Genetic Analysis Reveals Substantial Proportion of Non-targeted Tilapias among Farmed Stocks in Kilosa and Kibaha, Tanzania

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Abstract

Nile tilapia farmers in Tanzania have been complaining about slow growth and low yields. Since some farmers obtain their seeds from unreliable sources, they may potentially be dealing with various non-targeted tilapias that require different environments and treatments to enhance growth and yields. Thus, this study analysed fragments (600 base pairs) of the cytochrome oxidase subunit I gene (COI) from 74 farmed tilapias in Kilosa and Kibaha, Tanzania to determine the presence of non-targeted tilapias. An additional 42 samples from the Magadu Fish Farm, Mindu dam, and Lake Victoria were included for comparison. The findings revealed that non-targeted tilapias accounted for 22.22–31.41% of the farmed stock, supporting the hypothesis that farmers are unknowingly dealing with a variety of non-targeted tilapias. Furthermore, pairwise F_{ST} comparison indicated genetic relatedness among the farmed fish, suggesting the sharing of fingerlings from the same broodstock or collection from the same wild locality. Therefore, farmers are advised to ensure they source seeds from certified hatcheries to minimize the risk of stocking non-targeted species. Additionally, due to the observed low genetic diversity in Kilosa samples, it is recommended that the country should establish a national breeding program for tilapia to provide farmers with access to high-quality seeds.

Keywords: DNA barcoding, Unintended Cichlids, fish ponds, Gene flow, Tanganyika.

Introduction

The Nile tilapia (*Oreochromis niloticus)* is one of the world's most widely farmed fish, accounting for about 75% of total tilapia production (Eknath and Hulata 2009, Munguti et al. 2014). The fish species is increasingly becoming popular in Africa due to its rapid growth, efficient food conversion, high fecundity, tolerance to diverse environmental conditions, and excellent table quality (Klett and Meyer 2002, Kaliba et al. 2006). Although tilapia farming in Tanzania is primarily conducted on a small scale by rural communities and a few urban dwellers, Nile tilapia accounts for over 95% of the country's total tilapia production (Kaliba et al. 2006, URT 2015). Despite the government's efforts to promote tilapia farming in order to reduce pressure on wild stocks, the sector's contribution to total fish production remains low, accounting for only 3.9% (URT 2022). Yet, the sector provides households with an average yearly income of USD 222, accounting for 13% of total household income (Mulokozi et al. 2020).

However, the development of tilapia farming in Tanzania is constrained by several factors. These include insufficient quality seeds and feeds, low adoption of appropriate technologies including biotechnology and bio-safety, inadequate extension services, limited technical skills in seed and feed production, lack of farming equipment, insufficient capital, and limited access to markets (Shoko et al. 2023). Despite the fact that the country's production of fish seeds has increased from 21,676,187 in 2020 to 35,967,180 fingerlings in 2023 (URT 2020, 2023), most fish farmers lack access to reliable seed sources. As a result, the majority of farmers obtain seeds or broodstock either from wild sources (Lakes, rivers, or dams) or from fellow farmers (other ponds) with little or no prior knowledge of their characteristics and expected performance (Shoko et al. 2023). Hence, there have been fears that most of the fingerlings used in the small and medium-scale aquaculture are not identified correctly, are inbred, and have low genetic diversity (Bradbeer et al. 2018) leading to poor yields (Shoko et al. 2011). Given the limited taxonomic expertise among fish farmers, there is a possibility that they may be working with various non-targeted tilapia species (Kajungiro et al. 2019). Therefore, the main objective of this study was to elucidate the characteristics of various species of tilapia cultured in Kilosa and Kibaha and determine whether they all belong to *O. niloticus*, as previously assumed. We hypothesized that there are non-targeted tilapias among the farmed fish that warrant attention from farmers and necessitate management interventions. Some data on phenotypic characterization have been extensively collected and are available (Hassanien et al. 2011, Rumisha and Nehemia 2013).

Materials and Methods
Study area
The study was carried out in two Districts of Mainland Tanzania, namely Kilosa (Morogoro Region, Eastern Tanzania) and Kibaha (Pwani Region, East Coast of Tanzania) (Figure 1). The districts were purposively selected based on their history of fish farming evidenced by availability of fish ponds, presence of active small-scale fish farmers, and previous involvement in aquaculture research led by scientists from Sokoine University of Agriculture (SUA), based in Morogoro Tanzania. In the sites, farmers who were willing to participate in the study were asked for their fish for sampling. Additionally, natural water bodies (Lake Victoria) as well as two man-made water sources such as the Mindu dam and research fish ponds at Magadu Fish Farm Unit (MFFU), a facility of SUA were included among the study areas (Figure 1). The three sites are found at 6.8670357°S and 37.6150417°E (Mindu), 0.7558° S and 33.4384° E (Lake Victoria) and -6.8527°S, 37.6503°E (MFFU). Wild sources were purposively included because of natural sources of Tilapia species, hence helps in genetic diversity comparison with cultured species.

