ASSESSMENT OF GENETIC PURITY AND DIVERSITY OF FARMED TILAPIA FISH IN TANZANIA MAINLAND

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN HEALTH OF AQUATIC ANIMAL RESOURCES OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Fish farming in Tanzania is done in ponds and cages by commercial and smallholder farmers, mostly using tilapia species. The productivity of fish farming is believed to be strongly influenced by the quality of the strain cultured. In Tanzania tilapia culture is mainly dominated by *Oreochromis* and *Coptodon* spp, and most authorities consider the fast-growing *Oreochromis niloticus* to be the most appropriate species for aquaculture in various parts of the country. However, there other species that are farmed in some areas such as *Oreochromis urolepis* and *Oreochromis leucostictus*. These species are known to hybridise with *Oreochromis niloticus*. This study assessed the purity and genetic diversity of tilapia fish in hatcheries and farms in Tanzania Mainland. The study focused on three specific objectives which were; i) assessment of sources of tilapia broodstocks and fingerlings in hatcheries and farms, ii) identification of farmed tilapia fish species and hybrids using single nucleotide polymorphism (SNPs) and iii) assessment of within-and between-population genetic diversity of Nile tilapia broodstock in hatcheries and farms. Information on the sources of tilapia broodstock and fingerlings in hatcheries and farms in Tanzania Mainland was collected through face-to-face interview using structured questionnaires. A total of 248 and 16 respondents from fish farms and hatcheries, respectively, were interviewed. Data on sources of tilapia broodstock and fingerlings were analyzed using Statistical Package for Social Sciences (SPSS.Version, 21) to derive descriptive and inferential statistics. Significant differences were judged at a probability level of $p \leq 0.05$. Tilapia species and hybrids cultured in hatcheries and farms were determined through SNP analysis. Species identification was facilitated by inclusion of reference samples of *Oreochromis niloticus* and *O. leucostictus* from Lake Albert in Uganda and *O. urolepis* from the lower Wami River in Tanzania in the analysis. A total of 536 tilapia pectoral fin samples were collected from 10 fish hatcheries and 59 fish farms

located in seven agro-ecological zones of Tanzania Mainland. Following euthanasia, clips of the pectoral fin were cut and placed into labelled vials containing 95% ethanol. The vials were put in a cool box packed with ice and transported to a laboratory where they were stored at -20^{o}C, until DNA extraction. Overall, analysis was carried out on 190 fin clips, which were selected from the initial collection of 536 tilapia specimens. DNA extraction from fin clips was done using the BioArk extraction kit and SNP genotyping was performed by LGC Genomics GmbH campany in Berlin. The SNP assay was originally calibrated with three species of *Oreochromis* (*O. niloticus, O. urolepis and O. leucostictus*), not with *Coptodon*. A pseudo-reference set for *Coptodon* was generated using four specimens for which clear photographs of *Coptodon* were available. These were then coded as reference samples and used to identify the genotypic profile of *Coptodons* in the analysis, in an attempt to identify *Coptodon* specimens among the non-photographed samples. Principal component analysis (PCA) was done using SNPRelate package in R v4.01 software and used to preliminary identify the species and hybrids present in the collected tilapia samples. Ancestry analysis was done using Admixture v1.3.0 software to determine the proportion of ancestral admixture for assigning appropriately the different types of species and their hybrid present in both fish hatcheries and farms. A threshold of 80% cluster membership was used to classify samples as pure species. Individuals with cluster membership of less than 80% were considered to be potential hybrids. Based on cluster membership criterion, a total of 50 *O. niloticus* were identified out of 190 genotyped samples, whereby 31 and 19 *O. niloticus* individuals were from fish hatcheries and farms, respectively. These were then selected as samples for the study of assessment of within and between population genetic diversity of Nile tilapia broodstocks in hatcheries and farms. The population structure of the fish samples was analyzed using Structure v.2.3.4 Software with K values ranging from 2 to 5. Markov

Chain Monte Carlo (MCMC) of 100 000 iterations with a burn-in period of 10 000 was carried out for each K value.

Observed (Ho) and expected (He) heterozygosity parameters were used to assess the within population genetic diversity for the hatchery populations. Similarly average individual inbreeding coefficient (F_{IS}) for *O. niloticus* identified in fish hatcheries were estimated using stacks v2 software. The R package StAMPP was used to perform Analysis of Molecular Variance (AMOVA) using 1 000 permutations. Also, the principal component analysis (PCA) was carried out using the R package Adegenet version 2.1.1.

The results on sources of tilapia fingerlings and broodstock indicate that, the main sources of tilapia broodstocks in fish hatcheries were other hatcheries within the country (45.5%). However, some fish hatchery managers (12.3%) were importing broodstock from Uganda and Thailand. The results revealed that fish hatcheries and natural water bodies were used as sources of fingerlings in all agro-ecological zones, but the extent of utilization differed significantly among the agro-ecological zones. Fingerlings from the wild were more utilized in the Northern, Western and Lake Zones while the use of fingerlings from hatcheries predominated in the Eastern zone. The results on tilapia fish species and hybrid identification in fish farms and hatcheries, indicate that 91.52% of the fish farms and 70% of the hatcheries were not culturing pure *O. niloticus*, but hybrids or tilapia of other species. The pure *O. niloticus* (percentage of pure individuals in brackets) were observed at Ruvu fish farm (12.90%), Safina bigfish (12.90%) and 821KJ-Bulamba (51.61%). Likewise the results for within and between population genetic diversity of Nile tilapia broodstocks in hatcheries and farms indicated that pairwise F_{ST} values varied from 0.072 to 0.359 in some of fish hatcheries. The lowest F_{ST} values were found between 821KJ-Bulamba and populations from Mwamapuli, Ruvu fish farm, and Safina bigfish. The highest F_{ST} values were observed between 821KJ-Bulamba and Faiza fish farm. Admixture was detected at Mwamapuli, Faiza fish farm, Ruvu fish farm and Safina bigfish, but was not detected at 821KJ-Bulamba. In fish farms admixture was detected at SUA-Morogoro and Babati populations. Result on Analysis of molecular variance (AMOVA) show that 17.71% of genetic variation was found among populations, -7.69% among individuals within populations and 89.98% of variation was within individuals. Principal component analysis indicated one genetic group of *O. niloticus* clustering to *O. niloticus* reference with no any individual clustering with either *O. urolepsi* reference or *O. leucostictus* reference. This study concluded that, the main sources of tilapia broodstock for fingerlings production in hatcheries are other hatcheries within and outside Tanzania as well as wild sources. The main sources of tilapia fingerlings for stocking fish farms in Tanzania mainland are hatcheries within Tanzania, wild sources and recruits from other fish farms. A large number of sampled fish farms (91.52%) and hatcheries (70%) do not culture pure *Oreochromis niloticus,* instead they culture hybrids and a mixture of either *Oreochromis niloticus* with other species of tilapia or unknown tilapia species. There is high number of hybrid individuals both in fish farms and hatcheries than any pure single species. The high level of genetic impurity in farmed tilapia fish in both hatcheries and farms is contributed by existence of *O. leucostictus, O. jipe, and O. urolepsi* and *Coptodon* species. There is high genetic diversity within populations than among Nile tilapia populations. Based on the findings of this study it is recommended to establish certified hatcheries in each agro-ecological zone to increase accessibility of quality seeds. Also it is recommended to establish a practical tilapia breeding program for maintaining the purity of different tilapia strains as well as conducting regular training to fish farmers and hatchery managers on how to handle and manage tilapia broodstock.

DECLARATION

I, MASHAKA SHABANI, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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LIST OF ABBREVIATION AND SYMBOLS

AMOVA	Analysis of Molecular Variance
COSTECH	Tanzania Commission for Science and Technology
DSM	Dar es Salaam
FAO	Food and Agriculture Organization of the United Nations
FETA	Fisheries Education Training Agency
F_{ST}	The fixation index
JRS	Jacob Richard Schramm
MCMC	Markov Chain Monte Carlo
MSc	Master of Science
^{O}C –	Degree Celsius
PCA	Principal Component Analysis
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
URT	United Republic of Tanzania
%	Percentage

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Fish is used in many countries as a primary source of protein, contributing up to 17% of the total global animal protein intake and about 6.5% of all protein consumed in the world (FAO, 2018). Fish is also a major source of livelihood and income, particularly in developing countries. In recent years fish production from natural waters or capture fisheries has shown tremendous declining trend (FAO, 2020), this has stimulated more efforts to be put on fish production from aquaculture. Currently, fish production from aquaculture represents 47% of the total production (FAO, 2018). Aquaculture has the potential of enhancing food security directly through producing fish for household consumption, improving the supply and reducing fish price in the market. Currently, aquaculture is promoted as a mechanism for rural development with a focus on poverty alleviation in developing countries (Lowell *et al.*, 2016).

Among the fish species used in aquaculture, tilapia is the world's second most popular farmed fish after carps. Tilapias are farmed in at least 85 countries, with most production coming from the developing countries of Asia and Latin America (Martinez and Pedini, 1998). In Tanzania, tilapia is the most cultured species and accounts for 75% of the total fish production from aquaculture (Rukanda and Sigurgeirsson, 2018). The word "Tilapia" is commonly used to refer to a group of relatively deep bodied African fish species that occupy lakes or slow-moving rivers, and have a generalist diet including plankton, aquatic plants, vegetative detritus and benthic invertebrates. Tilapias form the foundation of most small-scale inland fisheries and aquaculture initiatives in the country. Tanzania's tilapia capture fisheries yield was 64 740 tonnes in 2016 while fish production from aquaculture was 3 800 tonnes, forming approximately 20% of fish production in the country (FAO, 2018).

In natural water bodies of Tanzania, there are mainly three “Tilapia” tribes. The most species rich tribe in the country is the *Oreochromini*, which has at least 20 species (*O. tanganicae, O. chungruruensis, O. shiranus, O. urolepis, O. hunteri, O. malagalasi, O. korogwe, O. esculentus, O. niloticus, O. karomo, O. karongae, O. rukwaensis, O. squaminnis, O. leucostictus, O. amphimelas, O. variabilis, O. pangani, O. macrochir, O. ruvumae and O. spilurus*) (Genner *et al.*, 2018). The tribe *Coptodini* is represented by two species (*Coptodon rendalli* and *Coptodon zillii*), and the tribe *Tilapiini* is represented by one species (*Tilapia sparrmanii*) (Genner *et al.*, 2018).

Tanzania is a hotspot for *Oreochromis* species biodiversity with about 20 species, some of which are endemic to particular catchments (Shechonge *et al*., 2018a). The native tilapia species may harbor important genetic information that could be useful in aquaculture production, including a wide range of trophic and ecological adaptations, high disease tolerance, high growth rate, high reproduction performance, tolerance to harsh environments like extreme salinity and temperature. However, the native tilapia species are threatened by introduced species stocked in fish farms. The introduced non-native tilapia species, presents a big challenge to tilapia fish farming in Tanzania as they escape from aquaculture farms and interbreed with the native species in natural water bodies, leading to genetic dilution (Shechonge *et al*., 2018a). Because of lack of proper breeding programme, poor management of broodstock, fingerlings, and misidentification of farmed tilapia species, most farms and hatcheries in Tanzania are raising tilapias of mixed species, and thus are experiencing low fish production (Kajungiro *et al*., 2019).

1.2 Aquaculture Industry in Tanzania

Aquaculture in Tanzania mainland started in the late 1920s, following the introduction of trout from Scotland to the streams of Kilimanjaro and Mbeya regions (Balarin, 1985). In 1950s, fish farming started using experimental ponds at Korogwe in Tanga Region and Malya in Mwanza Region (Nilsson and Wetengere, 1993; FAO, 2012). During those times, tilapia fingerlings were supplied from wild stocks in Lake Victoria and the Congo and Pangani Rivers (Rothuis *et al.*, 2014). Later, Nile tilapia fingerlings were supplied by Hombolo Center across mainland Tanzania (Coche *et al.*, 1994). These fingerlings were distributed by the government to fish farms (both public and private) and to public water reservoirs (Madalla, 2008).

