genetic information of catfish populations in the country to inform management decisions. Informed decisions will contribute to better management of broodstock and also to conservation management of the species in wild populations.

1.4 Objective

The study seeks to define and understand the population diversity, morphological and genetic differences of these fish species in their natural habitats from which brood stock is obtained and in hatcheries.

1.4.1 Specific objectives

1. Characterization of African catfish populations from selected areas in Kenya using morphometric data.

2. Mitochondrial DNA variation assessment of selected wild and cultured African catfish populations

CHAPTER TWO

LITERATURE REVIEW

2.1 African Catfish Biology

The African catfish (*Clarius gariepinus*) have an elongated sub-cylindrical body (Bruton, 1996). The African catfish have dorsal and anal fins being extremely long. The head is highly ossified (Agnese *et al.,* 2005) and the body has no scales. The skin has dark pigments along its body. They have four pairs of barbels. The major function of the barbels is for prey detection (Bruton, 1996).

Catfish have a preference for shallow running water environments; frequented habitats are floodplain swamps and pools due to ability to breath in air (Bruton, 1979; Clay, 1979). Catfish are generally bottom dwellers and are rather immobile. Catfishes occupy the bottom substrates, hence the selection for dorsoventrally flattened body shapes (Lujan and Conway, 2015). The flattened head also facilitates habitation of water bottoms as it reduces perturbation effect under flowing water and makes it suitable for grubbing of prey and uprooting plants along water beds.

2.2 African catfish (*Clarius gariepinus)* in aquaculture

In Kenya, aquaculture started in the 1920s (Vernon and Someren, 1960) when it first began with culture of trout species for recreational fishing. Later, trout aquaculture was supplemented by other fish for food such as tilapia and the African catfish. Aquaculture production in Kenya has

grown over the years as shown in Figure 2. The spike in production being attributed to the Economic Stimulus Programme in 2009 run by the government to encourage aquaculture production in Kenya (Musa *et al.,*2012)

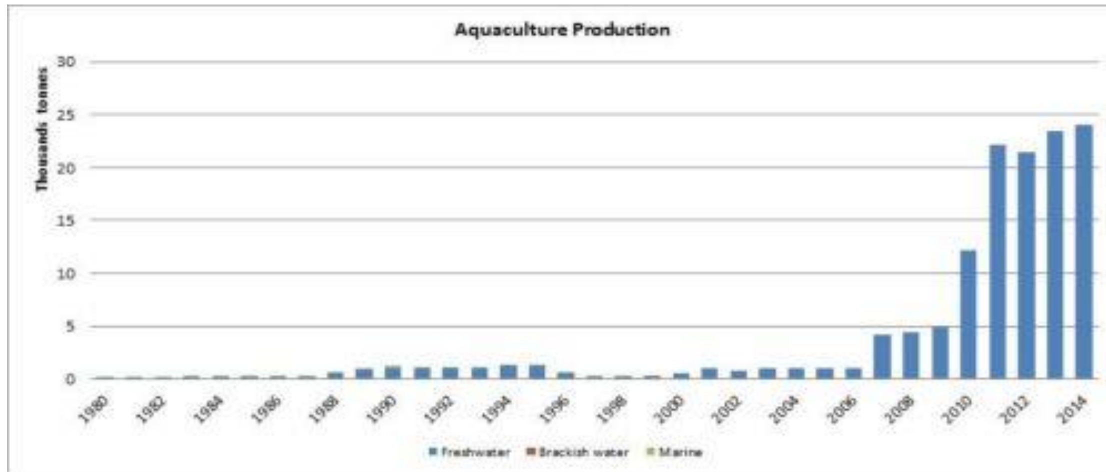

Figure 2.Fishery production in Kenya from 1980 (FAO Fishery Statistic, 2015)

Clarias gariepinus is one of the major aquaculture fish species in Kenya accounting for a fifth (Otieno, 2011) of the total fish produced in the country as shown in Figure 3 closely after tilapia. The figure indicates the percentage contribution of catfish to aquaculture production in Kenya.

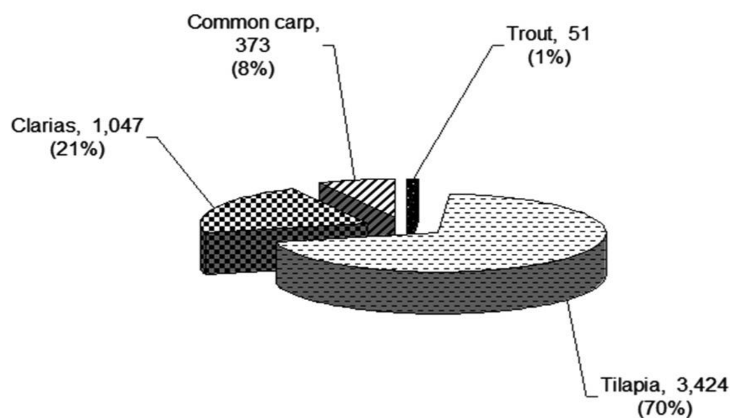

Figure 3**.** Aquaculture production by species in 2009 (adapted from Otieno, 2011).

The fish are becoming very popular with farmers due to the high growth rates, resistance to diseases and their ability to utilize atmospheric air hence survive harsh environments (Teugels, 1996). However, there has been occasional losses by grow out farms due to poor quality seed supply and other stresses (Orina *et al.,* 2014).

2.3 Catfish Genetics

Genetics deals with variation of heritable characters in living organisms. DNA which is found in both the nucleus and mitochondria is the main hereditary molecule (Avise, 1984) which have been used in the studies of populations. DNA is inherited although not all heritable variations are phenotypically expressed. Species can be described and distinguished using phenotypically expressed or molecular markers that are not expressed. Morphological and molecular markers are used in population genetic studies to depict status and trends of species populations. Morphological markers indicate variations in phenotype of the fish while molecular markers indicate genetic variations.

The molecular markers are based on polymerase chain reaction for amplification a revolutionary tool in genetics since its invention. There is a number of varying molecular markers available for the genetic study of fish. Several chemical and biochemical methods like restriction fragment length polymorphism (RFLP), isozyme electrophoresis and microsatellites are often applied (Dunham *et al.,* 2001). Different techniques have been used in analyzing genetic diversity and similarity and in genetics and breeding research involving fish. Markers that are more powerful compared to traditional markers include random amplification of polymorphic DNA (Liu *et al.,* 1998), microsatellite (Dunham *et al.,* 2001), amplified fragment length polymorphism (Vos *et al.,* 1995, Liu *et al.,* 1998), expressed sequence tags (Liu *et al.,* 1999) and single nucleotide

polymorphism (Kocabas *et al.,* 2002). Different markers have different applications in population genetics of fish (Liu and Cordes, 2004).

2.3.1 Morphological markers

Morphological markers are used to describe the phenotype of fish species (Agnese *et al.,* 1997). Such markers allow determination of exploitive stock and stocks to be separately managed allowing optimal harvest (Erguden and Turan, 2005; Salini *et al.,* 2004). Linear distances such as lengths of different fins and ratios to total lengths (Turan *et al.,* 2005) and number of fin rays are used to differentiate populations. Such measurements have been used to assess intra-specific differences in sampled populations as well as inter specific-differences in various fish species. Due to many variables in morphology, multivariate analysis is used in morphological variation studies of a variety species (Veasey *et al.*, 2001). Khayyami *et al. (*2014) used morphological variability to analyse *Liza aurata* along the southern Caspian Sea. Turan *et al. (*2006) used morphological and meristic variations to characterize stocks of Bluefish from the Mediterrenean Sea. Principal Component and canonical analysis were used to distinguished fish stocks from the different sites along the Sea (Khayyami *et al.,* 2104; Turan *et al.,* 2006).

Research studies have looked at the differences in specific organ sizes and shapes in catfish such as by Maina and Maloiy(1986) but this study looked at the general body morphology differences of the African catfish in Kenya. Morphometric characters are used to describe aspects of shape of a fish's body. This can characterize fish morphologically thus enabling their identification. Variation in morphology between populations can provide a basis for the stock structure and is relevant for studying environmentally induced variation (Begg *et al.,* 1999).

The diagnostic measurements for characterisation of the African catfish (Agnese *et al.,* 1997) to be taken for analyses were; prepelvic length; occipital process length; standard length (SL) (mm); anal fin length; head length (HL); predorsal length; dorsal fin length; preanal length; prepectoral length; premaxillary toothplate width; interorbital width; occipital process width; and vomerine toothplate width. Canonical variates analysis was used to find linear combinations of the set of variables thereby giving functions that can be used to discriminate between the groups.

2.3.2 Isozymes

The isozymes are multiple molecular forms of individual enzymes. The variation in their amino acids is detected through the resulting differences in their electrophoretic mobility. Isozymes were widely used to study populations (Daugherty *et al.,* 1990) and assay genetic variations in populations (O'Brien *et al.,* 1983, Lesica *et al.,* 1988). Since, allozymes possess low allelic variation and possible non-neutrality (O'Brien *et al.,* 1983) they do not reveal much about the underlying DNA sequence variations. Isozymes have been used in catfish and other species but they are limited in both the numbers of loci available and polymorphism e.g. low isozyme variation in fish (Agnese *et al.,* 1997) and the variation is associated with performance (Dunham *et al.,* 2001). Since the amount of their polymorphism is limited to performance they are not the assay of choice for the present study of the different populations, but in the study of local adaptation.

2.3.3 Microsatellites

Microsatellites are short and are highly polymorphic regions of the DNA. Microsatellites are fragments of the DNA where a simple pattern, generally 1-6 bp long, such as $(AC)_n$, $(AAT)_n$ or $(GATA)_n$, is tandemly repeated along the DNA. They are codominatly inherited. Microsattelites markers have been applied in catfish studies and other fishes (DeWoody and Avise, 2000;

Ilaboya, 2011) giving insight in genetic variation. Microsattelite markers are convenient tools in population variation (Abdul-Muneer, 2014). However, the presence of null alleles and homoplasy may be a challenge as that would demand very high sample size for analysis (Dunham *et al.,* 2001).

2.3.4 Expressed Sequence Tags

Expressed Sequence Tags (EST) are short single pass complementary DNA (cDNA) sequences reverse transcribed from mRNA. The single pass sequencing is at both the upstream and the downstream segments of cDNAs. EST analysis is an efficient way to identify genes and very powerful for the analysis of their expression. Microsatellites have been discovered in ESTs generated from a variety of tissues in channel catfish (Kocabas *et al.,* 2002). ESTs are however very expensive to run as advanced sequencers are required.

2.3.5 Single Nucleotide Polymorphism

Single Nucleotide Polymorphism (SNP) is caused by base variation among individuals at any site of the genome. They are highly polymorphic as it identifies every single difference. However SNP analysis has need for sequence information and is very expensive (Kocabas *et al.,* 2001).

