

MAKERERE



UNIVERSITY

**EVOLUTIONARY HISTORY OF THE NILE PERCH IN ITS NATIVE
HABITAT - THE NILOTIC SYSTEM VERSUS ITS NEW HAVEN – THE
LAKE VICTORIA REGION WATERS**

BY

MWANJA TENYWA MATTHEW

BSc, P.G.D.E, MSc (MAK)

**A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE
TRAINING FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY OF
MAKERERE UNIVERSITY**

OCTOBER 2013

DECLARATION

This study is original and has not been submitted for any other degree award to any other University before.

Signature

Date

Mwanja Tenywa Matthew

APPROVAL

This thesis/dissertation has been submitted for examination with the approval of the following supervisors:

Signature

Dr. Vincent Muwanika,

BSc., MSc., PhD

Date

Signature

Assoc. Prof. Dr. Silvester Nyakaana

BSc. Dip Ed., MSc., PhD

Date



ABSTRACT

Nile perch is an important food fish species of the genus *Lates*, belonging to family Latidae. It widely occurs in the Afro-tropical region in Africa, and in East Africa it is native to Lakes Albert and Turkana and River Albert Nile. It was introduced to lakes Kyoga, Nabugabo and Victoria where it successfully established itself. Nile perch became the leading fishery, bolstering economies of Uganda, Kenya and Tanzania, countries that are engaged in Nile perch processing and export, to the value of US \$250m annually. But recently Nile perch stocks have been reported to be on the decline. Characterisation of Nile perch populations of E. Africa and understanding the species' evolutionary history are important for both capture fisheries management and culturing efforts of Nile perch. Earlier studies were mostly based on morphological characters and were all less or not definitive. This study combined morphological and molecular characterisation to establish the phylogenetics, genetics and evolutionary history of Nile perch sampled from lakes – Albert, Kyoga, Nabugabo, Victoria (Uganda), Turkana (Kenya) and Chamo (Ethiopia) and Senegal River (Senegal). The aims of this study were to determine phyletic and phylogeographic relationships among populations of Nile perch in its extant range in E. Africa and to establish the species evolutionary history on the African continent. Morphological phylogenetics of E. African Nile perch revealed existence 2 morphotypes. Analysis of genetic variation among populations in E. Africa, based on 9 polymorphic microsatellite loci and partial sequence (463bp) of mtDNA showed that introduced Nile perch had higher genetic diversity than populations from the putative native origins. In addition, findings revealed that all sampled water bodies had 2 underlying populations of Nile perch, which most probably represent 2 different subspecies. Evolutionary history studied using mtDNA CR partial sequence (476bp) revealed 37 distinct haplotypes in 124 samples. The haplotypes clustered into 2 major groups, one composed of individuals sampled from eastern and the other from western Africa. The 2 most probably belong to 2 different Nile perch lineages that may have developed in geographical isolation during the Pleistocene times and have remained largely allopatric. The 2 groupings should be considered as different ESUs since depletion of individuals from one lineage cannot easily be replenished by natural migration by the other. Therefore, the ESUs should be conserved as such and genetic exchange due to translocations should never be allowed

DEDICATION

On his sick bed in hospital when we were little boys just 6 years old, our father while holding our hands firmly asked God to keep him alive to see his twin boys Wilson and Matthew through their education, which the Almighty granted, as 17 years later we graduated with bachelors. But he was not given the chance to see his twin doctoral graduands! For his love and commitment to our education and investment therein I dedicate this work to my parents – Isabirye ni Nabirye

Mwanja and all Glory I give to God!

TABLE OF CONTENTS

DECLARATION	ii
ABSTRACT	iv
DEDICATION	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES	xi
LIST OF FIGURES.....	xiii
LIST OF ABBREVIATIONS	xv
ACKNOWLEDGEMENTS	xviii
CHAPTER ONE.....	1
1.0 General introduction.....	1
1.1 Background.....	1
1.2 Statement of the problem.....	2
1.3 General Objective/Purpose	3
1.4 Specific Objectives.....	4
1.5 Major findings of this study.....	4
1.6 Applicability of research findings	5
1.7 Suggestions for future research	6
1.8 Thesis organisation.....	7
1.9 Brief description of each of the Chapters:.....	7
1.10 References	11
CHAPTER TWO	16
<i>The Evolutionary history, ecology, socioeconomics, consequences of introductions into new environs and the management challenge of the Nile perch (Lates spp. Linnaeus 1758) of East Africa</i>	<i>16</i>
2.0 Abstract.....	16
2.1 Introduction.....	17

2.2 Socioeconomics of the Nile perch in LVR.....	19
2.3 Ecology and ecological genetics of the Nile perch.....	21
2.4 Evolutionary history, fossil record and taxonomic status of the Nile perch.....	23
2.5 Can a balance be struck between fish biodiversity conservation and Nile perch harvests that make economic sense in LVR?	24
2.6 Conclusions	26
2.7 Acknowledgements.....	27
2.8 References	28
CHAPTER THREE.....	33
<i>Population Morphological Variation of the Nile perch (Lates niloticus, L. 1758), of East African Lakes and their associated waters</i>	33
3.0 Abstract.....	33
3.1 Introduction	34
3.2 Study area, materials and methods.....	35
3.2.1 Study area	35
3.2.2 Sample collection.....	36
3.3 Biometric measurements and data analysis	41
3.4 Results.....	42
3.5 Discussion	47
3.6 Acknowledgements.....	49
3.7 References	50
CHAPTER FOUR	54
<i>Microsatellite DNA analyses reveal population subdivision among the recently introduced Nile perch (Lates spp. L.1758) of Lake Victoria</i>	54
4.0 Abstract.....	54
4.1 Introduction	55
4.2 Study area, materials and methods.....	57

4.2.1 Study area	57
4.2.2 Sampling.....	59
4.2.3 DNA extraction.....	59
4.2.4 Microsatellite DNA Analysis	59
4.3 Data Analysis.....	60
4.3.1 Genetic diversity.	60
4.3.2 Genetic structure.	60
4.4 Results.....	61
4.4.1 Microsatellite DNA polymorphisms	61
4.4.2 Genetic diversity	63
4.4.3 Population structure.	70
4.5 Discussion	76
4.5.1 Genetic diversity	76
4.5.2 Population structure.....	76
4.6 Implications for fisheries management	78
4.7 Acknowledgements.....	78
4.8 References	80
CHAPTER FIVE.....	87
<i>Genetic evidence of successful establishment of the Nile perch (Lates spp. L.) in East African lakes and implications for management</i>	<i>87</i>
5.0 Abstract.....	87
5.1 Introduction	88
5.2 Materials, methods and study area	89
5.2.1 Study area	89
5.2.2 Sample collection and DNA extraction.....	90

5.2.3 Mitochondrial DNA Sequence Analysis.....	90
5.2.4 Microsatellite M Analysis.....	91
5.3 Data Analyses.....	93
5.3.1 Mitochondrial DNA.....	93
5.3.2 Microsatellites	93
5.4 Results.....	94
5.4.1 Mitochondrial DNA Sequence Analyses	94
5.4.2 Microsatellite Marker Analyses	97
5.4.3 Demographic Changes	101
5.5 Discussion	108
5.5.1 Genetic diversity and genetic differentiation	108
5.5.3 Demographic changes.....	109
5.6 Implications to Fisheries Management	110
5.7 Acknowledgements.....	111
5.8 References	112
CHAPTER SIX.....	122
<i>Evolutionary History of Nile Perch Lates sp. Inferred from Mitochondrial DNA Variation Analyses</i>	122
6.0 Abstract.....	122
6.1 Introduction	123
6.2 Materials and Methods.....	125
6.2.1 Sample collection and DNA extraction.....	125
6.2.2 DNA amplification and Sequencing.....	125
6.2.3 D-loop Sequence Analyses.....	127
6.2.4 Nucleotide diversity, haplotype diversity and population differentiation	127
6.2.5 Genetic diversity and phylogenetics analyses	127

6.2.6 Mismatch analysis and demographic history	128
6.3 Results.....	129
6.3.1 Nucleotide diversity, haplotype diversity and population differentiation	129
6.3.2 Geographical distribution of Nile perch lineages, Hierarchical Genetic Diversity and Phylogenetics analyses	130
6.3.3 Mismatch Analysis and Demographic history	140
6.4 Discussion	142
6.4.1 Nucleotide diversity, haplotype diversity and population differentiation	142
6.4.2 Phylogenetics relationships among Nile perch populations	142
6.4.3 Mismatch analysis and demographic history	143
6.4.4 Taxonomic status of the Nile perch.....	144
6.5 Conclusions	144
6.6 Acknowledgements.....	145
6.7 References	146
CHAPTER SEVEN.....	151
7.0 General Discussion.....	151
7.1 Conclusions and Recommendations.....	153
7.2 References	155
APPENDIX (Published papers)	Error! Bookmark not defined.

LIST OF TABLES

TABLE 2.1. Sampling sites, number of fish collected from each site, eco-zone of the sample site, and depth at the start and end of each fishing trawl.....	38
TABLE 2.2. Eigen values and distribution of variance among different components.....	45
TABLE 3.1. Primers used to amplify the nine microsatellite loci in Nile perch and a number of alleles per locus for the sister species <i>Lates calcarifer</i>	62
TABLE 3.2. Genetic variability indices for nine microsatellite loci of the Nile perch collected from 22 different geographical locations within L. Victoria.....	64
TABLE 3.3. Summary descriptive statistics – microsatellite data for the samples collected from the different geographical locations of L. Victoria.....	71
TABLE 3.4. AMOVA by distance method using number different of alleles (F_{ST}) for the Nile perch microsatellite data.....	72
TABLE 4.1. Primers used to amplify the nine microsatellite loci in Nile perch and number of alleles per locus for the sister species <i>Lates calcarifer</i>	92
TABLE 4.2. List of 35 polymorphic sites of 27 mtDNA D-loop Nile perch haplotypes.....	96
TABLE 4.3. Mitochondrial DNA (mtDNA) sequence variability data in control region (CR) for the Nile perch of East Africa.....	98
TABLE 4.4. Summary descriptive statistics – microsatellite data for the samples from the different lakes of East Africa.....	99
TABLE 4.5. Assessment of bottleneck effects at nine microsatellite loci in 27 Nile perch populations collected from five East African lakes.....	100

TABLE 4.6. Analysis of genetic differentiation among the different populations of the different lakes of East Africa.....	102
TABLE 4.7. Pairwise Φ_{ST} values computed from haplotype frequencies for the mtDNA pooled data.....	103
TABLE 4.8. AMOVA by distance method Tamura & Nei (1993) distances for the Nile perch sequence data across lakes.....	104
TABLE 4.9. Pairwise F_{ST} values by distance method using number of different alleles for the microsatellite pooled data.....	105
TABLE 4.10. Summary statistics for genetic variation of the Nile perch at nine microsatellite Loci in five East African lakes.....	106
TABLE 4.11. AMOVA by distance method using number of alleles (F_{ST}) for the Nile perch microsatellite data.....	107
TABLE 5.1. List of 35 polymorphic sites of 37 mtDNA D-loop Nile perch haplotypes.....	135
TABLE 5.2. Nile perch population statistics.....	136
TABLE 5.3. Observed 95% confidence intervals of simulated number of polymorphic sites S , demographic parameters (95% CI) among Nile perch lineages.....	137
TABLE 5.4. Analysis of molecular variance in Nile perch using the 7 sampled water bodies populations as the top level grouping.....	138
TABLE 5.5. mtDNA sequence characteristics of the two Nile perch lineages.....	139

LIST OF FIGURES

FIGURE 2.1. Map showing the East African lakes from which Nile perch was sampled.....	37
FIGURE 2.2. Map of Lake Victoria showing the sites from where Nile perch samples were collected.....	39
FIGURE 2.3. Nile perch photo showing morphological characters taken for biometric measurements.....	40
FIGURE 2.4. A graph of frequency Vs PD/TL% of all data from the principal component showing normal curves of the probable 2 major groupings of sampled Nile perch generated after PCA under 'PAST'.....	43
FIGURE 2.5. Scores of the first two most significant principal components, 1 representing PD/PL percentage ratio and 2 representing BD/TL percentage ratio.....	44
FIGURE 2.6. Regression graph for pooled data for Log_{10}TL versus Log_{10}Wt showing regression coefficient ' R^2 ' and regression equation.....	46
FIGURE 3.1. Map of Lake Victoria showing the different sampling sites for Nile perch and number of samples collected from each site.....	58
FIGURE 3.2. Multidimensional scaling plot of pairwise F_{ST} values among samples of Nile perch collected from 22 different geographical locations within L. Victoria.....	73
FIGURE 3.3. ΔK values as a function of K, the number of putative genetic groupings of the Nile perch in Lake Victoria, $K=2$	74
FIGURE 3.4. Bayesian analysis of the genetic structure based on nine microsatellite loci.....	75
FIGURE 4.1. Map showing East African lakes from which Nile perch was sampled and the	

number of samples collected from each lake.....	118
FIGURE 4.2. Phylogenetic tree showing evolutionary relationships of taxa - the evolutionary history was inferred using the Neighbor-Joining method (Saitau and Nei 1987).....	119
FIGURE 4.3. mtDNA haplotype networks A and B estimated with 95% statistical limits of parsimony using the algorithm in Templeton et al. 1992.	120
FIGURE 4.4. Description of the Evanno et al. (2005) 4 step graphical method used in detecting the true number of population groups K.	121
FIGURE 5.1. Map showing the Afro-tropical region of Africa and the water bodies from which Nile perch was sampled.	126
FIGURE 5.2. N-J tree describing the phylogenetic relationships among 37 mtDNA CR haplotypes of Nile perch.	132
FIGURE 5.3. Consensus tree based on maximum-parsimony analysis relating to the two major evolutionary lineages of Nile perch.	133
FIGURE 5.4. Minimum spanning network analysis of haplotypes for African Nile perch.....	134
FIGURE 5.6. Frequency distributions of pairwise number of mutational differences between Nile perch observed in both Nile perch mtDNA evolutionary lineages.	141

LIST OF ABBREVIATIONS

- ABI – Applied Biosystems
- KU – Off Kaiso landing site Uganda of L. Albert
- ARDC – Aquaculture Research and Development Center
- UT – Off St. Augustine Island Uganda of L. Victoria
- BU – Off Bugabu Island Uganda of L. Victoria
- BD – Body depth
- ECA – Biosciences Eastern and Central Africa
- SU – Off Bumangi Ssesse Island Uganda of L. Victoria
- CAB – Centre for Agricultural Bioscience
- CIFA – Central Institute of Freshwater Aquaculture
- AFRO – East African Research Organisation
- ED – Environment for development
- PT – Off Emin Persha Island Tanzania of L. Victoria
- ESU – Evolutionary significant unit
- GS – GeneScan
- HET – Haplochromines Ecology Survey Team
- ICES – International Council for the Exploration of the Sea
- IK – Off Ikondo Island Tanzania of L. Victoria
- MFRI – Kenya Marine and Fisheries Research Institute
- KU – Off Kagege landing site Uganda of L. Victoria

IU – Off Kifudwe landing site Uganda of L. Victoria
 IT – Off Kishoka landing site Tanzania of L. Victoria
 T – Off Kerebe landing site Tanzania of L. Victoria
 YU – Lake Kyoga Uganda
 UT – Off Lukuba Island Tanzania of L. Victoria
 VR – Lake Victoria Region
 IIG – Off Migingo Island Kenya of L. Victoria
 ISI – Millennium Science Initiatives
 IT – Off Mazinga Island Tanzania of L. Victoria
 IWU – Off Mwena landing site Uganda of L. Victoria
 FIRRI – National Fisheries Resources Research Institute
 AU – Nabugabo lake Uganda
 AST – PAlaeontology STatistical software
 D – Peduncle Depth
 L – Peduncle Length
 CU – Roseberry Channel Uganda of L. Victoria
 PT – Ruega Point Tanzania of L. Victoria
 UT – Off Rubafu landing site Tanzania of L. Victoria
 K – Off Saga landing site Kenya of L. Victoria
 N – Senegal River
 G – Science Initiative Group

AE – Lake Chamo Ethiopia

AFIRI – Tanzania Fisheries Research Institute

CS - Templeton, Crandall and Sing phylogenetic network estimation using statistical parsimony

LK – Turkana Lake Kenya

KT - Off Ukara Island Tanzania of L. Victoria

ST - Off Ushengere landing site Tanzania of L. Victoria

IC – Lake Victoria

WIT - Off Wiru Island Tanzania of L. Victoria

ACKNOWLEDGEMENTS

would like to thank my supervisors Dr. Vincent Muwanika and Assoc. Prof. Silvester Nyakaa and my advisors Dr. Charles Masembe, Dr. Justus Rutaisire and Dr. Wilson Mwanja for their mentoring right from proposal development, field sampling, laboratory analysis, data analysis, manuscript development to the thesis write up. Thank you all for always being there for me, I learnt a lot both theoretically and practically.

This work was majorly sponsored under the project MSI-Nile perch, for this I want to thank my supervisor – Dr. Justus Rutaisire and all the other team members for securing the grant. I am very grateful to the funders – the Worldbank and the local managers of the fund - the Uganda National Council of Science and Technology. I also want to extend my gratitude to the Göteborg University, Department of Economics, Environmental Economics Unit for the partial funding of the laboratory analysis of genetic samples of the Nile perch from L. Victoria.

I am also grateful to all staff and student colleagues in the genetics laboratory of the College of Agriculture and Environmental Sciences of Makerere University for their support and advice. Special mention to Dr. Allen Nalugwa for taking me through a number of genetics analysis packages. I also want to thank the following persons who assisted in field sampling – the crew of Explorer vessel of TAFIRI and members of LVFO hydroacoustics team 2007/2008 for sampling on L. Victoria, Mr. Odada of KEMFRI for sampling on L. Turkana, the MSI-Nile perch team for sampling on L. Albert, Mutebi of Nabugabo, Seye Mouhamadane of Dakar, Senegal and Dereb of Addis Ababa, Ethiopia for their efforts during sampling from L. Nabugabo, River Senegal and L. Chamo respectively.

To my wife, thanks for being patient and always being there for me. Through your contacts, you introduced me to the Environmental Economics Unit, Department of Economics, Göteborg University, I am grateful for your unwavering support.

To my girl Philipa, I should have given you more time, for it was in prime years during your undergraduate law course but thank you for your understanding, I know my success is your inspiration.

to my twin girls – Martyna and Maryna, welcome to the world! You came when my hands were
all, but I promise to make it up to you and thanks to you, the conclusion of my studies was made
wonderful and hopeful for whenever the going became tough I had you two to think about to
push through.

to the big African family – BaMwanja, the terms ‘field’ and ‘laboratory’ should have bored you
to the marrow for whenever you wanted me, I was either in the field or in the laboratory.
anyhow thanks for being patient and I know my success is gratifying to you all.

to my old woman – Maama Nabirye, I missed those many weekends at Mayuge – your food,
priorities, advice, music that a mother of twins does for her offsprings. I had to stay away in order to
accomplish this noble cause, thanks for your understanding and blessings.

CHAPTER ONE

1.0 General introduction

1.1 Background

Nile perch belongs to Lates genus of the teleost fishes. Nile perch is native to Lakes Albert and Turkana in East Africa (Azeroual, Entsua-Mensah, Getahun, Lalèyè, Moelants, and Ntakimazi, 2009), they are also found widely distributed in Afro-tropic region of the African continent. Nile perch are found in major river basins including the Nile, Congo, Senegal, Volta, and Niger basins. Nile perch is also naturally occurring in Lake Chad and Lake Marriott in Nile delta, River Setit in Eretria (Azeroual et al. 2009) , in Lakes Chamo, Abaya and Gambella and River Baro in Ethiopia (Golubtsov and Hebtelassie, 2010), and numerous small river systems in Central and West Africa (Moritz & Linsenmair, 2005). Nile perch was introduced into Lakes Victoria, Kyoga and Nabugabo in the 1950s and 1960s with intention of bolstering the then failing fishery of these Lakes and as a potential for sport fishing (Gee, 1969, Acere, 1985; Barlow & Lissle, 1987; Achieng, 1990). After its introduction, the Nile perch slowly colonised its new environs and then exploded in the 1980s (Arunga, 1981; Okaronon, Acere, and Ocenodengo, 1985; Goudswaard & Ligtoet, 1988). This increase in Nile perch population coincided with the disappearance of close to 50% of the endemic fish species in the new environs and Nile perch was labelled the villain (Barel et al. 1985). Nile perch has established itself in the new environs and currently dominates the fishery in these Lakes. Despite this ecological wrath, due to the lucrative international and regional markets, the Nile has boosted the economies of the three riparian countries of Lake Victoria – Kenya, Uganda and Tanzania, where most Nile perch is harvested, bringing in a hefty US \$250 million from the international markets alone (Odongkara, Abila, and Luomba, 2009). But because it's a now much sought after fish due to the lucrative market, the Nile perch fishery has been subjected to overfishing, exacerbated by deteriorating environment, and is showing signs of a declining fishery with resurgence of few of the native species that were thought to be extirpated (Balirwa et al. 2003; Witte, Wanink, and Kishemachumu, 2007; EFD, 2008). There have been postulations of the possibilities of Nile perch population crash if management of the fishery were not improved (Lowe-McConnell, 1994), which may lead to the eventual collapse of the fishery with socio-economic reverberations.

Ugandan public agricultural research institute for fisheries (NaFIRRI) reacted to the possible crash of Nile perch by proposing the domestication and culture of the Nile perch, and embarked on the preliminary studies including the species reproductive biology, feeding biology, genetic characterisation, hormonal physiology and digestive system biology (Anderson, 2010).

Specific plans for management of Nile perch have been developed (LVFO, 2010) in response to the continuing decline of stocks. The species socioeconomic significance and the need to maintain the apparent resurgence of part of lost fisheries biodiversity have to be balanced in the new plan. Nile perch fish cannot easily be eradicated from the introduced environs to reverse to the old status quo, the generally agreed plausible way out is effective management of the fishery to allow for its sustainable exploitation and resurgence of some of the endemic fish species to provide ample prey without extirpation of the latter by the Nile perch fish (Mwanja & Mwanja, 2008; Ogutu-Ohwayo, 1994).

For both the domestication efforts and effective management of the Nile perch it is a prerequisite that we find out what occurs in the native waters and what has happened to the genetic structure of Nile perch in the novel environments five decades after introductions. There is need to establish which species/sub-species successfully established itself and may be responsible for the unprecedented socioeconomic performance and associated ecological 'wrath' of the Nile perch in the introduced environs. It is also necessary to determine the most suitable taxon for use in domestication efforts and culture of the species. This study involved use of both morphological and molecular markers to characterise the Nile perch of East Africa and study of its evolutionary history on the African continent.

2 Statement of the problem

The lack of clear delineation of stocks, populations, presumed sub-lineages and lineages as well as lack of understanding of their phyletic and phylogeographic relationships present difficulty for effective management of this species. With the ecological and socioeconomic significance of Nile perch especially in East Africa and the apparent efforts to limit some of its detrimental ecological effect and yet harness its natural production potential and socioeconomic advantages requires very good understanding of the population genetic structure of Nile perch. It is important to establish what occurs in the native lakes and what is occurring in the introduced

akes as the species seemed to have performed much better in the new environs, especially as to whether there is a genetic and evolutionary basis for differences in establishment and ecological performance in the new environs. Lack of knowledge about the definite historical and evolutionary relationships and differentiations of Nile perch populations within and between water bodies limits our understanding of the current situation. With reported declining trends in the introduced environs, domestication of the species has been proposed. Given the varied stock parameters within and between water bodies domestication of Nile perch requires establishing whether the apparent differences between stocks have a genetic basis and hence aid in the choice of the best broodstock of Nile perch for use in culture systems. On the other hand, it is important that the roles of the different introduced sub-species and species in ecological and economic performance of the Nile perch is clarified given the current understanding that are two different taxa of Nile perch may have been introduced from lakes Albert and Turkana (Harrison, 1991). Lake Victoria currently contributes most to the produced Nile perch in East Africa (Bagumire, 2009) yet the species' population structure in this vast lake is not defined. This study sets out to confirm what occurs in the native lakes using a combination of both morphological and molecular analyses, and determine whether the same situation is developing in the introduced environs. In addition, Nile perch is widely distributed in the Afro-tropical region of the African continent (Lipton, 2003) that has experienced a number of palaeoclimatic and palaeoenvironmental changes (Lévêque, 1997). The phyletic relationships among the different Nile perch populations and how these historical changes have affected the recent evolution of the Nile perch on the African continent are not known. Therefore this study sets out to unravel the recent as well as historical evolution of the Nile perch on the African continent in order to delineate the different evolutionary lineages that may warrant separate management and conservation strategies, and that may guide in the domestication of the Nile perch as well as further expansion of natural and culture fisheries production of this species.

3 General Objective/Purpose

to generate information for improved management of the Nile perch fishery, protection of region's fisheries biodiversity and development of the species' aquaculture.

1.4 Specific Objectives

1. To review works so far done on evolution and genetics of Nile perch as well as the ecological and socioeconomic changes of this species.
2. To determine whether there are morphological variations associated with the different geographical populations of the Nile perch of East Africa.
3. To assess the genetic diversity and establish the population structure of the Nile perch of Lake Victoria.
4. To determine, compare and contrast the genetic variation between the native and introduced environments to evaluate the species establishment success.
5. To explore the evolutionary history of Nile perch in perspective of the palaeoclimatic and paleo-environmental changes that have occurred in the species extant range on the African continent.

1.5 Major findings of this study

- Nile perch of East Africa is of 2 morphotypes apparently one more dominant than the other. The 2 morphotypes are consistently found in all lakes in East Africa where Nile perch exists with the same distributional patterns.
- The different Nile perch geographical populations of L. Victoria exhibited genetic differentiation ($F_{ST} = 0.0234$, $P < 0.5$) indicating that there is a tendency of Nile perch population structuring (population subdivision) in the L. Victoria
- Nile perch in East African lakes had genetic diversity above average for freshwater species ($H_E = 0.71, 0.62, 0.75, 0.65$ and 0.69 for lakes Victoria, Albert, Kyoga, Nabugabo and turkana respectively; the freshwater fish (0.62 , averaged from 105 species, Mccusker and Bentzen, 2010) based on microsatellite DNA analyses. The analyses also revealed a total of 28 haplotypes among 104 samples of the East African Nile perch indicating high levels of genetic diversity.
- Nile perch in novel environments had genetic diversity relatively higher than the native environments (H_E introduced average = 0.70 , H_E native average = 0.66). This indicate a better performing fishery in the novel environments

- East African Nile perch clustered in 2 genetic groupings, which 2 genetic groupings were present in all East African lakes, one consistently more dominant than the other.
- Phylogenetic and phylogeographic analyses clearly showed the presence of two distinct Nile perch lineages among the Nile perch of the African continent, one East African lineage and the other West African lineage that have recently diverged.
- The two lineages may have developed in geographical isolation in the Pleistocene times and have remained largely allopatric without genetic exchange between the two lineages most probably caused by evolution of the drainage system of the African continent.

1.6 Applicability of research findings

The effective management of an exploited fish species requires the resource managers to ensure sustainability in harvesting fish stocks and to avoid a depletion of these stocks (Reiss, Hoarau, Dickey-Collas, and Wolff, 2009). Understanding the population genetic structure, that is, the genetic diversity, composition, and divergence among its component populations of a fishery is essential for its rational management (Waples & Naish, 2009; Reiss et al., 2009; Tudela, Garca-Marn Pla, 1999; Coyle, 1998). For these measures are used to assess migration patterns, biogeographic variability, recruitment success, and the effects of anthropogenic exploitation and habitat loss for the fishery (Zhang, Cai, and Huang, 2006; Kenchington, 2003). In addition, molecular genetic techniques offer the ability to identify and delineate fish stock structure where it may not be apparent from phenotypic or behavioural characteristics (Shaklee & Currens, 2003; Coyle, 1998). Population genetic structure is also essential in providing information to underpin resource recovery and to aid delineating and monitoring populations for fishery management (Roldan et al., 2000). But a holistic approach for fisheries management and stock assessment is required for an exploited species (Trenkel, 2007; Caddy, 2002) such as the Nile perch of East Africa, where genetics forms part of the wider range of suitable indicators of population demography and evolutionary changes. Ascertaining the evolutionary history of a species enables us to determine how and why organisms have become what they are and understand the processes that were and are currently responsible for the action to modify these organisms. In addition for the successful selective breeding in aquaculture of a species there must be significant amounts of genetic variation for the production-related traits in the fish populations (Law, 2000).

The findings of this study that include will greatly aid management in identifying and delineating the lineages, sub-lineages and/or stocks responsible for varied performance of the Nile perch fishery of the East African region. The established genetic diversity characteristics and distribution will be used in determining the source of broodstock for the on-going efforts of Nile perch domestication and culture. In addition, the findings will also provide the genetic benchmark for future genetic monitoring of the Nile perch resource in the wild in all its extant range in the region and for monitoring the effects of the proposed domestication and culture of the Nile perch.

1.7 Suggestions for future research

For the population structure of Nile perch in L. Victoria, there need for routine sampling and genetic analyses to determine the changes in population structure with changes in fishing activity. The sampling should be both spatial and temporal following similar transects.

In this study because of limited resources, we used microsatellite DNA markers characterized and developed for a sister species *Lates calcarifer*, its more suitable to develop species specific markers for following the changes in the different stocks, sub-lineages and lineages of the Nile perch.

Both morphological and genetics analyses revealed that the Nile perch of East Africa exists in 2 major underlying populations, there is need for further study and characterisation of these two populations. Nile perch of E. Africa should be reclassified using techniques such as barcoding using coenzyme I of mtDNA (COI), DNA–DNA hybridization and other biochemical tests.

For the evolutionary history studies of the Nile perch on the African continent, the sampling in this study was not as fine over the natural range of the species, as such there is need for finer sampling especially from West and along the River Nile as well as use of other more definitive molecular markers such as the COI and more advanced genetical analytical methods such as genomics to get a better resolution of the evolutionary relationships of the Nile perch.

1.8 Thesis organisation

The thesis begins with review of the literature and works on Nile perch collating the latest findings and providing the state-of-the-art knowledge on the subject, bringing together research findings from over 115 highly rated scientific publications.

The study investigated the morphological phylogenetics of the Nile perch of East Africa from both introduced and native lakes to get insights into how the species is morphologically varied in the region and to test the hypothesis by ecologists that Nile perch existed in two cryptic populations in its native environs.

Since Lake Victoria is a vast lake, the second biggest in the world, and of all lakes in East Africa contributes most of Nile perch production, the study set out to establish whether Nile perch in Lake Victoria is a single panmictic population and determine the patterns of genetic diversity in this population. The source populations used for the introductions are said to have two cryptic populations (Harrison, 1991), this study set out to determine the validity of this scenario and whether Lake Victoria Nile perch population maintains the two cryptic population.

Invasiveness of the Nile perch in the introduced environs in East Africa has been a puzzle because of its very high success in such a short time. This study attempts to unravel the puzzle by establishing and comparing the genetic diversity in both the native and introduced environs. This study also investigated how the patterns of palaeoclimatic and palaeoenvironmental changes on the African continent influenced the historical and modern distribution of Nile perch.

1.9 Brief description of each of the Chapters:

Chapter 2 ('The Evolutionary history, ecology, socioeconomics, consequences of introductions into new environs and the management challenge of the Nile perch (*Lates spp.* Linnaeus 1758) of East Africa.') contributes to the state of art knowledge of the Nile perch, with particular reference to the species' genetics and evolution, ecology and socioeconomic importance especially in the East African region where the species is both native and introduced (Mwanja & Mwanja, 2008). It brings out the two sides that argue for and against the current Nile perch status in the introduced environs and the need to strike a balance between the two sides and how this can be done. This chapter discusses ways for the possible sustainable exploitation of the Nile

perch fishery and at the same time allowing for possible recovery of the disappeared native fish species in the introduced environs as a balance between the two antagonistic sides.

Chapter 3 ('Population Morphological Variation of the Nile perch (*Lates niloticus*, L. 1758), of East African Lakes and their associated waters') investigates whether the different Nile populations within and among different lakes both native and introduced are morphologically varied and whether these findings agree with works of earlier ecologist who characterised the East African Nile perch basing on morphological analyses. Morphological variation was also used to detect possible differentiation within and among the different lakes and for stock identification. This work is compared and contrasted to findings in subsequent chapters whose analyses are based on genetic markers to determine the congruence of the two methods. The study was based on 864 Nile perch sampled from 25 different locations from different lakes in the region and 10 morphological characters. Also determined were the Length – Weight relationships and condition factor of sampled Nile perch. The log transformed data of all sampled Nile perch were subjected to multivariate analysis. Multivariate analysis of morphological characters is an effective method for defining population units and differentiating between genera, species, sub-species and groups of animals (Kusznierz, Kotusz, Kazak, Popiolek, and Witkowski, 2008; Bektas & Belduz, 2009). The chapter examines the morphological variation among Nile perch of East Africa and determines which morphological characteristic contributes most to this variation as well as deciphering its morphological phylogenetics.

Chapter 4 ('Microsatellite DNA analyses reveal population subdivision among the recently introduced Nile perch (*Lates spp.* L.1758) of Lake Victoria') deals with assessment of the genetic variation and establishment of the population of Nile perch within and among different geographical samples of Lake Victoria. Nile perch ($n = 432$) was sampled from 22 different geographical locations within Lake Victoria and genotyped at 9 microsatellite loci. Microsatellite markers are highly polymorphic with codominant inheritance (Chistiakov, Hellemans, and Volckaert, 2006), their polymorphism has enabled revelation of adequate information for effective management of fish stocks (Alam & Islam, 2005). The hypervariability of microsatellites makes them especially useful for recently isolated populations and detection of fine-scale genetic differentiation (van Oppen, Turner, Rico, Deutsch, Ibrahim, and Robinson,

1997). Microsatellite variations reveal the amount of genetic diversity, which is expected to decrease during rapid population size reductions, so called genetic bottlenecks common with invasive species introductions, and increase when the primary introduction expands and establishes a population in the new environs (Rollins, Woolnough, Wilton, Sinclair, and Sherwin, 2009). The Nile perch introduced just over five decades, using a few hundred individuals that slowly colonised, then later exploded, currently having a Lake-wide distribution and established a dominant fishery in Lake Victoria, suits this category of invasive species and its genetic diversity can be best explored using the microsatellite DNA markers.

Chapter 5 ('Successful establishment of the Nile perch (*Lates spp.* L.): genetic variation in the invasive populations versus that in the native source populations of Nile perch in East Africa') explores the genetic diversity of the Nile perch and its distribution in both the native and introduced environs to infer the mechanism of establishment of the Nile perch in its novel environs. The chapter also establishes, compares and contrasts the number of taxa of Nile perch in both native and introduced environs to in a bid to define the taxonomy of this species within East Africa and find out which of the introduced taxa is got itself established and is responsible for the ecological wrath and socioeconomic boom within the East African region. Populations introduced using small numbers are expected to suffer from low genetic variation, which lowers their ability to adapt to the new conditions in the novel environs (Dlugosch & Parker 2008). Small numbers are said to have been used for the introduction of the Nile perch in East Africa, the official record has it that 382, 585 and 295 Nile perch fish were used for the introductions into lakes Victoria, Kyoga and Nabugabo respectively (Hauser, Carvalho, Pitcher, Ogutu-Dhwayo, 1998). These small populations were expected to experience reduced genetic variability resulting from founder effects, population bottlenecks and genetic depletion. This did not happen with the introduced Nile perch in East Africa, as the introduced fish went ahead to colonise its novel environs, multiplied its populations and expanded its range to cover almost all habitats and became the leading fishery in the novel environs (Witte et al., 2007).

Chapter 6 ('Evolutionary History of Nile perch inferred from mitochondrial DNA variation analyses') investigates the evolutionary history of the Nile perch using mtDNA sequence data. For effective management and conservation of an exploitable species such as the Nile perch, it is important to define its taxonomic status and its historical distributions throughout its extant range

delineate its different evolutionary lineages that may warrant separate management strategies (Guitierrez & Gitzendanner 1999). A 476bp sequence of the control region of the mtDNA was analysed for distinct number of haplotypes and their distribution. The inferred phylogeny was established by analysis of bootstrapped neighbour-joining and maximum-parsimony trees of sequences of the revealed haplotypes to determine the possible extant Nile perch lineages on the African continent.

References

- Eng, A. P. (1990). The impact of the introduction of Nile perch, *Lates niloticus* (L) on the fisheries of Lake Victoria. *Journal of Fish Biology* (Supp. A), 17-23.
- Fre, T.O. (1985). Observations on the biology, age, growth, maturity and sexuality of Nile perch, *Lates niloticus* (Linne). and the growth of its fishery in the northern waters of Lake Victoria. pp. 42-61. In: CIFA report of the third session of the subcommittee for the development and management of the fisheries of Lake Victoria. Jinja, Uganda. 4-5 October 1984. Fish. Rep. No. 1. FAO, Rome.
- Ham, S & Islam, S. (2005). Population genetic structure of *Catla catla* (Hamilton) revealed by microsatellite DNA markers. *Aquaculture* 246, 151-160.
- Maderson, A. (2010). MSI: Fighting to save a huge fish. Science Initiative Group (SIG). Retrieved from <http://sig.ias.edu/node/396>.
- Munga, J. O. (1981). A case study of Lake Victoria Nile perch *Lates niloticus* (Mbuta) fishery. 165-184. In: Proceedings of the workshop of the Kenya Marine and Fisheries Research Institute on aquatic resources of Kenya, 13-19 July 1981, Mombasa. Kenya Marine and Fisheries Research Institute and Kenya National Academy for Advancement of Arts and Sciences.
- Meroual, A., Entsua-Mensah, M., Getahun, A., Lalèyè, P., Moelants, T., & Ntakimazi, G. (2010). *Lates niloticus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. Retrieved from www.iucnredlist.org.
- Mugumire, A. (2009). Impact of the Global Financial and Economic Crisis on the Fish industry in Uganda and Tanzania. A report prepared for UNIDO. Retrieved from http://www.unido.org/fileadmin/user_media/Services/LDC_SSC/Tanzania%20%26%20Uganda%20Fisheries%20study%20-%20draft.PDF.
- Mwalirwa, J. S., Chapman, C. A., Chapman, J. L., Cowx, J. I., Geheb, K., Kaufman, L., Lowe-McConnell, H. R., Wanink, H. J. O., Welcomme, L. R., & Witte, F. (2003). Biodiversity and

- fishery sustainability in the Lake Victoria Basin: An unexpected marriage? *BioScience* 53, 703-715.
- Barrel, C.D.N., Dorit, R., Greenwood, P. H., Fryer, G., Hughes, N., Jackson, P.B. N., Kawanabe, M., Lowe-McConnell, R. H., Nagoshi, M., Ribbink, J. A., Trewavas, E., Witte, F., & Yamaoka, K. (1985). Destruction of fisheries in Africa's lakes. *Nature* 315: 19-20.
- Barlow, G. C., & Lissle, A. (1987). Biology of The Nile perch *Lates niloticus* (Pisces: Centropomidae) with Reference to its Proposed Role as a Sport Fish in Australia. *Biological Conservation* 39, 269 – 289.
- Bektas, Y & Belduz, A. O. (2009). Morphological variation among atlantic horse mackerel, *Trachurus trachurus* populations from Turkish coastal waters. *Journal of Animal and Veterinary Advances* 8(3) 511-517.
- Bwathondi, P. O. J. (1985). The future of the fisheries of the Tanzania part of Lake Victoria, in view of the predominance of the Nile perch *Lates niloticus*. F.A.O. fisheries reports 335, pp 143-145.
- Caddy, J. F. (2002). Limit reference points, traffic lights, and holistic approaches to fisheries management with minimal stock assessment input. *Fisheries Research* 56, 133-137.
- Coyle, T. (1998). Stock identification and fisheries management: the importance of using several methods in a stock identification study. In: D. A. Hancock (Ed). *Taking Stock: defining and managing shared resources*. Australian Society for Fishery Biology, Sydney pp. 173-182.
- Chistiakov, D. A; Hellemans, B., & Volckaert, F. A. M. (2006). Microsatellites and their genomic distribution, evolution, function, and applications: a review with special reference to fish genetics. *Aquaculture* 255, 1-29.
- Dologosch, M. K., & Parker, M. I. (2008). Founding effects in species invasions: genetic variation, adaptive evolution, and the role multiple introductions. *Molecular Ecology* 17, 431-449.
- Environment for Development (EfD). (2008). Over fishing of Nile perch in Lake Victoria. EfD Tanzania press release 02nd- 11 – 2008. Retrieved from http://www.efdinitiative.org/news-press/copy_of_job-openings/job-openings-archive/press-releases-2009/over-fishing

- e, J. M. (1969). A comparison of certain aspects of the biology of *Lates niloticus* (Linne 58) in some East African lakes. Rev. Zool. Bot. Afr. 80: 244-262.
- ubstov, S. A., & Habteselassie, R. (2010). Fish faunas of the Chamo-Abaya and Chew Bahir ins in southern portion of the Ethiopian rift valley: origin and prospects of survival. Aquatic osystem Health and Management 13(1) 47-55.
- udswaard, P. C., & Ligtoet, W. (1988). Recent developments the fishery of Haplochromines sces: Cichlidae) and the Nile perch *Lates niloticus* (L.) (Pisces: Centropomidae) in Lake toria. pp.101-112. In: CIFA. Report of the fourth session of the subcommittee for evelopment and management of the fisheries of Lake Victoria, 6-10 April,1987, Kisumu, aya. FAO Fish. Rep.388. FAO, Rome
- rison, K., (1991). The taxonomy of East African Nile perch, *Lates* spp.(Perciformes, ntropomidae). Journal of Fisheries Biology 38, 175-186.
- user, L., Carvalho, R. G., Pitcher, J. T., & Ogutu-Ohwayo R. (1998). Genetic affinities of an oduced predator: Nile perch in Lake Victoria, East Africa. Molecular Ecology 7, 849-857.
- ehington, E. (2003). The effects of fishing on species and genetic diversity. In: Responsible heries in the Marine Ecosystem, Vol. Chapter 14 (eds M. Sinclair and G. Valdimarsson). FAO d CAB international, Rome and Wallington, pp. 235–253.
- sznierz, J., Kotusz, J., Kazak, M., Popiolek, M., & Witkowski, A. (2008). Remarks on the rphological variability of the Arctic charr, *Salvelinus alpinus* (L.) from Spitsbergen. Polish ar Research 29(3), 227-236.
- w, R. (2000). Fishing, selection, and phenotypic evolution. – ICES Journal of Marine ence, 57, 659-669.
- vêque, C. (1997). Biodiversity dynamics and conservation: the freshwater fish of tropical ica. United Kingdom: Cambridge University Press.
- ton, D. (2003). "Lates niloticus" (On-line), Animal Diversity Web.Retrieved from http://animaldiversity.ummz.umich.edu/site/accounts/information/Lates_niloticus.html.

- Lowe-McConnell R. (1994). The changing ecosystem of Lake Victoria, East Africa. *Freshwater Forum* 4: 76–89.
- LVFO. (2010). Lake Victoria and fisheries co-management plan. Retrieved from http://www.lvfo.org/index.php?option=com_content&view=article&id=56&Itemid=62.
- Moritz, C. (1994). Defining evolutionary significant units for conservation. *Trends in Ecology and Evolution* 9(10) 373-375.
- Mwanja, T. M., & Mwanja, W.W. (2008). Preliminary results of the analysis of the population structure of the Nile perch (*Lates niloticus* Linnaeus, 1758) of Lake Victoria using microsatellite markers. *African Journal of Biotechnology* 7(16) 2885-2892.
- Odongkara, O. K., Abila, O. R., & Luomba, J. (2009). The contribution of Lake Victoria Fisheries to National Economies. *African journal of Tropical Hydrobiology and Fisheries* 12, 47-51.
- Ogutu-Ohwayo, R.(1993). The impact of predation by Nile perch, *Lates niloticus* L., on the fishes of Lake Nabugabo, with suggestions for conservation of endangered endemic cichlids. *Conservation Biology* 7, 701-711.
- Ogutu-Ohwayo R. (1994). Adjustments in fish stocks and in life history characteristics of the Nile perch *Lates niloticus* L. in Lakes Victoria, Kyoga, and Nabugabo. Ph.D. Thesis
- Okaranon, J. O., Acere, T. O., & Ocenodongo, D. L. (1985). The current state of the fisheries in the northern portion of lake Victoria. pp. 89-98. In: CIFA. Report of the third session of the subcommittee for development and management of the fisheries of Lake Victoria, 4-5th October 1984, Jinja, Uganda. FAO Fish. Rep. 335. FAO, Rome.
- Reiss, H., Hoarau, G., Dickey-Collas, M., and Wolff, W. J. (2009). Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries* 10(4) 361–395.
- Roldan, M.I., Perrotta, R.G., Cortey, M., & Pla, C. (2000). Molecular and morphologic approaches to discrimination of variability patterns in chub mackerel, *Scomber japonicus*. *Journal of Experimental Marine Biology and Ecology* 253, 63-74.

- Collins, A. L., Woolnough, P. A., Wilton, N. A., Sinclair, R., & Sherwin, B. W. (2009). Invasive species can not cover their tracks: using microsatellites to assist to assist management of starling (*Sturnus vulgaris*) populations in Western Australia. *Molecular Ecology* 18, 1560-1573.
- Flakelee, J. B., & Currens, K. P. (2003). Genetic stock identification and risk assessment. In: Smith, L. I. 2003. A tutorial on principal components analysis. [Online]. Available: <http://www.sn1.salk.edu/~shlens/pub/notes/pca.pdf>. Last accessed on 04/06/2012.
- Smith, L. I. 2002. A tutorial on Principal Component Analysis. Retrieved from http://www.sccg.sk/~haladova/principal_components.pdf.
- Holtis, P. S., & Gitzendanner, A. M. (1999). Molecular systematic and conservation of rare species. *Conservation Biology* 13, 471-483.
- Krenkel, V. M., Rochet, M. J., & Mesnil, B. (2007). From model-based prescriptive advice to indicator-based interactive advice. *ICES Journal of Marine Science* 64, 768-774.
- Mudela S, Garca-Marn JL., & Pla C. (1999). Genetic structure of the European anchovy, *Engraulis encrasicolus*, in the north-west Mediterranean. *Journal of Experimental Marine Biology and Ecology* 234, 95-109.
- van Oppen, M. J. H., Turner, G. F., Rico, C., Deutsch, J. C., Ibrahim, K. M., & Robinson, R. L. (1997). Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes. *Proceedings of Royal Society of London, Biological Sciences* 264, 1803-1812.
- Waples, R. S., & Naish, K. A. (2009). Genetic and evolutionary considerations in fishery management: research needs for the future. *Fish and Fisheries Series*, 2009 31, 427-451.
- Witte, F., Wanink, H. J & Kische-Machumu. (2007). Species distinction and the biodiversity crisis in Lake Victoria. *Transactions of the American Fisheries Society* 136, 1146-1159.
- Zhang, J., Cai, Z., & Huang, L. (2006). Population genetic structure of crimson snapper *Lutjanus erythropterus* in East Asia, revealed by analysis of the mitochondrial control region. *Journal of Marine Science*, 63: 693-704.

CHAPTER TWO

Evolutionary history, ecology, socioeconomics, consequences of introductions into new environs and the management challenge of the Nile perch (*Lates spp.* Linnaeus 1758) of East Africa

Phew. T. Mwanja, Wilson. W. Mwanja, Sylvester Nyakaana and Vincent Muwanika.

Abstract

Evolutionary biologists and conservationists have loathed the human facilitated expansion of Nile perch (*Lates spp.*) into other fish species rich waters of Lake Victoria Region (LVR) for its ecological wrath. Since the introduction of Nile perch it is estimated that over 50% of native fish species in LVR waters have been extirpated. On the other hand the socio-economists are all in favour and even protective of the Nile perch in LVR waters for its bolstering of the economies of the three riparian countries to Lake Victoria. Explosion of the Nile perch fishery in the novel environs led to the establishment of industrial fish processing and export industry in the three countries based on the expanded Nile perch fisheries production. Over this period hundreds of key evolutionary and ecological species mostly cichlids were thought to have been predated to extinction by the Nile perch. Recently heightened fishing pressure has led to signs of collapse of the Nile perch stocks with real fears for sustainability of the fishery. Among the recent changes reported has been a notable resurgence of some native species previously reported to have disappeared. To the evolutionary biologists and conservationists the resurgence was welcome news for natural restoration of the fisheries biodiversity. To the socio-economists the decline in Nile perch fishery production was perceived as a significant threat to the fishing industry upon which millions of livelihoods were dependent and serious detrimental impact to the national economies of the three riparian countries. The question is whether a balance could be struck where a fair amount of ecological integrity of Lake Victoria fisheries is ensured while maintaining socioeconomically exploitable Nile perch stocks in LVR? In this paper, we review work so far done on evolution and genetics of Nile perch as well as the ecological and socioeconomic changes of this species.

