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Reduction of the "ngege", *Oreochromis esculentus* (Teleostei: Cichlidae) populations, and resultant population genetic status in the Lake Victoria Region

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Abstract

Ngege, *Oreochromis esculentus*, originally formed the mainstay of the Lake Victoria Region (LVR) fisheries. Together with its indigenous congener *O. variabilis*, it was displaced from Lakes Victoria and Kyoga of LVR and was found to survive as isolated small populations within the peripheral minor lakes and reservoirs around the two lakes. Displacement of the two LVR indigenous tilapiines was thought to be principally driven by changed lake environment and predation by the introduced Nile perch, but also competition and genetic swamping by the closely related introduced and comparatively more ecologically versatile tilapine species. In a study carried out in the LVR between 1993 and 2003, micro satellites and RAPD markers were used to analyse the remnant populations so as to establish the population structure and extant genetic diversity of *O. esculentus*. Analyses indicated that the surviving *O. esculentus* retained a high proportion of genetic diversity with high differentiation between units an indication of genetic exchange between indigenous and introduced Nile tilapia where the two forms co-existed. While this heightened concern for genetic swamping of the remnant population units by the introduced tilapiines it was noteworthy that in a few of the satellite lakes where the *O. esculentus* was dominant evidence for introgression was weak.

Key words: Genetic interaction, genetic swamping, Ngege, Nile tilapia

Introduction

Oreochromis esculentus (Trewavas, 1983), formerly *Tilapia esculenta* (Graham, 1928), was a prized food fish endemic to the Lake Victoria and Lake Kyoga basins of East Africa (Fryer and Iles, 1972). *Oreochromis esculentus*, the true 'ngege' as it was locally known around Lake Victoria in Uganda together with *Oreochromis variabilis* (Trewavas, 1983) locally known as *mbiru*, are the only endemic tilapiine species of the Victoria and Kyoga basins - both of which constitute the major components of the Lake Victoria Region (LVR). The two species were abundant open-water phytoplanktivores and detrivores, respectively (Fryer and Iles, 1972). Previously, ngege was the mainstay of the fisheries of the two lakes (Graham, 1928; Garrod, 1959; Welcomme, 1965; Fryer and

Iles, 1972). This changed with the introduction and establishment of non-indigenous tilapiines and a voracious predator, the Nile perch; *Lates niloticus* (Linnaeus, 1758) in the 1960s (Welcomme, 1966, 1967; Balirwa, 1992). The two LVR native tilapiine species were found to have been extirpated from the main Lakes; Victoria and Kyoga, and survived only as small isolated pockets within the satellite lakes surrounding the two main lakes (Balirwa, 1992; Mwanja *et al*., 1995; Mwanja 1996, 2000). At best, the two species were a highly local component of the fisheries of the satellite lakes, and were faced with increasing pressure from fishing mortality, and from the effects of the ecological and genetic interaction with the closely related introduced tilapiines in these lakes. The satellite lakes in which the ngege still occurred include Malimbe and Kubegena Lakes in Tanzania (Msuku, 2004; Nagl *et al.*, 2001), Lake Kanyaboli and some reservoirs in the Siaya region in Kenya east of Lake Victoria, and in a number of minor lakes in Uganda including the Kyoga satellite lakes of Kawi, Nawampasa, Nyaguo and Lemwa, the Nabugabo Lakes (Kayanja, Kayugi and Manywa), and the Kooki Lakes (Mburo, Kijanebalola, and Kachera) of the Kagera basin southwest of Lake Victoria (Mwanja, 1996).

Earlier work on *O.esculentus* dealt with the taxonomy (Graham, 1928), biology and ecology (Garrod, 1959) of this species and the interaction between tilapiine species as in Lowe McConnell (1958, 1959) on the suspected hybridisation between *O. esculentus* and Nile tilapia. Previously, the Nile tilapia was considered a sister species to *O. esculentus* (Trewavas, 1983); however, recent phylogenetic studies by Nagl *et al.* (2001) and Klett and Meyer (2002) have disputed this claim. Welcome (1967), Fryer and Iles (1972), Ogutu-Ohwayo (1988, 1990), Balirwa (1992), Kaufman (1992), Kaufman *et al*. (1997), Bugenyi and Balirwa (2003) highlighted the changes in the ecology of the ngege with the changes in lake limnology and ecology including the introduction and establishment of the non-indigenous tilapiine species and the Nile perch. Hecky *et al.* (1994) attributed the decline in ngege to the ecological changes that took place from the late 1970s.