Currently, aquaculture in Tanzania seems to be a high potential sub-sector for investment for both foreign and domestic investors. This is due to the availability of quality water, huge fish markets, security and good climate conditions that favors growth of different fish species (Shoko *et al*., 2011; Rukanda and Sigurgeirsson, 2018). Aquaculture in Tanzania is an emerging and fastest growing industry which is dominated by pond culture of tilapia species and African catfish. Currently, it is estimated that there are about 30 032 fish ponds and 431 tilapia cages scattered across Tanzania mainland (URT, 2021).

Most fish farmers prefer to culture Nile tilapia (*Oreochromis niloticus*) in earthen ponds under mixed-sex culture system. But there are other tilapia species that are used in aquaculture in different parts of the country such as *Oreochromis urolepis*, *Oreochromis leucostictus* and *Oreochromis shiranus*. Moreover, different hatcheries in the country have been importing various tilapia species from different countries (Rukanda and Sigurgeirsson, 2018). Uncontrolled introduction of different tilapia species, farming of mixed tilapia strains and collection of fingerlings from natural water bodies have resulted into increased level of interspecific hybridization among the cultured tilapia species in fish hatcheries and farms (Clavero and Garcia-Bethou, 2005). Hybridization causes loss of genetic purity of the cultured tilapia species and overall decrease in productivity.

1.3 Tilapia Fish Farming in Tanzania

Tilapia is the collective name for a group of cichlids belonging to the genera *Oreochromis, Tilapia*, *Coptodons* and *Sarotherodon* (Trewavas, 1983; Genner *et al.*, 2018). Among the *Oreochromis* species only *Oreochromis niloticus* and *Oreochromis urolepis* are produced commercially in Tanzania (Rukanda and Sigurgeirsson, 2018). Tilapia culture is increasing exponentially in the country, but its growth has been constrained by improper breeding practices including intentional introduction of non-native tilapia species, despite the presence of more than 20 tilapia species in natural water bodies available in different catchments in the country (Shechonge *et al.*, 2018a). Most tilapias cultured by small-scale farmers are of low genetic potential in terms of traits of economic importance such as growth rate and size at maturation.

1.4 Tilapia Broodstock Management in Tilapia Farming

Tilapia production in aquaculture depends on availability of quality fingerlings produced from quality broodstock. However, the majority of fish producers in Tanzania do not have access to improved and good quality tilapia fingerlings that can reach market size within a short period. The current pond cultured strains of tilapia reach market size at an average period of eight to nine months with the average weight of 250 grams. This prolongs the culture period (Chenyambuga *et al.*, 2012; Hall *et al.*, 2016). For better management of broodstock, importation of exotic species and strains should be discouraged. This is due to the fact that the introduced exotic species are known to pose threats to indigenous species and the whole aquaculture industry. According to Bartley *et al.* (2000), the introduction of any strain of *Oreochromis* to places where currently it is not found should be undertaken with greatest caution. This is because the introduced populations are likely to escape into the wild and interbreed with the native species. Similarly, the transfer and culture of *Oreochromis niloticus* into new watersheds should be avoided (Bartley *et al.*, 2000).

1.5 Effect of Introduction of Non–native Species of Tilapia

Tilapia fish hybridization in natural water bodies is a process that may result from either human influence or natural phenomena. Hybridization between introduced and indigenous species may lead to loss of unique genetic resources, leading to extinction of the native species (Levine and D'Antonio, 2013). In Tanzania, Nile tilapia (*Oreochromis niloticus*) and blue spotted tilapia (*Oreochromis leucostictus*) have been introduced to different non-native habitats for aquaculture for the purpose of enhancing production from fish farming. This practice poses a threat to the tilapiine biodiversity through hybridization and replacement of the native species, as the introduced species may out compete the natives (Genner *et al.*, 2018).

1.6 Problem Statement and Justification of the Study

At the moment there is no properly established breeding programme for tilapia in the country. Lack of practical tilapia breeding programme in the country (Kajungiro *et al.*, 2019), imposes challenges such as unavailability of good quality fingerlings and increased mixing of different tilapia species in fish farms due to collection of fingerlings and broodstocks from wild sources as well as fish hatchery stations within and outside the country. Therefore, in most hatcheries and farms, there are diverse tilapia species, both native and introduced. The interbreeding of introduced species with the native species may results into loss of uniqueness of the tilapia species, and this precipitates extinction of native species through hybridization and inbreeding. This translates into economic loss through reduced growth and reproductive performance (D'Amato *et al.*, 2017).

In Tanzania little is known with regard to the sources of tilapia fingerlings for stocking fish farms and broodstock for fingerlings production in hatcheries. Likewise, there is no information about the number of farms with pure farmed tilapia species and hybridization status in various fish farms and hatcheries. Moreover, the genetic diversity and structure of the most cultured tilapia species (*O. niloticus*) is not well documented. The availability of such information would assist in designing conservation and genetic improvement programmes of farmed tilapia populations in Tanzania Mainland.

1.7 Objectives of the Study

1.7.1 General objective

The main objective of this study was to assess the purity and genetic diversity of Tilapia fish in hatcheries and farms in Tanzania mainland.

1.7.2 Specific objectives of the study

Specifically, the study intended to;

i) Assess the sources of tilapia broodstock and fingerlings in hatcheries and farms in Tanzania mainland.
ii) Identify the farmed tilapia fish species and hybrids in fish farms and hatcheries in Tanzania mainland.
iii) Assess within and between population genetic diversity of Nile tilapia broodstock in hatcheries and farms.

CHAPTER TWO

2.0 MANUSCRIPT I

Assessment of sources of tilapia broodstock and fingerlings in fish hatcheries and farms in Tanzania mainland

Status of the Manuscript: In Preparation

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Abstract

Tilapia fish farming in Tanzania is increasing rapidly and accounts for 80 percent of fish production from aquaculture. This has led to a high demand of good quality tilapia broodstocks and fingerlings. This study assessed the sources and availability of tilapia broodstocks and fingerlings in hatcheries and farms in Tanzania Mainland. Information was collected through structured questionnaires from a total of 248 and 16 respondents from fish farms and hatcheries, respectively, in seven agro-ecological zones. The agro-ecological zones surveyed were Lake, Eastern, Western, Southern highland, Northern, Southern and Central zones. Results indicate that the majority of the respondents in hatcheries (62.5%) and fish farms (80.6%) produced only tilapia species. There was a positive and significant correlation between the number of fish ponds owned and the number of fingerlings produced per year ($r = 0.54$: $p<0.05$). The main sources of broodstocks in fish hatcheries were other hatcheries within the country (45.5%). However, some fish hatchery managers (12.3%) were importing broodstocks from Uganda and Thailand. The results revealed that fish hatcheries and natural water bodies were used as sources of fingerlings in all agro-ecological zones, but the extent of utilization differed significantly among the agro-ecological zones ($\chi^2 = 22.87$, df = 6: $p < 0.05$). Fingerlings from the wild sources were more used in the Northern, Western and Lake Zones, while the use of fingerlings from hatcheries predominated in the Eastern zone. Most of the respondents (62.5%) in fish hatcheries and some of fish farmers in the seven agro-ecological zones were mixing tilapia broodstocks and fingerlings from different sources into one fish pond without knowing the consequences on genetic purity of the farmed tilapia species. The majority of the respondents (63.7%) were not capable of distinguishing different species of tilapia in their farms. Availability of pure and good quality fingerlings was reported to be the major problem in all agro-ecological zones. This study has revealed that main sources of tilapia broodstock and fingerlings for the hatcheries in the country are

other hatcheries within the country, breeding stations in Uganda and Thailand, while fish farms in all agro-ecological zones depend on hatcheries and natural water bodies as sources of fingerlings. The study has shown that the practice of mixing tilapia broodstock and fingerlings from different sources into one fish pond is a common practice in most fish farms. Increasing the accessibility to quality seeds by establishing more hatcheries that are distributed in all seven agro-ecological zones of the country could be a sustainable solution for the shortage of quality broodstocks and fingerlings in the country, provided that good production methods are employed, such as maintaining single-species ponds and avoiding inbreeding and accidental selection of small-maturing fish.

Keywords: agro - ecological zone, fish farmers, quality broodstock, wild source

2.1 Introduction

Since 1950s, tilapias have been considered to be of great importance for fish farming in African countries (Mallya, 2007; FAO, 2012). This is because of their high growth rate, even under high stocking densities and excellent meat quality. Tilapias are also very resistant to diseases and can survive under poor water quality, and thus are appreciated in many African countries (FAO, 2007b). Globally, tilapia production from aquaculture has increased tremendously from 5 881 000 metric tons in 2017 to 6 276 000 metric tons in 2018 and 6 800 000 metric tons in 2019 (Bostock *et al.*, 2010).

In Tanzania, farmed tilapia production is increasing rapidly and accounts for 80 percent of fish production from aquaculture. The number of farmers involved in tilapia farming has increased from 17 725 farmers owning 20 235 operating fish ponds with the total production of 3 942 tons in 2014 to 20 348 farmers owning 26 445 fish ponds and producing around 18 018.6 metric tons in 2019 (Shoko and Kamugisha, 2018; URT, 2019). In Tanzania, tilapias are reared extensively or semi-intensively for subsistence in earthen ponds, hapas and cages and fed on locally available feeds (Nguyen, 2008; Chenyambuga *et al.*, 2014; Shechonge *et al.,* 2018b).

Nile tilapia are known for their tolerance to a wide range of environmental conditions, resistance to stress and disease, ability to reproduce in captivity, and acceptance of artificial feeds immediately after yolk-sac absorption (Ansah *et al.,* 2014). Despite the mentioned advantages which make the Nile tilapia to be a suitable species for farming in developing countries, like other tilapia species, it is prone to early maturity, especially when cultured in ponds, where they spawn before reaching market sizes (Nkhoma and Musuka, 2014). Early sexual maturation results into overcrowding in ponds which, in turn, causes stunted growth. This has disappointed many fish farmers in Tanzania and led to some farmers and private owned hatcheries to import Nile tilapia fingerlings and broodstocks from Thailand, Uganda, Zambia and Malawi, believing that the imported fish would perform better than the native species (Rukanda, 2018; Shechonge *et al.,* 2018b). Most of these imports are illegal, and put the aquaculture sector in Tanzania at risk from genetic contamination and introduction of diseases (Kajungiro *et al.*, 2019b).

The rapid growth of aquaculture in Tanzania has resulted into high demand of quality fish seeds for the commonly cultured species (Nile tilapia and African catfish) to meet the increasing demand for production of table size fish as well as broodstock for sustainable development of aquaculture industry in the country (Rukanda, 2018). This high demand, in turn, has led to increased investment in seed production by private fish hatcheries resulting into an increase in the number of fish hatcheries producing Nile tilapia and African catfish seeds from 2015 to 2019 (URT, 2019). Tilapia and Catfish fingerlings production from both private and public hatcheries have increased from 3 039 775 in 2017/2018 to 6 221

076 fingerlings in 2018/2019 (URT, 2019) and 21 676 187 fingerlings in 2019/2020 (URT, 2020).

Currently, there are 12 active registered tilapia hatcheries in Tanzania that are either government or privately owned (Rukanda, 2018). These hatcheries produce fingerlings for distribution to the local farmers across the seven agro-ecological zones of the country. However, the number of fingerlings produced in hatcheries is low compared to the prevailing demand of 40 000 000 fingerlings per year (URT, 2019). Because of shortage of fingerlings, some farmers have reported to collect fingerlings from the lakes and rivers. Collection of fingerlings from the wild is unreliable due to possibility of introducing diseases, Seasonality of availability of fingerlings, variability in size, quality and difficult in determining the age of the fingerlings (Hecht, 2006: Charo-Karisa *et al.*, 2012).