Keywords: Nile perch, genetics, translocations, ecology, socioeconomics

Introduction

Though the ecological dominance of the Nile perch was associated with the disappearance of hundreds of species (Barel et al., 1985), socioeconomically the introduction of the Nile perch is regarded as one of the most successful fisheries bio-manipulations in recent times. It gets this credit for having led to the development of a very lucrative international and regional Nile perch based fish export trade. Nile perch boosted the economies of the three riparian countries of Lake Victoria that include Kenya, Tanzania and Uganda bringing in an estimated US \$250 million annually from the international markets alone (LVFO, 2005) from the previous less than US \$10 million gotten from the local-based subsistence fishery before 1980 (Ogutu-Ohwayo (1994), Kaufman, and Mwanja (2000), and Mwanja & Mwanja (2008) recognizing the difficulty of eliminating the Nile perch or reversing the obtaining ecological situation in waters where the Nile perch was introduced and became ecologically dominant, recommended that the most plausible management option was manipulation of the stocks through controlled fishing of the Nile perch to allow for resurgence of naturally occurring species as has recently been reported (Balirwa et al. 2003; Ogutu-Ohwayo et al. 2007). For further protection of the native species, Mwanja et al. (2003) and Mwanja & Mwanja (2008) called for protection of the minor satellite lakes in LVR. Recognising the Nile perch's successful establishment, its dominant ecological role and important socioeconomic status in the Nile perch fishery in the major water bodies of LVR, called for recognition and protection of the sizeable component of the original fish species biodiversity of these major water bodies. It was found to still occur in the minor lakes that surround the major lakes, as an alternative to reduced cropping of the Nile perch. These minor lakes thought to have played key role as life support systems during the episodes of repeated drying up and refilling associated with hydrological history of the LVR (Kaufman, 1992) are envisaged as ecological safe havens or natural refugia for the native fish species against the predation from Nile perch.

Though the resurgence of cichlid species at time when Nile perch biomass in the LVR is falling (Balirwa et al. 2003) has made a case for management interventions to keep the Nile perch biomass in check through fishing. The socioeconomic concerns are about the collapse of the Nile perch fishery. The ever increasing fishing pressure that may eventually lead to collapse, has thus threatens the maintaining the lucrative Nile perch based fish export trade and consequently the millions of livelihoods (LVFO, 2005; Lowe-McConnell, 1994). The three riparian countries of the major Nile perch fisheries production water body, L. Victoria, called for special

ventions to bolster the Nile perch fishery to its former ecological dominance level (LVFO Council of Ministers Communiqué, 2008). These measures would put the continued existence of other species that largely form Nile perch prey, the native fish species, at risk of complete extinction. Native species resurgence data indicates that heavy fishing of Nile perch may indeed enhance biodiversity (Balirwa et al. 2003), creating a debate between conservation ecologists and economists as to the continued role of the Nile perch in the LVR water bodies. The latter considering the Nile perch as a bane where overfishing restores the native fisheries biodiversity; while the former called it a boom to the socio-economic fabric of the region that should not be allowed to collapse into a bust, but should only be exploited sustainably. To find an acceptable solution, the resources managers need to design and implement appropriate management regimes. These regimes should be based on identified and mapped units of Nile perch in the different waters of the region. This will allow for ample harvests of Nile perch that make economic sense, allow for recruitment into the fishery to support sustainable harvests while at the same time allowing for continued resurgence of threatened native fish species. The native species, which are indeed needed to maintain healthy stocks of Nile perch by providing the species' prey. Such fisheries management measures require a combination of ecological, evolutionary and genetics studies for both the hunter – Nile perch and the hunted – the native species, especially the cichlids, Nile perch's most favoured prey (Mkumbo & Ligotvoet, 2002). For the Nile perch, efforts have been focused mainly on its ecology and much less on its evolution and genetics, with only a few genetic studies done (Hauser et al. 1998; Mwanja & Mwanja, 2008) yet the general view is that both ecological and evolutionary factors need to be taken into account when elucidating the success of establishment and designing management regimes for the introduced non-indigenous species (Hanfling, 2007). One key debate is whether Nile perch is composed of a number of species – *Lates macrophalamus*, *Lates albertianus*, *Lates niloticus* having two subspecies, *L. niloticus niloticus* and *L. niloticus longipinus*, or whether these groups are simply different morphs of the same species inhabiting different parts of the water bodies without clear genetic distinction to a level of sub-speciation (Harrison, 1991; Mwanja & Mwanja, 2008). Earlier ecologists noted that the Nile perch in its native environs occurs in two populations a leaner, deep open water dwelling and big deeper bodied, shallow shore water dwelling sub species (Harrison, 1991). Bwathondi (1985) also suspected that the

the Nile perch in the introduced waters of L. Victoria exhibited similar distinction. But the classification of the Nile perch to subspecies level, both in the native and introduced environs, has not been wholly accepted with the Nile perch being managed in every lake where it exists, as a single panmictic population.

In this paper we review the socioeconomic issues related to the Nile perch fishery in East Africa, its ecology and ecological genetics, the species taxonomy, recent evolutionary history and the dispersal record of the genus *Lates*. We also discuss the causes of the successful establishment of the Nile perch in the introduced environs. Furthermore, we discuss the possible use of population genetics tools in the management of the Nile perch in East Africa and the feasibility of striking a balance between the sustainable exploitation of the Nile perch and the resurgence and maintenance of the biodiversity of the native fisheries of the introduced environs that form the Nile perch prey.

2 Socioeconomics of the Nile perch in LVR

The Nile perch introductions into lakes Victoria, Kyoga and Nabugabo were intended to boost the declining native fisheries of these lakes and creating a sports fishery (Pringle, 2005; Ogutu-Ematawa, 1993). The riparian communities initially perceived the establishment of the Nile perch negatively in most aspects – cultural, nutrition requirements, employment and income provision with the development and dominance of the new Nile perch fishery. Despite the ecological wrath associated with the introduction of the Nile perch in its novel environs and the initial negative socioeconomic impacts on the artisanal fisher folk and the riparian communities, the Nile perch became a darling of the fisheries sector. Fish processing, with two players, the artisanal fish processors who mostly smoke or dry fish mostly for local and regional markets and the industrial processors of chilled fresh fillets and frozen head and gutted fish targeting mostly the international markets, provides employment to good number of people (Nyombi and Bolwig, 2004). Setting up of fish processing industries provided hundreds of thousands with both direct and indirect employment opportunities in the three L. Victoria riparian countries. Most importantly, the Nile perch industry became a sure source of foreign exchange, greatly contributing to the balance of payments of the three National economies (Nyombi and Bolwig, 2004).

Each fishery came with its peculiar challenges to the riparian communities, the fisher folk, fishing industries, fish ecologists, resource managers and the economies of the riparian areas (Ikwaput-Nyekko, Kirema-Mukasa, Odende, Mahatane, 2009). To the riparian communities, fishing was mostly done for food and as a cultural activity, but it became commercialised and controlled in that permission to fish must be sought before through licensing from respective government bodies. To riparian communities and the artisanal fisher folk the Nile perch fishery required strong and more expensive fishing gear, and required use of sizeable quantities of the permitted gillnets and long lines to realise a good return on investment. The artisanal fisher folk who mostly process the fatty Nile perch by smoking for the local market, have to venture into surrounding forests for provision of required firewood, which has led to unchecked deforestation, soil run offs and increased siltation as well nutrient loading (Hecky et al., 1993). Nile perch was also resented by the artisanal fisherfolk because of marketing problems created by the overabundance of Nile perch on the local market, a situation where supply always exceeded the demand of such an easily perishable and hard to preserve good that consequently led to fetching lower prices compared to the then scarce native fish species (Barel et al., 1985). For the fishing industries the increased value of Nile perch and reported declining stocks led to increased competition for raw material with a number of the processing factories having to close down for lack of raw materials – the Nile perch (LVFO, 2005). To the resource managers, the challenge became one of how to design a management regime that fits a species whose attributes such as spawning mode, spawning ground, larvae feed, stock differentiation, declining sizes at first maturity, sex ratios and the causes of life history changes of the Nile perch at different developmental stages and reaction to stress were not well understood. Resource managers also faced the challenge of policing the vast water bodies to stem the increasing fishing malpractices as fishermen go out for ‘big kills’ to make quick gains. To the fish ecologists the challenge was how to restore the native species when the option of totally eliminating the Nile perch was not viable and regional governments want to maintain Nile perch’s ecological dominance (Mwambi et al. 2003; LVFO, 2008). To the national economies of the East African countries, the challenge was how to maintain a steady or even increasing inflow of foreign exchange from the Nile perch trade with the reported decline in Nile perch stocks (Arnason, 2009). To tackle and overcome the above challenges requires a good understanding of the Nile perch stocks in addition

stock assessment, fisheries ecology, and socioeconomic analyses. Knowledge of responses of specific stocks of Nile perch to human and natural pressures would guide the design and development of socioeconomic and ecological interventions that are needed for sustainable exploitation of Nile perch resource.

Ecology and ecological genetics of the Nile perch

Nile perch (*Lates spp.*) is known to occur in the major river basins within the Afro-tropic region including the Nile, Congo, Senegal, Volta, and Niger basins (Lipton, 2003). Nile perch also occurs naturally in a number of lakes that are linked to these river systems including L. Albert, L. Tanganyika (Harrison, 1991), L. Chad and L. Mariout in Nile delta, River Setit in Eritrea (Azeroual et al., 2010), and in Lakes Chamo, Abaya and Gambella and River Baro in Ethiopia (Golubtsov and Hebrteselassie, 2010). The species also occurs in numerous small river systems in Central and East Africa (Moritz and Linsenmair, 2005). Nile perch was also introduced into lakes Kyoga and Nabugabo in Uganda starting in the 1950s to 1960s with the putative origins as lakes Tanganyika and Turkana (Hauser et al., 1998). Nile perch slowly colonised its new environs and exploded in population size in the early 1980s, ecologically and socioeconomically dominating the fisheries of these new environs (Mwanja & Mwanja, 2008). The population explosion of the Nile perch in the novel environs coincided with disappearance of over 50% of the native fish species, especially the haplochromines. For this, the Nile perch was labelled the villain (Barel et al., 1985) even though stocks of a number of the native fish species had been reported to be declining way before the time of Nile perch introduction (Graham, 1929). In addition, eutrophication due to increased farming activities and urbanisation around the introduced water bodies, which is also thought of as one of the causes of cichlids species disappearance, had already been reported and still continued after the introduction of the Nile perch (Welcomme, 1997; Hecky, 1993). Prior to its introduction, the prospect of introducing a large predator into Lake Kyoga was very controversial with the proponents arguing that the Nile perch would feed on the relatively unexploited and abundant haplochromines in the novel environs thereby creating a new fishery for the region, whereas the opponents argued that the top predator would extirpate the native fish species and cause disruption of the food chain and consequently the ecology of the new environs (Fryer, 1960). A settlement was found in the introductions trials of Nile perch into lakes Kyoga and Nabugabo to find out the effect of the introduced Nile perch on the native

as in these two lakes, which had similar fish biodiversity to that of L. Victoria (Gee, 1964). Before the evaluation of the introduction trials, Nile perch was found in the fish catches of Victoria in 1960 and the authorities then decided to officially allow more introductions into the (Ogutu-Ohwayo, 1993). Nile perch became the mainstay of the capture fisheries in the novel environs until recently with the reported signs of collapse of Nile perch fisheries (LVFO, 2005; Irwa et al. 2003).

The cause of the population explosion of the Nile perch after a dormancy or staying for between 10 to 30 years as minor component in the fisheries of the introduced environs is unknown (Mufman, 1992). The successful establishment and ecological explosion of the Nile perch in the environs (Mwanja et al. 2011) creates a genetic paradox – ‘How did the Nile perch overcome low genetic diversity and consequently low evolutionary potential, associated with introductions based on small numbers (Dlugosch and Parker, 2008) and establish its self in the novel environs where it did not exist before?’ Like other invasive species, the answer to this paradox is most probably found in multiple introductions (Dlugosch and Parker, 2008) that may have repeatedly augmented genetic diversity of the introduced Nile perch. Official records have that only a few fish were used for the introductions (Hauser et al., 1998), however, according to Pringle (2005) there were repeated stealthy introductions into these new environs that were carried out for over a decade from the early 1950s. This is further proven by the fact that even before introductions trials were evaluated and concluded, the Nile perch had already found its way into L. Victoria (Ogutu-Ohwayo, 1993).

It has been hypothesized that one of the major drivers in Nile perch fishery is the changes in availability of prey (Ogutu-Ohwayo, 1994, Mkumbo and Ligvoet, 1992). Some life history characteristics of Nile perch have been found to vary with the size (Ogutu-Ohwayo, 1994), like growth type, size, and number of prey ingested in most habitats varies with the length, one of the survival mechanisms of this species. It is of scientific and ecological interest to establish the effect of resurgence of haplochromines, the preferred prey and related cichlids on the Nile perch fisheries in the introduced environs. Chapman et al. (2003) observed that in L. Nabugabo with the resurgence of the haplochromines, the presence of haplochromines in the diet of Nile perch increased and that the Nile perch switched from a predominantly invertebrate diet to an ivory diet at a smaller size (5-10cm) than when the haplochromines were rare (30 cm total

...; the last four are endemic to L. Tanganyika, whereas two species, *L. albertianus* and *L. ...* ...
...*g*is, were found to be synonymous with *L. niloticus* (Otero, 2004). Fossil record has it that
Lates has been around since early Miocene to the Pleistocene eras with extensive presence
... and North Eastern Africa (Elmer, 2009) and its members have also been found in
... areas in Europe (Otero, 2004). There is an argument that Nile perch introduction into
... is a second home coming since Miocene fossil deposits for the genus *Lates* were found
... Island at the entrance to the Nyanza Gulf of L. Victoria (Greenwood, 1951). This
...ent, though, is countered by one that the current L. Victoria is a relatively new lake, just
...15,000 years old, formed due to tectonic movements and changes in hydrology, whereas
... Miocene fossil deposits belonged to a totally different lake system (Leveque, 1997).
...ly there were reported early Pleistocene fossil deposits of Nile perch on the shores of both
...esent day lakes – Albert and Edward, lakes formed only recently through tectonic
...ents and rifting of the African continent (Elmer, 2009, Otero, 2004). Zoogeographically,
... (2009) argues that the freshwater *Lates* is a marine immigrant known only from Africa
... such may not be a very close relative of the *Lates* of other regions such as *L. calcarifer* of
... Australian region.

Can a balance be struck between fish biodiversity conservation and Nile harvests that make economic sense in LVR?

...ng a balanced approach to the management of Nile perch requires in-depth understanding
... evolutionary, ecological and socioeconomic aspects of this species. Critical in these
... would be the need for genetics assessment of the species past history, its introduction,
...shment and ecological dominance of this species in the new environs. The new demands
... defining with precision the taxonomic status of the Nile perch, stock characterization,
...cation of the best material and taxa/strains for aquaculture development and assessment of
...ological viability of the Nile perch in the LVR.

...sue in fisheries socioeconomics is whether the Nile perch fisheries should be managed as
...resource or as a controlled resource. For controlled fishing of the Nile perch will allow for
...ainable exploitation, which will maintain the species in the introduced environs balanced
...e need to protect the resurging remnant native species. On the other hand, due to the high
...atiable demand for Nile perch on the market, if its exploitation is not controlled or poorly

managed, the resulting immense fishing pressure will lead to the collapse of the fishery and most probably allow for the full resurgence of disappeared native species as is already being reported with the Nile perch declining stocks (Balirwa et al. 2003; Chapman et al. 2003). There is an urgent need to strike a balance between the socioeconomic side that advocates for sustainable exploitation of the Nile perch and the ecological biologists that advocate for the species elimination or Nile perch stocks reduction through increased fishing pressure in the introduced environs to protect the remaining native fish species and allow for resurgence of disappeared native species. The question though is whether the resurgence is a result of collapsing Nile perch stocks or it is as a result of the changes in the lake environment?

Just concluded MSI-Nile perch project by NaFIRRI, Uganda for domestication of Nile perch has laid the groundwork for the species culture as an alternative for reduced wild stocks in the region. However, the major undoing to the culturing of such a voracious and ecologically versatile species is the re-entry into the wild through escape from the production systems as it has been found to happen with farmed tilapias (Mwanja & Mwanja, 2009), which may lead to further catastrophic effects in the whole of East African region including the satellite water bodies that have acted as safe havens and natural refugia for the native species following the Nile perch introductions (Mwanja et al, 2001).

Currently the regional Nile perch markets are haphazard and responsible for most illegal and immature Nile perch fishing (Benkenstein, 2011). The three East African countries processing and exporting Nile perch – Kenya, Tanzania and Uganda working together with the industrial fish processors ought to re-invest part of the incomes from Nile perch fish trade into the fisheries management, fisheries and aquaculture research and the proposed alternatives of increasing Nile perch production outlined above. Furthermore, since both the native fish species and the Nile perch in the LVR can only thrive in quality environment, the East African governments must work to ensure good water quality and environment management for the region's inland fishery to thrive.

Sound knowledge and use of evolutionary and ecological genetics are crucial in the management and control of non-indigenous and invasive species (Allendorf & Lundquist, 2003). In addition, it has been established that effective fisheries management is only possible with knowledge of identifiable self-sustaining population units (Hauser & Ward, 1998). The classification of Nile

and delineation of its associated geographical ranges is fundamental to the effective management of its fishery and understanding of its role in the whole ecosystem. The traditional fisheries management system (Arlinghaus et al. 2002) though generally successful in the short term, over the long term it is problematic as it may not reveal the impact of the management measures on genetic viability of stocks. Under the conventional fisheries management system the Nile perch in such a vast water body like Lake Victoria is taken as one uniform and panmictic population. Such an understanding ordinarily employs uniform management measures across the lake. However, results of molecular variation analyses so far question such a stand (Mwanja & Mwanja, 2008). Confirming of genetic based differences in populations and subpopulations allows for designing of targeted management measures for the specific units and monitoring of these respective groupings in a more direct and focused manner.

According to Povh et al., (2008), use of population genetics to assess biological diversity through genetic monitoring is important in maintaining fish diversity. Introduced Nile perch, like other populations established by translocations, should be periodically surveyed for genetic diversity (Stockwell, Mulvey, and Vinyard, 1996) so as to enable accurate identification and monitoring of locally adapted populations as well guiding of actions that have to be taken to maintain the species genetic biodiversity.

Conclusions

Nile perch remains a key fisheries species in East Africa which has impacted greatly on the ecology, population dynamics and evolution of other species. Socioeconomically though slow growth Nile perch has to be managed carefully as millions of people and national economies depend on it. Ecologically the Nile perch remains the most dominant but recent findings of emergence of a number of native species in waters where the Nile perch was introduced is ecologically and evolutionary good news. Definition of the population structure and extant genetic diversity of the Nile perch is key not only in the ecological and evolutionary studies but also in the effective management of this species. Such studies are also a big complement to the efforts to manage the Nile perch under controlled conditions.

7 Acknowledgements

Research on the evolution and genetics of the Nile perch was funded by the World Bank under the Millennium Science Initiatives through the Uganda Council of Science and Technology and the Uganda government. We are grateful to Dr. Margaret Banga Tenywa and Dr. Charles Masembe for their contributions towards literature search for this review.

8 References

- Allendorf, F. W., & Lundquist, L. L. (2003). Introduction: Population Biology, Evolution, and Control of Invasive Species. *Conservation Biology* 17, 24-30.
- Arlinghaus, R., Mehner, T., & Cowx, G. I. (2002). Reconciling traditional inland fisheries management and sustainability in industrialised countries, with emphasis on Europe. *Fish and Fisheries* 3, 261-316.
- Arnason, R. (2009). Rents drains in fisheries: the case of Lake Victoria Nile perch fishery. *African Journal of Tropical Hydrobiology and Fisheries* 12, 2-8.
- Azeroual, A., Entsua-Mensah, M., Getahun, A., Lalèyè, P., Moelants, T., Ntakimazi, G., 2010. *Lates niloticus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. www.iucnredlist.org. Downloaded on 22 February 2012.
- Balirwa, J. S., Chapman, C. A., Chapman, J. L., Cowx, J. I., Geheb, K., Kaufman, L., Lowe-McConnell, H. R., Wanink, H. J. O., Welcomme, L. R., & Witte, F. (2003). Biodiversity and fishery sustainability in the Lake Victoria Basin: An unexpected marriage? *BioScience* 53, 703-715.
- Barel, C.D.N., Dorit, R., Greenwood, P. H., Fryer, G., Hughes, N., Jackson, P.B. N., Kawanabe, H., Lowe-McConnell, R. H., Nagoshi, M., Ribbink, J. A., Trewavas, E., Witte, F., & Yamaoka, K. (1985). Destruction of fisheries in Africa's lakes. *Nature* 315: 19-20.
- Benkenstein, A. (2011). 'Troubled Waters' Sustaining Uganda's Lake Victoria Nile Perch Fishery. In: Governance of Africa's Resources Programme, Research Report 9, October 2011. Retrieved from http://www.saiia.org.za/images/stories/pubs/report/saia_rpt_09_benkenstein_20111031.pdf
- Bwathondi, P. O. J. (1985). The future of the fisheries of the Tanzania part of Lake Victoria, in view of the predominance of Nile perch *Lates niloticus*. F.A.O. Fisheries reports 335, 143-145.

- man, J. L., Chapman, A. C., Schofield, J. P., Olowo, P. J., Seehausen, O., Ogutu-Ohwayo, R. (2003). Fish faunal resurgence in Lake Nabugabo, East Africa. *Conservation Biology* 17 (2), 111-118.
- Wright, R. K., Reggio, C., Wirth, T., Verheyen, E., Salzburger, W., Meyer, A. (2009). Pleistocene glaciation in East Africa bottlenecked but it did not extirpate the adaptive radiation of Lake Victoria haplochromine cichlid fishes. *Proceedings of National Academy of Sciences* 106 (32), 13404-13409.
- Worm, B., & Pauly, D. (Eds.). (2004). Species of Lates in FishBase. October 2004 version.
- Wright, G., 1960. Concerning the proposed introduction of Nile perch into Lake Victoria. *East African Agriculture Journal* 25(4), 267-70.
- Wright, G. A., Kaufman L., & Mwanja, W. W. (2000). Lake Victoria Region: Large lakes and surrounding minor water bodies as a natural laboratory for adaptive ecology, speciation and evolution of fish species. *Ohio Journal of Science* 100(1), A44.
- Wright, J. M. (1964). Nile perch investigations. *Annual Reports of EAFRO*, 1962/63; pp 14-24.
- Wright, J. M., & B. (2007). Understanding the establishment success of non-indigenous fishes: lessons from population genetics. *Journal of Fish Biology*, 71, 115-135.
- Wright, J. M., & K. (1991). The taxonomy of East African Nile perch, *Lates* spp. (Perciformes, Cichlidae). *Journal of Fisheries Biology* 38, 175-186.
- Wright, L. G., Carvalho, G.R., Pitcher, T. J., & Ogutu-Ohwayo, R. (1998). Genetic affinities of introduced predator: Nile perch in Lake Victoria, East Africa. *Mol Ecol*. 7, 849-857.

L., & Ward, R.D. (1998). Population identification in pelagic fish: the limits of genetic markers. G.R. Carvalho (Ed.), *Advances in Molecular Ecology*. Oxford: IOS Press: 1-10.

R. E. (1993). The eutrophication of Lake Victoria. *Verhandlungen Vereinigung für Limnologie*, 25, 39-48.

-Nyeko, J., Kirema-Mukasa, T. C., Odende, T., & Mahatane, T. A. (2009). African Journal of Tropical Hydrobiology and Fisheries 12, 67-73.

, F. J., Schindler, E. D., Ogutu-Ohwayo, R., & Reinthal, N. P. (1997). The Nile perch in Lake Victoria: Interactions between predation and fisheries. *Ecol Appl* 7(2), 653-664.

P. N., Shuter, J. B., & Abrahams, A. P. (2004). Interpreting the von Bertalanffy model of growth in fishes: the cost of reproduction. *Proceedings of Royal Society of London, Biological Sciences* 271, 1625-1631.

, C., (1997). (Ed). *Biodiversity dynamics and conservation: the freshwater fish of Africa*. Press Syndicate, University of Cambridge, UK.

D. (2003). "Lates niloticus" (On-line), Animal Diversity Web. Retrieved from animaldiversity.ummz.umich.edu/site/accounts/information/Lates_niloticus.html.

McConnell, R. (1994). The changing ecosystem of Lake Victoria, East Africa. *Freshwater Biology*, 76-89.

(2005). The state of the fisheries resources of Lake Victoria and their management. *Proceedings of Entebbe Regional Stakeholder's conference, 24-25th February 2005, Entebbe, Uganda*. ISBN: 9970-713-10-2.

(2008). Council of Ministers Communiqué. Retrieved from <http://www.unhcr.org/refugees/council-of-ministers-communication.html>.

www.afdevinfo.com/htmlreports/org/org_38021.html.

kumbo, C. O., & Ligtoet, W. (1992). Changes in diet of Nile perch, *Lates niloticus* (L.), in the Mwanza Gulf, Lake Victoria. *Hydrobiologia* 232, 79-83.

Mwanja, T. M., Muwanika, V., Nyakaana, S., Masembe, C., Mbabazi, D., Rutaisire, J., & Mwanja, W. W. (2011). Population morphological variation of the Nile perch (*Lates niloticus* Linnaeus, 1758) of East African lakes and their associated waters. *African Journal of Environmental Science and Technology* 5(11), 941-949.

Mwanja, T. M., & Mwanja, W. W. (2009). Escape of farmed tilapiines into the wild and entry of wild forms in fishponds, and possible interaction between wild and farmed tilapiines from a sample of smallholder farms in Central Uganda. *African Journal of Ecology*, 47, 469-475.

Mwanja, M. T., & Mwanja, W. W. (2008). Preliminary results of the analysis of the population structure of the Nile perch (*Lates niloticus* Linnaeus, 1758) of Lake Victoria using microsatellite markers. *African Journal of Biotechnology* 7(16), 2885-2892.

Mwanja, W.W., Armoudlian, A. S., Wu, L., Wandera, S. B., Booton, G. C., Kaufman L., & Fuerst A. P. (2001). Bounty of minor lakes: role of small satellite water bodies in evolution and conservation of fishes in Lake Victoria region, East Africa. *Hydrobiologia* 458(1-3), 55-62.

Mwanja, W.W., & Fuerst, A. P. (2003). How issues of genetic diversity affect management of African inland water fisheries: the example of Lake Victoria Region. In: T.L. Crisman, L. Chapman, C.A. Chapman, & L. Kaufman, *Conservation of Aquatic Biodiversity of Africa*, pp 288-300. University Press of Florida Press, Gainesville, Florida.

Nyombi, K., & Bolwig, S. (2004). A quantitative analysis of alternative development strategies for Ugandan fisheries. Last accessed on 04th – March – 2012. Retrieved from <http://ussp.ifpri.info/files/2011/09/fisheries-development-strategies-nyombi-and-bolwig-04.pdf>.

- wayo, R. (1994). Adjustments in fish stocks and in life history characteristics of the Nile perch, *Lates niloticus* L. in lakes Victoria, Kyoga, and Nabugabo. Ph.D. Thesis – Ontario Canada.
- wayo, R. (1993). The impact of predation by Nile perch, *Lates niloticus* L., on the fish community of Lake Nabugabo, with suggestions for conservation of endangered endemic cichlids. *Conservation Biology* 7, 701-711.
- Smith, A. (2004). Anatomy, Systematics and phylogeny of both recent and fossil latid fishes (Perciformes, Latidae). *Zoological Journal of the Linnaean Society* 141, 81-133.
- Alves, A., Lopera-Barrero, N. M., Ribeiro, R. P., Lupichinski, Jr, E., Gomes, P. C., & Lopes, T. (2003). Genetic monitoring of fish repopulation programs using molecular markers. *Ciência e Tecnologia Agrária*, 35(1), 5-15.
- Smith, R. M. (2005). The origins of the Nile perch in Lake Victoria. *BioScience* 55(9), 780-787.
- Ward, A. C., Mulvey, M., & Vinyard, G. L. (1996). Translocations and the preservation of biodiversity. *Conservation Biology* 10, 1133-1141.
- Smith, R., Wanink, H. J., & Kische-Machumu. (2007). Species Distinction and the Biodiversity of Lake Victoria. *Transactions of the American Fisheries Society* 136, 1146-1159.

CHAPTER THREE

Population Morphological Variation of the Nile perch (*Lates niloticus*, L. 1758), of East African Lakes and their associated waters

Authors: T. Mwanja, Vincent Muwanika, Sylvester Nyakaana, Charles Masembe, Dismas Mubizi, Justus Rutasire and Wilson. W. Mwanja

Abstract

In this study an attempt was made to determine whether there are morphological variations associated with the different geographical populations of the Nile perch of East Africa. Analyses of the levels of morphological differentiation based on morphological characterisation and statistical analysis were carried out. The study was based on 864 Nile perch individuals sampled from 25 different locations from different Lakes in the region and 5 morphological characters. Also determined were the Length – Weight relationships (LWRs) and condition factor of sampled Nile perch. The log transformed data of all sampled Nile perch were subjected to multivariate analysis, using ‘PAST’ statistical software. The findings showed that peduncle form and size, of the variables used in this study, contributed most to the variance. The analysis clustered the Nile perch into two groups, which were found to be as characterised by earlier morphological description of this species and most probably are representatives of two distinct taxa of Nile perch in the East African waters. The LWRs and condition factor conformed to the fish isometric growth formula $W = aL^b$ with the value for the pooled data being $W = 0.6664L^{0.3264}$. The existence of 2 morphs agrees with earlier taxonomists who proposed that Nile perch exists in two populations – a bigger shallow water dwelling morph and a smaller deep open water dwelling morph. This calls for identification and mapping of the 2 populations in the different waters of East Africa that may require application of different management regimes.

Keywords: Nile perch, taxa, morphological variation, East Africa

Introduction

Nile perch *Lates niloticus* (Linnaeus 1782) is an important commercial freshwater fish species in Africa. It is endemic to Lakes Albert and Turkana and was introduced into Lakes Victoria, Uganda and Nabugabo over five decades ago (Hamblyn, 1961; Gee, 1964; EAFRO, 1967; Comme, 1988; Pringle, 2005) with intention of bolstering the then failing fishery of these lakes and as a potential for sport fishing (Gee, 1969, Acere, 1985; Barlow & Lissle, 1987; Leng, 1990). After its introduction, the Nile perch slowly colonised its new environs and then exploded in the 1980s (Arunga, 1981; Okaronon et al. 1985; Goudswaard & Ligtvoet, 1988). The increase in Nile perch population coincided with the disappearance of close to 50% of the endemic fish species in the new environs and Nile perch has been labelled the villain (Barel et al. 1985). Nile perch has established itself in the new environs and currently dominates the fishery of these Lakes. Despite this ecological wrath, due to the lucrative international and regional markets, the Nile has boosted the economies of the three riparian countries of Lake Victoria – Kenya, Uganda and Tanzania, where most Nile perch is harvested, bringing in a hefty US \$250 million from the international markets alone (Odongkara et al. 2009).

Due to its high demand in the market, this fish species has been put under increased fishing pressure in this region, leading to a great decline in its stocks (EfD, 2008). The decline in Nile perch stocks is further compounded by habitat degradation, water pollution and changes in prey of Nile perch (Ogutu-Ohwayo, 1990; Mkumbo and Ligtvoet, 1992). One way to mitigate this problem is to domesticate and culture this species so as to supplement the supplies from the wild and reduce the pressure on the natural stocks. This study is part of a bigger project whose overall aim is to domesticate, breed and culture the Nile perch based at ARDC – Kajjansi, Uganda.

When to culture any species from the wild it is prudent that the species is characterized taxonomically, ecologically and geographically as the first step. This enables one to define this species and to determine the performance of the different taxa that are in the wild so as to aid selection of the best suited taxon for culturing (Liao & Haung, 2000). Earlier efforts failed to unambiguously define the Nile perch in this region (Worthington, 1929, 1932; Harrison, 1991; Hauser et al. 1998). For example what was being taken as one species – *Lates niloticus*, turned out to be considered as two different species - *Lates albertienseis* and *Lates macrophthalmus* for the Lake Albert Nile perch (Worthington, 1929; Worthington, 1940), and interestingly the Lake Turkana

Nile perch were taken to be two different subspecies - *Lates niloticus rudolfianus* and *Lates niloticus longispinus* of the riverine species *Lates niloticus* (Worthington, 1932; Greenwood, 1955). Later this grouping was revised to subspecies level and not species of *Lates niloticus* as specimens from Lakes Albert and Turkana were seen as intermediates and also thought to interbreed (Worthington, 1940). The other school of thought is that the observed differences in morphology of the Nile perch may be arising from the extremities of a normal population curve. According to Harrison (1991) the Lake Victoria Nile perch has been and is wrongly referred to as *Lates niloticus*, yet its taxonomic status has never been resolved. This paper is part of a bigger study that is attempting to taxonomically characterise the Nile perch using a combination of both morphological and molecular techniques. The paper examines the morphological variation among Nile perch of East Africa and determines which morphological characteristic contributes most to this variation as well as deciphering its morphological phylogenetics. In this paper, results of the analysis of the Nile perch populations and subdivisions based on measurement of morphological characteristics, done using the 'PAST' statistical software are presented and discussed.

2 Study area, materials and methods

2.1 Study area

Nile perch samples were taken from Lakes Albert in Uganda and Turkana in Kenya where the fish is endemic and the putative origins for the introduction of this species into Lakes Victoria, Kyoga and Nabugabo in Uganda. Lakes Albert and Turkana are rift valley lakes, with Lake Albert found on the western border of Uganda with Democratic Republic of Congo and Lake Turkana on the north western border of Kenya with Ethiopia. Lake Victoria is the second largest fresh water lake in the world, and is shared between three countries: Uganda (41%), Kenya (6%) and Tanzania (53%). Lake Kyoga is in Central Uganda connected to both Lake Victoria and Lake Albert by the Victoria Nile River. There are waterfalls on either side of Lake Kyoga - Owen Falls and Murchison Falls, which act as a buffer to the movement of adult Nile perch due to their size. Lake Nabugabo is a small (24 km²) satellite lake, formerly a bay on the western shore of Lake Victoria separated from the main lake by a sand bar more than 5,500 years ago (Chapman et al. 1996). It is alleged that before introduction of Nile perch from Lake Albert into

the Victoria, the fish were first rested and tried out in Lake Nabugabo before eventually being introduced into Lake Victoria (Barlow and Lissle, 1987).

2.2 Sample collection

Lakes Albert, Turkana, Kyoga and Nabugabo (**FIGURE 1**) fish were caught using seine nets. In these lakes the sampling followed different eco-zones (shallow offshore waters and the deep open waters) because different Nile perch morphs are believed to inhabit different eco-zones. In Lake Victoria, samples were caught from 22 different geographical locations (**FIGURE 2**) by bottom trawls. Sampling followed transects pre-determined by hydroacoustics experts after consideration of a number of factors including traditional fishing grounds, history as told by the local fisher folk, previous knowledge of Nile perch distribution, findings of tagging experiments, and the Lake topography. Trawls were done after the hydroacoustics experts on board detected signals indicating abundance of relatively big size fish on their computer screen. For the trawls the depth of sampled sites ranged from as low as 4.5m to a maximum 50.4m (**TABLE1**). The towing of Nile perch from both deep open and shallow offshore waters and trawling from the deep open waters toward the shallow offshore waters ensured that if there are differences in habitat for different morphs as alleged, then all morphs would be sampled and included in the analyses. A total of 864 Nile perch fish were sampled from all lakes (**TABLE 1**).

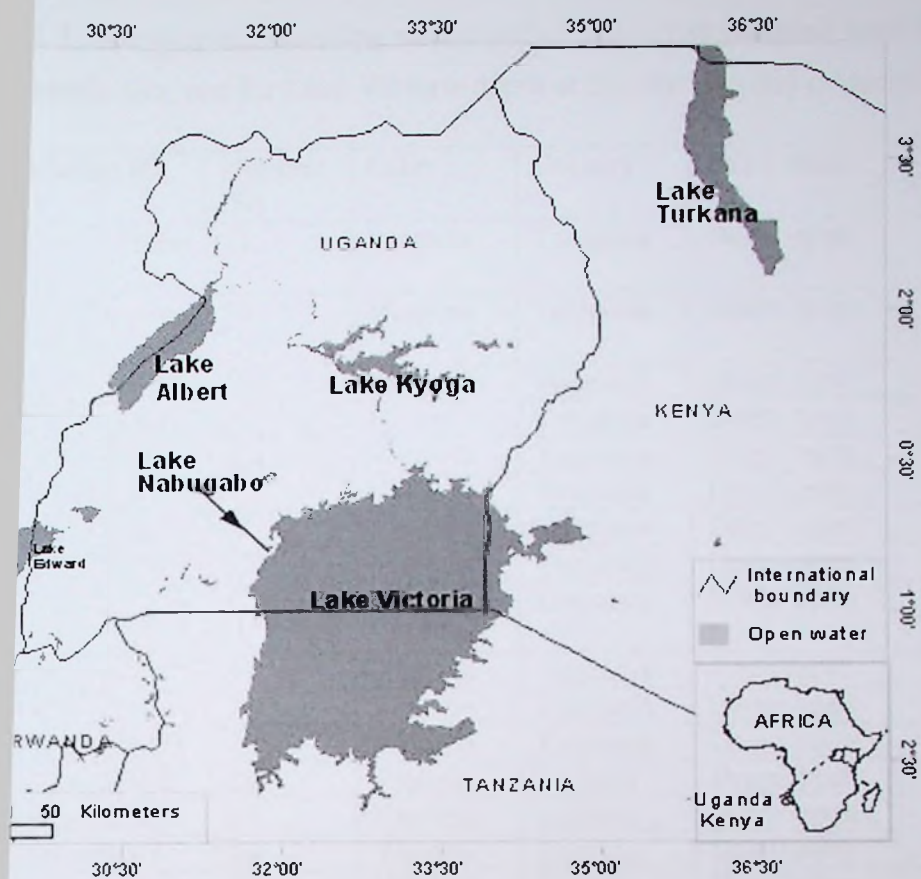


FIGURE 1. Map showing the East African lakes from which Nile perch was sampled for this study.

Showing the sampling sites, number (N) of fish sampled from each site, the eco-zone of the site, and for Lake Victoria depth at the start and end of each fishing trawl.

Station ID	Number (N)	Lake	Country	Eco - zone	Depth Start - End of trawl
Pasha	22	Victoria	Tanzania	Deep/ open	9.0m - 9.0m
point	50	Victoria	Tanzania	Deep/ open	25.0m - 24.0m
3/MT	38	Victoria	Tanzania	Deep/ open	45.0m - 48.0m
KT	31	Victoria	Tanzania	Deep/ open	50.0m - 50.4m
JT	40	Victoria	Tanzania	Deep/ open	30.0m - 30.0m
3/KIT	40	Victoria	Tanzania	Deep/ open	33.0m - 29.0m
4/RUT	20	Victoria	Tanzania	Deep/ open	9.4m - 9.4m
5/LUT	50	Victoria	Tanzania	Deep/ open	30.2m - 30.2m
6/ere	42	Victoria	Tanzania	Deep/ open	40.0m - 37.5m
7/UKT	33	Victoria	Tanzania	Deep/ open	32.5m - 32.0m
8/MIT	20	Victoria	Tanzania	Deep/ open	40.0m - 40.0m
9/us /AUT	20	Victoria	Tanzania	Deep/ open	13.2m - 14.0m
10/a /MWU	28	Victoria	Uganda	Deep/ open	32.0m - 31.0m
11/e /KGU	15	Victoria	Uganda	Deep/ open	5.5m - 4.5m
12/BBU	46	Victoria	Uganda	Deep/ open	4.8m - 4.5m
13/gi	39	Victoria	Uganda	Deep/ open	8.0m - 8.0m
14/BSU	16	Victoria	Uganda	Deep/ open	24.0m - 24.6m
15/ry el/RCU	41	Victoria	Uganda	Deep/ open	20.0m - 24.0m
16/e/KIU	12	Victoria	Uganda	Deep/ open	20.0m - 18.0m
17/SGU	50	Victoria	Kenya	Deep/ open	30.0m - 25.0m
18/AK	35	Victoria	Kenya	Deep/ open	43.0m - 43.0m
19/o/ MIK	19	Turkana	Kenya	Deep/open & Shallow/offshore	-
20/a /TLK	95	Albert	Uganda	Deep/open & Shallow/offshore	-
21/Kaiso	50	Kyoga	Uganda	Deep/open & Shallow/offshore	-
22/gu /KYU	18	Nabugabo	Uganda	Deep/open & Shallow/offshore	-
23/abo					

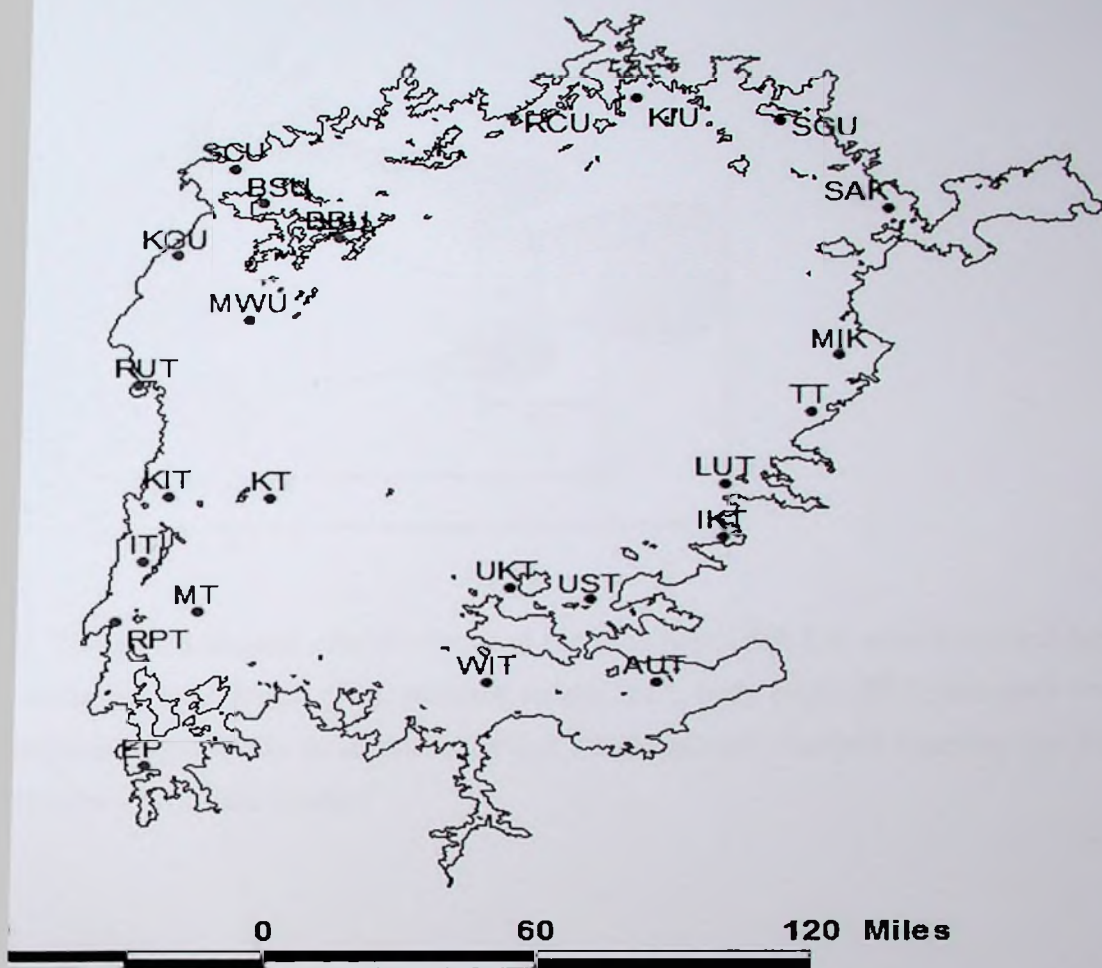


Fig 2: Map of Lake Victoria showing the sampled sites

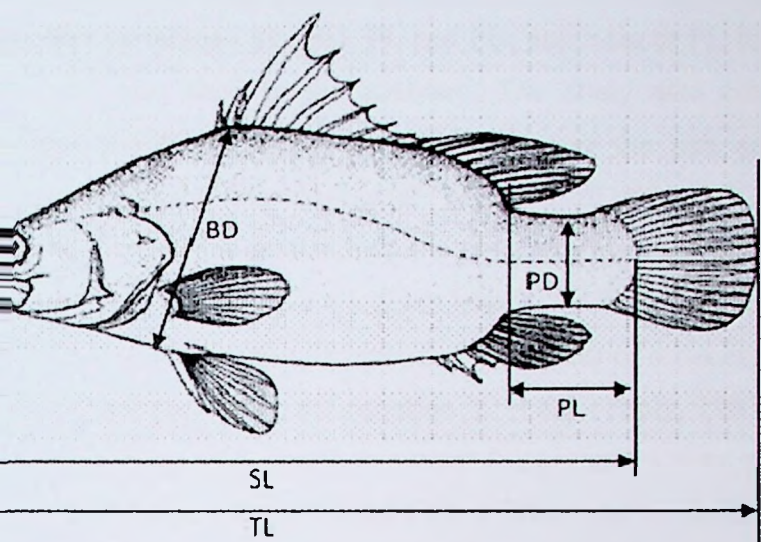


FIGURE 3. The morphological characteristics of the Nile perch fish that were measured during the study including total length (TL), standard length (SL), body depth (BD), peduncle length (PL) and peduncle depth (PD). In addition, the wet weight of each sampled fish was also taken. (Source of photo – FAO Fish Finder)

Biometric measurements and data analysis

Morphological characters studied included - Total Length (TL), Standard Length (SL), Body Depth (BD), Peduncle Length (PL) and Peduncle Depth (PD) (**Figure 3**), so for every sampled fish, measurements of these characteristics were taken immediately after capture/trawl. Ratios of all other variables - SL, BD, PL and PD, and ratio of PL to PD for each sampled fish were calculated and also used in the analyses. The study also considered the Length – Weight relationships (LWRs) and the condition factor of the fish and establish whether these are affected or affect the morphological variation of this fish. Condition factor refers to a mathematical formula for determining the physiological state of a fish, including its reproductive capacity. The heavier a fish is for a given length, the higher its condition factor (K), which is one factor that is considered when determining the health of a fishery. The LWRs of pooled data for fish were estimated using the equation $W = aL^b$ (Pauly, 1983), where W = weight of fish in grams, L = TL in cm, b = length exponent (slope) and a = proportionality constant (intercept). The 'a' and 'b' values were obtained from a linear regression of length and weight of fish. The correlation or degree of association between length and weight (r^2) was calculated from linear regression analysis, $R = r^2$. The condition factor 'K' was determined using the equation, $K = 100W/L^3$. In order to standardize the data, all variables taken including SL, BD, PL and PD were expressed as a percentage of TL. Also since different strains of the Nile perch are thought to differ in PL and PD, the ratios of these two variables were expressed as percentage and the condition factor 'K' was determined for each sample. To ensure normality and homogenous variances, all values were $\log_{10}$ – transformed before statistical analyses were performed. The outcomes (PL/PD%, BD/TL%, BD/SL% and 'K') were then used as the variables in the subsequent multivariate data analysis using the PAST software. Principal Component Analysis (PCA) was used to extract principal components from the four variables. The 'PAST' (Paleoecology and Archaeology STatistical) software package was originally aimed at palaeontology but currently has become popular in ecology and other fields (Hammer et al., 2001). Using PAST, the measured morphological characters were subjected to multivariate analysis, a tool highly recommended for determining relationships between populations of a species (Thorpe, 1987) and for use in stock identification of freshwater fish and investigating taxonomical problems in sympatric populations (Cawdery and Ferguson, 1988). The most significant component that contributed most to the variance was then used in determining the morphological phylogenetic

relationships of the Nile perch in the East African waters. Based on the outcome of the mixture analysis in 'PAST', all data were subjected to K-means clustering to determine non-hierarchical grouping of all the samples into the number of groups (2) specified under the mixture analysis. The significance level considered was 0.05.

Results

The allometric relationship of Nile perch was found to be $\text{Log}_{10}W = 0.6664 + 0.3264\text{Log}_{10}TL$, corresponding to $W = 0.6664L^{0.3264}$, indicating an isometric growth pattern. The regression coefficient for the pooled data was $r^2 = 0.9832$ (FIGURE 6). The relative condition factor ranged from 0.57 to 2.28 with an average for pooled data at 1.16. Principal Component Analysis of the data from the four samples revealed that approximately 96.8% of the total variation was explained along two components 1 and 2, that is PD/PL% and BD/TL% respectively. With most of the variance explained by the component 1 - PD/PL% (73.457%) followed by BD/TL% (23.300%) then TL% (3.2239%) and condition factor 'K' (0.017%) making the smallest contribution to the morphological variation of the Nile perch (TABLE 2). All the Eigen values for all components were positive (Table 2) indicating all used variables have some effect on the morphological variation of the Nile perch with the most significant contribution from the variable percentage PD/PL (Figure 5). When subjected to mixture analysis, the fish clustered into two groups (Figure 4). From the K-means clustering, the majority of the individuals (583) belonged to one group, that is, cluster 1 and the rest (281) belonged to the other group, that is, cluster 2. The mixture analysis curve (Figure 4) for all data pooled together showed two major groupings. The Neighbour-Joining clustering using Euclidean similarity measure generated by 'PAST' using the square-root transformed data from the principal component 1 also revealed two clades.