2.3.6 Randomly amplified polymorphic DNA

Randomly amplified polymorphic DNA (RAPD) technique can sample a large number of loci and no prior DNA sequence information is needed to perform the assay (Christopher *et al.,* 2004). RAPD markers are polymorphic sequences and are isolated by gel electrophoresis after PCR (Liu *et al.,* 1998). Polymorphisms are a result of changes in the primer binding sites or of sequence length changes caused by indels or rearrangements. RAPDs DNA are expressed as dominant alleles. RAPD technique is used in distinguishing between species or subspecies in a

variety of organisms, comprising fishes (Qiubai *et al.,* 2006; Huang *et al.,* 2003; Huang and Chen 2003; Hung *et al.,* 2005). Saad *et al.* (2009) analysis of RAPD data succeeded in screening the variations among the *C. gariepunius* populations. Popoola, *et al. (*2014) used RAPDs to assess the genetic variability in cultured and wild population of *Clarias gariepinus*. This form of polymorphism is used for parentage determination; follow segregating populations produced by crosses; construction of genetic maps and for phylogenetic relationships especially at the intraspecific level (Dunham *et al.,* 2001).

2.3.7 Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) is efficient as it combines the specialties of RFLP and RAPD. The genomic DNA is cleaved with two restriction enzymes e.g. EcoR1 and Mse1, adaptors are ligated to the DNA fragments which are then amplified with different primers. These markers are highly polymorphic. AFLP markers have been used in genetic linkage and quantitative trait locus mapping (Liu *et al.,* 1999). These markers are highly reproducible. Liu *et al.* (1998; 1999) tested the reproducibility in channel and blue catfish by using DNA templates from different individual fish isolated at different times. High levels of reproducibility were observed as tested individuals always exhibited identical banding patterns. Bands of 50-500 base pairs exhibit the highest reproducibility in AFLP. The markers are dominant and are used in assessing independently evolving lineages and for individual identification and parentage analysis (Mueller *et al.,* 1999).

2.3.8 Mitochondrial DNA

The mitochondrion is the major site of cellular respiratory metabolism and a source of maternal effect from mitochondrial DNA. Genetic improvement programmes thus should also be concerned with mtDNA.

Mitochondrial DNA (mtDNA) are genetic materials found in the mitochondria; small organelles in the cells of organisms. The size of mtDNA in fish and most animals range from 16,000bp to 19000bp. An example of a complete sequenced mitochondrial genome is that of the channel catfish with 16,497bp (Waldbieser *et al.,* 2003). Mitochondrial genome is a haploid, circular DNA structure that encodes 13 proteins, 2 ribosomal ribonucleic acids (rRNAs), 22 transfer ribonucleic acids (tRNAs) and has a regulatory control region.

Mitochondrial DNA polymorphisms are length polymorphisms, restriction site polymorphisms and those caused by base-pair additions, deletions or both and heteroplasmy. In restriction fragment length polymorphism, endonuclease enzymes are used in this method to directly cut the DNA at restriction sites. Base substitutions at the restriction sites, insertions, deletions or DNA fragments rearrangements can cause variations. The mitochondrial variation of the control regions was used in this study as the molecular markers.

The control region is a non-coding region and the most hyper-variable in the mtDNA (Saccone *et al.,* 1991). The control region has a high mutation rate as it lacks repair mechanisms in replication thus high levels of variation making it useful when looking for patterns of genetic differentiation (Moritz *et al.,* 1987). The mtDNA is maternally inherited and transmission occurs without recombination hence advantageous in ascertaining phylogeny and evolutionary

significant units. Mitochondrial DNA analysis is convenient for lineage studies, it often revealed genetic differences among populations such as in the Asian catfish (Nazia *et al.,* 2010) and pleisomorphy demonstrated by Barasa *et al.,* 2014 in the African Catfish in Kenya.

Different aspects of genetic diversity of *C.gariepinus* have been assessed in Kenya. Barasa *et al.,* 2014 using mitochondrial markers observed levels of gene flow among Lake Victoria and Lake Kanyaboli African catfish. They observed shared haplotypes between the two populations.

Genetic information of natural populations allows the monitoring of stocks in the wild as well as in hatcheries. Reusing broodstock may cause inbreeding caused by mating of closely related individuals. Inbreeding depression is well documented in fish (Dunham *et al.,* 2001). Majority of inbreeding experiments on fish have been done in aquaculture environments. Turan *et al.,* 2005 studied *C. gariepinus* population differences indicating morphologic differentiation among *C. gariepinus* populations as a result of differential environmental conditions but suggested mtDNA analysis, highlighting the importance of genetic analysis. Popoola *et al.,* 2014 reported that the high mean heterozygosity observed in their studies confirmed high genetic variability in *C. gariepinus* both within and between populations; emphasizing on the importance of diversity studies in population distinction.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Areas

Sampling was done from six sites in the country: four hatcheries and two lakes. Below is the Kenyan map showing the sites (Figure 4).

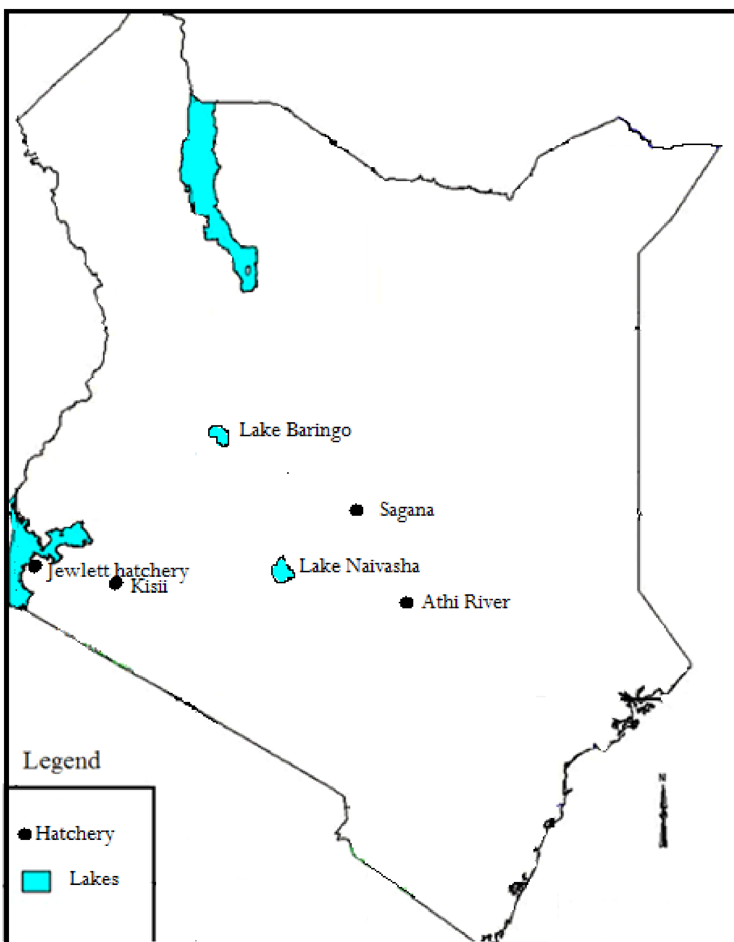

Figure 4. Map of Kenya showing location of the six study sites: Lake Baringo, Lake Naivasha, Athi River, Sagana, Kisii and Jewlet hatcheries.

3.1.1 Cultured catfish-Hatcheries

Samples for analysis were collected from the four hatcheries namely Athi River hatchery, Jewlett, Kisii and Sagana in different locations in Kenya. Athi River is a privately owned hatchery located in Machakos County and was established in 2013. The hatchery produces both catfish and tilapia. Jewlett hatchery, located in Homa Bay County, was established in 2011 and is a private enterprise that produces both tilapia and catfish. The hatchery serves farmers from all around Homa Bay county and other regions. The Kisii Fingerling Production Centre is located in Kisii County and was established in 1987 as a Kenyan government institution that produces both catfish and tilapia. The Kisii Fingerling Production Centre had however started off with only tilapia production. Sagana Fisheries Research Centre was established in 1948 and is also a research centre run by the government producing both tilapia and catfish.

3.1.2 Wild catfish - Lake Naivasha and Lake Baringo

Samples for the study were obtained from Lake Baringo and Naivasha. Both lakes Naivasha and Baringo occur in the Kenyan Rift valley.Lake Naivasha is a shallow lake with an approximate area of 160 km^2 .Lake Naivasha is averagely 6m deep and the deepest point is at 30m. It is majorly fed by the perennial River Malewa and the lake experiences fluctuations in water level which influences the lakes productivity (Betch and Harper, 2002). The lake lies at an altitude of 1890 m above sea level and is located 100km North West of Nairobi. The lake forms habitat for diverse flora and fauna. The fish composition of the lake is influenced by changes in climate, fishing effort and the introduction of invasive species. Common carp accounts for majority of the mass of fish caught in the lake as reported at KEMFRI, Naivasha Station (Fish Landings summary Naivasha, 2015, personal communication).

Lake Baringo has a surface area of approximately 130 km^2 located north of Lake Bogoria. The lake is fed by rivers Molo and Perkerra and is at an altitude of 990m above sea level with a maximum depth of 12m. The lake has a number of introduced fish species. The marbled lungfish (*Protopterus aethiopicus*) an introduced species provides the majority of fish output from the lake. Fish in this lake include lungfish, African catfish, guppy, two tilapia species and two undescribed species (Nyingi *et al.,* 2013).

3.2 Data Collection

3.2.1 Morphometric measurements

Data was first collected by use of the questionnaire as shown in Appendix 1. One on one interviews were carried out with the respondents at the hatchery site. The data obtained informed the decision on the choice of hatcheries as the study areas. The catfish samples were collected and preserved for analysis in 4% formalin at the collection site in separate containers and transported to the laboratory.

One hundred and forty-five samples of fish were measured in the Animal Production departmental laboratory. Fourteen measurements (Appendix 2) were taken on each specimen using vernier calipers. The diagnostic measurements included were; prepectoral length (PPEL); standard length (SL); anal fin length (AFL); head length (HL); occipital process length (OPL); predorsal length (PDL); total length (TL); preanal length (PAL); premaxillary toothplate width (PMW); prepelvic length (PPL); dorsal fin length (DFL); interorbital width (IOW); occipital process width (OPW) and vomerine toothplate width (VMW). These measurements were taken from one hundred and forty-five sex-recorded fish. Measurements were selected on their diagnostic value as demonstrated by Teugels (1982; 1986).

3.2.2 DNA Extraction and Polymerase Chain Reaction of D-loop (PCR)

DNA Extraction

A sterile scalpel was used to excise tissue from each sample to avoid cross contamination. The tissue samples were preserved in 96% ethanol in 1.5ml tubes and transported to the Animal Production Department Laboratory University of Nairobi.

