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Population genetics structure of wild and domesticated African catfish (*Clarias gariepinus*) in Victoria and Albertine Drainage Basins

BY

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DECLARATION

This study is original and has not been submitted for any other degree award to any other University before. Whenever contribution of others is involved, every effort was done to indicate this clearly, with due reference.

This work was done under the guidance of Dr. Charles Masembe from the department of biology, college of natural sciences, Makerere University-Kampala and Dr. Vincent Muwanika from the department of environmental management, college of agriculture and environmental sciences of Makerere University.

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Date: _____

DEDICATION

I dedicate the dissertation to my lovely mother and father who raised me to be the man I am today. To my beloved fiancée Ms Deborah and our precious daughter Mirielle who stood by me in bad and good times. To my sister Martha, brothers; Perez, Simon and Moses for their guidance and unconditional love they have always given me to succeed. Finally, I dedicated the dissertation to my mentors in genetics and bioinformatics Charles Masembe (PhD) and Peter Akoll (PhD) from the Department of Biological Sciences, College of Natural Sciences of Makerere University. Thank for giving me the confidence that I can achieve anything once I put my mind to it.

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ABSTRACT

Wildlife capture fisheries have declined tremendously due to overfishing resulting from high demand. Aquaculture is a viable option to meet this demand. The African catfish (*Clarias gariepinus*) is one of the major species cultured in several fish farms in Uganda. However, this catfish domestication has been done without knowledge on the genetic characteristics of this fish. Indeed profiling the genetic integrity of *C. gariepinus* populations on farms and in the wild would enable the establishment of potential source of broodstock within Albertine and Victoria drainage basins for optimum performance. In this study, genetic diversity, differentiation and demographic history and evolutionary relationships of wild and domesticated *C. gariepinus* were determined. Caudal fins from 180 catfish individuals were collected from four fish farms, Victoria wild (lakes Wamala and Victoria and River Rwizi) and Albertine wild (lakes Albert, Edward and George). They were sequenced for partial mitochondrial DNA control region. The mtDNA control region revealed 45 haplotypes in 68 polymorphic sites, two distinct groupings (i.e., Albertine and Victoria groups) and a relatively high overall genetic diversity. Apart from Kabeihura fish farm (h =0.222), a generally high genetic diversity was observed among Albertine wild (L. George (h=0.935), L. Edward (h=0.958) and L. Albert (h=1.000)), Victoria wild (L. Wamala (h=0.742) and R. Rwizi (h=0.600)) fish farms (POCIFF (h=0.897), KFF (h=0.800) and SIFFA (h=0.756)) *C. gariepinus* strains. No significant genetic differentiation was observed between Victoria wild and Albertine wild indicative of events of the late Pleistocene. Similarly, no significant genetic differentiation between Victoria wild and fish farm strains showing that all broodstock for farms included in this study were most likely obtained from Lake Victoria. The results from this study indicate that most domesticated fish are not genetically depauperate and further reveal that fish farmers pick brood stock from other farms and the wild irrespective of the locality basin. Our results provide a guideline and basis for genetic considerations while sourcing and locating broodstock in aquaculture enterprises for improving fish production.

Keywords: domestication, aquaculture, lineages, evolutionary relationships, drainage basins

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The African hydrological landscape was modified by historical climate change (Hay, *et al.*, 2002), orogeny (Chadwick, *et al.*, 2013), rifting (Baker & Wohlenberg, 1971) and volcanism (Maslin & Trauth, 2006). These processes produced distinct landforms and diversity of environmental features. Complex vegetation, high and low altitude freshwater streams, rivers, and lakes exemplify the features. East African Victoria and Albertine drainage basins are such a paradigm, which form major freshwater ecosystem (Darwall, *et al.*, 2005). The Albertine Drainage Basin (ADB) lies within the western arm of the East African rift valley, stretching from Uganda in the North to Malawi in the South (Wood & Guth, 2013). This basin comprises of major lakes Albert, Edward and George, rivers Semliki, Rwizi and lower Nile valley and wetland systems in Uganda. The Victoria Drainage Basin (VDB) on the other hand is located at the center of East Africa, and shared amongst five repatriate East African states of Uganda, Kenya, Tanzania Rwanda and Burundi. The VDB is endowed with rivers; majorly the upper Nile valley, Kagera, Katonga and Nzoia; lakes Victoria, Nabugabo, Wamala, Sare, Namboyo; and extensive network of wetlands.

Victoria and Albertine drainage basins were connected in the past (William, *et al.*, 2006). Lakes Victoria, Nabugabo, Wamala, Edward and George, rivers Kagera and Katonga from both basins were all part of the greater Nile catchment (Worthington & Lowe-McConnell, 1994), connected by uplifted and rifted rocks of Precambrian age . During up-warping of western part of Lake Victoria in mid Pleistocene, studies theorize that rivers Kagera and Katonga spilled over their banks draining into the lakes Edward and George complex (Beadle, 1981). Today, the Katonga River still flows back and forth between lakes George and Victoria. In addition, fish species such as *Oreochromis niloticus, Bagrus sp, Clarias sp. and Haplochromis sp* are closely related and endemic in lakes Victoria, George. Edward, Wamala and Nabugabo (Greenwood, 1966; Stager & Johnson, 2007). Similar fish species perhaps provide evidence on the linkage of both Albertine and Victoria drainage basins.

Though lakes Albert and Edward are situated within the Albertine basin, connected by River Semliki and have some shared fauna such as *Clarias sp, Bagrus sp, and Oreochromis sp*

(Greenwood, 1961), Albert has unique fish species and families absent in Edward. These amongst others are *Hydrocynus sp,* and *Mastacembelus sp,* and Characidae and Schilbeidae families (Hughes, 1992). Devaere (2007) attributes this faunal differences to the presence of 300m long rapids on a section of the Semliki River. Semliki rapids are characterized by relatively steep riverbed gradient, which results into an increase in water velocity and turbulence. Rapids are considered barriers to fish movement whose effect produces differing assemblage structures in ecosystems (Devaere, *et al.*, 2007; Torrente-Vilara, *et al.*, 2011). Studies show that the Semliki was formed between the lakes Albert and Edward in the early Pleistocene (Lærdal, *et al.*, 2004; William, *et al.*, 2006), allowing for habitat connectivity. However, formation of rapids on the Semliki affected upstream full colonization of Lake Edward by fish from Lake Albert. Catfishes are found not only in Edward and Albert but also in all lakes and rivers in both Albertine and Victoria drainage basins. Most are air-breathing and migratory (Teugels, 1996). In addition, they are widely distributed in Africa, Asia Minor and South-East Asia.

Species diversity of catfishes is highest in Africa, where 12 genera with 74 species are known; only three genera with 18 species occur in Asia (Devaere, *et al.*, 2007). North African catfish *Clarias gariepinus* (Burchell, 1822) is arguably the most commercially important catfish in Africa. The catfish is source of wild food to fishing communities (Gunder, 2004), potential aquaculture species (Hogendoorn, 1979; Roodt-Wilding, *et al.*, 2010) and invasive in its non-endemic environments (Vitule, *et al.*, 2006). However over-fishing, environmental degradation and re-distribution of surface water underplay its commercial potential (Balirwa, *et al.*, 2003). Recent wild fish stock assessments conducted by National Fisheries Resource Research Institute on major lakes in Uganda affirm that wild catfish are generally on a decline (DFR, 2012). Government noticed the decline, and responded through formulating policies promoting fish farming. Fish farming (aquaculture) is taking shape and providing an alternative to over-burdened natural water bodies.

The number of commercial *C. gariepinus* farmers is steadily rising across Uganda (FishstatPlus, 2013). They are responding to an increase in demand for fish and its products created by declining catches from most natural water bodies. Farmers prefer *C. gariepinus* (Ssebisubi, 2010) because it attains a bigger size and has higher gross margin in comparison to Nile Tilapia *Oreochromis niloticus* and Common Carp *Cyprinus carpio* (Halasi, *et al.*, 2012). Its production technologies are well understood, following recent infusion of research breakthrough and financial aid from development partners (DFR, 2012). However, *C.*

gariepinus do not easily reproduce in captivity (Hogendoorn, 1979), rather it requires specialized facility, fish hatchery, for artificial propagation. Birth of commercial catfish hatchery operators is part a response to demand for seed from grow-out farmers.

The majority of catfish hatcheries lie within the Ugandan Albertine and Victoria drainage basins (Aulanier, *et al.*, 2011). They are located adjacent to wetlands perhaps for supply of water and fish brood stock. However, hatchery operators are producing *C. gariepinus* fry below their estimated capacities mainly because of mortalities (Akoll, *et al.*, 2012), slow growth rates (Schram, *et al.*, 2012) and increasing cost of fish feeds (Nyina-wamwiza, *et al.*, 2012). In addition, fish exhibit slow growth rates and has placed operators at a risk of losing their clientele (grower out fish farmers). Slow growth makes fish stay longer on fish farms, increasing cost of production. Consequently, farmed fish is not competitive on local and international markets (Halasi, *et al.*, 2012).

The ancestry of all farmed *C. gariepinus* can be traced to the wild. Ironically, farmed fish are smaller, more susceptible to disease, adverse environmental conditions and have relatively lower fecundity rate than their counterparts in the wild. Studies on domesticated brown trout revealed low survive rate in the wild due to lost fitness (Maric, *et al.*, 2010). Probable causes of loss of fitness in domesticated individual could be bottleneck effect from unconscious selection and use of small number of parent stock (An, *et al.*, 2011). Farmed *C. gariepinus* may undergo bottleneck effect once they loss their genetic diversity because of selection pressure and adaptation to artificial environment.

1.2 Statement of the problem

Smallholder fish farmers constitute the largest category of catfish farmers in Uganda. They obtain catfish seed from medium sized commercial hatchery operators (Aulanier, *et al.*, 2011). However, the smallholder farmers are demanding for less and lesser of farmed catfish seed because; they yield comparatively smaller table sized fish than those from the wild. In addition, farmed catfish have low growth rates increasing cost of production. Consequently, fish stays longer on farms, affecting enterprise profitability and competitiveness. Farmed *C. gariepinus* could be experiencing effects of loss of fitness, resulting from uncertified brood stock source and management. Commercial hatcheries source brood fish from other farms and natural water bodies yet their genetic characterization is unknown. Detection and screening for the threat of loss of fitness amongst populations is absent in commercial catfish

production in Uganda. There is a need therefore to profile the genetic profiles of farmed and wild catfish populations to ascertain family-based pedigrees.

1.3 Objectives

1.3.1 General objective

The aim of the study is to assess genetic profile of current farmed North African catfish in Uganda.

1.3.2 Specific objectives

1. Determine the genetic diversity and differentiation of farmed and wild *Clarias gariepinus*.
2. Elucidate phylogenetics and evolutionary relationships among farmed and wild *Clarias gariepinus* in the Albertine and Victoria Drainage Basins.

1.4 Hypotheses

Ho. Farmed *Clarias gariepinus* strains are genetically more diverse than wild strains.

1.5 Significance of the study

The genetic characterization of the farmed and wild *Clarias gariepinus* strains can be a good management tool for guiding production of farmed catfish. Catfish hatcheries will precisely select broodstock with beneficial mutations for seed production following genetic characterization. Informed hatchery operators will source broodstock from high genetic diversity natural water bodies and fish farms. They will easily maintain broodstock family pedigree paving way for catfish seed traceability and quality assurance. Traceability could foster building of trust between hatcheries and smallholder farmers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Albertine Drainage basin

Albertine Drainage Basin lies within the Albertine rift (AR). The AR is the western branch of the Great Rift Valley of Africa and it extends from the northern end of Lake Albert to the southern end of Lake Tanganyika (ARCOS, 2012). It encompasses lands on both sides of the rift and spanning several countries: the Democratic Republic of Congo (DRC), Uganda, Rwanda, Burundi, Tanzania and Zambia. The AR is endowed with a variety of ecosystems ranging from the snow-capped Mountain Rwenzori, mid altitude and lowland forests, savannahs and woodlands, several streams and rivers that drain into the numerous wetlands lakes.