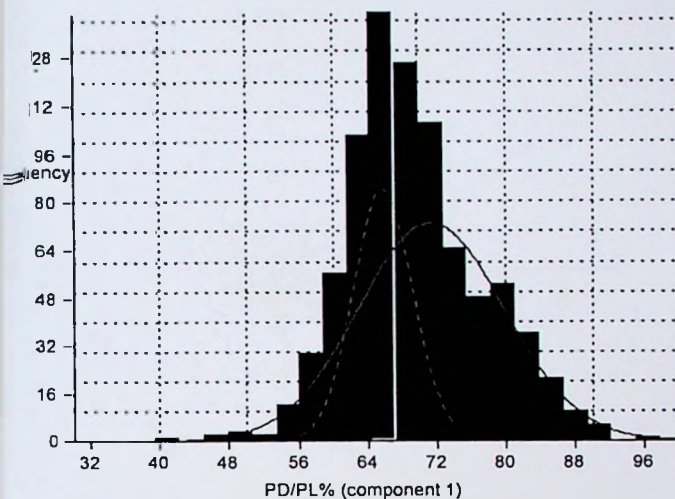


FIGURE 4: A graph of Frequency Vs PD/TL% of all data from the principal component (component one) showing normal curves of the probable 2 major groupings of sampled Nile fish generated after PCA under 'PAST'. The unbroken line curve represents cluster one the most dominant and the broken line represents cluster 2

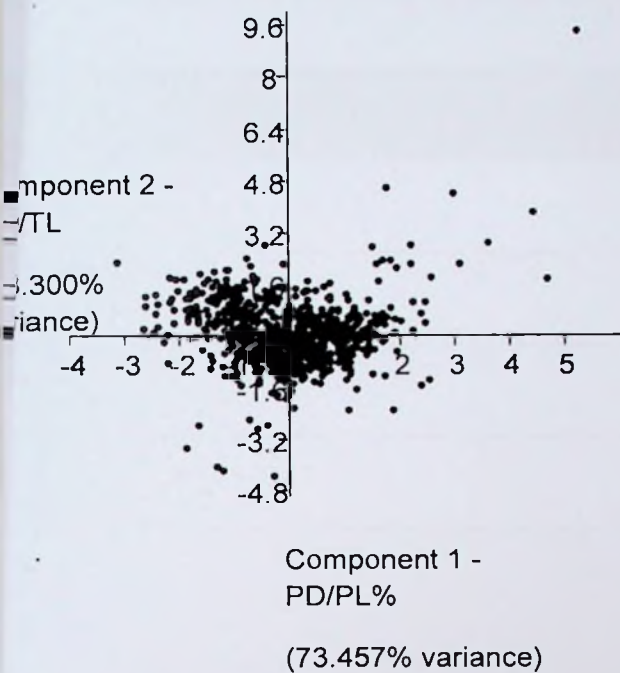


FIGURE 5. Scores of the first two most significant principal components in this study. Component 1 represents the PD/PL percentage ratio whereas Component 2 represents the BD/TL percentage ratio.

BLE 2. Eigen values and the distribution of variance among the different components

orphological character	Component ranking	Eigen value	% varaince
PD/PL%	1	71.965	73.457
BD/TL%	2	22.827	23.300
BD/SL%	3	3.160	3.226
K	4	0.016	0.017

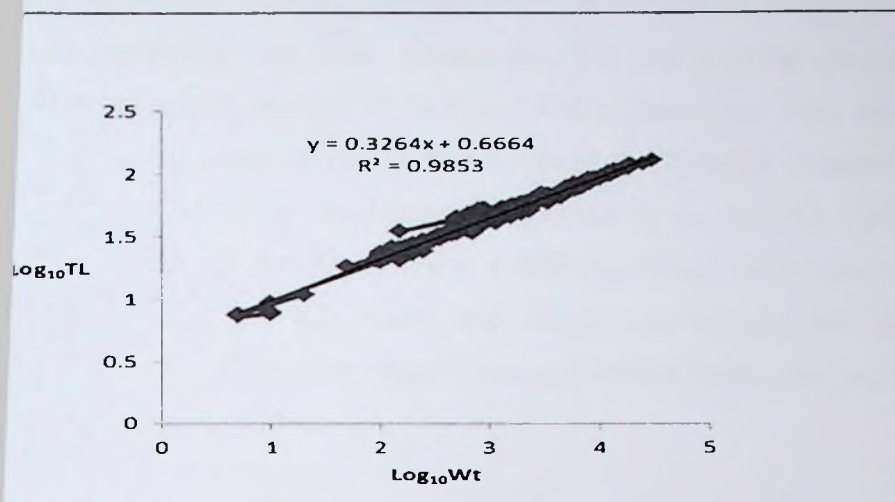


FIGURE 6. Regression graph for pooled data for Log_{10}TL versus Log_{10}Wt showing regression coefficient ' R^2 ' and regression equation.

Acknowledgements

This study is part of the bigger project Nile perch technologies sponsored by the World Bank Millennium Science Initiatives (MSI) under the Uganda National Council of Science and Technology (UNCST). We are thankful to Dr. Rob Skilton of BECA - Nairobi, Kenya for helping in correcting the final manuscript. We are grateful to Lake Victoria Fisheries Organisation for having allowed us on board during their Lake wide stock assessment on Lake Victoria and to the crew of the Explorer vessel of Tanzania Fisheries Research Institute (TAFIRRI) that was used during the bottom trawls on Lake Victoria. We also indebted technicians Mapunda and Ogaro from TAFIRRI, Odada from Kenya Marine and Fisheries Research Institute (KEMFRI), Opiri and Mutebi of Uganda for their assistance during bathymetric measurements on Lakes Victoria, Turkana, Nabugabo and Kyoga respectively.

References

- ..., T.O. (1985). Observations on the biology, age, growth, maturity and sexuality of Nile perch, *Lates niloticus* (Linne.) and the growth of its fishery in the northern waters of Lake Victoria. pp. 42-61. In: CIFA report of the third session of the subcommittee for the development and management of the fisheries of Lake Victoria. Jinja, Uganda. 4-5 October 1984. Fish. Rep. 1984. FAO, Rome.
- ..., A. P. (1990). The impact of the introduction of Nile perch, *Lates niloticus* (L) on the fisheries of Lake Victoria. J. Fish Biol. (Supp. A), 17-23.
- ..., J. O. (1981). A case study of lake Victoria Nile perch *Lates niloticus* (Mbuta). pp. 165-184. In: Proceedings of the workshop of the Kenya Marine and Fisheries Research Institute on aquatic resources of Kenya, 13-19 July 1981, Mombasa. Kenya Marine and Fisheries Research Institute and Kenya National Academy for Advancement of Arts and Sciences.
- ..., C.D.N., Dorit, R., Greenwood, P. H., Fryer, G., Hughes, N., Jackson, N. B. P., Kawanabe, M., Lowe-McConnell, H. R., Nagoshi, M., Ribbink, J. A., Trewavas, E., Witte, F., & Yamaoka, K. (1985). Destruction of fisheries in Africa's lakes. Nature 315: 19-20.
- ..., G. C., & Lissle, A. (1987). Biology of The Nile perch *Lates niloticus* (Pisces: Cichlidae) with Reference to its Proposed Role as a Sport Fish in Australia. Biological Conservation 39, 269 - 289.
- ..., P. O. J. (1985). The future of the fisheries of the Tanzania part of Lake Victoria, in view of the predominance of the Nile perch *Lates niloticus*. F.A.O. fisheries reports 335, pp 143-155.
- ..., S. H. and Ferguson, A. (1988) Origins and differentiation of three sympatric species of trout (*Salmo trutta* L.) in Lough Melvin, Ireland. Polskie Archiwum Hydrobiologii, 35, 267-277.
- ..., M. L. (2004). Phylogenetics analysis of morphological data (Review). In: Human Biology, 76 (1), 165-168.

- man, L. J., Chapman, A. C., Ogutu-Ohwayo, R., Chandler, M., Kaufman, L., & Keiter, A. (1996). Refugia for Endangered Fishes from an Introduced Predator in Lake Nabugabo, Uganda. *Conservation Biology*, 10(2), 554-561.
- EAFRO. (1967). East Africa Freshwater Fisheries Organization annual reports, 1961-1967; Kampala, Uganda.
- Environment for Development (EfD). (2008). Over fishing of Nile perch in Lake Victoria. EfD Uganda press release 02nd- 11 - 2008. Retrieved from http://www.efdinitiative.org/news-s/copy_of_job-openings/job-openings-archive/press-releases-2009/over-fishing
- J.M. (1964). Nile perch investigations. Annual Reports of East African Freshwater Research Organisation, 1962/63; pp 14-24.
- J. M. (1969). A comparison of certain aspects of the biology of *Lates niloticus* (Linne 1758) in some East African lakes. *Rev. Zool. Bot. Afr.* 80, 244-262.
- Greenwood, P. H. (1966). the Fishes of Uganda. Kampala (Uganda): Uganda Society.
- Woudswaard, P.C., & Ligtvoet, W. (1988). Recent developments the fishery of Haplochromines (Pisces: Cichlidae) and the Nile perch *Lates niloticus* (L.) (Pisces: Centropomidae) in Lake Victoria. pp.101-112. In: CIFA. Report of the fourth session of the subcommittee for development and management of the fisheries of Lake Victoria, 6-10 April, 1987, Kisumu, Kenya. FAO Fish. Rep.388. FAO, Rome.
- Embalyn, E. L. (1961) the Nile perch project. EAFFRO annual report, 1960, pages 26-32.
- Hammer, O, Harper, D. A. T., & Ryan, P. D (2001) Paleontological statistics software package for education and data analysis. In: *Paleontol Electronica*, Vol. 4 (1); art. 4: 9pp; 178kb
- Harrison, K. (1991) the taxonomy of East African Nile perch, *Lates spp.* (*Perciformes centropomidae*). *Journal of Fish Biology*, Vol. 38 (2); Pages 175 - 186.
- Busser, L. G; Carvalho, G.R; Pitcher, T. J and Ogutu-Ohwayo, R; (1998) genetic affinities of an introduced predator: Nile perch in Lake Victoria, East Africa. *Molecular Ecology*, 7; pages 849-857.

- ...n, M. J. (1967) the systematics of the genus *Lates* (Teleostei: Centropomidae) in L. Albert Africa Journal of Zoology (London), 151; 329-342.
- ...on, A. J. (1972) a study of the Nile perch, *Lates nilotics* (L.), in Lake Chad. HMSO Seas Research Publications, 19; Foreign and Commonwealth Office, London.
- ...I. C and Haung, Y. S. (2000) methodological approach used for the domestication of potential candidates of aquaculture. In: Recent advances in Mediterranean aquaculture finfish species diversification . Zaragoza : CIHEAM-IAMZ, 2000; pages 97-107.
- ...ambo, O. C and Ligtoet, W. (1992) changes in the diet of Nile perch, *Lates niloticus* (L), the Kazinga Gulf Lake Victoria. In: Hydrobiologia, Vol. 232 (1); pages 79-83.
- ...ngkara, O. K; Abila, O. R. and Luomba, J. (2009). The contribution of Lake Victoria fisheries to National Economies. African journal of Tropical Hydrobiology and Fisheries 12, 51.
- ...utu-Ohwayo, R. (1990). Changes in prey ingested and the variations in the Nile perch and other fish stocks of Lake Kyoga and Northern waters of Lake Victoria (Uganda). Journal of Fish Biology, Vol. 37 (1); pages 55-63.
- ...aranon, J.O; Acere, T.O. & Ocenodongo, D.L. (1985). The current state of the fisheries in the northern portion of lake Victoria. pp. 89-98. In: CIFA. Report of the third session of the committee for development and management of the fisheries of Lake Victoria, 4-5th October 1984, Jinja, Uganda. FAO Fish. Rep. 335. FAO, Rome.
- ...ngle, R. M. (2005) the origins of the Nile perch in Lake Victoria. In: BioScience, Vol. 55(9); pages 780-787.
- ...orpe, R. S. (1987) geographic variation: a synthesis of cause, data pattern and congruence in relation to subspecies, multivariate analysis and phylogenies. Bollettino di Zoologia, 54; 3-11.

me, R. L. (1988) international introductions of inland aquatic species. FAO Fish
al paper, 249, FAO Rome.

ngton, E. B. (1929) a Report on the Fishing Survey of Lakes Albert and Kyoga, March to
28. London: Crown Agents for the Colonies

ngton, E. B. (1932) scientific results of the Cambridge expedition to the East African
pages 1930–31

ngton, E. B. (1940) geographical differentiation in freshwaters with special reference to
the *Systematics* (J. Huxley, ed.), pp 287–302. London: Oxford University Press.

CHAPTER FOUR

satellite DNA analyses reveal population subdivision among the recently introduced Nile perch (*Lates spp. L.1758*) of Lake Victoria

w. T. Mwanja; Vincent Muwanika; Charles Masembe, Justus Rutaisire, Wilson. W. a and Silvester Nyakaana.

Abstract

Nile perch introduced into Lake Victoria only over five decades ago has become the leading fish in the East African region with significant socioeconomic and ecological importance. Knowledge of Nile perch population structure in such a vast lake with high micro-geographical variation that creates a potential for genetic differentiation is important in the effective management of the species. In this study, microsatellite DNA variability was used to assess the genetic diversity and define the population structure of the Nile perch collected from 22 different geographical locations within Lake Victoria. A total sample of 432 individuals were genotyped at 10 microsatellite loci. Analyses of the multilocus microsatellite genotypes revealed that genetic diversity of Nile perch of Lake Victoria is relatively high ($H_E = 0.68$). They also reveal shallow and significant genetic differentiation ($F_{ST} = 0.029$; $P < 0.05$) within the lake. STRUCTURE analysis indicated that Nile perch of Lake Victoria was made up of two ($K=2$) populations. These results are surprising given that Nile perch was only recently introduced in the lake and is known to be monophyletic by nature. We interpret these results in light of the multiple introductions from two different source populations, which could have been from two different source subspecies of Nile perch and discuss the implications of these results for the region's fisheries management.

Keywords: Lake Victoria, Nile perch, genetic structure, microsatellites

roduction

Nile perch is an important food fish that currently dominates the fisheries of three East African countries riparian to Lake Victoria – Uganda, Kenya and Tanzania despite the former having a vast coastline with a very high potential for marine fisheries production (Mwanja et al. 2009). Nile perch, caught mostly from Lake Victoria, foreexport to the international markets, in a hefty USD \$250 million returns annually to the three economies with even much revenues accruing from local and regional markets (Odongkara et al. 2009).

Nile perch is not native to Lake Victoria; it was introduced into the lake beginning in the mid 1950s (Mwanja & Mwanja, 2008). Before the introductions of none-native fish species, Lake Victoria's ecology had high levels of fish faunal biodiversity, with over 500 fish species, of which 90% were cichlids (genus haplochromis) (Graham, 1929; Witte et al. 1992). In East Africa, Nile perch are native to Lakes Albert and Turkana (Worthington and Ricardo, 1936), which are the putative origins of introductions into lakes Victoria, Kyoga and Nabugabo (Ogututu-Ohwayo, 1993; Hauser 1998). Nile perch and four exotic tilapias (originally not found in the region including *Oreochromis niloticus*, *O. leucostictus*, *Tilapia zillii*, and *T. rendalli*) were introduced into Lake Victoria beginning in the 1950s for purposes of bolstering then failing fisheries of the Lake and for the possible use of Nile perch as a sport fishery on the vast Lake Victoria (Ogututu-Ohwayo, 1993). Nile perch were first introduced into Lakes Kyoga and Nabugabo, which had similar fauna like Lake Victoria for trial purposes before introduction into Lake Victoria (Gee, 1964). But before the trials were concluded Nile perch had found its way into the lake and subsequently the then authorities ordered for more Nile perch introductions into Lake Victoria in early 1960s (Ogututu-Ohwayo, 1993). For this purpose, about 400 Nile perch fish individuals were used for the introductions into Lake Victoria in the early 1960s (Gee, 1969; Hauser et al. 1998).

Nile perch slowly colonised its new habitat for the first 20 years and then exploded in the 1980s (Mwanja, 1981; Okaronon et al. 1985; Goudswaard & Ligtvoet, 1988), currently has a Lake-wide distribution and dominates the fishery of the lake. The Nile perch together with Nile tilapia (*Oreochromis niloticus*) the other introduced species and mukene (*Rastrineobola argentea*), are the major commercial fisheries of Lake Victoria (Taabu et al. 2010). In the novel environs, Nile perch genetics and distribution may have been affected by factors such as the vast size of the lake, microgeographic variation within the Lake, the mode of colonisation, extension of

at range by the Nile perch to cover the whole Lake, prey distribution and abundance and other effects due to small numbers used for the introductions. These factors could have reduced Nile perch's genetic variation causing possible population structuring and genetic differentiation within a seemingly uniform lake without physical barriers to fish dispersal (Mwanja & Mwanja, 2008).

Earlier attempts to define the population structure of Nile perch in Lake Victoria by Hauser et al. (1998) and Mwanja & Mwanja, (2008) indicated that the species was genetically differentiated, but the markers they employed were limited in power to completely define the species genetic population structure in the lake. Similarly Bwathondi (1985) suspected population structuring of Nile perch in Lake Victoria, just as was alleged to be occurring in the native environs by earlier evolutionary ecologists (Worthington & Ricardo, 1936; Gee, 1969; Ribbink, 1987; Harrison, 1991). These ecologists believed Nile perch in its native environs, lakes Albert and Turkana, were structured into two different populations, one a smaller morph, deep open water dwelling and the other a bigger morph, shallow water, inshore dweller (Worthington & Ricardo, 1936; Gee, 1969; Ribbink, 1987; Harrison, 1991). Unfortunately to date there is no systematic study that has definitely clarified the population structure of the Nile perch in Lake Victoria yet this is important for the effective management of the region's fisheries considering that the lake is not only extensive in area, but also shared and managed by three different riparian countries.

Genetic resources can be exploited sustainably only when the fishery stocks are defined (Munierjee et al. 2008, Carvalho & Hauser, 1994) and the first step to conserve, manage and sustainably exploit genetically divergent stocks of fish species is to identify such stocks (Worthington, 2003). Population genetic analysis is a useful tool in fisheries management with a number of applications including ability to determine whether a fishery is panmictic or is composed of different subunits/stocks, the spatial distribution of genetic variation, elimination of influence of environment that affects meristic and morphometric characters, genetic stock identification, studying impacts of fish introductions, genetic marking, forensics and population viability analysis (Shaklee & Bentzen, 1998; Utter, 2003).

Microsatellite markers are highly polymorphic with codominant inheritance (Chistiakov et al. 2006). Their polymorphism has enabled revelation of adequate information for effective management of fish stocks (Alam & Islam, 2005). The hypervariability of microsatellites makes

especially useful for recently isolated populations and detection of fine-scale genetic differentiation (van Oppen et al. 1997). Microsatellite DNA variations reveal the amount of genetic diversity, which is expected to decrease during rapid population size reductions (genetic bottlenecks) common with invasive species introductions, and increase when the primary introduction expands and establishes a population in the new environs (Rollins et al. 2009). The Nile perch introduced just over five decades ago, using about 400 individuals slowly colonised novel environments and later exploded its population with a Lake-wide distribution. This kind of species suits the above category of invasive species and its genetic diversity can be best explored using the microsatellite DNA markers.

In this study, we assessed the microsatellite variability within Nile perch populations, the extent of hierarchical genetic differentiation at intrapopulation, regional, and whole-species levels, and the comparison of genetic relationships with geographical relationships among populations in Lake Victoria. We also discuss how the outcomes can be used to design better management strategies for the sustainable exploitation of the Nile perch fishery, to stem its decline and the predicted population crash (Lowe-McConnell, 1994) and devise ways for possible turn round for the fishery.

Study area, materials and methods

1 Study area

Lake Victoria (**Figure 1**) with a highly indented shoreline of 3,440 Km and many isles has a surface area of 68,800 Km², making it Africa's largest lake by area, the largest tropical lake and second largest freshwater lake in the world (Kendall, 1969). It is situated between longitude 33° 40' E to 34° 50' E and latitude 3° 40' S and 0° 30' N. The lake is shared by three riparian countries in the following order, Tanzania (51%), Uganda (43%), and Kenya (6%). Its average depth is 40m and maximum depth 84m. The lake's fish fauna previously included mostly cichlid species as the endemic species (Graham, 1929). Nile perch introduced into the lake only over five decades ago currently dominates the fishery of Lake Victoria with a Lake-wide distribution, occurring in almost every habitat (Witte et al. 1992).

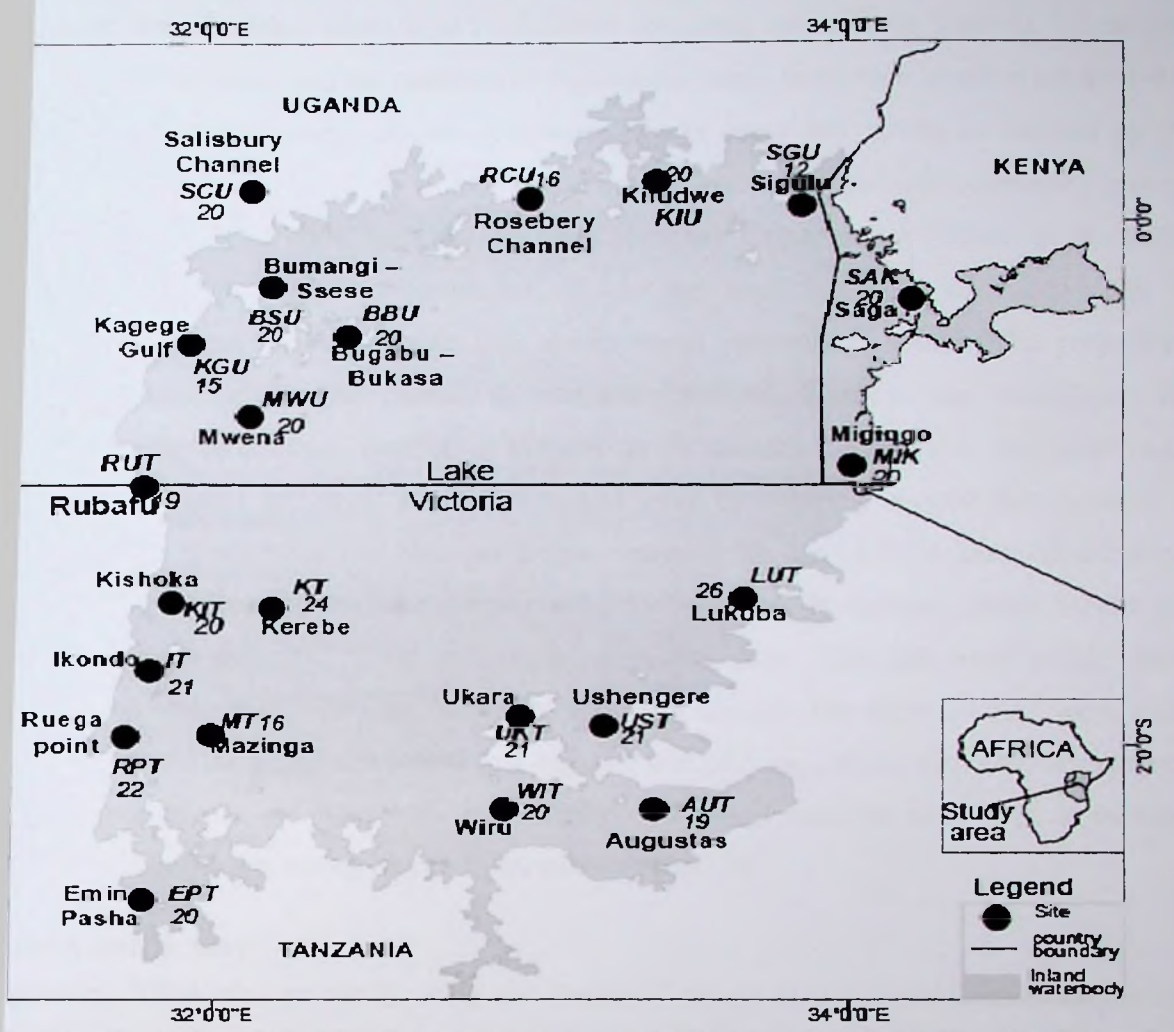


Figure 1: Map of Lake Victoria showing the different sampling sites (round black dots) for Nile and number of samples collected from each site.

Sampling

Nile perch samples were taken from 22 different locations within Lake Victoria, whose map of the different locations and the numbers of individuals taken from each location are shown in Figure 1. These locations were chosen following clearly identified stocks as defined by the hydroacoustics preliminary findings during bi-annual hydroacoustic and environmental surveys carried out from 2000 to 2006 by Lake Victoria Fisheries Organisation (Taabu et al. 2010). Fisheries hydroacoustics involves estimation of biomass and abundance of pelagic fish by determining parameters of the pelagic fish stocks using transmitted sound. The parameters provide initial data for forecast models to estimate fish size, location, age, abundance and biologically sustainable catch quantities (Taylor & Maxwell, 2007). The transects/routes followed by the vessel we used, were determined after considering several factors such as traditional fishing grounds of the Nile perch, previous knowledge of Nile perch distribution, previous tagging studies and the lake's topography (Tumwebaze & Getabu, 1999). Stocks and their locations were delimited using prior hydro-acoustics data. The fish were caught using bottom trawling technique following the hydro acoustics signals. Fin clips 2-4 cm² were taken from each sampled Nile perch fish stored in a separate vial of 5 cm², filled with absolute ethanol, which was drained off one hour later and refilled. On board samples were kept at ambient temperature and in the laboratory before DNA extraction at -80°C.

2.3 DNA extraction

Total genomic DNA was extracted using the Dneasy™ tissue kit (QIAGEN) according to the manufacturer's instructions. The DNA extracts were dissolved in 300 µl of elution buffer and stored at -20°C. To test for success of the DNA extracts, 4µl of the total genomic DNA was electrophoresed on 2 % agarose gel stained with Ethidium bromide (EtBr) and then visualized under ultra violet (u.v) light for clear DNA bands.

2.4 Microsatellite DNA Analysis

Microsatellite markers originally developed for sister species *Lates calcarifer* were used for the amplification and identification of polymorphic products in the Nile perch. Thirty seven different microsatellite loci developed for *Lates calcarifer* (Zhu et al. 2006a, 2006b; Genhua Yue et al. 2001) were screened and optimised for use on the Nile perch, only nine were found to be

polymorphic. These nine that were used include *Lca70*, *Lca74*, *Lca98*, *Lca147*, *Lca165*, *Lca171*, *Lca185*, *Lca193* and *LcaM01*. Amplification of each sample was performed in 20 µl reaction mixture containing 1X PCR buffer, 1.15 mM MgCl₂, 1 mM dNTP for both forward and reverse primers, 0.08 U Taq DNA polymerase and 50ng template genomic DNA. Amplifications were carried out in an Eppendorf master cycler under the following conditions that involved a Touchdown (TD) PCR. TD protocol consisted of 1 cycle at 94 °C for 5 min, 1 cycle of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s, followed by 9 cycles reducing the annealing temperature from 65 °C to 55 °C at 1 °C steps for each cycle, and finally 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. Samples were genotyped by BioScience (UK), on an ABI 3730 (Applied Biosystems) using GS-500 (Liz) in each capillary as a size standard. Allele sizes were estimated on GeneMapper version 3.7 (Applied Biosystems).

4.3 Data Analysis

The software MICRO-CHECKER 2.2.1 (van Oosterhout et al. 2004) was used for identifying possible genotyping errors (i.e. stuttering, large allele dropout and null-alleles) within the microsatellite data set by performing 1000 randomizations.

4.3.1 Genetic diversity. This was assessed by evaluating of Hardy-Weinberg (H-W) expectations, linkage disequilibrium, inbreeding coefficients, total number of alleles per locus, heterozygosity both expected and observed and the allelic richness per locus per sample. The GENEPOP 4.0 software package (Rousset, 2008) was used to test for deviations of genotypic distributions from H-W (exact tests; Guo & Thompson, 1992), linkage disequilibria (LD), and for estimating inbreeding coefficients F_{IS} . The program CONVERT 1.3.1 (Glaubitz, 2004) was used to calculate allele frequencies. ARLEQUIN 3.5 (Excoffier and Lischer, 2010) was used for estimation of the observed (H_O) and expected (H_E) heterozygosities. The software FSTAT 2.9.3.2 (Goudet, 2001) was used to estimate number of alleles (A) at each locus of each sample and the allelic richness per locus per sample (AR), which represents the number of alleles independent of sample size (Mousadik & Petit, 1996).

4.3.2 Genetic structure. ARLEQUIN 3.5 (Excoffier & Lischer, 2010) was used to test for heterozygosity excess and deficiency (exact tests), to determine allele frequency divergence and to calculate Analysis of Molecular Variance (AMOVA). AMOVA was used to partition genetic

variance hierarchically within and among populations using F -statistics (Weir & Cockerham, 1984). For AMOVA the populations were grouped according to geographical distribution into Uganda, Kenya and Tanzania populations. The significance of the genetic structure generated from AMOVA was tested by comparing observed values to null distribution generated by permutation using 10,000 replicates. The genetic relationships among samples were visualised by multidimensional scaling (MDS) of F_{ST} values using the program PAST version 2.13 (Hammer et al. 2001). Populations were ordinated according to the first and second axes.

To further analyse the Lake Victoria Nile perch genetic structure, we used the Bayesian based model-clustering in the program STRUCTURE 2.3.1 (Falush et al. 2003), which involved conducting genotypic population assignment (MCMC = 10,000 generations; burnin = 10,000; and iterations = 20). To infer the genetic groups sampled we analysed the results of STRUCTURE using ΔK method (Evanno et al. 2005). We used the admixture model with correlated allele frequencies, 20 replicates and set the number of genetic groups from $K = 1$ to $K = 25$ for each level of analysis. The modal value of ΔK was taken as the indicator of ancestral population clusters or genetic groups (Evanno et al. 2005). For calculating ΔK we used median rather than mean likelihoods to minimise the effects of outlier runs. After determining the number of genetic groups (the chosen K value), each sampled individual Nile perch was assigned to one of the groups according to the highest ancestry proportion assigned by STRUCTURE.

4.4 Results

4.4.1 Microsatellite DNA polymorphisms

Out of the 37 microsatellite loci screened, 30 (81.08%) were successfully amplified, but only 9 were found to be polymorphic and therefore usable on the Nile perch. Their primer sequences, number of alleles, allele sizes and references are listed in **Table 1**.

Table 1: Primers used to amplify the 9 microsatellite loci in Nile perch (*Lates spp.*), A is number of alleles per locus for the sister species *Lates calcarifer*.

Microsatellite DNA locus	Motif	Primers (5' → 3')	A	Size range (Asian sea bass)	Size range (Nile perch)	References
Lca70	(CAG) ¹⁷	CATCATTACACTCTGTTGCCTC GACAGA CAG GTG TTT TAG CCT ATT TG	7	288 - 308	204 - 303	Zhu et al., 2006a
Lca165	(TG) ¹²	TCCCCTGATGAGAGGAGGTCA GTTACAGCCGTGGATGGACAC	8	258 - 290	202 - 310	Zhu et al., 2006b
Lca98	(TG) ¹⁴	CAA AGG GGC CAC TGC ACA TAA T CTC CAG CTC ACC CAG GTT CAC T	10	186 - 242	184 - 228	Zhu et al., 2006a
Lca147	(TC) ³⁵	TGCCCTTAATGTATTCTTTCCACT GCTCCACCTCTCATTCATTATTC	16	164 - 226	152 - 256	Zhu et al., 2006b
Lca185	(CA) ⁶ (GA) ¹⁹	TTCGCCCTGTTTGCAAAATACATTAG ACACCCAGCATGGCTTCCTAT	10	175 - 215	152 - 232	Zhu et al., 2006b
Lca193	(TC) ²³	CCTCTGCCCTTTTTCATCTATATTGC CACATCGCACAAATGGACTGA	12	140 - 176	148 - 230	Zhu et al., 2006b
Lca171	(TG) ¹³	ATTGCGTTACCAAGAGGTGAA TGCTTTGAAGGCTGAAAACTG	14	294 - 336	250 - 340	Zhu et al., 2006b
Lca74	(CA) ¹³	CAT CAT TTA CAC TCT GTT TGC CTC AT GAC AGA CAG GTG TTT TAG CCT ATT TG	4	160 - 170	160 - 190	Zhu et al., 2006a
LcaM01	(CG) ⁴ (CA) ⁸	AT TCG CTC TTT ATT CTC CC GTC GGC CTT TCA TAT GCA AT	3	260 - 264	250 - 276	Genhua Yue et al., 2001

1.4.2 Genetic diversity. Genotypic distribution at all loci showed significant deviation from H-W (heterozygosity excess) in a few populations, in 21 out of the 198 tests (**Table 2**). At locus Lca70 in MT and UKT, Lca165 in BBU, KT, MT, RCU, and UST, Lca98 in RCU, SCU and UST, Lca147 in MIK, MWU, SAK, SCU, UKT and WIT, Lca185 in AUT, MWU and UST, Lca185 in AUT, Lca193 in UST, Lca171 in WIT, Lca74 in MT, LcaM01 in AUT (**Table 2**). All these outcomes were due to heterozygosity deficiency. The global test for all locus and all populations was significant ($P=0.000$, $S.E=0.000$). MICRO-CHECKER software revealed possible existence of null alleles for 6 loci but in 11 out of the 22 populations. These included Lca147 in UKT, SAK, MWU and UST, Lca193 in UST and MT, Lca165 in UST, RCU, MIK, KT and BBU, Lca185 in UST, Lca98 in EPT and AUT, LcaM01 in AUT. In all these cases there were fewer heterozygotes than expected, we therefore adjusted for null allele homozygotes following van Oosterhout et al. (2004) on a locus by locus basis coding them as missing data based on adjusted genotypes by MICRO-CHECKER. However, we did not find any evidence of stuttering or big allele dropout affecting our results, for when we used the adjusted genotypes to calculate test statistics and compared to the original values the overall results did not change. Examination of LD using Fisher's exact test showed no disequilibrium in these loci. Therefore all the 9 loci were included in all analyses. A total of 161 alleles were detected at the 9 loci across the 22 data sets among the 432 individuals. The number of alleles per microsatellite locus within samples varied from 2 at loci Lca70 and Lca147 to 17 at locus Lca165 (**Table 2**). Locus Lca165 was most polymorphic with a total of 30 alleles, while Lca70 was least variable with a total of 12 alleles. Allelic richness (AR) per locus and sample varied from 1.46 at locus Lca147 in RUT to 7.41 at locus Lca171 in SGU, (**Table 2**). The average AR over all loci for the different populations ranged from 3.65 for population KGU to 4.78 for population MT. Average observed heterozygosities, H_O , varied from 0.07 in AUT at LcaM01 to 1.00 in RUT and RPT at locus Lca171 and in KIT at locus Lca98 and average expected heterozygosities, H_E , varied from 0.10 in RUT at locus Lca147 to 0.93 in MT at locus Lca165 (**Table 2**). The average H_E over all loci for the different populations ranged from 0.64 for populations BSU, MWU and RUT to 0.76 for population MT. The highest level of genetic diversity was detected in population MT ($AR = 4.78$, $H_E = 0.76$). The H_E over all samples was 0.68 and the H_O over all samples was 0.64 (**Table 3**), indicating a general deficiency in heterozygosity.

Table 2: Genetic variability indices for nine microsatellite loci of the Nile perch sampled from 22 different geographical locations within L. Victoria. A: number of alleles; AR: allelic richness; H_E expected heterozygosity; H_O observed heterozygosity; F_{IS} inbreeding coefficient; n: sample size; HW: probability values of concordance with Hardy-Weinberg expectations

Population		Locus										Average		S.E
(n)		Lca70	Lca165	Lca98	Lca147	Lca185	Lca193	Lca171	Lca74	LcaM01				
AUT (19)	A 2	5	3.76	4.80	3.05	3.93	3.71	3.74	5.35	5	5.33	0.71		
	AR	1.99								3.50	3.76	0.32		
	Size range	294-297	212-294	204-214	164-236	154-174	154-182	268-330	162-190	254-272				
	H _E	0.50	0.69	0.82	0.51	0.69	0.70	0.73	0.79	0.45	0.65	0.05		
	H _O	0.42	0.68	0.56	0.37	0.58	0.50	0.53	0.90	0.07	0.52	0.07		
	F _{IS}	0.16	0.02	0.33	0.28	0.17	0.29	0.28	-0.14	0.82	0.25	0.26		
	HW	0.402	0.639	0.005	0.023	0.001	0.134	0.041	0.983	0.000				
BBU (20)	A 2	11	5	3	3	3	4	9	8	5	5.56	1.03		
	AR	2.0	6.27	3.68	2.45	2.79	3.33	4.65	5.02	2.89	3.68	0.46		
	Size range	294-297	206-296	194-212	232-236	156-170	154-182	256-336	162-190	258-276				
	H _E	0.52	0.88	0.73	0.37	0.59	0.67	0.77	0.82	0.50	0.65	0.06		
	H _O	0.55	0.50	0.71	0.43	0.63	0.74	0.85	0.95	0.65	0.67	0.06		
	F _{IS}	-0.05	0.44	0.03	-0.16	-0.07	-0.11	-0.11	-0.16	-0.30	-0.05	0.21		
	HW	0.787	0.000	0.397	1.000	0.370	0.745	0.907	0.920	1.000				
BSU (20)	A 4	8	6	3	3	5	5	7	9	5	5.78	0.64		
	AR	2.87	4.36	3.63	2.41	3.86	3.27	4.16	5.72	3.04	3.70	0.33		
	Size range	294-303	204-294	194-212	156-234	156-170	154-182	256-336	162-190	258-276				
	H _E	0.57	0.75	0.70	0.39	0.72	0.61	0.72	0.86	0.43	0.64	0.05		
	H _O	0.71	0.75	0.55	0.35	0.80	0.70	0.80	0.85	0.35	0.65	0.06		
	F _{IS}	-0.25	0.00	0.22	0.10	-0.12	-0.14	-0.11	0.01	-0.11	-0.04	0.14		
	HW	0.927	0.183	0.113	0.031	0.808	0.873	0.872	0.323	0.042				
EPT (20)	A 2	9	5	5	2	5	7	7	11	5	5.89	0.99		
	AR	2.00	5.62	4.46	1.95	3.19	4.73	4.29	6.19	2.65	3.90	0.51		
	Size range	294-297	206-298	204-214	232-234	156-196	152-182	268-336	162-190	252-276				
	H _E	0.51	0.85	0.80	0.36	0.62	0.77	0.76	0.88	0.33	0.65	0.07		

H ₀	0.55	0.75	0.50	0.44	0.60	0.75	0.75	0.90	0.32	0.02	0.17
F _{is}	-0.08	0.12	0.38	-0.26	0.04	0.03	0.03	-0.03	0.06	0.03	0.17
HW	0.786	0.374	0.006	1.000	0.418	0.077	0.598	0.440	0.459		
IT (21)	A	3	8	7	5	4	7	8	9	4	0.72
AR	2.34	4.46	4.50	3.27	3.05	3.91	4.98	5.60	2.87	3.89	0.36
Size range	294-303	204-296	190-214	230-238	156-196	152-182	268-236	162-190	268-276		
H _E	0.53	0.75	0.76	0.63	0.62	0.68	0.80	0.84	0.44	0.67	0.04
H ₀	0.57	0.80	0.70	0.95	0.57	0.57	0.76	0.95	0.29	0.68	0.07
F _{is}	-0.08	-0.07	0.08	-0.52	0.09	0.16	0.04	-0.13	0.36	-0.01	0.08
HW	0.724	0.794	0.241	1.000	0.163	0.032	0.134	0.703	0.063		
KGU (15)	A	2	5	4	5	6	8	7	3	4.89	0.63
AR	2.00	3.52	3.24	3.49	3.77	3.71	5.30	5.02	2.79	3.65	0.34
Size range	294-297	210-286	204-214	156-236	156-172	154-186	268-336	162-186	268-272		
H _E	0.52	0.65	0.64	0.69	0.71	0.68	0.80	0.81	0.55	0.67	0.03
H ₀	0.60	0.73	0.64	0.79	0.80	0.87	0.93	0.73	0.60	0.74	0.04
F _{is}	-0.17	-0.13	-0.00	-0.14	-0.14	-0.29	-0.17	0.10	-0.11	-0.12	0.04
HW	0.881	0.870	0.589	0.433	0.764	0.953	0.696	0.353	0.308		
KIT (20)	A	3	8	6	7	5	6	11	5	6.56	0.77
AR	2.59	3.93	4.15	4.86	3.87	3.60	5.10	6.14	2.88	4.12	0.37
Size range	294-300	210-296	194-214	154-236	156-172	154-182	250-336	162-188	268-276		
H _E	0.57	0.68	0.75	0.81	0.72	0.67	0.82	0.87	0.39	0.70	0.05
H ₀	0.50	0.60	1.00	0.74	0.85	0.84	0.80	0.95	0.39	0.74	0.07
F _{is}	0.13	0.12	-0.35	0.09	-0.18	-0.26	0.03	-0.09	0.01	-0.06	0.06
HW	0.044	0.088	1.000	0.041	0.740	0.984	0.017	0.816	0.640		
KIU (20)	A	2	8	5	6	7	5	9	4	5.56	0.73
AR	1.97	4.75	4.06	4.14	4.78	2.94	3.30	5.83	2.59	3.82	0.41
Size range	294-297	206-298	204-214	156-236	156-198	152-182	268-330	162-190	268-276		
H _E	0.39	0.77	0.77	0.74	0.80	0.59	0.65	0.87	0.38	0.66	0.06
H ₀	0.30	0.65	0.80	0.85	0.90	0.45	0.78	0.95	0.44	0.68	0.08
F _{is}	0.23	0.16	-0.04	-0.15	-0.13	0.24	-0.17	-0.10	-0.17	-0.02	0.06
HW	0.320	0.112	0.671	0.369	0.210	0.158	0.945	0.960	1.000		

KT (24)	A	3	10	8	2	10	9	9	12	
AR	2.21	5.12	5.54	1.99	4.88	5.11	5.20	6.22	3.81	4.45
Size range	294-303	232-296	200-214	232-236	154-232	152-182	266-336	106-188	250-276	
H _E	0.52	0.80	0.85	0.46	0.79	0.81	0.77	0.88	0.54	0.71
H _O	0.54	0.43	0.90	0.68	0.83	0.83	0.84	0.88	0.48	0.71
F _{IS}	-0.04	0.47	-0.06	-0.50	-0.05	-0.04	-0.09	0.01	0.24	-0.01
HW	0.676	0.000	0.827	1.000	0.505	0.035	0.181	0.552	0.022	
LUT (26)	A	2	5	8	3	9	4	9	12	4
AR	1.99	3.22	5.06	2.61	4.80	3.12	4.86	6.07	2.62	3.82
Size range	294-297	270-296	190-214	232-236	156-198	156-182	266-336	162-190	268-274	
H _E	0.46	0.64	0.81	0.57	0.79	0.67	0.77	0.87	0.44	0.67
H _O	0.31	0.68	0.76	0.90	0.92	0.59	0.71	0.92	0.45	0.69
F _{IS}	0.34	-0.07	0.06	-0.60	-0.17	0.11	0.09	-0.06	0.16	-0.02
HW	0.095	0.058	0.186	1.000	0.981	0.234	0.289	0.871	0.591	
MIK (20)	A	4	6	7	4	7	5	8	9	5
AR	3.16	4.16	5.12	3.34	4.63	3.52	4.95	5.68	2.78	4.15
Size range	291-300	206-294	202-218	156-234	156-174	154-182	266-336	162-190	252-272	
H _E	0.67	0.74	0.83	0.63	0.78	0.68	0.76	0.85	0.48	0.71
H _O	0.58	0.50	0.89	0.41	0.70	0.58	0.84	0.95	0.44	0.65
F _{IS}	0.14	0.33	-0.08	0.35	0.10	0.15	-0.11	-0.13	0.29	0.12
HW	0.029	0.007	0.158	0.001	0.110	0.299	0.397	0.971	0.434	
MT (16)	A	3	12	5	5	8	5	6	11	3
AR	2.93	7.30	4.39	5.00	4.72	4.64	4.68	6.72	2.65	4.78
Size range	294-300	204-298	202-214	156-236	156-210	156-230	254-340	160-190	254-272	
H _E	0.66	0.93	0.80	0.82	0.78	0.82	0.73	0.90	0.39	0.76
H _O	0.23	0.62	0.73	0.40	0.75	0.43	0.67	0.67	0.43	0.55
F _{IS}	0.66	0.35	0.09	0.54	0.04	0.50	0.09	0.27	-0.13	0.27
HW	0.001	0.001	0.099	0.021	0.554	0.013	0.120	0.000	1.000	
MWU(20)	A	3	8	6	5	4	5	7	10	4
AR	2.42	4.71	4.70	2.99	3.54	3.98	4.07	5.44	2.54	3.82
Size range	291-297	268-298	202-214	154-242	156-170	154-182	268-336	162-190	270-274	

H _E	0.45	0.75	0.81	0.43	0.70	0.73	0.72	0.83	0.53	0.04	0.09
H ₀	0.26	0.60	0.79	0.17	0.70	0.65	0.85	0.95	0.37	0.59	0.09
F _{IS}	0.42	0.21	0.03	0.62	0.00	0.11	-0.19	-0.14	0.31	0.15	0.09
HW	0.007	0.177	0.319	0.001	0.605	0.382	0.962	0.971	1.000		
RCU (16) A	3	12	6	7	5	6	8	7	5	6.56	0.84
AR	2.56	6.07	4.14	4.01	3.88	3.95	4.94	5.20	3.30	4.23	0.35
Size range	291-297	202-308	184-214	152-236	156-170	152-182	266-336	162-190	254-274		
H _E	0.58	0.85	0.70	0.56	0.69	0.72	0.80	0.82	0.54	0.70	0.04
H ₀	0.40	0.50	0.47	0.42	0.75	0.63	0.88	0.81	0.63	0.61	0.06
F _{IS}	0.32	0.42	0.34	0.26	-0.09	0.13	-0.10	0.01	-0.16	0.13	0.07
HW	0.015	0.000	0.000	0.056	0.266	0.105	0.511	0.559	0.927		
RPT (22) A	4	8	6	4	4	6	8	11	5	6.22	0.80
AR	3.04	3.92	4.04	2.86	3.52	3.74	4.11	5.81	3.36	3.82	0.29
Size range	267-303	208-298	194-214	156-236	156-170	152-182	252-330	162-190	256-274		
H _E	0.58	0.68	0.76	0.50	0.68	0.70	0.72	0.85	0.54	0.67	0.04
H ₀	0.86	0.57	0.73	0.58	0.73	0.64	1.00	0.86	0.64	0.73	0.05
F _{IS}	-0.52	0.16	0.04	-0.18	-0.07	0.09	-0.41	-0.01	-0.19	-0.12	0.08
HW	0.998	0.107	0.338	0.924	0.711	0.410	1.000	0.755	0.974		
RUT (19) A	3	9	5	2	7	5	9	8	5	5.89	0.84
AR	2.46	5.01	4.01	1.46	4.69	3.25	5.40	5.16	2.92	3.82	0.46
Size range	294-300	204-296	204-214	232-234	152-172	154-182	256-336	162-190	256-276		
H _E	0.55	0.81	0.76	0.10	0.79	0.65	0.83	0.83	0.46	0.64	0.08
H ₀	0.47	0.74	0.90	0.11	0.84	0.68	1.00	0.84	0.28	0.65	0.10
F _{IS}	0.14	0.09	-0.18	-0.03	-0.07	-0.05	-0.22	-0.01	0.40	0.01	0.06
HW	0.019	0.324	0.965	1.000	0.385	0.708	1.000	0.648	0.052		
SAK (20) A	2	10	8	6	8	6	10	7	6	7.00	0.82
AR	2.00	5.07	4.54	3.65	4.66	3.94	5.72	4.78	3.38	4.19	0.36
Size range	294-297	202-298	194-228	156-232	152-180	152-182	252-332	162-190	252-274		
H _E	0.48	0.80	0.78	0.62	0.78	0.71	0.86	0.80	0.53	0.71	0.04
H ₀	0.45	0.80	0.70	0.26	0.95	0.58	0.85	0.79	0.55	0.66	0.07
F _{IS}	0.07	-0.00	0.10	0.58	-0.22	0.19	0.01	0.01	-0.05	0.08	0.07

Size range	291-300	202-298	204-214	156-234	156-198	154-182	250-336	162-190	208-274
H _E	0.71	0.88	0.78	0.70	0.69	0.63	0.52	0.85	0.22
H ₀	0.40	0.60	0.68	0.20	0.70	0.63	0.28	0.71	0.12
F _{is}	0.44	0.33	0.12	0.72	-0.01	0.01	0.47	0.18	0.48
HW	0.007	0.007	0.090	0.002	0.074	0.612	0.002	0.104	0.091

4.3 Population structure. Pairwise F_{ST} estimates for all loci ranged from -0.00045 (between KT and UST) to 0.0754 (between KT and WIT). Out of 231 pairwise comparisons 131 (6.71%) F_{ST} values were significant ($P < 0.05$). The global tests for genic and genotypic differentiation for all populations across all loci were all significant ($X^2 = \text{infinity}$, $df = 18$) with global $F_{ST} = 0.029$. When samples were pooled into three different groups according to the region or country waters of the lake from which they were sampled, results of AMOVA analysis indicated that there was no genetic clustering according to the different regions – Kenya, Tanzania and Uganda, with 0.07% of the genetic variation attributed to among groups ($P = 0.33$). There was slight indication of genetic differentiation among populations within the groups ($P = 0.00$) with 2.34% of the genetic variation, and 95.83% of the genetic variation attributed to within individuals ($P = 0.00$) (**Table 4**). Likewise, the derived stimulus Euclidean distance model for quick analysis using F_{ST} in a MDS procedure, which shows related samples together and differentiated ones apart, its plot based on pairwise F_{ST} values among all samples did not indicate any regional clustering (**Figure 2**). Some populations that are geographically far from each other and different regions of the Lake such as KIU in Uganda and EPT in Tanzania, RCU in Uganda and RUT in Tanzania, EPT and SAK in Kenya (**Figure 1**) were indicated as genetically near each other and clustered together (**Figure 2**), indicating absence of regional structuring of Nile perch populations in Lake Victoria. The MDS plot also indicates that the most related were RUT in Tanzania and RCU in Uganda, farthest from each other on the plot and therefore least related were KIT & UKT both in Tanzania, though not the most geographically separated. The two principal axes together explained 69.18% of the total genetic variation. The first principal axis explained 51.77% of the variance among populations and the second axis accounted for 17.41% of the variance. The median maximum likelihood (ML) of the data given a modal estimate of $K = 1-25$ populations is shown in Figure 3. The ΔK Bayesian clustering analysis following the procedure of Evanno et al. (2005) indicates that a $K = 2$ model best fits the data ($\Delta K = 26.98$ for $K = 2$, $\Delta K < 5.88$ for all other K values). This suggests that the Nile perch of Lake Victoria clusters into two genetic groups. According to the highest ancestry proportion assigned by STRUCTURE, one group was more dominant (**Figure 4**) in the catch than the other, with one cluster assigned 259 and the other 173 individuals. All geographical populations had both individuals from both genetic groupings.