Moreover, collection of fingerlings from the wild increases the chance of genetic contamination of farmed tilapia species in the country. This is because some catchments in the country contain more than one species of tilapia, for example Lake Victoria has *O. variabilis, O. niloticus* and *O. leucostictus* and their hybrids (Shechonge *et al.*, 2018b). Therefore, collecting tilapia fingerlings in these catchments and mixing with other fingerlings from others sources increases the chance of losing tilapia biodiversity in fish farms. Furthermore, some fish farmers still obtain fingerlings from recruits in their ponds after harvesting large mature fish. If farmers harvest the large fish and breed from the remaining small fish, it may lead to accidental selection for small size, early maturation and slow growth, leading to poor quality stocks (FAO, 2007b).

Therefore, an assessment of sources and availability of tilapia broodstocks and fingerlings in hatcheries and farms in Tanzania is required in order to estimate the quantity and quality of fingerlings produced and determine the main sources of tilapia broodstocks and fingerlings. This is important, because Tanzania is a hotspot of biodiversity, with more than 20 native species of tilapia (Genner *et al.*, 2018). The present study aimed at determining the sources of tilapia broodstocks and fingerlings in fish farms and hatcheries in Tanzania and document on how they are managed within the industry, with the aim of recommending improved management practices of farmed tilapia.

2.2 Materials and Methods

2.2.1 Ethics statement

This study was carried out in accordance with the law on the protection of animals against cruelty (Act no.12/1974 of the United Republic Tanzania) upon its approval by the Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture (Appendix 5). All the permits required for collecting information in fish farms and hatcheries were sought. Research clearance was obtained from SUA on behalf of Tanzania Commission for Science and Technology (COSTECH) (Appendix 6) and district authorities in the locality where sample collection was done.

2.2.2 Description of the study area

The study was carried out between November, 2018 and April, 2019 in seven agro-ecological zones of Tanzania; namely Eastern (7), Northern (6), Southern (3), Central (2), Southern highland (4), Western (6) and Lake (7) zones; the number of districts is indicated in brackets. The numbers of districts in each zone were selected depending on the availability of fish farms and hatcheries. All surveyed districts are presented in Figure 2.1 and Table 2.1.

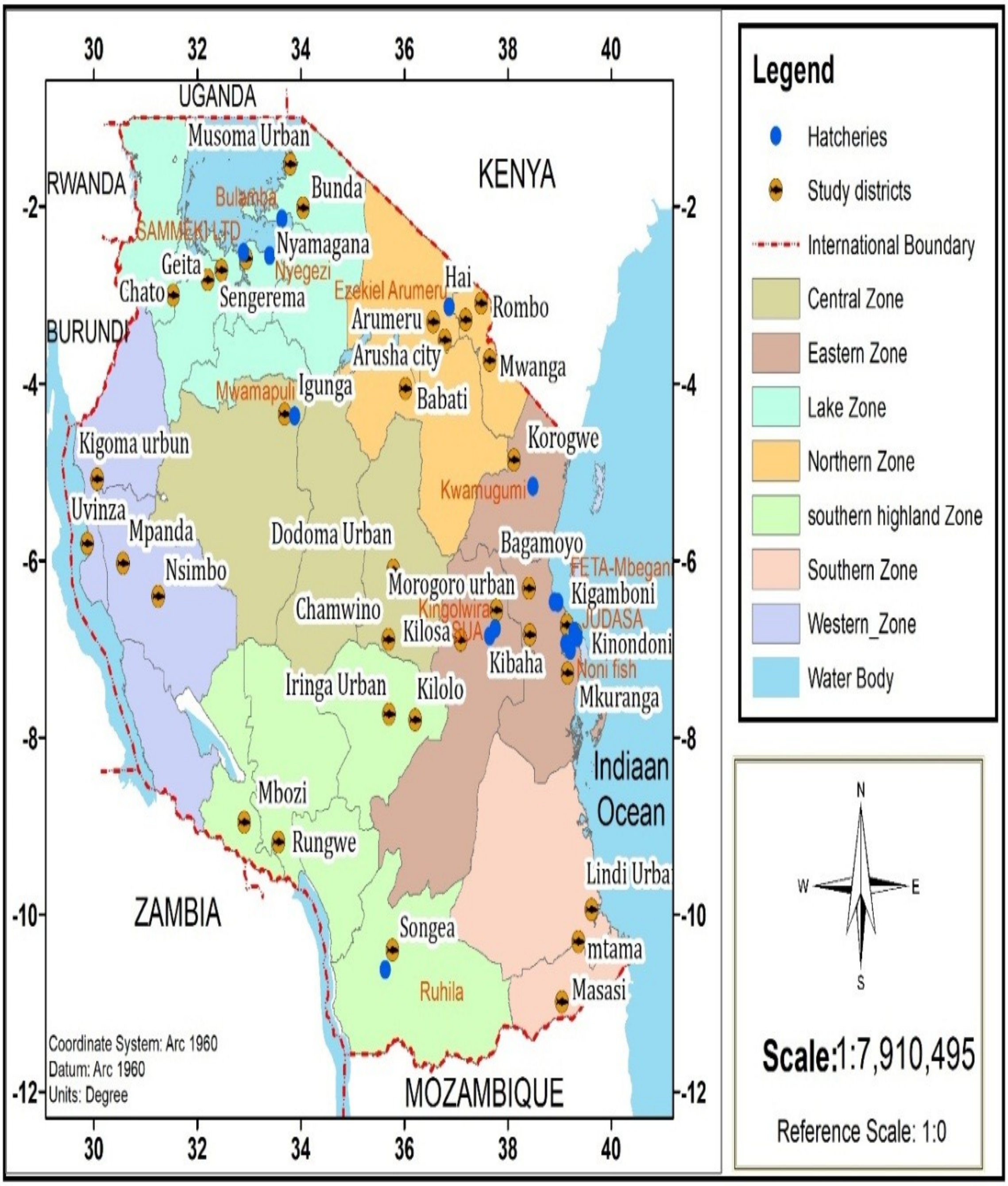

Figure 2.1: Map of Tanzania showing study districts and fish hatcheries in seven agro - ecological zones

Table 2.1: Districts surveyed in seven agro-ecological zones

Agro-ecological zone	Number of districts	Name of districts
Lake zone	7	Bunda, Musoma urban, Geita, Chato, Bukoba, Nyamagana and Sengerema
Northern zone	6	Arusha urban, Arumeru, Rombo, Mwanga, Babati and Hai
Eastern zone	7	Bagamoyo, Kibaha, Mkuranga, Morogoro urban, Kigamboni, Korogwe and Kinondoni
Southern zone	3	Lindi urban, Mtama and Masasi
Southern highland zone	4	Iringa urban, Kilolo, Rungwe and Songea
Central zone	2	Dodoma urban, Chamwino
Western zone	6	Uvinza, Kigoma urban, Mpanda, Igunga Nsimbo and Kasulu

2.2.3 Sampling procedures

In this study a purposive sampling procedure was used to select all seven agro-ecological zones of Tanzania in which fish farming is predominantly practiced. In each zone, two to seven districts were randomly selected, making a total number of 35 districts. Within a district, two to ten farmers were randomly selected from the list of fish farmers (obtained from Livestock and Fisheries department in each district), depending on the number of fish farmers in the district, making a sample size of 248 fish farms from all districts. Likewise, a purposive sampling was used to select all 16 operating freshwater fish hatcheries in the country from both registered and unregistered fish hatcheries which included public (8) and private (8) hatcheries; their numbers in bracket.

2.2.4 Method of data collection

A household survey was conducted in the selected districts (n=35). In each district, face to face interviews with the selected farmers were conducted using structured questionnaires having both closed and open-ended questions. The questionnaire was designed to gather information on households' socio-economic characteristics, number of fish ponds owned, and pond size, sources of tilapia broodstock and fingerlings, also hatchery capacity (Appendix 1). During the survey, heads of the households and managers were the main respondents for fish farms and fish hatcheries, respectively. However, other members of the households were requested to attend the interview so as to provide supplementary information.

2.3 Data Analysis

Data from questionnaires were coded and recorded into the Statistical Package for Social Sciences (SPSS. Version, 21) computer software. The data were analyzed to generate frequencies and percentages. Chi-square analysis was carried out to test if there were significant differences among the agro-ecological zones in terms of sources of fingerlings, and utilization of fish hatcheries as well as wild sources. Pearson correlation coefficient was used to analyze the relationship between quantity of fingerlings produced and the number of fish ponds in hatcheries.

2.4 Results

2.4.1 Fish hatcheries

2.4.1.1 Socio-economic characteristics of the respondents in fish hatcheries

Table 2.2 shows the socio-economic characteristics of the respondents from the fish hatcheries. The results show that 87.5% of the hatchery managers were men and most of the managers (62.5%) had the age ranging from 31 to 40 years, followed by those with the age of 21 to 30 years (25%) (Table 2.2). Most of the interviewed respondents had a bachelor degree in fisheries (68.8%), followed by those who had diploma in fisheries (18.8%). Work experience among the hatchery managers varied greatly; the majority had experience of four to five years, followed by those with two to three years.

Table 2.2: Characteristics of the respondents from the fish hatcheries

Variable	Frequency	Percent of the respondents
Gender		
Male	14	87.5
Female	2	12.5
Age (years)		
21 - 30	4	25
31 - 40	10	62.5
41 - 50	1	6.3
51 - 60	1	6.3
Education		
Primary School	1	6.3
Diploma in fisheries	3	18.8
BSc. in fisheries	11	68.8
Postgraduate studies	1	6.3
Experience (years)		
2 – 3	6	37.5
4 – 5	7	43.8
6 – 10	1	6.3
11 – 15	2	12.5

2.4.1.2 Fish hatchery characteristics and production practices

Fish hatcheries owned different numbers of ponds for broodstock and fingerlings production. It was found that 50% of the fish hatcheries owned 11 to 15 fish ponds (Table 2.3). Most of the respondents (43.8%) reported that the stocking density of tilapia broodstock in their ponds ranged from eight to nine fish per m^2, followed by those having stocking density ranging from four to five tilapia broodstock per m^2 (37.5%). About two thirds (62.5 %) of the hatcheries were producing tilapia fingerlings only while the rest produced both tilapia and African catfish fingerlings (Table 2.3).

Table 2.3: Fish hatchery characteristics and production practices

Variable	Frequency	Percent (%) of the respondents
Number of fish ponds		
1 – 5	1	6.3
6 – 10	5	31.3
11 – 15	8	50.0
16 – 21	2	12.5
Size of Fish Ponds (m^2)		
50 -100	4	25.0
150 - 200	7	43.8
250 – 300	3	18.8
350 – 400	2	12.5
Stocking Density (Broodstock/m^2)		
4 – 5	6	37.5
6 –7	3	18.8
8 – 9	7	43.8
Fish species farmed (Broodstock)		
Tilapia only	10	62.5
Tilapia and African catfish	6	37.5

2.4.1.3 Sources of tilapia broodstock in fish hatcheries

According to hatchery managers, the major sources of broodstocks for hatcheries were other hatcheries and wild sources (Table 2.4). Most of the hatcheries obtained broodstock (45.5%) from other hatcheries within the country. Some hatcheries obtained broodstock from outside the country, specifically Uganda (85.7%) and Thailand (14.3%). The major reasons mentioned by the interviewed managers for importing tilapia broodstock were i) Obtaining broodstock of good quality (67%), and ii) shortage of quality broodstocks within the country (33%). Wild sources of broodstock included rivers, lakes and dams, whereby the predominant wild source was Lake Victoria (Figure 2.2). Most of the respondents (62.5%) reported that they were intentionally mixing broodstock from both hatcheries and wild sources into one fish pond during seed production. The majority of hatchery operators (68.8%) were capable of distinguishing different species of tilapia.