The DNA extraction was done using the Qiagen extraction kit (Qiagen Valencia, CA USA) following the manufacturer's instructions (Appendix 5) with a few modifications. Briefly, twenty five (25) mg of catfish skeletal tissue was macerated, lysed and digested for 2 hours at 56°C. The centrifuge for spinning down digested content was at 10000rpm except for the final wash at 14000rpm. The sample was eluted with 50 *u*l of AE elution buffer for all the samples.

Presence and quality of the extracted genomic DNA was assessed using gel electrophoresis on a gel apparatus. Gel (Appendix 3) was prepared using 1% agarose gel in 1× TAE Buffer and run on an electrophoresis apparatus for 30 minutes at 75 volts.

Polymerase Chain Reaction of D-loop region

Amplification of the targeted mitochondrial region in the extracted DNA, ~550bp, was by conventional polymerase chain reaction .The primer set used were forward primer L16473 (5′-CTAAAAGCATCGGTCTTGTAATCC-3′); reverse primer H355 (5′-CCTGAAATGAGGAGGAACCAGATG-3′) (Nazia *et al.,* 2010).

The PCR reaction was with a master mix prepared in the laboratory at the Institute of Primate of Research. To make a 20 *u*l reaction for each PCR reaction; sterile deionised water 12.3 *u*l, 2 *u*l of 5X PCR buffer, 2 *u*l of 10um dNTPs, 0.5 *u*l of 5 *u*m each of the forward and the reverse primers,

0.2 *u*l of Taq DNA polymerase and 2.5 *u*l of DNA template were used. The amplification was done in the DNA 480 Thermal cycler, Applied Biosystems USA.

The protocol for amplification of the D-loop region adapted from Barasa *et al.* (2014) and Nazia *et al.* (2010) was as follows:

Initial denaturation was for 2 minutes at 94°C, 29 cycles of denaturation, annealing and extension for 94°C for 1 minute, 56°C for 1 minute 10 seconds and 72°C for 2 minutes, respectively and the final extension at 72°C for 5 minutes. After PCR, 5 *u*l of each of the reaction was run on 1% agarose gel stained with ethidium bromide (5 mg/*u*l) to verify amplification. The gel was visualized and photograph taken as shown in figure 5.The samples were then purified using a Qiagen PCR purification kit (Qiagen Valencia, CA USA) following manufacturer's instructions (Appendix 4). Ninety-six PCR products were selected with correct band size and good quality and were sequenced using an automated BigDye Terminator cycle chemistry (Sanger sequencing) by Genewiz® United Kingdom.

Figure 5. Image of 500bp PCR products run on 1% agarose gel. Lane a is the molecular ladder lane, x is the 500bp marker of the 1kbplus molecular ladder.1-19 represents PCR samples from Athi River.

3.3 Data Analysis

The survey, morphological and DNA data in this research study was analysed by methods as outlined below.

3.3.1 Morphological Analysis

The survey data was analyzed using descriptive statistics in SPSS Version 23. Figure 6 below shows the survey data keyed in SPSS version 23 for analysis.

4	AthiFish	private	both	7000	4000	yes	10.00	catfish
5	Thongoni	private	both	0	0	yes	3.00	tilapia
6	Kibos	government	both	.	.	yes	50.00	tilapia
7	Dominion	private	both	15000	30000	yes	32.00	tilapia
8	KisiiFPC	government	both	.	.	yes	30.00	tilapia
9	Sang'oro	other	both	.	.	yes	50.00	tilapia
10	Lutonyi	government	both	10000	30000	yes	40.00	tilapia
11	Jambo	private	both	1500	.	yes	10.00	catfish
12	Jewlett	private	both	200	.	yes	10.00	tilapia

Figure 6. Screen shot of survey data in SPSS version 23

The multiple response question was transformed in SPSS into a defined set to allow for descriptive analysis.

SPSS version 23 and Genstat Discovery were used to carry out morphological analysis. To attribute the variations in this study to differences in body shape, and not to the sizes of the fish, the effects of size were eliminated by standardizing the morphometric parameters using the allometric formula by Elliott *et al.,* (1995):

$$M_{adj} = M \times (Ls \times Lo^{-1})^{b}$$

In the formula, M_{adj} is the size-adjusted measurement, M is the original measurement, Ls is the overall mean of the TL for all fish from all samples and Lo is the TL of the fish. Parameter b was estimated for each character as the slope of the regression of log M on log Lo using all the fish in all the groups.

The measurements were subjected to principal component analysis (PCA) and canonical variate analysis (CVA) (Humphries *et al.,* 1981 and Bookstein, 1985) in SPSS and Genstat software respectively. Principal Component Analysis has been used to morphologically differentiate populations (Khayyami *et al.,* 2014).

An analysis of variance (ANOVA) was carried out to test the significance of morphometric differences between populations using SPSS .The possible sources of variation included sex of the fish, sources from where the samples were obtained and the age. However, the age was not considered as five sites had no records of age of fish.

The measurements were standardized and analysed without distinguishing the sexes because of the insignificant effect due to sex with $p>0.3$. After standardization of the data, they were submitted to PCA, a statistical method used to analyse the dimensions resulting from the variables taken from the samples. The analysis used transformation to convert the set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables i.e. principal component (PC).

The measurements were subjected to CVA using Genstat discovery version. The canonical variate 1 (CV1) and canonical variate 2 (CV2) were plotted to allow visualization of the distribution of each sample along the canonical variate axis.

3.3.2 Genetic Analysis

The resulting sequences were retrieved from Genewiz, the sequencing laboratory in multifasta formats. The sequences were edited using Bioedit version 7.1.9 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and then aligned by using ClustalW.

The generated sequences were then compared with the already published nucleotide sequences in the GenBank using the Basic Local Alignment Search Tool (BLAST) to confirm the identity of species.

MEGA V 7.0 (Kumar *et al.,* 2015) was used to construct the evolutionary phylogenetic trees. The data was then exported to DNASP V5.10.01 (Librado and Rozas, 2009) where the haplotype diversities, nucleotide diversities and the standard errors were computed. The data was then saved in arlequin and roehl file formats for use in arlequin and network softwares respectively. The arlequin software version 3.5 (Excoffier and Lischer, 2010) was used to group the data for analysis of molecular variance (AMOVA). DNASP V5.10.01 (Librado and Rozas, 2009) was used for genetic differentiation estimates. The programme Network 5.0 version 8 was used to visualize the haplotypes in the populations using median joining tree.

CHAPTER FOUR

RESULTS

4.1 Preliminary Results

There are a number of hatcheries that have been established in the country. The current study gathered information on fish farming practices in twelve hatcheries in Kenya using a questionnaire. The types of farms were private, public and jointly owned. The farms that were privately owned (50%), followed by public owned (42%) while the jointly owned (8%). The commonly cultured fish species in the study hatcheries were tilapia and catfish. Despite efforts by hatcheries to produce catfish and tilapia, tilapia are largely preferred by consumers, recording 83% preference of tilapia and 17% catfish.

The breeding practices of the hatcheries were varied; 67% reported to have randomly picked fish for their broodstock while the remaining 33% did not randomly pick. Once in the hatchery, 91% of catfish were selected based on size. Broodstock was brought in from different sources including lakes, rivers, swamps, other hatcheries or imported improved broodstock. Twenty-five percent (25%) of hatcheries obtained the first batch of their broodstock from Lake Victoria.

All the hatcheries (100%) used artificial mating for fingerling production, one hundred percent. Catfish does not breed in captivity; breeding of the species in the wild is influenced by natural environmental factors making it almost impossible in captivity. The respondents occasionally mated catfish from different locations with the intention to improve quality of fry.

All the respondents had at least heard the term inbreeding and crossbreeding, 91% of the respondents believed that they do have an effect on the quality of fingerlings. There were a number of deformities observed in hatcheries. These deformities included; albinism, bent backbones, deformed tails, eyesight loss, dented/ swollen head. The hatcheries were sampled and fifty eight (58%) had cases of deformities. The most frequent was bent backbone with two cases. Forty one (41%) percent have had cases of diseases, this was however not confirmed as postmortem was not done in some cases.

All the hatcheries acknowledged encountering a number of challenges in production as shown in Table 1. The least constraint was broodstock exchange cycle at 4.1%.

Table 1. Challenges faced in catfish culture production

Challenges	Percent
Poor climatic conditions	18.2
Inadequate Funds	18.2
Poor Nutrition	18.2
Predation	18.2
Low Demand	13.6
Poor Handling	9.1
Broodstock	4.1

4.2 Morphological Results

4.2.1 Morphological variance results

The morphometric variation was evident in the collected samples. The variation due to sex was insignificant and the interaction between morphometric characteristics indicated that sex was not significant, $p > 0.05$ (Table 2), thus not considered in subsequent grouping analyses. Analysis of

variance indicated significant differences in means of variables from different groups as shown in Table 3 with p values <0.05.

Table 2.Source of morphological variation and interaction among the wild and cultured catfish populations

Source	Variance ratio	Fpr
Group	14.33	<0.001
Sex	6.27	0.3
Group. Sex	0.29	0.835

Variation of all the collected samples indicated significant differences among populations as shown in the Table 3. The means were significantly different in all variables.

Table 3. Analysis of variance showing differences in variables among populations (p<0.01).

Variable	N	Mean± SD	F value	p value
IOW	171	36.49± 14.95	41.727	0.000
Weight	171	368.44± 490.64	11.918	0.000
DFL	171	195.65± 55.91	23.448	0.000
TL	171	355.42± 111.06	30.213	0.000
HL	171	86.80± 35.01	60.523	0.000
SL	145	289.67±63.72	11.986	0.000
PPEL	145	61.66±16.59	33.611	0.000
PAL	145	152.72±36.54	32.155	0.000
PPL	145	129.86±30.07	28.282	0.000
PDL	145	97.76±22.47	27.031	0.000
OPL	145	11.85±4.17	69.231	0.000
OPW	145	18.64±5.88	37.534	0.000
PMW	145	41.24±11.59	28.022	0.000
VMW	145	33.11±17.13	158.468	0.000
AFL	145	127.56±28.81	3.939	0.005

4.2.2 Morphological variations

The morphological variations were based on ratios from individual groups as revealed by analysis of variance indicated significant differences of p<0.05 for all the variables.

Table 4.The standardized variables by ratios grouped by source of the catfish.