Table 3: Summary descriptive statistics of microsatellite data for the samples from the different geographical locations of L. Victoria showing sample size (N), mean number of alleles (A), expected heterozygosity (H_E), observed heterozygosity (H_O), mean allelic size range (AS), Garza-Williamson index (M).

Population	Statistics					
	N	A	H_E	H_O	AS	M
UT	19	5.33	0.67	0.51	18.33	0.48
BU	20	5.56	0.65	0.67	15.78	0.51
SU	20	5.78	0.64	0.65	20.11	0.44
PT	20	5.89	0.65	0.62	16.44	0.56
T	21	6.11	0.67	0.69	16.89	0.52
GU	15	4.89	0.67	0.74	17.33	0.51
IT	20	6.56	0.70	0.74	19.56	0.54
IU	20	5.56	0.66	0.68	19.67	0.48
ET	24	8.11	0.71	0.71	17.56	0.63
UT	26	6.22	0.67	0.69	12.67	0.64
MIK	20	6.11	0.71	0.66	19.67	0.48
MT	16	6.44	0.76	0.55	25.11	0.40
WU	20	5.78	0.64	0.59	15.44	0.57
RCU	16	6.56	0.70	0.61	21.44	0.43
RPT.	22	6.22	0.67	0.73	21.22	0.38
RUT	19	5.89	0.64	0.65	15.78	0.57
SAK	20	7.00	0.71	0.66	22.11	0.44
SCU	20	6.22	0.71	0.65	19.89	0.51
SGU	12	6.11	0.69	0.64	12.22	0.61
UKT	21	7.00	0.71	0.63	22.11	0.45
UST	21	7.33	0.73	0.62	21.44	0.45
WIT	20	6.89	0.67	0.48	24.22	0.44
Overall	432	6.25	0.68	0.64	18.86	0.50

Table 4: Analysis of molecular variance by distance method using number different of alleles (F_{ST}) for the Nile perch microsatellite data (Wier & Cockerham, 1984; Excoffier, Smouse, and Quattro 1992).

Source of variation	df	SS	Variance components	% variation	F-statistics	P
Among groups	2	4.809	0.00081	0.07	$F_{CT} = 0.0007$	0.3294
Among populations within groups	19	41.981	0.02702	2.34	$F_{SC} = 0.0234$	0.0000
Among individuals within populations	410	471.332	0.02040	1.76	$F_{IS} = 0.0181$	0.1139
Within individuals	432	479.000	1.10880	95.83	$F_{IT} = 0.0417$	0.0020
Total	863	997.123	1.15703	100		

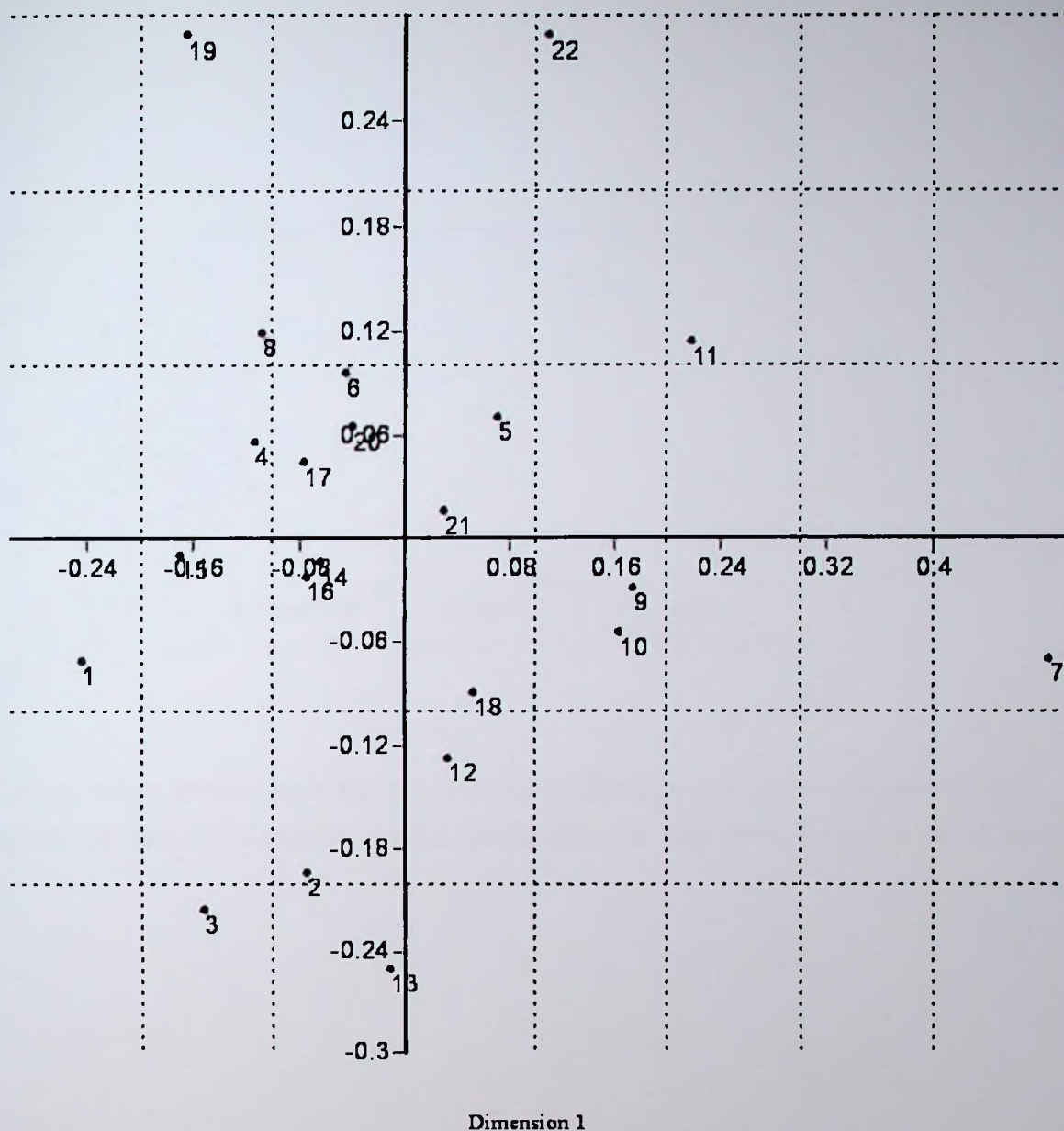


Figure 2: Multidimensional scaling plot of pairwise F_{ST} values among samples of Nile perch collected from 22 different geographical locations within Lake Victoria. Each point represents a geographical population serially numbered as follows: AUT-1, BBU-2, BSU-3, EPT-4, 1T-5, KGU-6, KIT-7, KIU-8, KT-9, LUT-10, MIK-11, MT-12, MWU-13, RCU-14, RPT-15, RUT-16, SAK-17, SCU-18, SGU-19, UKT-20, UST-21, WIT-22.

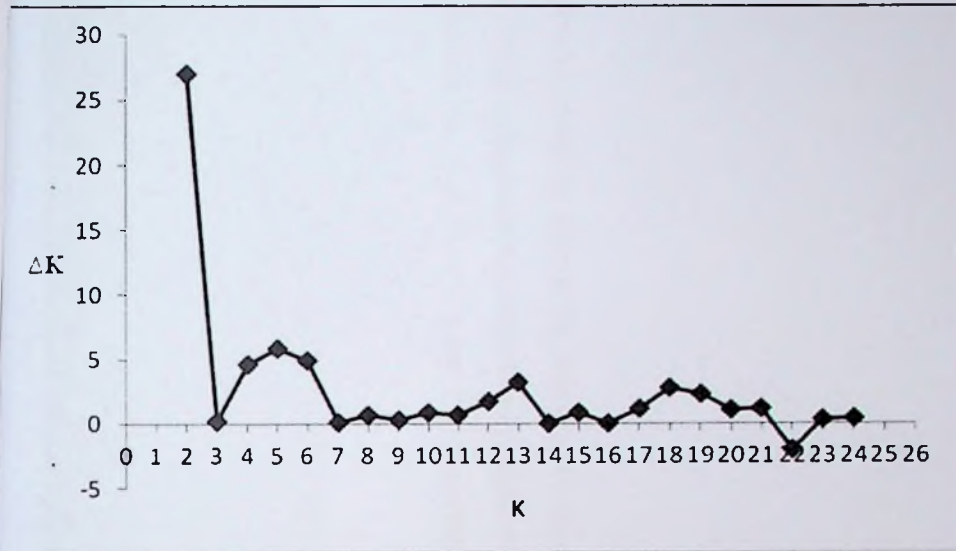


Figure 3: ΔK values (a measure of the rate of change in the structure likelihood function) as a function of K , the number of putative genetic groupings of the Nile perch in Lake Victoria, $K=2$.

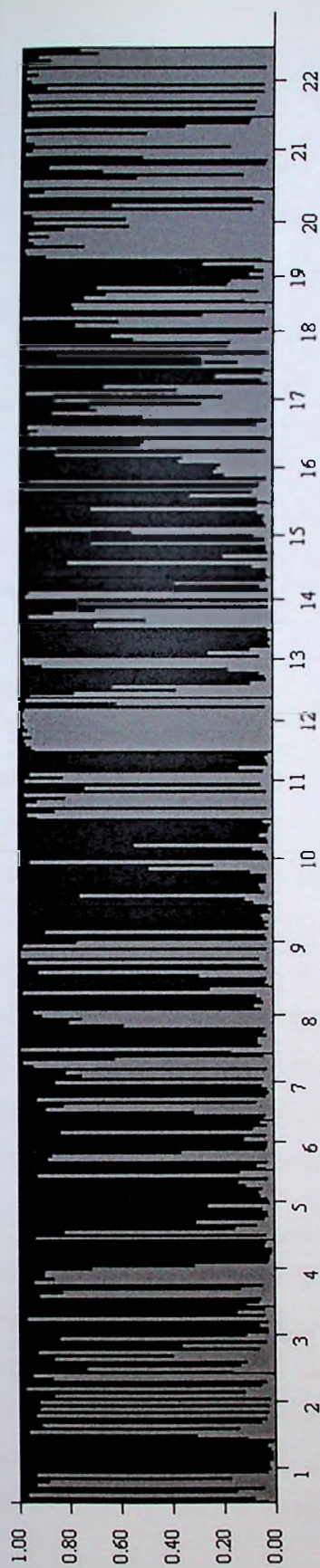


Figure 4: Bayesian analysis of the genetic structure based on nine microsatellite loci. Each individual is represented by a vertical line, which is coloured according to the proportion of membership of each pre-defined population in each of the 2 clusters assigned groups at the estimated $K = 2$ (See **Figure 3**). X-axis represents the ancestry proportion whereas the Y-axis are the 22 populations serially numbered as indicated in **Figure 2**.

4.5 Discussion

4.5.1 Genetic diversity

The Nile perch, having been introduced into Lake Victoria only five decades back and using a small founding population, its genetic diversity is expected to be low due to founder effects and population bottlenecks characterised by reduced heterozygosity, loss of rare alleles, genetic drift and selection processes (Allendorf and Lundquist, 2003; Dlugosch & Parker, 2008). However, the findings in this study are not consistent with this expectation. In this study the findings indicated that Nile perch had relatively high genetic diversity. Significant heterogeneity was found among the collections of Nile perch from the different geographical locations of the vast Lake Victoria. The microsatellite markers used were all polymorphic with an average allele number alleles/locus across all data sets comparable to the average found in freshwater fishes (DeWoody & Avise, 2000) suggesting that there is substantial genetic diversity within the Nile perch. The overall gene diversity as measured by expected heterozygosity (H_E) of the Nile perch of Lake Victoria was also above average when compared to other freshwater fishes (0.62, averaged from 105 species) (Mccusker & Bentzen, 2010), further confirming that there is relatively high genetic diversity among the Nile perch of Lake Victoria. This relatively high genetic diversity is most probably attributed to stealth repeated introductions of the Nile perch into the lake (Pringle, 2005). Multiple introductions are known to augment genetic diversity of invasive species (Darling et al, 2008; Dlugosch & Parker, 2007) and thereby aiding their establishment. In addition the versatile and migratory nature of the Nile perch (Mkumbo and Ligtvoet, 1992), the then abundant prey, with the preferred prey species being the originally abundant cichlids (Graham, 1929; Gee, 1969) and the species high fecundity may have contributed to the species successful establishment and its ecological dominance.

4.5.2 Population structure

Identification of management units is very important to the effective management of wild resources and is crucial to monitoring anthropologic effects on the species abundance (Darwall, 2005; Ferguson, 2004). The lack of biological information to delineate appropriate management units hinders the effective management of a fishery especially one like the Nile perch fishery whose yields are said to be declining and facing intense fishing pressure (Taabu, 2010; EfD, 2008; Witte et al. 2007). In this study our main purpose was to detect genetic variation and its

distribution in a bid to definitely define the population structure of the Nile perch fish of Lake Victoria. We found significant genetic differentiation among the Nile perch populations of Lake Victoria, which is consistent with that reported in previous studies (Hauser et al. 1998; Mwanja & Mwanja, 2008). The observation of genetic heterogeneity among Nile perch in Lake Victoria suggests existence of independent sub-populations of Nile perch with limited genetic exchange. The existence of relatively high levels of genetic diversity and the indication of genetic differentiation among the Nile perch of Lake Victoria are interesting as well as perplexing findings. For Nile perch has only had limited time for isolation in its novel environs, having been introduced only over five decades back (Mwanja & Mwanja, 2008). In addition, Nile perch was introduced using small numbers (Hauser et al. 1998) that slowly colonised and exploded into a very large population (Ogutu-Ohwayo, 1994) that may not be very sensitive to genetic drift (DeWoody & Avise, 2000). This further compounded by the very high fecundities of the Nile perch of several millions of eggs per female spawn in the open waters (Ogutu-Ohwayo, 1994), the migratory nature of this species (Mkumbo & Ligetvoet, 1992) and the long range dispersal typical of large pelagic fishes (Waples, 1998), factors work against population structuring. The cause of the population structuring of the Nile perch of Lake Victoria may be more of cryptic processes such as water mixing/circulation patterns, prey distribution patterns and the heightened human exploitation rather than physical barriers for Lake Victoria is largely open (Kendall, 1969).

Two underlying genetic groupings detected in the Nile perch with all sampled populations leaving both groupings in differing proportions most probably is an indicator that Nile perch is structured into two distinct genetic groupings. These findings are consistent with our earlier morphology findings (Mwanja et al. 2011) and those other ecological biologists who suggested existence of two populations in the putative origins of the Nile perch – Lakes Turkana and Albert (Harrison, 1991; Gee, 1969; Worthington & Ricardo, 1936) and Bwathondi (1985) who suspected formation of two underlying populations of Nile perch in Lake Victoria. After the assignment of individuals to the two groups we found that one of the two genetic groups had more individuals making it more dominant than the other, which is consistent with our earlier Nile perch morphology studies (Mwanja et al. 2011). These findings suggest that most probably two taxa of Nile perch became established in Lake Victoria after its introduction. Most probably the Nile perch exists as two sub-species in Lake Victoria, taxa that require mapping and further

studying to determine whether they are following same or different trends of population structuring and would require separate management regimes.

The analyses of Garza & Williamson (2001) indicate that M values < 0.68 support the occurrence of a past reduction in population size. In a bottleneck population the reduction in number of alleles is expected to be much more than the reduction in allelic range and therefore the ratio of between number of alleles and allelic size range is expected to be smaller in recently reduced populations than in equilibrium populations (Garza & Williamson, 2001). The M value for all of the Lake Victoria Nile perch geographical populations combined was below 0.68 supporting a past genetic bottleneck. Indeed Nile perch of Lake Victoria just recently introduced using small founder population should have experienced a population bottleneck.

4.6 Implications for fisheries management

In most cases fisheries managers would require hard stock boundaries or stock boundaries that are concordant with pre-existing fisheries jurisdictions (Broderick, et al. 2011), which is hard to achieve in vast open waters such as Lake Victoria with especially a migratory species like Nile perch (Mkumbo & Ligtoet, 1992). From the findings of this study there is no regional population subdivision of the Nile perch or partitioning according to state jurisdictions. Therefore the most efficient and sustainable way to manage the Nile perch fishery of Lake Victoria is to do it cooperatively. A coordinated management of the fishery by the three riparian countries is best way to sustainably exploit the Nile perch fishery of this lake. Nile perch in Lake Victoria is genetically differentiated and most probably belongs to two subspecies, which calls for urgent mapping of the different subspecies and stocks and design appropriate management regimes for the fishery in Lake Victoria. The genetic diversity of Nile perch in Lake Victoria is relatively high to support sustainable exploitation. But there is need to routinely genetically monitor the Nile perch and other species (endangered prey) to follow their genetic diversity trends for the sustainable exploitation of the Nile perch and conservation of the native fishery of Lake Victoria.

4.7 Acknowledgements

This study was part of a bigger project Nile perch technologies sponsored by the World Bank Millennium Science Initiatives (MSI) under the Uganda National Council of Science and Technology (UNCST) – the preliminary study to domesticate the Nile perch. We are thankful to

Environmental Economics unit of Gothenburg University, Sweden for their partial sponsorship to support part of the genotyping services. We are grateful to Lake Victoria Fisheries Organisation for having allowed us on board the Explorer vessel during their Lake wide stock assessment on Lake Victoria and to the crew of the Explorer vessel from Tanzania Fisheries Resources Research Institute (TAFIRRI) that was used during the bottom trawls on Lake Victoria. We also indebted to technicians Mapunda and Ogaro from TAFIRRI, Odada from Kenya Marine and Fisheries Research Institute, for their assistance during the genetics sampling during the Lake-wide survey and sampling.

4.8 References

- Alam, S., & Islam, S. (2005). Population genetic structure of *Catla catla* (Hamilton) revealed by microsatellite DNA markers. *Aquaculture* 246, 151-160.
- Allendorf, F. W., & Lundquist, L. L. (2003). Introduction: Population Biology, Evolution, and Control of Invasive Species. *Conservation Biology*, 17: 24-30.
- Arunga, J. O. (1981). A case study of lake Victoria Nile perch *Lates niloticus* (Mbuta) fishery.165-184. In: Proceedings of the workshop of the Kenya Marine and Fisheries Research Institute on aquatic resources of Kenya, 13-19 July 1981, Mombasa. Kenya Marine and Fisheries Research Institute and Kenya National Academy for Advancement of Arts and Sciences.
- Banerjee, T., Raj, K. D., & Misra, V. (2008). Conservation of Natural Fish Population. Sengupta, M and Dalwani, R (eds). Proceedings of Taal2007: The 12th World Lake Conference: 562-567.
- Billington, N. (2003). Mitochondrial DNA. E. M. Hallerman (ed), pp 59-100. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland, USA.
- Broderick, D., Ovenden, R. J., Buckworth, C. R., Newman, J. S., Lester, G. J. R., & Welch, J. D. (2011). Genetic population structure of grey mackerel *Scomberomorus semifasciatus* in Northern Australia. *Journal of Fish Biology* 79, 633-661.
- Bwathondi, P. O. J. (1985). The future of the fisheries of the Tanzania part of Lake Victoria, in view of the predominance of the Nile perch *Lates niloticus*. F.A.O. fisheries reports 335, pp 143-145.
- Cawdery, S. H. and Ferguson, A. (1988) Origins and differentiation of three sympatric species of trout (*Salmo trutta* L.) in Lough Melvin, Ireland. *Polskie Archiwum Hydrobiologii*, 35; 267-277.
- Carvalho, G. R., & Hauser, L. (1994). Molecular genetics and the stock concept in fisheries. Special Issue of Reviews in Fish and Fisheries Biology (GR Carvalho and TJ Pitcher, eds.), 4, 351-373.

- Chistiakov, D. A., Hellemans, B., & Volckaert, F. A. M. (2006). Microsatellites and their genomic distribution, evolution, function, and applications: a review with special reference to fish genetics. *Aquaculture* 255, 1-29.
- Darling, A. J., Bagley, J. M., Roman, J., Tepolt, K. C., & Geller, B. J. (2008). Genetic patterns across multiple introductions of the globally invasive crab genus *Carcinus*. *Molecular Ecology* 17, 4992-5007.
- Darwall, W. R. T. (2005). Identifying important sites for conservation of freshwater biodiversity: extending species-based approach. *Fisheries Management and Ecology* 12, 287-293.
- DeWoody, A. J., & Avise, C. J. (2000). Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. *Journal of Fish Biology* 56, 3, 461-473.
- Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and role of multiple introductions. *Molecular Ecology* 17, 431-449.
- Environment for Development (EfD), (2008) over fishing of Nile perch in Lake Victoria. EfD Tanzania. Retrieved from http://www.efdinitiative.org/news-press/copy_of_job-openings/job-openings-archive/press-releases-2009/over-fishing.
- Evanno, G., Regnaut, S & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14, 2611-2620.
- Excoffier, L., Smouse, P. E & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes-application to human mitochondrial DNA restriction data. *Genetics*, 131, 479-491.
- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10, 564-567.
- Falush, D., Stephens, M., & Pritchard, J. K., (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164, 1567-1587.

- Ferguson, A. (2004). The importance of identifying conservation units: brown trout and pollan biodiversity in Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy* 104B, 3, Threatened Irish freshwater fisheries, 33-41.
- Garza, J. C., & Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology* 10, 305-318.
- Gee, J. M. (1964). Nile perch investigations. *Annual Reports of East African Freshwater Research Organisation*, 1962/63; pp 14-24.
- Gee, J. M. (1969). A comparison of certain aspects of the biology of *Lates niloticus* (Linne 1758) in some East African lakes. *Rev. Zool. Bot. Afr.* 80, 244-262.
- Genhua, Y., Li, Y., & Orban, L. (2001). Characterisation of Microsatellites in the IGF-2 and GH Genes of the Asian Seabass (*Lates calcarifer*). *Marine Biotechnology* 3, 1-3.
- Glaubitz, J. C. (2004). CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology notes* 4, 309-310.
- Guo, S., & Thompson E. (1992). Performing the exact test of Hardy-Weinberg proportion of multiple alleles. *Biometrics* 48, 361-372.
- Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from www2.unil.ch/popgen/softwares/fstat.htm.
- Goudswaard, P.C., & Ligetvoet, W. (1988). Recent developments the fishery of Haplochromines (Pisces: Cichlidae) and the Nile perch *Lates niloticus*(L.) (Pisces: Centropomidae) in Lake Victoria. pp. 101-112. In: CIFA. Report of the fourth session of the subcommittee for development and management of the fisheries of Lake Victoria, 6-10 April 1987, Kisumu, Kenya. FAO Fish. Rep.388. FAO, Rome.
- Graham, M. (1929). The Victoria Nyanza and its fisheries. A report on fishing surveys of Lake Victoria (1927-28). Crown Agents Colonies, London.

- Hammer, Ø., Harper, D.A.T., & Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4(1): 9pp. Retrieved from http://palaeo-electronica.org/2001_1/past/issue1_01.htm
- Harrison, K. (1991). the taxonomy of East African Nile perch, *Lates spp.* (*Perciformes Centropomidae*). *Journal of Fish Biology*, Vol. 38 (2); Pages 175 – 186.
- Hauser, L. G., Carvalho, G.R., Pitcher, T. J., & Ogutu-Ohwayo, R. (1998). Genetic affinities of an introduced predator: Nile perch in Lake Victoria, East Africa. *Molecular Ecology*, 7, 849-857.
- Kendall, L. R. (1969). An Ecological History of Lake Victoria Basin. *Ecological Society of America* 39(2) 121-176.
- McCusker, M., & Bentzen, P. (2010). Positive relationships between genetic diversity and abundance in fishes. *Molecular Ecology* 19, 4852-4862.
- Lowe-McConnell, R. (1994). The changing ecosystem of Lake Victoria, East Africa. *Freshwater Forum* 4, 76–89.
- Mkumbo, O. C., & Ligtvoet, W. (1992). Changes in the diet of Nile perch, *Lates niloticus* (L), the Mwanza Gulf Lake Victoria. In: *Hydrobiologia* 232(1), 79–83.
- Mouşadik, A., & Petit, R. J. (1996). High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L) Skeels] endemic to Morocco. *Theoretical Applied Genetics* 92, 832-839.
- Mwanja, T. M., & Mwanja, W.W. (2008). Preliminary results of the analysis of the population structure of the Nile perch (*Lates niloticus* Linnaeus, 1758) of Lake Victoria using microsatellite markers. *African Journal of Biotechnology* 7(16), 2885-2892
- Mwanja, M. T., Muwanika, V., Nyakaana, S., Masembe, C., Mbabazi, D., Rutaisire, J., & Mwanja, W. W. (2011). Population morphological variation of the Nile perch (*Lates niloticus*) of East African Lakes and their associated waters. *African Journal of Environmental Science and Technology* 5(11), 941-949.

- Odongkara, O. K., Abila, O. R., & Luomba, J. (2009). The contribution of Lake Victoria Fisheries to National Economies. *African journal of Tropical Hydrobiology and Fisheries* 12, 47-51.
- Ogutu-Ohwayo, R. (1993). The impact of predation by Nile perch, *Lates niloticus* L., on the fishes of Lake Nabugabo, with suggestions for conservation of endangered endemic cichlids. *Conservation Biology* 7, 701–711.
- Ogutu-Ohwayo, R. (1994). Adjustments in fish stocks and in life history characteristics of the Nile perch *Lates niloticus* L. in lakes Victoria, Kyoga, and Nabugabo. Ph.D. Thesis.
- Okaranon, J.O., Acere, T.O., Ocenodongo, D. L. (1985). The current state of the fisheries in the northern portion of lake Victoria. pp. 89-98. In: CIFA. Report of the third session of the subcommittee for development and management of the fisheries of Lake Victoria, 4-5th October 1984, Jinja, Uganda. FAO Fish. Rep. 335. FAO, Rome.
- Pringle, M. R. (2005). The origins of Nile perch in Lake Victoria. *BioScience* 55(9), 780-787.
- Ribbink, A. J. (1987). African Lakes and their fishes: conservation scenarios and suggestions. *Environmental Biology of Fishes* 19, 3-26.
- Rollins, A. L., Woolnough, P. A., Wilton, N. A., Sinclair, R., & Sherwin, B. W. (2009). Invasive species can not cover their tracks: using microsatellites to assist to assist management of starling (*Sturnus vulgaris*) populations in Western Australia. *Molecular Ecology* 18, 1560-1573.
- Rousset, F. (2008). GENEPOP '007: a complete reimplementation of genepop software for Windows and Linux. *Molecular Ecology Resources* 8, 103-106.
- Shaklee, J. B., & Bentzen, P. (1998). Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science* 62, 589-621.
- Taabu, A. M., Kayanda, R., Nyamweya, C. S., Ezekiel, C., Wabeya, U., Odada, E., & Magezi, D. (2010). A report of the Lake-wide hydro – acoustic and Environmental Survey 17th – March to 16th – April 2010. LVFO. Retrieved from

http://www.firi.go.ug/tech_reports/REPORT%20OF%20THE%20LAKE-WIDE%20HYDRO-ACOUSTIC%20AND%20ENVIRONMENTAL%20SURVEY%2017th%20March%2016th-April%202010.pdf

Taylor, J. C., & Maxwell, S. L. (2007). Hydroacoustics: Lakes and Reservoirs. In: Johnson, D.H., Shrier, B.M., O'Neal, J.S., Knutzen, J.A., Augerot, X., O'Neil, T.A. and Pearsons, T.N. (eds.): Salmonid Field Protocols Handbook: Techniques for Assessing Status and Trends in Salmon and Trout Populations. American Fisheries Society, Bethesda, pp. 153 – 172.

Tumwebaze, R., & Getabu, A. (1999). Results of the first hydroacoustic survey of the Ugandan waters of Lake Victoria. In: Tweddle, D. and Cowx, I. G. (eds) Report on third fisheries data working group (FIDAWOG) workshop held at the Triangle Hotel, Jinja, 29 March to 1 April 1999. Fisheries Data Working Group of the Lake Victoria Fisheries Research Project, pp. 91-97. LVFRP Technical Document, 6.

Utter, F. (2003). Genetic impacts of fish introductions. In E. M. Hallerman (ed), Population genetics: principles and applications for fisheries scientists, pp357-378. Bethesda, American Fisheries Society.

van Ooster, C., Hutchinson, W. F., Wills, D. P. M., Shipley, P., (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology notes 4, 535-538.

van Oppen, M. J. H., Turner, G. F., Rico, C., Deutsch, J. C., Ibrahim, K. M., & Robinson, R. L. (1997). Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes. Proceedings of Royal Society of London, Biological Sciences 264, 1803-1812.

Waples, R. S. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity 89, 438-450.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. Evolution 38, 1358-1370.

Witte, F., Golgschmidt, T., Wanink, J., Oijen, M., Goudswaard, K., & Bouton, N. (1992). The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. *Environmental Biology of Fishes* 34(1), 1-28.

Witte, F., Wanink, H. J., & Kische-Machumu. (2007). Species distinction and the biodiversity crisis in Lake Victoria. *Transactions of the American Fisheries Society* 136, 1146-1159.

Worthington, E. B., & Ricardo, C. K. (1936). Scientific results of the Cambridge expedition of the East African lakes, 1930 – 1- 15. The fish of Lake Rudolf and Lake Baringo. *Journal of Linnaean Society of London, Zoology* 39, pp 354-389.

Zhu, Y. Z., Lin, G., Lo, C. L., Xu, X. Y., Feng, F., Chou, R., & Yue, H. G. (2006a). Genetic Analyses of Asian seabass stocks using novel polymorphic microsatellites. *Aquaculture* 256, 167-173.

Zhu, Y. Z., Wang, M. C., Lo, C. L., Feng, F., Lin, G., & Yue, H. G. (2006b). Isolation, characterisation and linkage analyses of 74 novel polymorphic microsatellites in Baramundi (*Lates calcarifer*) *Genome* 49, 969-976.

CHAPTER FIVE

Genetic evidence of successful establishment of the Nile perch (*Lates* spp. L.) in East African lakes and implications for management

Matthew Tenywa Mwanja, Sylvester Nyakaana, Vincent Muwanika, Charles Masembe and Wilson Waiswa Mwanja

5.0 Abstract

Nile perch establishment in novel ranges in East Africa is one of the most successful freshwater invasion stories in the recent history. It became ecologically dominant and well established in several lakes in the Lake Victoria Region in a period of less than 5 decades. Genetic diversity of both introduced and native populations were assessed and patterns compared in order to establish the genetic consequences of their introductions. Genetic variation was surveyed at both the mitochondrial control region (CR) and at nine microsatellite loci. A total of 527 Nile perch fish were sampled from 5 East African lakes: native source populations were examined from lakes Albert and Turkana while introduced populations were sampled from lakes Kyoga, Nabugabo and Victoria. Both types of markers revealed higher average genetic diversity for invasive species ($H_E = 0.70$, $h = 0.81$) than for native source populations ($H_E = 0.66$, $h = 0.69$). Both populations scored relatively higher than the average for freshwater fishes ($H_E = 0.62$). Both native and introduced populations had 2 underlying genetic groupings in similar proportions as revealed by the STRUCTURE program. The high genetic diversity was most probably a consequence of higher numbers of propagules than outlined by official records of introductions. Use of high number of individuals at stocking is probably the reason for apparently minimized 'founder effects' of Nile perch in the introduced ranges. The two underlying populations revealed through genetic analysis may be representatives of the two subspecies of Nile perch previously reported in other studies. Implications to the management of this fishery in the East African region is that with the relatively high genetic diversity, the species could be sustainably exploited if it were effectively managed. In addition, further studies of the life histories and other attributes of the two genetic groupings of Nile perch in the region are recommended, as they may require different management plans.

Keywords: Nile perch, East Africa, invasiveness, genetic diversity

5.1 Introduction

Genetic characteristics of invasive species are important in their successful establishment and range expansion (Lee, 2002). Explanation of the importance of evolutionary and genetic processes during establishment of invasive fish species has received little consideration with most research focused on the victims of translocations, i.e. the native fish species (Hanfling, 2007). From the evolutionary and genetics perspectives, the potential for a species to adapt to a novel or changing environment depends on the quantity of genetic variability of the species (Lavergne & Molofsky, 2007). Yet introduced species are expected to undergo loss of genetic diversity during introduction and establishment due to the founder effect caused by genetic drift resulting from small number introductions, which greatly lowers the adaptability of the population in novel habitats (Dlugosch & Parker, 2008). In East Africa Nile perch (*Lates spp.*) occurs naturally in Lake Albert and the White Nile River in Uganda, and in Lake Turkana in Kenya. It was introduced into lakes Kyoga and Nabugabo from Lake Albert and into Lake Victoria from lakes Albert and Turkana (Mwanja & Mwanja, 2008).

Lake Albert was said to contain two perch species, namely *Lates niloticus* and *Lates macrophthalmus* (Holden, 1967). Welcomme (1967), who considered the two as sub-species rather than species, presumed that the two sub-species were introduced together into Lake Victoria either accidentally or intentionally. In addition, some introductions are said to have been from the Lake Turkana, which is said to have a sub-species of *L. niloticus*, i.e. *Lates niloticus longispinus* (Greenwood, 1976). The putative origins of Nile perch were reported to be composed of two characteristic populations; one having a smaller body form and found dwelling in deep open waters while the other population have a deeper bodied morph and inhabit inshore and shallow waters (Harrison, 1991; Holden, 1967; Worthington & Ricardo, 1936). This population structuring has also been suspected to be occurring in the introduced ranges (Bwathondi 1985); and is being questioned if it may signify different taxa of the Nile perch.

Official introductions were reported to be done using small numbers totalling to 382, 295 and 585 individuals for introductions into lakes Victoria, Nabugabo and Kyoga respectively (Hauser et al. 1998). These fish were intended to bolster the then failing fisheries in the non-native ranges of Nile perch, and for possible use as a sport fishery (Ogutu-Ohwayo, 1994). Nile perch slowly colonised its new habitat for the first 20 to 30 years and then its population exploded in

the 1980s (Mwanja & Mwanja, 2008). The cause of the population explosion of the Nile perch after staying for the lag time as a minor component in the fisheries of the introduced environments is unknown. The successfully establishment of the Nile perch and extension of its geographical range to cover all the new introduced ranges, despite the expected loss of genetic variation due to founder effects, creates a genetic paradox (Mwanja & Mwanja, 2008).

Both introduced and the putative source populations were examined for genetic variation in the mitochondrial CR and at nine microsatellite loci. Comparison of the patterns of genetic diversity of these populations was necessary to understand the mode and mechanism of the species' successful establishment. It also gave insight into the relationships of the different Nile perch taxa in both introduced and native ranges. Genetic diversity patterns of the species were also compared to other freshwater exploited species to determine how the species has been affected by the reported overfishing and to recommend how the species may be effectively managed to best conserve its genetic integrity in the region.

5.2 Materials, methods and study area

5.2.1 Study area

Nile perch samples were taken from five lakes – Victoria, Kyoga and Nabugabo in Uganda, the introduced environs; and from their native ranges of Lake Albert in Uganda and Lake Turkana in Kenya. Lakes Albert and Turkana are considered as the putative origins for the introduction of Nile perch into the novel ranges (**Figure 1**). Lakes Albert and Turkana are rift valley lakes, with Lake Albert found on the western border of Uganda with the Democratic Republic of Congo forming part of the Nile system while Lake Turkana is on the north western border of Kenya with Ethiopia (**Figure 1**). Lake Turkana is said to have had a connection with the River Nile for more than once during the wet periods of the Pleistocene, with the most recent connection occurring approx. 7,000 ago (Dgebuadze et al. 1994). Lake Kyoga is located in Central Uganda and connected to both Lake Victoria (the second largest fresh water lake in the world with varied habitat types and ranges) and Lake Albert by the Victoria Nile River. There are a number of areas with rapids which culminate into spectacular waterfalls on either side of Lake Kyoga along Victoria Nile River, the Owen Falls and Murchison Falls. The rapids and falls act as impediments to the free movement of fish such as Nile perch between two watersheds. Lake Nabugabo is a relatively smaller lake (24 km²) commonly classified as a satellite lake to Lake

Victoria. Lake Nabugabo was formerly a bay on the western shore of Lake Victoria which was separated from the main lake by a sand bar more than 5,500 years ago (Chapman et al. 1996). It is alleged that Nile perch from Lake Albert were first held and tried out in Lake Nabugabo before eventually being introduced into Lake Victoria (Barlow & Lissle, 1987).

5.2.2 Sample collection and DNA extraction

A total of 527 Nile perch fish were sampled randomly along representative transects in the 5 lakes, using bottom trawls for Lake Victoria and seining for lakes Albert, Kyoga, Nabugabo and Turkana. Transects were made in such a way as to cut across all key habitat types in each lake. Small (2 cm x 4 cm) fin clips were taken from each sampled fish and stored in absolute ethanol. Total genomic DNA was extracted using the DneasyTM tissue kit (QIAGEN) according to manufacturer's instructions.

5.2.3 Mitochondrial DNA Sequence Analysis

D-loop region of mtDNA of Nile perch was amplified using primers LN20 (ACCACTAGCACCCAAAGCTA) and HN20 (GTGTTATGCTTTAGTTAAGC) (Bernatchez & Dänzmann, 1993). PCR reactions consisted of the following: 5 µL genomic DNA; 25 µL AmpliTaq Gold[®] Master Mix, 5 µL of a 10 µM solution of each of two primers, and ddH₂O added for a final volume of 50 µL. Touchdown PCR conditions used for amplification included an initial denaturing step of 10 min at 95°C, followed by one cycle each of 94°C for 1 min, 67 - 51°C for 1 min 30s, and 72°C for 2 mins for each of the annealing temperatures starting at 67°C and stepping down by 2°C from each preceding cycle up to 51°C. This was followed by 25 cycles of 94°C for 1 min, 61°C for 1 min 30s and 72°C for 2 mins with a final extension of 72°C for 7 mins. Double stranded PCR products were cleaned following the manufacturer's protocol (QIAquick). The sequencing reactions were carried out by Macrogen Inc. (South Korea) using Applied Biosystems 3730xl DNA analyser. Samples were sequenced in both forward and reverse directions to guarantee accuracy of nucleotide identification. Standard chromatographic curves of forward and reverse sequences were imported into the program ChromasPro 1.41 (Technelysium Pty Ltd, Tewantin QLD, Australia) and were manually aligned and edited. Consensus sequences were exported to the program BioEdit version 7.0.9 (Hall, 1999) and aligned with other sequences using the CLUSTALW algorithm (Thompson et al. 1994).

5.2.4 Microsatellite M Analysis

Microsatellite markers developed for sister species *Lates calcarifer* (Zhu et al. 2006a, 2006b; Genhua Yue et al. 2001) were used for screening of Nile perch. Nine microsatellite markers that consistently gave scoreable results and exhibited polymorphism were used for genotyping of the Nile perch samples (**Table 1**). Samples were genotyped by BioScience (UK), on an ABI 3730 (Applied Biosystems) using GS-500 (Liz) in each capillary as a size standard. Allele sizes were estimated on GeneMapper version 3.7 (Applied Biosystems).

Table 1: Primers used to amplify the 9 microsatellite loci in Nile perch (*Lates spp.*), A is number of alleles per locus for the sister species *Lates calcarifer*.

Locus	Motif	Primers (5' - 3')	A	Size range	References
Lca70	(CAG)17	CATCAATTACACTCTGTTGCCTC GACAGA CAG GTG TTT TAG CCT ATT TG	7	288 - 308	Zhu et al. 2006a
Lca165	(TG)12	TCCCTGATGAGAGGAGTCA GTTACAGCCGTGGATGGACAC	8	258 - 290	Zhu et al. 2006b
Lca98	(TG)14	CAA AGG GGC CAC TGC ACA TAA T CTC CAG CTC ACC CAG GTT CAC T	10	186 - 242	Zhu et al. 2006a
Lca147	(TC)35	TGCCCCTAATGTATTCTTTCACCT GCTCCCACCTCTCATTCATTATTC	16	164 - 226	Zhu et al. 2006b
Lca185	(CA)6(GA)19	TTCGCCCTGTTTGCAAAATACATTAG ACACCCAGCATGGCTTCCTAT	10	175 - 215	Zhu et al. 2006b
Lca193	(TC)23	CCTCTGCCCTTTTCACTCTATATTGC CACATCGCACAAATGGACTGA	12	140 - 176	Zhu et al. 2006b
Lca171	(TG)13	ATTGCGTTACCAAGAGGTGAA TGTCCTTTGAAGGCTGAAAAC TG	14	294 - 336	Zhu et al. 2006b
Lca74	(CA)13	CAT CAT TTA CAC TCT GTT TGC CTC AT GAC AGA CAG GTG TTT TAG CCT ATT TG	4	160 - 170	Zhu et al. 2006a
LcaM01	(CG)4(CA)8	AT TCG CTC TTT ATT CTC CC GTC GGC CTT TCA TAT GCA AT	3	260 - 264	Genhua Yue et al. 2001

5.3 Data Analyses

5.3.1 Mitochondrial DNA

The software program ARLEQUIN 3.5 (Excoffier & Lischer, 2010) was used to perform intra- and inter-population analysis, calculating haplotype diversity (h) and nucleotide diversity (π) for the mtDNA sequence data, within sampling areas. Sequence based statistics (Hst , Kst^* , Z^* & Snn) together with gene flow (Nm), raggedness static, neutrality tests including Fu and Li's D , Fu and Li's F , Fu's F_s & Tajima's (1989) D were calculated using DnaSP 5.0 (Librado & Rozas, 2009). The distribution of pairwise differences between sequences was also calculated to investigate demographic changes based on three parameters, tau (τ), which is time since expansion expressed in units of mutational time, theta (θ_0) and (θ_1), which is time before and after population expansion (Rogers & Harpending, 1992). Due to lack of calibrated mutation rate for the Nile perch mtDNA control region, a divergence rate of 3.6% per million years estimated using geminate species of snook from a sister family, Centropomidae (Kim & Ronald 1999) was used in the calculations. The concordance of observed with the expected under the sudden expansion model of Rogers was tested using Harpending's Raggedness Index (Hri), and Sum of Squared deviation (SSD). The mismatch distributions were all calculated using ARLEQUIN 3.5.

The software program MEGA 5.05 (Tamura et al. 2011) was used to generate a table of variable sites and for constructing a neighbour-joining tree based on the Tamura & Nei (1993) distance. The T92 model, with invariable sites parameter (T92+I, $I = 0.90$) was selected as best fitting model using MEGA 5.05 (Tamura et al. 2011). A bootstrap analysis with 5000 replicates was used to evaluate support of phylogenetics relationships. In addition, a maximum parsimony network was constructed using TCS 1.6 (Clement, Posada, and Crandall, 2000); a program that provides a 95% plausible set for all haplotype linkages in an un-rooted tree.

5.3.2 Microsatellites

The software MICRO-CHECKER 2.2.1 (van Oosterhout et al. 2004) was used for identifying possible genotyping errors within the microsatellite data set by performing 1000 randomizations. Microsatellite data were checked for departures from Hardy-Weinberg (HW) and linkage disequilibrium (LD) using GENEPOP 4.0 (Rousset, 2008). Genetic variation in the population samples were quantified by the 'expected heterozygosity' and number of alleles at the loci. ARLEQUIN 3.5 was used for calculations of observed (H_o) and expected (H_E) heterozygosities,

and to test for heterozygosity excess and deficiency (exact tests) for microsatellite data, and for estimating global single locus F_{IS} . Furthermore, the software FSTAT 2.9.3.2 (Goudet, 2001) was used on the microsatellite data set to estimate allele richness per locus and sample (AR), which represents the number of alleles independent of sample size (Mousadik & Petit, 1996). F_{ST} and R_{ST} were estimated using GENEPOP 4.0. The statistical significance of population pairwise F_{ST} was analysed using permutation with 10,000 replicates. The genetic structure was assessed through the use of Bayesian based model-clustering in the program STRUCTURE 2.3.1 (Falush et al. 2003), by conducting genotypic population assignments (MCMC = 50,000 generations; burnin = 50,000 and iterations = 20). To infer the genetic groups sampled, STRUCTURE results were analysed using the ΔK method (Evanno et al. 2005). The admixture model with correlated allele frequencies, 20 replicates and set the number of genetic groups from $K = 1$ to $K = 10$ for each level of analysis. The modal value of ΔK was taken as the indicator of ancestral population clusters or genetic groups (Evanno et al. 2005). After determining the number of genetic groups, each individual sampled Nile perch was assigned to one of the groups, according to the highest ancestry proportion assigned by STRUCTURE.

5.4 Results

5.4.1 Mitochondrial DNA Sequence Analyses

Out of the 104 Nile perch mtDNA sequences analysed, they were a total of 35 variable/segregating sites (no indels) of which 24 were parsimony informative with a 2.2:1 transition to transversion ratio (Table 2). There were 27 haplotypes detected with hap-5 found in four out of the five lakes in East Africa, and noted as the most dominant of the identified haplotypes of Nile perch (Table 2). They were eight shared haplotypes - hap-1, hap-5, hap-9, hap-10, hap-11, hap-15, hap-20 and hap-23, which were all found in samples of L. Victoria. 2 haplotypes, hap-24 and hap-25, were unique to L. Albert; 2 haplotypes, hap-26 and hap-27, were unique to L. Nabugabo; haplotypes 2, 3, 4, 6, 7, 8, 12, 13, 14, 16, 17, 19, 21 and 22 were unique to Lake Victoria; whereas Lakes Turkana and Kyoga did not have any unique haplotypes (Table 2). Overall haplotype diversity (Hd) was high (0.86 ± 0.03), and ranged from 0.58 to 0.93. The overall nucleotide diversity (π) was 0.00855 ± 0.00139 and ranged from 0.0020 to 0.0153 (Table 3). The Nile perch control region had a nucleotide composition of A = 26.8%, C = 18.3%, G = 21.4%, and T = 33.5% making it A/T rich with A+T content = 0.603 and G+C content = 0.397.

Haplotype sequences were deposited in the GenBank, accession numbers JQ778220 – JQ778247.

Reconstructing of phylogenies by both Neighbour-Joining and parsimony methods revealed that Nile perch of East Africa belong to 2 clades A and B (**Figure 2**). The minimum spanning haplotype network of genealogical relationships among haplotypes revealed a network for Nile perch of E. Africa that was characterised by a high number of unique haplotypes with a star-like general pattern, rooted in the central most common haplotype, hap-5 (VIC6), possibly suggesting a recent population expansion (**Figure 3**). The geographical distribution of haplotypes was non-random with some of the samples having private haplotypes.

Table 2: List of 35 polymorphic sites of 27 mtDNA D-loop Nile perch haplotypes. Dots represent nucleotide variants identical to the first sequence. VIC, NAU, KYU, AKU, and TLK represent lake populations of lakes - Victoria, Nabugabo, Kyoga, Albert and Turkana respectively.