Sampling of fish
Sampling of fish was conducted between 2020 and 2021. A total of 84 farmed fish were collected from fish ponds in Kilosa (35 samples), Kibaha (39 samples), and MFFU (10 samples) using seine nets. In each locality, a total of two random fish ponds were selected, with at least five samples being taken from each pond. Furthermore, 32 wild tilapias were collected from local fishermen at one landing site in Lake Victoria (17 samples) and the Mindu dam (15 samples). The sampled fish were examined to satisfy that they were the ones regarded as tilapia based on phenotypic characteristics. Sex of each fish was determined through external examination of genital papilla located immediately behind the anus to ensure that approximately even sex ratios of males to females were sampled. If proved that the fish were not tilapia, the same were returned into the water. Then fin clips (about 1 cm × 2 cm) were dissected from each fish using a sharp and sterile pair of scissors and immediately preserved in 2 mL microcentrifuge tubes containing 99% ethanol. All the samples were transported on

the same day within a space of 6 to 10 hours to the Biosciences Laboratory at Sokoine University of Agriculture (SUA), and stored at – 20°C until DNA extraction (2 to 3 weeks later).
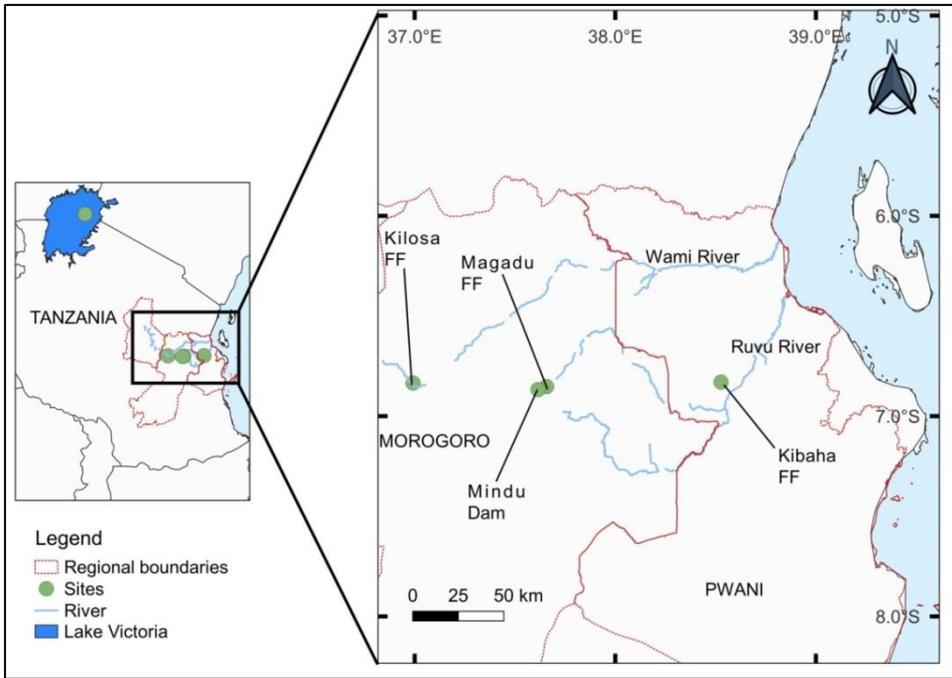
Figure 1: Location of fish farms and natural water bodies where tilapias were sampled. The map was created with quantum GIS software ver. 3.28 and shapefiles from the Database of Global Administrative Areas (https://gadm.org/maps/TZA.html. Accessed 10 June 2022).