Population	Athi River	Kisii	Naivasha	Jewlett	Sagana	Baringo
Variable	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd
Weight	57.12±29.39	25.95±6.42	20.71±13.10	27.86±9.84	39.75±12.94	120.33±138.02
IOW	39.23±2.64	40.64±14.95	54.71±9.74	39.11±1.98	37.89±1.40	43.46±2.38
DFL	54.29±2.81	55.60±1.83	60.91±2.98	55.24±2.18	51.38±1.90	53.74±3.75
HL	25.26±2.40	25.16±.79	18.11±2.14	23.59±1.20	25.98±.92	27.38±1.73
PPEL	19.53± 1.93	19.93±0.99	15.99±1.45	18.77±85.27	20.03±1.07	
PPL	40.37±4.35	41.60±1.49	36.57±1.89	39.92±2.46	41.11±1.62	
AFL	36.71±3.28	37.95±1.79	46.72±1.31	38.33±2.47	34.64±1.97	
PAL	48.52±3.47	48.78±1.42	42.64±1.94	46.99±2.60	46.82±1.18	
PDL	30.09±2.07	29.93±1.01	28.63±2.00	29.38±1.53	31.75±1.18	
OPL	16.46±5.59	15.06±1.51	13.70±2.29	17.32±2.20	15.61±2.06	
OPW	24.58±3.89	21.20±1.95	27.37±4.32	24.00±1.70	24.30±3.24	
PMW	34.00± 6.93	60.31±2.78	75.40±7.16	56.51±4.79	59.25±6.73	
VMW	61.04±4.94	31.23±2.87	40.49±4.35	34.45±2.67	29.88±1.83	
SL	86.08±4.73	89.22±1.28	90.59±2.26	89.03±0.75	89.26±1.56	

From the ratios in Table 4 Baringo samples had the largest heads and Naivasha the smallest heads as derived from the lengths. Inter-obittal width is widest in Naivasha meaning the eyes were more laterally placed in Naivasha samples. Pre maxillary toothplate width was narrowest in

Athi River and widest in Naivasha. The vomerine toothplate widest in Athi River and narrowest in Sagana samples.

The pectoral fin is most anteriorly positioned in Naivasha catfish compared to the others.

Naivasha have the longest anal fin lengths and Sagana the shortest. Preanal fin lengths were shortest in Naivasha and almost similar in the others. Dorsal fin length was longest in Naivasha.

4.2.3 Canonical variate analysis (CVA)

Canonical Variate Analysis revealed population clustering as in Figure 7. Canonical variate 1 accounted for 73.99% of the variability while canonical variate 2 accounted for 23.35% of the variation.

All the sample means were different from each other. However, individuals from Kisii, Kendu Bay and Sagana fish populations showed overlap. For CVA, the Baringo was not computable since some variables were not measured. This was not possible due to constraints in the field. The population from Athi River and Naivasha were considerably isolated from the other three populations that appeared to overlap. Sagana and Naivasha population were the most distant as observed from Figure 7 and from the inter-group distances in Table 5.

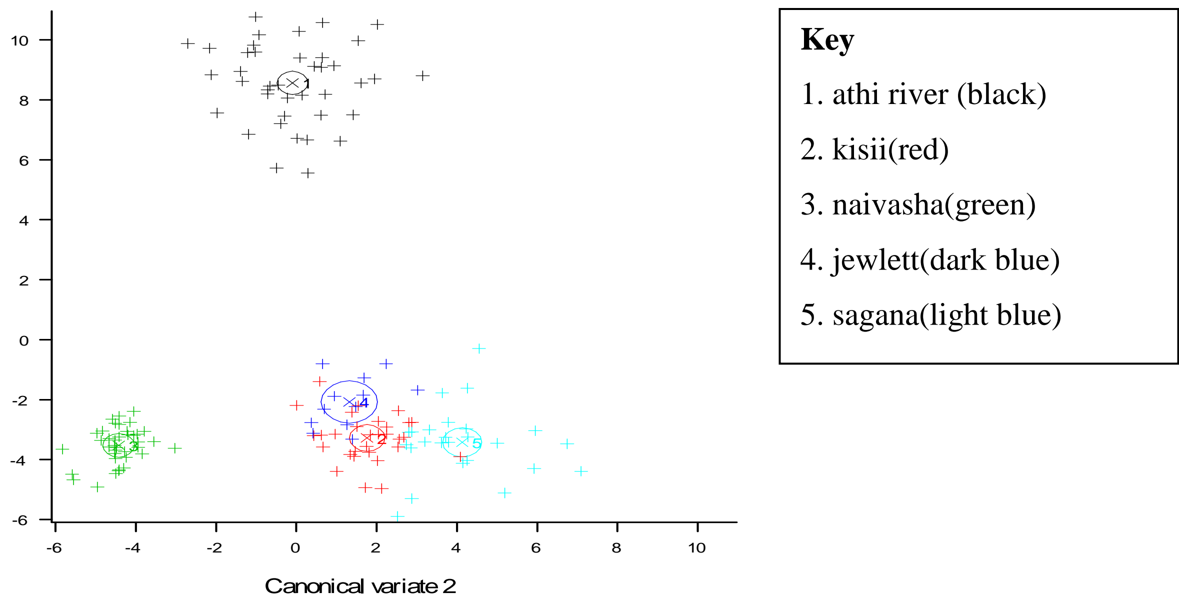

Figure 7.Canonical variate analysis of catfish from Athi River, Kisii, Naivasha. Jewlett and Sagana

4.2.4 Inter-group distances between catfish populations

The populations with shortest distance was between Kisii and Jewlett hatcheries with a distance of 1.2840 this is supported by the two populations overlapping as depicted in Table 5 above and the mean points being short distant apart.

Table 5. Distance between populations Athi River, Kisii, Naivasha, Jewlett and Sagana

	Athi River	Kisii	Naivasha	Jewlett	Sagana
Athi River	0.000				
Kisii	11.992	0.000			
Naivasha	12.842	6.195	0.000		
Jewlett	10.736	1.284	5.925	0.000	
Sagana	12.714	2.374	8.560	3.121	0.000

4.2.5 Principal component analysis (PCA)

Principal components were examined to illustrate the contribution of variables to clustering.

The analysis revealed two dimensions. The two components had eigenvalue above 1.0. The first component attributed to six of thirteen variables (head length, occipital process length, predorsal length, prepectoral length, preanal length, prepelvic length) and the second component represented by three morphometric variables- (vomerine toothplate width, standard length and premaxillary toothplate width) as shown in Figure 8.

Distance of the variables from the origin showed that the contribution of variations were mostly from the head measurements. These include the vomerine toothplate width, premaxillary toothplate width, interorbittal width and head lengths.

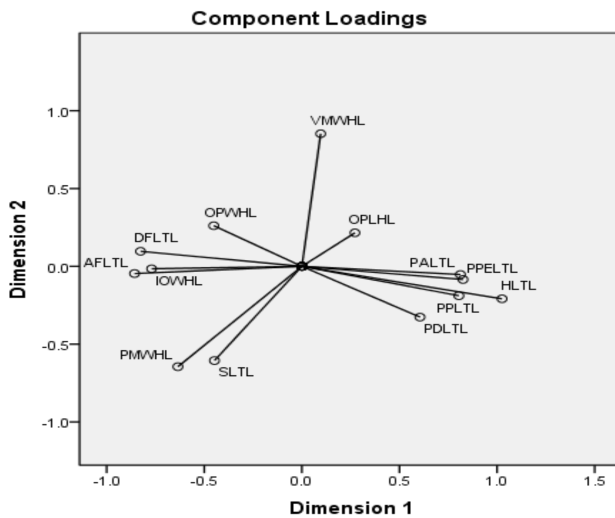

Figure 8. Component plot loadings of the variables: HL (head length), IOW(interorbittal width), PDL(predorsal length), VMW(vomerine toothplate width), OPW(occipital process width), OPL(occipital process length), PPEL(prepectoral length), PAL(preanal length), PPL(prepelvic length), PMW(premaxillary width), SL(standard length), AFL(anal fin length), DFL(dorsal fin length)

The population clustered as shown below (Figure 9) using optimal scaling. The Naivasha and Athi river samples were a distant apart from the other four populations. Baringo samples

overlapped with Kisii and Sagana samples similar to canonical variate overlaps (Figure 7). The binning shows concentration of the samples in the bottom half of the chart shown by the large centroids and the scale (Figure 9). Figure 10 shows combined plot of variables and objects plots.

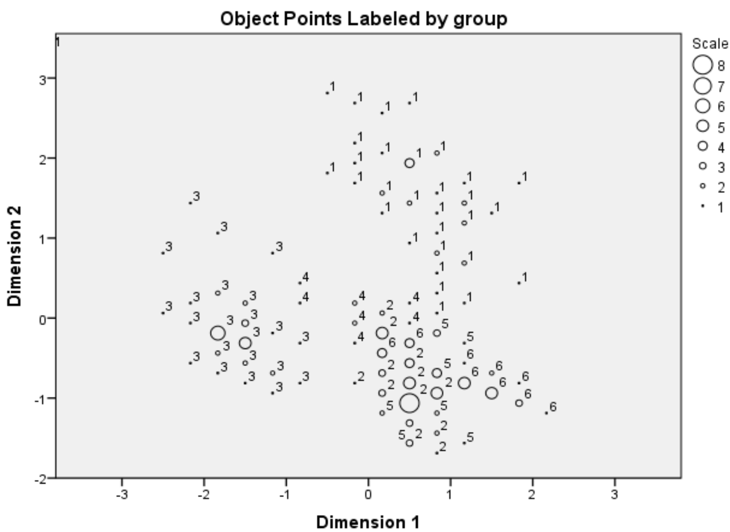

Figure 9. Binned Samples labeled by source (1.Athi River, 2. Kisii,, 3. Naivasha,4. Jewlett,5. Sagana and 6.Baringo)

A combined variables and sample plotting as Figure 10 shows how the objects points related with the variables. The individual plot loadings are as shown in appendix 6.

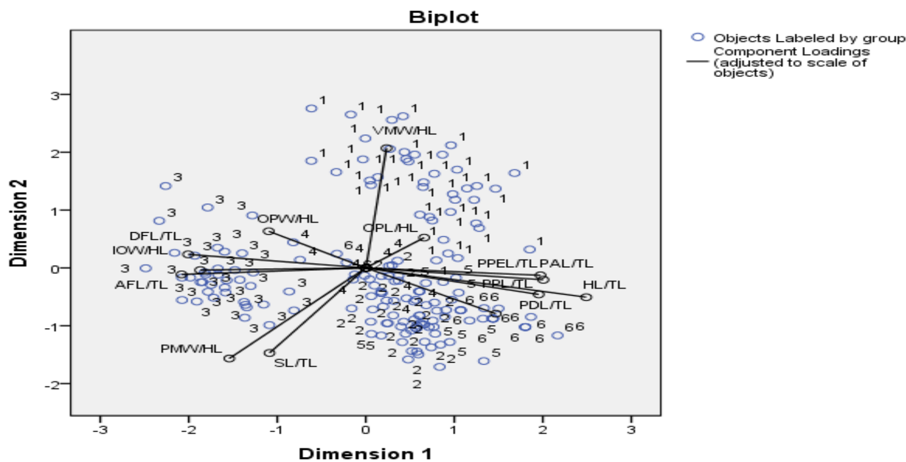

Figure 10. Combined variables and object points by sources of fish

4.3 Genetic diversity using Mitochondrial D-Loop Analysis

The mitochondrial DNA sequences constituted 433 nucleotides that were used in the subsequent analysis. One hundred and twenty three (123) sequences of samples were aligned and then trimmed to 433bp long as shown in figure 11.