35 Polymorphic sites					Haplotype distribution and frequency					Totals
					VIC	NAU	KYU	AKU	TLK	
		111 1111111111 122222222 22234								
		3355569033 4444466789 9001122235 55943								
		1824787338 1234556750 6581606751 69645								
1 VIC1		TCGATGTAAA CTCTCGTCCC TCCCGAACGC AAATA	5	1						6
2 VIC2		CG..CA...GTAT ..G.....A	1							1
3 VIC3		T... ..G.A.....	2							2
4 VIC5		.G.....T... ..G.....	1							1
5 VIC6		G.....	24	1	5			3		33
6 VIC8		.G..... ..G.....	1							1
7 VIC11		G.....	3							3
8 VIC13		C....AGG.T... ..G.....	1							1
9 VIC14		T... G.....	8	2			6			16
10 VIC15		C..... ..G.....	6		4					10
11 VIC17		T...	5		1			3		9
12 VIC20		CG...AG.G TCTCT..TAT .TG...T...	1							1
13 VIC22		CG..... ..G.....	2							2
14 VIC48		C..GC.AG.G TCTCT..TAT .TG....A.G	1							1
15 VIC49		T... ..G.....	2					1		3
16 VIC52		CG..C.AG.G TCTCT..T... .TG..G.... ..G..	1							1
17 VIC53		CG..C.AG.G TCTCT..T... .T...G...A G....	1							1
18 VIC54		C..... ..G..G....	1							1
19 VIC55		CG.G..AG.GT.T .TG..... ..G.	1							1
20 VIC67		C.....GG.....	1				1			2
21 VIC69		CG..C.AG.G TCTCTTGT.T .TGT..... ..G..	1							1
22 VIC70		..A.....	1							1
23 VIC71		..A..... ..G.....	1					1		2
24 AKU1		C.....T... ..G.....					1			1
25 AKU5		CG..C.AG.G ...CT..T... ..G..GT.AA					1			1
26 NAU4		...GC.AG.G TCTCT..TAT .T...G..A. .G...		1						1
27 NAU7		G.G.....		1						1
Number of bases		2222222222 2222222222 2222222222 22222								
Totals			71	6	10	9	8		104	

5.4.2 Microsatellite Marker Analyses

MICRO-CHECKER software did not detect errors in the genotypic data matrix, indicating there were no scoring errors associated with null alleles, stuttering bands, or large allele drop outs in the 9 loci used in the genotyping; consequently all 9 were included in the analysis. A total of 499 alleles were detected at the 9 loci across the 19 data sets, Lca165 was the most polymorphic microsatellite with 32 alleles, while Lca70 and LcaM01 were least variable with 13 alleles each. Number of alleles per locus varied from 2 at alleles Lca70 and Lca185 to 17 at Lca165. The mean number of alleles varied from 9.44 ± 1.47 for KYU samples from Lake Kyoga to 3.67 ± 0.44 for ANU samples from Lake Albert, and the allelic richness varied from 4.30 ± 0.41 for MT samples from Lake Victoria to 3.33 ± 0.39 for AKU samples from Lake Albert. Pooled samples according to lakes from which they were collected revealed allelic richness ranging from 7.25 ± 1.75 for Lake Kyoga to 5.20 ± 2.21 for Lake Nabugabo (**Table 4**). Private allelic richness ranged from 0.05 for RUT samples for Lake Victoria to 0.41 for MT samples for Lake Victoria. The private allelic richness average over all loci for the pooled lake populations was 0.34, 0.25, 0.16, 0.13, and 0.35 for lakes Kyoga, Nabugabo, Victoria, Albert and Turkana respectively. The allele frequency for 9 loci varied significantly among the different populations but all loci had one most common allele for all populations. Observed heterozygosity (H_O) ranged from 0.08 to 1.00, whereas the expected heterozygosity (H_E) ranged from 0.10 to 0.93. The H_E average over all loci ranged from 0.60 for AKU population from Lake Albert to 0.76 for MT population from Lake Victoria, whereas the H_O overall loci ranged from 0.48 for WIT population from Lake Victoria to 0.76 of KGU population from Lake Victoria. 16 populations exhibited heterozygosity deficiencies, 9 exhibited heterozygosity excesses and 2 populations had same values for both H_E and H_O . For the different pooled populations, 4 lake populations all had heterozygosity deficiencies (Kyoga - H_E 0.75 and H_O 0.64; Victoria - H_E 0.68 and H_O 0.65; Albert - H_E 0.63 and H_O 0.54; Turkana - H_E 0.69 and H_O 0.61) save for Lake Nabugabo (H_E 0.65 and H_O 0.68) with excess heterozygosity (**Table 4**). Three out of 243 tests for conformance to Hardy-Weinberg expectations differed significantly ($P < 0.001$) after corrections for multiple tests (data not shown). The IT and LUT samples had genotypic distributions that differed significantly at locus Lca147. RPT samples had a genotypic distribution that differed at locus Lca171. For most samples ($\approx 70\%$) the value of the inbreeding index was positive.

Table 3: mtDNA sequence variability in the control region for the Nile perch of East Africa: sample size (N), number of segregating sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), demographic expansion parameters, tau (τ), theta at time $0 - (\theta_0)$ and at time $1 - (\theta_1)$, Harpending's Raggedness Index (Hri), Sum of Squared deviation (SSD)

Lake	Statistics										
	N	HE	HO	S	h	Hd	π	τ	θ_0	θ_1	SSD
Victoria	71	0.71	0.65	33	23	0.86	0.0090	2.347	0.157	61148.63	0.0087
Albert	09	0.62	0.50	15	04	0.58	0.0094	20.96	0.257	25010.47	0.1263
Kyoga	10	0.75	0.64	03	03	0.64	0.0020	0.996	0.003	86059.55	0.0119
Nabugabo	06	0.65	0.68	19	05	0.93	0.0153	2.310	0.082	81007.12	0.0856
Turkana	08	0.69	0.61	03	04	0.79	0.0029	1.555	0.027	79912.00	0.0109
Overall	104	0.68	0.62	35	27	0.86	0.0086	5.525	0.526	54762.76	0.0543

Table 4: Summary descriptive statistics – microsatellite data for the samples from the different lakes of East Africa showing sample size (N), mean number of alleles (A), expected heterozygosity (H_E), observed heterozygosity (H_O), Theta (H) under stepwise mutation model, mean allelic size range (A_S), Garza-Williamson index (M).

Lake	Statistics						
	N	A	HE	HO	Theta (H)	AS	M
Victoria	432	17.67	0.71	0.65	1.912	49.22	0.49
Albert	35	7.11	0.62	0.50	1.620	15.57	0.62
Kyoga	25	9.44	0.75	0.64	2.138	25.44	0.47
Nabugabo	18	6.33	0.65	0.68	1.688	16.89	0.52
Turkana	17	7.11	0.69	0.61	1.830	17.78	0.50
Overall	527	9.53	0.68	0.62	1.837	24.98	0.52

Table 5: Assessment of bottleneck effects from the BOTTLENECK software at 9 microsatellite loci in 27 Nile perch populations in 5 East African lakes – Albert, Victoria, Nabugabo, Kyoga and Turkana. Significant P- values have * ($P < 0.05$).

Population	Sign			Standardised			Wilcoxon			G-W
	IAM	TPM	SMM	IAM	TPM	SMM	IAM	TPM	SMM	M
AKU	0.562	0.109	0.005*	0.479	0.023*	0.000*	0.500	0.875	0.995	0.58
ANU	0.237	0.409	0.376	0.208	0.444	0.300	0.285	0.367	0.787	0.43
AUT	0.171	0.566	0.034*	0.044*	0.335	0.079	0.010*	0.326	0.850	0.54
BBU	0.409	0.434	0.311	0.044*	0.320	0.107	0.014*	0.367	0.715	0.52
BSU	0.209	0.026*	0.026*	0.245	0.206	0.001	0.065	0.850	0.997	0.43
EPT	0.159	0.387	0.132	0.076	0.420	0.021	0.082	0.285	0.787	0.56
IT	0.447	0.282	0.028*	0.186	0.242	0.000*	0.082	0.850	0.995	0.46
KGU	0.047*	0.428	0.539	0.017*	0.149	0.329	0.002*	0.082	0.633	0.48
KIT	0.206	0.456	0.100	0.096	0.452	0.007*	0.082	0.367	0.875	0.50
KIU	0.187	0.436	0.117	0.048*	0.294	0.090	0.014*	0.248	0.820	0.47
KT	0.593	0.307	0.121	0.168	0.193	0.000*	0.180	0.752	0.976	0.62
KYU	0.519	0.086	0.029*	0.464	0.047*	0.000*	0.455	0.898	0.995	0.46
LUT	0.044*	0.571	0.116	0.027*	0.281	0.063	0.005*	0.285	0.918	0.61
MIK	0.212	0.217	0.109	0.036*	0.300	0.075	0.005*	0.285	0.850	0.47
MT	0.222	0.490	0.487	0.036*	0.199	0.382	0.014*	0.213	0.590	0.40
MWU	0.208	0.281	0.101	0.370	0.107	0.000*	0.285	0.674	0.976	0.55
NAU	0.551	0.106	0.005*	0.315	0.143	0.000*	0.213	0.875	0.995	0.51
RCU	0.439	0.094	0.026*	0.430	0.027*	0.000*	0.367	0.849	0.995	0.43
RPT	0.530	0.027*	0.029*	0.304	0.076	0.000*	0.285	0.936	0.997	0.37
RUT	0.178	0.452	0.122	0.110	0.487	0.031*	0.065	0.367	0.976	0.57
SAK	0.432	0.287	0.032*	0.139	0.311	0.001*	0.082	0.674	0.986	0.43
SCU	0.201	0.209	0.279	0.022*	0.236	0.155	0.005*	0.285	0.545	0.47
SGU	0.426	0.426	0.115	0.210	0.347	0.020*	0.285	0.500	0.820	0.60
TLK	0.534	0.102	0.000*	0.483	0.051	0.000*	0.367	0.976	1.000	0.50
UKT	0.215	0.269	0.283	0.122	0.440	0.013*	0.082	0.590	0.936	0.41
UST	0.528	0.527	0.527	0.125	0.283	0.004*	0.082	0.752	0.820	0.45
WIT	0.215	0.284	0.005*	0.243	0.005*	0.000*	0.285	0.674	0.995	0.41

5.4.3 Demographic Changes

ARLEQUIN analyses of mtDNA revealed large differences between θ_1 and θ_2 within all samples suggesting rapid population expansion (Table 3). Tajima's $D = (-1.26001, P > 0.10)$, Fu and Li's D^* test statistic $(-1.26266, P < 0.02)$, Fu and Li's F^* test statistic $(-1.51239, P > 0.10)$, and Fu's F_s statistic (-9.735) were all negative, all of which confirm the recent introduction and resultant population explosion for the Nile perch of East Africa, which is typical of recent natural population expansion (Fu, 1997). The overall Harpending's raggedness Index, $H_{ri} = 0.119$ and overall sum of squares, $SSD = 0.0543$ (Table 3). Results for assessment of bottleneck effects from the BOTTLENECK software at 9 microsatellite loci in 27 Nile perch populations in 5 East African lakes – Albert, Victoria, Nabugabo, Kyoga and Turkana for all 3 tests, revealed some evidence in 25 out of the 27 populations (results not shown). Most evidence was revealed under the standardised test where 8 populations had significant heterozygosity excess under IAM model, 4 populations had heterozygosity excess under TPM model and 16 populations had heterozygosity excess under SMM model. The assessment of Garza-Williamson index, M , (results not shown) for the different lakes, values ranged from 0.47 for L. Kyoga to 0.62 for L. Albert. For the individual populations the M values ranged from 0.37 for RPT population to 0.62 for KT population.

Table 6: Analysis of genetic differentiation among the different populations of the different lakes of East Africa where Nile perch is found. Values for mean haplotype diversity (Hd), and for haplotype (Hst) and sequence based statistics (Kst^* , Z^* and Snn) were calculated according to Hudson et al. (1992) and Hudson (2000); ns not significant; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$.

Lake populations	Hd	π	Hst	Kst*	Z*	Snn	Nm	FST
Victoria Vs Albert (n1 = 71, n2 = 9)	0.87	0.0093	0.040 (P=0.00***)	0.039 (P=0.002**)	7.02 (P=0.00***)	0.84 (P=0.03*)	2.25	0.10
Victoria Vs Kyoga (n1 = 71, n2 = 9)	0.84	0.0083	0.005 (P=0.173 ns)	0.003 (P=0.258 ns)	7.13 (P=0.146 ns)	0.79 (P=0.368 ns)	3.30	0.07
Victoria Vs Nabugabo (n1 = 71, n2 = 9)	0.87	0.0095	0.001 (P=0.32 ns)	-0.003 (P=0.64 ns)	7.03 (P=0.28 ns)	0.86 (P=0.32 ns)	-15.65	-0.02
Victoria Vs Turkana (n1 = 71, n2 = 9)	0.86	0.0085	0.002 (P=0.26 ns)	-0.004 (P=0.62 ns)	7.09 (P=0.43 ns)	0.82 (P=0.40 ns)	6.51	0.04
Albert Vs Kyoga (n1 = 9, n2 = 10)	0.82	0.0070	0.248 (P=0.01**)	0.209 (P=0.003**)	3.80 (P=0.002**)	0.84 (P=0.001**)	0.52	0.33
Albert Vs Nabugabo (n1 = 9, n2 = 6)	0.73	0.0114	0.031 (P=0.327 ns)	-0.025 (P=0.616 ns)	3.73 (P=0.248 ns)	0.64 (P=0.068 ns)	-5.58	-0.05
Albert Vs Turkana (n1 = 9, n2 = 8)	0.85	0.0071	0.200 (P=0.001**)	0.106 (P=0.019*)	3.69 (P=0.005**)	0.89 (P=0.001**)	1.09	0.19
Kyoga Vs Nabugabo (n1 = 10, n2 = 6)	0.82	0.0077	0.093 (P=0.049*)	0.098 (P=0.011*)	3.69 (P=0.016*)	0.64 (P=0.060 ns)	1.43	0.15
Kyoga Vs Turkana (n1 = 10, n2 = 8)	0.74	0.0027	0.046 (P=0.169 ns)	0.095 (P=0.086 ns)	4.04 (P=0.101 ns)	0.59 (P=0.101 ns)	1.02	0.20
Nabugabo Vs Turkana (n1 = 6, n2 = 8)	0.89	0.0084	0.051 (P=0.131 ns)	0.053 (P=0.089 ns)	3.51 (P=0.127 ns)	0.64 (P=0.096 ns)	8.67	0.03

Table 7: Pairwise Φ_{ST} values computed from haplotype frequencies for the mtDNA pooled data. Below diagonal are the Φ_{ST} values and above diagonal are the P -values.

Lake	ALBERT	VICTORIA	KYOGA	NABUGABO	TURKANA
ALBERT	***	0.00	0.12	0.37	0.23
VICTORIA	0.18373	***	0.00	0.37	0.00
KYOGA	0.03229	0.38520	***	0.08	0.13
NABUGABO	0.00310	0.04000	0.15591	***	0.14
TURKANA	0.01598	0.31865	0.08046	0.08757	***

Table 8: Analysis of molecular variance by distance method Tamura & Nei (1993) distances for the Nile perch sequence data across lakes (Wier & Cockerham 1984; Excoffier et al. 1992; Wier, 1996).

	AMOVA					
Source of variation	df	SS	Variance components	% variation	F-statistics	P
Among groups	3	8.61	-0.31789	-15.35	$\Phi_{ST} = 0.05$	0.06
Among populations within groups	1	5.13	0.42229	20.39	$\Phi_{SC} = 0.18$	0.01
Within populations	99	194.67	1.96641	94.96	$\Phi_{CT} = -0.15$	0.89
Total	103	208.42	2.07080			

Table 9: Pairwise F_{ST} values by distance method using number of different alleles for the microsatellite pooled data. Below diagonal are the F_{ST} values and above diagonal are the P-values.

Lake	ALBERT	VICTORIA	KYOGA	NABUGABO	TURKANA
ALBERT	***	0.00	0.00	0.00	0.00
VICTORIA	0.05129	***	0.00	0.00	0.00
KYOGA	0.09760	0.02923	***	0.03	0.00
NABUGABO	0.07078	0.02444	0.01882	***	0.00
TURKANA	0.15918	0.07246	0.03533	0.05733	***

Table 10: Summary statistics for genetic variation of the Nile perch at 9 microsatellite loci in 5 East African lakes. A: number of alleles; AR: allelic richness; H_E expected heterozygosity; H_O observed heterozygosity; F_{IS} inbreeding coefficient; F_{IS} probability of significant deviation from Hardy-Weinberg equilibrium; n: sample size.

Lake (n)	Locus													
	Lca70	Lca165	Lca98	Lca147	Lca185	Lca193	Lca171	Lca74	LcaM01	mean	SE			
Albert (35)	A 4	6	5	6	3	8	11	11	10	7.11	1.01			
	AR	2.91	4.51	4.20	2.35	4.54	8.60	8.48	6.36	5.20	0.74			
	HE	0.52	0.63	0.74	0.41	0.53	0.87	0.82	0.70	0.62	0.06			
	HO	0.29	0.59	0.56	0.36	0.37	0.81	0.77	0.45	0.50	0.06			
	FIS	0.45	0.06	0.25	0.12	0.09	0.07	0.06	0.36	0.20	0.05			
Victoria (432)	HW	0.01	0.15	0.11	0.37	0.69	0.00	0.40	0.00	0.22	0.08			
	A 10	30	17	16	18	14	24	16	14	17.67	1.99			
	AR	3.29	8.31	6.28	4.88	6.45	7.64	8.49	4.75	6.19	0.58			
	HE	0.55	0.80	0.80	0.57	0.76	0.82	0.86	0.51	0.71	0.04			
	HO	0.48	0.65	0.74	0.48	0.76	0.80	0.86	0.48	0.66	0.05			
Kyoga (25)	FIS	0.13	0.19	0.08	0.16	0.01	0.03	-0.01	0.11	0.09	0.02			
	HW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
	A 5	17	7	6	8	6	15	11	10	9.44	1.41			
	AR	4.14	11.23	6.24	5.61	5.86	10.16	9.13	7.73	7.25	0.82			
	HE	0.44	0.89	0.79	0.71	0.77	0.83	0.88	0.65	0.75	0.05			
Nabugabo (18)	HO	0.40	0.76	0.90	0.53	0.72	0.84	0.88	0.42	0.64	0.08			
	FIS	0.09	0.15	-0.14	0.26	0.07	-0.02	0.01	0.37	0.15	0.07			
	HW	0.14	0.01	0.31	0.00	0.04	0.01	1.00	0.00	0.17	0.11			
	A 3	5	4	4	4	5	12	10	8	6.3	1.01			
	AR	2.67	4.33	3.97	3.61	4.33	10.60	8.52	7.00	5.57	0.87			
Turkana (17)	HE	0.37	0.66	0.70	0.32	0.67	0.88	0.85	0.72	0.65	0.06			
	HO	0.33	0.61	0.89	0.24	0.94	0.88	0.89	0.65	0.68	0.09			
	FIS	0.09	0.07	-0.27	0.27	-0.43	-0.00	-0.04	0.10	-0.03	0.07			
	HW	0.63	0.03	0.01	0.07	0.01	0.47	0.53	0.09	0.25	0.09			
	A 4	7	5	5	7	4	12	11	7	7.11	0.94			
	AR	3.64	6.25	4.57	7.00	3.69	10.70	9.77	6.50	6.47	0.82			
	HE	0.29	0.77	0.56	0.77	0.60	0.89	0.88	0.66	0.69	0.06			
	HO	0.19	0.56	0.53	0.83	0.69	0.80	0.88	0.53	0.61	0.07			
	FIS	0.36	0.27	0.04	-0.10	-0.15	0.10	-0.01	0.19	0.12	0.07			
	HW	0.08	0.02	0.68	0.79	0.23	0.00	0.38	0.07	0.25	0.10			

Table 11: Analysis of molecular variance by distance method using number of alleles (F_{ST}) for the Nile perch microsatellite data (Wier and Cockerham; 1984; Excoffier et al. 1992; Wier, 1996)

AMOVA I (across populations)										AMOVA II (across lakes)				
Source of variation	df	SS	Variance components	% variation	F-statistics	P	df	SS	Variance components	% variation	F-statistics	P		
Among groups	5	22.187	0.03332	2.84	FCT = 0.03	0.00	3	17.434	0.00958	0.81	FCT = 0.01	0.60		
Among populations within groups	21	46.791	0.02799	2.39	FSC = 0.03	0.00	1	2.699	0.03638	3.08	FSC = 0.03	0.00		
Among individuals within populations	500	565.035	0.01807	1.54	FIS = 0.02	0.11	522	613.880	0.04104	3.48	FIS = 0.04	0.00		
Within Individuals	527	576.500	1.09393	93.23	FIT = 0.07	0.00	527	576.500	1.09393	92.63	FIT = 0.07	0.00		
Total	1053	1210.512	1.17331	100			1053	1210.512	1.18093					

5.5 Discussion

5.5.1 Genetic diversity and genetic differentiation

The overall genetic diversity of the introduced ranges was found to be higher than that of the putative origins. This scenario is most probably explained by the introductions from multiple sources that were repeatedly done for a period of close to a decade from the mid 1950s to early 1960s (Pringle, 2005). Repeated introductions are known to augment genetic diversity of invasive species (Dlugosch & Parker, 2008, Lavergne & Molofsky, 2007). In addition, even though Nile perch may have experienced bottlenecks these do not necessarily lead to population crash or stop evolution provided a population grows rapidly (Frankham 2005). Nile perch is known to have very high fecundity - said to be as high as 17 millions eggs per female (Ogutu-Ohwayo, 1994). The high fecundity and prolific reproduction may have kept the species' population growth rate positive and growing despite the expected bottlenecks during the colonisation period in the introduced ranges. The unsuspecting and then most abundant prey, the haplochromines (cichlids), in the introduced ranges helped to easily provide the required energy bolstering of the population of the colonisers, as evidenced in the differences in the bigger and deeper bodied introduced Nile perch when compared to the source populations (EAFRO, 1969).

Both markers, the mtDNA CR and the microsatellite loci, revealed relatively high levels of genetic polymorphisms among native and introduced Nile perch of East African lakes. The availability of the high genetic variation in Nile perch offers a good reprieve in the management efforts of the fishery given the recent reports of fast dwindling yields and natural stocks of this species in the region. Both markers, the mtDNA CR and the microsatellite loci, showed Lake Albert as having the least genetic diversity in spite of being the putative origin and the alleged biggest contributor to the introduced environs – lakes Victoria, Kyoga and Nabugabo during the translocations. This may be partly explained by the nature of Lake Albert whose major inflows either do not have Nile perch - Semiliki River, or have rapids or falls - Murchison falls that are effective barriers to faunal interchange, thereby preventing any gene flow from outside Lake Albert.

The putative origins, lakes Albert and Turkana, though they had relatively lower genetic diversity than the introduced ranges had genetic diversities which were way above the average

for freshwater fish species, suggesting that the Nile perch fisheries of these two lakes are still healthy and stable enough to support management and conservation efforts for the species.

According to structure analysis, there were two genetic groupings of the East African Nile perch and all lakes had both groupings but in differing proportions. There was no trend in the different proportions of the genetic clusters in different lakes, which may be attributed to geographical location. These findings indicate that Nile perch in East Africa is structured into two distinct genetic groupings found in all the lakes where Nile perch is found. These findings agree with our earlier morphology findings (Mwanja et al. 2011) and earlier studies by other ecological biologists who suggested the existence of two sub-species in the region (Harrison, 1991; Welcomme, 1967; Gee, 1964; Worthington, 1940). Therefore there are most probably two Nile perch sub-species in East African lakes.

Phylogenetic reconstruction did not support the existence of more than one species of the Nile perch in East Africa as proclaimed by earlier fish ecologists based on morphological description (Harrison, 1991), instead it revealed 2 underlying clades found in all 5 lakes. What may have been considered as separate species are most likely synonyms of the same species. Most probably Nile perch of East African lakes belongs to one species having two subspecies.

5.5.3 Demographic changes

Grant & Bowen (1998) proposed that fish with high h and low π probably might have undergone population expansion after a period of low effective population size. Our findings on the genetic variation of the Nile perch are in agreement with this scenario, with high haplotype diversity and low nucleotide diversity (h , 0.62 – 0.71; π , 0.002 – 0.009). Similarly the star-like TCS parsimonious haplotype network is an indicator of recent population expansion with the central common haplotype being the hypothetical ancestral haplotype (Sophie et al. 2007) and the large negative neutrality tests further support the phenomenon of recent population expansion (Fu, 1997). The haplotype network for Nile perch of East Africa formed a star-like network suggesting recent population expansion. This was further supported by the small SSD and H_{ri} values, which are indicators of a historically expanding population model (Roggers, 1995). In the introduced ranges, the Nile perch, which was translocated using small numbers (Hauser et al. 1998), and said to have slowly colonised its novel habitats and later exploded in the 1980s to

dominate their fisheries (Ogutu-ohwayo, 1994) fits the above recent expansion model. For the native ranges, the possible explanation for recent population expansion, is most probably attributed to oscillation of water levels from the time when these lakes – Albert and Turkana were still small lakes (20,000 years to 3000 years ago) and are thought to have stabilized at their current levels (Beuning, Talbot, and Kelts, 1997). The reduction in population size and recent population expansion of the population model of the Nile perch of East Africa as described above is further supported by the findings of the bottleneck analysis and the low M values. According to Garza and Williamson (2001) M values < 0.68 support occurrence of a past reduction in population size. Both the BOTTLENECK software analysis and the Garza-Williamson indices indicate that the Nile perch of East Africa had undergone reduction in genetic diversity and population expansion thereafter. The possible explanations for this scenario for the introduced environs are the recent Nile perch translocations and the palaeo-environmental changes in water levels in the lake basins of lakes Albert and Turkana.

5.6 Implications to Fisheries Management

The relatively high genetic diversity, despite the reported declining stocks, both in the native and introduced ranges of Nile perch provides the requisite evolutionary flexibility that may support efforts toward the effective management and sustainable exploitation of the Nile perch resource in the region. There should be no need for new translocations of Nile perch from the native to the introduced environs for the sake of augmenting the species diversity. We advocate for effective management that allows controlled fishing effort and for improvement of water quality in the region. These factors may allow for the sustainable exploitation of the Nile perch and the possible resurgence of the native species in the introduced ranges that form the Nile perch prey as evidenced by recent revelations (Balirwa et al. 2003). There is urgent need to map out and further study the life histories and other attributes of the two underlying populations of Nile perch in both native and introduced ranges that were revealed in this study. The two populations also supported by works of earlier scientist (Harrison, 1991) may require different management approaches. From the demographic history the Nile perch has shown that it has high prospects for recovery after bottleneck episodes, it is pertinent to enhance the scientific knowledge of the mechanisms of this fishery's recovery in order to formulate recommendations for the species long term management plans and their implementation. Lastly, there is urgent need to initiate

routine genetic monitoring of both the Nile perch and the native fish species that form the species' prey in all Nile perch ranges in this region. The outcome will aid in designing appropriate and contemporary management regimes for creating a healthy sustainable fishery in the region using our current findings as a benchmark.

5.7 Acknowledgements

This study was part of the bigger project Nile perch technologies sponsored by the World Bank Millennium Science Initiatives (MSI) through the Uganda National Council of Science and Technology (UNCST). We are grateful to Lake Victoria Fisheries Organisation for aiding the sampling on Lake Victoria. We are thankful to technicians Sophia of NaFIRRI, Uganda, Mapunda and Ogaro from TAFIRRI, Tanzania, Odada from KEMFRI, Kenya and to the Lake beach management officials - Opiri and Mutebi of lakes Kyoga and Nabugabo, Uganda for their assistance during sampling from lakes Albert, Victoria, Turkana, Kyoga and Nabugabo respectively. Lastly we wish to acknowledge Dr. Monica R. Sullivan for her assistance with editing English writing.

5.8 References

- Balirwa, J. S., Chapman, C. A., Chapman, J. L., Cowx, J. I., Geheb, K., Kaufman, L., Lowe-McConnell, H. R., Wanink, H. J. O., Welcomme, L. R., & Witte, F. (2003). Biodiversity and fishery sustainability in the Lake Victoria Basin: An unexpected marriage? *BioScience* 53, 703-715.
- Barlow, G. C., Lissle, A. (1987). Biology of The Nile perch *Lates niloticus* (Pisces: Centropomidae) with Reference to its Proposed Role as a Sport Fish in Australia. *Biological Conservation* 39, 269-289.
- Bernatchez, L., Danzmann, G. R. (1993). Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of Brook charr (*Salvelinus fontinalis* Mitchell). *Molecular Biology and Evolution* 10, 1002-1014.
- Beuning, M. R. K., Talbot, R. M., Kelts, K. (1997). A revised 30,000-year paleoclimatic and paleohydrologic history of L. Albert, E. Africa. *Palaeogeography, palaeoclimatology, Palaeoecology* 136, 259-279.
- Bwathondi, P. O. J. (1985). The future of the fisheries of the Tanzania part of Lake Victoria, in view of the predominance of the Nile perch *Lates niloticus*. *FAO Fish. Rep.* 335, 143-145.
- Cawdery, S. H., & Ferguson, A. (1988). Origins and differentiation of three sympatric species of trout (*Salmo trutta* L.) in Lough Melvin, Ireland. *Polskie Archiwum Hydrobiologii*, 35, 267-277.
- Chapman, L. J., Chapman, A. C., Ogutu-Ohwayo, R., Chandler, M., Kaufman, L., Keiter, A. E. (1996). Refugia for Endangered Fishes from an Introduced Predator in Lake Nabugabo, Uganda. *Conservation Biology* 10(2), 554-561.
- Clement, M., Posada, D., Crandall, K. A. (2000). TCS: a computer program to estimate genegenealogies. *Molecular Ecology*, 9, 1657-1660.
- Dgebuadze, Y. Y., Golubstov, A. S., Mikeev, V. N., Mina, M. V. (1994). Four fish species new to the Omo-Turkana basin, with comments on the distribution of *Nemacheilus abyssinicus* in Ethiopia. *Hydrobiologia* 286, 125-128.

- Dlugosch, M. K., & Parker, M. I. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol Ecol* 17, 431-449.
- EAFRO. (1969). East Africa Freshwater Fisheries Organization annual reports, 1961-1967; Entebbe, Uganda.
- Evanno, G., Regnaut, S., Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14, 2611-2620.
- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10, 564-567.
- Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164, 1567-1587.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39, 783-791.
- Frankham, R. (2005). Resolving the genetic paradox in invasive species. *Heredity*, 94, 385. doi:10.1038/sj.hdy.6800634.
- Fu, Y. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147, 915-925.
- Garza, J. C., & Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. *Mol Ecol* 10, 305-318, doi:10.1046/j.1365-294x.2001.01190.x
- Gee, J. M. (1964) Nile perch investigations. Annual Reports of East African Freshwater Research Organisation, 1962/63; 14-24.
- Genhua, Y., Li, Y., & Orban, L. (2001). Characterisation of Microsatellites in the IGF-2 and GH Genes of the Asian seabass (*Lates calcarifer*). *Marine Biotechnology* 3, 1-3.
- Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Retrieved from www2.unil.ch/popgen/softwares/fstat.htm.

- Grant, W. S., Bowen, B. W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes insights from sardines and anchovies and lessons of conservation. *Journal of Heredity* 89, 415-426.
- Greenwood, P. H. (1966). *The Fishes of Uganda*. Kampala (Uganda): Uganda Society.
- Greenwood, P. H. (1976). A review of the family Centropomidae (Pisces, Perciformes). *Bulletin of the British Museum (Natural History)* 29, 1-81.
- Hall, T. A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid symposium series* 41, 95-98
- Harrison, K. (1991). The taxonomy of East African Nile perch, *Lates spp.* (Perciformes Centropomidae). *Journal of Fish Biology* 38(2), 175-186.
- Hanfling, B. (2007). Understanding the establishment success of non-indigenous fishes: lessons from population genetics. *Journal of Fish Biology* 71, 15-135.
- Hauser, L. G., Carvalho, G. R., Pitcher, T. J., Ogutu-Ohwayo, R. (1998). Genetic affinities of an introduced predator: Nile perch in Lake Victoria, East Africa. *Molecular Ecology* 7, 849-857.
- Holden, M. J. (1967). The systematics of the genus *Lates* (Teleostei: Centropomidae) in L. Albert East Africa. *Journal of Zoology (London)* 151, 329-342.
- Kaufman, L. S. (1992). Catastrophic species-rich Ecosystems. *BioScience* 42(11), 846-858.
- Kim, A. D., & Raymond, R. W. (1999). Amphi-panaimic Geminates of Snook (Percoidei: Centropomidae) provide a calibration of the divergence rate in mtDNA control region of fishes. *Molecular phylogenetics and Evolution* 13(1), 208-213.
- Lavergne, S., & Molofsky, J. (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *PNAS* 104(10), 3883-3888. doi: 10.1073/pnas.0607324104.
- Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in Ecology and Evolution* 17(3), 386-391.

- Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451-1452 | doi: 10.1093/bioinformatics/btp187.
- Mousadik, A., & Petit, R.J. (1996). High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L) Skeels] endemic to Morocco. *Theoretical Applied Genetics* 92, 832-839.
- Mwanja, T. M., & Mwanja, W. W. (2008). Preliminary results of the analysis of the population structure of the Nile perch (*Lates niloticus* Linnaeus, 1758) of Lake Victoria using microsatellite markers. *African Journal of Biotechnology* vol. 7 (16), 2885-2892.
- Mwanja, M. T., Muwanika, V., Nyakaana, S., Masembe, C., Mbabazi, D., Rutaisire, J., & Mwanja, W. W. (2011). Population morphological variation of the Nile perch (*Lates niloticus*) of East African Lakes and their associated waters. *African Journal of Environmental Science and Technology* 5(11), 941-949.
- Ogutu-Ohwayo, R. (1994). Adjustments in fish stocks and in life history characteristics of the Nile perch *Lates niloticus* L. in Lakes Victoria, Kyoga, and Nabugabo. Ph.D. Thesis.
- Pringle, R. M. (2005). The origins of Nile perch in Lake Victoria. *BioScience* 55(9), 780-787.
- Rogers, A. R., & Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic difference. *Molecular Biology and Evolution* 9, 552-569.
- Rousset, F. (2008). GENEPOP '007: a complete reimplementation of genepop software for Windows and Linux. *Molecular Ecology Resources* 8, 103-106.
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406-425.
- Sophie von der, H., Lipinski, R. M., & Matthee, A. C. (2007). Mitochondrial DNA analyses of the Cape hakes reveal an expanding, panmictic population of *Merluccius paradoxus*. *Molecular Phylogenetics and Evolution* 42, 517-527.
- Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585-595.

- Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10, 512-526.
- Tamura, K., & Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* 101, 11030-11035.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28, 2731-2739.
- Templeton, A. R., Crandall, K. A., & Sing, C. F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132, 619-633.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673-4680.
- van Ooster, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology notes* 4, 535-538.
- Welcomme, R. L. (1967). Observations on the biology of the introduced species of *Tilapia* in Lake Victoria. *Revue de Zoologie Africaine* 76(3-4), 249-279.
- Worthington, E. B., & Ricardo, C. K. (1936). Scientific results of the Cambridge expedition of the East African lakes, 1930 – 1- 15. The fish of Lake Rudolf and Lake Baringo. *Journal of Linnaean Society of London, Zoology* 39, 354-389.
- Worthington, E. B. (1940). Geographical differentiation in freshwaters with special reference to fish. In the *Systematics* (J. Huxley, ed.), pp 287-302. London: Oxford University Press.

Zhu, Y. Z., Lin Lo, C. L., Xu, X. Y., Feng, F., Chou, R., & Yue, H. G. (2006a). Genetic Analyses of Asian sea bass stocks using novel polymorphic microsatellites. *Aquaculture* 256, 167-173.

Zhu, Y. Z., Lin Lo, C. L., Xu, X. Y., Feng, F., Chou, R., & Yue, H. G. (2006b). Isolation, characterisation and linkage analyses of 74 novel polymorphic microsatellites in Baramundi (*Lates calcarifer*) Genome 49, 969-976.

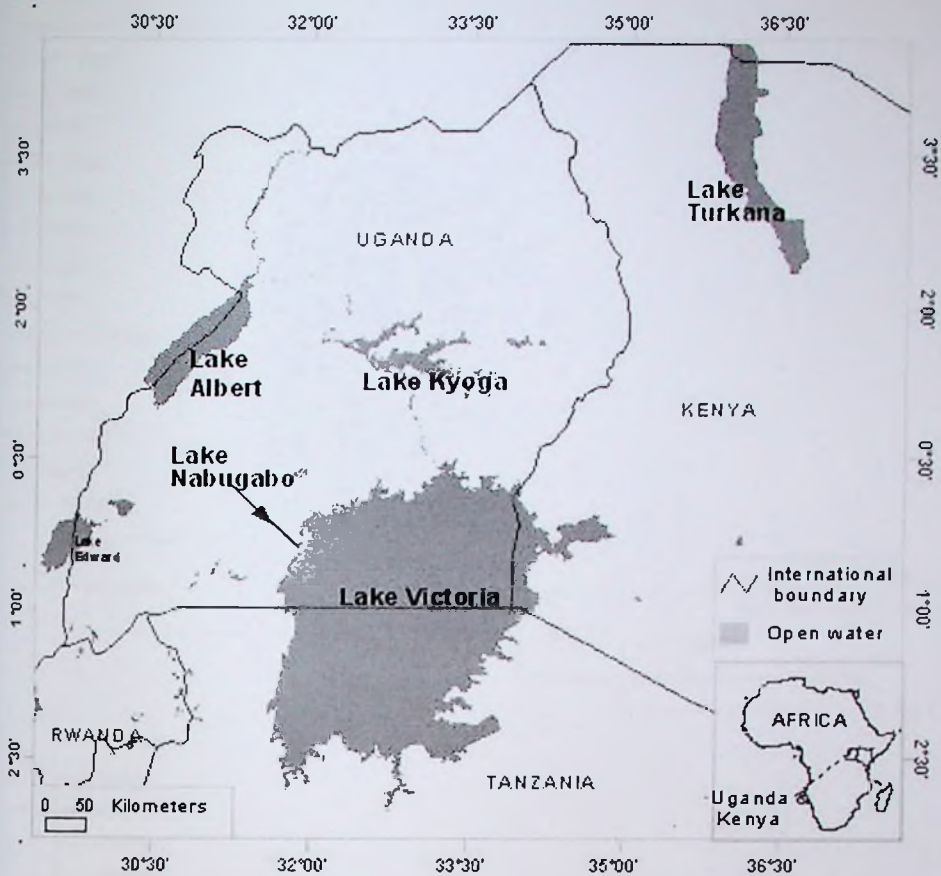


FIGURE 1: Map showing East African lakes – Albert, Kyoga, Nabugabo, Turkana and Victoria where Nile perch is found and where Nile perch samples were collected.

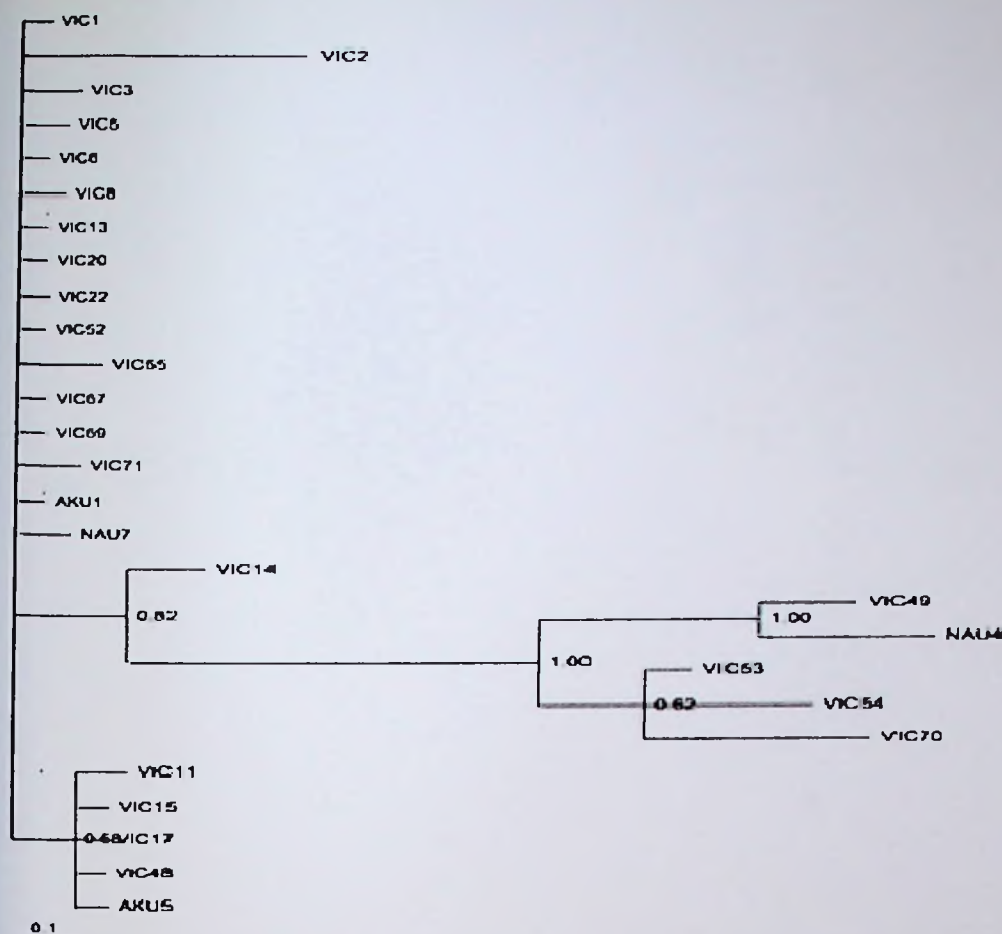


Figure 2. Evolutionary and phylogenetics relationships of taxa - was inferred using the Neighbor-Joining method (Saitau and Nei 1987). The optimal tree with the sum of branch length = 0.12482215 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 104 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 163 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011)

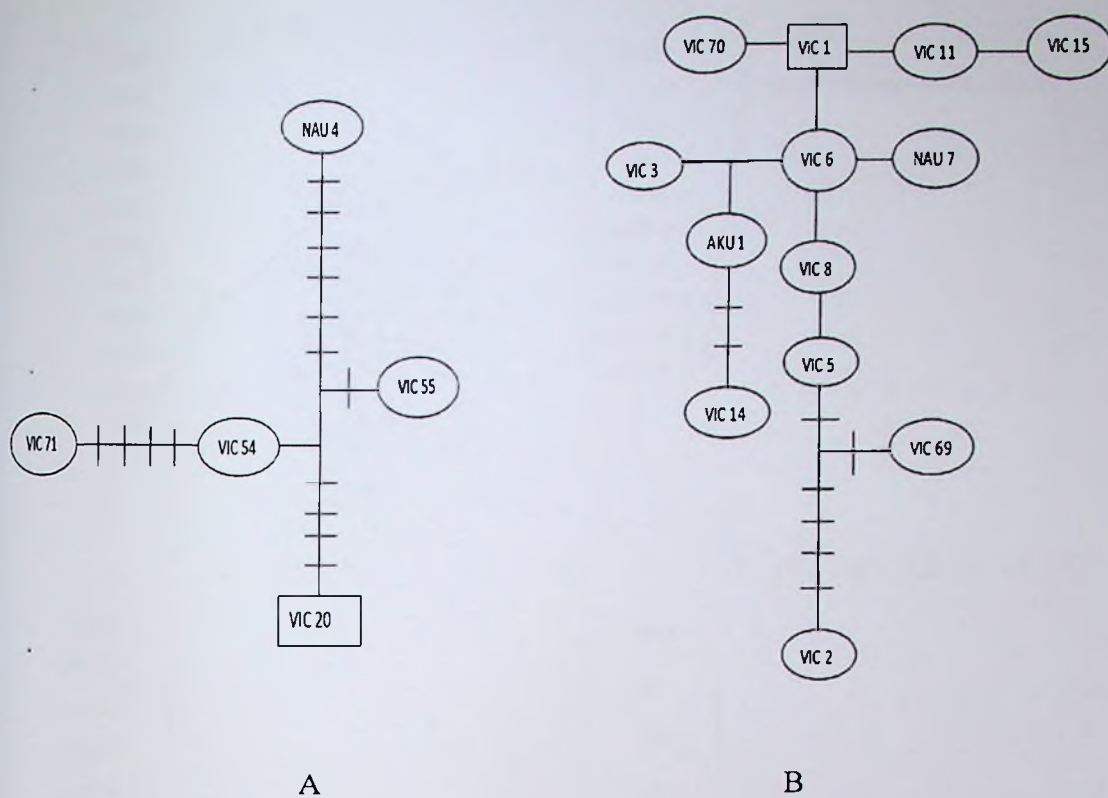


Figure 3: mtDNA haplotype networks A and B estimated with 95% statistical limits of parsimony using the algorithm in Templeton et al. 1992. Haplotypes are represented as rectangle and circles. Each node represents an additional mutational event.

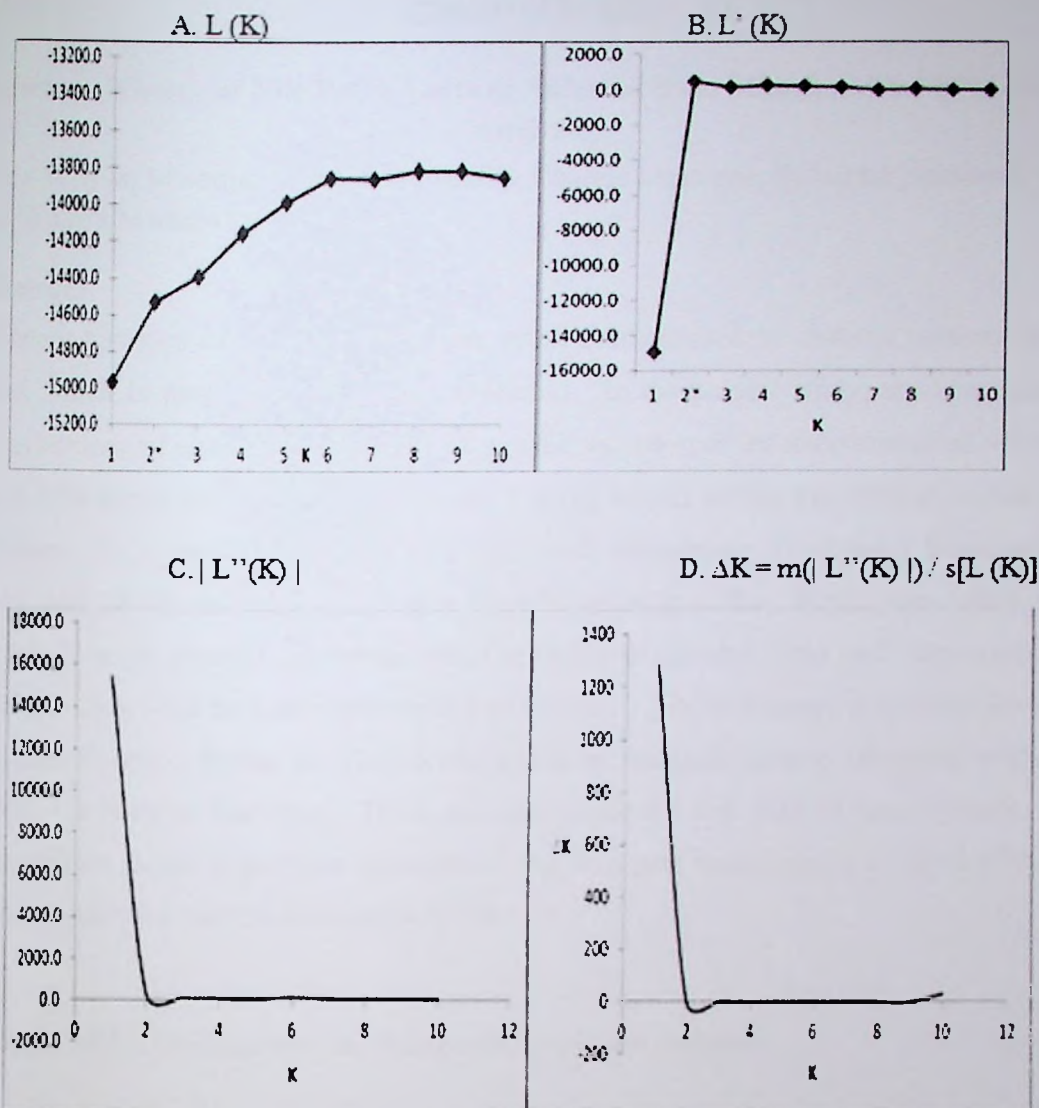


Figure 4: Description of the Evanno et al. (2005) 4 step graphical method used in detecting the true number of population groups K^* . (A) Mean $L(K)$ over 20 runs for each K -value, (B) rate of change of likelihood distribution calculated as $L'(K) = L(K) - L(K-1)$. (C) Absolute values of the second order rate of change of likelihood distribution calculated as $C. |L''(K)| = |L'(K+1) - L'(K)|$. (D) ΔK calculated as $\Delta K = m |L''(K)| / s[L(K)]$. Modal value of this distribution was taken as the true K^* or upper most level of structure, here two clusters.

CHAPTER SIX

Evolutionary History of Nile Perch *Lates* sp. Inferred from Mitochondrial DNA Variation Analyses

Matthew Tenywa Mwanja, Vincent Muwanika, Charles Masembe, Sylvester Nyakaana and Wilson Waiswa Mwanja

6.0 Abstract

Evolutionary histories of aquatic species are often characterized by distinct patterns of genetic variation, which in part, reflect drainage evolution. In the present study, the consequences of paleo-environmental changes on patterns of genetic variation of the mitochondrial DNA control region in Nile perch *Lates* sp. sampled from 7 water bodies across the African continent were investigated. In a total sample of 124 individual sequences, 37 distinct haplotypes were observed, and 78.4% of these haplotypes were location specific. Haplotypes were found to cluster into 2 major groups, one composed of individuals sampled from East Africa and another from West Africa, with no haplotypes shared in between. These lineages may have developed in geographical isolation during the Pleistocene and have remained largely allopatric without gene flow ($Nm = 0.0$) since that time. There was also evidence that both of these genetic lineages have undergone recent population expansions. We interpret these results in light of the recent evolution of Africa's modern drainage network.

Key words: Africa drainage system, Nile perch, Evolution, mtDNA.

6.1 Introduction

Construction of spatiotemporal details of the evolutionary histories of fishes can reveal key events of drainage evolution (Cotterill & de Wit, 2011; Goodier, Cotterill, O’Ryan, Skelton, and de Wit, 2011). This is because the biogeography of extant freshwater fishes represents responses to landscape evolution. Since the ecology of these fishes incorporates responses to habitat change, these responses leave corresponding signatures in the genetics of these fishes (Balon 1974; Lévêque, 1997; Zengeya, Decru, and Vreven, 2011). Therefore, the evolutionary history of each aquatic species is characterized by a distinct pattern of genetic variation, which includes persisting relics of drainage evolution (Craw, Burrige, Anderson, and Waters, 2007).

