Tanzania Journal of Science 46(1): 31-41, 2020 ISSN 0856-1761, e-ISSN 2507-7961

Genetic Diversity Among Tilapia Species Farmed at Chita in Kilombero, Morogoro Region, Tanzania

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Received 27 Aug 2019, Revised 16 Jan 2020, Accepted 29 Jan 2020, Published 31 Mar 2020

Abstract

The development of aquaculture industry in Tanzania cannot cope with the expected substantial increase in demand for fish and fish products due to several bottlenecks including unavailability of good quality feeds and fingerlings. Fish farmers often complain about slow growth rate of the farmed fish necessitating genetic characterization. This study identified and determined the genetic diversity of tilapia species farmed in Kilombero Districts at Chita earthen ponds. Fish samples were collected from nine earthen ponds belonging to small-scale fish farmers for genetic characterization. Total DNA was extracted by using the phenol-chloroform technique. The genetic diversity was calculated by using the Arlequine version 3.01. The study found that, 90% of the sampled fish at Chita were *Oreochromis niloticus*, while 5.5% were *Bathybates minor*. There was low genetic diversity in the farmed tilapia, suggesting the possibility of inbreeding effects and a limited number of founder broodstock in the populations. Therefore, successful aquaculture development in Tanzania requires well-managed and effective fish breeding programs for production of good quality fingerlings through fish genetic resources management.

Keywords: Aquaculture, *Oreochromis niloticus*, identification, genetic marker.

Introduction

Tilapia species collectively belong to the Cichlid family of fishes. They are the most spectacular of extant vertebrates, comprising several hundred morphologically and behaviourally diverse species (Fryer and Iles 1972, Trewavas 1983, Sültmann et al. 1995, Klett and Meyer 2002). Tilapia is an excellent fish species for aquaculture because of its rapid growth rate, high survival rate, easy breeding and ability to feed low on the aquatic food chain (Abdel-Hamide 1998, Kapinga et al. 2019). Moreover, the species are easy to handle, resistant to diseases and tolerant to a wide range of environmental conditions (Balarin and Hatton 1979).

Tilapia is the common name for four fish genera: *Oreochromis*, *Sarotherodon*, *Coptodon* and *Tilapia* (Dunz and Schliewen 2013). These fishes are indigenous to tropical and sub-tropical fresh waters of Africa, Mediterranean and Middle East (Trewavas 1983). The four genera are taxonomically distinguished based on their parental care patterns (Mjoun and Rosentrater 2010). In the genera *Tilapia* both parents (male and female) guard the eggs, wriggler, and free-swimming fry, and hence they are referred to as bi-parental caring substrate spawners (Trewavas

1983, Mjoun and Rosentrater 2010). On the other hand, in the genus *Oreochromis*, the females incubate the fertilized eggs and the young fry in their mouths, hence they are known as arena-spawning maternal mouth brooders (Trewavas 1983). The genus *Coptodon* comprises of substrate-brooding, benthopelagic fish, guarding eggs as a form of parental care is done by both male and female (Dunz and Schliewen 2013). The Cichlids of the genus *Sarotherodon* are paternal, maternal or bi-parental brooders, in which either male or female or both parents, protect and carry the eggs, wriggler and free swimming fry in their mouths (Mjoun and Rosentrater 2010, Canonical et al. 2005).

Most classifications of Tilapia species are based on reproduction, development, feeding, structural characteristics and biogeography (Trewavas 1983). Although such characteristics are valuable for identification purposes, yet none of them, either singly or in combination, can be used to unambiguously identify an individual fish because the differences among species are imperceptible and overlap (Fryer and Iles 1972). Species differentiation becomes more complicated when interspecies hybridization occurs; hence the correct identification of the species is imperative. Genetic markers are fundamental tools for monitoring fish populations (Rashed et al. 2011) and fish species genetic variability (Saad et al. 2012). Different molecular methods have been used in characterization and identification of tilapia species since the adoption of DNA based classification technique in the 1980s. These methods include restriction fragment length polymorphism (RFLP) (Agnése et al. 1999), isozymes (Agnése et al. 1999) and random amplified polymorphic DNA–RAPD. Application of microsatellite markers for genetic diversity studies in Tilapia culture lines has been described by Romana-Eguia et al. (2004).