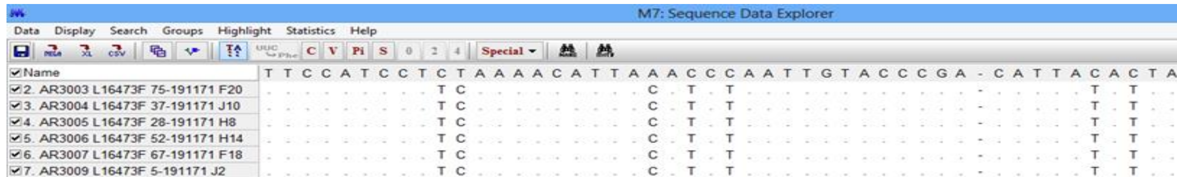

Figure 11. Sample sequences aligned and trimmed in Mega 7

BLAST (basic local alignment search tool) (Zheng *et al*., 2000) was used to identify similar alignments with the sequences. There were hits with sequence identity of 99% corresponding to *Clarius gariepinus* mitochondrial D-Loop partial sequence verifying that the sequences were of the D-loop region of the African catfish DNA.

4.3.1 Evolutionary History

A phylogenetic tree of the 123 catfish samples is as shown below (Figure 12). The Naivasha population samples occurred in a single clade. Nine from Athi River and five from Sagana formed a distinct sub clade as well while the other remaining samples in Athi River occurred with the other catfish from other sites.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei (Tamura & Nei, 1993) model with the bootstrap support included on the branches. There were a total of 428 positions in the final dataset with 122 polymorphic sites. Evolutionary analyses were conducted in MEGA7 (Kumar S. *et al.,* 2015).

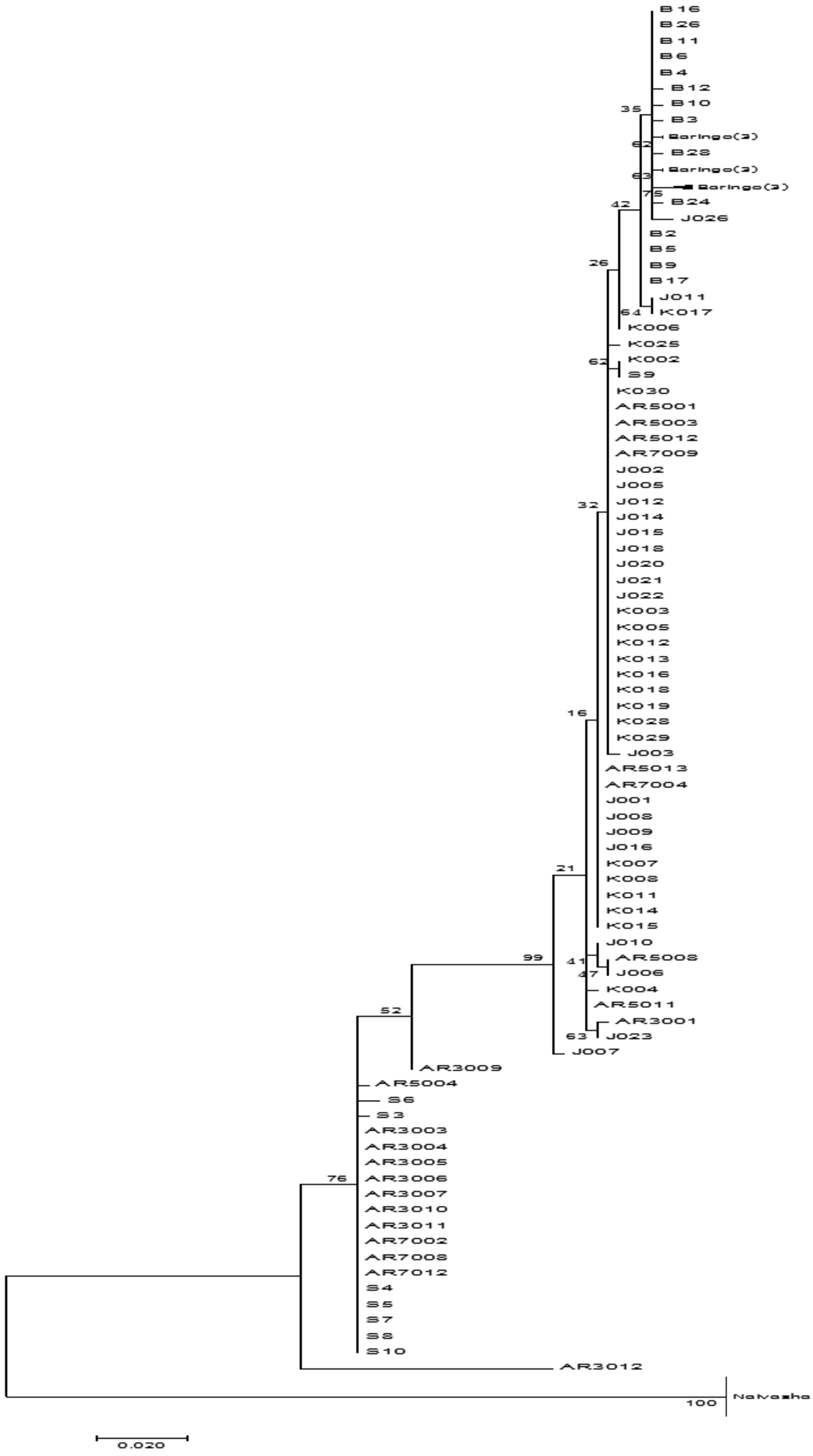

Figure 12.Evolutionary relationships of the African catfish from Athi River (AR), Jewlett(J), Kisii(K) ,Sagana(S), Baringo (B) and Naivasha.

4.3.2 Genetic Diversity

A total of 34 haplotypes of the 122 polymorphic sites (Table 6) were observed from the 433 nucleotide sites of the partial D-Loop region. The haplotype diversity of all the samples was Hd: 0.884 ±0.017 and the nucleotide diversity (π) was 0.09018± 0.10414 with 20 singleton variation sites and 102 parsimony informative sites.

Table 6. Table showing haplotype diversity of the catfish populations

Population	N	Polymorphic sites	Haplotypes	Haplotype diversity ± SD	Nucleotide diversity± SD
Athi river	22	47	9	0.775±0.081	0.03035 ±0.00503
Kisii FPC	20	8	7	0.711 ±0.089	0.00307 ±0.00088
Jewlett	20	14	9	0.779 ± 0.085	0.00579 ±0.00153
Sagana	8	23	4	0.643±0.184	0.01337±0.00966
Baringo	23	15	12	0.913±0.035	0.00526±0.00384
Naivasha	30	0	1	0.000±0.000	0.0000 ±0.0000
[Victoria	24	14	11	0.754 ±0.093	0.008 ±0.002]Barasa *et al.,* 2014
[Kanyaboli	28	11	10	0.741 ±0.064	0.005 ±0.001]Barasa *et al.,* 2014
Total	123	122	34	0.811±0.026	0.10227 ±0.00624

Athi River population was the most diverse followed by Sagana among the cultured populations while Lake Baringo populations was the most diverse of the wild populations. Lake Baringo had the highest number of haplotypes followed by Athi River and Jewlett.

4.3.3 Haplotype Distribution

The population at Athi River has the same number of haplotypes as Jewlett although of different types. Lake Baringo had the highest number of haplotypes and Lake Naivasha had the least with only one haplotype. Six haplotypes: two, five, seven, nine, thirteen, sixteen occurred in more than one population as shown in Table 7. Haplotypes 5 and 9 were shared in three populations of Athi River, Kisii and Jewlett. Haplotype 2 occurred in Athi River and Sagana. Haplotype 7 occurred in Athi River and Jewlett. Haplotype 13 occurred in Jewlet and Kisii populations. Haplotype 16 occurred in Kisii and Sagana. Naivasha had one haplotype 20 while haplotypes 21 to 32 all occurred in the Baringo catfish.

The median joining tree was used to illustrate the haplotype distribution and linkage of the haplotypes among the populations as shown in Figure 13.

Table 7. Haplotype distribution among the sampled catfish populations

Haplotype	No	Athi river	Jewlett	Kisii	Sagana	Baringo	Naivasha
Hap_1	1	AR3001					
Hap_2	15	AR3003, AR3004, AR3005, AR3006, AR3007, AR3010, AR3011, AR7002, AR7008, AR7012			S4 ,S5, S7 S8, S10		
Hap_3	1	AR3009					
Hap_4	1	AR3012					
Hap_5	23	AR5001, AR5003, AR5012, AR7009	J002, J005,J012 J014,J015, J018,J020, J021,J022	K003,K005, K012, K013,,K016, K018, K019,K028, K029,K030			
Hap_6	1	AR5004					
Hap_7	2	AR5008,	J006				
Hap_8	1	AR5011					
Hap_9	11	AR5013, AR7004	J001,J008, J009,J016	K007,K008,K0011, K014, K015			
Hap_10	1		J003				
Hap_11	1		J007				
Hap_12	1		J010				
Hap_13	2		J011	K017			
Hap_14	1		J023				
Hap_15	1		J026				
Hap_16	2			K002	S9		
Hap_17	1			K004			
Hap_18	1			K006			

Haplotype	No	Athi river	Jewlett	Kisii	Sagana	Baringo	Naivasha
Hap_19	1			K025			
Hap_20	30						N001,N042, N302,N303,N304,N305, N506, N401, N512 ,N509, N062,N072,N082,N092, ,N306, N102,N301, N402,N406 N508, N507, N504,N504,N505, N502,N403,N404,N405, N407, N511, N510
Hap_21	4					B2,B5,B9,B17	
Hap_22	1					B3	
Hap_23	5					B4,B6,B11,B16,B26	
Hap_24	1					B7	
Hap_25	1					B7	
Hap_26	1					B12	
Hap_27	3					B13,B14,B22	
Hap_28	3					B15,B18,B19	
Hap_29	1					B23	
Hap_30	1					B24	
Hap_31	1					B25	
Hap_32	1					B28	
Hap_33	1				S3		
Hap_34	1				S6		