Goodier et al. (2011) studied the phylogeography of the African tigerfish of the genus *Hydrocynus* and found that the diversity and distribution of *Hydrocynus* reflects a complex history of vicariance and dispersal. Moreover, range expansions in this particular species testify to changes in drainage basins, and the principal divergence events in *Hydrocynus* have closely interfaced with evolving drainage systems across tropical Africa. Agnèse et al. (1997) using molecular marker polymorphisms found that the Nile tilapia *Oreochromis niloticus* with an almost similar natural distribution on the African continent as the Nile perch, *Lates* sp. can be divided into 2 groups: one in East Africa and the other comprising the Nile and West African populations. They attributed the subdivisions to tectonic movements and volcanic activity in East Africa that played a major role in the formation of the different basins on the continent, although they also postulated that the alternating dry and humid phases across the continent in the Pleistocene may have had large influences on the expansion and contraction of populations and their speciation.

Nile perch occurs naturally in the Afro-tropical region of the African continent, in the major river basins of the Nile, Congo, Volta, Niger, and Senegal Rivers (Fishbase, 2010). It also occurs naturally in Albert Lake in Uganda, Turkana Lake in Kenya (Harrison, 1991), Chamo, Abaya, and Gambella Lakes and the Baro River in Ethiopia (Golubtsov & Hebtelassie, 2010), Lake Chad in Central Africa, Marriott Lake in the Nile Delta in Egypt, and in scores of small rivers in tropical West Africa (Moritz & Linsenmair, 2005). Several introductions of Nile perch into non-native waters resulted in successful establishment as reported in East Africa in Victoria, Kyoga,

and Nabugabo Lakes (Ogutu-Ohwayo, 1993, 1990). For effective management and conservation of this fishery, it is prudent to understand its evolutionary history throughout its extant range and also in environs into which it was introduced.

The rate of change in genetic markers allows investigations of diversity on temporal scales resulting from recent history to deep evolutionary time (Nielsen, 1998). For conservation efforts, the evolutionary history of the Nile perch will assist in deciphering historical congruencies responsible for the genetic subdivision throughout its extant range, in both endemic and introduced environs. The evolutionary history can also be used to test congruence between distributional histories and paleo-environmental changes that may have led to biological evolutionary diversification (van Tuinen, Ramakrishnan, and Hadly 2004; Dominguez-Dominguez, Alda, Perez-Ponce de Leon, Garcia-Garitagoitia, and Doadrio, 2008), and address broader ecological and evolutionary processes like changes in populations, demographics, and genetics over time (Ramakrishnan & Hadly, 2009). In addition, it can also describe how fish populations expanded or contracted in environments they occupy and provide possible successful establishment mechanisms in environs into which they were introduced (Tzeng, 2006). Influences of patterns of post paleo-environmental changes on species distributions and historical demographics of the Nile perch have not yet been studied. For effective management of an exploitable species such as the Nile perch, it is important to define its taxonomic status and historical distributions throughout its extant range and delineate its different evolutionary lineages that may warrant separate management strategies and should be considered different evolutionarily significant units (ESUs) (Soltis & Gitzendanner, 1999).

In this study, we analyzed the genetic diversity of mitochondrial (mt)DNA and combined phylogeographic, phylogenetic, and mismatch analyses to infer spatial dynamics of the distributional and demographic history of the Nile perch throughout its native and introduced ranges on the African continent. Based on the results, we provide proposals for effective management to ensure its continued and sustainable exploitation in the future.

6.2 Materials and Methods

6.2.1 Sample collection and DNA extraction

Nile perch samples were collected from Albert, Turkana, Kyoga, Nabugabo, and Victoria Lakes in East Africa, Chamo Lake in Ethiopia, and the Senegal River of West Africa (Fig. 1). In the field, fin clips (about 2 x 4 cm each) were taken from fish samples, and these were stored in separate 5-ml vials filled with absolute ethanol. The ethanol in the vials was drained off 1 h later, and the vials were refilled with absolute ethanol; in the field, samples were kept at room temperature and in the laboratory at -80 °C before DNA extraction.

Total genomic DNA was extracted using the DneasyTM tissue kit (Qiagen, Germantown, Maryland, USA) according to the manufacturer's instructions. This involved maceration of part of the fin tissue, cell lysis, digestion with proteinase K at 55 °C for 2~3 h, followed by binding of the DNA, and then washing and eluting of the DNA. A negative control that contained no tissue was also prepared. DNA extracts were dissolved in 300 µl of elution buffer and stored at -20 °C. To test for success of DNA extraction, 4 µl of total genomic DNA was electrophoresed on a 2% agarose gel stained with ethidium bromide and then visualized under ultraviolet light for clear DNA bands.

6.2.2 DNA amplification and Sequencing

The mtDNA D-loop region of Nile perch was amplified using primers LN20 (ACCACTAGCACCCAAAGCTA) and HN20 (GTGTTATGCTTTAGTTAAGC) respectively located in the proline and phenylalanine transfer RNA genes (Bernatchez & Danzmann, 1993). Polymerase chain reactions (PCRs) consisted of 5 µL genomic DNA, 25 µL AmpliTaq Gold[®] Master Mix, 5 µL of a 10 µM solution of each of the 2 primers, and double-distilled H₂O added to a final volume of 50 µL. Touchdown PCR conditions were used for amplification, which included an initial denaturation step of 10 min at 95 °C, followed by 1 cycle each of 94 °C for 1 min, 67~51 °C for 1.5 min, and 72 °C for 2 min for each of the annealing temperatures starting at 67 and stepping down by 2 °C from each preceding cycle to 51 °C. This was followed by 25 cycles of 94 °C for 1 min, 61 °C for 1.5 min, and 72 °C for 2 min with a final extension at 72 °C for 7 min. Double-stranded PCR products were cleaned following the manufacturer's protocol (QIAquick, Qiagen).

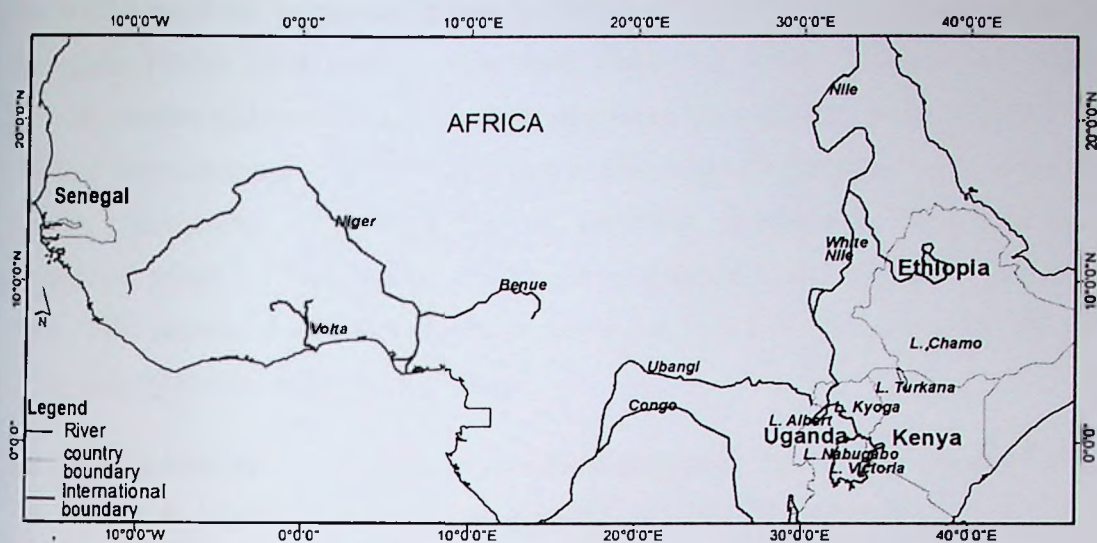


Figure 1: Map showing the Afro-tropical region of Africa and the water bodies from which Nile perch was sampled.

6.2.3 D-loop Sequence Analyses

Sequencing reactions were carried out by Macrogen (Seoul, South Korea) using an Applied Biosystems 3730xl DNA analyzer (Carlsbad, California, USA). Samples were sequenced in both the forward and reverse directions to guarantee the accuracy of nucleotide identification. Standard chromatographic curves of forward and reverse sequences were imported into the program ChromasPro 1.41 (Technelysium, Tewantin, Queensland, Australia) and manually aligned and edited. Consensus sequences were exported to the program BioEdit vers. 7.0.9 (Hall, 1999), aligned with other sequences using the CLUSTALW algorithm (Thompson et al. 1994), and adjusted by eye.

6.2.4 Nucleotide diversity, haplotype diversity and population differentiation

The number of haplotypes, haplotype diversity (h), number of polymorphic sites based on methods of Nei (1987), an estimate of nucleotide diversity (π), the average number of nucleotide differences (k ; Tajima, 1983), and the average number of nucleotide substitutions per site (d_{xy} ; Nei, 1987) were calculated using DnaSP 5.0 (Librado & Rozas, 2009). The MEGA 5.0 software (Tamura et al. 2011) was used to generate a table of variable sites. The number of haplotypes was counted for all polymorphic sites in all populations. Two measures of population differentiation, namely (a) F_{ST} calculated using ARLEQUIN 3.5 (Excoffier & Lischer, 2010) and (b) N_{ST} calculated using Dnasp 5.0 (Librado & Rozas, 2009), were considered. Population subdivisions were further tested by calculating the nearest neighbor statistic (S_{nn} , Hudson, 2000) for mtDNA control region sequences (10^4 permutations and gaps excluded in pairwise comparisons), using DnaSP 5.0 (Librado & Rozas, 2009). In addition, to visualize genetic heterogeneity, a minimum spanning haplotype network was estimated using the TCS program (Clement et al. 2000), which implements the statistical parsimony method of Templeton et al. (1992).

6.2.5 Genetic diversity and phylogenetics analyses

Levels of genetic diversity within and among the different geographical populations were compared using haplotype diversity and a maximum-likelihood (ML) estimation of the average number of nucleotide substitutions per site within and among groups (Nei, 1987) using MEGA 5.0 (Tamura et al. 2011). A hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed using ARLEQUIN 3.5 (Excoffier & Lischer, 2010) to compare the

imputable component genetic diversity to the variance among major lineages of the Nile perch with that observed among populations within each lineage. Φ_{ST} analyses were performed using a matrix of Tamura & Nei (1993) distances. The significance of the variance components associated with different levels of genetic structure was tested using 10^4 permutations.

Phylogenetic trees were constructed using Neighbor-joining (NJ) and ML methods in MEGA5.0 (Tamura et al. 2011), and the Bayesian inference (BI) method using MRBAYES (Huelsenbeck & Ronquist, 2001, Ronquist & Huelsenbeck, 2003). The tree topology was tested by a bootstrap analysis with 5×10^6 pseudo-replicates to evaluate support for the phylogenetic relationships (Felsenstein, 1985). These were further supported by construction of a minimum-spanning haplotype network using TCS vers. 1.6 (Clement et al. 2000) as described in Templeton et al. (1992). MODELTEST 3.6 (Posada & Crandall, 1998) was used to determine the optimal substitution model for the mtDNA data. Parameter values estimated by MODELTEST were adopted for further analysis, including phylogenetic relationships of the haplotypes, analysis of molecular variance (AMOVA), and estimates of gene flow. The NJ and ML trees used the Nile perch's putative most closely related sister species, barramundi *L. calcarifer* (GenBank DQ012415.1) as an outgroup. The robustness of the NJ and ML tree branches were estimated based on 2000 bootstraps, using the complete deletion of gaps.

6.2.6 Mismatch analysis and demographic history

The history of demographic changes in the Nile perch among the identified major lineages was investigated by a mismatch distribution analysis and neutrality tests. Since the population structure has a limited effect on the mismatch distribution (Harpending, 1994), different populations within the same lineage were pooled together. Tajima's (1989) and Fu's (1997) tests of selective neutrality were used to examine haplotypes for the effect of selection by checking deviations from neutrality on the total number of segregated sites as a means to assess evidence of population expansion. The population demographic history was examined by calculating mismatch distributions (Harpending 1994; Rogers 1995). Both the neutrality tests and mismatch distribution analysis were conducted using ARLEQUIN 3.5 (Excoffier & Lischer, 2010). The pairwise frequency distribution of individuals was used to determine species population expansion. Demographic expansion parameters, tau (τ), theta at time 0 - (θ_0) and theta at time 1 - (θ_1), Harpending's Raggedness Index, the simulated sum of squared deviation, and the observed

sum of squared deviations were calculated using ARLEQUIN 3.5. The raggedness index was included in the mismatch analysis to determine the goodness of fit to a unimodal distribution (Harpending, 1994). Following the method of Schneider & Excoffier (1999), the moment estimators of time to expansion τ , and the mutational parameters before ($\theta_0 = 2\mu N_0$) and after expansion ($\theta_1 = 2\mu N_1$) were determined, and were expressed in units of mutational time, where N_0 and N_1 are respective female effective population sizes before and after an expansion that occurred τ generations ago. An estimate of time since population expansion, t , was calculated as $t = \tau/2u$; τ was determined as in Roger & Harpending's (1992) equation, $u = 2\mu_0k$, where k is the number of nucleotides, and μ_0 is the mutation rate per site per nucleotide. In this study, since the molecular clock for the control region of fishes seems to vary among different taxa of fishes, we used a sequence divergence rate of 3.6% per 10^6 yr (Kim & Raymond, 1999) estimated using the geminate species of snook from a sister family, the Centropomidae, to that of the Nile perch, the Latidae, for the Nile perch mtDNA control region analysis.

6.3 Results

6.3.1 Nucleotide diversity, haplotype diversity and population differentiation

Sequencing of the mtDNA control region of 124 individuals of Nile perch (**Table 2a**) produced a PCR fragment from which 476 nucleotides could be analyzed, among which 260 sites were variable. Thirty-seven unique haplotypes were found. Observed nucleotide frequencies were A = 0.283, T = 0.336, C = 0.178, and G = 0.203. Overall, haplotype diversity (h) was 0.893 ± 0.018 , and nucleotide diversity (π) was 0.12408. The most common haplotype, i.e., haplotype 5, was shared by 33 individuals and was found in most sampled water bodies except for Albert Lake, Chamo Lake, and the Senegal River (**Table 1**). In addition, 78.4% of the haplotypes were restricted to their sampling locality (sampled water bodies) (**Table 1**). Haplotype sequences were deposited in GenBank with accession numbers JQ778220~56.

Haplotype diversities (h) for the different collections were relatively high, ranging from 0.583 ± 0.183 for the Albert Lake population to 0.933 ± 0.122 for the Nabugabo Lake population, whereas nucleotide diversities, π , ranged 0.00207~0.01570 (**Table 2a**). The level of genetic diversity varied among the different populations, but were comparable among the 2 major lineages: the East and West African lineages (**Table 2a**).

Population differentiation assessed using the AMOVA showed highly significant heterogeneity ($\Phi_{ST} = 0.99$, $N_{ST} = 0.99$) when the populations were grouped in 2 geographic regions: East and West Africa (Tables 3, 4). The grouping was undertaken following the bifurcation pattern created by the high elevation of sub-basins of the Nile River that determined its northerly flow course. Even when the populations were grouped according to the sample sources, the AMOVA revealed highly significant heterogeneity ($\Phi_{ST} = 0.94$) among the sampled water bodies (Table 3), most probably caused by a lack of shared haplotypes between East and West African Nile perch populations, which may have led to an overestimation of genetic differentiation. To further test the samples' homogeneity, the nearest neighbor statistic (Snn) was calculated for D-loop sequences. When samples were grouped into East and West African populations, the overall test statistic revealed a significant association between D-loop sequence similarity and the geographical location ($Snn = 1.000$, $p = 0.001$) (Table 4).

The minimum spanning haplotype network obtained using TCS 1.21 revealed that both the East and West African Nile perch collections formed a star-shaped network as the general pattern, rooted in the central-most common haplotype which is possibly the ancestral haplotype (Fig. 4). This suggests the existence of relationships between populations from either region and recent population expansion in the Nile perch of both regions.

6.3.2 Geographical distribution of Nile perch lineages, Hierarchical Genetic Diversity and Phylogenetics analyses

The optimal substitution model of GTR (Tavere, 1986) for Nile perch was obtained with MODELTEST 3.6 (Posada and Crandall 1998). The parameters for this model were as follows, $\alpha = 1.46$; base frequencies were A = 0.290, T = 0.329, C = 0.179, and G = 0.202. The NJ tree presented a brush-shaped topology (Fig. 2). Nile perch haplotypes clustered into 2 distinct groups (henceforth referred to as the East and West African lineages). The distribution of the major Nile perch lineages was exclusive to where samples were collected (East or West Africa) with no overlap in distribution. Haplotypes of Nile perch collected from lakes in East Africa were not found in West African collections and vice versa (Table 2). Most of the molecular variance observed in Nile perch was imputable to differences among lineages, $\Phi_{ST} = 0.9345$ (Table 3). The phylogenetic trees showing the 37 haplotypes observed in African Nile perch are shown in figs. 2 and 3. The 2 clusters were separated by an observed 98.06% nucleotide

divergence (**Table 4**) with 99% bootstrap value support for the NJ tree (**Fig. 2**) and a bootstrap value of 1.00 for the 50% majority-rule consensus tree based on the BI analysis (**Fig. 3**). Haplotypes of East and West Africa defined separate monophyletic clades (**Figs. 2, 3**). The genetic distance by the simple P method, $P = Z_d/Z_t$ (Avice 1994), where, Z_d is the total number of mutations, deletions, and substitutions, and Z_t is the total number of bases compared, produced a genetic distance of $P = 58.2\%$ between the 2 lineages.

The relationship among haplotypes and the frequency of each haplotype of the African Nile perch were further visualized by constructing a minimum spanning network (**Fig. 4**). In the network, 2 lineages of East and West Africa were recognized. There was substantial structuring of groups among lineages with an overall Φ_{ST} of 0.0648 (**Table 3**).

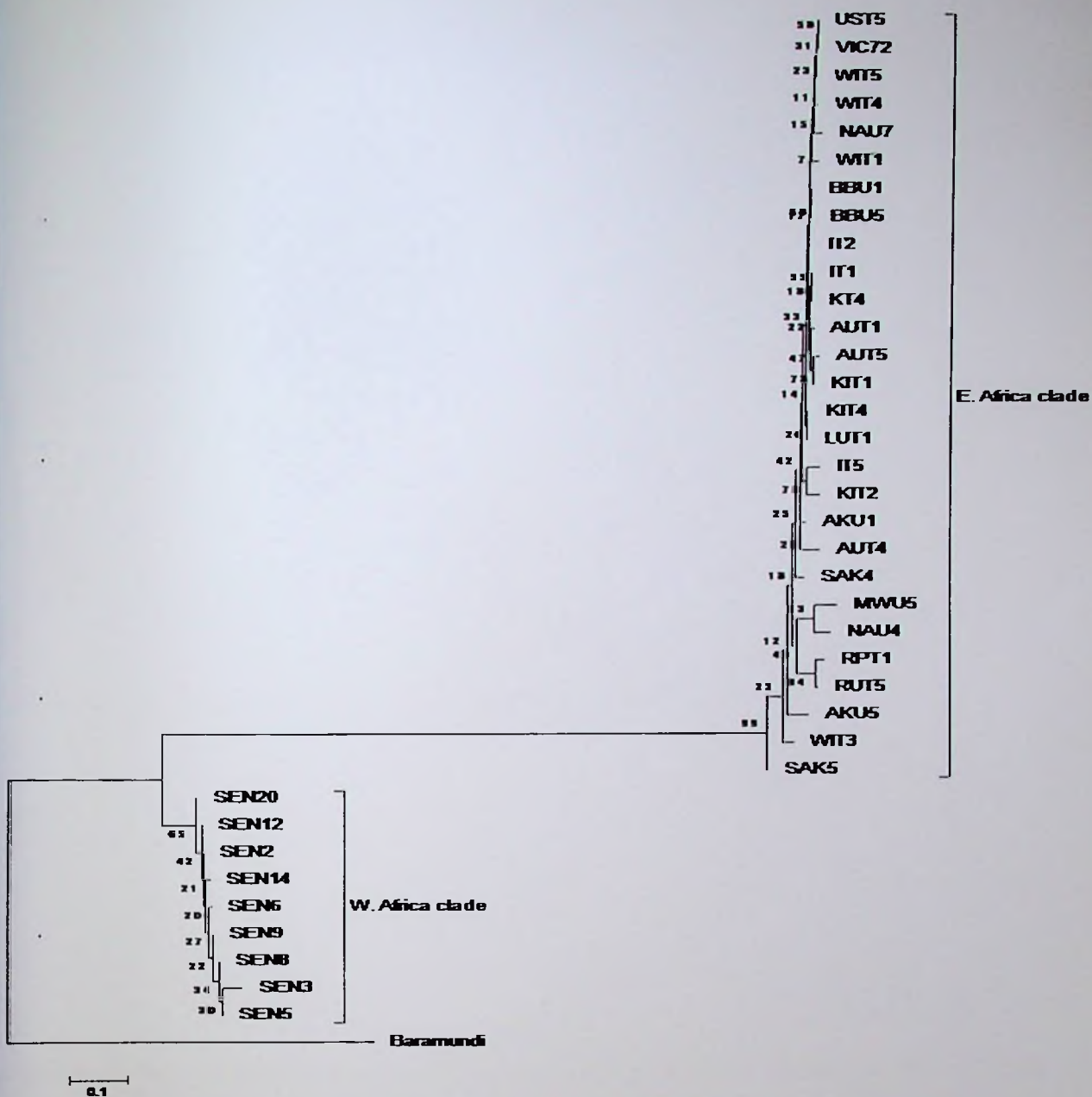


Figure 2: Neighbour-joining tree describing the phylogenetics relationships among 37 mtDNA CR haplotypes of Nile perch. Branch lengths are expressed as observed percentage nucleotide substitutions. Bootstrapped values based on 5000 replications are reported at the internodes. The tree was rooted using the mtDNA CR sequence of *Lates calcarifer* (Baramundi)

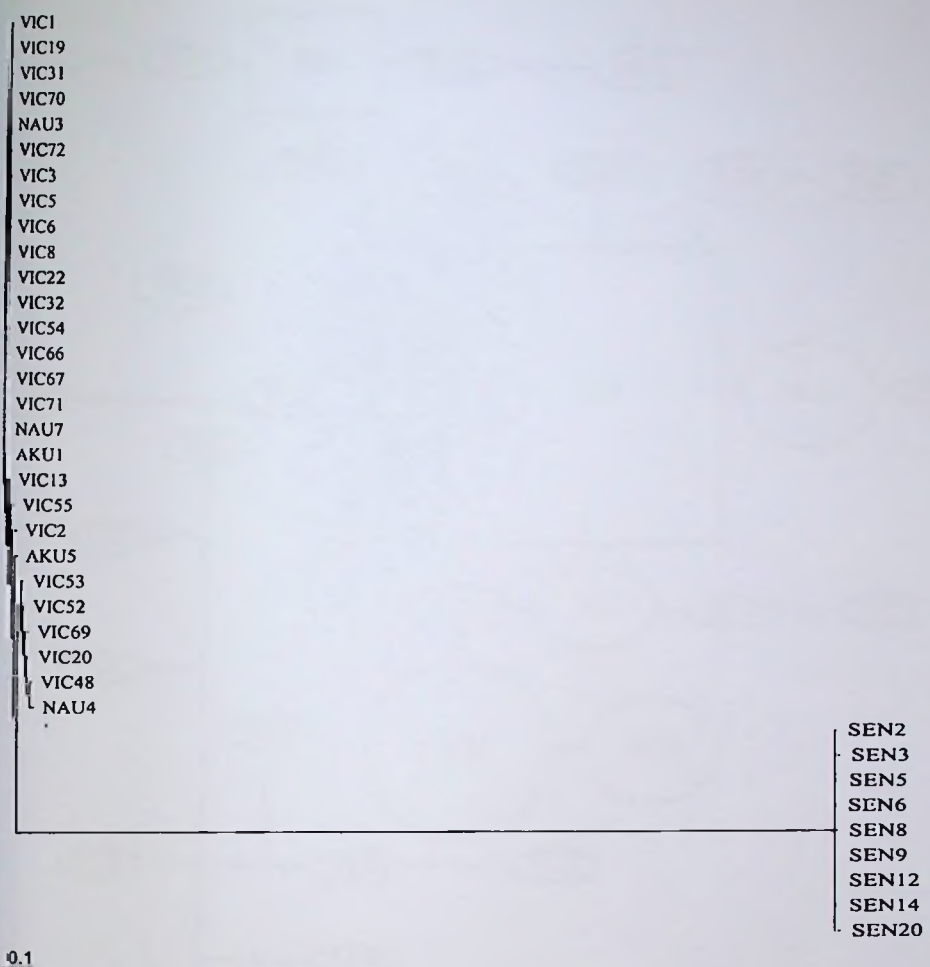


Figure 3: Consensus tree based on maximum-parsimony analysis relating to the two major evolutionary lineages of Nile perch. Relationships among haplotypes within each lineage was based on the 476-bp segment located at the 5' end of mtDNA control region

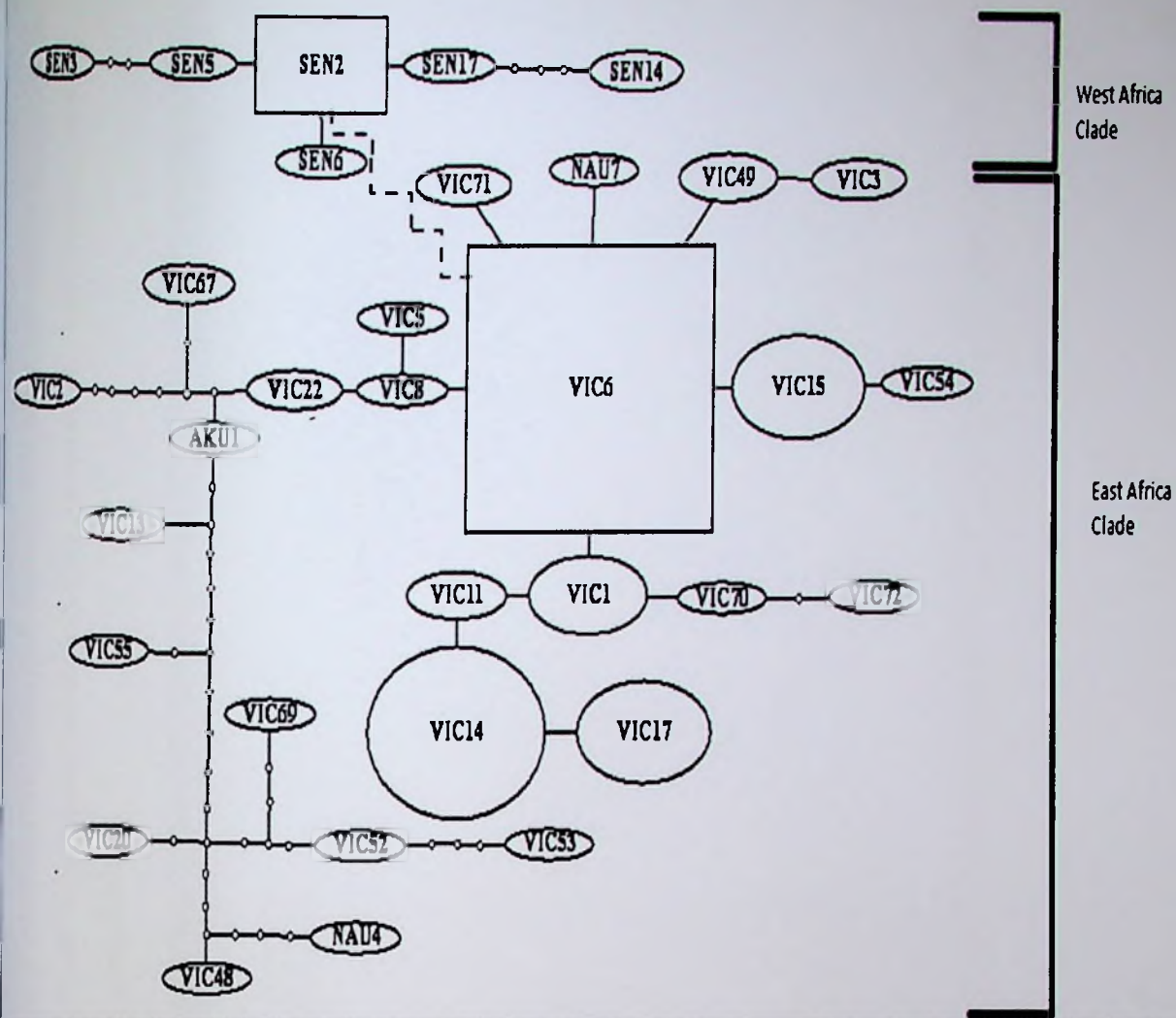


Figure 4: Minimum spanning network analysis of haplotypes for the African Nile perch. The sizes of circles and rectangles are representative of the haplotype frequency and the rectangles represent the ancestral haplotypes in both clades. Haplotypes are marked by names that correspond to Table 1. The dots on lines represent putative mutational steps between haplotypes obtained using the TCS program (Clement et al. 2000)

Table 1: List of 35 polymorphic sites of 37 mtDNA D-loop Nile perch haplotypes. Dots represent nucleotide variants identical to the first sequence. VIC, NAU, KYU, AKU, TLK, TAE and SEN represent populations from water bodies – lakes Victoria, Nabugabo, Kyoga, Albert, Turkana, Chamo and Senegal River respectively

260 Polymorphic sites in 37 haplotypes										Haplotype distribution and Frequency						TOTALS
	10	20	30	40	50	60	VIC	NAU	KYU	AKU	TLK	TAE	SEN			
1 VIC1	1111	1122222233	3333344455	5555556666	667777778	888999990	1									
2 VIC2	1234562346	7802678901	2457802901	2356780125	7912456893	6892456891		1				1				7
3 VIC3	GTATAGCACC	TGTTCTCTT	GCCTCGAAT	TGACTTAGC	GCGAGCACT	ACTCTGTGA										1
4 VIC4		C	G	C	A											2
5 VIC5																2
6 VIC6																3
7 VIC7		C				A										33
8 VIC8		C	G			A										1
9 VIC9		C	G													10
10 VIC10																2
11 VIC11		C														2
12 VIC12		C	G	C		A										18
13 VIC13		C	G	C		A										10
14 VIC14		C	G													1
15 VIC15		C														1
16 VIC16		C	G	C												3
17 VIC17		C	G	C		A										2
18 VIC18			A													1
19 VIC19			G													1
20 VIC20			G													2
21 VIC21		C		G		A										1
22 VIC22		C														1
23 VIC23		C	G			A										1
24 AKU1		C														1
25 AKU5		C	G	C		A										1
26 NAU4				G		A										1
27 NAU7				C												1
28 VIC22				A												1
29 SEN2	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			6
30 SEN3	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			1
31 SEN5	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			1
32 SEN6	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			1
33 SEN8	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			1
34 SEN9	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			1
35 SEN12	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			1
36 SEN14	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			1
37 SEN20	AGTCATGTT	CTCAATGCA	TAT.AAGC	CA.TA.TG	TGAGCTTTATG	TTGGAAAGT							1			2
Number of bases	2222222222	2222222223	2222322222	2222222222	2222222222	2222222222	71	6	10	9	8	4	15			124
TOTALS																

Table 2a: Nile perch population statistics: n sample size, N number of haplotypes, h haplotype diversity, π nucleotide diversity, D Tajima's test, P P-value of Tajima's test, F_s Fu's F_s test, P_{Fs} P-value of F_s test, τ tau, θ_0 theta at time 0, θ_1 theta at time of recorded measure, Hri Harpending raggedness index

Group	Lake	n	N	h	π	D	P	F_s	P_{Fs}	τ	θ_0	θ_1	N_0	N_1	Hri
Population															
East	Albert	9	4	0.583	0.00963	-1.037	0.16	2.217	0.88	20.43	0.250	25513.639	70.90	8386680.09	0.339
Africa															
	Kyoga	10	3	0.644	0.00207	-0.431	0.32	0.345	0.48	1.048	0.006	89037.437	1.17	25557965.10	0.127
	Nabugabo	6	5	0.933	0.01570	-0.940	0.23	0.240	0.46	2.251	0.080	81810.182	25.97	24164312.56	0.164
	Chamo	4	3	0.833	0.00259	0.592	0.83	-0.658	0.15	2.408	0.255	90394.584	44.64	26747561.57	0.250
	Turkana	8	4	0.786	0.00302	0.717	0.80	-0.621	0.20	1.622	0.064	82186.554	14.59	23426793.30	0.087
	Victoria	72	24	0.865	0.00934	-1.222	0.08	-8.328	0.01	2.279	0.203	50336.038	69.74	15089660.07	0.021
West	Senegal	15	9	0.845	0.00461	-1.489	0.07	-4.319	0.01	1.606	0.034	79119.289	11.09	23117407.51	0.104
Africa															

Population age ranges are given with 95% confidence intervals of τ for 1000 mismatch distribution replicates

Table 2b: Observed 95% confidence intervals (CI) of simulated number of polymorphic sites S , demographic parameters (95% CI) among Nile perch lineages τ , θ_0 , and θ_1 are the age of expansion and population size before and following expansion, expressed in units of mutational time. $P(SSD_{obs})$ is the probability of observing by chance a less good fit between the observed and the mismatch distribution for a demographic history of the lineage defined by estimated τ , θ_0 , and θ_1 parameters. $P(Hri)$ is the probability of observing by chance a higher value of the raggedness index than the observed one, under the hypothesis of population expansion

Group	Lake Population	S	95% CI	τ	θ_0	θ_1	$P(SSD_{sim})$	$P(SSD_{obs})$	Hri	$P(Hri)$
East Africa	Albert	15	1-14	13.81(0.04-68.81)	0.00(0.00-3.57)	2.11(0.16-99999.00)	0.240	0.126	0.339	0.20
	Kyoga	3	1-10	0.97(0.00-2.45)	0.00(0.00-0.05)	99999.00(1.35-99999.00)	0.580	0.012	0.127	0.60
	Nabugabo	19	2-12	2.20(0.04-4.18)	0.00(0.00-1.40)	99999.00(2.41-99999.00)	0.130	0.085	0.164	0.48
	Chamo	2	0-7	1.51(0.00-4.30)	0.00(0.00-2.53)	99999.00(16.84-99999.00)	0.650	0.037	0.250	0.74
	Turkana	3	3-12	1.60(0.00-3.23)	0.00(0.00-0.97)	99999.00(4.79-99999.00)	0.680	0.011	0.087	0.76
	Victoria	33	19- 41	1.25(0.24-5.65)	1.25(0.00-2.16)	26.25 (4.05-99999.00)	0.300	0.008	0.021	0.85
West Africa	Senegal	11	6-18	1.56(0.48-2.75)	0.00(0.00-0.53)	99999.00(2.92-99999.00)	0.170	0.028	0.104	0.34

Population age ranges are given with 95% confidence intervals of τ for 1000 mismatch distribution replicates

Source of variation	df	Sum of Squares	variance components			squares	components	
Among groups	6	6026.765	76.46248	93.45	0.9345	1	6465.984	245.03121
Among populations within groups	14	31.644	-0.83513	-1.02	-0.1557	5	16.596	0.12678
Within populations	103	638.323	6.19732	7.57	0.9243	117	214.153	1.83037
Total	123	6696.733	81.82467			123	6696.733	123.40335

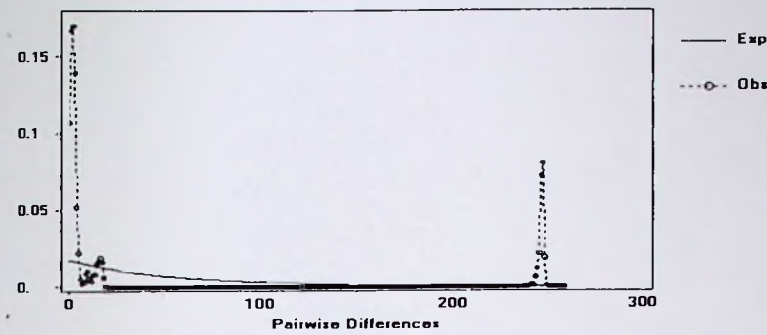
Table 4: mtDNA sequence characteristics of the two lineages - m, number of sequences; S number of segregating sites; N, number of haplotypes; h, haplotype diversity; K, average proportion of nucleotide differences between the lineages; Kt, average number of nucleotide differences; π , nucleotide diversity; Snn, Hudson's statistic; Nm, Gene flow (Hudson, 2000); D, Tajima's neutrality test; FS, Fu's neutrality test, P, probability values of the tests

Statistic	East Africa lineage	West Africa lineage	Overall
M	109	15	124
S	35	11	260
N	28	9	37
H	0.864	0.848	0.893
Kt	3.91	2.08	55.83
π	0.0087	0.0046	0.1241
Snn	-	-	1.00
K	-	-	0.9806
Nm	-	-	0.00
Φ_{ST}	-	-	0.99
N_{ST}	-	-	0.99
D	-1.259 (P = 0.08)	-1.489 (P = 0.06)	-0.545 (p=0.36)
FS	-10.685 (P = 0.00)	-4.319 (P = 0.01)	-1.589 (P = 0.32)

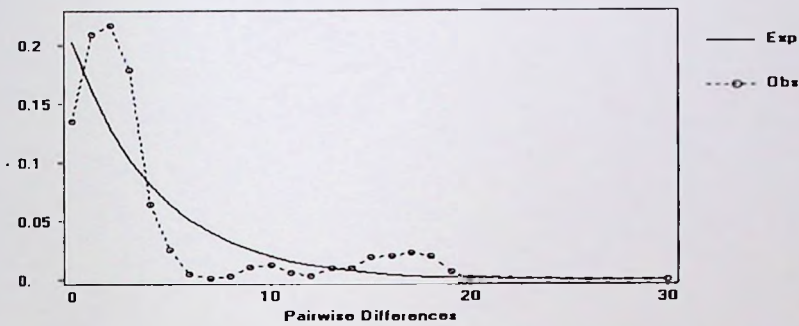
6.3.3 Mismatch Analysis and Demographic history

The mismatch distribution was clearly bimodal with 1 mode corresponding to the number of differences between lineages (~240) and the other to differences among individuals within lineages (~2) (**Fig. 5**). The goodness of fit test showed that no mismatch distributions within the 2 lineages significantly deviated ($\chi^2 = 0.224$, $p > 0.05$) from predicted values under the sudden expansion model (**Table 5**). Considering the 95% confidence interval generated by simulations, values of the observed numbers of polymorphic sites were consistent with simulated sites, meaning that the parameters estimated by the model were sufficiently accurate to account for the observed polymorphism within the 2 Nile perch lineages. Observed values for the age expansion parameter, τ (**Table 2B**), did not substantially differ among populations of the different sampled water bodies, and respective values for the 2 lineages were 1.691 and 1.563 for the East and West African lineages. The respective estimated times since expansion before the present, for the 2 Nile perch lineages were 49,603 and 45,576 yr. The expansion model was further supported by the fact that its findings for all collections agreed with the expectations of a historically expanding population model (Roggers 1995), where $P[\text{simulated sum of squared deviations } (SSD_{sim})] \geq P[\text{observed } SSD(SSD_{obs})]$ (**Table 2B**), the low Harpending's raggedness indices ($Hri = 0.156$, $P = 0.570$), and negative values of Tajima's D and Fu's F ($D = -0.550$, $FS = -1.589$) were consistent with population expansion.

All groups combined (a)



East Africa (b)



West Africa (c)

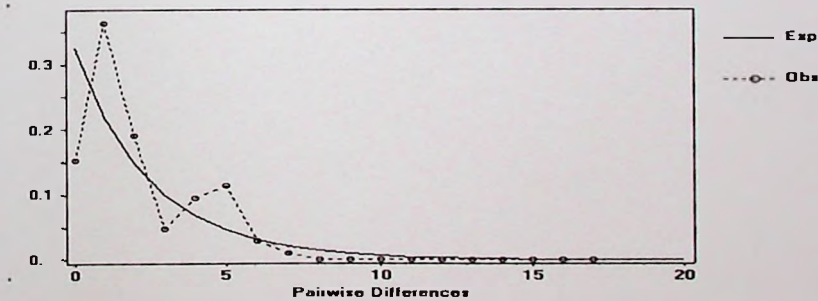


Figure 5: Frequency distributions of pairwise number of mutational differences between Nile perch observed in both Nile perch mtDNA evolutionary lineages combined, graph (a), and separately in the two Nile perch lineages defined by mtDNA analysis: East Africa, graph (b) and West Africa, graph (c). Dots represent observed data, bold curve is the model fitted to data.

6.4 Discussion

Results of the phylogeographic, phylogenetic, and mismatch analyses revealed that Nile perch on the African continent could be divided into 2 lineages that most likely identify ancestral populations of Nile perch that evolved separately as a result of allopatric separation. This discussion focuses on the species' evolutionary history as may have been dictated by various paleo-environmental changes on the African continent in the species' extant range that is most compatible with differential patterns of genetic diversity observed within and among the major evolutionary lineages beginning with the Pliocene-Pleistocene epoch to modern times.

6.4.1 Nucleotide diversity, haplotype diversity and population differentiation

The overall haplotype diversity was relatively high compared to those of other freshwater fishes (McCusker & Bentzen 2010). This high haplotype diversity can be explained by the presence of divergent lineages, the East and West African Nile perch lineages. In addition, the genetic signature of high haplotype diversity and low nucleotide diversity found in the African Nile perch can be attributed to rapid population expansion after a reduction in the effective population size (Ludt, Bernal, Bowen, and Rocha, 2012). The rapid population size increases immediately after the bottlenecks and a possibility that the populations may have had high pre-bottleneck genetic diversities, factors known to augment genetic variation (Avice, 1994), may have countered any negative bottleneck effects. The high genetic heterogeneity as exhibited by the high nucleotide divergences and the lack of shared haplotypes between the 2 major Nile perch lineages can be explained by the long separation of the 2 lineages (McCracken & Sorenson 2005) caused by formation of the novel Nile River basin through rifting, tilting, and climate changes on the African continent in the Late Pleistocene (Talbot, Williams, and Adamson, 2000).

6.4.2 Phylogenetics relationships among Nile perch populations

Nucleotide variability of control region sequences showed geographical structuring in Nile perch. Two major haplotype groups were found on the African continent, and these were well supported. The 2 groups showed high bootstrap values, clearly resolving the Nile perch into East and West African groupings that were monophyletic in the MP, NJ, and ML analyses. The high sequence divergence between the 2 groups and high number of sequences analyzed explain the high bootstrap value on the node, separating the East and West African haplotypes. The genetic distance between the 2 lineages was high, and there were no

haplotypes shared between the 2 lineages, suggesting historical interruption of gene flow for a number of generations, a period long enough for the species to reach a stage of reciprocal monophyly (Avisé et al. 1987, Kazirian & Donnelly 2004).

The parsimonious network of mtDNA haplotypes of the Nile perch D-loop for the 2 lineages, where the most common haplotypes were rooted in the center and shared with or connected to all of the other haplotypes, is an indicator of a recent population expansion in both lineages. Theoretical and empirical expectations suggest that mtDNA which shares a single recent ancestor shows a strong tendency for the ancestral haplotype (often most frequent) to be most interior followed in declining frequency by more-distantly related haplotypes (Avisé, Ball, and Arnold, 1988).

6.4.3 Mismatch analysis and demographic history

In the current study, both Nile perch lineages, the East and West African lineages, fit the sudden expansion model, and when comparing the number of generations as revealed by τ values, the expansions may have occurred around the same time. The population expansion model was further supported by the fact that both lineages had relatively high haplotype diversity (0.864 and 0.848, respectively) in contrast to having low nucleotide diversity (0.0087 and 0.0046, respectively), a scenario which suggests that the 2 lineages experienced population bottlenecks followed by population expansions (Avisé, 2000). The mismatch distribution topologies were very similar, and expansion times were close, suggesting that the expansion events may have occurred in the same period, most probably during the late Pleistocene, when the African continent went through alternating dry and wet climatic phases that may have led to bottlenecks and expansions in fish populations and speciation (Agnese, Adepo-Gourene, Abban, and Fermon, 1997; Beuning et al. 1997). The negative values of Fu's index and Tajima's D further support the hypothesis of recent population expansion. The formation of the novel Nile basin from previous sub-basins through rifting, tilting, and climatic changes in the late Pleistocene may have cut off all outflows from the Nile to West African lakes and river basins, thereby stemming any possible genetic exchange between East and West African fish. This bifurcation was associated with the rifting and tilting of the African continent that led to the rise of the left escarpment of the river Nile basin separating the Nilo-Sudan ichthyofaunal province into West and East Africa (Adamson, 1982; Adamson, McEvedy, and Williams, 1993; Woodward, Macklin, Krom, and William, 2007). Our findings are similar to those of previous studies that worked on Nile tilapia

phylogenetics, a species with a similar distribution to that of the Nile perch on the African continent (Agnese et al. 1997, Rognon & Guyomard, 1997). Those studies also indicated that populations of the Nile tilapia can be divided into East and West African lineages.

6.4.4 Taxonomic status of the Nile perch

The amount of genetic variation as measured by F_{ST} , genetic distance, and phylogenetic nodal support (bootstrap scores) found among East African Nile perch populations was insufficient to support the earlier classification of Nile perch by ecological biologists into 4 different species, *L. niloticus*, *L. macrothalamus*, *L. longispinis*, and *L. albertianus*, in the East African region (Harrison, 1991). Our findings do suggest that there are 2 genetic groupings of Nile perch on the African continent, including 1 East African group with 2 genetically divergent lineages and 1 West African group. Speciation into separate East and West African groups may have been caused by long geographical separation with no genetic exchange. Apparently, phylogenetic relationships among East African Nile perch populations are still problematic and require further investigation.

6.5 Conclusions

MtDNA control region data indicate that there are 2 genetically divergent lineages of Nile perch on the African continent, an eastern lineage defined by populations from East African and Ethiopian waters and a western lineage consisting of populations from the Senegal River. Genetic differences between these lineages are likely indicative of 2 different species and 2 ESUs.

Since depletion of one species cannot easily be replenished by natural migration by the other, the ESUs should be conserved as 2 separate management units, and genetic exchange due to anthropologic activities between the two should be avoided. The status of fisheries of the Nile perch on the African continent, although listed as of 'least concern' because of its wide distribution and there being no major threats, is vulnerable to overexploitation (Azeroual et al. 2010). The Nile perch fishery on the African continent has relatively high genetic diversity compared to other freshwater fish species, meaning the fishery is healthy and if effectively managed can ably support both the species' sustainable exploitation and conservation efforts.

6.6 Acknowledgements

This study was sponsored by the government of Uganda and the World Bank through its Millennium Science Initiatives (MSI). We are grateful to the staff and students of the Genetics Laboratory in the College of Agricultural and Environmental Sciences, Makerere University where most of the work was done. Our thanks go to technicians on board of the Explorer vessel of TAFRRI, Tanzania, Mr Odada of KEMFRI, Kenya, Mutebi of Nabugabo, Seye Mouhamadane of Dakar, Senegal, and Dereb of Addis Ababa, Ethiopia for their efforts during sampling of Nile perch in different parts of the continent. DNA sequencing was carried out in the laboratory of Macrogen (GeumCheon-Gu, Seoul, South Korea). The authors thank Prof. Dean Jerry for proofreading an earlier copy of the manuscript.

6.7 References

- Adamson, D. A. (1982). The integrated Nile. In MAJ Williams, DA Adamson, eds. A land between two Niles: Quaternary geology and biology in Central Sudan. Rotterdam: AA Balkema, pp. 221-234.
- Adamson, D., McEvedy, R., & Williams, M. A. J. (1993). Tectonic inheritance in the Nile basin and adjacent areas. *Isr. J. Earth Sci.* 41, 75-85.
- Agnese, F. J., Adepo-Gourene, B., Abban, K. E., & Fermon, Y. (1997). Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei Cichlidae). *Heredity* 79, 88-96.
- Awise, J. C., Arnold, J., Ball, R. M., Jr Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., Saunders, N. C. (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematic. *Annu. Rev. Ecol. Systemat.* 18, 489-522.
- Awise, J. C., Ball, M. R., & Arnold, J. (1988). Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory of neutral mutations. *Mol. Biol. Evol.* 5(4), 331-344.
- Awise, J. C. (1994). Molecular markers, natural history and evolution. New York: Chapman and Hall, 511 pp.
- Awise, J. C. (2000). Phylogeography: the history and formation of species. Cambridge, MA: Harvard Univ. Press, 447 pp.
- Azeroual, A., Entsua-Mensa, M., Getahun, A., Lalèyè, P., Moelants, T., Ntakimazi, G. (2010). *Lates niloticus*. In IUCN 2013. IUCN red list of threatened species. Version 2013.1. Retrieved from www.iucnredlist.org.
- Balon, E. K. (1974). Fish production of a tropical ecosystem. In EK Balon, AG Coche, eds. Lake Kariba: a man-made tropical ecosystem in Central Africa, *monographiae biologicae* 24. The Hague: Dr W Junk Publishers, pp. 248-748.
- Bernatchez, L., & Danzmann, G. R. (1993). Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of Brook charr (*Salvelinus fontinalis* Mitchill). *Mol. Biol. Evol.* 10: 1002-1014.
- Beuning, K. R. M, Talbot, M. R., & Kelts, K. (1997). A revised 30000 year paleoclimatic and paleohydrologic history of Lake Albert, East Africa. *Paleogeogr. Paleoclimatol. Palaeoecol.* 136(3-4), 259-279.
- Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9, 1657-1660.