More than 95% of fish farmers in Tanzania grow mixed-sex Tilapia species in earthen ponds (Kaliba et al. 2006). Most fish farmers obtain their Tilapia seeds for stocking in ponds either from the wild or neighbouring fish farmers with less experience and improper identification knowledge. Consequently, chances of stocking incorrectly identified stocks are common. Thus, Tilapia species cultured in earthen ponds in Tanzania are inbred leading to subsequent loss of genetic diversity (Bradbeer et al. 2019). This has caused poor yields from Tilapia species cultured in the country (Shoko et al. 2011). Therefore, studies aimed at using appropriate methods to identify correctly Tilapia fingerlings for stocking in earthen ponds are required to ensure increased fish production. The present study used simple sequence repeats technique to differentiate Tilapia species farmed at Chita in Kilombero District.

Materials and Methods

Study site

Kilombero District is situated in a vast floodplain, between the Kilombero River in the south-east and the Udzungwa-Mountains in the north-west. The area is predominantly rural with the semi-urban district headquarters Ifakara as major settlement. The majority of the villagers are subsistence farmers of maize and rice complemented with fish farming (Limbu et al. 2016). This study was conducted at Chita, one of the wards of the Kilombero District situated in the east of the region, between latitude 8° 33" and longitude 35° 58' (Figure 1).

Collection of fish samples

The fish used in this study were collected from earthen ponds owned by four small-scale fish farmers namely; Mponzi (three ponds), Ngaiza (two ponds), Soliyambingu (two ponds) and Lubagula (two ponds). Before fish collection, each pond was standardized to fit 200 m^2 and stocked with Tilapia species at a stocking density of 3 fish m^{-2}. This study was part of a large project, other growth parameters and fish management practices have been published elsewhere (Limbu et al. 2016, Limbu et al. 2017, Shoko et al. 2019).

At least 30 fish specimens were collected randomly from each fish pond by using a small mesh seine net from September to November 2013. Thereafter, one hundred and thirty four (134) fish samples were pooled from the collection, labelled, chilled on ice box and brought to the University of Dar es Salaam laboratory. In the laboratory, the fish were stored at –70 °C prior to genetic analysis. A fragment of muscle of approximately 1 x 1 cm from the caudal fin tissue was excised from each fish in a sample of 90 fish specimens selected randomly, and placed individually in labelled vials containing 1 ml of 95% non-denatured ethanol (70% isopropanol) and held at 4 °C for subsequent DNA extraction.

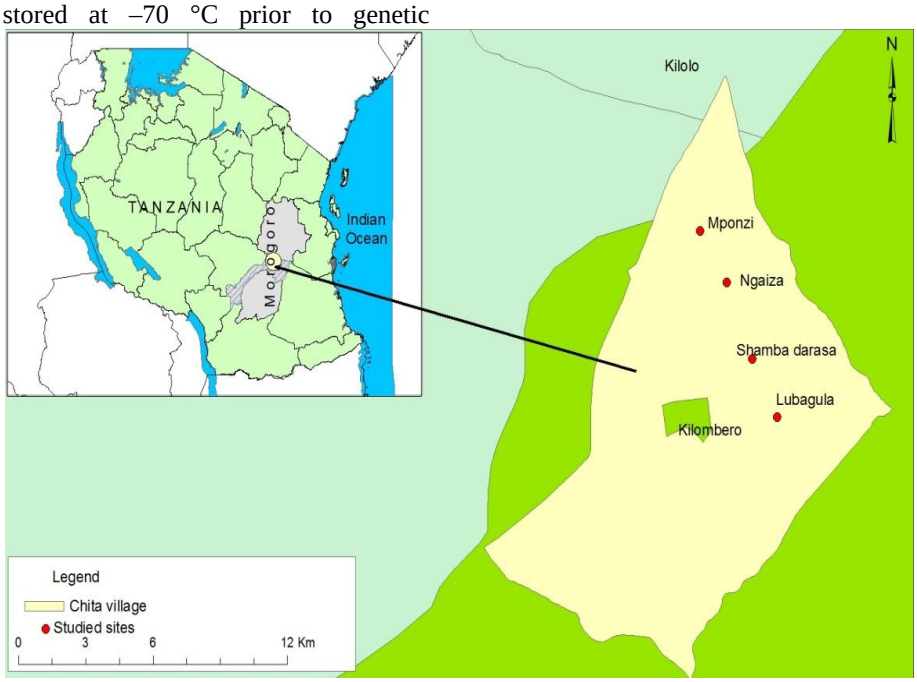

Figure 1: The map of Kilombero District, Morogoro region showing the study site. (Source: UDSM Cartographic Unit, 2016).

