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Research Article

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

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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 underling 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 macrophthalamus* (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 km^2) 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

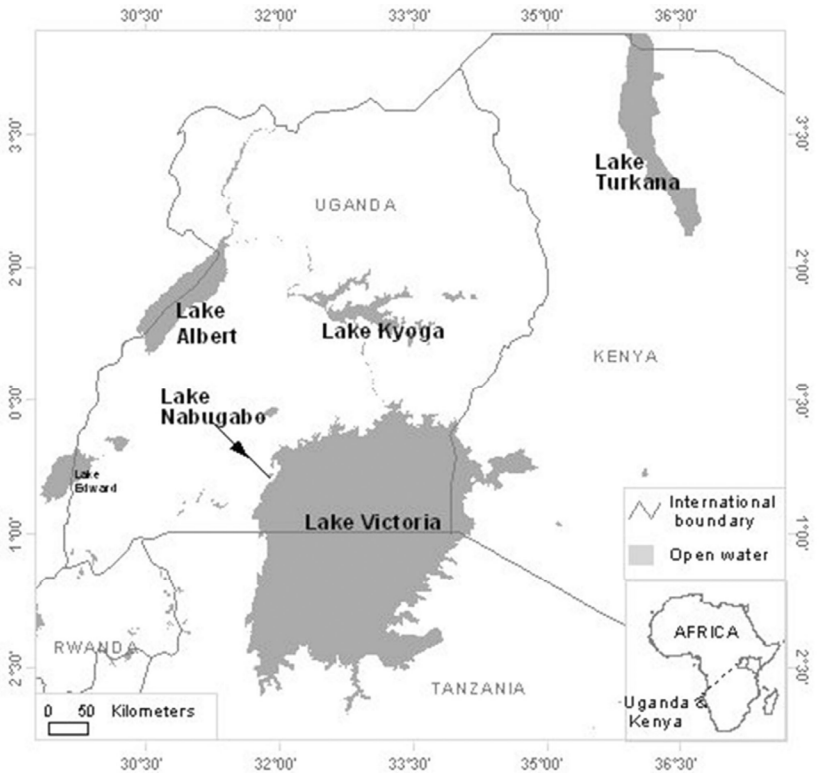

Figure 1. Map showing the East African lakes from which Nile perch was sampled for this study.

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 Dneasy™ tissue kit (QIAGEN) according to manufacturer's instructions.

Mitochodrial 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 ddH_2O 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 chromate-graphic 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	$(TC)_{35}$	TGCCCCTAATGTATTCTTTCCACT GCTCCCACCTCTCATTCATTATTC	16	164 - 226	Zhu et al. 2006b
Lca185	$(CA)_6(GA)_{19}$	TTCGCCTGTTTGCAAATACATTAG ACACCCAGCATGGCTTCCTAT	10	175 - 215	Zhu et al. 2006b
Lca193	$(TC)_{23}$	CCTCTGCCTTTTCATCTATATTGC CACATCGCACAAATGGACTGA	12	140 - 176	Zhu et al. 2006b
Lca171	$(TG)_{13}$	ATTGCGTTACCAAGAGGTGAA TGTCTTTGAAGGCTGAAAACTG	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 Fs 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 micro-satellite data set by performing 1000 randomi-zations. 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 (*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.

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 ± 0.41for 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	
	111 1111111111 1222222222 22234						
	3355569033 4444466789 9001122235 55943						
	1824787338 1234556750 6581606751 69645						
1 VIC1	TCGATGTAAA CTCTCGTCCC TCCCGAACGC AAATA	5	1				6
2 VIC2	CG..CA...G ....TAT ..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.G ....T.T .TG..... ...G.	1					1
20 VIC67	C........G ....G.....	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

5.20±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 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) 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 (≈ 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**