Amplification of fragments of the COI gene

Genomic DNA was extracted from about 25 mg of each sample using the Quick-DNA^{TM} Miniprep plus Kit (Zymo Research Inc, CA, USA) according to the instructions of the manufacturer. Quality of the DNA extracts was checked on 1% agarose gel and diluted samples were stored at -20 °C until time of analysis. Fragments of 600 base pairs of the cytochrome oxidase subunit I gene (COI) were amplified in a T100^{TM} Thermal cycler machine (Bio-Lab Inc, GA, USA) using the Forward primer FishF1: 5'TCAACCAACCACAAAGACATTGGCAC3' and the reverse primer FishR2: 5'ACTTCAGGGTGACCGAAGAATCAGAA3' (Ward et al. 2005). Each reaction was in a total volume of 60 μL containing 2.5 μL of the DNA template, 1 x *Taq* 2x Master mix,

0.3 μM of each primer, and 0.4 mg of bovine serum albumin. The reaction was firstly denatured at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 35 s, annealing at 54 °C for 45 s, and extension at 72 °C for 1minute. These processes were followed by a final extension at 72 °C for 10 minutes. The quality of the PCR products was checked on a 1% agarose gel. After all procedures, 25 μL of each PCR product was sent to the Macrogen Europe Laboratory for Sanger sequencing. Each sample was sequenced twice on an ABI 3730 DNA Analyzer (Applied Biosystems) using the forward primer FishF1 and the reverse primer FishR2.

Data analysis

For each sample, the obtained forward and reverse sequences were edited to trim the

ends and aligned using the ClustalW algorithm as implemented in the software MEGA ver. 11 (Tamura et al. 2021) to generate a consensus sequence. Each consensus sequence was translated with the same software into amino acid sequences using the vertebrate mitochondrial genetic code to identify and remove nuclear pseudogenes and sequencing artifacts from the data set (Fabiani et al. 2023, Rumisha et al. 2023b). The sequences were then submitted to the GenBank nucleotide database and given accession numbers OL440976-OL441031, OM763773-OM763800, and OK602703-OK602736. The taxonomic identity of each fish was revealed by comparing each consensus sequence with the published sequences in the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov). A total of 62 COI sequences identified as *O. niloticus* were selected and aligned using the software MEGA ver. 11 to create a multiple alignment. The multiple alignment was collapsed into haplotypes according to Rumisha and Kochzius (2023). The indices of genetic diversity such as haplotype diversity and nucleotide diversity were estimated with the software Arlequin ver. 3.5.2.2 (Excoffier and Lischer 2010). The same software was used

to test for population differentiation by running the AMOVA (Analysis of Molecular Variance) routine and then comparing genetic distances between pairs of populations (F_{ST}) (Rumisha et al. 2023a). The Holm-Bonferroni Sequential procedure was used to correct type one errors (Holm 1979).

Results
Proportion of non-targeted tilapias among the farmed stock

A total of 116 nucleotide sequences blasted against the published sequences in the NCBI database showed that the sampled fish were highly mixed with different tilapia species such as Nile tilapia (*O. niloticus*), blue-spotted tilapia (*O. leucostictus*), Wami tilapia (*O. urolepis*), Singida tilapia (*O. esculentus*), and redbreast tilapia (*Coptodon rendalli*). Of these, non-targeted tilapias accounted for 22.22–31.41% of the tilapia sampled from each fish farm (Table 1). Our sequences showed a query cover between 92 and 100% as well as maximum identity between 99.25 and 100%. In contrast, Nile tilapias accounted for 90.01% and 96.67% of the wild tilapia stock in Mindu dam and Lake Victoria, respectively, which is consistent with the annual fisheries statistics reports (URT 2021).

Table 1: Percentage of each tilapia species in wild and farmed samples of fish collected from Tanzania between 2020 and 2021. FF = fish farms, N = number of samples

Site	Number of sample	Targeted tilapia (%)		Non-targeted tilapia (%)				Total
		Nile tilapia (*Oreochromis niloticus*)	Blue-spotted tilapia (*O. leucostictus*)	Wami tilapia (*O. urolepis*)	Singida tilapia (*O. esculentus*)	Redbreast tilapia (*Coptodon rendalli*)		
Kilosa FF	35	68.5	17.14	2.85	11.42	0		**31.41**
Kibaha FF	39	73	3.33	16.67	6.67	0		**26.67**
Magadu FF	10	77.78	0	0	0	22.22		**22.22**
Mindu dam	15	90.01	6.67	0	0	3.33		
Lake Victoria	17	96.67	3.33	0	0	0		

Genetic stock structures of wild and farmed Nile tilapia

The 62 analysed COI sequences of Nile tilapias from Kibaha FF, Kilosa FF, Mindu dam and Lake Victoria showed a total of five haplotypes (Table 2). The sequences showed high haplotype diversity and low nucleotide diversity (Table 2). The highest haplotype and nucleotide diversity was detected in samples from Kibaha FF, whereas samples from Kilosa FF showed the lowest haplotype and nucleotide diversity.