In this study we investigated the ecological and genetic interactions between *O. esculentus* and Nile tilapia. The Nile tilapia known for its good fisheries and aquaculture attributes, has been introduced and got established all over the tropics and subtropics (Kocher *et al*., 1998). It is ecologically dominant and aggressive compared to other tilapiine species where they coexist, tending to swamp its relatives both ecologically and through genetic introgression (Leveque, 1997;Liem, 1981; Lowe-McConnell, 1959, 1987; Mwanja and Kaufman, 1995). This makes it urgent to evaluate what is left of the two endemic tilapiine species of the Lake Victoria and Lake Kyoga basins and enact a policy that ensures an adequate hedge against extinction. It is anticipated that such information may guide the salvage of what is left of the original highlights of the fisheries of Lakes Victoria and Kyoga. An important part of this study was to describe the population genetic status of *O. esculentus*, particularly in light of Lowe-McConnell's (1959, 1987) early warnings concerning the *O. esculentus* hybridisation with *O. niloticus*. We profiled the remnant populations of the ngege using the RAPDs technique and analysed the current genetic status of the remnant *O. esculentus* populations using microsatellite markers.

This study was carried out between 1993 and 2000 as part of a wider international project led by Ohio State University for conservation of cichlid fishes of Lake Victoria Region.

Materials and methods

Fishing survey and sample collection

Fish samples were collected from seven lakes in the Lake Victoria basin in East Africa (Figure 1, Table 2) from 1992 to 1996. Fish were caught using experimental fishing nets of varying mesh sizes mounted into 3 fleets set starting at 2 m depths and thereafter one fleet for every 100 m offshore. Each fleet had 2 nets measuring 20 m long by 1.5m wide per mesh size of 1.5 (38.1 mm), 2 (50.8 mm), 3 (76.2 mm), 4 (101.6 mm) and 5 (127.0 mm) inches. Nets were set between 17.00 and 18.00 hrs at dusk and checked every 2 to 3 hrs for 24-hour cycle per site of the lake studied. This allowed for getting the fish alive and fresh, and enabled clear identification and taking of uncontaminated samples for molecular analysis. Nets were set based on fishermen's knowledge of the occurrence of the fish and using physical and biological (floral) characteristics of shoreline. After retrieval of the fish from the nets, all tilapias were identified, sexed, weighed, and measured (length, body depth), and DNA samples (2 to 3 g of tissue from the right epaxial musculature placed in 95% ethanol) were taken from specimens that were unambiguously identified on site. The tissue sample for each individual fish sampled was placed in an individual vial containing 95% ethanol. After one hour, the ethanol was poured off and replaced with fresh ethanol, and the vials sealed and labelled for shipment to the laboratory at Ohio State University for DNA extraction. For each site, up to 20 individuals were sampled for molecular analysis and the voucher specimens archived at the National Fisheries Resources Research Institute in Jinja, Uganda. Some specimens were shipped to Boston University, Massachusetts to confirm ID based on morphological criteria.

Molecular analysis - RAPD analysis

DNA extraction was performed using the standard phenol/chloroform extraction method (Sambrook *et al*., 1989). PCR reaction mixtures of 25 µl final volume containing 50 ng of genomic DNA, and final concentration of 25 µM of dNTP, 0.6 µM of primer 2.5 µl of a reaction buffer, and 0.1 µl of 5 U/µl Taq polymerase enzyme from BRL technologies. RAPD decamer primers (Operon Technologies, Alameda, California) were used in a Perkin-Elmer thermocycler at the following sequence: a hot start for 3 min at 94^{o}C, then 45 cycles for 30 seconds at 94^{o}C, 1 min at 35^{o}C, and 2 min at 72^{o}C, with a ten minutes extension at 72^{o}C at the end of the 45 cycles. Repeatability and potential contamination of reaction conditions were checked using both a positive and a negative control for every reaction set for each primer. All sets of reactions were based on the same stock DNA extract diluted independently to 50 ng of genomic DNA for each PCR reaction mixture. Amplifications were separated by 1.6% agarosesynergel electrophoresis and visualized under UV light after ethidium bromide staining.

RAPDs band scoring and data analysis

The primers used were the 10-mer from Operon Technologies, Inc. The products were indexed using the primer code followed by a band number scored relative

to the position of a standard DNA ladder marker bands (123 bp Ladder DNA from BRL Life technologies) electrophoresed together with each reaction set. Bands were scored as '1' for present, and '0' if absent for each individual lane of each sample/population for every primer used. Each band position scored in all amplifications for each primer for all populations was taken as a single locus. Polymorphism was estimated as proportion of polymorphic loci (loci at an allele frequency of less than 0.95) in each population. All populations were assessed for population specific alleles.