The major rivers in the AR include Semliki connecting Lake Albert and Edward, rivers Akanyaru and Mukungwa that drain into River Nyabarongo in Rwanda, river Rusizi linking lakes Kivu and Tanganyika, and the Malagarasi River in south-eastern Burundi and north-western Tanzania (ARCOS, 2012). On the DRC side, major rivers of the AR include River Lubero and Rutshuru that drain into the Lake Edward basin. The Albertine rift also habours a diversity of wetland types both at high elevation and low lying areas (Karp, *et al.*, 2004). Examples of high altitude wetlands include the Rugezi marsh and Kamiranzovu in Rwanda, the Mucuya and Mubwindi swamps in Uganda. Major lakes within the AR include, Albert, Edward and George, Kivu, and Tanganyika (Lwanga, *et al.*, 2003).

These lakes are a source of livelihood, providing employment through fishing, food and transport (Scholz, et al., 2007). Most lake basins are trans-boundary and their management is collaborative sustainable management of the resources. Threats to the lakes include pollution and sedimentation (Lwanga, *et al.*, 2003) from unsustainably managed catchments, overfishing (Balirwa, *et al.*, 2003). Recent oil and gas exploration in the region will likely exacerbate the already fragile ecosystem (NPA, 2010).

2.2 The Victoria Drainage Basin

Lake Victoria lies within coordinates $0^{o}20'$ N to $3^{o}00'$S and $31^{o}39'$E to $34^{o}53'$W in East Africa (Verschuren et al., 2002), and is shared among three riparian countries including Kenya (6%), Uganda (43%), and Tanzania (51%) (Odada, *et al.*, 2004). It's drainage basin stretches to an approximate area of 193,000 km^2 (Okonga, et al., 2005). According to Stager

and Johnson (2007), the basin is large enough to create its own weather system. The basin receives 1,060 mm average annual rainfall (Odada, *et al.*, 2004). It supports a vast array of ecosystems including flourishing forests, vegetated wetlands, rivers and satellite lakes. River Kagera is the basin's principal affluent inflow, starts from the highlands of Rwanda and drains in Lake Victoria (Odada, *et al.*, 2004). Whereas River Nile is the main Lake Victoria outflow and drains into the Mediterranean Sea in Egypt.

Over 600 endemic haplochromine cichlids have been documented to exist in Lake Victoria, representing one of the most extensive recent radiations of vertebrates known (Greenwood, 1974; Kaufman, *et al.*, 1997). In addition, the Lake has a rich assemblage of non-cichlid fauna (Balirwa, *et al.*, 2003) including amongst others the large catfish *Bagrus docmak* and *Clarias gariepinus*, Mormyrids *Mormyrus Kanume*, African lungfish *Protopterus aethiopicus*. Therefore, Lake Victoria is the single most economically and ecologically important inland freshwater fishery in East Africa. For instance, fish became one of Uganda's leading export commodities by 2008 following maintenance of fish sanitary and phyto-sanitary conditions by fish processing plants (DFR, 2012).

However, the ecological and economical functionality of Lake Victoria drainage basin are threatened. Anthropogenic activities such as overfishing, siltation due to erosion of deforested watersheds, species introduction, and industrial pollution and climate change are by far the principal threats to the basin (Balirwa, *et al.*, 2003; Odada, *et al.*, 2004). Human occupancy in basin has dramatically increased over last century, replacing shoreline riparian forests with agriculture (Aloo, 2003). The Lake fishery during most of the 20th century was multi-species resting on its diverse ecosystems (Ogutu-Ohwayo, 1990). By the end of the century, Lake Victoria had much more productive fishery but one in which three species including Nile perch *Lates niloticus*, Silver fish *Rastrineobola argentea* and Nile tilapia *Oreochromis niloticus* made up most of the catches (Kaufman, 1992). Unfortunately, the once productive three species Lake Victoria fishery recently declined largely because of increase in fishing effort (DFR, 2012). In addition, the dramatic disappearance of haplochomine communities due to excellent Nile perch predatory tendencies contributed to fishery decline (Balirwa, *et al.*, 2003). Similar to Lake Victoria fishery, are Niger delta (Lae, 1995) and Ouema delta in Benin (Welcomme, 2003), where increased effort resulted into composition change. Decline in fish yields in heavily exploited systems is usually

accompanied by marked shift in species composition towards individuals and species that mature at very small size and age (Welcomme, 2003).

Lake Victoria is no stranger to desiccation and extinction of its endemic species. Well-documented evidence shows that Lake Victoria situated at an altitude of 1,134 m above sea level (Sixtus & Sven, 2006), dried out in the late Pleistocene and has since refilled (Verschuren, *et al.*, 2002). During this period, endemic fauna was wiped out and some extant taxa forced into refugia into low laying areas (Karp, *et al.*, 2004). Large lakes in the Albertine rift situated at an altitude range of 680-1000 m above sea level (WWF, 2006) were recipients of Victorian species. Albertine rift landscape morphology constitutes of lowland surface, deeply incised by rejuvenated rivers and lakes.

2.3 African Catfish (*Clarias gariepinus*, Burchell 1822)

The African catfish, has an elongate body and four pairs of barbells (Devaere, *et al.*, 2007). It possesses a unique supra-branchial respiratory organ, formed by arborescent structures derived from the second and fourth gill arches (Greenwood, 1961). Clariidae are air-breathing catfishes, naturally occurring in Africa, Asia Minor (Jordan, Lebanon, Syria, Israel and Southern Turkey) and South-East Asia (Roodt-Wilding, *et al.*, 2010; Shyama, 2013). Their diversity is highest in Africa, where 12 genera with 74 species are known; only three genera with about 18 species occur in Asia (Teugels, 1996). The genus *Clarias* has 35 species and is the third most diverse catfish genus in Africa (Roodt-Wilding, *et al.*, 2010). *Clarias gariepinus* (Burchell, 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 (Roodt-Wilding, *et al.*, 2010). It has therefore been introduced in many parts of the World where it did not exist before such as China (Shyama, 2013), South America (Vitule, *et al.*, 2006) and Europe (Gunder, 2004).

African Catfish inhabits lakes, rivers, streams, wetlands and flood plains. The commonest habitat is flood plains, where they can survive during the dry season due to their air-breathing accessories (Teugels, 1996). In addition, they are relatively poor swimmers and that they spend most of the time on the bottom of lakes and rivers (Gunder, 2004). The African catfish are however, able to move across land to another water source during damp conditions. They simply extend their strong pectoral fins and spines and begin crawling through shallow pathways (Islam, *et al.*, 2007).

The African catfish undertakes lateral migration from larger water bodies, where they feed and mature, temporarily or permanent vegetated margins of streams, flood plains, lakes to breed (Gunder, 2004). These reproductive migrations take places at the onset of the rainy season. *Clarias gariepinus* are therefore classified as an egg-scatterer reproductive guild, which await suitable environmental conditions before spawning (Teugels, 2003). The final gonad maturation in catfish is associated with raising water levels. Under ideal conditions, adult catfishes have mature gonads year round. The females may lay up 20,000 to 30,000 eggs /kg of body weight (Sheasby, 2009). The African catfish is omnivorous. Their stomach contents usually contain; insects, gastropods, crustaceans, small fish, aquatic plants and debris, larvae exclusively depend on zooplankton in the first week of exogenous feeding (Shyama, 2013).

2.4 Domestication and farming of African catfish

The African catfish *Clarias gariepinus* are invasive (Roodt-Wilding, *et al.*, 2010), known to trail along the extensive and elaborate wetland network systems that exist within Albertine rift and Victoria basins. *Clarias gariepinus* is an excellent aquaculture species and are widely farmed in the Uganda (Aulanier, *et al.*, 2011). The authors rests on the facts that *C. gariepinus* are hardy, tolerate captivity conditions, attain bigger table-size in ponds, more fecund than other farmed fish species and are highly marketable.

However, *C. gariepinus* do not easily reproduce under captivity (Hogendoorn, 1979). Consequently, *C. gariepinus* artificial reproduction methods have developed, giving raise to commercial hatchery catfish operators (Ssebisubi, 2010; Aulanier, *et al.*, 2011). Efforts to domesticate the African Catfish began in the 1970s, with trials by Hogendoorn, following Pillay's principles in the domestication of *C. gariepinus* in Cameroon (Hogendoorn, 1979). At Wageningen University, Hogendoorn was able to establish a population of C. *gariepinus* without further supply from the wild (in Africa) (Bilio, 2007). Domestication required continuous reproduction of progeny and kept free from any wild input. It is widely accepted that domestication is defined as breeding and raising organisms in captive environment at least for part of their life history (Araki, *et al.*, 2008). Conversely, studies on fish domestication suggest that wild inputs can still serve to avoid inbreeding and increase heterogeneity (Araki, *et al.*, 2008; An, *et al.*, 2011; Christie, *et al.*, 2012). For instance, salmonids in captivity often undergo rapid and significant evolutionary and phenotypic changes in behaviour, morphology and physiology (Wang, *et al.*, 2002). These changes may occur at genetic level, such as growth rate, which has a strong heritable component.

Alternatively, at phenotypic level, such as predator avoidance behaviour is not necessary passed on to their offspring. In both situations, domestication conditions fish to captive environment by increasing frequency of a particular mutations that are favourable in captivity but maladaptive in the wild (Araki, *et al.*, 2008).

Adaptive research at Aquaculture Research and Development Centre, Kajjansi is considered by many as the precursor for domestication of *C. gariepinus* in Uganda (Ssebisubi, 2010; Aulanier, *et al.*, 2011). The centre is charged with developing technologies and generating information through research (DFR, 2012). It were established in the 1960s. It promoted fish farming in Uganda. Catfish production in Uganda is estimated at 63,000 tons by 2010 (Dalsgaard, *et al.*, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the Ugandan Albertine Drainage Basin (ADB) and Victoria Drainage Basin (VDB) (Figure 1). Enclosed in the ADB are major water bodies of interest were; Lakes Albert, Edward and George that were studied. Similarly, two established fish farms operating catfish hatcheries were studied, including Kabeihura Farmers Limited (KFL) and Pukure Orphan Care Integrated Farm (POCIF). The farms are located in southwestern and northern Uganda respectively. While in the VDB, this study focused on Lakes Victoria, Wamala, River Rwizi and two fish farms operating hatcheries, including Kireka Fish Farm (KFF) and Salaama Integrated Fish Farm Association (SIFFA).