Table 2.4: Sources of tilapia broodstock in fish hatcheries

Sources of tilapia broodstock	Responses	
	Frequency	Percentage
River	1	3.0
Lake	8	24.2
Natural dam	3	9.1
Hatcheries outside Tanzania	4	12.1
Recruits from other farmers	2	6.1
Other fish hatcheries in Tanzania	15	45.5
Total	**33**	**100.0**

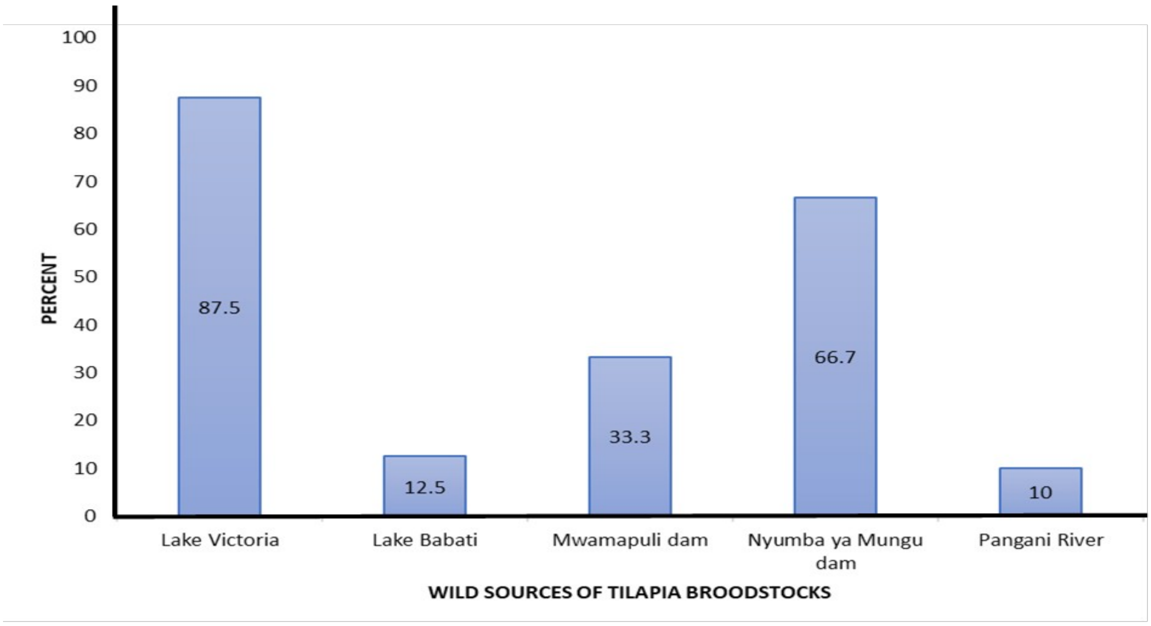

Figure 2.2: Wild sources of tilapia broodstock for fish hatcheries

2.4.2 Production capacities of the surveyed fish hatcheries

The largest tilapia fingerlings producers in 2019/ 2020, number of fingerlings produced in bracket, were FETA-Nyegezi (1 955 242), Ruvu fish farm (1 370 000), FETA- Mbegani (814 158) and Safina big fish farm (795 000) (Table 2.5). There was a significant and positive correlation between the number of fish ponds owned and the number of fingerlings produced per year, with hatcheries that had many ponds reporting higher production levels ($r=0.54$; $p<0.05$).

Table 2.5: Annual production of Tilapia fingerlings in various hatcheries in the year of 2019/2020

Name of fish hatchery	Ownership type	Number of tilapia fingerlings
Ruvu fish farm	Private	137 000
Noni fish farm	Private	80 432
Faiza fish farm	Private	474 343
FETA - Mbegani Campus	Public	814 158
FETA - Nyegezi Campus	Public	1 955 242
Mwamapuli Aquaculture Center	Public	173 034
Sokoine University of Agriculture (SUA)	Public	233 000
Kingolwira - Aquaculture Center	Public	544 566
JUDASA fish farm	Private	376 170
Safina big fish -Kigamboni	Private	795 000
Eden Agri- Aqua Limited	Private	760 000
SAMMEKI Limited	Private	453 216
Ezekieli Mahuna farm	Private	340 050
Ruhila - Aquaculture Center	Public	341 840
821JKT-Bulamba	Public	270 000
Kwamugumi Prison fish farm	Public	190 434

Source: URT (2020)

2.4.3 Fish farms

2.4.3.1 Socio-economic characteristics of the respondents from fish farms

Most of the respondents (83.5%) were males in all the seven agro-ecological zones. The majority of them had the age ranging from 41 to 50 years (34.7%) and a larger proportion of the respondents had attained secondary school level of education (60.5%). Details of the socio-economic characteristics of the fish farmers are shown in Table 2.6.

Table 2.6: Households socio-economic characteristics of the respondents in fish farms

Variable	Frequency	Percent of the respondents
Gender		
Male	207	83.5
Female	41	16.5
Age (years)		
20 – 25	1	4
26 – 30	18	7.3
31 – 40	68	27.4
41 – 50	86	34.7
51 – 60	56	22.6
Above 60	19	7.7
Education		
No formal education	20	8.1
Primary School	78	31.5
Secondary School	150	60.5

2.4.3.2 Fish farms characteristics and management practices

The number of ponds per farm ranged from one to five for most (90.7%) of the interviewed fish farmers in the seven agro-ecological zones of Tanzania (Table 2.7). Most of the ponds (68.7%) had size ranging from 150 to 200 m^2. Smaller ponds with size ranging from 50 to 100 m^2 comprised 23.4% of the total number of ponds visited and these were frequently located near the homesteads. A large proportion of the respondents (80.6 %) were farming only tilapias, whereas the remaining few included the African catfish. The stocking density for most of the farms (54.8%) was between 5 and 8 fish per m^2, followed by those having a stocking density of 1 - 4 fish per m^2 (27%). Also, most (76.61%) of the farms were feeding fish with diets made from locally available ingredients.

Table 2.7: Fish farms characteristics and management practices

Variable	Frequency	Percent of the respondents
Number of Fish Ponds		
1 – 5	225	90.7
6 – 10	22	8.7
11 – 15	1	4
Size of Fish Ponds (m^2)		
50 -100	58	23.4
150 - 200	158	68.7
250 – 300	19	7.7
350 – 400	13	5.2
Stocking Density (Fish/m^2)		
1 – 4	67	27.0
5 – 8	136	54.8
9 – 12	42	16.9
13 – 15	3	1.2
Fish species farmed		
Tilapia only	200	80.6
Tilapia and African catfish	48	19.4
Fish feeds used		
Commercial feed	52	20.97
Farm made (Locally made)	190	76.61
Natural feed in the pond	6	2.42

2.4.3.3 Sources of tilapia fingerlings in fish farms

The sources of tilapia fingerlings for fish farms found in the seven agro-ecological zones are shown in Table 2.8. Fish farms obtained fingerlings from hatcheries and the wild (natural water bodies). In some few incidences, farmers obtained fingerlings from fish ponds of other fish farmers (recruits). Results indicate that the utilization of fish hatcheries and wild sources depended on the location in which the hatcheries and natural water bodies are located in the country. There was a significant difference in the utilization of fish hatcheries and wild sources among the agro-ecological zones (χ^2 =22.87, df = 6, $p < 0.05$). In-country hatcheries serving as sources of fingerlings are listed in Table 2.9. Twelve farmers from Lake zone (7), Eastern (1), Western (2), and Southern highland zone (2) mentioned that they obtain fingerlings from outside the country in the following order; Uganda (58.3%), Zambia (33.3%) and Malawi (8.3%). Specific natural water bodies that were used as wild sources for tilapia fingerlings are indicated in Figure 2.3. Table 2.10 displays the number of fingerlings obtained from fish hatcheries and wild sources. Details on zone specific fingerlings contributions from fish hatcheries and wild sources are shown in Table 2.10. Overall, fish hatcheries contributed more fingerlings (81.92%) across all agro-ecological zones, except in the Western zone, whereby 61.26% of the respondents obtained fingerlings from the wild (natural water bodies). Mixing of tilapia fingerlings from different sources into one fish pond during the same production cycle was evident in all the zones (Table 2.11). A few farmers (36.3%) were able to distinguish the different species of tilapia while the majority of the respondents (63.7%) were not capable to distinguish the different species of tilapia in their farms.

Table 2.8: Utilization of fish hatcheries, natural water bodies and recruits from fish farms as sources of fingerlings

S/N	Agro-ecological zone	Sources of fingerings for fish farms (%)		
		Hatchery source	Wild source	Recruits from fish Farm
1.	Lake zone	33 (43.42%)	35 (46.1%)	8 (10.53%)
2.	Eastern zone	63 (50.81%)	33 (26.61%)	28 (22.58%)
3.	Northern zone	10 (34.48%)	12 (41.37%)	7 (24.14%)
4.	Western zone	10 (28.57%)	15 (42.86%)	10 (28.57%)
5.	Southern highland zone	23 (47.92%)	12 (25%)	13 (27.1%)
6.	Southern zone	16 (33.33%)	17 (35.42%)	15 (31.25%)
7.	Central zone	11 (36.67%)	10 (33.33%)	9 (30%)
Total for each fingerlings source		**166 (42.55 %)**	**134 (34.35 %)**	**90 (23.10%)**

Table 2.9: Fish hatcheries used as sources of tilapia fingerlings

S/N	Name of fish hatchery	Registration status	Agro–ecological zone	Farmers using the hatchery	
				Frequency	Percent
1	Kingolwira Aquaculture Center	Registered	Eastern zone	14	6.6
2	Ruhila Aquaculture Center	Registered	Southern zone	21	9.9
3	Mwamapuli Aquaculture Center	Registered	Central zone	7	3.3
4	Big fish-Kigamboni	Registered	Eastern zone	15	7.1
5	Eden Agri-Aqua Limited	Registered	Eastern zone	35	16.5
	Ruvu fish farm	Registered	Eastern zone	33	15.6
6	Kwamugumi Prison-Korogwe	Registered	Eastern zone	5	2.4
7	821 KJ -Bulamba fish hatchery	Registered	Lake zone	5	2.4
8	SAMMEKI-Limited	Registered	Lake zone	31	14.6
9	FAIZA fish farm	Registered	Eastern zone	7	3.3
10	NONI fish farm	Unregistered	Eastern zone	8	3.8
12	JUDASSA fish farm	Unregistered	Eastern zone	2	0.9
13	Sokoine University of Agriculture	Unregistered	Eastern zone	7	3.3
14	Ezekiel Mahuna fish hatchery	Unregistered	Northern zone	7	3.3
15	FETA - Mbegani	Unregistered	Eastern zone	12	5.7
16	FETA - Nyegezi	Unregistered	Lake zone	3	1.4
	Total			**212**	**100**

***Note*: FETA = Fisheries Education Training Agency**

Table 2.10: The contribution of fish hatcheries and natural water bodies to total production of fingerlings in each agro-ecological zone

Agro-ecological zone	Sources of Tilapia fingerlings				Total
	Fish hatcheries		Natural water bodies		
	Number of fingerlings	Percent	Number of fingerlings	Percent	
Lake zone	89 510	62.43	53 870	37.57	143 380
Northern zone	49 915	66.23	25 450	33.77	75 365
Eastern zone	356 042	92.70	28 040	7.3	384 082
Southern zone	87 800	88.38	11 540	11.62	99 340
Southern Highland zone	95 279	90.81	9 640	9.19	104 919
Central zone	34 400	82.24	7 431	17.76	41 831
Western zone	15 680	38.74	24 790	61.26	40 470
Total	**728 626**	**81.92**	**160 761**	**18.08**	**889 387**