Table 2: Indices of molecular diversity of farmed and wild Nile tilapias sampled from Tanzania between 2020 and 2021

Sites	Number of samples	Number of haplotypes (nh)	Haplotype diversity (h)	Nucleotide diversity (π (%))	Number of polymorphic sites
Kibaha FF	16	4	0.716 ± 0.070	0.145	3
Kilosa FF	18	3	0.451 ± 0.117	0.076	2
Lake Victoria	16	4	0.575 ± 0.115	0.104	3
Mindu dam	12	3	0.621 ± 0.086	0.111	2

Analysis of Molecular Variance (AMOVA) showed that 81.21% of the measured genetic variations were within sites (Table 3). The estimated F_{ST} value was significantly different from zero ($F_{ST} = 0.187$, $p < 0.05$), implying that there was restricted genetic connectivity between the sites. Pairwise F_{ST} comparison showed that the farmed fish were not genetically distinct from one another and from the fish in the Mindu dam. In contrast, farmed fish from Kilosa were genetically distinct from the fish in Lake Victoria (Table 4). The observed pattern of genetic connectivity was also revealed in the haplotype network (Figure 2). The network revealed that the studied populations shared two common haplotypes (h2 and h3). It also showed that two haplotypes were found exclusively in Lake Victoria and the Kibaha fish ponds, while one was found exclusively in the Kilosa fish ponds and the Mindu dam (h1).

Table 3: Analysis of molecular variance (AMOVA) among farmed and wild Nile tilapias sampled from Tanzania between 2020 and 2021. DF = degree of freedom, Va = Variation among population, Vb = Variation within population

Source of variaton	DF	Sum of squares	Variance components	Percentage of variation
Among population	3	3.99	0.07 Va	18.79
Within population	58	16.93	0.29 Vb	81.21
Total	61	20.93	0.36	
Fixation Index (F_{ST}) = 0.187				
P-value < 0.05				

Table 4: Pairwise F_{ST} values among farmed and wild Nile tilapias sampled from Tanzania between 2020 and 2021. Bolded values are significant after Holm-Bonferroni Sequential correction. FF = fish farm

Site	Kilosa FF	Kibaha FF	Mindu dam	Lake Victoria
Kilosa FF	0			
Kibaha FF	0.13	0		
Mindu dam	0.11	-0.03	0	
Lake Victoria	**0.44**	0.11	0.16	0
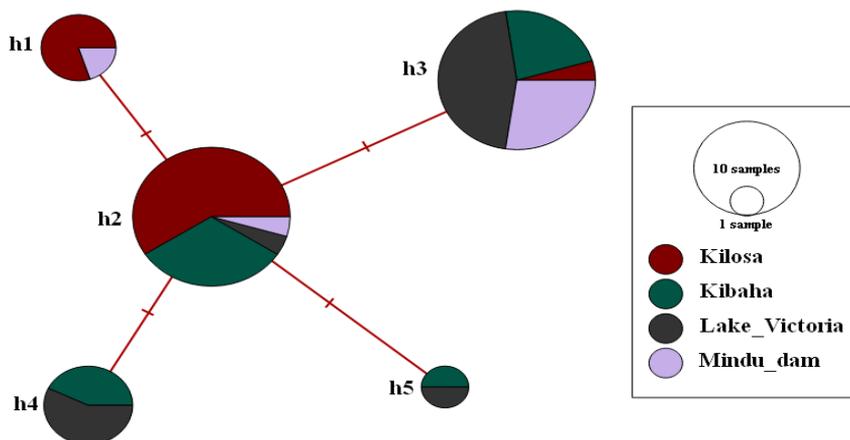
Figure 2: The minimum spanning haplotype network of partial cytochrome oxidase subunit I sequences of wild and farmed Nile tilapias sampled from Tanzania between 2020 and 2021.