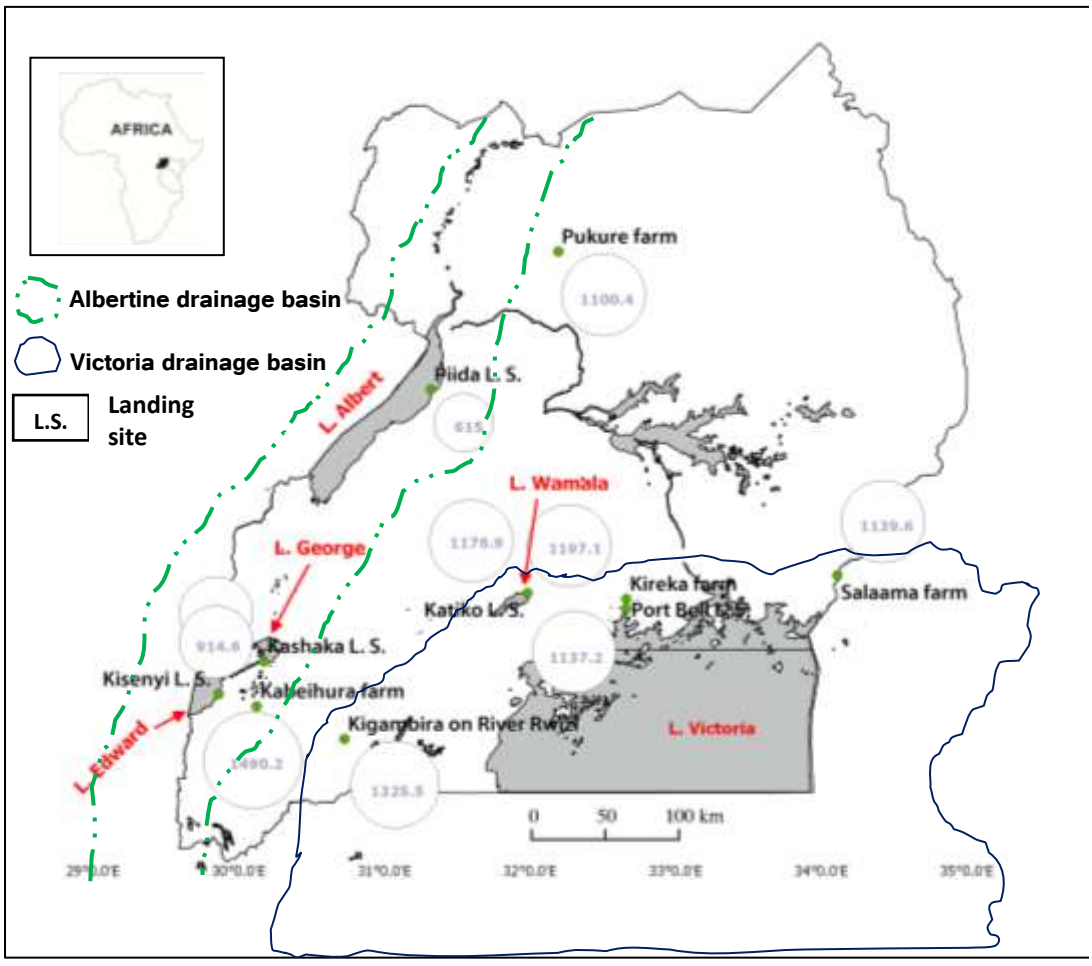


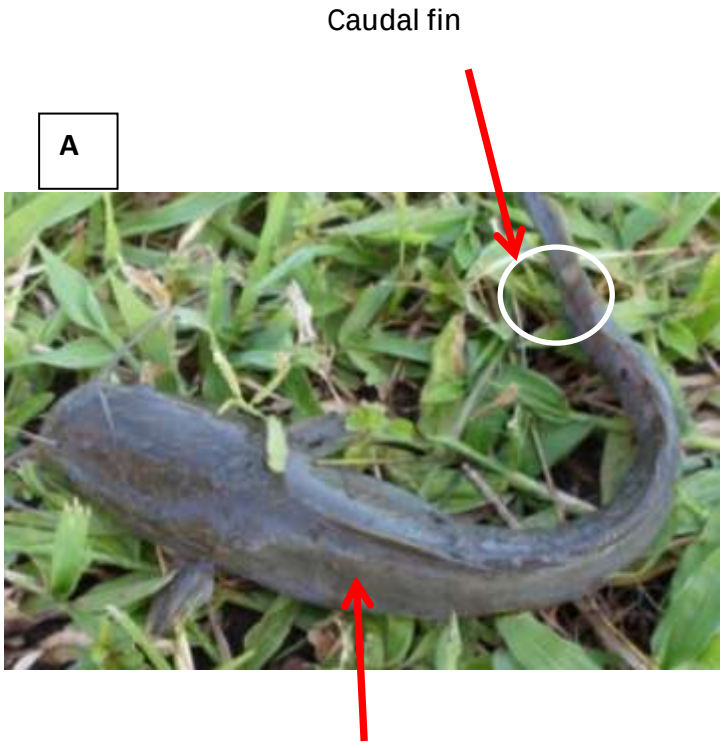
Figure 1: Collection sites of *Clarias gariepinus* samples in the basins of Albertine and Victoria. Samples were collected from fish farms and landing sites on shores of selected lakes and rivers within the basins. Altitudes measured at sampling sites are showed in both basins.

3.2 Study population, samples and sampling techniques

The target populations were North African Catfishes *Clarias gariepinus,* endemic in both lakes and rivers of Albertine rift and Victoria Basins. Wild *C. gariepinus* samples were drawn from catches landed at landing sites on lakes Albert, Edward, George, Wamala and Victoria, and river Rwizi. The sites were chosen based on cost-effectiveness and details of samples obtained are given in Table 1. Whereas farmed *C. gariepinus* were obtained from selected commercial fish hatchery operators (KFL, POCIF, SIFFA and KFF). Farmed samples were drawn from broodstock and nursery ponds using stratified random sampling technique.

3.2 Collection of samples and DNA extraction

Caudal fin clips from 180 individual *C. gariepinus* were collected from natural water bodies and fish farms. The fish fins were clipped using sterilized blade (size 22) and capped into collection tube filled with ethanol (70%) see plate 1. Tissues were transported from the field to Molecular laboratory at Makerere University for storage under -20^{o}C.



Clarias gariepinus

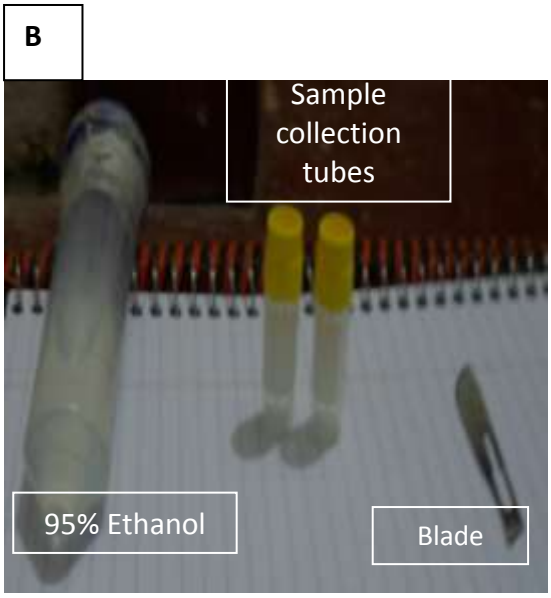


Plate 1: Sample specimen of *Clarias gariepinus* collected and items used to take tissue samples in the field. Plate A. shows the site of fin tissue clipping site. Plate B. shows the an ethanol vial, collection tubes and a size 22 blade.

Table 1: Collection details of the African Catfish providing for locations and sample sizes

Field ID	Sampling Area	Drainage Basin	Sampling Locality	Coordinates	Elevation (m)	Sample Size
	Wild Clarias gariepinus					
B-PID	Lake Albert	ADB	Piida-B Landing Site	1.897N, 31.320 E	615.0	25
K-GEO	Lake Edward	ADB	Kisenyi Landing Site	0.309S, 29.863E	914.0	20
K-EDW	Lake George	ADB	Kashaka Landing Site	0.080S, 30.173E	923.2	20
B-VIC	Lake Victoria	VDB	Port Bell Landing Site	0.287N, 32.661E	1,137.0	25
K-WAL	Lake Wamala	VDB	Katiko Landing Site	0.397N, 31.983E	1,176.9	20
K-RWI	Rwizi Wetland System	VDB	Kigambira Site	0.622S, 30.727E	1,325.5	10
	Domesticated Clarias gariepinus					
R-KFL	Kabeihura Farmers Ltd	ADB	Catfish Hatchery	0.398S, 30.149E	1,490.2	15
O-POC	Pukure Orphan Child Integrated Fish Farm	ADB	Catfish Hatchery	2.779N, 32.195E	1,100.4	15
O-SAL	Salaama Integrated Fish Farm Association	VDB	Catfish Hatchery	0.482N, 34.089E	1,139.6	15
K-KFF	Kireka Fish Farm	VDB	Catfish Hatchery	0.355N, 32.662E	1,197.1	15

The fin clips were thawed and sliced in preparation of DNA extraction. Total genomic DNA extracted from each fish fin clips using the GeneJet™ DNA purification kit following the manufacturer's instructions see Plate 2.

3.3 Polymerase Chain Reaction (PCR) amplification of Mitochondrial DNA

The entire Mitochondrial DNA (mtDNA) control region was Polymerase Chain Reaction (PCR) amplified using a pair of primers: LN20 (5'ACCACTAGCACCCAAAGCTA-3') and HN20 (5'-GTGTTATGCTTTAGTTAAGC-3') (Bernatchez & Danzmann, 1993). The 50µl PCR reaction mixtures contained 20pmol of each primer (HN20 and LN20), 25µl of AmpliTaq® Gold 360 Master Mix (Applied Biosystems, USA), 10µl of double distilled water (ddH_2O) and 5µl of genomic DNA template.

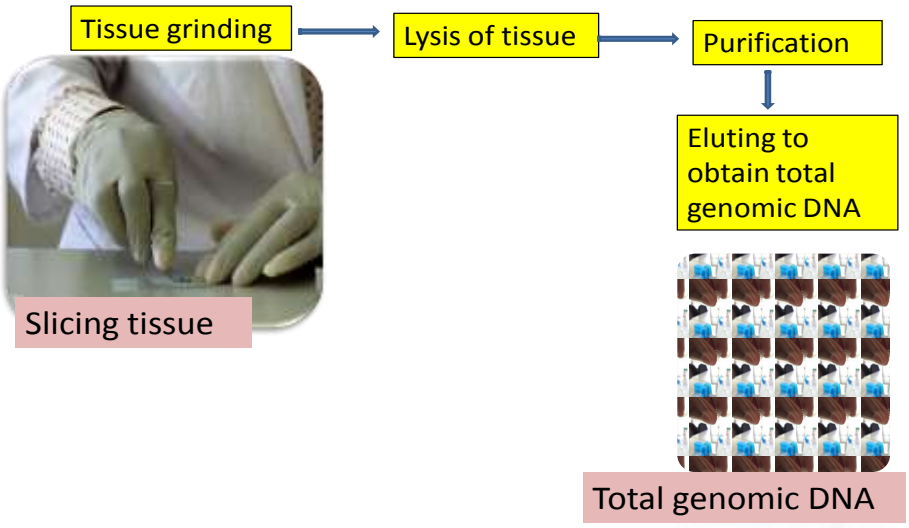


Plate 2: Illustration of extraction and purification of catfish genomic DNA in the Makerere University molecular laboratory

The PCR cycling conditions was as follows; initial denaturation at 95°C for 1min, 35 cycles of denaturation at 94°C for 30s, primer annealing at 51.3°C for 1min, initial extension at 72°C for 50s and followed by final extension for 10min. All PCR reactions were performed in a Thermal Mastercycler (Eppendorf, Germany) PCR system 9700. In every PCR, a DNA free template was included as a negative control. The PCR products were separated by electrophoresis (100v, 45min); size compared against lambda DNA ladder and visualized on 2% Nusieve agarose gel containing Ethidium Bromide. The Amplicons were purified using

GeneJet^{TM} columns following manufacturer's instructions. The products were visualized on 2% agarose gel containing Ethidium Bromide. The purified products were cycle-sequenced and analyzed on ABI 3100 automated-sequencer.

3.4 Data analyses

3.4.1 Sequence analysis

The chromatograms, both forward and reverse were refined and contigs from consensus generated in Geneious program v5.0.4 (Biomatters, 2010). The contigs were imported into BioEdit v7.1.11 (Hall, 1999) and edited. The sequences were aligned using the complete alignment application in Clustal X v 2.0 (Larkin, et al., 2007).