Microsatellite marker analysis

DNA extraction was done using the standard proteinase K, phenol/chloroform protocol (Sambrook *et al.*, 1989) and the NaOH extraction method (Zhang and Tiersch, 1994). A total of 45 pairs of microsatellite primers developed by Lee and Kocher (1996) from *Oreochromis niloticus* DNA library were screened, among which we chose a set of 10 primer pairs for genetic population structure analysis of *Oreochromis esculentus* populations. The primers chosen were those that gave clear and reproducible amplifications, within a size range that could be run and read on 6% polyacrylamide gel, and that worked for all populations all the time. Choice of primer pairs was also dependent on their use in other tilapiine species in the Lake Victoria Region (LVR) since, in another unpublished manuscript, we compared the genetic structures of all the tilapiine species in this region (taken to comprise of Lake Victoria Basin, Lake Kyoga Basin, and the Lake Edward-George System). The sequence, annealing temperature and number of cycles of amplification used are shown in Table 2. For PCR analysis, each forward primer was end-labelled with P32 radioisotope using T4 polynucleotide kinase (GIBCO BRL). PCR reactions were done in a final volume of 10 ❑l containing 25ng of genomic DNA, 0.3 ❑M of each primer, 25 ❑M of each dNTP, 3 mM of $MgCl_2$, and 0.375 units of *Taq* polymerase (GIBCO BRL). Amplification conditions were 5 minutes of hot start at 95°C, 30 cycles at following the sequence: 45 sec at 94°C and 30 sec at appropriate annealing temperature (Table 1), and 30 sec at 72°C. This was followed at the end of the 30 cycles by a 6 minutes extension at 72°C. Amplicons were electrophoresed in 6% polyacrylamide sequencing gels with 7M urea, dried and visualized using autoradiography. Sizing of the amplification products was based on the sequencing of pUC18 along with the microsatellite PCR products.

Microsatellite data analysis

Microsatellite loci variability was measured by the number of alleles amplified for each locus, allele size range, allelic frequency distribution, and level of differentiation among individuals. The loci were also evaluated for both the intra- and inter-population variability based on level of observed and expected heterozygosity (Tables 6 and 7), proportion of specific alleles and degree of differentiation. Population subdivision was estimated based on F statistics (Weir and Cockham, 1984). Comparison was made to the Fstanalogue,Rst (Goldstein *et al.*, 1995; Slaktin, 1995) based on the infinite allele model and a stepwise mutation model respectively and run using Genepop 3.1 software (Raymond and Rousset, 1995). Phyletic relationships among populations were estimated using three genetic distance measures: Fst, Rst, and on the

Table 1. Microsatellite primer sequences and reaction conditions for 10 loci used for *Oreochromis esculentus*

Locus	Primer pair sequences	Annealing temp	Cycles
UNH231	A: GCCTATTAGTCAAAGCGT	56°C	30
	B: ATTTCTGCAAAAGTTTTCC		
UNH222	A: CTCTAGCACACGTGCAT	54°C	30
	B: TAACAGGTGGGAACTCA		
UNH104	A: GCAGTTATTTGTGGTCACTA	54°C	30
	B: GGTATATGTCTAACTGAAATCC		
UNH118	A: CAGAAAGCCTGATCTAATATT	56°C	30
	B: TTTCAGATACATTTTATAGAGGG		
UNH136	A: TGTGAGAATTCACATATCACTA	51°C	30
	B: TACTCCAGTGACTCCTGA		
UNH142	A: CTTTACGTTGACGCAGT	58°C	30
	B: GTGACATGCAGCAGATA		
UNH169	A: GCTCATTCATATGTAAAGGA	57°C	30
	B: TATTTTTTGGGAAGCTGA		
UNH176	A: GATCAGCTCTCCTCTACTTA	58°C	30
	B: GATCTGATTTCTTATTACTACAA		
UNH178	A: GTCACACCTCCATCATC	58°C	30
	B: AGTTGTTTGGTCGTGTAAG		
UNH149	A: TTAAAACCAGGCCTACC	58°C	30
	B: GTTCTGAGCTCATGCAT		

proportion of shared alleles standardized as 1-ps as modified from Nei (1972) following Goldstein *et al*. (1995). Standard genetic including number of alleles, polymorphic loci, and expected heterozygosity were calculated using Arlequin 3.11 computer software programme while Genetic distances were calculated using Microsat 1.5 computer software program developed by Minch E, and previously anchored at the website: http://hpgl.stanford.edu/projects/microsat/microsat.html. The dendograms were constructed based on genetic distances using neighbour joining method (Saitou and Nei, 1987). Fisher's exact test was used in analysing and testing population differences in genic and genotypic variations aided by the Genepop 3.1 computer software.

Results

Species occurrence

Oreochromis esculentus was not recorded in its native range but only in satellite lakes (Table 2). In a number of the satellite lakes *O. esculentus* was recorded to occur together with Nile tilapia, and in a few of these satellite lakes the *O. esculentus* was the most prevalent and dominant tilapiine (Table 2).