Genomic DNA extraction

Total genomic DNA was extracted from 90 previously excised caudal fin tissue samples by using phenol/chloroform method as described by Ullrich et al. (1977). The presence of DNA was verified by agarose gel (0.8% w/v) electrophoresis and its concentration and purity were determined by a Nanodrop spectrophotometer (Thermo Scientific, Inc).

The polymerase chain reaction (PCR) and sequencing

The polymerase chain reaction (PCR) used the primers UNH 172, Primer sequence (5′–3′) (AATGCCTTTAAATGCCTTCA and CTTTTATAGTCGCCCTTTGTTA) (Kocher et al. 1989). The PCR was performed in final volume of 50 ml containing 0.25 mM $MgCl_2$, 0.2 mM of each dNTP, 1 mM of each primer, 5 ml of 10x buffer and 10 units of Taq polymerase (Promega). The PCR reaction conditions were: 3 minutes pre-heating at 93 °C followed by 40 cycles of 30 seconds at 93 °C, 30 seconds at 62 °C and 1 minute at 72 °C, with a final elongation phase of 72 °C for five minutes. Sequencing of the PCR products was done at Macrogen (Korea). Sequences were aligned using BioEdit 5.09 (Hall 1999).

Tilapia species identification

Tilapia species were identified by using conventional morphological method and microsatellite DNA based technique.

Morphological identification

A total of eighty nine male and female samples were identified using FAO identification sheet for fishery purposes (Eccles 1992). The *Oreochromis niloticus* species were identified based on morphometric variables (*M*) and three meristic variables (*m*), which were directly counted. The morphometric variables were measured to the nearest 0.1 cm using a measuring ruler and 0.001 g using digital weighing scale (OHAUS^{TM} 30035436 Model V41PWE6T). All measurements were taken on the left side of the fish. The morphometric variables determined included body weight (BW, g) and total length (TL, cm). External meristic characters examined included number of dorsal and caudal fin spines (DFS and CFS, respectively). Internal meristic characters examined were gill rackers.

Microsatellite DNA based identification

The forward and reverse sequences were aligned in one direction by using BioEdit 5.09 (Hall 1999) in order to avoid editing ambiguities that occurred during sequencing. The edited microsatellites sequences were separately aligned with the published Genbank National Center for Biotechnology Information (NCBI) sequences for *Oreochromis* species through Basic Local Alignment Search Tool (BLAST). This was necessary in order to compare the query sequences at hand with the data base sequences (http://blast.ncbi.nlm.nih.gov retrieved 24th may 2016 11:36). The sequences with maximum likelihood were aligned by MUSCLE software (Edgar 2004) using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (Tamura et al. 2011) in order to determine their similarities and differences.

Genetic diversity of Tilapia species

The number of haplotypes and nucleotide diversity (n) were calculated for the entire population using ARLEQUIN version 3.01 (Librado and Rozas 2009) in order to examine the extent of genetic diversity of the species studied.

Phylogenetic tree analysis

Phylogenetic tree for the microsatellite sequences were constructed by using the Maximum Likelihood (ML) algorithm in MEGA 7.0 software based on Kimura 2-Parameter model to depict the genetic relationships of the sampled individuals. The robustness of the nodes in each phylogeny was assessed by bootstrap analysis (Felsenstein 1985) for 100 replicates. The resulting phylogenetic trees were edited by using Tree Graph 4.0.

Results

Morphological identification

Fish collected from earthen ponds at Chita in Kilombero District were characterized by long spiny dorsal fins anteriorly (Plate 1). Other features included dorsal fin with 16 to 17 spines and 11 to 15 soft rays, anal fin with 3 spines and 10 to 11 rays. The nostril is on each side and uninterrupted lateral line with the first section being slightly above and curved following the dorsal profile of the body. The fish had a laterally compressed body with cycloid scales and caudal fin with numerous black bars. These morphological features and meristic counts were congruent to those from the family Cichlidae and the genus *Oreochromis* as described by Trewavas (1983).

The colour of the collected fishes ranged from grey-brownish to dark-grey, with some fish having vertical bars on their trunks (Plate 1). Sampled fish had 14 to 28 gill rackers portraying the characteristic features of *Oreochromis niloticus* as described by Trewavas (1983).