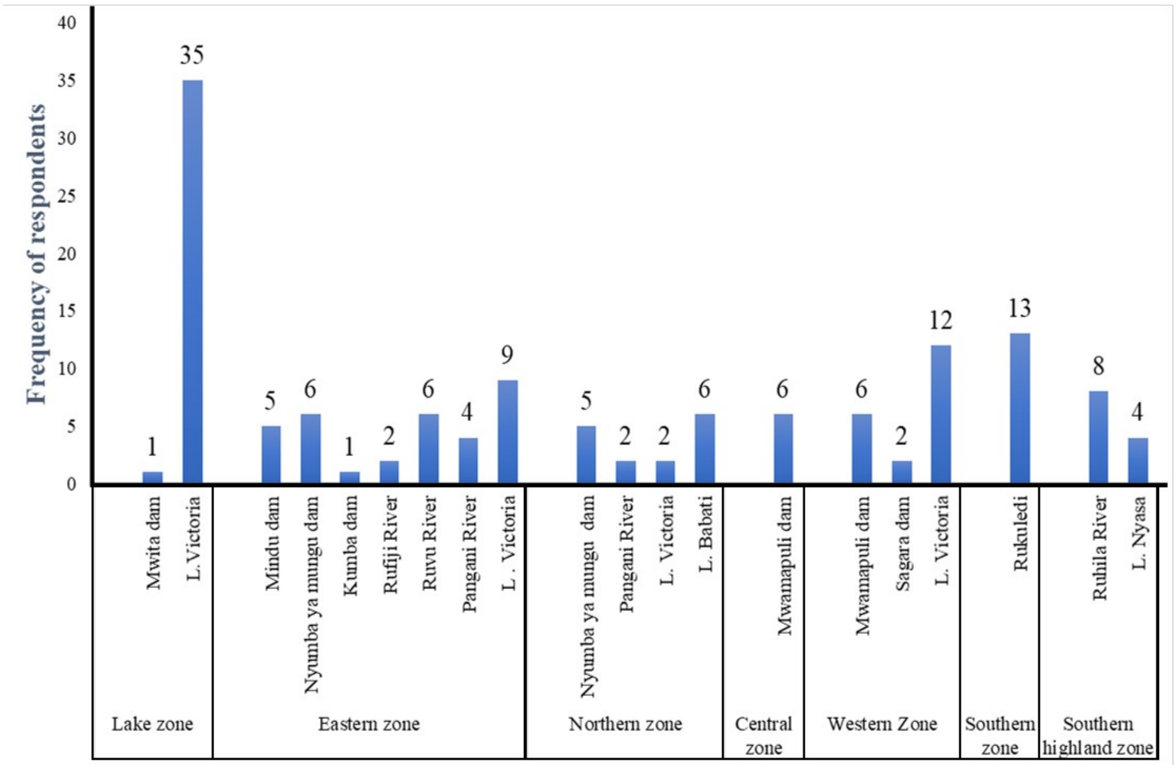

Figure 2.3: Natural water bodies used as wild sources of fingerlings in seven agro-ecological zones of Tanzania mainland

Table 2.11: Percentage of farmers mixing fingerlings from different sources into one fish pond

Agro-ecological zone	Frequency of respondents	
	Number	Percentage
Lake zone	18	42.86
Eastern zone	19	22.35
Northern	10	50
Western zone	5	16.13
Southern highland zone	17	56.67
Southern zone	20	74.07
Central zone	9	69.23
Total	**90**	**36.3**

2.5 Discussion

2.5.1 Socio-economic characteristics of the respondents in fish farms and hatcheries

In this study, most of the respondents were men for both fish hatcheries and farms in all the seven agro-ecological zones of Tanzania. This may be due to the local customs and cultural practices in most of the farming communities in Tanzania. In many areas of the country, women are restricted to own assets and land which are acquired mainly through inheritance which favors men. The results of this study are in agreement with the findings of Chenyambuga *et al.* (2014) who reported that 76.7% of fish ponds in Morogoro are owned by men who are the household heads. Also, similar results have been reported by Benard *et al*. (2018), Mulokozi *et al*. (2020), Mmanda *et al.* (2020), and Mwaijande and Lugendo (2015) who stated that the fish-farming sub-sector in Tanzania is dominated by men. According to FAO (2014), 20% of fish farming operations in Africa are run by women, this compares well with the results of the present study in hatcheries and farms. The high number of respondents with secondary school level of education suggests that educated people are now embarking on fish farming activities. According to URT (2016) the lack of enough skilled personnel for providing extension services and adequate knowledge and skills for farmers to practice aquaculture commercially are the limiting factors for this young sub-sector in Tanzania. But interestingly, high number of graduates with specialization in fisheries were identified in this study, working as managers in some fish hatcheries indicates that fingerlings production is currently done by people with relevant knowledge and skills. This is in line with FAO recommendation (FAO, 2012), that fish hatchery must be managed by personnel having technical know how in aquaculture. This is important because fish farming is an activity which offers opportunities for both employment and poverty reduction among communities. It has been reported that fish production contributes substantially to the income and indirectly to food security of more than 10 % of the world population, essentially in developing and emergent countries (Brummet *et al.*, 2008; FAO, 2014; Béné *et al.*, 2015; Bogard *et al.*, 2017; Kassam and Dorward, 2017).

2.5.2 Fish hatcheries and farms characteristics, management practices and production

Results on fish farming characteristics revealed that most farmers owned one to five fish ponds. A relatively higher number of respondents owning one to five fish ponds compared to those who owned more than six fish ponds might be due to small size of land they owned and low knowledge on the importance of fish farming (Hatchery managers, Personal communication, 2019). This observation is in line with the report of FAO (2012), which states that most of small-scale fish farmers in Tanzania own small ponds of an average size of 150 m^2, covering an estimated area of 221.5 ha. Also, similar results have been reported by Shoko *et al.* (2016) who indicated that the majority of farms in Tanzania have limited number of fish ponds ranging from one to three ponds per farm. Lack of quality fish seed, lack of fish feeds, poor extension services or lack of appropriate fish farming information, and poverty are among the factors for low expansion of fish farming in Africa (Brummet, 2008; FAO, 2013; Nasr-Allah *et al.*, 2014).

This study found that tilapias pre-dominate in both fish hatchery and farms, probably due to the fact that tilapias are traditional and favorite dish in almost all countries of Sub-Saharan Africa (FAO, 2014; Hellerman and Hilsdorf, 2014). Some even call it a "democratic fish" in the sense that the fish is consumed as an affordable source of protein in poor rural communities, and it is a premium product for the affluent in urban centers. The average annual per capita fish consumption in Sub- Saharan Africa is approximately 8.9 kg and it is difficult to quantify demand trends, specifically for tilapia due to scanty data. However, nearly all tilapias produced in Sub-Saharan Africa are locally consumed, with very limited exports to overseas markets (FAO, 2014). Also, according to FAO (2016) fish farming in the United Republic of Tanzania is almost totally dominated by the tilapias.

The results in the present study revealed that fingerlings production capacities of the surveyed fish hatcheries were considerably low compared to the actual demand. This might be due to low investment capacity among hatchery operators (Rukanda, 2018). Also inadequate skills on fingerlings production have been reported in Sub-Saharan Africa by Bhujel (2014), and this could be linked to the low fingerlings production capacities in the hatcheries surveyed in the present study. This observation is in agreement with the report by Shoko *et al.* (2011) who found that fingerlings production is increasing in Zambia and Tanzania, but still low compared to the national demand. According to URT (2020) the current fingerlings production in Tanzania is 21 676 187, which is low compared to the existing demand of 40 million fingerlings. This study found that there is a significant and positive correlation between the number of fish ponds owned and number of fingerlings produced per year. The hatcheries that had more ponds reported higher production levels than hatcheries with few fish ponds, this is an indication of low intensification among hatcheries in the country. Similar results have been reported by Nyonje *et al.* (2018) who stated that the quantity of fingerlings produced in each hatchery correlates positively with the number of facilities in the Kenyan fresh water fish hatcheries. Beside the observed increase in fingerlings production, there is a need of embarking seriously on fingerlings production of farmed tilapia species in the country, in order to remove the existing fingerlings production gap with the aim of promoting fish farming sub-sector in Tanzania.

2.5.3 Sources of tilapia broodstock and fingerlings in hatcheries and farms

The study showed that in Eastern zone more fingerlings are obtained from the hatcheries than from the wild sources. This may be due to the presence of more hatcheries in this zone compared to other zones. Most of the respondents both in fish hatcheries and farms mentioned fingerlings from Eden Agri-Aqua limited, Ruvu fish farm, Sammeki Limited, Ruhila aquaculture development Centre, Safina big fish, Kingolwira aquaculture development center and Sokoine University of Agriculture. These hatcheries are located in the Eastern zone and are the major producers and suppliers of tilapia fingerlings in the Eastern zone and beyond. According to Rukanda (2018), the main sources of fingerlings in the country are Safina big fish, Ruvu fish farm, Kingolwira, Ruhila and Mwamapuli aquaculture development center. These hatcheries have higher fingerlings production capacities and well-established facilities such as egg hatching jars, fry rearing facilities and water quality parameter kits. The presence of Ruhila fish hatchery and a long history of fish farming in Ruvuma region may have contributed to the increased utilization of fish hatcheries in the Southern highlands zone (Rukanda, 2018). According to Kajungiro *et al.* (2019b) some fish farmers depend on the available hatcheries in the country, because it is easy to obtain high number of fingerlings from hatcheries compared to wild sources.

On the other hand, the high contribution of wild sources to fingerlings production in Western zone may be due to lack of well-established hatcheries in this zone. Similar result has been observed by Shoko and Kamugisha (2018) who reported high utilization of natural water bodies as the major source of fingerlings in the Western zone of Tanzania. The lack of hatcheries in this zone necessitates the transportation of fingerlings over long distances from Eastern zone, the situation which may contribute to high mortality of fingerlings among fish farmers in Western zone. The relatively high use of wild sources of fingerlings in the Lake zone may be due to proximity of Lake Victoria which acts as the major source of Nile tilapia broodstock and fingerlings in the country. This is due to the perception that Lake Victoria contains high-quality, fast growing Nile Tilapia. Also lack of nearby hatcheries in some regions in this Lake zone forces farmers to depend on Lake Victoria as the source of fingerlings. During the survey, it was found that there is no operational fish hatchery in Kagera region. This concurs with the observation made by Shoko and Kamugisha (2018) that about 40 percent of farmers in the country source their tilapia fingerlings from the wild sources because of lack of reliable hatcheries.

Apart from Lake Victoria other important wild sources of broodstocks and fingerlings mentioned by respondents from different zones were Mwamapuli dam, Lake Babati, Pangani River systems, Rukuledi River and Ruhila River. According to Opiyo *et al.* (2017) obtaining broodstocks from the wild sources is cheaper compared to fish hatchery sources, especially for small-scale fish farmers in Africa. Wild sources of fingerlings are considered as unsustainable sources of fingerlings for tilapia fish farming development, because poor quality fingerlings result into poor harvests (Shoko *et al.*, 2016).