RAPD Band Amplification and Band Sharing

A total of 140 RAPD band markers were generated using 8 primers (Table 3). Bands produced per primer for each population ranged between 2-16 and a size range of 120 to 1700 bp. In general, there was a higher proportion of shared bands

Table 2. Occurrence of the ngege (*O. esculentus*) and the Nile tilapia (*O. niloticus*) in the LVR. The table includes information on the original status before the recorded introductions: N = native; I = introduced; and the current status: D = dominant; A = absent; E = extirpated or displaced; C = common but not the dominant tilapiine species; R = Rare

Lake/location	*O. esculentus*		*O. niloticus*	
	Original	Current	Original	Current
Napolean Gulf - Lake Victoria	N	E	I	D
Lake Kyoga	N	E	I	D
Lake Nabugabo	N	E	I	D
Lake Kayanja - Nabugabo lakes	I	D	A	A
Lake Manywa - Nabugabo lakes	I	D	A	A
Lake Kayugi - Nabugabo lakes	I	D	A	A
Lake Kachira - Kooki lakes	I	C	I	D
Lake Mburo - Kooki lakes	I	C	I	D
Lake Kijanebalola - Kooki lakes	I	C	I	C
Lake Nyaguo - Kyoga lakes	N	D	I	R
Lake Nawampasa - Kyoga lakes	N	C	I	C
Lake Lemwa - Kyoga lakes	N	C	I	R
Lake Kawi - Kyoga lakes	N	D	A	A
Lake Bisina - Kyoga lakes	N	R	I	R
Lake Edward - Edward/George basin	I	R	N	D
Lake Kanyaboli -Yala basin	I	D	I	C

Table 3. Number of RAPD bands that were amplified by specific primers for each population of the seven ngege (*O. esculentus*) populations of Lake Victoria basin (OPM 2,7,11,12,14,15,17,19)

Lake/Primer	Kachira (N=10)	Mburo (N=10)	Kanyaboli (N=10)	Kayugi (N=10)	Kayanja (N=10)	Manywa (N=10)	Kijanebalola (N=05)
OPM2	8	6	9	8	4	4	5
OPM7	5	10	5	7	8	2	2
OPM11	13	13	7	3	13	3	8
OPM12	9	16	12	11	5	10	8
OPM14	7	6	7	8	3	4	5
OPM15	6	5	7	6	12	3	6
OPM17	2	14	5	9	7	5	8
OPM19	5	3	8	10	4	6	6
Total	55	63	60	62	56	37	48

within than between populations (Table 5). Table 4 shows the number of private bands for the respective populations indicating continuing differentiation between remnant populations of *O. esculentus*, while Table 5 and Figure 1 show band sharing and relatedness of populations. Fish from three main geographical areas were genotyped at RAPD markers: the Koki lakes (Mburo, Kachira, and Kijanebalola); the Nabugabo lakes (Kayanja, Kayugi, Manywa); and Lake Kanyaboli, Kenya. The Lake Kanyaboli population exhibited higher allele sharing with Nabugabo populations than with the Koki Lakes populations. Lake Mburo had the highest within population band sharing, followed by Lake Kayugi and Lake Kanyaboli. The dendogram (Figure 2) based on RAPD band sharing indices (Table 5) shows clustering of populations based on geographical location (Figue 1).

Population structure and variability of the ngege based on Microsatellite marker analysis

All populations were highly polymorphic with a mean of 7.3 alleles per locus. Lake Nawampasa population had the largest number of alleles with a mean of 13.2 alleles per locus. Lake Kayanja population had the least allele number with 3.5 alleles per locus. Populations had significant differentiation (Fisher's exact test, Genepop3.1) in both genic and genotypic variation, and were highly heterozygous (Table 6) with a mean of 0.596 observed heterozygosity within populations. Lake Kayanja population had the least heterozygosity (0.339) while Lake Nawampasa population had the highest (O.840). Table 7 shows standard diversity indices produced using the Arlequin 3.11 version genetic analysis program

Table 4. Number of population-specific (unique or private alleles) bands found in *O. esculentus* populations of Lake Victoria basin asit relates to the occurrence of *O. niloticus*

Lake/Parameter	Kachira (N=10)	Mburo (N=10)	Kanyaboli (N=10)	Kayugi (N=10)	Kayanja (N=10)	Manywa (N=10)	Kijanebalola (N=05)
Presence of *O. niloticus*	+++	+++	-+	-+	-	-	-+
Population specific bands/alleles	5	13	6	9	2	4	4

Presence of *O. niloticus*: +++ dominant, -+ rare, -absent.

Table 5. Proportion of shared RAPD bands within and between sites (below the bold diagonal) and similarity indices (above the bold diagonal) derived from the band sharing proportions between individuals

	Kachira	Mburo	Kanyaboli	Kayugi	Kayanja	Manywa	Kijanebalola
Kachira	**0.735**	0.737	0.625	0.534	0.762	0.541	0.599
Mburo	0.376	**0.562**	0.605	0.604	0.684	0.600	0.531
Kanyaboli	0.433	0.327	**0.882**	0.554	0.588	0.623	0.601
Kayugi	0.262	0.246	0.397	**0.722**	0.574	0.544	0,784
Kayanja	0.551	0.386	0.450	0.356	**0.840**	0.474	0.549
Manywa	0.289	0.262	0.445	0.286	0.276	**0.840**	0.517
Kiganebalola	0.379	0.225	0.455	0.522	0.383	0.311	**0.826**