Discussion

Proportion of non-targeted tilapias among the farmed stocks

Availability of quality and correctly identified tilapias has remained a major challenge to most fish farmers in developing countries to date (Munguti et al. 2014). The genetic and phenotypic characteristics of the majority of cultured fish in these countries remain unclear, which hinders the implementation of breeding and conservation programs and can result in suboptimal performance (Rukanda and Sigurgeirsson 2016). Hence, we have taken the initial step of identifying fish from various farms and determining whether they belong to the targeted tilapia. Additionally, we have conducted an assessment of the genetic diversity of the sampled fish, which is crucial for providing valuable information for selective breeding. This information becomes especially significant when prioritizing the production of superior quality seeds (Hilsdorf and Hallerman 2017). Based on the evidence from our study, non-targeted tilapias accounted for 22.22–31.41% of the farmed stocks. This implies that although farmers thought that they were culturing Nile tilapias, in fact they were unknowingly working with different species possibly interbreeding in the same ponds. Non-targeted tilapias found in the study sites included *O. leucostictus, O. Urolepis, O. esculentus,* and *Coptodon rendalli.* These findings are consistent with a recent study that discovered that 10% of the fish raised in Chita, Morogoro, are non-targeted tilapias (Mndeme et al. 2020). The presence of other non-targeted tilapias in Nile tilapia farms is possibly due to the high demands for Nile tilapia fingerlings which have caused uncontrolled purchases and movements of unknown tilapia species from different places possibly shifting them outside their natural geographical locations (Shechonge et al. 2019). The Nile tilapia has been recommended for best performance than any other tilapia species in Tanzania (Chenyambuga et al. 2011). Because fish farmers in the study area were complaining about the poor growth performance of fish, it is possible that this was due to an unclear mixture of various tilapia species and uncontrolled breeding. The most likely cause of poor growth and yield in farmed Nile tilapias is nutritional deficiency and stress caused by intra-specific competition for food and space from non-targeted tilapias (Genner et al. 2018, Shechonge et al. 2019). A previous study showed that poor feeding and irregular pond fertilization are common in the study area (Chenyambuga et al. 2012), further complicating the situation. Therefore, aquaculture should be undertaken seriously, taking on board all management aspects including genetic characterization and breeding.

Genetic stock structures of wild and farmed Nile tilapias

Nucleotide diversity indices highlight low levels of mitochondrial genetic diversity, with Lake Victoria, Mindu dam and Kibaha species displaying higher diversity than Kilosa (Table 2). The measured indices of genetic diversity are comparable to the values reported by other researchers (Wu and Yang 2012, Mndeme et al. 2020). The low genetic diversity observed in Kilosa samples suggests that the ponds were stocked with low quality fingerlings. This scenario could also imply that farmers share fish seeds from the same broodstock or from the same locality in the wilderness. Given that low genetic diversity may contribute to poor performance (Petit-Marty et al. 2022), the observed low genetic diversity in fish from Kilosa could also account for poor growth and yield at the site. AMOVA showed that over 80% of the genetic variations were within populations (Table 3). Genetic variations in a group of organisms enable some organisms to survive better than others in the environment in which they live in. Higher genetic variations within a population may potentially be important positive parameters for conducting selection within a population and tools for cross breeding among populations (Kajungiro et al. 2019, Moses et al. 2020). Pairwise F_{ST} comparisons showed that with the exception of Lake Victoria and Kilosa, all other F_{ST} comparisons were not significant (Table 4). This suggests that the farmed fish in Kilosa and Kibaha were related to one another and to the wild fish in the Mindu Dam, but that the farmed fish in Kilosa were not genetically related to the fish in Lake Victoria. The fact that the farmed fish were related to one another suggests that fish farmers may be obtaining their seeds or broodstock from the same sources. The fact that farmed fish were genetically related to the fish in Mindu dam indicated that broodstock or seeds were obtained from the dam or rivers that are connected to the dam. The results of the haplotype network showed that the studied populations shared two common haplotypes (Figure 2). Because Nile tilapia has been translocated to diverse habitats across the country since its introduction into Lake Victoria in the 1950s (Tibihika et al. 2022), it is probable that the two frequently observed haplotypes (h2 and 3) originated from Lake Victoria. Similarly, the two haplotypes (h4 and 5) found exclusively in Kibaha and Lake Victoria could be Lake Victoria haplotypes that were introduced into Kibaha.

Conclusion

Nile tilapia farmers in Tanzania have been complaining about poor growth and low yields. The findings of this study showed that non-targeted tilapias accounted for 22.22 to 31.41% of the farmed fish in the study area. This implies that although farmers thought that they were culturing Nile tilapias, they were unknowingly working with several untargeted tilapia species that require different environments and treatments for enhanced growth and yields. Therefore, it is advised that farmers should ensure that the seeds they use for aquaculture are from certified hatcheries, as this will reduce the likelihood of stocking non-targeted species. Additionally, given the low genetic diversity observed in samples from Kilosa, it is advised that the country should develop a national breeding program for tilapia to ensure farmers have access to quality seeds. Lastly, efforts should be taken to improve the taxonomic expertise of farmers to avoid stocking of incorrectly identified fish.

Declaration of Interest

The authors affirm that they have no conflict of interest to declare.

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