3.4.2 Analysis of genetic diversity, structure, differentiation and demographic history of population of *Clarias gariepinus*

Genetic diversity within samples of wild and domesticated *Clarias gariepinus* populations in the Victoria and Albertine Drainage Basins were quantified using haplotype diversity (h) and nucleotide diversity (π) (Nei, 1987) in Arlequin v3.1 (Excoffier, *et al.*, 2005). More diversity tests including Theta S (θ_s) and Theta Pi (θ_π) were performed in Arlequin. Similarly in Arlequin, the distribution and identification of shared of haplotypes was performed according to segregated sites.

Hierarchical distribution of genetic structure of *C. gariepinus* in Albertine and Victoria basins was analyzed by standard analysis of molecular variance (AMOVA) computations (Gaggiotti & Excoffier, 2000) performed in Arlequin v3.1. Populations were grouped into three based wild and farmed *C. gariepinus,* which included the Albertine wild (Lakes Albert, George and Edward), Fish farms (Kabeihura Farmers Ltd, Pukure Orphan Care Integrated farm, Kireka Fish Farm and Salaama Integrated Fish Farm Association), and Victoria wild (Lakes Victoria and Wamala and River Rwizi). The AMOVA partitioned total variance into covariance components due to intra-individual differences, inter-individual differences and inter population differences. The covariance computed fixation indices included F_{CT} ,F_{SC} and $F_{ST,}$ respectively.as defined by Wright (1965) in terms of inbreeding coefficient or later in terms of coalescent times by Slatkin (1991).

Genetic differentiation among farmed and wild populations of *C. gariepinus* two basins were computed using in AMOVA. Further, the extent of genetic differentiation was based on genetic heterogeneity. Genetic heterogeneity of Albertine and Victoria basins wild and

farmed *C. gariepinus* was quantified using pairwise F_{ST} among genetic groups. Their significant differences computed via 10,000 permutations performed in Arlequin v3.1 (Excoffier, *et al.*, 2005). F_{ST} described genetic differentiation produced by non-random distribution of individuals among sub populations relative to total population.

The account of the demographic history of *C. gariepinus* population expansion and decline within and between Albertine and Victoria basins was simulated in (coalescent simulation graphs) DNASP v 5.10 (Librado & Rozas, 2009). Mismatch distribution analysis was used to explain demographic changes in the *C. gariepinus* populations. Further, neutrality tests; Fu's F_s(Fu, 1997) and Tajima's D (Tajima, 1989) were estimated in DNASP.

3.4.3 Phylogenetics analysis

Phylogenetics relationships among *C. gariepinus* control region nucleotide sequence haplotypes was estimated using Maximum Likelihood (ML) method. The best fit model DNA evolution was H-K-Y+G, performed in MEGA v 5.10 (Tamura, *et al.*, 2007). To test phylogeny, 1000 bootstrapping replications were used and inferred tree in Subtree-Pruning-Regrafting (SPR) ML heuristic method, performed in MEGA. Consensus tree was generated in MEGA, visualized in FigTree v 1.4 (Rambaut, 2012).

CHAPTER FOUR

4.0 RESULTS

4.1 Sequence analysis

A fragment of the mtDNA was PCR amplified from 115 samples obtained from both Victoria and Albertine basin populations. Consensus sequences of 830 base pairs (bp) resulted after editing the sequenced amplified fragments. The 830bp corresponding to the control region (D-loop) of the mtDNA was used for analysis.

4.2 Genetic diversity of farmed and wild *Clarias gariepinus* populations from Albertine and Victoria basins

In the 830bp fragment of the control region, 68 segregating sites defined 45 haplotypes as showed in Table 2, many (30 out of 45) of which occurred as singletons. Albertine and Victoria basins' wild and farmed *Clarias gariepinus* populations shared some haplotypes. The basins populations which shared the haplotypes, were grouped into three basing on wild and farmed individuals including Albertine wild (lakes George, Edward and Albert), Victoria wild (lakes Wamala and Victoria and River Rwizi) and fish farms (POCIF, KFL, SIFFA and KFF). Fish farms shared only one haplotype (*68_K_GEO*) with the Albertine wild. However, with the Victoria wild, fish farms shared three haplotypes (*99_K_RWI, 93_K_WAL, 51_K_WAL*). For Albertine and Victoria wild shared only one haplotype (68_K_GEO). The commonest haplotypes was *68_K_GEO* shared amongst the three groups representing 10 individuals (see Table 2). Haplotype *99_K_RWI* was most frequent only to fish farms and Victoria wild, representing 22 individuals. Fish farms had the highest number (50) of individuals sharing unique haplotypes followed by Albertine wild (41) and the least were in the Victoria wild (24).

Genetic diversity of *C. gariepinus,* based on haplotype diversity (h) and nucleotide diversity (π) showed in Table 3, was generally high across Albertine and Victoria basins. Haplotype diversity in both basins was higher than nucleotide diversity. Notably, the basins were presented as groups based on either *C. gariepinus* being farmed or wild in Table 3. The values of h and π were closely similar in farmed group as compared to Albertine wild and Victoria wild. Albertine wild group revealed the highest haplotype diversity, i.e. L. George (h=0.935), L. Edward (h=0.958) and L. Albert (h=1.000). While the highest nucleotide diversities were observed in most fish farms, i.e. SIFFA (π=0.0050), KFF (π=0.0071) and POCIFF (π=0.0085) except for KFL (π=0.0008). Within the Victoria wild group, genetic

Table 2: Distribution of 68 segregating sites from the 45control region sequences in a total sample of 115 *Clarias gariepinus* individuals. R. Rwizi=River Rwizi, L. Geo=Lake George, L. Edw=Lake Edward, L. Vic=Lake Victoria, L. Wal=Lake Wamala, L. Alb=Lake Albert, POCIF farm=Pukure Orphan Care Integrated Fish farm, KF farm=Kireka Fish farm, KFL farm=Kabeihura Farmers Limited farm, SIFFA farm=Salaama Integrated Family Fish Association farm. Vertical numbers show the positions of polymorphic sites relative to haplotypes (101_K.RWI); - denotes a deletion introduced to optimize alignment.

Hap #	Sequences	10 20 30 40 50 60 1111111111111111122222222222222333333344444555555556666667777788 1890222444566778889900122224556679000145901559135688992256781111120 7169478023308346790959002485293683179463349343220419783898611234555 0	Haplotype Frequencies										Sum
			R. Rwizi	L. Geo.	L. Edw.	L. Vic.	L. Wam.	L. Alb.	POCIF farm	KF farm	KFL farm	SIFA farm	
1	101_K_RWI	CTCACCTCCAGCCCGCGAACCCTTCCCTACAAGCTACCGCGCACTCCCGCTTAAAACACT----GCCC	4										4
2	99_K_RWI	.....T.....T.....T.....C.....	1				5		2	4	8	2	22
3	105_K_RWI	.....C.....	1										1
4	17_K_GEO	TCTG..C.T.....AG.TTT...T...T.G...G...T.....T...T...GC-.....		1									1
5	18_K_GEO	TCTG..C.T....T..AG.TTT...T...T.G.....T.....T...T...GC-.....		5	2								7
6	20_K_GEO	TCT...C.T....T..AG.TTT...T...T.G.....T.T.....T...T...GC-.....		1									1
7	11_K_GEO	TCTG..C.T.....AG.TTT...T...T.G.....T.....T...T...GC-.....T.		1									1
8	65_K_GEO	TCTG..C.T....T.TAG.TTT...T...T.G.....AT.....T...T...GC-.....		1									1
9	68_K_GEO	.....T.....T.....T.....C.....		1			7		2				10
10	69_K_GEO	TCTG....TGA..T..AG.TTT...T...T.G..C....T.....T..AT...GC-.....-		1									1
11	70_K_GEO	TCTG..C.T.....AG.TTT...T...T.G.....T.....T...T...GC-.....		1	4								5
12	71_K_GEO	TCTG..C.T....T..AG.TTT.C.T...T.G..C....T.....T...T...GC-.....-		1	0								1
13	72_K_GEO	TCTG..C.T....T..AG.TTT...T..TT.G.....T.....T...T...GC-.T.....		1	0								1
14	73_K_GEO	T.TG....T.A..T..AG.TTTC..T...T.G..C....T.....T...T...GC-.....		1	0								1
15	74_K_GEO	TCTG..C.T....T..AG.TTT...T...T.G.....T.....T...TC..GC-.....		1	0								1
16	12_K_GEO	TCTG..C.T....T..AG.TTT...T...T.G....T..T.....T...T..GGC-.....		1	0								1
17	13_K_GEO	TCT.T.C.T....T..AG.TTT...T...T.G.....AT.....T...T...GC-.....		1	0								1
18	10_K_EDW	TCTG....T.A..T..AG.TTT...T...T.G..C....T.....T...T...GC-.....			1								1
19	59_K_EDW	TCTG..C.T....T.TAG.TTT...T...T.G.....T.....T...T...GC-.....			1								1
20	55_K_EDW	T.TG..C.T....T..AG.TTT...T...T.G..C.....T...T...GC-.....			1								1
21	56_K_EDW	TCTG..C.T....T..AG.TTT...T...T.G.....T.....T...T.C.GC-.....			2								2

Hap #	Sequences	10 20 30 40 50 60 1111111111111111122222222222222333333344444555555556666667777778 1890222444566778889900122224556679000145901559135688992256781111120 7169478023308346790959002485293683179463349343220419783898611234555	Haplotype Frequencies										Sum
			R. Rwizi	L. Geo.	L. Edw.	L. Vic.	L. Wam.	L. Alb.	POCIF farm	KF farm	KFL farm	SIFA farm	
22	57_K_EDW	TCTG..C.T....T..AG.TTT..TT...T.G.......T.....T...T...GC-....ACGC....			1								1
23	58_K_EDW	TCTG..C.T....T..AG.TTT...T...T.G..C....T...TCT...T...GC-.....			1								1
24	7_K_EDW	T.TG..C.T....T..AG.TTT...T...T.G.......T.....T...T...GC-.....			1								1
25	8_K_EDW	TC.G..C.T....T..AG.TTT...T...T.G.......T.....T...T...GC-.....			1								1
26	9_K_EDW	TCTG..C.T.A.....AG.TTT...T...T.G.......T.....T-..T...GC-.....			1								1
27	1_K_EDW	TCTG..CTT....T..AG.TTT..TTT..T.G.......T.....T...T...GC-.....			1								1
28	4_K_EDW	TCTGT.C.T....T..AG.TTT...T...T.G......AT.....T...T...GC-........A...			1								1
29	6_K_EDW	T.TG..C.T..T.T..AG.TTT...T...T.G.T.....T.....T...T...GC-.....			1								1
30	64_K_EDW	TCTG..C.T....T..AG.TTT.......T.GA......T.....T.T.T...GC-.....			1								1
31	43_B_VIC	............T.............................G...........C.....				2							2
32	51_K_WAL	..T.....................T.............T.........T.C..C.....					1				1		2
33	79_K_WAL	.........................T.............T.......T......C.....					1						1
34	84_K_WAL	......C.......A..G.T.....T...T.........T.............C.....					1						1
35	93_K_WAL	............T.....G.....................................C.....					1		1				2
36	144_B_PID	T.TG..C.T....T..AG.TTT...T...T.G.......T.....T...T...GC-..-.........						1					1
37	145_B_PID	TCTG..C.T....T..AG.TT....T...T.G.......T.....T...T...GC-.....						1					1
38	146_B_PID	T.TG..C.T..T.T..AG.TTT...T.C.T.G.......T.....T...T...GC-.........T..						1					1
39	137_O_POC	.....TC......TA....T..........-.........TAT.......T....C.T..C........							3	4		1	8
40	133_O_POC	.........................T.............T.........T....C.....							1			1	2
41	147_O_POC	......C.............T.....T...T.........T.............C.....							3	4			7
42	135_O_POC	.....TC......TA....T...C......-.........T.T...T.........C....C........							1				1
43	154_K_KFF	......C.............T.....T...T.........T...........C..C.....								3			3
44	32_O_SAL	......C.............T.....T...T.........T.........T....C.....										6	6
45	33_O_SAL	.........................T.............T.......T......C...-.........										3	3
No. of bases		2222222222222222222222222222221222222222222222212222222221221211112221	6	18	20	2	16	3	13	15	9	13	115

diversity was computed for all water bodies except Lake Victoria because it had only two samples. River Rwizi in the Victoria wild had lower genetic diversity (h=0.6000, π=0.0015) to Lake Wamala (h=0.742, π=0.0029).