Some fish farmers were using recruits from fellow fish farmers as the source of fingerlings. This might be due to lack of alternative sources of fingerlings. The Eastern zone, Southern zone and Southern highland zone had the highest number of respondents who were using recruits from other fish farms as source of tilapia fingerlings compared to

Western zone, Lake zone, Northern zone and Central zone. In general, this indicates that some fish farmers in all seven agro - ecological zones of Tanzania obtain fingerlings from fish farms of other farmers or their own production ponds. Similar results have been reported by FAO (2007a), that Tanzania has a number of fish hatcheries, but some fish farmers still obtain fingerlings from recruits in their ponds after harvesting mature fish. Also, Shoko and Kamugisha (2018) reported that about 30 percent of fish farmers in Tanzania collect recruits from other farmers or own ponds. Turner *et al*. (2017) reported that 44% of fish farmers in Zambia obtain fingerlings from fellow farmers. However, in most cases, the fish seeds collected from recruits in fish ponds are stunted and inadequate to meet the prevailing demand of fish seeds in the country (FAO, 2007b). Normally it is not recommended to use recruits as source of fingerlings in tilapia farming because of early sexual maturity of tilapia and prolific breeding that leads to overcrowding and "stunting" where adult fish can sometimes mistakenly be stocked as fingerlings (Shoko *et al.*, 2016).

Generally, in the current study it was found that the contribution of fish hatcheries to fingerlings production is higher compared to wild sources and fellow farmers across all seven agro – ecological zones in the country. This might be due to the perception of farmers that hatcheries are sources of good quality tilapia fingerlings compared to fellow farmers and wild sources. Wild-caught fingerlings may be viewed as inferior, as well as being difficult to source at some times of the year (FAO, 2007a). While sourcing fingerlings from fellow farmers may lead into getting stunted mature fish which are mistaken with juvenile, this practice can lead to accidental selection for genotypes that mature early at small sizes.

Likewise, the study has revealed that some hatchery managers were importing broodstock from Uganda and Thailand, while some fish farmers were importing tilapia fingerlings from Uganda, Zambia and Malawi. This may be due to the fact that, some fish hatchery operators believe that tilapia broodstock from outside the country are of better quality and grow faster than those produced from the fish hatcheries and those obtained from the natural water bodies within the country. The results of this study agree with the report by Rukanda (2018) and Shechonge *et al.* (2018b) who reported that broodstock and fingerling sources in Tanzania are both from the wild and fish hatcheries within and outside the country.

2.5.4 Mixing different tilapia species in fish hatcheries and farms

Wild sources of fingerlings are believed to contain different species of tilapia. The use of fingerlings from wild sources may lead to production of hybrids in production farms. Most of the hatchery managers claimed to be able to distinguish different species of tilapia, although this was not independently verified, for example, by showing them unlabeled photographs of tilapia of known species. If they are able to distinguish species, it might be due to the long experience they had in managing tilapia farms. All managers had two or more years of working experience at their respective hatchery, hence, they had acquired knowledge through daily activities in hatchery operations. Also, the level of education can be considered as the main reason for their ability to distinguish tilapia species. During the survey it was revealed that most fish hatchery managers had formal education such as diploma and bachelor degrees in fisheries or related fields. Moreover; it may be that as experienced qualified professionals, they felt they ought to claim to possess expertise of this nature.

Most of the respondents in fish farms were unable to distinguish different species of tilapia in their respective farms. This could be due to lack of formal education relating to aquaculture as most of them in all agro-ecological zones had primary and secondary school education only. The inability of farmers to distinguish tilapia species has resulted into having a mixture of different species in hatcheries and farms. The use of wild stock and mixed tilapia species in the hatchery may have contributed to genetic contamination and lack of purity of the cultured species. There is evidence of hybridization of some species in the catchments, for example, the cross-breeding between O. *urolepis* and *O. niloticus, O. urolepis* and *O. leucostictus* has been reported in the Mindu and Kidatu dams and in Lake Victoria (Shechonge *et al.*, 2018a).

This study found that most of the hatchery managers were intentionally mixing broodstocks from different sources into one fish pond during the same cycle of seeds production. This was done in order to improve growth performance of the fingerlings which were produced (Hatchery managers, Personal communication, 2019). Moreover, most of the farmers in fish farms were unintentionally mixing tilapia fingerlings from different sources into one fish pond during the same cycle of fish production. There were more farmers in the Southern zone, Eastern zone, Lake zone and Southern highland zone who were mixing tilapia fingerlings from different sources compared to Western zone, Northern zone and Central zone. This may be due to shortage of fingerlings in those zones. The shortage of fingerlings forced most of the fish farmers to collect fingerlings from any source and mix together in one fish pond in order to attain the number of fingerlings required according to the pond size. Moreover, the similarity of different tilapia species at the fingerling stage increased the probability of having mixed stocks in fish ponds because of the difficulties in separating the fish belonging to different species (Charo-Karisa *et al.,* 2009). The rearing of mixed species into one pond may not result into the expected profitable production (Kajungiro *et al.*, 2019a). This observation conforms to the observation made by Shoko *et al.* (2011) who found that fish farmers collect fingerlings from different sources in order to meet their demand for tilapia fingerlings, especially in the Western zone of Tanzania. This finding indicates that, there is high level of intentional and unintentional hybridization of farmed tilapia species in both fish hatcheries and farms in the country. This may be attributed to the fact that Tanzania is a hotspot for tilapia biodiversity in wide range of natural water bodies which are commonly used as sources of broodstock and fingerlings (Genner *et al.,* 2018).

Several studies have shown advantages of intentional hybridization including production of all males, for example, *Oreochromis niloticus* x *Oreochromis aurea* (FAO, 2016); *Oreochromis niloticus* x *Oreochromis urolepis* (Mbiru *et al*., 2016). However, in the long-term, hybridization has been found to have a negative impact on biodiversity, because it can lead to the erosion of unique genetic resource (Todesco *et al.*, 2016). Hybrids have been shown to possess noval traits that enhance their potential to have deleterious impacts on indigenous population (Gaskin and Schaal, 2002; Facon *et al.*, 2005). Furthermore, in freshwaters, genetic or demographic swamping during hybridization is currently considered as the major driver of biodiversity loss, alongside habitat loss and pollution (Scribner *et al*., 2000; Perry *et al.*, 2002). To avoid the negative impact of mixing different species of tilapias from different ecological zones within the country or outside the country aquaculture zonation should be adopted.

2.6 Conclusions

The current study has found that fish hatcheries within and outside Tanzania mainland, natural water bodies and fish farms of neighbor farmers are the main sources of tilapia broodstock and fingerlings for stocking fish farms and hatcheries in the country. The study has shown clearly that tilapia fingerlings production from fish hatcheries is low compared to the prevailing demand in the country. Moreover, the study has found that both intentional and unintentional mixing of tilapia broodstock and fingerlings from different sources into one pond during the same production cycle is a common practice in some of the hatcheries and fish farms in all agro-ecological zones of Tanzania.

2.7 Recommendations

From the results of this study it is recommended to:-

i. Establish certified hatcheries that can produce fingerlings of pure single tilapia species in each agro-ecological zone to increase accessibility of quality seeds.
ii. Establish specific guidelines and perform regular inspection for control of the quality of broodstocks and fingerlings produced from fish hatchery in order to ensure that the fingerlings produced are of high quality.
iii. Restrict or rigorously control importation of both broodstocks and fingerlings in order to avoid mixing of different species in the established hatcheries and farms.
iv. Train fish farmers and hatchery managers on how to handle and manage broodstocks and fingerlings to avoid unintentional mixing of tilapia species from different sources.

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CHAPTER THREE

3.0 MANUSCRIPT II

Species and hybrid identification of farmed tilapia in Tanzania mainland using single nucleotide polymorphism (SNP) DNA markers

Status of the Manuscript: In Preparation

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Abstract

Identification of farmed tilapia species and hybrids in fish farming subsector is essential for both efficient aquaculture production and wild population management. This study was conducted to identify tilapia species and their hybrids in fish farms and hatcheries in Tanzania using single nucleotide polymorphism (SNPs) DNA markers. A total of 336 and 200 tilapia pectroral fins were collected from fish farms and hatcheries, respectively, making a total of 536 fish samples collected from farms and hatcheries, but due to budget limitation only 81 and 109 tilapia samples were selected for genotyping from 10 fish hatcheries and 59 fish farms, respectively. A total of 120 SNPs were selected for genotyping of tilapia species sampled from fish hatcheries and farms. Also reference samples which consisted of six specimens of *Oreochromis niloticus* and *Oreochromis leucostictus* from Lake Albert in Uganda and 10 specimens of *Oreochromis urolepis* from Wami River in Tanzania were genotyped. The tribe *Coptodon* was identified using Pseudo-reference from collected samples because the SNP chip was originally calibrated with three species of *Oreochromis* (*O. niloticus, O. urolepis and O. leucostictus*), and not with *Coptodon*. Therefore, a pseudo-reference set for *Coptodon* was generated, using four specimens for which clear photographs were available. A threshold of 80% cluster membership was used to classify samples as pure species. Individuals with cluster membership less than 80% were considered to be potential hybrids. The results indicated that more than half (70%) of the fish hatcheries were not stocking pure *O. niloticus*, but hybrids or other tilapia species. This was found in seven out of the ten hatcheries surveyed. The pure *O. niloticus* (percent of pure individuals in brackets) were observed at Ruvu fish farm (12.90%), Safina bigfish (12.90%) and 821KJ-Bulamba (51.61%). Admixture of tilapia species was detected at Faiza fish farm, Ruhila, Kingolwira, Sammeki farm Ltd, Eden IAGRI-AQUA-Ltd and Kwamngumi Prison. In addition, a unique genetic cluster which didn't cluster to any of the reference *Oreochromis* species was identified at Mwamapuli. Admixture of species was detected in most of the farms in all agro-ecological zones, with high number of hybrids being between *O. niloticus* and unknown tilapia species which formed unique genetic clusters that did not cluster to any of the reference *Oreochromis* species. It is concluded that most of the sampled fish farms and hatcheries do not culture pure *O. niloticus* instead they stock a mixture of *O. niloticus, O. leucostictus, O. urolepsis, Coptodon*, unknown tilapia species and high number of hybrids of tilapia species. Also, most of the fish farms in Rungwe district culture *Coptodons* instead of Nile tilapia. The study recommends to establish more hatcheries that can produce more fingerlings of a single pure species and fish farmers should farm a single pure tilapia species rather than mixed tilapia species. Tilapa fish farmers are adviced to obtain fingerlings from hatcheries which have pure single species such as Ruvu fish farm, Safina bigfish and 821KJ-Bulamba.

Keywords: aquaculture, broodstock, fingerlings, genetic markers, hybridization

3.1 Introduction

Aquaculture is a critically important sector which contributes substantially to food security and income generation (Rothuis *et al*., 2014; FAO, 2018). Since the late 1980s, capture fishery production has not increased in terms of annual output, while aquaculture production continues to increase and its contribution to total global fish production reached 47% in 2016 (FAO, 2018). Aquaculture in Tanzania is mainly practiced at a small-scale in earthen ponds (Shoko *et al*., 2011), mainly under extensive and semi-intensive farming systems (Chenyambuga *et al*., 2014).

Tilapia cichlids are the main species cultured in Tanzania. Tilapia is an umbrella term including several taxonomic tribes of fish important to aquaculture. The most species rich tilapia tribe in Tanzania is the *Oreochromini*, which has at least 20 species. The other tilapia tribes include *Coptodini* represented by two species (*Coptodon rendalli* and *Coptodon zillii*), and *Tilapiini* represented by one species (*Tilapia sparrmanii*) (Genner *et al*., 2018; Shechonge *et al*., 2018b). Native tilapia species may harbor important genes for adaptation, growth, disease resistance, temperature and salt tolerance. These genes are likely to be useful for aquaculture production (Genner *et al.,* 2018). Maintaining genetic diversity is important to enable populations to adapt in the face of fluctuating environmental conditions (Markert *et al.,* 2010).