(Excoffier *et al*., 2005), with expected heterozygosity estimates in agreement with the above analysis. Table 8 shows that 39.1% of the alleles were private alleles, reinforcing the idea of growing population subdivision and remnant population differentiation. However, populations from similar lake basins and/ or minor lake complexes were in general, less differentiated from each other than distant populations. Though on a fine scale the differentiation is less discernable, on a larger scale the structure is more apparent. For example, genetic differentiation was clear when comparing populations from the Kyoga lakes (NYE, NWE, LME, KWE, BSE) to populations from the Nabugabo lakes (MNE, KJE, KGE). Nabugabo lakes had a mean pairwise Fst of 0.12 while that for Kyoga populations was 0.14. Nabugabo and Lake Mburo population of the Koki lakes (Southwest of Lake Victoria) had a mean pairwise Fst of 0.33 and 0.24 respectively, when compared to the population of Lake Kanyaboli, Yala system (East of Lake Victoria). The Kyoga populations were closer to Kanyaboli (with a Mean pairwise Fst = 0.16) than Lake Mburo (Mean pairwise Fst = 0.22).

Phyletic relationships using microsatellite markers

The relationships among populations based on Rst are shown in Figure 3. The populations were clustered largely along sub-regional groupings, similar to what was depicted in Figure 2. There was a good degree of segregation between the Lake Kyoga basin and the Lake Victoria basin populations, as well as division among populations from the Nabugabo lakes (KJE, MNE & KGE), the Yala system (KNE) and the Koki lakes (MBE). The grouping reflected and rhymed with the

Table 6. Number of microsatellite loci, allele number, private alleles, observed heterozygosity for Lake Victoria Region *Oreochromis esculentus* populations enforceable

Population		Sample size	Loci revealed	Allele number	Alleles per locus	Private Alleles	Observed H_o
Nyaguo	NYE	11	10	71	7.1	5	0.66
Nawampasa	NWE	20	9	119	13.2	39	0.84
Lemwa	LME	17	10	83	8.3	9	0.63
Mburo	MBE	21	10	53	5.3	6	0.43
Kanyaboli	KN	20	10	88	8.8	14	0.61
Manywa	MNE	20	10	61	6.1	2	0.53
Kayugi	KGE	11	10	46	4.6	0	0.53
Kayanja	KJE	20	10	35	3.5	1	0.34
Kawi	KWE	20	10	67	6.7	3	0.60
Edward/George	EDE	11	9	84	9.3	20	0.68
Bisina	BSE	9	10	74	7.4	8	0.70

relative abundance of Nile tilapia when compared to that of *O. esculentus* (Table 3, Figure 3).

Discussion

Oreochromis esculentus was not found within the major water bodies of its endemic ranges but occurred as isolated populations within the satellite minor lakes around Lakes Victoria, Kyoga and Nabugabo. Previous references make little mention of the *O. esculentus* in the satellite water bodies (Greenwood, 1966, 1974; Fryer and Iles, 1972) and assume that the species was moved to the satellite lakes by fisheries managers to protect it against introduced closely related tilapiine species and the voracious Nile perch. Indeed, some of the satellite lakes in the LVR are themselves not natural but artificial impoundments. For example, some of the best remnant *O. variabilis* populations are in one or two artificial impoundments in Kenya's Siaya district, near Lake Kanyaboli. Lake Sare, a haplocromine refugium that also houses Nile tilapia is also artificial as a result of back-ponding in the lower swamp following the diversion of the Sare River. However, most of the satellite lakes are papyrus swamp ponds, ephemeral in size and shape, though entirely natural. Anthropogenic movement of tilapiine species in the Lake Victoria region has been very extensive since the 1930s. In this study, *O. esculentus* was found as far as Kazinga Channel of Lake Edward, a remarkable record in a place where it had never been recorded before, while the Nile tilapia was found in nearly all the lakes. *Oreochromis esculentus* was found to still occur commonly within the satellite lakes. *Oreochromis esculentus* is evidently threatened in many of these refugia by hybridization with the introduced Nile tilapia where the two coexist. Also the preference of Nile tilapia by fisheries managers and fish farmers puts *O. esculentus* at increased risk of ecological competition, and the postulated genetic swamping with the escape and spread of the Nile tilapia to the *O. esculentus* refugia.