Microsatellite DNA based identification

A total of ninety (90) nucleotide sequences blasted against the published sequences in the NCBI database. The sequences showed the query cover of 33 to 100% and maximum identity of 97 to 100%. About 90.1% of the samples were identified as *O. niloticus*, whereas a 5.5% samples were identified as *Bathybates minor* and the remaining 4.4% of the sequences were not identified.

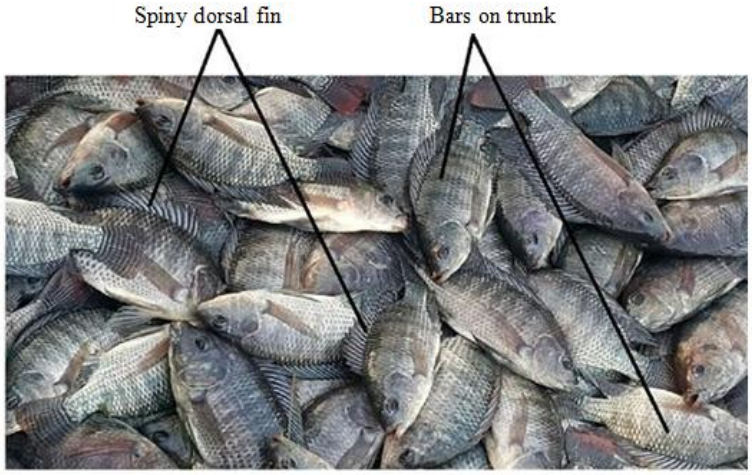

Plate 1: A harvest of *Oreochromis niloticus* from one of the ponds at Chita (Photo: Mndeme). Note: Dark stripes on the body are distinctive of the species, colour ranged from grey-brownish to dark-grey.

Genetic diversity of Tilapia fish species

The results of microsatellite sequences produced from Arlequine 3.1 analysis were eighty nine haplotypes (Table 1) with allele sizes ranging from 119 to 194 bp. The nucleotide diversity (average per loci) and gene diversity were 0.5885 and 1.0000 ± 0.0017, respectively.

Table 1: Standard diversity indices: (pop_1)

Number of gene copies	89
Number of sequences	89
Number of allele	89
Number of loci	1
Number of usable loci	218
Number of polymorphic site	218
Number of heterozygosity	1.000

Phylogenetic tree analysis

Similarities among fish collected from different ponds at Chita were checked via a phylogenetic tree constructed by the Maximum Likelihood algorithm based on the Tamura-Nei model using MEGA 7 (Kumar et al. 2012). The inference involved 91 nucleotide sequences. We used the sequences for *Oreochromis mossambicus* (GenBank accession number AB601825) and *Oreochromis aureus* (GenBank accession number AB195552.1) as out-groups. We constructed the tree by using the highest log likelihood (-3803.1956; Figure 2). The tree shows the percentage relatedness of the collected Tilapia species. The maximum likelihood produced a tree with ten clusters (groups) separated by insignificant bootstrap values. In addition, individuals collected from different ponds were randomly intermingled in the different clusters. Notably, *B. minor* was also observed pairing with *O. niloticus* at some point in the tree (Figure 2).