Currently there are 12 active tilapia hatcheries in Tanzania which produce fingerlings for distribution to local farmers. These hatcheries are either owned by the government or private companies. The hatcheries have minimal biosecurity precautions in place to prevent fish entering or escaping from the ponds, this poses risk for disease transmission and genetic contamination (Kajungiro *et al.,* 2019a). Furthermore, the capacities of the hatcheries are insufficient to meet the increasing demand of fingerlings due to low level of investment and limited electric power supply (Rukanda, 2018). The estimated country demand is 40 000 000 fingerlings, but the current supply is 21 173 226 fingerlings per year (URT, 2019). To counterbalance the huge demand of tilapia fingerlings in the country, some fish farmers collect both broodstock and fingerlings from natural water bodies such as lakes, rivers and natural dams for stocking in their farms (Shoko and Kamugisha, 2018; Shabani *et al*., 2020 unpublished data).

Therefore, there is a high possibility of finding a wide range of tilapia species – native, introduced, or their hybrids in tilapia hatcheries and farms in Tanzania, due to the practice of collecting tilapia fingerlings from different water bodies containing different species and incorrect identification (Shechonge *et al.,* 2018a). Uncontrolled breeding in ponds under farm settings may result in hybridization of tilapia species, some of which may have low growth potential and mature at small size. This may result in tilapia hybrids with early sexual maturation and stunted growth and hence, low productivity. The situation has disappointed many fish farmers in Tanzania, and some farmers, government and private owned hatcheries have resorted to importation of Nile tilapia fingerlings and broodstock from other countries, such as Kenya, Uganda, Zambia and Thailand, believing that they would perform better than the native strains (Rukanda, 2018; Shechonge *et al.*, 2018a; Mbiru *et al.,* 2020 and Shabani *et al.,* 2020 unpublished data). Most of these imports are illegal and put the country at a risk of genetic pollution and introduction of disease.

Furthermore, the genetically improved Nile tilapia strains with high growth and yields have been optimized for intensive farming systems, with high quality feed, cohort breeding systems to minimize inbreeding, and curation of broodstock to prevent selection for maturity at small sizes. These strains have not been tested in low input extensive small-scale pond systems, and may not perform better than the native species under these settings. Moreover, importation of broodstock from outside the country may lead to population admixture between the native populations and the imported strains, consequently diluting the uniqueness of the native populations. Many tilapia species are able to interbreed and produce fertile hybrids (Shechonge *et al.,* 2018b). In a long run, it may result in extinction of some native species through hybridization (Rhymer and Simberloff, 1996).

Intentional stocking into natural dams and tilapia escapees have been reported to contaminate the wild population. The escapees from farms enter into wild either through the normal activities of changing nets, water flushing or harvesting. A good example is the wide spread of non-native *O. niloticus* that has dominated and threatened the existence of indigenous tilapia species in many places (Champneys *et al*., 2020). The non-native species typically originated from intentional stocking events or escapees from commercial aquaculture farms (Canonico *et al.,* 2005). The introduction of non-native species into wild water bodies and subsequent interbreeding with native species results into loss of uniqueness of the native tilapia species.

Many closely related tilapia species are difficult to phenotypically identify, so broodstock in fish hatcheries could be incorrectly identified, leading to having mixed stocks with different species and hybrids. This increases the likelihood of having stunted and poorly performing offspring. Genotyping with molecular markers is a more reliable method for species identification for aquaculture. Single nucleotide polymorphism (SNP) markers are a good candidate for genetic analysis of tilapia species and their hybrid identification because of their abundance, high polymorphism and reproducibility. Recently it has been shown that a panel of 96 targeted SNPs can reliably distinguish several native *Oreochromis* species in Tanzania (Ciezarek *et al*., 2021). SNP markers have been used successfully for identification of broodstock, and strains used in aquaculture, as well as identifying candidate genes for traits and quantitative trait loci (QTL) useful in aquaculture (Oyarzún *et al.*, 2013; Yáñez *et al.*, 2015). Molecular markers have been used to study the population structure and genetic diversity of Nile tilapia strains cultured in fish farms in Tanzania (Kajungiro *et al.,* 2019b) and characterization of the genetic structure of introduced tilapia strains has been done using double digest RAD sequencing (Mbiru *et al.,* 2020). However, currently there is no information on the purity and hybridization status of tilapia in Tanzanian fish farms and hatcheries. The current study intended to identify tilapia species and their hybrids in both hatcheries and farms in the country by using SNPs. Determining the tilapia species and hybridization status of cultured tilapia species in Tanzania mainland is important for ensuring broodstocks purity in farms and hatcheries as well as establishing broodstocks of known genetic background. This will be helpful in the establishment of sustainable and well-maintained breeding program for aquaculture improvement in the country.

3.2 Materials and Methods

3.2.1 Ethics statement

This study was carried out in accordance with the law on the protection of animals against cruelty (Act no.12/1974 of the United Republic Tanzania) upon its approval by the Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture (Appendix 5). All the permits required to sample farmed tilapia species were obtained and the sampling protocol adhered. Research clearance was obtained from SUA on behalf of Tanzania Commission for Science and Technology (COSTECH) (Appendix 6) and district authorities in the locality where sample collection was done.

3.2.2 Fish sample collection and preparation

The study was carried out between October 2019 and April 2020. A total of 336 and 200 tilapia pectroral fins were collected from fish farms and hatcheries respectively, making a total of 536 fish samples collected from farms and hatcheries. But due to budget limitation only 109 tilapia samples were selected for genotyping from 59 fish farms located in seven agro-ecological zones of Tanzania; namely Eastern, Northern, Southern, Central, Southern highland, Western and Lake zones (Appendix 3). In addition, due to budget limitation only 81 pectoral fins samples were selected for genotyping out of 200 collected samples from ten fish hatcheries (five Public and five Private) found in different parts of the country (Figure 3.1).

Both farms and hatcheries were selected purposively based on the criterion that the farm or hatchery cultures tilapia. Following euthanasia, clips of the pectoral fins approximately 1.5 cm long were obtained from each fish and placed into a labelled vial containing 95% ethanol and the vials were put in a cool box packed with ice. The fish fin samples were transported to a laboratory at Sokoine University of Agriculture within 24 – 72 hours, and kept at 4°C. A photograph of each fish sampled was taken then uploaded into the TilapiaMap app for species identification. Photographs of sampled tilapia fish in the tilapia map app were identified based on morphological characteristics using tilapia fish species identification guide by Trewavas (1983) and Genner *et al.* (2018). In particular, *O. niloticus* were distinguished from other tilapia species by having large deep-bodied size with relatively small heads and presence of regular vertical stripes throughout the depth of caudal fin. *O. urolepis* were identified by having grey head, brownish-golden upper parts, or sometimes with pinkish unpaired fins. Males tend to have uniform black body and fins, with reddish-pink dorsal and tail fin margins. Mature males develop enlarged jaws and a concave head profile. Females and immatures vary from light grey to dark brownish background, with dark flank patches and an anal fin with faint vertical bars. *Oreochromis jipe* were identified with a slender body and small head and mouth. Males and females are characterized by rows of blotched scales across the flanks. Males have a pale blue head with dark spots, dark fins with pale spots, and orange margins to the dorsal and caudal. *Oreochromis leucostictus* were identified with a relatively deep, flattened body and small head and jaws. Males are black with white spots on the flanks and fins. Females are more olive colored, with pale ventral regions, faint vertical barring and dark anal and tail fins. *Coptodon* species were identified with a steep head profile, narrow head and small mouth. Often appearing brownish with a white belly, some individuals (both adults and juveniles) have bright red bellies.

Photographic records were available for half of the specimens. A total of 190 specimens were selected for genotyping (81 of which had labelled photographs). The samples covered all hatcheries and farms in all locations and a few reference samples from natural water bodies. As the aim was to estimate the genetic purity of farmed tilapia species, using photograph records, individuals that appeared phenotypically to be pure specimens of *O. niloticus* were selected. The SNP assay included reference samples consisting of six specimens each of *O. niloticus* and *O. leucostictus* from Lake Albert in Uganda and 10 specimens of *O. urolepis* from Wami River in Tanzania.

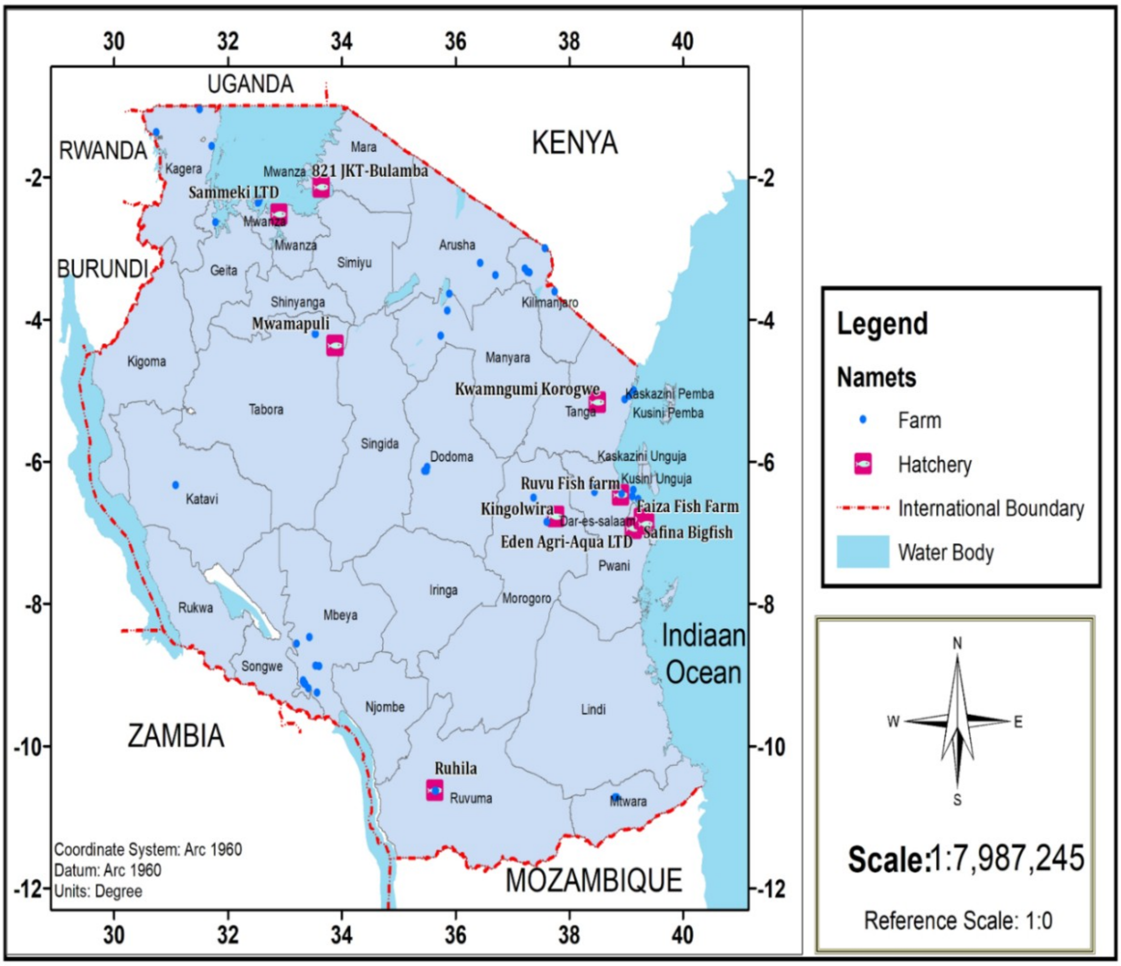

Figure 3.1: Map of Tanzania showing fish hatcheries and farms from which samples were collected

3.2.3 Development of SNP panel for hybrid identification

The SNP panel was developed based on an existing panel optimised for distinguishing *Oreochromis* species and hybrids (Ciezarek *et al.*, 2021), but was modified as some of the SNPs did not perform well in quality control testing for Illumina sequencing (having previously only been used for Agena genotyping). In this case, SNPs for which high specificity oligo probes could not be designed in silico were replaced by selecting the nearest available SNP by base pair position with the same F_{ST} scoring. The original panel and replacement SNPs were then tested in a final panel of 202 SNPs, with 120 SNPs then selected for genotyping (Appendix 2).