Table 2: Molecular diversity indices for *Clarias gariepinus* populations. Indices include Nucleotide (π) and haplotype (h) diversities. The populations consist of wild ADB (wild Albertine Drainage Basin), wild VDB (wild Victoria Drainage Basin) and fish farms.

Populations	n	Haplotypes	π	h	Theta S (θ_s)	Theta Pi (θ_π)
Albertine wild						
Lake Albert	3	3	0.0048	1.000	3.333	2.323
Lake Edward	20	13	0.0043	0.958	6.201	2.423
Lake George	18	14	0.0066	0.935	10.466	3.955
Victoria wild						
Lake Victoria	2	1				
Lake Wamala	16	4	0.0029	0.742	3.917	1.720
River Rwizi	6	3	0.0015	0.600	1.314	0.910
Fish farms						
POCIF	13	4	0.0085	0.897	5.478	2.390
KFL	9	0	0.0008	0.222	1.104	0.738
KFF	15	1	0.0071	0.800	3.998	1.773
SIFFA	13	2	0.0050	0.756	4.189	1.901

Exploring other genetic diversity indices including Theta S (θ_s) and Theta Pi (θ_π), it were confirmed wild Albertine had the highest diversity (Theta S (θ_s) ranges from 3.333 to10.466, and Theta Pi (θ_π) from 2.323 to 3.955) followed by fish farms (θ_s from 3.998 to 5.479 and θ_π from 1.773 to 2.390) (See Table 3). Further, Lake George revealed highest diversity across all indices (h=0.0066, π=0.935, θ_s=10.466 and θ_π=3.955) in comparison to all other populations.

4.3 Genetic structure and differentiation among *C. gariepinus* populations

When *C. gariepinus* populations were grouped based on wild and farmed populations, three clusters were identified including Albertine wild (lakes George, Edward and Albert), Victoria wild (lakes Wamala and Victoria and River Rwizi) and fish farms (POCIF, KFL, SIFFA and KFF) for the analysis of molecular variance (AMOVA). The AMOVA results showed in Table 4, revealed significant (F_{CT} = 0.72701, P<0.0001) sub-divisions among groups of Albertine wild and fish farms. Similarly, the sub-division among populations within these groups and within populations contributed significantly (F_{SC} = 0.07987, P<0.05 and F_{ST} = 0.74881, P<0.0001 respectively) to the observed genetic structure.

Table 3: Analysis of Molecular Variance (AMOVA) among Clarias gariepinus haplotypes

Hierarchical level	Source of Variation	d.f	Sum of Squares	Variance components	% of variation	Fixation index	P
Albertine wild and Fish farms	Among groups	1	309.685	6.75150	72.70	F_{CT} = 0.72701	0.000 ***
	Among populations within groups	5	23.861	0.20248	2.18	F_{SC} = 0.07987	0.015 *
	Within populations	84	195.948	2.33271	25.12	F_{ST} = 0.74881	0.000 ***
	Total	**90**	**529.494**	**9.28669**			
Victoria wild and Fish farms	Among groups	1	20.046	0.40197	14.41	F_{CT} = 0.14414	0.085 **ns**
	Among populations within groups	5	29.517	0.40157	14.40	F_{SC} = 0.16825	0.001 *
	Within populations	67	133.005	1.98515	71.91	F_{ST} = 0.28814	0.000 ***
	Total	**73**	**182.568**	**2.78868**			
Albertine wild and Victoria wild	Among groups	1	248.521	8.04479	80.02	F_{CT} = 0.80017	0.112 **ns**
	Among populations within groups	4	14.803	0.21978	2.19	F_{SC} = 0.10939	0.004 *
	Within populations	59	105.568	1.78929	17.80	F_{ST} = 0.82203	0.000 ***
	Total	**64**	**368.892**	**10.05386**			

Note : ns Non significant, * Significant values (P<0.05), **Significant values (P<0.001), ***Significant values (P<0.0001)

However, sub divisions among Victoria wild & fish farms group and Victoria wild & Albertine wild group did not contribute significantly to the observed genetic structure (Table 4). But, sub divisions among populations within Victoria wild and fish farms groups and within the populations were significant at F_{SC} = 0.16825, P<0.05 and F_{ST} = 0.28814, P<0.0001 respectively. In addition, the sub divisions among populations within Victoria and Albertine wild groups and within the populations were significant as well at F_{SC} = 0.10939, P<0.05 and F_{ST} = 0.82203, P<0.0001 respectively.

Table 4: Genetic heterogeneity pairwise F_{ST} among groups of wild and farmed *Clarias gariepinus* populations from Victoria and Albertine basins

	Lake Albert	Lake Edward	Lake George	Lake Victoria	Lake Wamala	River Rwizi	POCIFF	KFL	KFF	SIFFA
Lake Albert										
Lake Edward	0.07 **ns**									
Lake George	0.01 **ns**	-0.01 **ns**								
Lake Victoria	0.89 **ns**	0.87**	0.79***							
Lake Wamala	0.88***	0.85***	0.79***	0.44*						
River Rwizi	0.92*	0.87***	0.80***	0.77 **ns**	0.27***					
POCIFF	0.67***	0.76***	0.69***	0.27**	0.17**	0.23**				
KFL	0.93***	0.87***	0.80***	0.87**	0.17*	0.67**	0.23**			
KFF	0.70***	0.76***	0.70***	0.44*	0.30***	0.36**	0.02 **ns**	0.30***		
SIFFA	0.73*	0.79***	0.72***	0.54***	0.29***	0.43***	0.07*	0.27***	0.05 **ns**	

Notes: * P<0.05, **P<0.001, ***P<0.0001 and **ns** Not significant

Consistent with AMOVA distribution findings, pairwise F_{ST} values between different populations revealed heterogeneity except for Albertine wild (lakes Edward, Albert and George), some fish farms (Kireka Fish Farm) and some Victoria wild (River Rwizi and Lake Victoria) showed in Table 5. Computed pairwise F_{ST} was based on Weir and Cockerham (Weir & Cockerham, 1984). For instance between Albertine and Victoria wild, all populations revealed significant genetic differentiation except lakes Victoria and Albert that showed homogeneity i.e. absence of genetic differentiation. The smallest significant genetic differentiation between Albertine and Victoria wild was between Lake Albert and River Rwizi F_{ST} = 0.92, P<0.05. The highest were between lakes Edward and Wamala (F_{ST} = 0.85, P<0.0001), Lake Edward and River Rwizi (F_{ST} = 0.87, P<0.0001), lakes Albert and Wamala (F_{ST} = 0.88, P<0.0001), lakes George and Victoria (0.79, P<0.0001), lakes George and Wamala (F_{ST} = 0.79, P<0.0001) and River Rwizi and Lake George (F_{ST} = 0.80, P<0.0001). Within individual clusters of Victoria and Albertine wild, there was generally homogeneity as showed in Table 4, except for River Rwizi and Lake Wamala that revealed significant genetic differentiation (F_{ST} = 0.27, P<0.0001). This was consistent with AMOVA distribution among populations within groups showed in Table 3.

Pairwise F_{ST} values between farmed and wild groups revealed heterogeneity (see Table 4). This was consistent with AMOVA distribution (Table 3) especially for Albertine wild and fish farms. For instance the lowest significant genetic differentiation was between Salaama Integrated Fish Farm Association (SIFFA) and Lake Albert (F_{ST} = 0.73, P<0.05). Victoria

wild and fish farms revealed significant genetic differentiation, the smallest were between Kabeihura Farmers Limited (KFL) and Lake Wamala (F_{ST} = 0.17, P<0.005) and Kireka Fish Farm (KFF) and Lake Victoria. Within farmed fish cluster, some farms showed homogeneity including KFF and SIFFA; KFF and Pukure Orphan Care Integrated Fish Farm (POCIFF).

4.4 Population demographic history

Mismatch distribution of observed number pairwise differences between haplotypes of *C. gariepinus* from Albertine and Victoria basins are presented as those from fish farms (Figure 2), Victoria wild (Figure 3) and Albertine wild (Figure 4). Similarly, their corresponding neutrality tests including Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) of random sample of DNA sequence are provided in Table 6.

The total mismatch distribution of farmed fish revealed an observed multi-modal shape (Figure 2). Fish farms including Pukure Orphan Care Integrated Fish Farm (POCIFF), Kabeihura Farmers Limited (KFL), Salaama Integrated Fish Farm Association (SIFFA) and Kireka Fish Farm (KFF) had clearly observable multi-modal shaped distributions, suggesting stable populations. This was however inconsistent with non-significant overall fish farm neutrality tests (Tajima's D and Fu's Fs) showed in Table 6. Neutrality tests generally revealed that farmed *C. gariepinus* stocks recently expanded and exhibited heterogeneity of mutation rates among sites. For instance negative non-significant Tajima's D were obtained in KFL (D= -1.513) and SIFFA (D= -0.438) farms, perhaps showed that these populations had acquired an excess of new mutations and undergoing purifying selection.