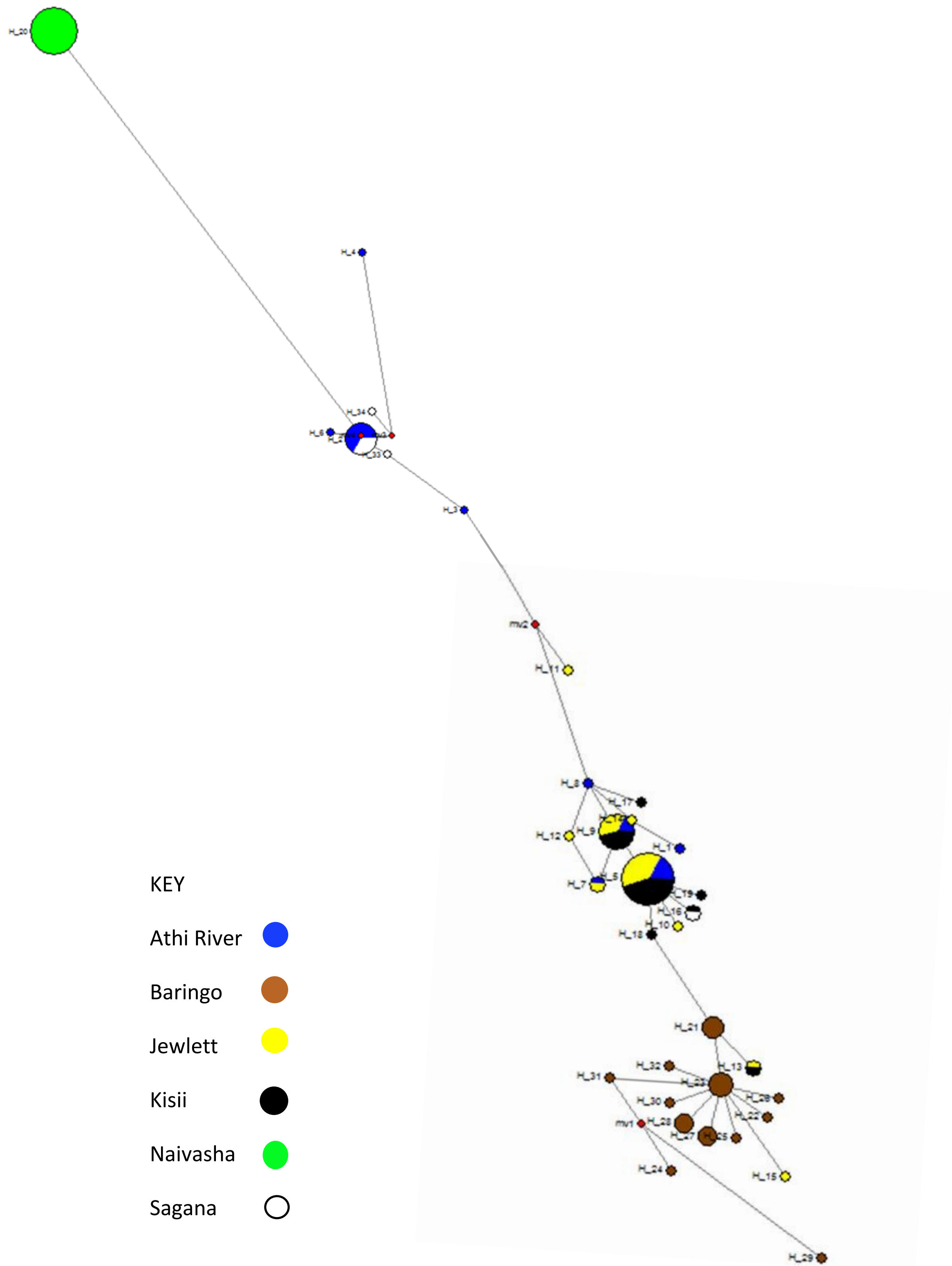

Figure 13. Median Network tree of African catfish mitochondrial DNA haplotypes(H) Athi River, Jewlett, Sagana, Kisii, Baringo and Naivasha populations

4.3.4 Genetic differentiation and AMOVA

Genetic Differentiation was based on both haplotype (Hs) and nucleotide statistics (Ks) (Hudson *et al*. 1992). Table 8 shows the pairwise differences. The overall Hs was 0.7425. The overall Ks was 21.3614. Chi-square test did reveal significant differentiation levels at $p < 0.001$ hence failure to accept null hypothesis which stated there was no genetic difference in populations.

Table 8. Pairwise genetic differences of the six sampled populations

Population 1	Population 2	Hs	Ks
Athi River	Jewlett	0.7768	8.0222
Athi River	Kisii	0.7444	7.4658
Athi River	Naivasha	0.3229	5.5220
Athi River	Baringo	0.8457	7.5365
Athi River	Sagana	0.7442	11.1048
Jewlett	Kisii	0.7447	1.9053
Jewlett	Naivasha	0.3048	0.9958
Jewlett	Baringo	0.8512	2.3672
Jewlett	Sagana	0.7449	3.4211
Kisii	Naivasha	0.2780	0.5284
Kisii	Baringo	0.8196	1.8238
Kisii	Sagana	0.6936	2.5865
Naivasha	Baringo	0.3913	0.98113
Naivasha	Sagana	0.1135	1.2105
Baringo	Sagana	0.8530	3.1613

The AMOVA estimated 92.36% variation among populations and 7.64% variation to be from within populations with a p value <0.05. The Fst value between the six populations was 0.9236 with $p<0.05$ indicating significant levels of differentiation in the population.

Table 9. Hierarchical analyses of molecular variance showing amount of population genetic structure.

Source of variation	df	Percentage of variation	P value
Among populations	5	92.36	0.000
Within populations	117	7.64	

4.3.5 Neutrality test results

The Tajima's test is used to measure neutrality levels of populations by deviations from zero. The overall Tajima's D value showed non-significant negative value. Tajima's D: -1.02103 at P > 0.05. The D value of each population considered individually were as shown in the table 12 below.

Table 10. Neutrality indices of sampled catfish populations

Statistics	Athi River	Jewlett	Kisii	Naivasha	Baringo	Sagana
Sample size	22	20	20	30	23	8
Polymorphic Sites	47	14	8	0	15	23
Pi	13.05195	2.48947	1.32105	0.0000	2.26087	5.75000
Tajimas D	0.04848	-1.35131	-1.39585	0.0000	-1.58323	-1.8443
P-value	0.575	0.084	0.086	1	0.04200	0.00000

Kisii and Jewlett populations had a relatively similar Tajima's D value of -1.39585 and -1.35131 respectively. Naivasha population had a value of 0.00 due to the absence of polymorphic sites from which Tajima's D value is derived. Athi River was the only population that had a positive tajima's value of 0.04848.

CHAPTER FIVE

DISCUSSION

5.1 Breeding and Management practices

The breeding and management practices of the hatcheries were varied. Fifty percent of hatcheries obtained broodstock from different sources and exchanged their broodstock after some time. This would mean hatcheries would probably have quality fry than others. There was more production of fingerling where original broodstock had been deliberately selected

Diseases and deformities recorded could be as a result of poor management levels such as poor handling, inadequate nutrition and inbreeding. The studies by Alarape *et al.,* 2015 and Orina *et al.,* 2014 show that occurrence of diseases and deformities are closely linked to management. Deformities are a manifestation of generations of inbreeding in a population and low genetic diversity. Inbreeding depression can cause reduced resistance to parasites (Coltman *et al.,* 1999), high vulnerability to environmental pressures (Keller *et al.,* 1998), body deformations and reduced reproductive fitness (Mehlis *et al.,* 2012) and high mortality rates.

There were challenges faced by farmers and hatcheries which included inadequate financial resources, climatic conditions and poor handling among others contributing to low production yields. The least frequent challenge was broodstock exchange cycle. This appeared not to be a concern to most hatcheries despite being perceived as a major challenge.

5.3 Morphology variation and biogeography

The source of fish was identified as the main source of morphological variation. There was a negligible effect of sex in the observed morphological variation as demonstrated in other studies (Agnese *et al.,* 1997; Turan *et al.,* 2005).

The pelvic and pectoral fins were most anteriorly positioned in the Naivasha samples, they also had the longest anal and dorsal fins with respect to their sizes. The dorsal fin position is related to the water column depth of the niche habitat occupied by the catfish, with posteriorly placed dorsal fins representing adaptation to surface habitats in non-flowing waters (Matthews W.J, 2012).

The contribution of variables revealed that morphometric variation between samples was largely located in the head of *C. gariepinus* also seen in other catfish studies (Agnese *et al.,* 2005; Turan *et al.,* 2005). The eyes were most laterally placed in Naivasha population (the widest inter-orbittal width). In relation to the occupation of habitats by fish species, Gatz (1979) demonstrated that eye position reflected the stratum occupied by a species in the water column (Cunico and Agostinho, 2006).

The samples from Baringo had the largest heads giving clues of larger prey size diets compared to Naivasha samples that would accommodate small prey size due to small heads (Gatz, 1979). Fish size appears to play a major role in determining diet; bigger fish tend to have bigger heads while the smaller fish have smaller heads. The smallest fishes often feed on small zooplankton, intermediate size fish on larger invertebrates like crayfish while the largest fish on other fish (Wainwright and Richard, 1995).

Catfish have a wide habitat range in freshwater but show preference for shallow running water environments and many inhabit large open water (Bruton, 1996). Morphological variations are majorly attributed to different habitat characteristics such as water turbidity and even fish diets. The diversification of fish in a habitat have been linked to attributes like light intensity (Witte *et al.,* 2008) and water flow (Langerhans, 2008) and predator densities among others (Hendry *et al.,* 2006, Langerhans *et al.*, 2009). Water regimes generally have the strongest effect on body shape (Franssen *et al.,* 2013).

Environmental factors that elicit plasticity of traits can result in canalization (Debat and David 2001) and may facilitate evolution of resident populations (Ghalambor *et al.,* 2007; Pfennig *et al.,* 2010). The catfish populations in Lake Naivasha could have risen from a hatchery or reservoir upstream River Malewa and then undergone plasticity pressures resulting in evolution into distinct phenotype population. A reservoir upstream represents a point from which introduced species can spread particularly in the downstream direction (Penczack and Gomes, 2000) and into a water body.

Morphological measures give insight into the bio-geographical elements of fish habitats as concluded from the results by Agnese *et al.* (2005) and Gatson *et al.* (2012). The measurements are useful in preliminary population characterization of catfish samples before genetic characterization for identity verification.

5.2 Genetic differentiation and diversity

There was high degree of separation of the fish samples suggesting high morphological differentiation. As shown previously in Figure 8, Athi River and Naivasha were clearly distinct from the others. Three populations Sagana, Kisii and Jewlet clustered together. The populations overlap signified probable similar source of original broodstock for the respective hatcheries.