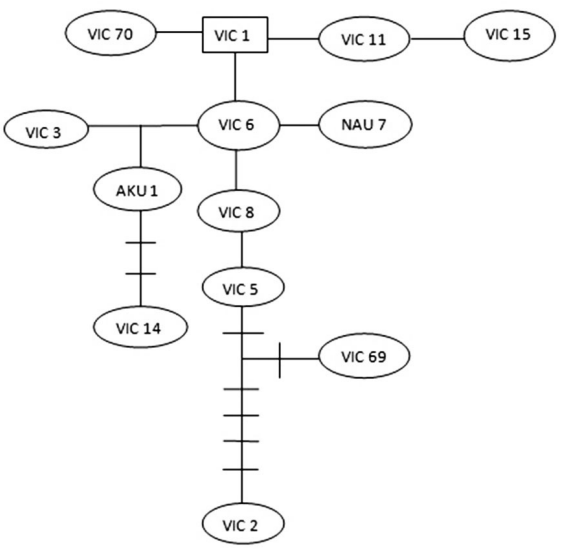

Figure 2. mtDNA haplotype networks A and B estimated with 95% statistical limits of parsimony using the algorithm in Templeton et al. 1992. Haplotypes are represented as rectangle and circles. Each node represents an additional mutational event.

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

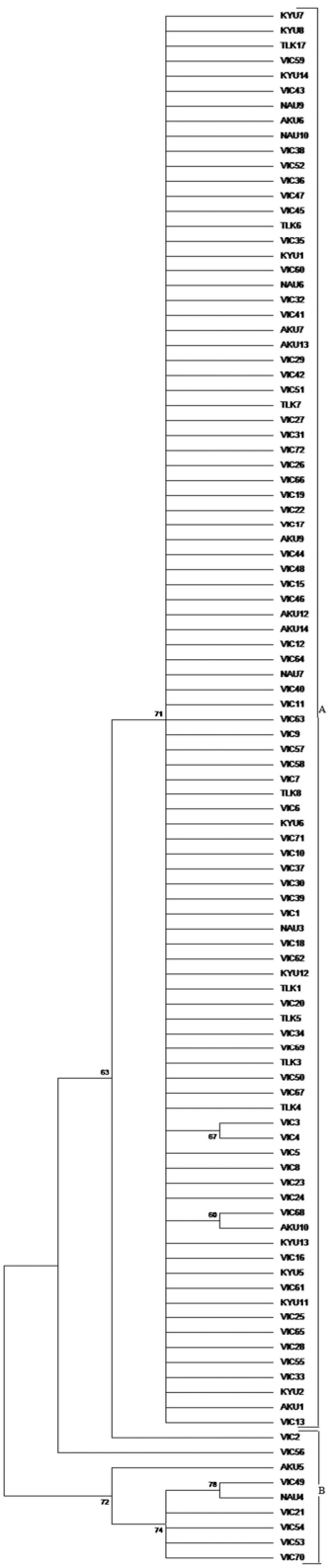

Figure 3. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 2000 replicates (Felsenstein 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions 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).

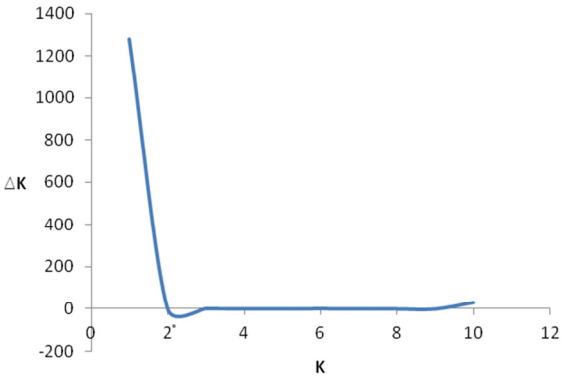

Figure 4. Description of the Evanno et al. (2005) 4 step graphical method used in detecting the true number of population groups K*. ΔK calculated as ΔK = m | L''(K) | / s[L(K)]. Modal value of this distribution was taken as the true K* or upper most level of structure, here two clusters.

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 *Hri* 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.

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