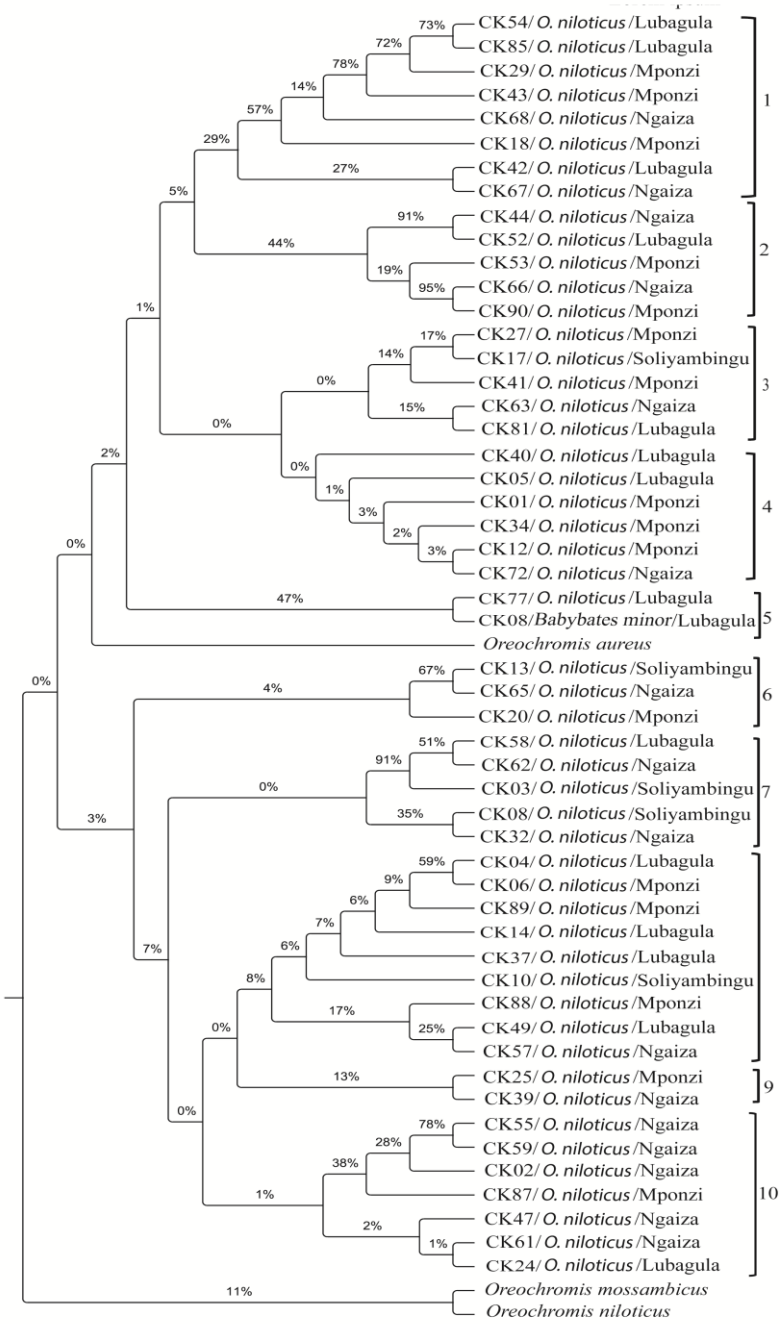

Figure 2: Maximum Likelihood consensus tree of microsatellite haplotypes based on Kimura's two-parameter sequence divergence between haplotypes of Chita earthen ponds.

Discussion

Identification of fish

The study was undertaken to understand the identity and genetic diversity of fish species farmed at Chita in earthen ponds in Kilombero valley. Fish sampled from the earthen ponds exhibited morphological and morphometric characteristics of the genus *Oreochromis* as previously described by Trewavas (1983) and Nyingi et al. (2009). The specimens had vertical dark stripes on the caudal fin, which according to Trewavas (1983) portrayed a characteristic feature for *Oreochromis niloticus*. Moreover, the lateral line was interrupted by 30 to 34 cycloid scales, which is an addition feature for this species (Trewavas 1983).

The study however, did not morphologically delineate *O. niloticus* to subspecies level mainly due to overlapping of meristic counts from the observed specimens. The present results are supported by the findings from Samaradivakara et al. (2012) in which meristic measures did not show sufficient divergence among Tilapia populations in Sri Lanka. Similar findings were reported from earlier study by Vidalis et al. (1997) who asserted that meristic characteristics have narrow variable range. The inability to delineate the fish samples at subspecific level was probably due to the fact that, many *O. niloticus* subspecies have an overlapping narrow range of meristic characteristics, which make them hard to classify when mixed. This limitation necessitated the use of molecular techniques.

A molecular approach for fish species classification is more accurate and practical compared with classical methods such as meristic characteristics. Different molecular tagging methods have been designed to study fish species characterization and population structure (Rashed et al. 2011, Saad et al. 2012). Among these, microsatellite or simple sequence repeats (SSR) marker is a convenient tool in studying genetic structure and phylogenetic relationships among fish species (Rashed et al. 2011). The potential uses of such methods in aquaculture include monitoring changes in genetic variation as a consequence of different breeding strategies, interactions between wild and cultured populations as in this study (Hutchings and Fraser 2008). Other aspects include parentage assignment and estimation of relatedness between potential breeding pairs (Hutchings and Fraser 2008).

Microsatellite sequences produced 90.1% identical to *O. niloticus*. However, individuals in these ponds related differently in terms of genotypes as observed from the genetic diversity indices values. This finding is further to confirm inbreeding events among the studied Tilapia species not necessarily during this study but from where the fingerlings for stocking in the current ponds were obtained.