The broodstock from the three were possibly intermingled. This is supported by Kisii FPC having got some broodstock from Jewlett and some from Sagana although at different times. This may increase chance of occurrence of similar diseases and deformities in farms with fish from similar broodstock.

The pairwise group distances revealed proximity of Jewlett to Kisii than to Sagana. This could be attributed to Kisii hatchery obtaining some of its broodstock from Jewlett and from Sagana when it initially began. The shorter the genetic distance between populations, the more probable there was some breeding between them and the less isolated they are from one another (Wright, 1943).

The catfish population from Baringo closely occurred to the Sagana population. This could be attributed to introduction of catfish into the lake from Sagana, a research station. The Naivasha population was distant from the others and occurred independently. The Naivasha population is less probable to have been introduced from these hatcheries illustrated from the distant cluster. However, it could be that the population drawn from either populations has undergone major morphological changes influenced by environmental pressures of Lake Naivasha causing the major drift illustrated as adaptive phenotypic plasticity (Debat and David, 2001).

The hierarchical analysis of molecular variance demonstrated significant levels of differentiation in the selected populations. Ninety-two point four 92.4% of variation was significantly accounted for among population variation. Nazia *et al.,* 2010 also observed high levels of within population varations but limited between-population variations. The variation between populations in the current study was low and although they are geographically isolated, a common origin of broodstock having transported by humans for aquaculture purposes may have resulted in the low between-population variation.

Genetic diversity analysis revealed 34 haplotypes and 122 polymorphic sites. The population haplotype diversities ranged from 0.913 to 0.643 accommodating 0.754 and 0.741 of Lake Victoria and Lake Kanyaboli respectively (Barasa *et al*., 2014). The nucleotide diversities ranged from 0.00307 to 0.03035. Lake Baringo population had slightly more haplotypes than Lake Kanyaboli and Lake Victoria and can be attributed to mixed broodstock introduced into the lake from Sagana station. Naivasha population had one haplotype, the least of all the sampled populations hence 0.00 haplotype and nucleotide diversities. Lake populations have higher diversity than cultured populations as demonstrated by Li Q *et al.,* (2004). The high diversity in Lake Baringo could be attributed to obtaining broodstock of catfish from different sources with potentially higher diversities including Sagana hence more haplotypes in the population.

5.4 Phylogenetic structure

Based on the phylogenetic tree two major clades were observed differentiating the Naivasha samples from all the others that divided into sub clades. Athi river samples dominated one of the two sub clades (Figure 13). The phylogenetic analysis showed there were differences between the catfish populations by the tree topology.

The phylogentic tree was congruent with the median joining tree. The Naivasha population had one haplotype, also seen in *Gambusia holbrooki* in Australia where a single haplotype existed (Ayres *et al.,* 2010) and the rest distributed in the four populations. As shown in the median joining tree the Kisii, Athi River and Jewlett samples clustered together. This indicates the strong haplotype relatedness between the three populations. Some haplotypes were shared between the three populations indicating that they could be sharing broodstock. The mixed haplotypes supports that some brooders in the hatcheries could have been obtained from the same source hence shared haplotypes such as Lake Victoria. The occurrence of Kisii and Jewlett populations

appear paraphyletic in the phylogenetic tree. This is as a result of common source of broodstock from Lake Victoria in the past years.

5.5 Population selection

The Athi River population had positive D value hence it could have experienced selection maintaining variation although at insignificant levels p>0.05. The Kisii, Jewlett, Sagana and Baringo populations had negative D value. The four populations with the negative value could have undergone selection removing variation although at insignificant levels of p>0.05. The neutrality test indicates how much a population has significantly deviated from neutral selection. In this case the deviations were present but insignificant except in Sagana. The Naivasha population had Tajima's value 0.000 at p>0.05 attributed to no polymorphic sites in the mitochondrial DNA region analysed. This suggested the occurrence of population size expansion after events of genetic bottleneck or founder events (Tajima, 1989; Maggio *et al.,* 2006) hence no polymorphism.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

-From the morphometric characterisation differences were evident, in line with other studies that found differences due to varied environmental conditions (Gatson *et al.*,2012 and Franssen *et al.,* 2013) emphasizing that environmental factors generally influence morphological differentiation. Different hatcheries have varying management and breeding practices (Orina *et al.,* 2014) influencing overall morphology.

-Mitochondrial DNA revealed maternal linkage of the population as in other studies of the African catfish (Barasa *et al.,* 2014). The most distant of the populations under study was the Naivasha population with no diversity in the sequenced d-loop region. This could mean that the current occurrence of catfish arose from a very small population implying a bottlenecked population in Lake Naivasha.

-Lake Baringo and Athi River had the highest diversities hence high potential for source of broodstock for farmers rearing catfish in Kenya. The broodstock in the hatcheries should be regularly changed to maintain high diversities.

6.2 Recommendations

1. The levels of inbreeding should be conclusively assessed in hatcheries. Inbreeding or crossbreeding could have far reaching effects on quality of fry hence the overall yield would be affected.

2. The recommendation from the study is that the management of cultured populations should change the broodstock regularly to minimize inbreeding.

3. There should be continued awareness on catfish consumption for increased markets and technical training on handling for better overall yield.

4. African catfish in different parts of Lake Naivasha should be examined for further variability using other markers like microsattelite markers.

5. Farmers should explore the Athi River hatchery population for their fingerlings due to its high diversity

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APPENDICES

Appendix 1: Questionnaire for Hatcheries

The aim of this survey is to understand hatchery breeding management practices of African catfish. Any information given is **confidential** and will not be used for any other purposes other than this study.

Please fill the questionnaire by ticking or filling the appropriate places.

Questionnaire No…….

Name of enumerator………………………

GPS Code…………………..……………..

Date………………………………Start of interview………. End of interview……

A. General Information

Name of respondent……………………………Position in farm/hatchery…………

Name of Hatchery…………………………………..

Respondent: ……………………………….....

Gender: Age: Phone/email:

Level of education; ………….. (1. Primary 2. Secondary 3.Tertiary)

Year of Establishment; …………….....

Years on operation: ………………..

Type of farm a) government__ b) private__ c) group__ d) other (specify) __

1. Which fish species do you sell?
    a. (Catfish)
    b. (Tilapia)
2. How many fish of each of the species do you produce in a year?

    a) Catfish…………….b tilapia …………
3. Do farmers buy fingerlings from here?

    Yes___/no___
    a. How many farmers purchase fingerlings from the hatchery in a month?____
    b. What is the most popular species? ______________
    c. What quantities of fingerlings do you sell (numbers) in a year?
        i. (Catfish)………………..
        ii. (Tilapia)………………..
4. What species of catfish do you produce in your hatchery?……………..
    a. Is there a broodstock?

    yes____ /no____

    If yes,
    b. What is the source of catfish broodstock?

    Lakes (yes/no) i.e. …………………………….

    Rivers (yes/no i.e. …………………………….

    Swamps (yes/no) i.e. …………………………..

    Others (specify) ……………………………….

c. When was catfish last stocked______

d. What was the number brought in______

e. Were they randomly picked

i. yes____/no_____

If the answer to e(i) above is no

ii. How were they chosen ……………………………….………

5. How is the broodstock selected once in the hatchery for breeding…………………………

a. size,

b. colour,

c. Other(specify)

6. Do you change the broodstock in the hatchery?

a. Yes___/no____

If the answer to question 6 above is yes

b. When was the broodstock last changed____

c. How often is the exchange cycle ____ (days/weeks/months)

d. From which source do you obtain the fish to change into the broodstock…………………………………………………

7. Facility

a. How many of the ponds do you have

i. Breeding ponds _____

ii. Nursery ponds_____

iii. Grow out ponds ______

b. How many fish do you put in each of the ponds

i. …………………..

ii. ………………….

iii. ………………….

8. Mating systems

a. Which mating systems do you use?

i. Natural controlled…………………..

ii. Natural uncontrolled………………..

b. List the reasons for prefereing this mating system……………………………………

c. How do you facilitate mating of males and females?

………………………………………………………………………………

d. Do you mate fish from different locations/places

yes___ /no___

If the answer to question 8 (d) above is yes -

i. From which different locations/places do you mate……………….,……….……….,………..….

ii. Why mate fish from these places

………………………………………………………………………………

e. What are the ratios of males: females in the broodstock?

……………………………………………………………………………………

f. How do you choose the males for mating

…………………………………………………………………………………….

g. How do you choose the females for mating……………….……..

9. Fish condition

   a. Have you encountered fish disease conditions in the hatchery?

      Yes_____ /no_____

      If the answer to question number 9 (a) above is yes, which diseases conditions have you encountered?

   b. Was postmortem done on the fish yes___/no____

      If the answer to question 9b above is yes _,

   c. What were the results……………………………

10. Have you encountered deformities in catfish

    a. Yes____/no____

    b. Which kind (If yes)_____

    c. In your opinion, what is the likely cause for the deformities? a) defective at source b) inadequate feeds c) poor quality of feeds d) inbreeding

11. What tilapia fingerlings do you produce

    a. Monosex___ Mixed sex___ Both___

    b. What influences this choice…………………………………………

12. If you produce monosex tilapia ,how do you produce them

    a. Manual sexing

    b. sex reversal

    c. Hybridization

13. What tilapia species do you produce?

14. Are you certain about the species of tilapia in the hatchery? a) Yes b) NO

15. Do you have any challenges in your operations

a. Yes___/no____

b. What are the main challenges

i.

ii.

(Technical knowhow/fish handling, Inadequacy of funds, Broodstock exchange cycle, Poor fish nutrition, Climatic condition of location)

16.

a. What is the quality of fingerlings?

i. good

ii. fair

iii. bad

b. How can the quality of fingerling production be improved?

i.

ii.

iii.

17. Year's respondent involved in fisheries………… In this hatchery: ………..

18. Are you aware/familiar of the following

a.

i. Pure breeds yes___/no____

ii. Cross breeds yes___/no____

iii. Inbreeding yes___/no____

b. Do they have or not have an effect on the catfish in general/fingerlings

Yes___/no____

Would you want to know with certainty the species you keep?