- Cotterill, F. P. D., & de Wit, M. J. (2011). Geocodynamics and the Kalahari epeirogeny: linking its genomic record, tree of life and palimpsest into a unified narrative of landscape evolution. *S. Afr. J. Geol.* 114, 493-518.
- Craw, D., Burridge, C., Anderson, L., & Waters, J. M. (2007). Late Quaternary river drainage and fish evolution, Southland, New Zealand. *Geomorphology* 84, 98-110.
- Dominguez-Dominguez, O., Alda, F., Perez-Ponce de Leon, G., Garcia-Garitagoitia, J. L., & Doadrio, I. (2008). Evolutionary history of the endangered fish *Zoogoneticus quitzeensis* (Bean, 1898) (Cyprinodontiformes: Goodeidae) using a sequential approach to phylogeography based on mitochondrial and nuclear DNA data. *BMC Evol. Biol.* 8, 161.
- Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes-application to human mitochondrial DNA restriction data. *Genetics* 131, 479-491.
- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite vers. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10, 564-567.
- Fu, X. Y. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147, 915-925.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, (783-791).
- FishBase. (2010). "Lates niloticus, Nile perch". FishBase. Retrieved from <http://fishbase.org.cn/Country/CountryList.php?ID=347&GenusName=Lates&SpeciesName=niloticus>
- Goodier, S. A, Cotterill, F. P., O'Ryan, C., Skelton, P. H., & de Wit, M. J. (2011). Cryptic diversity of African tigerfish (genus *Hydrocynus*) reveals palaeogeographic signatures of linked Neogene geotectonic events. *PLoS One* 6(12): e28775. doi: 10.1371/journal.pone.0028775.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acid Symp. Ser.* 41, 95-98.
- Harpending, R. C. (1994). Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. *Hum. Biol.* 66, 591-600.
- Harrison, K. (1991). The taxonomy of East African Nile perch, *Lates* spp. (Perciformes, Centropomidae). *J. Fish Biol.* 38, 175-186.
- Hudson, R. R. (2000). A new statistic for detecting genetic differentiation. *Genetics* 155, 2011-2014.

- Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17, 754-755.
- Kazirian, D., & Donnelly, A. M. (2004). The criterion of reciprocal monophyly and classification of nested diversity at the species level. *Mol. Phylogen. Evol.* 32, 1072-1076.
- Kim, A. D., & Raymond, R. W. (1999). Amphi-panaimic geminates of snook (Percoidae: Centropomidae) provide a calibration of the divergence rate in mtDNA control region of fishes. *Mol. Phylogen. Evol.* 13(1), 208-213.
- Ludt, B. W., Bernal, A. M., Bowen, W. B., & Rocha, A. L. (2012). Living in the past: phylogeography and population histories of Indo-Pacific wrasses (genus *Halichoeres*) in shallow lagoons versus outer reef slopes. *PLoS One* 7(6): e38042. doi:10.1371/journal.pone.0038042.
- Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451-1452.
- McCracken, K., & Sorenson, M. (2005). Is homoplasy or lineage sorting the source of incongruent mtDNA and nuclear gene trees in the stiff-tailed ducks (*Nomonyx-Oxyura*)? *Syst. Biol.* 54(1), 35-55.
- McCusker, R. M., & Bentzen, P. (2010). Positive relationship between genetic diversity and abundance in fishes. *Mol. Ecol.* 19, 4852-4862.
- Moritz, T., & Linsenmair, K. (2005). West African fish diversity – distribution patterns and possible conclusions for conservation strategies. *In*: [Huber, A. B., Sinclair, J. B., & Karl-Heinz Lampe] (eds.) *African biodiversity*, pp 187-196. New York, NY 10013, USA Springer US, printed in the Netherlands.
- Nei, M. (1987). *Molecular evolutionary genetics*. New York: Columbia Univ. Press.
- Nielsen, L. J. (1998). Population genetics and the conservation and management of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 55(S1), 145-152.
- Ogutu-Ohwayo, R. (1990). The decline of the native fishes of Lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environ. Biol. Fishes* 27, 81-96.
- Ogutu-Ohwayo, R. (1993). The effects of predation by Nile perch, *Lates niloticus*, L. on fish of Lake Nabugabo, with suggestions for conservation of endangered cichlids. *Conservation Biology* 7(3), 701-711.
- Posada, D., & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817-818.

- Ramakrishnan, U., & Hadly, E. A. (2009). Using phylochronology to reveal cryptic population histories: review and synthesis of four ancient DNA studies. *Mol. Ecol.* 18, 1310-1330.
- Rogers, A. R. (1995). Genetic evidence for a Pleistocene population explosion. *Evolution* 49, 608-615.
- Rogers, A. R., & Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic difference. *Mol. Biol. Evol.* 9, 552-569.
- Rognon, X. & Guyomard, R. (1997). Mitochondrial DNA differentiation among East and West African Nile tilapia populations. *J. Fish Biol.* 51: 204-207.
- Ronquist, F., & Huelsenbeck, J. P (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574.
- Soltis, P. S., & Gitzendanner, A. M. (1999). Molecular systematic and conservation of rare species. *Conserv. Biol.* 13, 471-483.
- Tajima, F. (1989). Statistical method for testing neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585-595.
- Talbot, R. M., Williams, J. A. M., & Adamson, A. D. (2000). Strontium isotope evidence for late Pleistocene reestablishment of an integrated Nile drainage network. *Geology* 28(4), 343-346.
- Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10, 512-526.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28, 2731-2739.
- Tzeng, C. S., Lin, Y. S., Lin, S. M., Wang, T. Y., & Wang, F. Y. (2006). The phylogeography and the population demographics of selected freshwater fishes in Taiwan. *Zool. Stud.* 45, 285-297.
- Tavere, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures Math. Life Sci. (Am. Math. Soc.)* 17, 57-86.
- Templeton, A. R., Crandall, K. A., & Sing, C. F. (1992). A cladistic analysis of phenotype associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132, 619-633.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22, 4673-4680.

van Tuinen, M., Ramakrishnan, U., & Hadly, E. A. (2004). Studying the effect of environmental change on biotic evolution: past genetic contributions, current work and future directions. *Phil. Trans. A Math. Phys. Engin. Sci.* 362: 2795-2820. doi: 10.1098/rsta.2004.1465.

Woodward, J. C., Macklin, G. M., Krom, D. M., & Williams, J. A. M. (2007). The Nile: evolution, Quaternary river environments and material fluxes. *In*: D Maddy, MG Macklin, JC Woodward, eds. *River basin sediment systems: archives of environmental change*. Rotterdam: A Balkema, pp. 261-292.

Zengeya, T. A., Decru, E., Vreven, E. (2011). Revalidation of *Hepsetus cuvieri* (Castelnau, 1861) (Characiformes: Hepsetidae) from the Quanza, Zambezi and southern part of the Congo ichthyofaunal provinces. *J. Nat. Hist.* 45(27), 1723-1744.

CHAPTER SEVEN

7.0 General Discussion

The aim of this study was to establish the evolutionary and phylogeographical relationships of the Nile perch –*Lates niloticus* in its extant ranges using both molecular and morphological characterisation. The basic principle of all molecular genetic methods is to use inherited stable markers to identify genotypes that characterize populations, after which the resulting data are used to test whether samples were drawn from a large, randomly mating population with equilibrium genotype frequencies, or from an assemblage of genetically distinct units (Coyle, 1998). To justify the continued use of morphological characteristics, Weins (2004) pointed out that since we have not reached a stage where all molecular phylogenies are reconstructed without error, it is still pertinent to carry out rigorous morphology-based phylogenies as a check on the molecular analysis results.

Molecular and morphological characterisation of organisms always produces contrasting outcomes in biology (Germann et al. 2010), but for the Nile perch the 2 types of data are congruent. Analyses using morphological characters resulted into 2 morphotypes and similarly genetics analyses revealed 2 clusters in East African Nile perch. If future research confirms stability of the established genetic and morphological structures and their mappings occur in the same locales this would further confirm the congruence. The two groupings most probably represent two subspecies of the Nile perch in East Africa. Further studying of the 2 subspecies should enable designing of appropriate regimes for their management and determining their attributes for that will aid selection of the best subspecies for use in the proposed domestication and culture of Nile perch.

In vast more or less open water bodies such as Lake Victoria, the lack of migration limits to dispersal and gene flow between populations especially of a migratory fish such as the Nile perch is expected to work against local genetic and morphological differentiation. But the findings of this study showed that the Nile perch of Lake Victoria is genetically differentiated though at a low level. This population structuring is most probably attributed to subtle or eriptic isolation mechanisms such as differential physic-chemical conditions and intense fishing pressure (Palumbi, 1994). There is need to determine what empirical factors are causing the Nile perch population structuring tendency, which will aid in designing the species' effective management.

Genetic analysis of the East African Nile perch revealed surprisingly high levels genetic diversity in all the different lakes' populations. The relatively high genetic diversity of the heavily exploited Nile perch of East African lakes as revealed by the study findings is contrary to the expectations where removal of biomass is expected to reduce the genetic diversity of the exploited species (Lee & Sarfina, 1995). The question is whether with the reported decline in Nile perch stocks (Balirwa et al. 2003; EfD, 2008) this high genetic diversity can be maintained for long before the predicted fishery crash (Lowe-McConnell, 1994). Or it may be deceitful by painting a good situation of 'high genetic diversity' as Nei, et al. (1975) population genetics theory suggests that genetic diversity is significantly reduced only in very small populations in that even crashed populations may consist of far too many fish to show declines in genetic diversity measurable with feasible sample sizes (Ryman, Utter, and Laikre, 1995).

The taxonomy of the Nile perch requires reappraisal, for according to this study's findings the East African Nile perch belongs to one species with two subspecies. It is also the finding of this study that the African continent has two ESUs - one East African and the other West African that may probably be equated to species. Yet according to Otero (2004) fossil record studies revealed a number of extant species including: *Lates niloticus*, *Lates macrophthalmus*, *Lates longispinis* with two species earlier thought to be independent, *Lates albertianus* and *Lates maliensis*, being found to be synonymous with *L. niloticus* within the East African region. Like *Lates albertianus* and *Lates maliensis* the other two species *Lates macrophthalmus* and *Lates longispinis* may be synonymous with *Lates niloticus* or just subspecies of the later. Like the phylogenetic relationships between East African Nile tilapia (*Oreochromis niloticus*) populations are still problematic and deserve additional investigation (Rognon & Guyomard, 1997) the Nile perch of East Africa require fresh reappraisal.

The findings of this study indicated that there were 2 lineages of Nile perch on the continent that may have been geographically separated since Late Pleistocene and stayed without genetic exchange between the two since then. The geographical separation may have been caused by paleoclimatic and paleoenvironmental changes that caused merging of smaller basins to form the bigger Nile basin cutting off westward flow to the Atlantic and Eastward flow to the Indian Ocean. This resulted into dividing and eventual separation of the Afro-tropical widely distributed Nile perch into East and West Africa lineages. The findings agree

with earlier studies of genetical characterisation of the Nile tilapia a species with almost similar distribution on the African continent like Nile perch that found similar clustering into East and West African lineages (Rognond & Guyomard, 1997).

7.1 Conclusions and Recommendations

Both morphological and genetics characterization of the Nile perch revealed two groupings of Nile perch in all lakes where the Nile perch occurs in E. Africa. These two groupings most probably are representatives of 2 subspecies of the Nile perch. It is recommended that the 2 be further studied and characterized to ascertain whether they may require different management approaches.

There is genetic differentiation among the Nile perch of L. Victoria, which shows that the species is undergoing population structuring. Therefore it is recommended that further studies be undertaken in order to enable the delineation of the different populations of Nile perch within L. Victoria to allow for effective management of this fishery. Such studies should first identify whether the various populations are conspecific prior to undertaking any assessment/confirmation of the population structure. Managing the Nile perch of Lake Victoria as one panmictic population may lead to overexploitation and eventual extinction of isolated locally adapted populations.

Nile perch of lakes of E. Africa is of relatively high genetic diversity compared to other freshwater fishes. I recommend that for non-connected water bodies the species should not be translocated to ensure continued integrity and high genetic diversity. For the proposed Nile perch culture the findings of this study will serve as a benchmark for assessing the impact of acitivity on the species genetics both on-farm and in the wild. Ecological and biological performance of two lineages within and stocks of the different water bodies need to be contrasted to establish if differences in ecological and biological performances have a genetic basis. Such a study will guide the choice of the appropriate sources of the parental stock in the domestication and aquaculture trials of the Nile perch. The Nile perch of the introduced environments had relatively higher genetic diversity than the putative native sources indicating that most probably there was higher propagule pressure resulting from multiple source and repeated introductions than the official record. It is therefore recommended that unofficial fish movements and translocations be strongly prohibited within the region.

Nile perch on the African continent belongs to 2 genetic groupings that have been separated for a very long time, which most probably represent 2 lineages. These 2 groupings should be considered as 2 different ESUs and since depletion of individuals from one lineage cannot easily be replenished by natural migration by the other, the ESUs should be conserved as such and genetic exchange due to anthropologic activities between the two should never be allowed.

7.2 References

- Balirwa, J. S., Chapman, C. A., Chapman, J. L., Cowx, J. I., Geheb, K., Kaufman, L., Lowe-McConnell, H. R., Wanink, H. J. O., Welcomme, L. R., & Witte, F. (2003). Biodiversity and fishery sustainability in the Lake Victoria Basin: An unexpected marriage? *BioScience* 53, 703-715.
- Bernatchez, L. (2001). The evolutionary history of brown trout (*salmo trutta* l.) Inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution* 55(2), 351-379.
- Chistiakov, D. A., Hellemans, B., & Volckaert, F. A. M. (2006). Microsatellites and their genomic distribution, evolution, function, and applications: a review with special reference to fish genetics. *Aquaculture* 255, 1-29.
- Coyle, T. (1998). Stock identification and fisheries management: the importance of using several methods in a stock identification study. In: D. A. Hancock (Ed). *Taking Stock: defining and managing shared resources*. Australian Society for Fishery Biology, Sydney pp. 173-182.
- EfD (Environment for Development). (2008). Over fishing of Nile perch in Lake Victoria. EfD Tanzania press release 02nd-11-2008. Retrieved from http://www.efdinitiative.org/news-press/copy_of_job-openings/job-openings-archive/press-releases-2009/over-fishing
- Germann, C., Pollet, M., Tanner, S., Backeljau, T., & Bernasconi, M. V. (2010). Legs of deception: disagreement between molecular markers and morphology of long-legged flies (Diptera, Dolichopodidae). *Journal of Zoological Systematics and Evolutionary Research* 48(3), 238-247.
- Lee, M., & Sarfina, C. (1995). The Effects of Overfishing on Marine Biodiversity. *The Journal of Marine Education*, 13, 5-9.
- Liu, Z. J., & Cordes, F. J. (2004). DNA marker technology and their applications in aquaculture genetics. *Aquaculture* 238, 1-37.
- Lowe-McConnell, R. (1994). The changing ecosystem of Lake Victoria, East Africa. *Freshwater Forum* 4, 76-89.

- Nei, M., Maruyama, T., & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution* 29, 1-10.
- Otero, O. (2004). Anatomy, Systematics and phylogeny of both recent and fossil latid fishes (Teleostei, Perciformes, Latidae). *Zoological Journal of the Linnaean Society* 141, 81-133.
- Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecological Systems* 25, 547-572.
- Rognon, X., & Guyomard, R. (1997). Mitochondrial DNA differentiation among East and West African Nile tilapia populations. *Journal of Fish Biology* 51, 204-207.
- Ryman, N., Utter, F., & Laikre, L. (1995). Protection of intraspecific biodiversity of exploited fishes. *Rev. Fish Biol. Fish.* 5, 417-446.
- Weins, J. J. (2004). The Role of Morphological Data in Phylogeny Reconstruction. *Systematic Biology* 53(4), 653-661.

Full Length Research Paper

Population morphological variation of the Nile perch (*Lates niloticus*, L. 1758), of East African Lakes and their associated waters

Matthew. T. Mwanja^{1*}, Vincent Muwanika³, Sylvester Nyakaana³, Charles Masembe⁴, Dismas Mbabazi⁵, Justus Rutasire¹ and Wilson. W. Mwanja²

¹ARDC – Kajjansi, Box 530 Kampala, , Uganda.

²Department of Fisheries Resources, Box 4 Entebbe, Uganda.

³College of Agricultural and Environmental Sciences, Makerere University, Box 7298 Kampala, Uganda.

⁴College of Natural sciences, Makerere University, Box 7062 Kampala, Uganda.

Accepted 24 October, 2011

Abstract

In this study an attempt was made to determine whether there are morphological variations associated with the different geographical populations of the Nile perch of East Africa. Analyses of the levels of morphological differentiation based on morphological characterisation and variation were carried out. The study was based on 864 Nile perch sampled from 25 different locations from different Lakes in the region and 10 morphological characters. Also determined were the length – weight relationships and condition factor of sampled Nile perch. The log transformed data of all sampled Nile perch were subjected to multivariate analysis, using 'PAST' statistical software. Findings showed that peduncle form and size, of all the variables used in this study, contributed most to the variance. The analysis clustered the Nile perch into two groups, which were found to be as characterised by earlier morphological description of this species and most probably are representatives of two distinct taxa of Nile perch in the East African waters. The LWRs and condition factor conformed to the fish isometric growth formula $W = aL^b$ with the value for the pooled data being $W = 0.6664L^{0.3264}$. The existence of 2 morphs agrees with earlier taxonomists (Harrison, 1991; Ribbink, 1987; Bwathondi, 1985; Holden, 1967) who proposed that Nile perch exists in two populations – a bigger shallow water dwelling morph and a smaller deep open water dwelling morph. This calls for identification and mapping of the 2 populations in the different waters of East Africa that may require application of different management regimes.

Key words: Nile perch, taxa, morphological variation, East Africa.

INTRODUCTION

Nile perch *Lates niloticus* (Linnaeus, 1782) is an important commercial freshwater fish species in East Africa. It is endemic to Lakes Albert and Turkana and was introduced into Lakes Victoria, Kyoga and Nabugabo five decades ago (Hamblyn, 1961; Gee, 1964; FRO, 1967; Welcomme, 1988; Pringle, 2005) with the intention of bolstering the then failing fishery of these

Lakes and as a potential for sport fishing (Gee, 1969, Acere, 1985; Barlow and Lissle, 1987; Achieng, 1990).

After its introduction, the Nile perch slowly colonized its new environs and then exploded in the 1980s (Arunga, 1981; Okaromon et al., 1985; Goudswaard and Ligtoet, 1988). This increase in Nile perch population coincided with the disappearance of close to 50% of the endemic fish species in the new environs and Nile perch has been labelled the villain (Barel et al., 1985). Nile perch has established itself in the new environs and currently dominates the fishery in these Lakes. Despite this

*Corresponding author. E-mail: mmtenywa@hotmail.com.

ological wrath, due to the lucrative international and national markets, the Nile has boosted the economies of the three riparian countries of Lake Victoria – Kenya, Uganda and Tanzania, where most Nile perch is exported, bringing in a hefty USD \$250 million from the international markets alone (Odongkara et al., 2009).

Due to its high demand in the market, this fish species has been put under increased fishing pressure in this region, leading to a great decline in its stocks (Environment Development, 2008). The decline in Nile perch stocks is further compounded by habitat degradation, water pollution and changes in prey for Nile perch (Mkumbo Lightvoet, 1992; Ogutu-Ohwayo, 1990). One way to mitigate this problem is to domesticate and culture this species so as to supplement the supplies from the wild and reduce the pressure on the natural stocks. This study is part of a bigger project whose overall goal is to domesticate, breed and culture the Nile perch based at the Aquaculture Research and Development Center – Jinja, Uganda.

To culture any species from the wild it is prudent that the species is characterized taxonomically, ecologically and geographically as the first step. This enables one to name this species and to determine the performance of different taxa that are in the wild so as to aid selection of the best suited taxon for culturing (Liao and Haung, 2000). Earlier efforts (Worthington, 1929, 1932; Harrison, 1941; Hauser et al., 1998) failed to unambiguously define the Nile perch in this region. For example what was being taken as one species – *Lates niloticus*, turned out to be considered as two different species – *Lates albertiensi* and *Lates macrophthalmus* for the Lake Albert Nile perch (Worthington, 1929; Worthington, 1940), and interestingly the Lake Turkana Nile perch were taken to be two different subspecies – *Lates niloticus rudolfianus* and *Lates niloticus longispinus* of the riverine species *Lates niloticus* (Worthington, 1932; Greenwood, 1966). Later this grouping was revised to subspecies level and not species of *Lates niloticus* as the specimens from Lakes Albert and Turkana were seen as intermediates and also thought to interbreed (Worthington, 1940). The other school of thought is that the observed differences in morphology of the Nile perch may be arising from the remoteness of a normal population curve. According to Harrison (1991) the Lake Victoria Nile perch has been and is wrongly referred to as *Lates niloticus*, yet its taxonomic status has never been resolved. This paper is part of a bigger study that is attempting to taxonomically characterise the Nile perch using a combination of both morphological and molecular techniques. The paper examines the morphological variation among Nile perch in East Africa and determines which morphological characteristic contributes most to this variation as well as deciphering its morphological phylogenetics. In this paper, results of the analysis of the Nile perch populations and subdivisions based on measurement of morphological characteristics, done using the 'PAST'

statistical software are presented and discussed.

METHODOLOGY AND STUDY AREA

Nile perch samples were taken from Lakes Albert in Uganda and Turkana in Kenya where the fish is endemic and the putative origins for the introduction of this species into Lakes Victoria, Kyoga and Nabugabo in Uganda. Lakes Albert and Turkana are rift valley lakes, with Lake Albert found on the western border of Uganda with Democratic Republic of Congo and Lake Turkana on the north western border of Kenya with Ethiopia. Lake Victoria is the second largest fresh water lake in the world, and is shared between three countries: Uganda (41%), Kenya (6%) and Tanzania (53%). Lake Kyoga is in Central Uganda connected to both Lake Victoria and Lake Albert by the Victoria Nile River. There are waterfalls on either side of Lake Kyoga – Owen Falls and Murchison Falls, which act as a buffer to the movement of adult Nile perch due to their size. Lake Nabugabo is a small (24 km²) satellite lake, formerly a bay on the western shore of Lake Victoria separated from the main lake by a sand bar more than 5,500 years ago (Chapman et al., 1996). It is alleged that before introduction of Nile perch from Lake Albert into Lake Victoria, the fish were first reared and tried out in Lake Nabugabo before eventually being introduced into Lake Victoria (Barlow and Lissle, 1987).

Sample collection

In Lakes Albert, Turkana, Kyoga and Nabugabo (Figure 1) fish were caught using seine nets. In these lakes, the sampling followed different eco-zones (shallow offshore waters and the deep open waters) because different Nile perch morphs are believed to inhabit different eco-zones. In Lake Victoria, samples were caught from 22 different geographical locations (Figure 2) by bottom trawls. Sampling followed transects pre-determined by hydroacoustics experts after consideration of a number of factors including traditional fishing grounds, history as told by the local fisher folk, previous knowledge of Nile perch distribution, findings of tagging experiments, and the Lake topography. Trawls were done after the hydroacoustics experts on board detected signals indicating abundance of relatively big size fish on their computer screen. For the trawls the depth of sampled sites ranged from as low as 4.5m to a maximum 50.4m (Table 1). The seining of Nile perch from both deep open and shallow offshore waters and trawling from the deep open waters toward the shallow offshore waters ensured that if there are differences in habitat for different morphs as alleged, then all morphs would be sampled and included in the analyses. A total of 864 Nile perch fish were sampled from all lakes (Table 1).

Biometric measurements and data analysis

Morphological characters studied included – total length (TL), standard length (SL), body depth (BD), peduncle length (PL) and peduncle depth (PD) (Figure 3), so for every sampled fish measurements of these characteristics were taken immediately after capture/trawl. Ratios of TL to all other variables – SL, BD, PL and PD, and ratio of PL to PD for each sampled fish were calculated and also used in the analyses. The study also considered the length – weight relationships (LWRs) and the condition factor of the fish and establishes whether these are affected or affect the morphological variation of this fish. Condition factor refers to a mathematical formula for determining the physiological state of a fish, including its reproductive capacity. The heavier a fish is for a given length, the higher its condition factor (K), which is one factor that is considered when determining the

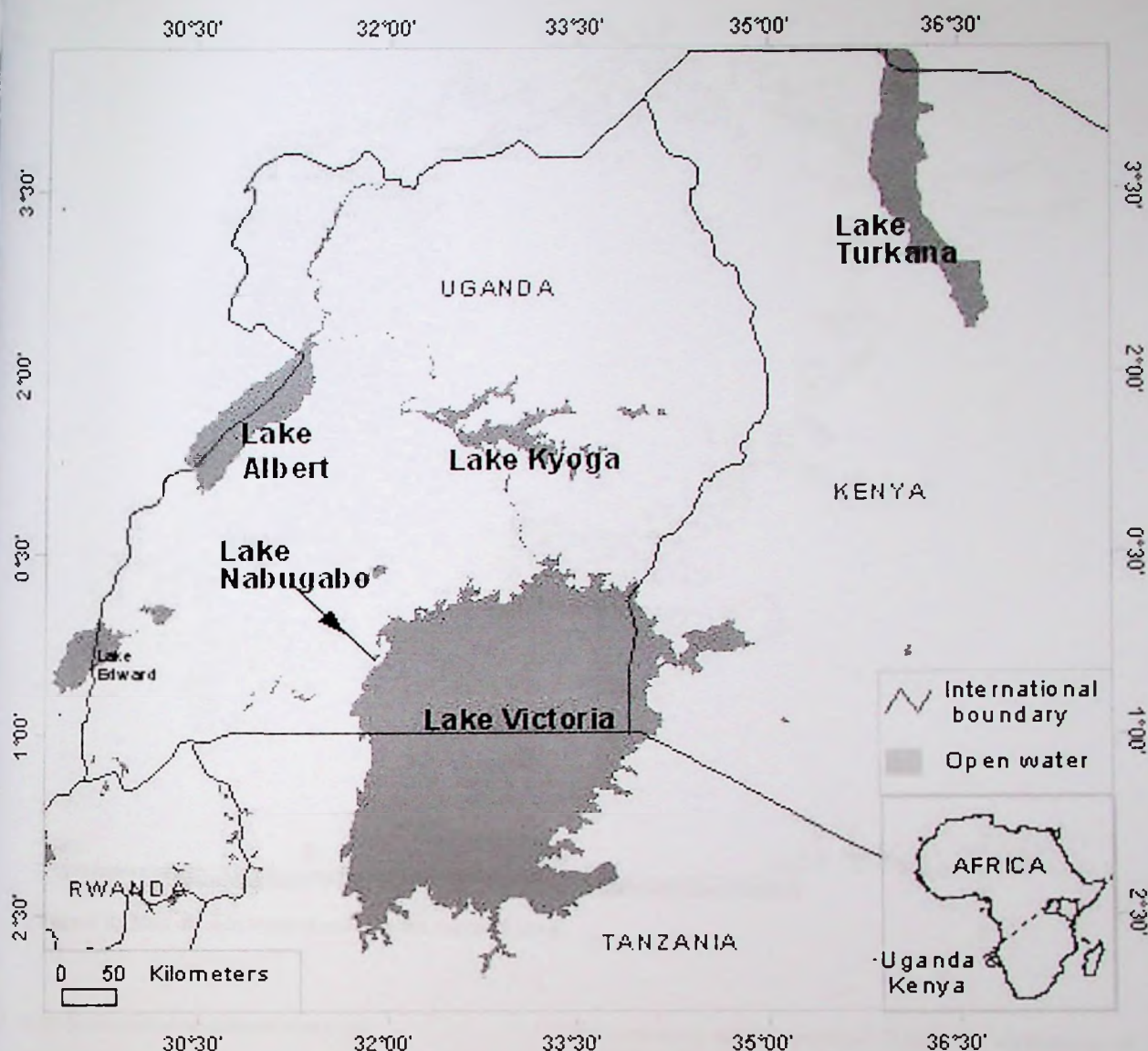


Figure 1. Map showing the East African lakes from which Nile perch was sampled for this study.

with a fishery. The LWRs of pooled data for all fish were estimated using the equation $W = aL^b$ (Pauly, 1983), where W = weight of fish in grams, L = TL in cm, b = length exponent (slope) and a = proportionality constant (intercept). The 'a' and 'b' values were obtained from a linear regression of length and weight of fish. The correlation or degree of association between length and weight was calculated from linear regression analysis, $R = r^2$. The condition factor 'K' was determined using the equation, $K = W/L^3$. In order to standardize the data, all variables taken including SL, BD, PL and PD were expressed as a percentage of TL. Also since different strains of the Nile perch are thought to differ in SL and PD, the ratios of these two variables were expressed as a percentage and the condition factor 'K' was determined for each sample. To ensure normality and homogenous variances, all values were $\log_{10}$ - transformed before statistical analyses were performed. The outcomes (PL/PD%, BD/TL%, BD/SL% and 'K') were then used as the variables in the consequent multivariate data

analysis using the PAST software. Principal component analysis (PCA) was used to extract principal components from the four variables. The 'PAST' (PALaeontology STATistical) software package was originally aimed at palaeontology but currently has become popular in ecology and other fields (Hammer et al., 2001). Using PAST, the measured morphological characters were subjected to multivariate analysis, a tool highly recommended for determining relationships between populations of a species (Thorpe, 1987) and for use in stock identification of freshwater fish and investigating taxonomical problems in sympatric populations (Cawdery and Ferguson, 1988). The most significant component that contributed most to the variance was then used in determining the morphological phylogenetic relationships of the Nile perch in the East African waters. Based on the outcome of the mixture analysis in 'PAST', all data were subjected to K-means clustering to determine non-hierarchical clustering of all the samples into the number of groups (2) specified under the mixture analysis. For all

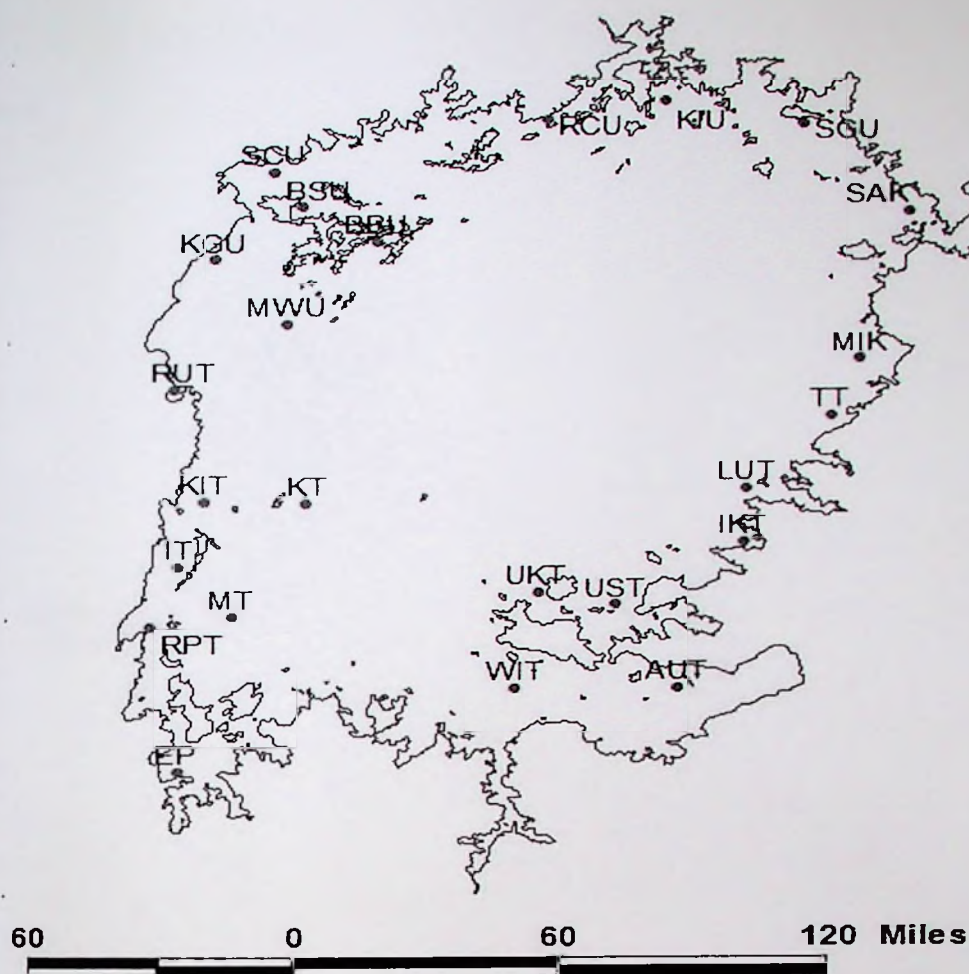


Figure 2. Map of Lake Victoria showing the sampled sites.

the significance level considered was 0.05.

RESULTS

WLRs of Nile perch was found to be $\log_{10}W = 4 + 0.3264\log_{10}TL$, corresponding to $W = 4 \times 10^4 L^{0.3264}$, indicating an isometric growth pattern. The regression coefficient for the pooled data was $r^2 = 0.9832$ (Figure 6). The relative condition factor ranged from 0.57 to 1.8 with average for pooled data at 1.16. Principal component analysis of the data from the four variables showed that approximately 96.8% of the total variation explained along two components 1 and 2, that is 96.8% and BD/TL% respectively. With most of the variance provided by the component 1 - PD/PL% (77%) followed by BD/TL% (23.300%) then BD/SL% (0.99%) and condition factor 'K' (0.017%) making the largest contribution to morphological variation of the Nile perch (Table 2). All the Eigen values for all

components were positive (Table 2) indicating all used variables have some effect on the morphological variation of the Nile perch with the most significant contribution from the variable percentage ratio PD/PL (Figure 5). When subjected to mixture analysis, the fish clustered into two groups (Figure 4). From the K-means clustering, the majority of the individuals (583) belonged to one group, that is, cluster 1 and the rest (281) belonged to the other group, that is, cluster 2. The mixture analysis curve (Figure 4) for all data pooled together showed two major groupings. The Neighbour-Joining clustering using Euclidean similarity measure generated by 'PAST' using the $\log_{10}$ -transformed data from the principal component 1 also revealed two clades.

DISCUSSION

These findings indicate that Nile perch fish growth is isometric and obeys the cube law ' $W = L^3$ ', that is, the

Figure 1. Showing the Sampling sites, number (N) of fish sampled from each site, the eco-zone of the sample site, and for Lake Victoria at the start and end of each fishing trawl.

Population ID	Number (N)	Lake	Country	Eco - zone	Depth start – End of trawl
in Pasha /EPT	22	Victoria	Tanzania	Deep/ open	9.0 – 9.0 m
nga point /RPT	50	Victoria	Tanzania	Deep/ open	25.0 – 24.0 m
zinga /MT	38	Victoria	Tanzania	Deep/ open	45.0 – 48.0 m
ebe/KT	31	Victoria	Tanzania	Deep/ open	50.0 – 50.4 m
ndo/IT	40	Victoria	Tanzania	Deep/ open	30.0 – 30.0 m
oka/KIT	40	Victoria	Tanzania	Deep/ open	33.0 – 29.0 m
afu /RUT	20	Victoria	Tanzania	Deep/ open	9.4 – 9.4 m
uba/LUT	50	Victoria	Tanzania	Deep/ open	30.2 – 30.2 m
engere /UST	42	Victoria	Tanzania	Deep/ open	40.0 – 37.5 m
ra /UKT	33	Victoria	Tanzania	Deep/ open	32.5 – 32.0 m
u /WIT	20	Victoria	Tanzania	Deep/ open	40.0 – 40.0 m
ustus /AUT	20	Victoria	Tanzania	Deep/ open	13.2 – 14.0 m
ena /MWU	28	Victoria	Uganda	Deep/ open	32.0 – 31.0 m
ege /KGU	15	Victoria	Uganda	Deep/ open	5.5 – 4.5 m
abu/BBU	46	Victoria	Uganda	Deep/ open	4.8 – 4.5 m
angi Sseese/BSU	39	Victoria	Uganda	Deep/ open	8.0 – 8.0 m
ebery Channel/RCU	16	Victoria	Uganda	Deep/ open	24.0 – 24.6 m
dwe/KIU	41	Victoria	Uganda	Deep/ open	20.0 – 24.0 m
ilu /SGU	12	Victoria	Uganda	Deep/ open	20.0 – 18.0 m
a/SAK	50	Victoria	Kenya	Deep/ open	30.0 – 25.0 m
ngo/ MIK	35	Victoria	Kenya	Deep/ open	43.0 – 43.0 m
kana /TLK	19	Turkana	Kenya	Deep/open and shallow/offshore	–
ert Kaiso /AKU	95	Albert	Uganda	Deep/open and shallow/offshore	–
ga - Bukungu /KYU	50	Kyoga	Uganda	Deep/open and shallow/offshore	–
ugabo /NAU	18	Nabugabo	Uganda	Deep/open and shallow/offshore	–

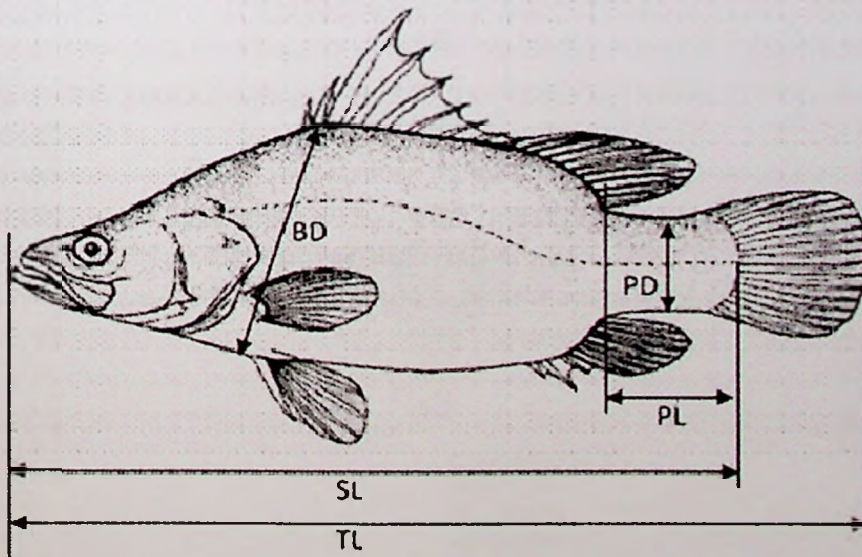


Figure 3. The morphological characteristics of the Nile perch fish that were measured during this study including total length (TL), standard length (SL), body depth (BD), peduncle length (PL) and peduncle depth (PD). In addition, the wet weight of each sampled fish was also taken. (Source of photo – FAO Fish Finder (1994).

Table 2. Eigen values and the distribution of variance among the different components.

Morphological character	Component ranking	Eigen value	Variance (%)
PD/PL%	1	71.965	73.457
BD/TL%	2	22.827	23.300
BD/SL%	3	3.160	3.226
K	4	0.016	0.017

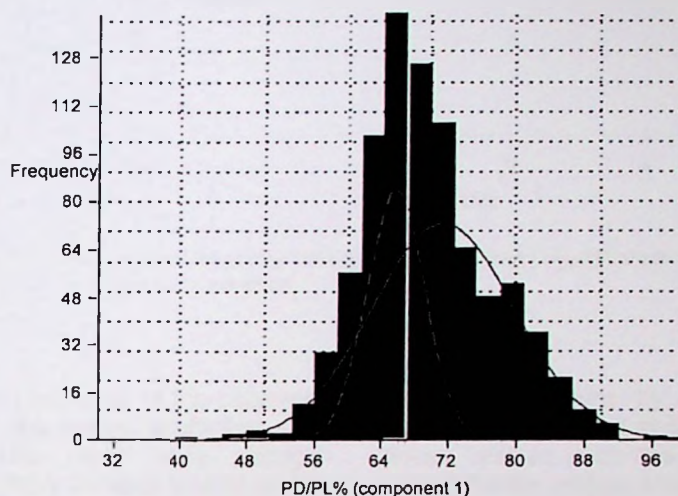


Figure 4. A graph of Frequency Vs PD/TL% of all data from the principal component (component one) showing normal curves of the probable 2 major groupings of sampled Nile perch generated after PCA under 'PAST'. The unbroken line curve represents cluster one the most dominant and the broken line represents cluster 2.

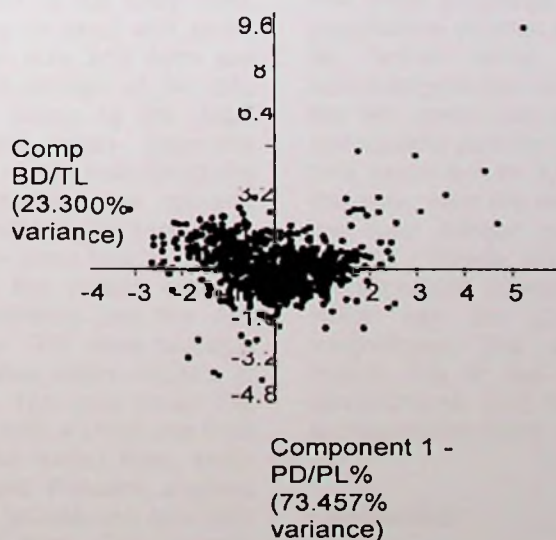


Figure 5. Scores of the first two most significant principal components in this study. Component 1 represents the PD/PL percentage ratio whereas Component 2 represents the BD/TL percentage ratio.

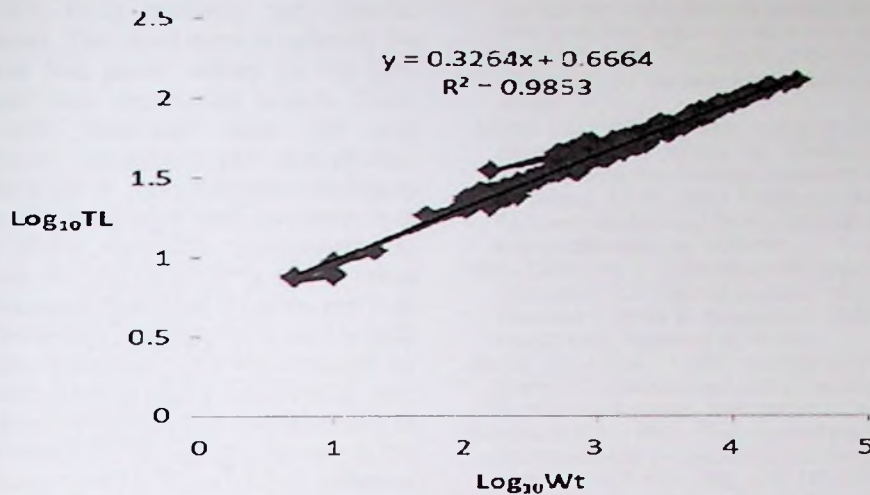


Figure 6. Regression graph for pooled data for Log_{10}TL versus Log_{10}Wt showing regression coefficient ' R^2 ' and regression equation.

nt of the Nile increases in proportion to the cube root of its length. Also from the regression coefficient $r^2 = 0.9853$ all the LWRs of Nile perch were strongly significant. The results of the PCA indicate that of all the variables used in this study, the percentage ratio of TL/BD (73.457%) contributed most to the variance in the Nile perch. This suggests that the different morphs of the Nile perch most probably may be largely be differentiated by their characteristic difference in the form and size of the peduncle. The ratio $\text{BD}/\text{TL}\%$ also contributes significantly (23.3%) to the variation in morphology of the Nile perch, this ratio is an indicator of the body form, whether the fish is lean and long or stout and short. Before the peduncle and body size and form are responsible for the variation in morphology of the Nile perch, which agrees with what is known by the local fisher folk around the different lakes (personal communication from the interviews conducted during the sampling). The fisher folk recognize two general morphotypes of Nile perch, one stout (deeper body) with short and thick peduncle, and the other lankier, with a lean and slender peduncle. From the mixture analysis using PAST software and the generated tree the fish were divided into two groups/branches. This most probably suggests that there are probably two major morphs of Nile perch in East African waters. The local fisher folk have attested that the stout form, with a short and thick peduncle, is more common than the lankier form, which agrees with the mixture analysis and K-means analysis which places the sampled fish in two groups with one (583 samples) more prominent than the other (281 samples). The lankier morph most probably uses its slender and long peduncle to swim faster and forages in the deep waters where rare availability of prey may not allow formation of a deep body, but this keeps it away

from easy capture by fishermen, whereas the stout morph with the short and thick peduncle is thought to be slower, and as such this morph forages in the shallower open offshore waters where it can hunt easily, a situation that leads to its easier capture by the fishermen and therefore its predominance in the catch. The two clusters/groups differentiated largely by variation in peduncle morphs, despite the spatial separation from the putative lakes of origin they are as clearly distinct in their new environments in which they were introduced over five decades ago. Therefore it is most likely that the two Nile perch groupings are most probably from two different populations of even taxa at subspecies level, thus calling for further work using a combination of both morphological and molecular markers to properly classify the Nile perch and provide the basic (taxonomic status) prerequisite tool for its effective management. Most of the Nile perch fish in the sampled lakes belonged to one of the taxa, with the stout morph as the most dominant in the East African waters of lakes - Albert, Turkana, Victoria, Kyoga and Nabugabo. The contribution to morphological variation of the Nile perch by the condition factor can be ignored as the value (0.017%) is insignificant. The differences in condition factor are mainly due to the wellbeing of the fishery in certain environments and how fish best exploit those particular environments rather than the morphology of the fish.

Conclusion

The exhibited isometric growth implies that Nile perch does not increase in weight faster than the cube of their total lengths. The Nile perch of East Africa has two distinct morphotypes, the stout and lankier forms, an

indicator that there are most probably two different species of this species. The stout form is currently the major contributor to the Nile perch fishery on the East African Great lakes and their associated waters. Given the morphological and molecular data are only complementary and not in competition with one another (Ang, 2004), this outcome of morphological phylogenetic analysis will be compared, contrasted and combined with on-going analysis of the molecular phylogenetics of the very same samples of Nile perch in a bid to more fully define the taxonomical status of the Nile perch of African waters. The findings of this study are largely in conformity with earlier descriptions of this species by Jen (1967), Hopson (1972), Bwathondi (1985) and Hopson (1991). The implications to the management of Nile perch fishery in the East African region is that the variable two populations or taxa may have different ecology, prey preferences, environmental conditions, growth at first maturity fecundity, life expectancy and other differences that will require developing different management regimes for each morph/population. About the choice of taxon of Nile perch most suitable for domestication and culturing in the region, there is need for further work to determine the comparative advantage of the two morphs such as easy of adaptability to on-farm environmental conditions, breeding in captivity, stress tolerance, disease resistance and uptake of artificial diets. The two morphs need to be first mapped in the different lakes and then further studied.

KNOWLEDGEMENTS

This study is part of the bigger project Nile perch technologies sponsored by the World Bank Millennium Science Initiatives (MSI) under the Uganda National Council of Science and Technology (UNCST). We are grateful to Dr. Rob Skilton of BECA - Nairobi, Kenya for helping in correcting the final manuscript. We are grateful to Lake Victoria Fisheries Organisation for having accommodated us on board during their Lake wide stock assessment on Lake Victoria and to the crew of the motor vessel of Tanzania Fisheries Resources Research Institute (TAFIRRI) that was used during the trawl on Lake Victoria. We are also indebted to technicians Mapunda and Ogaro from TAFIRRI, Odada from Kenya Marine and Fisheries Research Institute (KMRFI), Opiri and Mutebi of Uganda for their assistance during morphometrics measurements on Lake Victoria, Turkana, Nabugabo and Kyoga respectively.