All populations showed close similarity ranging between 0.474 (Lake Kayanja

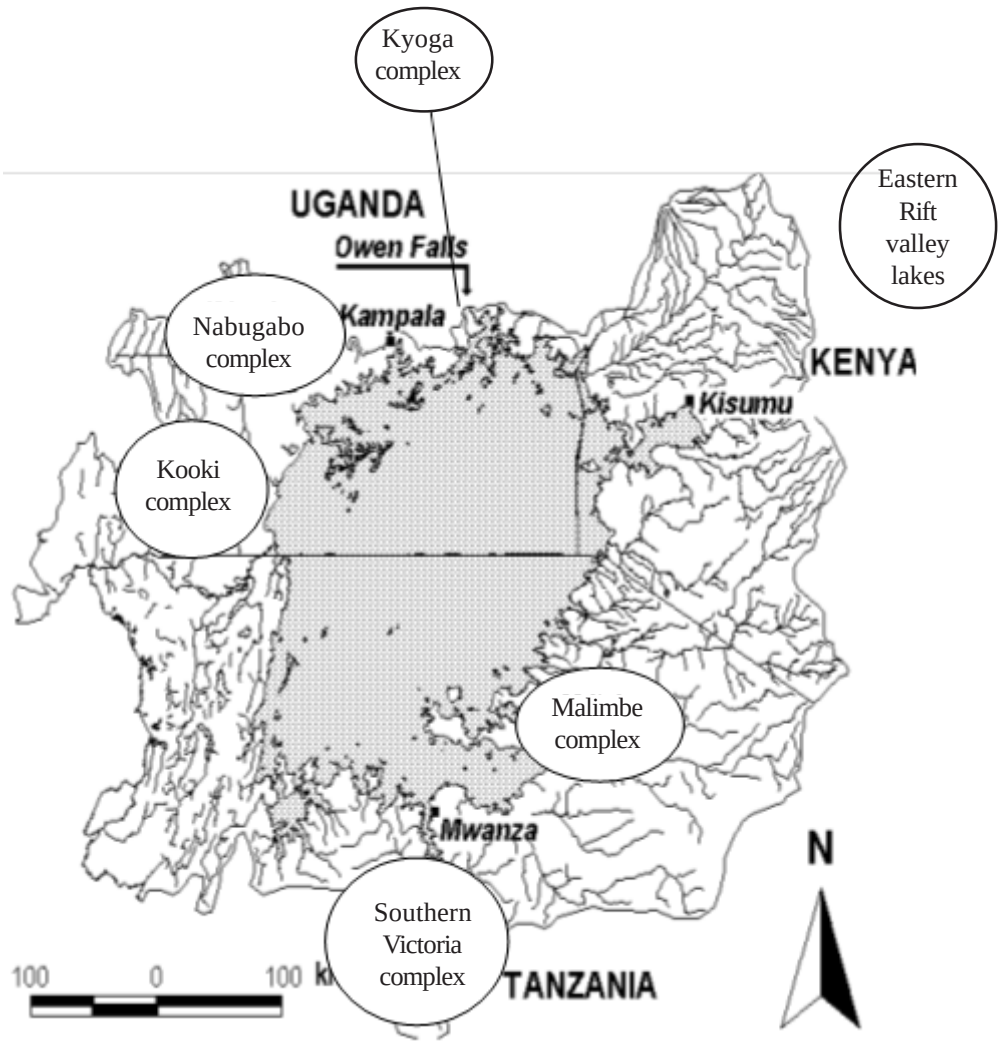

Figure 1. Lake Victoria Basin Systems.

and Lake Manywa populations) and 0.784 (for Lake Kijanebalola and Lake Kayugi populations). On average, the Koki lakes' populations had higher similarity indices within populations than Nabugabo populations, and exhibited a higher similarity with Nabugabo lakes' populations than those exhibited amongst Nabugabo lakes.Ecologically, the remnant *O. esculentus* populations were found to be subdivided and spatially isolated. Genetically, the *O. esculentus* populations were diverse with significant differentiation and subdivision between population units. We postulate that the geographical isolation of remnant *O. esculentus* populations, the occurrence of substantial numbers of private alleles and high genetic differentiation between populations indicate a low rate of genetic exchange between them and/or a likelihood of movement and introduction of *O. esculentus* based on small founder sizes. This is the first attempt to genetically