Morphological and genetic diversity

The present study found nucleotide diversity values of 0.588518 ± 0.282253 from fish samples collected in earthen ponds at Chita. This suggests existence of low genetic variability in the population studied, as previously reported by Wu and Yang (2012), who found nucleotide diversity of captive individual Tilapia fish ranging from 0 to 0.3874. The obtained low genetic variability is attributed to inbreeding effects and a possible limited number of founder broodstock in the population as reported previously (Wu and Yang 2012). Fish farmers at Chita, might be obtaining fish seeds from limited broodstock, which are shared haphazardly among them. Therefore, minimizing the mating of closely related individuals by maintaining large numbers of broodstock separately is recommended to restore the genetic diversity of the population as suggested by Martins et al. (2004).

The effect a small number of founder broodstock in aquaculture has been shown in other parts of the world. For example, the initial *O. niloticus* stock brought to the Philippines originated from a small number of fish imported from Thailand in 1972. Thailand's nationwide stock is, in turn,

derived from about 200 fish from Japan, and these fish were derived from ancestors collected from open waters in Egypt, Africa in 1962 (Watanabe 1997). As no pedigree records for the import and transportation of the Tilapia fish were available, these practices possibly resulted in inbreeding and associated low reproduction and growth performance. Indeed, Chita fish farming practices show that, fish farmers collect a few brooders from Lake Victoria and use them in the same way as for the Philippine's and Thailand cases.

Inbreeding undermines genetic gains and production performance if not controlled and monitored properly in aquaculture systems (De Donato et al. 2005). Inbreeding leads to a reduction in heterozygosity of genes and an increase in homozygosity on the allelic level, which may expose deleterious or lethal recessive alleles (Lande 1994) and their expression in the homozygote state (Barrett and Kohn 1991, Brock and White 1992). This negatively impacts life history traits and productive fitness causing reduced growth, low survival rate during the eggs, fry and adult stages, low reproductive performance with a higher proportion of biochemical disorders and deformities in fish population (Gjedrem 2005). The loss of fitness and productive performance in inbred individuals is referred to as inbreeding depression (Wright 1977, Shields 1987). This phenomenon might be contributing to slow growth of fishes as pointed out by the fish farmers in the present study site.

Phylogenetic relationship

In the present study, the phylogenetic tree depicted minor variations among individuals sampled from different ponds. Despite this, inbreeding effects seemed to be happening in this farming system. The tree points out that individuals of the same species are slowly being separated genetically (low bootstrap values in clusters 1 to 10, Figure 2). The varying degrees of introgression within the population observed in this study lead to low genetic diversity. The low genetic diversity consequently caused inbreeding, which is generally detrimental to fish, similar to previous results on introgression of *O. mossambicus* that resulted into reduced growth rates (Macaranas et al. 1995, Micha et al. 1996). Nevertheless, pairing of individuals from different ponds as observed in this study implies that farmers are sharing fish seeds from the same broodstock or rather collecting fingerlings from the same locality in the wilderness.

Interestingly, the present study reported that 5.5% of all the fish sampled were *Bathybates minor*. The presence of *B. minor*, a species endemic to Lake Tanganyika at Chita ponds needs further research. Fish farming history in Tanzania indicates that fish farmers have been obtaining fish seeds from various regions including Mbozi District, Mbeya and Lake Victoria in Mwanza (Pauly 1983). Possibly, the *B. minor* were first cultured in Mbozi farmers' ponds and later transferred to ponds in Chita via importation of fish seeds.

Conclusion

In summary, the cultured fish species at Chita were dominated by *Oreochromis niloticus* and few *Bathybates minor* with low genetic diversity. To improve fish farming in Tanzania, three strategies need to be implemented. First, DNA-based molecular markers should be used as a non-invasive method to identify and characterize Tilapia species for re-stocking. Secondly, periodic assessments of the standing genetic diversity of population should be conducted in order to evaluate the health of the population. Third, maintaining genetic heterogeneity in reproductive stocks to allow for constant and predictable Tilapia species production that satisfies the national demand. Future studies should consider using genetic markers to improve assessment and characterization of diversity for effective management of Tilapia species.

Acknowledgements

We thank the Government of the United Republic of Tanzania through Commission for Science and Technology (COSTECH) under "KILIMO KWANZA" and the Lake Victoria Research (VicRes) for funding the study. This study would be impossible without the assistance of Tilapia farmers from Chita Kilombero, who provided samples of their fish and spent many long hours discussing the history of those fish.

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