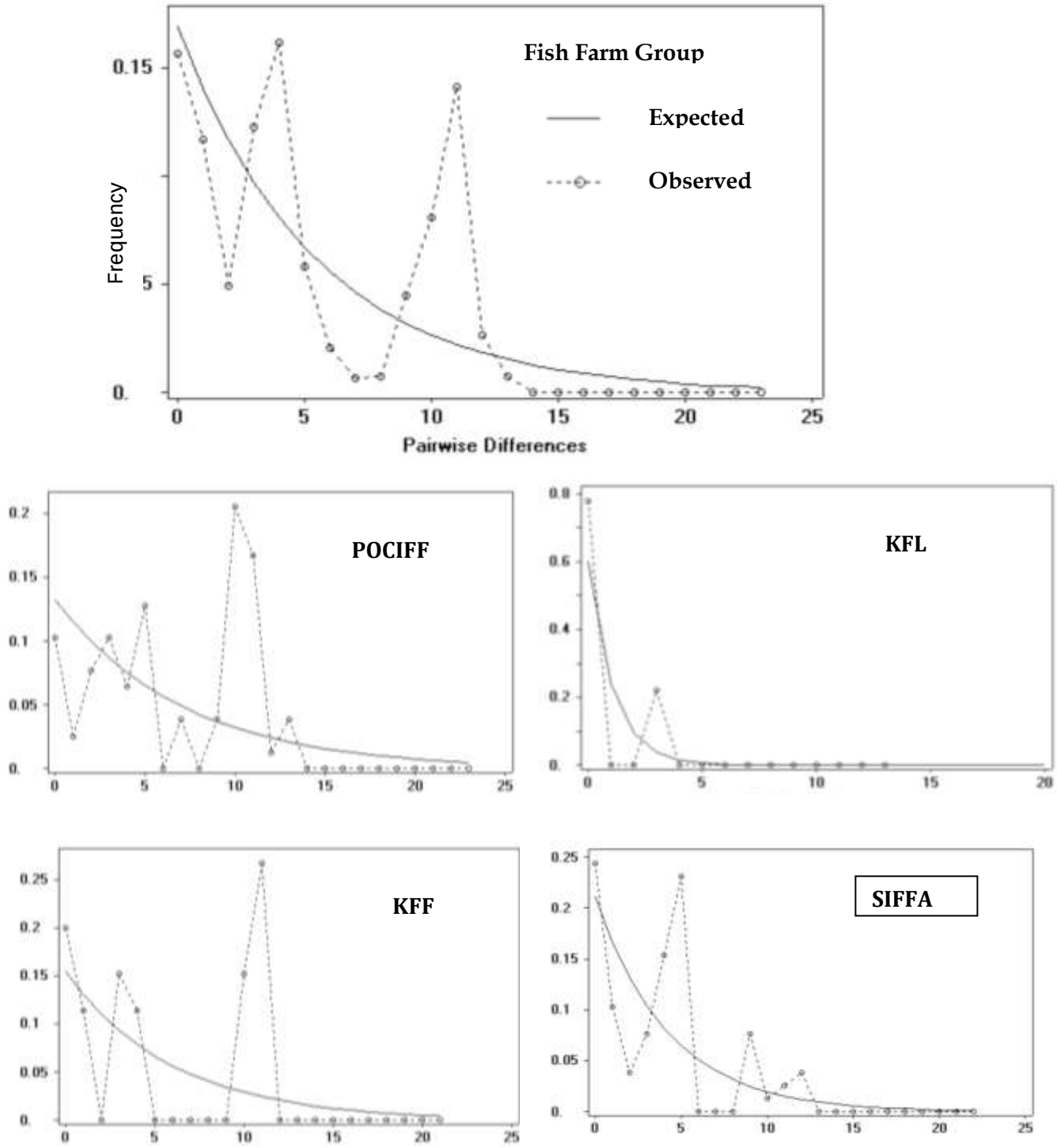


Figure 2: The mismatch distribution of individual *Clarias gariepinus* sequences control region from the fish farm group. Farms included; POCIFF = Pukure Orphan Care Integrated Fish Farm, KFL = Kabeihura Farmers Limited, KFF = Kireka Fish Farm, and SIFFA = Salaama Integrated Fish Farm Association. The plots were based on the constant population size change model.

The total mismatch distribution of Victoria wild was unimodal shaped as showed in Figure 3, suggesting recent demographic expansion. Similarly, Lake Wamala and River Rwizi observed graph revealed unimodal shapes. As for Lake Victoria, its mismatch distribution could not be plotted because of few samples obtained. Demographic expansion was consistent with overall non-significant Tajima's D (D= -1.121) and negative Fu's Fs (-0.546)

values showed in Table 6. Both the Rwizi and Wamala had negative Tajima's D suggesting purifying selection and an excess of new mutations.

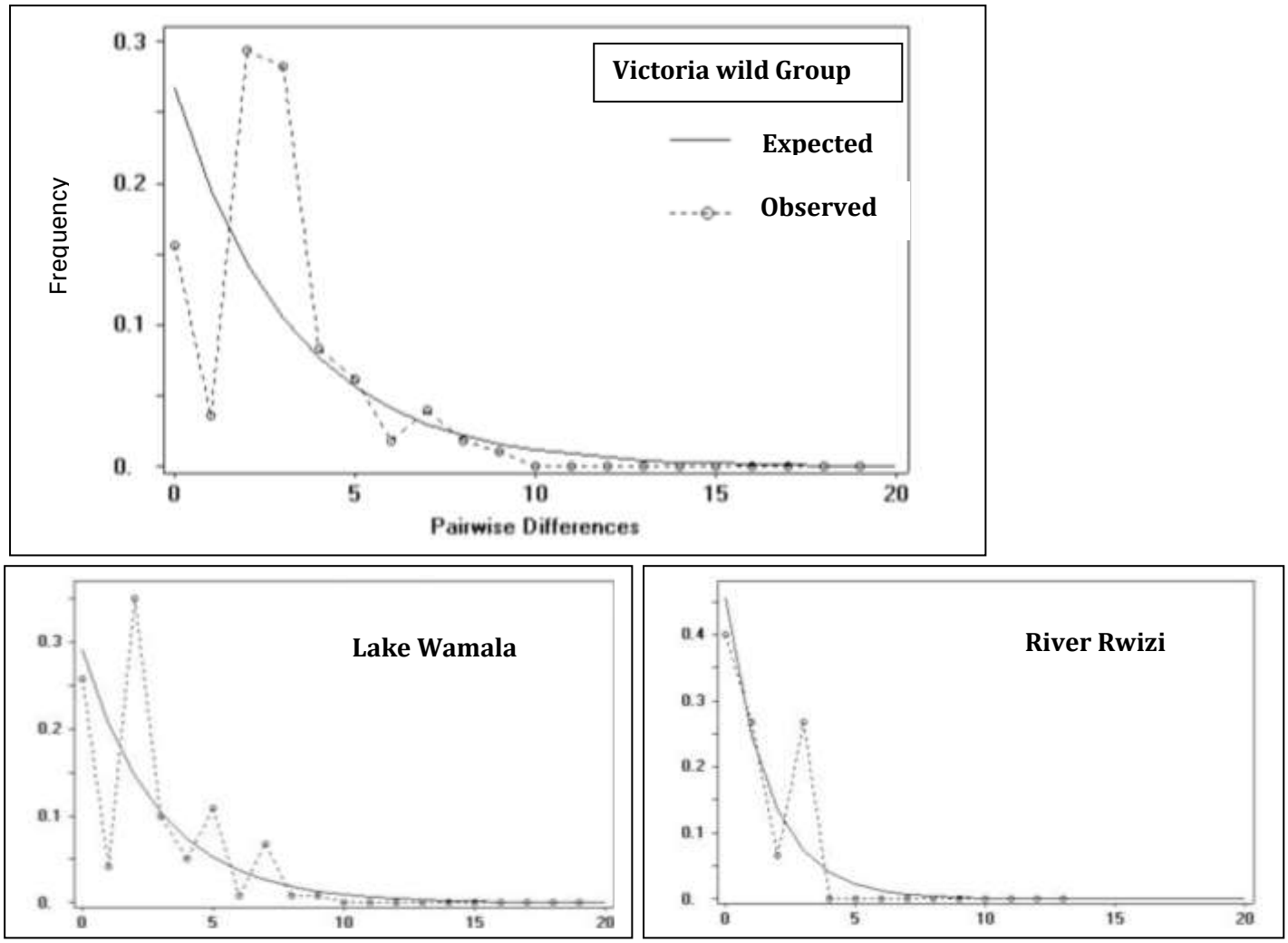


Figure 3: The mismatch distribution individual *C. gariepinus* sequences control region from Victoria wild group, which includes Lake Wamala and River Rwizi. Lake Victoria was excluded since its two sequences could not be plotted for mismatch distribution. The plots were based on the constant population size change model.

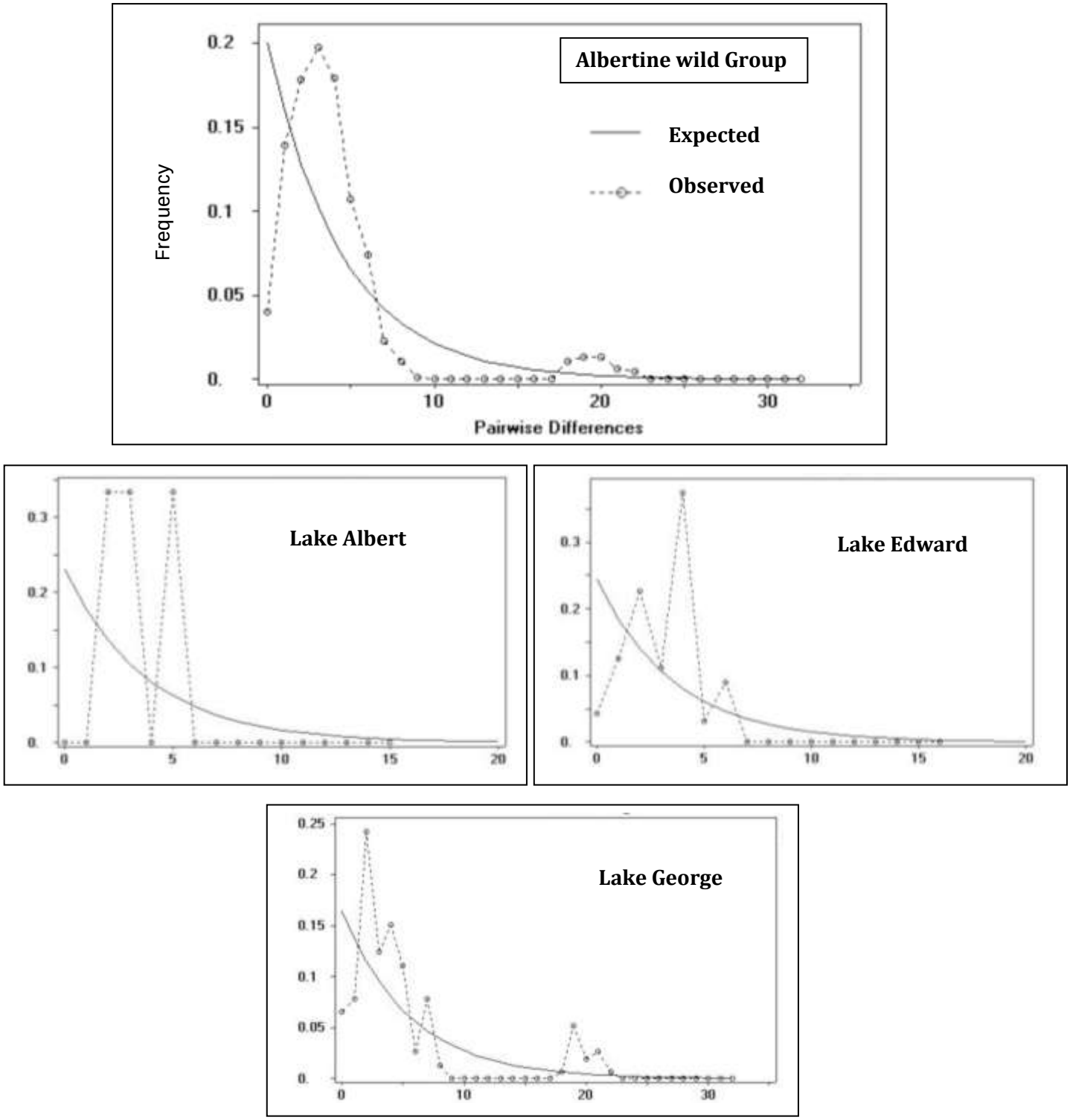


Figure 4: The mismatch distribution of individual *C. gariepinus* sequences control region from Albertine wild group, which includes Lake Albert, Lake Edward and Lake George. The plots were based on the constant population size change model.

Largely, Albertine wild revealed an observed multi-modal shaped mismatch distributions despite Lake Edward having a unimodal shape (Figure 4). Distinct multimodal shape were in lakes Albert and George suggesting that the populations had attained equilibrium and were

stable. Overall stability of Albertine wild populations was affirmed by significant Tajima's D value (D= -2.333, P<0.001) showed in Table 6. Significant Tajima's D values were also revealed in both lakes Edward and George, which were D= -1.916, P<0.05 and D= -2.082, P<0.05 respectively. The significant Tajima's D in Lake Edward was inconsistent with unimodal shaped mismatch distribution. This study could not obtain Tajima's D for Lake Albert because the samples were less than four.