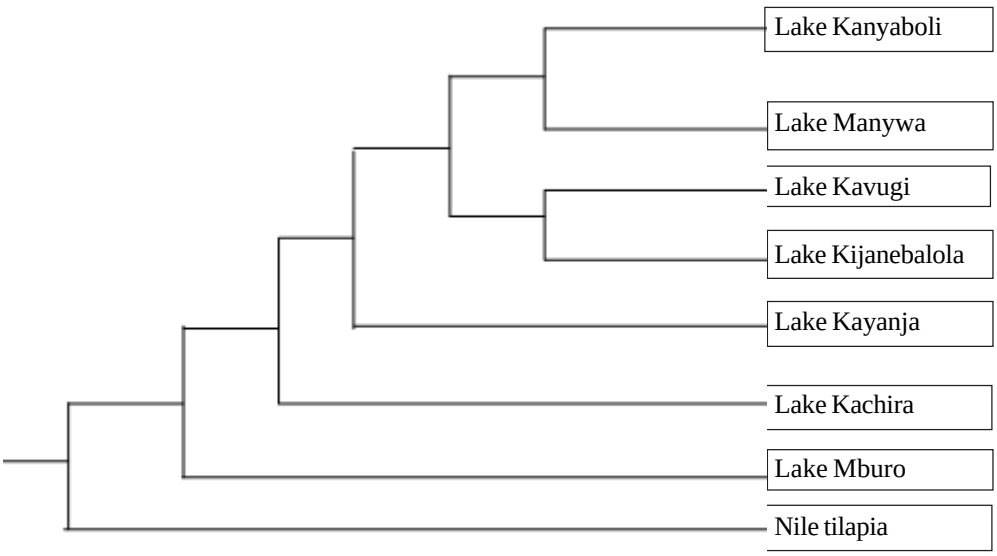

Figure 2. Dendogram (reproduced not to scale) showing the phylogeographical relations between the ngege populations based on RAPD Band sharing indices with *Oreochromis niloticus* (Nile tilapia) from Napoleon Gulf of Lake Victoria as an out group.

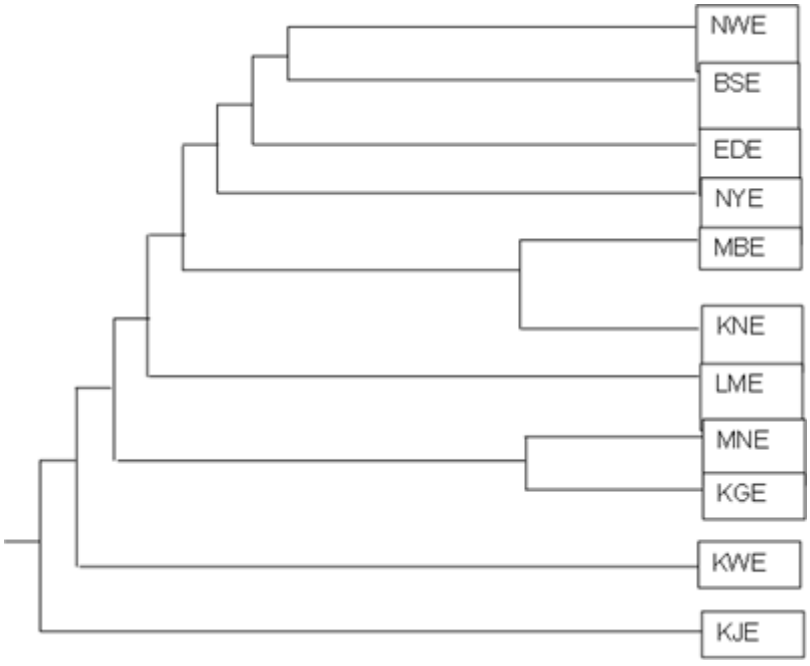

Figure 3. Phyletic relationships between remnant ngege populations of Lake Victoria Region based on Rst Genetic Distance measures. The dendogram is redrawn (not to scale) from Mwanja (2000).

Table 7. Standard diversity indices using Arlequin.311 computer software

Parameters	NYE	NWE	LME	MBE	KNE	MNE	KGE	KJE	KWE	EDE	BSE	Avg	sd	Tot.
Gene copies	10	20	17	21	20	20	11	20	20	10	08	16.1	5.1	N/A
No. Loci	10	10	10	10	10	10	10	10	10	10	10	10.0	0.0	N/A
No. Loci	09	04	02	08	08	09	09	10	08	08	09	7.6	2.4	N/A
Avg. Alls	4.30	7.60	5.30	4.30	5.90	4.40	3.10	2.80	4.40	4.50	4.50	4.7	1.3	20.3
Poly.Loci	08	04	01	05	07	07	07	06	05	06	08	5.8	2.0	N/A
Exp. Het	0.64	0.76	0.60	0.38	0.59	0.47	0.44	0.31	0.48	0.56	0.74	0.5	0.1	0.71
Theta	1.77	3.11	1.47	0.62	1.44	0.89	0.79	0.46	0.92	1.25	2.91	1.42	0.9	N/A

NYE = Lake Nyaguo population of Lake Kyoga Basin minor lakes complex; NWE = Lake Nawampasa population of Kyog Basin minor lakes complex; LME = Lake Lemwa population of Kyoga Basin minor lakes complex; MBE = Lake Mburo population of Kooki lakes Complex in southwestern part of Lake Victoria Basin; KNE = Lake Kanyaboli population of Yala Basin complex in the Northeastern part of Lake Victoria Basin; MNE = Lake Manywa population of western part of Lake Victoria Basin – Nabugabo lakes complex; KGE = Lake Kayugi population of Nabugabo lakes Complex in western part of Lake Victoria Basin ; KJE = Lake Kayanja population of Nabugabo lakes Complex in western part of Lake Victoria Basin; KWE = Lake Kawi from the Lake Kyoga Basin minor lakes complex; EDE = Lake Edward population; BSE = Lake Bisina population of Lake Kyoga Basin minor lakes complex