Yes____ /No____

c. What do you approve of/disapprove of in catfish breeding in this hatchery

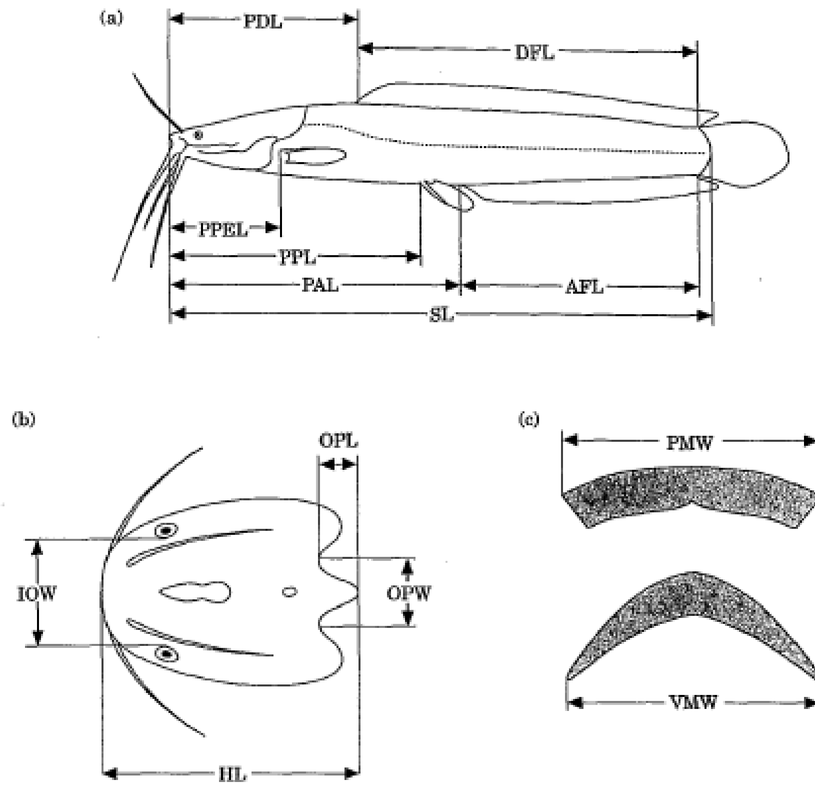
………………………………………………………….

d. What should be done

…………………………………………………………….

Thank you for taking the time to answer questions.

Appendix 2. Catfish measurements



Appendix 3: Laboratory reagent preparation procedure: Agarose gel preparation

Weigh 1g agarose powder into a conical flask. Add 1X TAE buffer to the conical flask to 100g. Boil in a microwave at 100° C for 55 seconds or until agarose is dissolved. Cool to about 50°C by gentle swirling over running cold water. Add 5µl of Ethidium Bromide and mix by gentle swirling.

Transfer the solution to a sealed casting tray with comb and allow to cool up to 45 minutes. When cooled remove the comb gently from the tray with gel. Then transfer the gel from casting tray into electrophoresis tank filled with 1X TAE.

Appendix 4: DNA extraction: Qiagen Kit protocol

1. 25mg tissue was cut into small pieces, and placed in 1.5ml microcentrifuge tube then added 180ul buffer ATL (tissue lysis buffer).

2. Added 20ul proteinase K (digestion enzyme) .Mixed by vortexing and incubated at 56°C until tissue was completely lysed. Vortexed occasionally during incubation to disperse the every 15minutes for 1 hour

3. Vortexed for 15 seconds and added 200ul buffer AL (lysis buffer) to the sample and mixed thoroughly by vortexing. Then added 200ul ethanol (96-100%) and mixed again thoroughly by vortexing

4. Pipetted the mixture from step 3 (including any precipitate) into the DNeasy spin column placed in a 2ml collection tube (provided). Centrifuged at 8000rpm for 1 min. Discarded flow through and collection tube.

5. Placed the DNeasy spin column in a new 2ml collection tube (provided) added 500ul buffer AW1, and centrifuged for 1 minute at 8000rpm (> 6,000 x g) . Discarded flow-through and collection tube

6. Placed the DNeasy spin column in a new 2ml collection tube (provided) added 500ul buffer AW2, and centrifuge for 3 minutes at 14000rpm (20,000 x g) to dry the DNEasy membrane. Discarded flow through and collection tube.

7. Placed DNeasy mini spin column (not provided) and pipette 50 ul buffer AE (elution buffer) directly onto the DNEasy membrane. Incubated for 1 min at > 6000 x g (8000 rpm) to elute.

Appendix 5: Table of means and significance levels

Variable	Athi river Mean± SD	KisiiFPC Mean±SD	Jewlett Mean±SD	Naivasha Mean±SD	Sagana Mean±SD	F value	p value
Weight	433.17 ±222.93	196.80 ±48.70	211.32±74.61	157.10 ±99.42	301.48± 98.12	23.33	0.000
TL	381.52 ±91.08	302.70 ±25.19	315.75±39.45	275.61 ±66.70	355.32 ±41.65	16.01	0.000
SL	327.63 ±77.38	270.13 ±23.45	281.17±35.65	249.79 ±61.41	317.08 ±36.84	11.99	0.000
PPEL	73.70 ±15.95	60.40 ±6.78	59.25 ±7.75	44.47 ±12.94	71.20 ±9.35	33.61	0.000
PAL	183.47 ±38.69	147.57 ±12.05	147.75±14.47	117.00 ±26.44	166.36 ±19.84	32.16	0.000
PPL	151.65 ±31.08	125.97 ±11.80	125.75±15.31	100.76 ±24.25	145.84 ±15.80	28.28	0.000
PDL	113.60 ±23.09	90.63 ±8.29	92.50 ±10.12	78.47 ±18.34	112.80 ±13.95	27.03	0.000
HL	95.1 ±19.65	76.20 ±7.33	74.33±9.05	50.00±14.32	92.36±11.93	57.53	0.000
IOW	37.23 ±7.54	30.67±9.46	29.00±3.08	26.76±6.95	35.00±4.69	12.20	0.000
OPL	15.20 ±3.62	11.47±1.50	12.83±1.90	6.63±1.42	14.40±2.50	69.23	0.000
OPW	23.55 ±6.45	16.10±1.65	17.75±1.60	13.34±3.12	22.32±3.60	37.53	0.000
PMW	32.35 ±8.90	45.90±4.39	41.92±5.53	38.00±11.92	54.48±7.91	28.02	0.000
VMW	57.85 ±11.66	23.80±3.24	25.58±3.45	20.47±6.68	27.52±3.26	158.47	0.000
AFL	140.08 ±36.13	114.80 ±10.17	121.75±22.10	129.08 ±33.00	123.32 ±18.12	3.94	0.005
DFL	206.65 ±48.18	168.20 ±13.50	174.58±24.23	168.24 ±41.50	182.48 ±21.84	7.47	0.000

Appendix 6: Individual sample loadings numbered by group

Object Scores								
Group	Dimension		Group	Dimension		Group	Dimension	
	1	2		1	2		1	2
1	0.874	0.532	2	0.04	-0.349	3	-1.285	-0.092
1	0.684	0.479	2	0.534	-0.383	3	-1.111	0.105
1	0.724	0.064	2	0.258	-0.083	3	-1.267	-0.029
1	0.648	0.094	2	0.227	-0.086	3	-1.446	-0.208
1	0.667	0.363	2	0.402	-0.543	3	-1.381	-0.063
1	1.021	0.514	2	0.068	-0.114	3	-0.565	-0.276
1	1.29	0.12	2	0.207	0.018	3	-0.886	-0.03
1	0.455	0.556	2	0.298	-0.349	3	-0.952	-0.323
1	0.892	0.259	2	0.277	-0.38	3	-1.3	-0.093
1	0.385	0.735	2	0.215	-0.308	3	-1.261	-0.043
1	1.17	0.615	2	0.313	-0.427	3	-0.906	-0.117
1	0.852	0.441	2	0.423	-0.308	3	-1.103	-0.16
1	0.501	0.33	2	0.588	-0.498	3	-1.095	-0.08
1	0.718	0.638	2	0.287	-0.481	3	-0.992	-0.077
1	0.523	0.308	2	-0.111	-0.263	3	-0.893	0.341
1	0.539	0.611	2	0.382	-0.333	3	-0.759	-0.371
1	0.041	0.538	2	0.401	-0.376	3	-1.198	-0.193
1	0.67	0.796	2	0.394	-0.207	3	-0.947	-0.24
1	-0.003	0.84	2	0.123	-0.052	3	-1.505	0.098
1	-0.232	0.622	2	0.173	-0.359	3	-1.217	-0.109
1	0.796	0.515	2	0.496	-0.383	3	-0.804	-0.238
1	0.426	0.345	2	0.396	-0.389	3	-1.33	-0.218
1	-0.428	1.034	2	0.054	-0.269	4	-0.518	0.053
1	-0.429	0.695	2	0.164	-0.212	4	0.182	0.013
1	0.603	0.734	2	0.333	-0.594	4	-0.571	0.166
1	0.031	0.567	2	0.136	-0.25	4	-0.009	-0.05
1	0.703	0.442	2	0.559	-0.278	4	0.32	-0.197
1	0.303	0.752	3	-1.164	-0.004	4	-0.1	-0.075

1	0.293	0.984	3	-1.242	-0.155	4	0.257	-0.05
1	0.336	0.69	3	-0.936	-0.256	4	0.182	-0.053
1	0.611	0.183	3	-1.109	-0.127	4	0.25	0.046
1	0.45	0.525	3	-1.446	-0.062	4	0.608	-0.09
1	0.555	0.049	3	-1.244	0.392	4	-0.151	0.035
1	0.205	0.96	3	-1.576	0.532	4	0.03	-0.073
1	-0.023	0.705	3	-0.958	-0.221	5	0.429	-0.358
1	0.095	0.589	3	-0.603	-0.153	5	0.399	-0.393
1	-0.118	0.995	3	-0.973	0.095	5	0.476	-0.242
1	0.189	0.772	3	-1.626	0.306	5	0.044	-0.428
1	0.868	0.289	3	-1.732	-0.002	5	0.929	-0.604
1	0.316	0.707	3	-1.03	-0.014	5	0.122	-0.438
2	0.042	-0.183	3	-1.163	-0.044	5	0.661	-0.481
2	0.526	-0.342	3	-1.346	0.079	5	0.546	-0.427
2	0.581	-0.642	3	-1.167	0.131	5	0.731	-0.16
5	0.414	-0.561	6	0.422	-0.152	6	1.255	-0.383
5	0.36	-0.405	6	0.422	-0.152	6	1	-0.329
5	0.472	-0.098	6	-0.227	0.092	6	1	-0.329
5	0.71	-0.064	6	0.816	-0.341	6	1.299	-0.318
5	0.421	-0.309	6	1	-0.329	6	0.632	-0.272
5	0.464	-0.479	6	1.045	-0.263	6	1.255	-0.383
5	0.459	-0.368	6	0.746	-0.274	6	0.377	-0.218
5	0.515	-0.515	6	-0.087	-0.043	6	0.746	-0.274
5	0.363	-0.539	6	0.123	-0.163	6	1	-0.329
5	0.623	-0.318	6	1	-0.329			
5	0.443	-0.366	6	0.123	-0.163			
5	0.165	-0.359	6	1.509	-0.438			
5	0.121	-0.154	6	0.93	-0.261			
5	0.22	-0.214	6	0.422	-0.152			
5	0.922	-0.329	6	0.676	-0.207			
5	0.54	-0.225	6	0.746	-0.274			