Table 5: Demographic statistic for the combined 830bp dataset of Albertine wild, Victoria wild and fish farms. Statistics include Tajima's D and Fu's Fs neutrality tests. Asterisk indicates significant statistic test (* P<0.05; ** P<0.001) while **ns** denotes not significant

Populations	Total mutations	Tajima's D	Fu's Fs
Albertine wild			
Lake Albert			-9.111 **ns**
Lake Edward	22	-1.916*	-10.213 **ns**
Lake George	36	-2.082*	-6.019 **ns**
Overall	**50**	**-2.333****	**-25.107 ns**
Victoria wild			
Lake Victoria			
Lake Wamala	13	-1.453 **ns**	-0.152 **ns**
River Rwizi	3	-0.447 **ns**	0.012 **ns**
Overall	**15**	**-1.121 ns**	**-0.546 ns**
Fish farms			
POCIFF	17	0.817 **ns**	0.996 **ns**
KFL	3	-1.513 **ns**	1.318 **ns**
KFF	13	1.463 **ns**	4.915 **ns**
SIFFA	13	-0.438 **ns**	1.567 **ns**
Overall	**20**	**0.033 ns**	**1.093 ns**

4.5 Phylogenetic analysis

Phylogenetics analysis was used to test hypothesis of evolutionary clustering in wild and farmed *C. gariepinus* in both Albertine and Victoria basins. The analysis revealed two clades including Albertine basin and Victoria basin as shown in mid-point rooted phylogenic tree (Figure 5). Relations in both clades were paraphyletic basing on bootstrapping values and red to blue colour gradient. The Victoria clade constituted of haplotypes largely from Victoria wild (lakes Wamala and Victoria, and River Rwizi) and fish farm (POCIFF, KFL, KFF, and SIFFA) and to a small extent one from Lake George. It revealed close relatedness of *C. gariepinus* in Victoria wild, fish farms and Lake George. Albertine clade constituted purely of Albertine wild (lakes Albert, Edward and George) haplotypes, showing their close relationships.

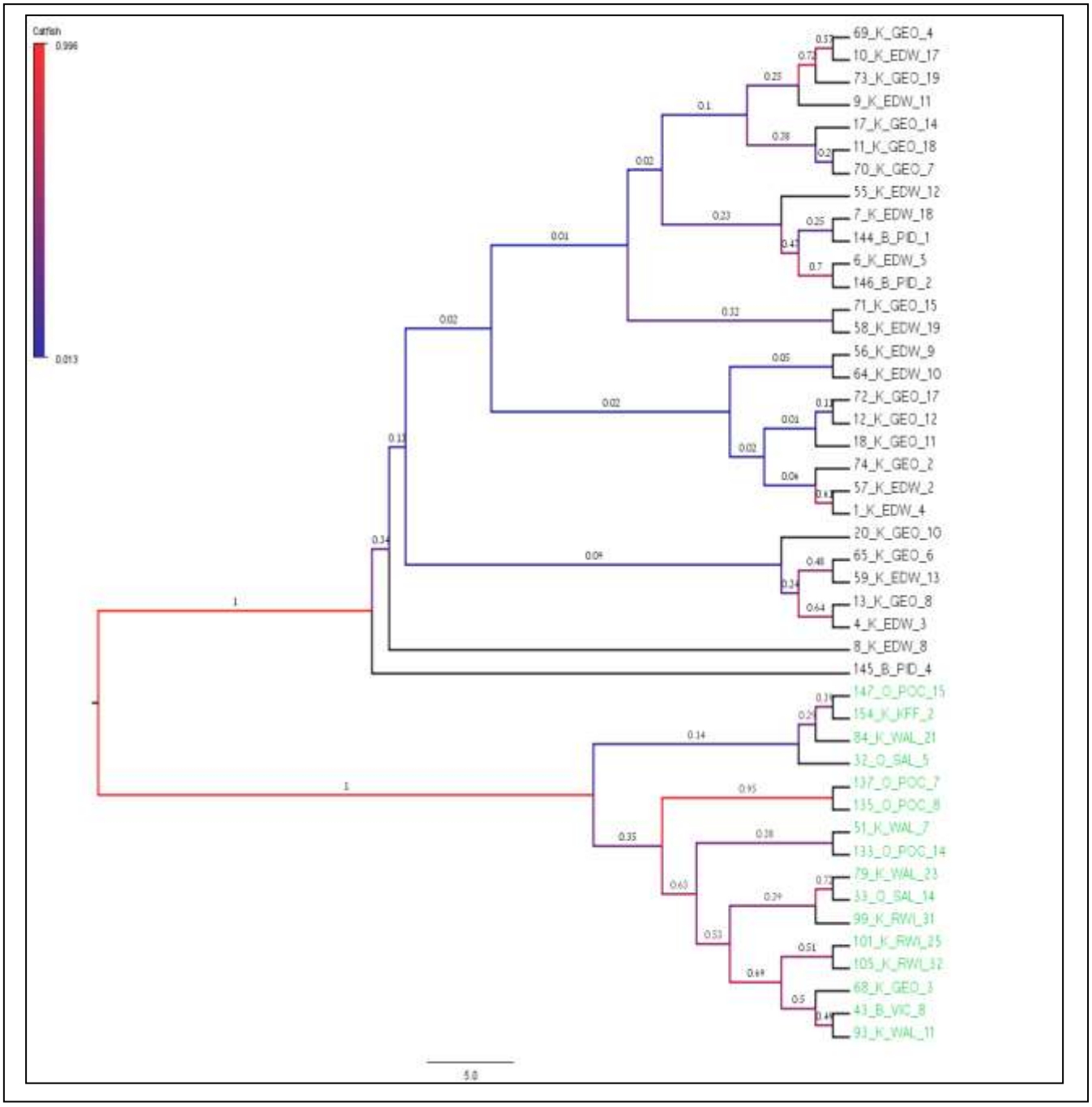


Figure 5: Maximum Likelihood tree based on Hasegawa-Kishino-Yano model for ten populations, grouped into two clades including the Albertine basin (K-GEO, K-EDW, and B-PID) and Victoria basin (B-VIC, K-RWI, K-WAL, O-POC, R-KFL, O-SAL and K-KFF). The numbers present in the branches are in decimals corresponding to bootstrap values based on 1000 replications. Similarly, the strength of bootstrap values are presented by gradient red to blue colours, where the highest is red and lowest blue. Clarias gariepinus in the Albertine and Victoria drainage basins

CHAPTER FIVE

5.0 DISCUSSION

5.1 Genetic diversity of *Clarias gariepinus* in the Albertine and Victoria drainage basins

Fragments of mtDNA control region 830bp of *Clarias gariepinus* from Albertine and Victoria basins revealed large amount (68) of polymorphic site. These are associated with high genetic variability. Studies in other fish species have linked the number of polymorphic sites to high genetic variability including Siruformes *Parauchenipterus galeatus* in Brazil (Lui, *et al.*, 2012) and Brown trout *Salmo trutta* in Europe (Bernatchez, et al., 1992). In this study, precise variability indices such as haplotype and nucleotide diversities and Theta S and Pi, revealed generally high genetic diversity in both farmed and wild populations.

Highest haplotype diversity was in the Albertine wild populations, explained by *C. gariepinus* life history, presence of elaborate waterways that interconnect lakes and rivers and events of the late Pleistocene. In addition, the Albertine wild had high haplotype and low nucleotide diversities, a pattern consistent with study on freshwater *Barbus* species (Muwanika, *et al.*, 2012) and marine *Sebastes* species (An, *et al.*, 2011). This pattern signals a recent radiation amongst Albertine wild populations. *Clarias gariepinus* is highly fecund (Sheasby, 2009), air breathing (Teugels, 1996) and migratory (Gunder, 2004), therefore it disperses easily in waterways. For instance, studies on Kazinga channel have showed it as a corridor that facilitates movement fish species between lakes Edward and George (ARCOS, 2012; Muwanika, *et al.*, 2012) and associated to reducing genetic variability (Roodt-Wilding, *et al.*, 2010). However, the high haplotype diversity in these lakes could be a case of adaptation that is reported to reduce gene flow between habitats by eliminating poorly adapted immigrants (Andrew, *et al.*, 2012). Unlike the Kazinga, River Semliki linking lakes Edward and Albert has rapids along its course (Devaere, *et al.*, 2007), a barrier that isolate populations. Still the genetic variability in both lakes Edward and Albert is high perhaps because they represent large ancestral populations which provided founder populations for re-colonization other waterways following climatic events of the late Pleistocene (Muwanika, *et al.*, 2012). One other explanation is that climate shaped migration patterns of fauna in the Pleistocene creating similar gradients in both lakes Albert and Edward.

Apart from Kabeihura Farmers Limited (KFL), highest nucleotide diversity in the study was observed in fish farms. Muwanika (2012) attributes high nucleotide diversity to persistence of ancestral strains within populations. Natural water bodies and other farms are the source of

ancestral or founding fish population on fish farms. The founding populations were subjected to series of selective breeding (Hogendoorn, 1979) to establish standing viable stocks. Established stocks could attain equilibrium maintaining old and adopting new mutations hence the high nucleotide diversity. Evidence supporting attainment of equilibrium was the multimodal shaped distribution curves revealed in all fish farms except KFL. Kabeihura Farmers Limited (KFL) farmed *C. gariepinus* expressed particularly a lower nucleotide and haplotype diversities than other fish farms and natural water bodies. It indicated that the population had gone through a bottleneck effect, resulting into loss in genetic diversity. Fish farming practices such as inherent massive selective breeding (Bilio, 2007; Araki, *et al.*, 2008; Christie, *et al.*, 2012) and maintenance of small economical and effective breeding stocks (Aulanier, *et al.*, 2011) could be responsible for loss in diversity. Studies on fish genetic diversities of farmed flounder (Shikano, *et al.*, 2008) and rock fish (An, *et al.*, 2011) revealed lower genetic diversity than in wild fish that was linked to farm practices. Therefore, the KFL farm practices could have trigger the loss of genetic diversity among *C. gariepinus*, consequently contributing to low growth rates among fish cited in increasing cost of producing farmed fish.

5.2 Assessment of genetic differentiation *C. gariepinus* among populations

Phylogenetic analysis (Figure 5) detected two genetic clusters of *C. gariepinus* populations in the Ugandan Albertine and Victoria drainage basins, including Albertine basin and Victoria basin clades. The Albertine basin clade constituted purely Albertine wild populations such as lakes Edward, George and Albert. Whereas the Victoria basin clade constituted both Victoria wild (Lake Wamala and River Rwizi) and fish farm (POCIF, KFL, SIFFA and KFF) populations. Based on hierarchical F-statistics analysis (Table 4), the levels of genetic differentiation varied between these two clusters. For instance while Victoria and Albertine basin clades were genetically distinct, some populations (Victoria wild and Albertine wild) within clades shared large amount of genetic information. The findings provide further evidence of the events of the late Pleistocene. During this period, endemic Victorian fauna was wiped out and some extant taxa forced into refugia into low laying Albertine basin (Karp, *et al.*, 2004). The extant fauna formed the basis of founder population that re-colonized Victoria basin. Similarly, this finding was consistent with results from studies on other fish species (Stager & Johnson, 2007; Muwanika, *et al.*, 2012), that revealed past linkage between Albertine and Victoria basins.

Wild sources of farmed C. gariepinus in the Victoria and Albertine basins

Albertine wild and fish farms revealed significant genetic differentiation (Table 4) indicative of none existent connectivity. Similarly, existence of pair wise (F_{ST}) genetic heterogeneity between populations in Albertine wild and fish farms affirmed isolation (see Table 5). The high level of the observed *C. gariepinus* isolation in Albertine wild and fish farms could be a function of the genesis of seed production around Lake Victoria crescent in Uganda dating back to the 1960s (FAO, 2005) and decline in relative fitness of farmed fish stocks (Araki, *et al.*, 2008; Christie, *et al.*, 2012). Indeed, for the former function, breeding of catfish and other fish species for development of aquaculture first started in a Government Aquaculture Research and Development Center (ARDC) center located within Lake Victoria crescent at Kajjansi-Wakiso district in Uganda. Farmed catfish seed was propagated artificially by simulating natural cues (Hogendoorn, 1979) since it does not naturally propagate in captivity. Therefore, parent *C. gariepinus* were perhaps not obtained from Albertine wild, i.e., lakes Albert, Edward and George, rather obtained from Victoria wild (see evidence in Table 4 and Figure 5). The ARDC distributed catfish seed to various farmers within the country until recently when private commercial fish hatcheries developed (Ssebisubi, 2010; Aulanier, *et al.*, 2011; Dalsgaard, *et al.*, 2012).