Table 8. Between population correlations using allele frequencies for *Oreochromis esculentus* populations (F-statistics) based on Genepop (3.1) computer software

	NYE	NWE	LME	MBE	KNE	MNE	KGE	KJE	KWE	EDE	BSE
Nyaguo	-										
Nawampasa	0.132										
Lemwa	0.109	0.176									
Mburo	0.264	0.285	0.162								
Kanyaboli	0.155	0.186	0.091	0.189							
Manywa	0.164	0.250	0.234	0.282	0.211						
Kayugi	0.151	0.230	0.218	0.282	0.194	0.004					
Kayanja	0.277	0.360	0.350	0.414	0.325	0.147	0.209				
Kawi	0.120	0.190	0.106	0.143	0.118	0.123	0.113	0.232			
Edward/George	0.223	0.180	0.245	0.339	0.239	0.282	0.270	0.429	0.237		
Bisina	0.123	0.086	0.145	0.242	0.167	0.238	0.225	0.390	0.164	0.124	-

F-statistics are estimated (Fwc) as in Weir and Cockerham (1984) as measure of population differentiation

characterise the ngege population structure in the Lake Victoria Region involving more than one water body, and in a sense, the horses are long since out of the barn. We did not have opportunity to compare the current situation with the original populations in Lakes Victoria and Kyoga before species introductions.

Both markers showed that populations of *O. esculentus* that coexisted with Nile tilapia were more polymorphic and heterozygous than populations that did not coexist with Nile tilapia. This situation points to a likely genetic exchange between two species where the two coexist. Nile tilapia has been known to hybridise readily with closely related species wherever it has been introduced (Lowe-McConnell, 1958; Fryer and Iles, 1972; Mather and Arthington, 1991; Balirwa, 1992; Leveque, 1997). In the Lake Victoria basin this has been a long held view with morphs intermediate between these two species found in the wild (Lowe-McConnell, 1958; Welcomme, 1966; Fryer and Iles, 1972; Balirwa, 1992; Mwanja, 1996). The Lake Mburo ngege population which coexisted with Nile tilapia, exhibited the highest genetic diversity with others showing varying levels probably attributable to the relative population sizes of these two species in the respective lakes.

The *O. esculentus* populations of Lake Manywa, Kayanja, and Kanyaboli were genetically the most distant from the Nile tilapia, and also encountered them in the wild the least, or not at all. Studies by Agnese *et al.* (1999) and Mwanja (2000) point to the continued dominance of *O. esculentus* over the Nile tilapia in Lake Kanyaboli, and purport that the *O. esculentus* remains pure. This is so for Lake Kanyaboli despite the existence of a fairly sizeable population of Nile tilapia in the same waters. This situation is curious and may be temporary. Nile tilapia populations have been known to lie low, later exploding and displacing species as in Lakes Victoria and Kyoga which Nile tilapia took over after more than 30 years of introduction (Mwanja 2000). It is therefore, imperative that ways and means are sought to enhance populations of the *O. esculentus* where it coexists with the Nile tilapia. A management option for *O. esculentus* in Lake Victoria basin would be to treat each remnant population as a distinct entity and to ensure the survival of each. Transfer of any fish stocks for aquaculture or reintroduction purposes may have to be genetically evaluated so as to monitor and control unwarranted genetic exchanges between these highly differentiated populations.

Populations of *O. esculentus*, which are coexisting with *O. niloticus*, should be considered as hybrid stocks until extensive genetic testing is carried out. Clearly, *O. esculentus* needs an organized management plan if it is to be shielded from extinction. The results of this study indicated that the species remains at a very high risk with increasing movement, occurrence and dominance of the Nile tilapia including within the satellite lakes, let alone the environment alterations that are occurring in all the LVR lakes including the satellite lakes. The plan must include elements of population and habitat conservation guided by these new data on genetic integrity, continued genetic tracking and perhaps, the establishment of additional pure stocks in self-supporting populations. If movement has to be done, it should be restricted to within and not between subregional groupings of the various satellite water bodies around the

major waters to minimize destabilising the already co-adapted remnant population units.

The long-term survival of *O. esculentus* will depend upon vigilant protection and nurturing of remnant wild populations against genetic swamping from the introduced Nile tilapia. This could be done through the establishment of additional refugium populations in waters devoid of exotic tilapiines but within the fish's native range.

Despite the limitations of the RAPD technique, its simplicity and low cost (Bardakci and Skibinski, 1994; Naish*et al*., 1995) makes it a good tool for fisheries management and for rapid stock monitoring . RAPDs markers were found to be a good first option in discerning troubled populations and species such as *O. esculentus*, as well as serving as a means of pointing out the evolutionary direction of genetically mixed populations of such species. On the other hand, microsatellite markers were found to be very good for detailed genetic analysis of tilapiines (Mwanja, 2000; D'Amato *et al*., 2007).

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