REFERENCES

Ang (1985). Observations on the biology, age, growth, maturity and sexuality of Nile perch, *Lates niloticus* (Linne), and the growth of fishery in the northern waters of Lake Victoria. In: CIFA report of the third session of the subcommittee for the development and

- management of the fisheries of Lake Victoria. Jinja, Uganda. 4-5 Oct. 1984. Fish. Rep. 335. FAO, Rome, pp. 42-61.
- Achieng AP (1990). The impact of the introduction of Nile perch, *Lates niloticus* (L) on the fisheries of Lake Victoria. J. Fish Biol., Supp. A: 17-23.
- Arunga JO (1981). A case study of lake Victoria Nile perch *Lates niloticus* (Mbuta) fishery. In: Proceedings of the workshop of the Kenya Marine and Fisheries Research Institute on aquatic resources of Kenya, 13-19 July, Mombasa. Kenya Marine and Fisheries Research Institute and Kenya National Academy for Advancement of Arts and Sciences, pp. 165-184.
- Barel CDN, Dorit R, Greenwood PH, Fryer G, Hughes N, Jackson PBN, Kawanabe H, Lowe-McConnell RH, Nagoshi M, Ribbink AJ, Trewavas E, Witte F, Yamaoka K (1985). Destruction of fisheries in Africa's lakes. Nature, 315: 19-20.
- Barlow GC, Lissle A (1987). Biology of The Nile perch *Lates niloticus* (Pisces: Centropomidae) with Reference to its Proposed Role as a Sport Fish in Australia. Biol. Conserv., 39: 269-289.
- Bwathondi POJ (1985). The future of the fisheries of the Tanzania part of Lake Victoria, in view of the predominance of the Nile perch *Lates niloticus*. F.A.O. Fish. Rep., 335: 143-145.
- Cawdery SH, Ferguson A (1988). Origins and differentiation of three sympatric species of trout (*Salmo trutta* L.) in Lough Melvin, Ireland. Polskie Archiwum Hydrobiologii, 35: 267-277.
- Chang ML (2004). Phylogenetics analysis of morphological data (Review). Hum. Biol., 76(1): 165-168.
- Chapman LJ, Chapman AC, Ogutu-Ohwayo R, Chandler M, Kaufman L, Keiter AE (1996). Refugia for Endangered Fishes from an Introduced Predator in Lake Nabugabo, Uganda. Conserv. Biol., 10(2): 554-561.
- EAFRO (1967). East Africa Freshwater Fisheries Organization Annual Reports, 1961-1967; Entebbe, Uganda.
- Environment for Development (EFD) (2008). Over fishing of Nile perch in Lake Victoria. EFD Tanzania press release 02 - 11 - 2008. At: http://www.efdtan.org/news-press/copy_of_job-openings/job-openings-archive/press-releases-2009/over-fishing last accessed on 05th/01/2009.
- FAO Fish Finder (1984). At: http://www.fao.org/fi/common/format/pop/Uplmage.jsp?xp_imageid=698261&xp_showpos=1.
- Gee JM (1964). Nile perch investigations. Annual Reports of East African Freshwater Research Organisation, 1962/63, pp. 14-24.
- Gee JM (1969). A comparison of certain aspects of the biology of *Lates niloticus* (Linne 1758) in some East African lakes. Rev. Zool. Bot. Afr., 80: 244-262.
- Greenwood PH (1966). The Fishes of Uganda. Kampala (Uganda): Uganda Society.
- Goudswaard PC, Ligthvoet W (1988). Recent developments the fishery of Haplochromines (Pisces: Cichlidae) and the Nile perch *Lates niloticus* (L.) (Pisces: Centropomidae) in Lake Victoria, pp. 101-112. In: CIFA. Report of the fourth session of the Subcommittee for Development and Management of the Fisheries of Lake Victoria, 6-10 April, 1987, Kisumu, Kenya. FAO Fish. Rep. 388. FAO, Rome.
- Hambalyn EL (1961). The Nile perch project. EAFRO Annual Report, 1960, pp. 26-32.
- Hammer O, Harper DAT, Ryan PD (2001). Paleontological statistics software package for education and data analysis. In: Paleontol Electronica, 4(1): 4-9.
- Harrison K (1991). The taxonomy of East African Nile perch, *Lates* spp. (*Perciformes Centropomidae*). J. Fish Biol., 38(2): 175-186.
- Hauser LG, Carvalho GR, Pitcher TJ, Ogutu-Ohwayo R (1998). Genetic affinities of an introduced predator: Nile perch in Lake Victoria, East Africa. Mol. Ecol., 7: 849-857.
- Holden MJ (1967). The systematics of the genus *Lates* (Teleostei: Centropomidae) in L. Albert East Africa J. Zool. (London), 151: 329-342.
- Hopson AJ (1972). A study of the Nile perch, *Lates niloticus* (L.), in Lake Chad. HMSO Overseas Research Publications, 19; Foreign and Commonwealth Office, London.
- Liao IC, Haung YS (2000). Methodological approach used for the domestication of potential candidates of aquaculture. In: Recent advances in Mediterranean aquaculture finfish species diversification Zaragoza: CIHEAM-IAMZ, pp. 97-107.
- Mkumbo OC, Ligthvoet W (1992). Changes in the diet of Nile perch,

- Lates niloticus* (L), the Mwanza Gulf Lake Victoria. In: *Hydrobiologia*, 32(1): 79-83.
- Muganyizi OK, Abila OR, Luomba J (2009). The contribution of Lake Victoria Fisheries to National Economies. *Afr. J. Trop. Hydrobiol. Fish.*, 12: 47-51.
- Munyaho R (1990). Changes in prey ingested and the variations in the Nile perch and other fish stocks of Lake Kyoga and Northern waters of Lake Victoria (Uganda). *J. Fish. Biol.*, 37(1): 55-63.
- Munyon JO, Acere TO, Ocenodongo DL (1985). The current state of the fisheries in the northern portion of lake Victoria, pp. 89-98. In: *FAO Report of the third session of the Subcommittee for Development and Management of the Fisheries of Lake Victoria*, 4-5th October, 1984, Jinja, Uganda. *FAO Fish. Rep.* 335. FAO, Rome.
- Nyong'o RM (2005). The origins of the Nile perch in Lake Victoria. *Hydrobiologia*, 55(9): 780-787.
- Thorpe RS (1987). Geographic variation: A synthesis of cause, data pattern and congruence in relation to subspecies, multivariate analysis and phylogenies. *Bull. Zool.*, 54: 3-11.
- Welcomme RL (1988). International introductions of inland aquatic species. *FAO Fish Technical Paper*, 249, FAO Rome.
- Worthington EB (1929). A Report on the Fishing Survey of Lakes Albert and Kyoga, March to July, 1928. London: Crown Agents for the Colonies
- Worthington EB (1932). Scientific results of the Cambridge expedition to the East African Lakes, pp. 30-31.
- Worthington EB (1940). Geographical differentiation in freshwaters with special reference to fish. In the *Systematics* (J. Huxley, ed.), London: Oxford University Press, pp. 287-302.

Research Article

Genetic evidence of successful establishment of the Nile perch (*Lates spp. L.*) in East African lakes and implications for management

Matthew Tenywa Mwanja¹, Wilson Waiswa Mwanja², Vincent Muwanika³, Charles Masembe^{1*} and Sylvester Nyakaana⁴

¹ Box 530 Kampala, ARDC - Kajjansi, Uganda

² Box 4 Entebbe, Department of Fisheries Resources, Uganda

³ Box 7298 Kampala, College of Agricultural and Environmental Sciences, Makerere University Kampala, Uganda

⁴ Box 7062 Kampala, College of Natural Sciences, Makerere University Kampala, Uganda

E-mail: mmtenywa@hotmail.com (MTM), wwwmwanja@yahoo.com (WWM), vmuwanika@mutenr.mak.ac.ug (VM), cmasembe@zoology.mak.ac.ug (CM), snyakaana@mutenr.mak.ac.ug (SN)

*Corresponding author

Received: 5 April 2012 / Accepted: 30 November 2012 / Published online: 15 December 2012

Handling editor: Vadim Panov, REABIC, Finland

Abstract

Nile perch establishment in novel ranges in East Africa is one of the most successful freshwater invasion stories in the recent history. It became ecologically dominant and well established in several lakes in the Lake Victoria Region in a period of less than 5 decades. Genetic diversity of both introduced and native populations were assessed and patterns compared in order to establish the genetic consequences of their introductions. Genetic variation was surveyed at both the mitochondrial control region (CR) and at nine microsatellite loci. A total of 527 Nile perch fish were sampled from 5 East African lakes: native source populations were examined from lakes Albert and Turkana while introduced populations were sampled from lakes Kyoga, Nabugabo and Victoria. Both types of markers revealed higher average genetic diversity for invasive species ($HE = 0.70$, $h = 0.81$) than for native source populations ($HE = 0.66$, $h = 0.69$). Both populations scored relatively higher than the average for freshwater fishes ($HE = 0.62$). Both native and introduced populations had 2 underlying genetic groupings in similar proportions as revealed by the STRUCTURE program. The high genetic diversity was most probably a consequence of higher numbers of propagules than outlined by official records of introductions. Use of high number of individuals at stocking is probably the reason for apparently minimized 'founder effects' of Nile perch in the introduced ranges. The two underlying populations revealed through genetic analysis may be representatives of the two subspecies of Nile perch previously reported in other studies. Implications to the management of this fishery in the East African region is that with the relatively high genetic diversity, the species could be sustainably exploited if it were effectively managed. In addition, further studies of the life histories and other attributes of the two genetic groupings of Nile perch in the region are recommended, as they may require different management plans.

Key words: Nile perch; East Africa; invasiveness; genetic diversity

Introduction

Genetic characteristics of invasive species are important in their successful establishment and range expansion (Lee 2002). Explanation of the importance of evolutionary and genetic processes during establishment of invasive fish species has received little consideration with most research focused on the victims of translocations, i.e. the native fish species (Hanfling 2007). From the evolutionary and genetics perspectives, the potential for a species to adapt to a novel or

changing environment depends on the quantity of genetic variability of the species (Lavergne and Molofsky 2007). Yet introduced species are expected to undergo loss of genetic diversity during introduction and establishment due to the founder effect caused by genetic drift resulting from small number introductions, which greatly lowers the adaptability of the population in novel habitats (Dlugosch and Parker 2008). In East Africa Nile perch (*Lates spp.*) occurs naturally in Lake Albert and the White Nile River in Uganda, and in Lake Turkana in Kenya. It was introduced into lakes Kyoga and Nabugabo from Lake

Albert and into Lake Victoria from lakes Albert and Turkana (Mwanja and Mwanja 2008).

Lake Albert was said to contain two perch species, namely *Lates niloticus* and *Lates macrophthalmus* (Holden 1967). Welcomme (1967), who considered the two as sub-species rather than species, presumed that the two sub-species were introduced together into Lake Victoria either accidentally or intentionally. In addition, some introductions are said to have been from the Lake Turkana and a sub-species of *L. niloticus*, i.e. *Lates niloticus longispinus* (Greenwood 1976). The putative origins of Nile perch were reported to be composed of two characteristic populations; one having a smaller body form and found dwelling in deep open waters while the other population have a deeper bodied morph and inhabit inshore and shallow waters (Harrison 1991; Holden 1967; Worthington and Ricardo 1936). This population structuring has also been suspected to be occurring in introduced ranges (Bwathondi 1985); and is being questioned if it may signify different taxa of the Nile perch.

Official introductions were reported to be done using small numbers totalling to 382, 295 and 585 individuals for introductions into lakes Victoria, Nabugabo and Kyoga respectively (Hauser et al. 1998). These introductions were intended to bolster the then failing fisheries in the non-native ranges of Nile perch, and for possible use as a sport fishery (Ogutu-Ohwayo 1994). Nile perch slowly colonised its new habitat for the first 20 to 30 years and then its population exploded in the 1980s (Mwanja and Mwanja 2008). The cause of the population explosion of the Nile perch after staying for the lag time as a minor component in the fisheries of the introduced environments is unknown (Kaufman 1992). The successfully establishment of the Nile perch and extension of its geographical range to cover all the new introduced ranges, despite the expected loss of genetic variation due to founder effects, creates a genetic paradox (Mwanja and Mwanja 2008).

Both introduced and the putative source populations were examined for genetic variation in the mitochondrial control region (CR) and at nine microsatellite loci. Comparison of the patterns of genetic diversity of these populations was necessary to understand the mode and mechanism of the species' successful establishment. It also gave insight into the relationships of the different Nile perch taxa in both introduced and native ranges. Genetic diversity

patterns of the species were also compared to other freshwater exploited species to determine how the species has been affected by the reported overfishing and to recommend how the species may be effectively managed to best conserve its genetic integrity in the region.

Materials and methods

Study area

Nile perch samples were taken from five lakes – Victoria, Kyoga and Nabugabo in Uganda, the introduced environs; and from their native ranges of Lake Albert in Uganda and Lake Turkana in Kenya. Lakes Albert and Turkana are considered as the putative origins for the introduction of Nile perch into the novel ranges (Figure 1). Lakes Albert and Turkana are rift valley lakes, with Lake Albert found on the western border of Uganda with the Democratic Republic of Congo forming part of the Nile system while Lake Turkana is on the north western border of Kenya with Ethiopia (Figure 1). Lake Turkana is said to have had a connection with the River Nile for more than once during the wet periods of the Pleistocene, with the most recent connection occurring approx. 7,000 ago (Dgebuadze et al. 1994). Lake Kyoga is located in Central Uganda and connected to both Lake Victoria (the second largest fresh water lake in the world with varied habitat types and ranges) and Lake Albert by the Victoria Nile River. There are a number of areas with rapids which culminate into spectacular waterfalls on either side of Lake Kyoga along Victoria Nile River, the Owen Falls and Murchison Falls. The rapids and falls act as impediments to the free movement of fish such as Nile perch between two watersheds. Lake Nabugabo is a relatively smaller lake (24 km²) commonly classified as a satellite lake to Lake Victoria. Lake Nabugabo was formerly a bay on the western shore of Lake Victoria which was separated from the main lake by a sand bar more than 5,500 years ago (Chapman et al. 1996). It is alleged that Nile perch from Lake Albert were first held and tried out in Lake Nabugabo before eventually being introduced into Lake Victoria (Barlow and Lissle 1987).

Sample collection and DNA extraction

A total of 527 Nile perch fish were sampled randomly along representative transects in the 5 lakes, using bottom trawls for Lake Victoria and

Figure 1. Map showing the East African lakes from which Nile perch was sampled for this study.



seining for lakes Albert, Kyoga, Nabugabo and Turkana. Transects were made in such a way as to cut across all key habitat types in each lake. Small (2 cm × 4 cm) fin clips were taken from each sampled fish and stored in absolute ethanol. Total genomic DNA was extracted using the DneasyTM tissue kit (QIAGEN) according to manufacturer's instructions.

Mitochondrial DNA Sequence Analysis

D-loop region of mtDNA of Nile perch was amplified using primers LN20 (ACCACTAG CACCCAAAGCTA) and HN20 (GTGTTATGCT TTAGTTAAGC) (Bernatchez and Danzmann 1993). PCR reactions consisted of the following: 5 µL genomic DNA; 25 µL AmpliTaq Gold[®] Master Mix, 5 µL of a 10 µM solution of each of two primers, and ddH₂O added for a final volume of 50 µL. Touchdown PCR conditions used for amplification included an initial denaturing step of 10 min at 95°C, followed by one cycle each of 94°C for 1 min, 67 - 51°C for 1 min 30s, and 72°C for 2 mins for each of the annealing temperatures starting at 67°C and stepping down by 2°C from each preceding cycle up to 51°C.

This was followed by 25 cycles of 94°C for 1 min, 61°C for 1 min 30s and 72°C for 2 mins with a final extension of 72°C for 7 mins. Double stranded PCR products were cleaned following the manufacturer's protocol (QIAquick). The sequencing reactions were carried out by Macrogen Inc. (South Korea) using Applied Biosystems 3730xl DNA analyser. Samples were sequenced in both forward and reverse directions to guarantee accuracy of nucleotide identification. Standard chromatographic curves of forward and reverse sequences were imported into the program ChromasPro 1.41 (Technelysium Pty Ltd, Tewantin QLD, Australia) and were manually aligned and edited. Consensus sequences were exported to the program BioEdit version 7.0.9 (Hall 1999) and aligned with other sequences using the CLUSTALW algorithm (Thompson et al. 1994).

Microsatellite Marker Analyses

Microsatellite markers developed for sister species *Lates calcarifer* (Zhu et al. 2006a, 2006b; Genhua Yue et al. 2001) were used for screening of Nile perch. Nine microsatellite

Table 1. Primers used to amplify the 9 microsatellite loci in Nile perch (*Lates spp.*). A is number of alleles per locus for the sister species *Lates calcarifer*.

Locus	Motif	Primers (5' – 3')	A	Size range	References
Lca70	(CAG) ₁₇	CATCATTTACACTCTGTTTGCCTC GACAGA CAG GTG TTT TAG CCT ATT TG	7	288 - 308	Zhu et al. 2006a
Lca165	(TG) ₁₂	TCCCCTGATGAGAGGAGGTCA GTTACAGCCGTGGATGGACAC	8	258 - 290	Zhu et al. 2006b
Lca98	(TG) ₁₄	CAA AGG GGC CAC TGC ACA TAA T CTC CAG CTC ACC CAG GTT CAC T	10	186 - 242	Zhu et al. 2006a
Lca147	(TC) ₃₅	TGCCCTAATGTATTCTTTCCACT GCTCCCACTCTCATTCAATTTC	16	164 - 226	Zhu et al. 2006b
Lca185	(CA) ₈ (GA) ₁₉	TTCCGCTGTTTGCAAAATACATTAG ACACCCAGCATGGCTTCCTAT	10	175 - 215	Zhu et al. 2006b
Lca193	(TC) ₂₃	CCTCTGCCTTTTCATCTATATTGC CACATCGCACAAATGGACTGA	12	140 - 176	Zhu et al. 2006b
Lca171	(TG) ₁₃	ATTGCGTTACCAAGAGGTGAA TGTCTTTGAAGGCTGAAAACCTG	14	294 - 336	Zhu et al. 2006b
Lca74	(CA) ₁₃	CAT CAT TTA CAC TCT GTT TGC CTC AT GAC AGA CAG GTG TTT TAG CCT ATT TG	4	160 - 170	Zhu et al. 2006a
LcaM01	(CG) ₄ (CA) ₈	AT TCG CTC TTT ATT CTC CC GTC GGC CTT TCA TAT GCA AT	3	260 - 264	Genhua Yue et al. 2001

markers that consistently gave scoreable results and exhibited polymorphism were used for genotyping of the Nile perch samples (Table 1). Samples were genotyped by BioScience (UK), on an ABI 3730 (Applied Biosystems) using GS-500 (Liz) in each capillary as a size standard. Allele sizes were estimated on GeneMapper version 3.7 (Applied Biosystems).

Mitochondrial DNA Data Analyses

The software program ARLEQUIN 3.5 (Excoffier and Lischer 2010) was used to perform intra- and inter-population analysis, calculating haplotype diversity (h) and nucleotide diversity (π) for the mtDNA sequence data, within sampling areas. Sequence based statistics (Hst , Kst^* , Z^* & Snn) together with gene flow (Nm), raggedness static, neutrality tests including Fu and Li's D , Fu and Li's F , Fu's F_s and Tajima's (1989) D were calculated using DnaSP 5.0 (Librado and Rozas 2009). The distribution of pairwise differences between sequences was also calculated to investigate demographic changes based on three parameters, tau (τ), which is time since expansion expressed in units of mutational time, theta (θ_0) and also (θ_1), which is time before and after population expansion (Rogers and Harpending 1992). Due to lack of calibrated mutation rates for the Nile perch mtDNA control region, a divergence rate

of 3.6% per million years estimated using geminate species of snook from a sister family, Centropomidae (Kim and Ronald 1999) was used in the calculations. The concordance of observed with the expected under the sudden expansion model of Rogers was tested using Harpending's Raggedness Index (Hri), and Sum of Squared deviation (SSD). The mismatch distributions were all calculated using ARLEQUIN 3.5.

The software program MEGA 5.05 (Tamura et al. 2011) was used to generate a table of variable sites and for constructing a neighbour-joining tree based on the Tamura and Nei (1993) distance. The T92 model, with invariable sites parameter (T92+I, I = 0.90) was selected as best fitting model using MEGA 5.05 (Tamura et al. 2011). A bootstrap analysis with 5000 replicates was used to evaluate support of phylogenetics relationships. In addition, a maximum parsimony network was constructed using TCS 1.6 (Clement et al. 2000); a program that provides a 95% plausible set for all haplotype linkages in an un-rooted tree.

Microsatellite Marker Data Analysis

The software MICRO-CHECKER 2.2.1 (van Oosterhout et al. 2004) was used for identifying possible genotyping errors within the microsatellite data set by performing 1000 randomizations. Microsatellite data were checked for

departures from Hardy–Weinberg (HW) and linkage disequilibrium (LD) using GENEPOP 4.0 (Rousset 2008). Genetic variation in the population samples were quantified by the 'expected heterozygosity' and number of alleles at the loci. ARLEQUIN 3.5 was used for calculations of observed (H_O) and expected (H_E) heterozygosities, and to test for heterozygosity excess and deficiency (exact tests) for microsatellite data, and for estimating global single locus F_{IS} . Furthermore, the software FSTAT 2.9.3.2 (Goudet 2001) was used on the microsatellite data set to estimate allele richness per locus and sample (AR), which represents the number of alleles independent of sample size (Mousadik and Petit 1996). F_{ST} and R_{ST} were estimated using GENEPOP 4.0. The statistical significance of population pairwise F_{ST} was analysed using permutation with 10,000 replicates. The genetic structure was assessed through the use of Bayesian based model-clustering in the program STRUCTURE 2.3.1 (Falush et al. 2003), by conducting genotypic population assignments (MCMC = 50,000 generations; burnin = 50,000 and iterations = 20). To infer the genetic groups sampled, STRUCTURE results were analysed using the ΔK method (Evanno et al. 2005). The admixture model with correlated allele frequencies, 20 replicates and set the number of genetic groups from $K = 1$ to $K = 10$ for each level of analysis. The modal value of ΔK was taken as the indicator of ancestral population clusters or genetic groups (Evanno et al. 2005). After determining the number of genetic groups, each individual sampled Nile perch was assigned to one of the groups, according to the highest ancestry proportion assigned by STRUCTURE.

Results

Mitochondrial DNA Sequence Analysis

Out of the 104 Nile perch mtDNA sequences analysed, they were a total of 35 variable/segregating sites (no indels) of which 24 were parsimony informative with a 2.2:1 transition to transversion ratio (Table 2). There were 27 haplotypes detected with hap-5 found in four out of the five lakes in East Africa, and noted as the most dominant of the identified haplotypes of Nile perch (Table 2). They were eight shared haplotypes - hap-1, hap-5, hap-9, hap-10, hap-11, hap-15, hap-20 and hap-23, which were all

found in samples of L. Victoria. 2 haplotypes, hap-24 and hap-25, were unique to L. Albert; 2 haplotypes, hap-26 and hap-27, were unique to L. Nabugabo; haplotypes 2, 3, 4, 6, 7, 8, 12, 13, 14, 16, 17, 19, 21 and 22 were unique to Lake Victoria; whereas Lakes Turkana and Kyoga did not have any unique haplotypes (Table 2). Overall haplotype diversity (H_d) was high (0.86 ± 0.03), and ranged from 0.58 to 0.93. The overall nucleotide diversity (π) was 0.00855 ± 0.00139 and ranged from 0.0020 to 0.0153 (Table 3). The Nile perch control region had a nucleotide composition of A=26.8%, C=18.3%, G=21.4%, and T=33.5% making it A/T rich with A+T content =0.603 and G+C content =0.397. Haplotype sequences were deposited in the GenBank, accession numbers JQ778220 – JQ778247.

Reconstructing of phylogenies by both Neighbour-Joining and parsimony methods revealed that Nile perch of East Africa belong to 2 clades A and B (Figure 2). The minimum spanning haplotype network of genealogical relationships among haplotypes revealed a network for Nile perch of E. Africa that was characterised by a high number of unique haplotypes with a star-like general pattern, rooted in the central most common haplotype, hap-5 (VIC6), possibly suggesting a recent population expansion (Figure 3). The geographical distribution of haplotypes was non-random with some of the samples having private haplotypes.

Microsatellite Marker Analysis

MICRO-CHECKER software did not detect errors in the genotypic data matrix, indicating there were no scoring errors associated with null alleles, stuttering bands, or large allele drop outs in the 9 loci used in the genotyping; consequently all 9 were included in the analysis. A total of 499 alleles were detected at the 9 loci across the 19 data sets, Lca165 was the most polymorphic microsatellite with 32 alleles, while Lca70 and LcaM01 were least variable with 13 alleles each. Number of alleles per locus varied from 2 at alleles Lca70 and Lca185 to 17 at Lca165. The mean number of alleles varied from 9.44 ± 1.47 for KYU samples from Lake Kyoga to 3.67 ± 0.44 for ANU samples from L. Albert, and the allelic richness varied from 4.30 ± 0.41 for MT samples from Lake Victoria to 3.33 ± 0.39 for AKU samples from Lake Albert. Pooled samples according to lakes from which they were collected revealed allelic richness ranging from 7.25 ± 1.75 for Lake Kyoga to

Table 2. List of 35 polymorphic sites of 27 mtDNA D-loop Nile perch haplotypes. Dots represent nucleotide variants identical to the first sequence. VIC, NAU, KYU, AKU, and TLK represent lake populations of lakes - Victoria, Nabugabo, Kyoga, Albert and Turkana respectively.

35 Polymorphic sites		Haplotype distribution and frequency					Totals
		VIC	NAU	KYU	AKU	TLK	
1 VIC1	111 111111111 122222222 22234						
2 VIC2	3355569033 4444466789 9001122235 55943						
3 VIC3	1824787338 1234556750 6581606751 69645						
4 VIC4	TCGATGTAAA CTCTCGTCCC TCCCGAACGC AAATA	5	1				6
5 VIC5	CG..CA..G ..TAT ..G..A	1					1
6 VIC6	T.G.A.....	2					2
7 VIC7	T.G.A.....	1					1
8 VIC8	T.G.A.....	1					1
9 VIC9	T.G.A.....	24	1	5		3	33
10 VIC10	T.G.A.....	1					1
11 VIC11	T.G.A.....	3					3
12 VIC12	T.G.A.....	1					1
13 VIC13	T.G.A.....	1					1
14 VIC14	T.G.A.....	8	2		6		16
15 VIC15	T.G.A.....	6		4			10
16 VIC16	T.G.A.....	5		1		3	9
17 VIC17	T.G.A.....	1					1
18 VIC18	CG...AG.G TCTCT..TAT ..TG...T.....	2					2
19 VIC19	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
20 VIC20	CG...AG.G TCTCT..TAT ..TG...T.....	2					2
21 VIC21	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
22 VIC22	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
23 VIC23	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
24 VIC24	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
25 VIC25	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
26 VIC26	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
27 VIC27	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
28 VIC28	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
29 VIC29	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
30 VIC30	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
31 VIC31	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
32 VIC32	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
33 VIC33	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
34 VIC34	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
35 VIC35	CG...AG.G TCTCT..TAT ..TG...T.....	1					1
Number of bases	2222222222 2222222222 2222222222 22222						
Totals		71	6	10	9	8	104

5.20±2.21 for Lake Nabugabo (Table 4). Private allelic richness ranged from 0.05 for RUT samples for Lake Victoria to 0.41 for MT samples for Lake Victoria. The private allelic richness average over all loci for the pooled lake populations was 0.34, 0.25, 0.16, 0.13, and 0.35 for lakes Kyoga, Nabugabo, Victoria, Albert and Turkana respectively. The allele frequency for 9 loci varied significantly among the different populations but all loci had one most common allele for all populations. Observed heterozygosity (H_O) ranged from 0.08 to 1.00, whereas the expected heterozygosity (H_E) ranged from 0.10 to 0.93. The H_E average over all loci ranged from 0.60 for AKU population from Lake Albert to 0.76 for MT population from Lake Victoria, whereas the H_O overall loci ranged from 0.48 for WIT population from Lake Victoria to 0.76 of KGU population from L. Victoria. 16 popula-

tions exhibited heterozygosity deficiencies, 9 exhibited heterozygosity excesses and 2 populations had same values for both H_E and H_O . For the different pooled populations, 4 lake populations all had heterozygosity deficiencies (Kyoga - H_E 0.75 and H_O 0.64; Victoria - H_E 0.68 and H_O 0.65; Albert - H_E 0.63 and H_O 0.54; Turkana - H_E 0.69 and H_O 0.61) for L. Nabugabo (H_E 0.65 and H_O 0.68) with excess heterozygosity (Table 4). Three out of 243 tests for conformance to Hardy-Weinberg expectations differed significantly ($P < 0.001$) after corrections for multiple tests (data not shown). The IT and LUT samples had genotypic distributions that differed significantly at locus Lca147.

RPT samples had a genotypic distribution that differed at locus Lca171. For most samples ($\approx 70\%$) the value of the inbreeding index was positive.

Table 3: mtDNA sequence variability in the control region for the Nile perch of East Africa: sample size (N), number of segregating sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), demographic expansion parameters, tau (τ), theta at time 0 (θ_0) and at time 1 (θ_1), Harpending's Raggedness Index (Hri), Sum of Squared deviation (SSD).

Lake	Statistics									
	N	S	h	Hd	π	τ	θ_0	θ_1	Hri	SSD
Victoria	71	33	23	0.86	0.0090	2.347	0.157	61148.63	0.023	0.0087
Albert	09	15	04	0.58	0.0094	20.96	0.257	25010.47	0.339	0.1263
Kyoga	10	03	03	0.64	0.0020	0.996	0.003	86059.55	0.127	0.0119
Nabugabo	06	19	05	0.93	0.0153	2.310	0.082	81007.12	0.164	0.0856
Turkana	08	03	04	0.79	0.0029	1.555	0.027	79912.00	0.087	0.0109
Overall	104	35	27	0.86	0.0086	5.525	0.526	54762.76	0.119	0.0543

Table 4: Summary descriptive statistics – microsatellite data for the samples from the different lakes of East Africa showing sample size (N), mean number of alleles (A), expected heterozygosity (H_E), observed heterozygosity (H_O), Theta (H) under stepwise mutation model, mean allelic size range (A_S).

Lake	N	A	H_E	H_O	Theta (H)	A_S
Victoria	432	17.67	0.71	0.65	1.912	49.22
Albert	35	7.11	0.62	0.50	1.620	15.57
Kyoga	25	9.44	0.75	0.64	2.138	25.44
Nabugabo	18	6.33	0.65	0.68	1.688	16.89
Turkana	17	7.11	0.69	0.61	1.830	17.78
Overall	527	9.53	0.68	0.62	1.837	24.98

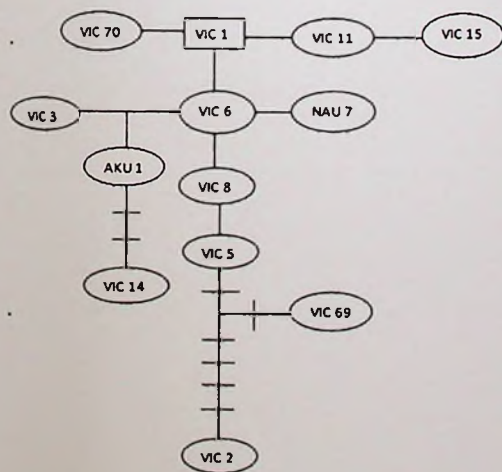
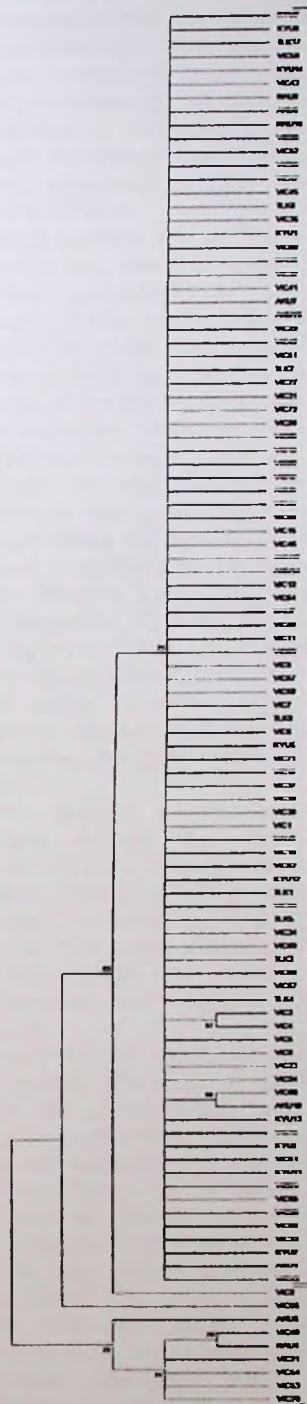


Figure 2. mtDNA haplotype networks A and B estimated with 95% statistical limits of parsimony using the algorithm in Templeton et al. 1992. Haplotypes are represented as rectangle and circles. Each node represents an additional mutational event.

Demographic Changes

ARLEQUIN analyses of mtDNA revealed large differences between θ_1 and θ_2 within all samples suggesting rapid population expansion (Table 3). Tajima's $D = (-1.26001, P > 0.10)$, Fu and Li's D^* test statistic $(-1.26266, P < 0.02)$, Fu and Li's F^* test statistic $(-1.51239, P > 0.10)$, and Fu's F_s statistic (-9.735) were all negative, all of which confirm the recent introduction and resultant population explosion for the Nile perch of East Africa, which is typical of recent natural population expansion (Fu 1997). The overall Harpending's raggedness Index, $Hri = 0.119$ and overall sum of squares, $SSD = 0.0543$ (Table 3). Results for assessment of bottleneck effects from the BOTTLENECK software at 9 microsatellite loci in 27 Nile perch populations in 5 East African lakes – Albert, Victoria, Nabugabo, Kyoga and Turkana for all 3 tests, revealed some evidence in 25 out of the 27 populations (results not shown). Most evidence was revealed under the standardised test where 8 populations had

Figure 3. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 2000 replicates (Felsenstein 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 104 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 463 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).



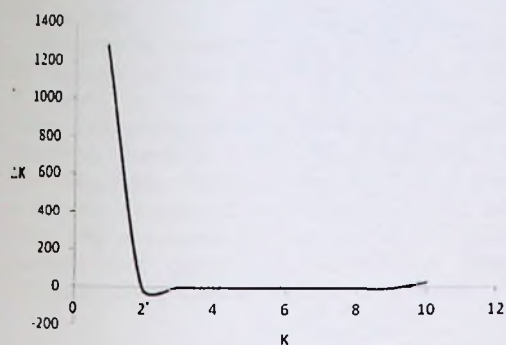


Figure 4. Description of the Evanno et al. (2005) 4 step graphical method used in detecting the true number of population groups K^* . ΔK calculated as $\Delta K = m | L''(K) | / s[L(K)]$. Modal value of this distribution was taken as the true K^* or upper most level of structure, here two clusters.

significant heterozygosity excess under IAM model, 4 populations had heterozygosity excess under TPM model and 16 populations had heterozygosity excess under SMM model. The assessment of Garza-Williamson index, M , (results not shown) for the different lakes, values ranged from 0.47 for L. Kyoga to 0.62 for Lake Albert. For the individual populations the M values ranged from 0.37 for RPT population to 0.62 for KT population.

Discussion

The overall genetic diversity of the introduced ranges was found to be higher than that of the putative origins. This scenario is most probably explained by the introductions from multiple sources that were repeatedly done for a period of close to a decade from the mid 1950s to early 1960s (Pringle 2005). Repeated introductions are known to augment genetic diversity of invasive species (Dlugosch and Parker 2008; Lavergne and Molofsky 2007). In addition, even though Nile perch may have experienced bottlenecks these do not necessarily lead to population crash or stop evolution provided a population grows rapidly (Frankham 2005). Nile perch is known to have very high fecundity - said to be as high as 17 millions eggs per female (Ogutu-Ohwayo 1994). The high fecundity and prolific reproduction may have kept the species' population growth rate positive and growing despite the expected bottlenecks during the

colonisation period in the introduced ranges. The unsuspecting and then most abundant prey, the haplochromines (cichlids), in the introduced ranges helped to easily provide the required energy bolstering of the population of the colonisers, as evidenced in the differences in the bigger and deeper bodied introduced Nile perch when compared to the source populations (EAFRO 1969).

Both markers, the mtDNA CR and the micro-satellite loci, revealed relatively high levels of genetic polymorphisms among native and introduced Nile perch of East African lakes. The availability of the high genetic variation in Nile perch offers a good reprieve in the management efforts of the fishery given the recent reports of fast dwindling yields and natural stocks of this species in the region. Both markers, the mtDNA CR and the microsatellite loci, showed Lake Albert as having the least genetic diversity in spite of being the putative origin and the alleged biggest contributor to the introduced environs - lakes Victoria, Kyoga and Nabugabo during the translocations. This may be partly explained by the nature of Lake Albert whose major inflows either do not have Nile perch - Semiliki River, or have rapids or falls - Murchison falls are effective barriers to faunal interchange, thereby preventing any gene flow from outside Lake Albert.

The putative origins, lakes Albert and Turkana, though they had relatively lower genetic diversity than the introduced ranges had genetic diversities which were way above the average for freshwater fish species, suggesting that the Nile perch fisheries of these two lakes are still healthy and stable enough to support management and conservation efforts for the species.

According to structure analysis, there were two genetic groupings of the East African Nile perch and all lakes had both groupings but in differing proportions. There was no trend in the different proportions of the genetic clusters in different lakes, which may be attributed to geographical location. These findings indicate that Nile perch in East Africa is structured into two distinct genetic groupings found in all the lakes where Nile perch is found. These findings agree with our earlier morphology findings (Mwanja et al. 2011) and earlier studies by other ecological biologists who suggested the existence of two sub-species in the region (Harrison 1991; Welcomme 1967; Gee 1964; Worthington 1940). Therefore there are most

probably two Nile perch sub-species in East African lakes.

Phylogenetic reconstruction did not support the existence of more than one species of the Nile perch in East Africa as proclaimed by earlier fish ecologists based on morphological description (Harrison 1991), instead it revealed 2 underlying clades found in all 5 lakes. What may have been considered as separate species are most likely synonyms of the same species. Most probably Nile perch of East African lakes belongs to one species having two subspecies.

Demographic changes

Grant and Bowen (1998) proposed that fish with high h and low π probably might have undergone population expansion after a period of low effective population size. Our findings on the genetic variation of the Nile perch are in agreement with this scenario, with high haplotype diversity and low nucleotide diversity (h , 0.62 – 0.71; π , 0.002 – 0.009). Similarly the star-like TCS parsimonious haplotype network is an indicator of recent population expansion with the central common haplotype being the hypothetical ancestral haplotype (Sophie et al. 2007) and the large negative neutrality tests further support the phenomenon of recent population expansion (Fu 1997). The haplotype network for Nile perch of East Africa formed a star-like network suggesting recent population expansion. This was further supported by the small SSD and H_{ri} values, which are indicators of a historically expanding population model (Roggers 1995). In the introduced ranges, the Nile perch, which was translocated using small numbers (Hauser et al. 1998), and said to have slowly colonised its novel habitats and later exploded in the 1980s to dominate their fisheries (Ogutu-ohwayo 1994) fits the above recent expansion model. For the native ranges, the possible explanation for recent population expansion, is most probably attributed to oscillation of water levels from the time when these lakes – Albert and Turkana were still small lakes (20,000 years to 3000 years ago) and are thought to have stabilized at their current levels (Beuning et al. 1997). The reduction in population size and recent population expansion of the population model of the Nile perch of East Africa as described above is further supported by the findings of the bottleneck analysis and the low M values. According to Garza and

Williamson (2001) M values < 0.68 support occurrence of a past reduction in population size. Both the BOTTLENECK software analysis and the Garza-Williamson indices indicate that the Nile perch of East Africa had undergone reduction in genetic diversity and population expansion thereafter. The possible explanations for this scenario for the introduced environs are the recent Nile perch translocations and the palaeo-environmental changes in water levels in the lake basins of lakes Albert and Turkana.

Implications to fisheries management

The relatively high genetic diversity, despite the reported declining stocks, both in the native and introduced ranges of Nile perch provides the requisite evolutionary flexibility that may support efforts toward the effective management and sustainable exploitation of the Nile perch resource in the region. There should be no need for new translocations of Nile perch from the native to the introduced environs for the sake of augmenting the species diversity. We advocate for effective management that allows controlled fishing effort and for improvement of water quality in the region. These factors may allow for the sustainable exploitation of the Nile perch and the possible resurgence of the native species in the introduced ranges that form the Nile perch prey as evidenced by recent revelations (Balirwa 2003). There is urgent need to map out and further study the life histories and other attributes of the two underlying populations of Nile perch in both native and introduced ranges that were revealed in this study. The two populations also supported by works of earlier scientist (Harrison 1991) may require different management approaches. From the demographic history the Nile perch has shown that it has high prospects for recovery after bottleneck episodes, it is pertinent to enhance the scientific knowledge of the mechanisms of this fishery's recovery in order to formulate recommendations for the species long term management plans and their implementation. Lastly, there is urgent need to initiate routine genetic monitoring of both the Nile perch and the native fish species that form the species' prey in all Nile perch ranges in this region. The outcome will aid in designing appropriate and contemporary management regimes for creating a healthy sustainable fishery in the region using our current findings as a benchmark.

Acknowledgements

This study was part of the bigger project Nile perch technologies sponsored by the World Bank Millennium Science Initiatives (MSI) through the Uganda National Council of Science and Technology (UNCST). We are grateful to Lake Victoria Fisheries Organisation for aiding the sampling on Lake Victoria. We are thankful to technicians Sophia of NaFIRRI, Uganda, Mapunda and Ogara from TAFIRRI, Tanzania, Odada from KEMFRI, Kenya and to the Lake beach management officials - Opiri and Mutebi of lakes Kyoga and Nabugabo, Uganda for their assistance during sampling from lakes Albert, Victoria, Turkana, Kyoga and Nabugabo, respectively. Lastly we wish to acknowledge Dr. Monica R. Sullivan for her assistance with editing English writing.

References

- Baliwa JS (2003) Biodiversity and fishery sustainability in the Lake Victoria Basin: An unexpected marriage? *BioScience* 53: 703–715, [http://dx.doi.org/10.1641/0006-3568\(2003\)053\[0703:BAFSIT\]2.0.CO;2](http://dx.doi.org/10.1641/0006-3568(2003)053[0703:BAFSIT]2.0.CO;2)
- Barlow GC, Lissle A (1987) Biology of The Nile perch *Lates niloticus* (Pisces: Centropomidae) with Reference to its Proposed Role as a Sport Fish in Australia. *Biological Conservation* 39: 269–289, [http://dx.doi.org/10.1016/0006-3207\(87\)90129-7](http://dx.doi.org/10.1016/0006-3207(87)90129-7)
- Bernatchez L, Danzmann GR (1993) Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of Brook charr (*Salvelinus fontinalis* Mitchill). *Molecular Biology and Evolution* 10: 1002–1014
- Beuning MRK, Talbot RM, Kelts K (1997) A revised 30,000-year paleoclimatic and paleohydrologic history of L. Albert, E. Africa. *Palaeogeography, palaeoclimatology, Palaeoecology* 136: 259–279, [http://dx.doi.org/10.1016/S0031-0182\(97\)00034-5](http://dx.doi.org/10.1016/S0031-0182(97)00034-5)
- Bwathondi POJ (1985) The future of the fisheries of the Tanzania part of Lake Victoria, in view of the predominance of the Nile perch *Lates niloticus*. F.A.O. fisheries reports 335, pp 143–145
- Cawdry SH, Ferguson A (1988) Origins and differentiation of three sympatric species of trout (*Salmo trutta* L.) in Lough Melvin, Ireland. *Polskie Archiwum Hydrobiologii* 35: 267–277
- Chapman LJ, Chapman AC, Ogutu-Ohwayo R, Chandler M, Kaufman L, Keiter AE (1996) Refugia for Endangered Fishes from an Introduced Predator in Lake Nabugabo, Uganda. *Conservation Biology* 10(2): 554–561, <http://dx.doi.org/10.1046/j.1523-1739.1996.10020554.x>
- Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate genealogies. *Molecular Ecology* 9: 1657–1660, <http://dx.doi.org/10.1046/j.1365-294x.2000.01020.x>
- Dgebuadze YY, Golubstov AS, Mikeev VN, Mina MV (1994) Four fish species new to the Omo-Turkana basin, with comments on the distribution of *Nemacheilus abyssinicus* in Ethiopia. *Hydrobiologia* 286: 125–128, <http://dx.doi.org/10.1007/BF00008502>
- Dlugosch MK, Parker MI (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431–449, <http://dx.doi.org/10.1111/j.1365-294X.2007.03538.x>
- EAFRO (1969) East Africa Freshwater Fisheries Organization annual reports, 1961–1967; Entebbe, Uganda
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611–2620, <http://dx.doi.org/10.1111/j.1365-294X.2005.02553.x>
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567, <http://dx.doi.org/10.1111/j.1755-0998.2010.02847.x>
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164: 1567–1587
- Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791, <http://dx.doi.org/10.2307/2408678>
- Frankham R (2005) Resolving the genetic paradox in invasive species. *Heredity* 94: 385, <http://dx.doi.org/10.1038/sj.hdy.6800634>
- Fu Y (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915–925
- Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology* 10: 305–318, <http://dx.doi.org/10.1046/j.1365-294x.2001.01190.x>
- Gee JM (1964) Nile perch investigations. Annual Reports of East African Freshwater Research Organisation, 1962/63, pp 14–24
- Genhua Y, Li Y, Orban L (2001) Characterisation of Microsatellites in the IGF-2, GH Genes of the Asian Seabass (*Lates calcarifer*). *Marine Biotechnology* 3: 1–3, <http://dx.doi.org/10.1007/s101260000043>
- Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from <http://www2.unil.ch/popgen/softwares/fstat.htm>
- Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes insights from sardines and anchovies and lessons of conservation. *Journal of Heredity* 89: 415–426, <http://dx.doi.org/10.1093/jhered/89.5.415>
- Greenwood PH (1966) The Fishes of Uganda. Kampala (Uganda): Uganda Society
- Greenwood PH (1976) A review of the family Centropomidae (Pisces, Perciformes). *Bulletin of the British Museum (Natural History)* 29: 1–81
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid symposium series* 41: 95–98
- Harrison K (1991) The taxonomy of East African Nile perch, *Lates* spp. (Perciformes, Centropomidae). *Journal of Fish Biology* 38(2): 175–186, <http://dx.doi.org/10.1111/j.1095-8649.1991.tb03104.x>
- Hanfling B (2007) Understanding the establishment success of non-indigenous fishes: lessons from population genetics. *Journal of Fish Biology* 71: 115–135, <http://dx.doi.org/10.1111/j.1095-8649.2007.01685.x>
- Hauser LG, Carvalho GR, Pitcher TJ, Ogutu-Ohwayo R (1998) Genetic affinities of an introduced predator: Nile perch in Lake Victoria, East Africa. *Molecular Ecology* 7: 849–857, <http://dx.doi.org/10.1046/j.1365-294x.1998.00399.x>
- Holden MJ (1967) The systematics of the genus *Lates* (Teleostei: Centropomidae) in L. Albert East Africa. *Journal of Zoology (London)* 151: 329–342
- Kaufman LS (1992) Catastrophic species-rich Ecosystems. *BioScience* 42 (11): 846–858, <http://dx.doi.org/10.2307/1312084>
- Kim AD, Raymond RW (1999) Amphipanaemic Geminates of Snook (Percoidae: Centropomidae) provide a calibration of the divergence rate in mtDNA control region of fishes. *Molecular Phylogenetics and Evolution* 13(1): 208–213, <http://dx.doi.org/10.1006/mpev.1999.0625>

- Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *PNAS* 104(10): 3883–3888, <http://dx.doi.org/10.1073/pnas.0607324104>
- Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology and Evolution* 17(3): 386–391, [http://dx.doi.org/10.1016/S0169-5347\(02\)02554-5](http://dx.doi.org/10.1016/S0169-5347(02)02554-5)
- Librad P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452, <http://dx.doi.org/10.1093/bioinformatics/btp187>
- Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L) Skeels] endemic to Morocco. *Theoretical Applied Genetics* 92: 832–839
- Mwanja TM, Mwanja WW (2008) Preliminary results of the analysis of the population structure of the Nile perch (*Lates niloticus* Linnaeus, 1758) of Lake Victoria using microsatellite markers. *African Journal of Biotechnology* 7 (16): 2885–2892
- Mwanja MT, Muwanika V, Nyakaana S, Masembe C, Mbabazi D, Rutaisire J, Mwanja WW (2011) Population morphological variation of the Nile perch (*Lates niloticus*) of East African Lakes and their associated waters. *African Journal of Environmental Science and Technology* 5(11): 941–949
- Ogutu-Ohwayo R (1994) Adjustments in fish stocks and in life history characteristics of the Nile perch *Lates niloticus* L. in Lakes Victoria, Kyoga, and Nabugabo. Ph.D. Thesis
- Pringle RM (2005) The origins of the Nile perch in Lake Victoria. *BioScience* 55(9): 780–787, [http://dx.doi.org/10.1641/0006-3568\(2005\)055\[0780:TOOTNP\]2.0.CO;2](http://dx.doi.org/10.1641/0006-3568(2005)055[0780:TOOTNP]2.0.CO;2)
- Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic difference. *Molecular Biology and Evolution* 9: 552–569
- Rousset F (2008) GENEPOP '007: a complete reimplementation of genepop software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106, <http://dx.doi.org/10.1111/j.1471-8286.2007.01931.x>
- Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425
- Sophie von der Heyden, Lipinski RM, Matthee AC (2007) Mitochondrial DNA analyses of the Cape hakes reveal an expanding, panmictic population of *Merluccius paradoxus*. *Molecular Phylogenetics and Evolution* 42: 517–527, <http://dx.doi.org/10.1016/j.ympev.2006.08.004>
- Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595
- Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512–526
- Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* 101:11030–11035, <http://dx.doi.org/10.1073/pnas.0404206101>
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731–2739, <http://dx.doi.org/10.1093/molbev/msr121>
- Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132: 619–633
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680, <http://dx.doi.org/10.1093/nar/22.22.4673>
- van Ooster C, Hutchinson WF, Wills DP M, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology notes* 4: 535–538, <http://dx.doi.org/10.1111/j.1471-8286.2004.00684.x>
- Welcomme RL (1967) Observations on the biology of the introduced species of *Tilapia* in Lake Victoria. *Revue de Zoologie Africaine* 76(3–4): 249–279
- Worthington EB, Ricardo CK (1936) Scientific results of the Cambridge expedition of the East African lakes, 1930 – 1–15. The fish of Lake Rudolf and Lake Baringo. *Journal of Linnean Society of London, Zoology* 39: 354–389
- Worthington EB (1940) Geographical differentiation in freshwaters with special reference to fish. In: Huxley J (ed), the Systematics. London: Oxford University Press, pp 287–302
- Zhu YZ, Lin G, Lo CL, Xu XY, Feng F, Chou R, Yue HG (2006a) Genetic Analyses of Asian sea bass stocks using novel polymorphic microsatellites. *Aquaculture* 256: 167–173, <http://dx.doi.org/10.1016/j.aquaculture.2006.02.033>
- Zhu YZ, Wang MC, Lo CL, Feng F, Lin G, Yue HG (2006b) Isolation, characterisation and linkage analyses of 74 novel polymorphic microsatellites in Baramundi (*Lates calcarifer*). *Genome* 49: 969–976, <http://dx.doi.org/10.1139/G06-080>