Distribution of farmed C. gariepinus within the Victoria drainage basin

While AMOVA distribution generally revealed no significant genetic differentiation between Victoria wild and fish farms (Table 4). This finding provided for more evidence about the origin of *C. gariepinus* having originated from the Victoria basin. However, heterogeneity was observed between individual natural water bodies within Victoria basin and fish farms (Table 5). For instance, smallest genetic differentiation F_{ST} P-values observed between Kabeihura Farmers Limited (KFL) and Lake Wamala; Kireka Fish Farm (KFF) and Lake Victoria, could be attributed to existence of minimal gene flow and adaptation to captivity environments. This finding was congruent with comparative studies conducted on genetic differentiation of wild and domesticated salmonids (Araki, *et al.*, 2008; Christie, *et al.*, 2012), that revealed existence of heterogeneity because the later had substantially lower fitness. The cause of reduced fitness in farmed fish stocks has remained elusive, though Araki, *et al.*, *(*2008*)* associated it to relaxed natural selection, environmental effects of captive rearing and inbreeding among close relatives.

Fish farms and lakes and rivers within the Victoria basin had minimal gene flow, facilitated by fish escape from farms (Roodt-Wilding, *et al.*, 2010) and broodstock sourced from a range

of lakes (Dalsgaard, *et al.*, 2012). However, in this study, heterogeneity persisted between farms and lakes, probably because fish in captivity had elevated frequencies of adaptations mutations favoring captive than natural environments. Such adaptations included high fecundity, high growth rates, suppressed resistance to diseases and low predator protection ability.

Homogeneity was also observed between some fish farms including Kireka Fish Farm (KFF) and Salaama Integrated Fish Farm Association (SIFFA); KFF and Pukure Orphan Care Integrated Fish Farm (POCIFF), indicative of farm as source for broodstock. Obtaining broodstock from farms has its merits such as increased control of diseases, fish are free from injuries associated with fishing (in the wild), sufficient quantities with less size disparities and traceability amongst others. In addition, economic risks are minimal when fish are sourced from other farms. However, genetic pedigree of farmed broodstock is often compromised and studies have reported farmed fish as being being inferior to wild counterparts (Wang, *et al.*, 2002; Blonk, *et al.*, 2009; Christie, *et al.*, 2012). Farms offer fish protection from predators and diseases and ensure availability food in turn under-developing mutations for innate defense against diseases and predators. Lack of developed intrinsic defense mutations make fish more susceptible to predators and disease, often under-performing in poorly managed farms. Sourcing broodstock from farms could be contributing to chronic slow growth rates frequently reported in Uganda's fish seed industry (Ssebisubi, 2010; Aulanier, *et al.*, 2011; Dalsgaard, *et al.*, 2012; DFR, 2012). Poor seed quality could therefore be partly linked to breeding practices in fish hatcheries (Blonk, *et al.*, 2009) and inadequate management by grow out farmers (Aulanier, *et al.*, 2011). Since grow out fish farms are modestly managed, hatcheries should improve quality of seed through regularly supplement the quality of broodstock by sourcing from the wild.

5.3 Examination of the evolutionary history of *C. gariepinus* in the Albertine and Victoria Drainage Basins

Mitochondria DNA control region marker was used to provide inference of the spatial distribution of *C. gariepinus* lineages across the Victoria and Albertine basins. The control region evolve faster than nuclear markers (Moritz, *et al.*, 1987). Rosetti and Remis (2012) suggests that markers with faster evolution likely enhances recent demographic events and changes, which could have saturated over long time periods. The control region haplotypes from *C. gariepinus* were used to analyze the demographic history. Demographic history was presented in mismatch distributions of observed pairwise differences between *C. gariepinus*

haplotypes and neutrality tests from Albertine and Victoria basins. Mismatch graphs were analyzed in three groups based on being farmed and wild namely; Albertine wild, Victoria wild and fish farms.

Overall mismatch distribution graphs showed multi-modal shape for fish farms (Figure 2) and Albertine wild (Figure 4), except Victoria wild (Figure 3) that was unimodal. Both fish farms and Albertine wild *C. gariepinus* populations attained demographic equilibria. Reference to demographic equilibrium theory, Walter and Hengeveld (2000) proposed that attainment of stability in populations was largely due to biotic processes and resource availability than ecological and evolutionary influences. In as much as biotic interactions drive much of the local-scale population stability, abiotic factors such as tectonic and climatic processes at time scales above 10^5 year could overwhelm these interactions (Benton, 2009). Unlike in fish farms, Albertine wild fish populations experienced historical climatic oscillations (Maslin & Trauth, 2006) and tectonic processes (Baker & Wohlenberg, 1971; Hay, *et al.*, 2002). These abiotic processes could have substantially altered local-scale adaptability of Albertine wild. Indeed overall, negative significant Tajima's D value observed in Albertine wild (see Table 6) was indicative of recent population size expansion after undergoing a bottleneck (Tajima, 1989). Existing heterogeneity within Albertine wild population had excess low frequency polymorphisms relative to expectation during abiotic processes. The population could have retained few old mutations in the face of an influx of immigrants hence undergoing purifying selection. Events of the Pleistocene forced Victorian faunal species into low laying Albertine wild (see Figure 1). Signature of the resultant gene flow is reflected in individual Albertine wild lakes including George and Edward similarly had negative significant Tajima's D values pointing to a recent purifying selection and population expansion. Elaborate water pathways within the Albertine basin linking to the Victoria basin could be reason for the expansion.

However, overall Fish farm Tajima's D value (Table 6) was positive and not significant linked to balancing selection within the population (Tajima, 1993), hence signaling stability. Fish farms' stability could be linked to stocking using genetically diverse fish increasing the pool of multiple alleles present. In addition, positive Tajima's D value could indicate sudden stock contraction that results in both low levels of both high and low frequency polymorphisms in the population (Tajima, 1989). Indeed commercial fish practices such as regular replenishing breeding stock, selective propagation, and sale of market size fish, clearly justify observed fish stock contraction. Studies on other domesticated fish species congruent with this finding, include Rock fish (An, *et al.*, 2011) and Brown trout (Ryman &

Ståhl, 1980) have revealed maintenance of considerable variation which was associated with number of broodstock used to produce stock. In addition, positive Tajima's D could indicate old mutations out-performing new mutations and maintaining stability of stocks

Kabeihura fish farm mismatch distribution graph for *C. gariepinus*, revealed unimodally shaped graph and negative not significant Tajima's D value. This suggested that Kabeihura had a small effective breeding population size (Santiago & Caballero, 1998) and high levels of farm to farm broodstock exchange (Excoffier, 2004; Aulanier, *et al.*, 2011). Incidences of farm-to-farm broodstock exchange have less beneficial for production of quality seed, because fish in captivity have reduced fitness (Christie, *et al.*, 2012). Consequently, they are prone maladaptation in more natural environments such as poorly managed growout earthen fishponds. Seed input users (growout farmers) are poor fish farm manager, leaving their farms to operate as those they in the wild. Grow out farmers rarely constitute a supplementary feeding regime, water quality monitoring system and control cannibalism (through regular fish grading). Management of *C. gariepinus* hatcheries in Uganda, particularly Kabeihura should consider infusion of new entrants from the wild particularly the Albertine lakes to improve broodstock heterogeneity, hence improving seed quality.

General Victoria wild unimodal distribution and not significant Tajima's D value excludes Lake Victoria. The results suggest probable recent population expansion in Victoria basin. This is consistent with other studies (Maslin & Trauth, 2006; Muwanika, *et al.*, 2012)and Pleistocene events (Scholz, *et al.*, 2007), fauna in basin expanded. Extensive network of waterways within basin could have facilitated expansion. For instance, Lake Wamala is linked to River Katonga system that spans from Albertine basin and possibly connects to Victoria basin. The Rwizi River on the other hand is connected to satellite lakes within the Victoria basin.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1Conclusion

In this study, populations were grouped based on the iIn this study, populations were grouped based on the individuals being either wild or domesticated. This study had Albertine wild (lakes Albert, Edward and George) Victoria wild (lakes Victoria and Wamala and River Rwizi) and Fish Farms (Pukure Orphan Care Integrated Fish Farm (POCIFF), Kabeihura Farmers Limited (KFL), Salaama Integrated Fish Farm Association (SIFFA) and Kireka Fish Farm (KFF)). The 830bp of mtDNA control region of *Clarias gariepinus* revealed 68 polymorphic sites defining 45 haplotypes, were used for analyses. Precisely, diversity indices measured were haplotype (h) and nucleotide ((π)) diversities and Theta S and Pi, revealed generally high genetic diversity in both farmed and wild populations. Albertine wild had high haplotype and low nucleotide diversities, a pattern which signals a recent radiation amongst Albertine wild populations. Apart from Kabeihura Farmers Limited (KFL), highest nucleotide diversity in the study was observed in fish farm, attributes to persistence of ancestral strains within populations since broodstock is sourced from ancestral farms and lakes. Victoria wild had relatively high genetic diversity in comparison to the other population groupings because they are founder populations following events of Pleistocene and were used to develop fish farming in Uganda.

Phylogenetic analysis (Figure 5) detected two genetic clusters of *C. gariepinus* populations in the Ugandan Albertine and Victoria drainage basins, including Albertine basin and Victoria basin clades. The Albertine basin clade constituted purely Albertine wild whereas the Victoria clade constituted Victoria wild and all fish farms. Based on hierarchical F-statistics analysis, the levels of genetic differentiation varied between these two clades. Despite being genetically distinct, individual lakes and farms shared large genetic information. For instance genetic differentiation among Albert wild and Victoria wild groups was not significant, revealed a shared past. Similarly Victoria wild and fish farms was not significant, which showed that fish farms sourced broodstock from Victoria basin. For pairwise F_{ST} values between Albert and Victoria was not significant neither.

Albertine wild, and fish farms overall multimodal mismatch distribution plots, signaling stability of populations. However, significant Tajima's D values for the Albertine wild were consistent with stability. Whereas negative Tajima's D could explain fish farms stability

values indicative of purifying selection. It could also show the population effective sizes on farms and exchange of broodstock for seed production. These practices could be causing poor quality seed production due to loss of fitness. Victoria wild-exhibited overall unimodal mismatch distribution plot and Tajima's D was not significant revealing a recent population expansion. The Victoria lakes are source of broodstock and have an elaborate waterway that communicates with farms and Albertine.

6.2 Recommendations

Farmers have not given attention to source of broodstock and have for long obtained it from other farms and from the natural water bodies. From this study, results show that fish obtained from other farms and the wild whose genetic pedigree is unknown could potentially affect seed quality. It would be contribute greatly to seed quality if hatchery operators establish genetic pedigree of broodstock from farm and wild before use.

Obtaining broodstock from natural water bodies has been showed in this study to increase fitness of farmed fish. In as much as other farms are sources of economical and disease free broodstock, it should be noted that after generations of use on the source farm, it reduces in fitness. It is therefore recommended that farmers obtain broodstock from the wild one in a while.

Not all is conclusive; further study employing molecular markers such as microsatellites (microsats) and more recent single nucleotide polymorphisms (SNPs) is required to explore the extent of inbreeding in domesticated fish populations. Challenges in the fish farming industry could be addressed once functional genes are identified affecting production.

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