3.3.4 Sample processing and genotyping

DNA extraction from fish fin clip was done using the BioArk extraction kit, following manufacturer's instructions. Genotyping was performed by LGC Genomics GmbH (https://www.lgcgroup.com/about-us/locations/). Genotyping used next generation sequencing based SeqSNP genotyping (NextSeq 500/550 v2), 1 x 75 base pair read length, at a depth of 200 X. Initial data filtering and quality control was performed by the LGC Genomics GmbH in Berlin. Demultiplexing of libraries was performed using bcl2fastq v2.20 software (Illumina). Sequence reads were clipped for adapter remnants and quality trimmed at the 3'-end to get a minimum average Phred quality score of 30 over a window of ten bases. Reads with Ns and those with a final length of less than 65 bases after quality trimming were discarded. The quality trimmed reads were aligned against the *O. niloticus* reference genome NCBI Orenil1.1 genome version GCA_000188235.2 (Brawand *et al*., 2014) using Bowtie2 v2.2.3 Software (Langmead and Salzberg, 2012). Genotyping at the target SNP sites was then performed using Freebayes v1.2.0 software (Garrison and Marth, 2012) with ploidy set to two and genotypes filtered for a minimum coverage of eight reads.

3.3.5 SNP genotyping

The NextSeq sequencing generated a total of 10 081 178 reads, with a mean of 53 058 reads per sample. A total of 8 064 943 (80%) of the reads were used in the downstream genotyping, with each target SNP having an average effective coverage of 357x. Of the 120 target SNPs, 106 passed quality threshold having greater than 85% of samples covered by a minimum of 8 reads. A total of 17 samples had a genotyping rate of less than 85%, these samples were subsequently identified as potential *Coptodon* individuals (Table 1) and removed from further analysis. When the genotyped dataset was combined with reference samples from the previously published genotype array (Ciezarek *et al.*, 2021), a total of 73 SNPs were overlapping, giving a dataset of 195 samples (173 in the present study that passed quality control, and 22 reference samples of *Oreochromis* species (*O. niloticus, O. urolepis and O. leucostictus*). These 73 SNPs were used in further analysis.

3.3.6 Development of a pseudo-reference set for *Coptodon* sampling and selection

The SNP assay was originally calibrated with three species of *Oreochromis* (*O. niloticus, O. urolepis and O. leucostictus*), but not with *Coptodon*. Therefore, a pseudo-reference set was generated for *Coptodon*, using four specimens for which clear photographs were available (Figure 3.2). These were then coded as reference samples in the initial Principal Components Analysis and used to identify the genotypic profile of *Coptodon* in the analysis, in an attempt to identify *Coptodon* specimens among the non-photographed samples. Pseudo-reference specimens of *Coptodon* **(**Figure 3.2**)** were collected from Ruhila Aquaculture Development hatchery at Songea (-10.622, 35.635) on 20 November 2020 (Top row-LH3), Kwamngumi Prison hatchery, Korogwe, Tanga (-5.162, 38.489) on 13 December 2019 (bottom left-Korogwe 7), Lake Kumba, Korogwe, Tanga (-4.806, 38.622) on 13 December 2019 (bottom right, Kumba-4) Figure 3.2. The probable

Coptodon specimens were identified in the samples and then removed from downstream analysis (Table 3.1).

Figure 3.2: **Pseudo-reference specimens for *Coptodon* identification**

3.3.7 Tilapia species and hybrid identification

Principal Component Analysis (PCA) in SNPRelate package in R v4.01 software was used to preliminarily identify the species and hybrids present in the Tanzanian tilapia samples. Also, ancestry analysis using ADMIXTURE v1.3.0 software was conducted (Alexander *et al.,* 2009) to determine the proportion of ancestral admixture for assigning appropriately the different types of species and their hybrid present in both fish hatcheries and farms to their likely species of origin. A threshold of 80% cluster membership was used to classify samples as pure species. Individuals with cluster membership of less than 80% were considered to be potential hybrids.

3.3.8 Population Genetic structure analyses in fish hatcheries and farms

The population structure of the fish samples was analyzed using STRUCTURE v.2.3.4 Software (Pritchard *et al*., 2000; Falush *et al.*, 2003; Hubisz *et al.*, 2009) with K values ranging from 2 to 5. Markov chain Monte Carlo (MCMC) of 100 000 iterations with a burn-in period of 10 000 was carried out for each K value. For each tested K value, three independent MCMC samplings were performed. The obtained posterior probability values (Pritchard *et al.*, 2000) were used to determine the optimal number of clusters. Structure results were interpreted using Structure Harvester (Earl, 2012) and CLUMPAK (Kopelman *et al*., 2015) for identifying the most probable number of clusters.

3.4 Results

3.4.1 Identification of *Coptodon* species from collected samples in hatcheries and farms

Clustering of samples was examined using a preliminary Principal Component Analysis of all 190 collected samples. Initial screening indicated that all four pseudo-reference specimens of *Coptodon* could be distinguished on the basis of the high positive scores on PC3, >0.20 (Figure 3.3). Non-photographed *Oreochromis* were identified with PC3 score above +0.073, while the reference samples of *O. niloticus, O. leucostictus* and *O. urolepis* all had PC3 scores of -0.036 to -0.125. An additional 13 specimens of the test samples had PC3 scores of +0.15 or more (Figure 3.3). All samples from these species also had slightly negative PC1 scores (-0.03 to -0.06) compared to high positive scores observed for the reference samples of *O. niloticus* (>+0.03) and high negative scores for *O. urolepis* (< -0.18). Also, they had PC2 scores around zero (-0.007 to +0.007), compared to high positive scores for the reference *O. leucostictus* (>0.18). All 17 specimens with PC3 score of +0.15 or greater did not belong to any of the reference *Oreochromis* species, and were likely to belong to *Coptodons*. These were then excluded from further analyses.

In addition to the pseudo-reference samples from the hatcheries at Songea and Korogwe, these specimens included samples from the wild in Lakes Kumba and Nyumba ya Mungu (Pangani system), as well as the Mara River, an afferent of Lake Victoria; along with 11 specimens from different farms in the South West of Tanzania, mainly in Mbeya District. PCA3 identified *Coptodon* samples, whereby all samples with PC3 score > 0.1 were identified as *Coptodon* individuals. Using this criterion, 17 individuals were identified as *Coptodon* (Table 3.1 and Figure 3.3) and removed from further analysis. All *Coptodon* had a PC3 score of +0.15 or greater, whereas samples with PC3 scores lower than +0.10 were believed to be *Oreochromis*.

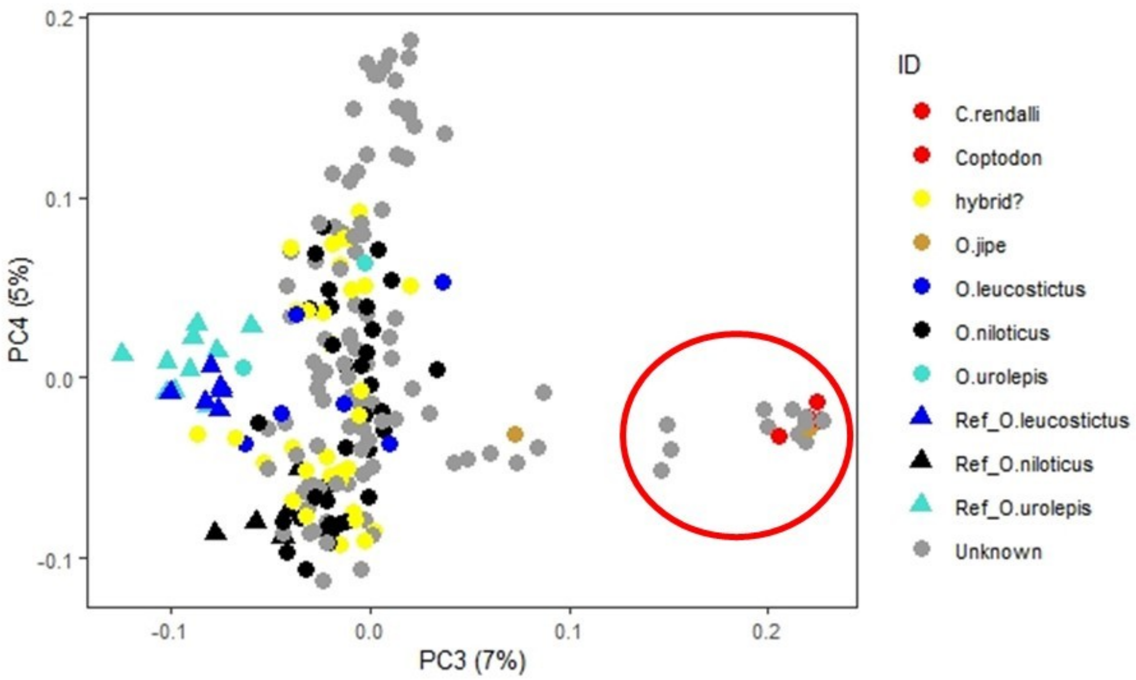

Figure 3.3: Principal components analysis (PCA) for 190 samples from hatcheries and farms (before removing all *Coptodons*) with reference *Coptodon* and provisionally identified *Coptodon* (in red circle).

3.4.2 Distribution of identified *Coptodon* individuals

Identified *Coptodon* individuals were collected from different parts of the country, most of the individuals were from different farms in Rungwe district and two individuals collected from Korogwe district in Tanga region and one sample identified at Ruhila-Songea district in Ruvuma region. Also one *Coptodon* individual was identified at Mara River in Mara region (Table 3.1).

Table 3.1: **Numbers of fish samples that were identified as *Coptodon* using PCA based on pseudo-reference samples**

S/N	Fish farm	Number of samples
1	Ushirika - Rungwe	3
2	Ndubi – Rungwe	3
3	Korogwe – Tanga	2
4	Songea – Ruvuma	2
5	Bujinga – Rungwe	2
6	Mwanga - Kilimanjaro	1
7	Bujera – Rungwe	1
8	Segera – Rungwe	1
9	Lubigi – Rungwe	1
10	Mara river - Mara	1
Total		**17**

3.4.3 Identification of pure tilapia species in fish farms using principal component analysis after removing identified *Coptodons*

After an initial screening of 17 *Coptodons* from data set of 190 sampled individuals, followed by removing 79 individuals from hatcheries (since 2 individuals were identified as *Coptodons* in hatcheries), leaving 94 tilapia samples from fish farms, which were then analyzed using Principal component analysis. Based on this analysis *Oreochromis niloticus* was best resolved using PC1. Reference samples of *O. niloticus* and other test samples had PC1 score of - 0.025 or less. The reference samples for *O. leucostictus* had scores of between +0.125 and < +0.175, while the reference samples for *O. urolepis* and one sample from Mbeya had scores of +0.175 or greater. It seems that the specimens with PC1 scores of - 0.025 or less were pure or nearly pure *O. niloticus* (Figure 3.4). Therefore, all *O. niloticus* shared a PC1 score of - 0.025 or less, whereas samples with PC1 scores ranging between -0.025 and +0.1 were believed to be hybrids of tilapia species, while specimens with PC1 score greater than +0.175 were believed to be *O. urolepis*.

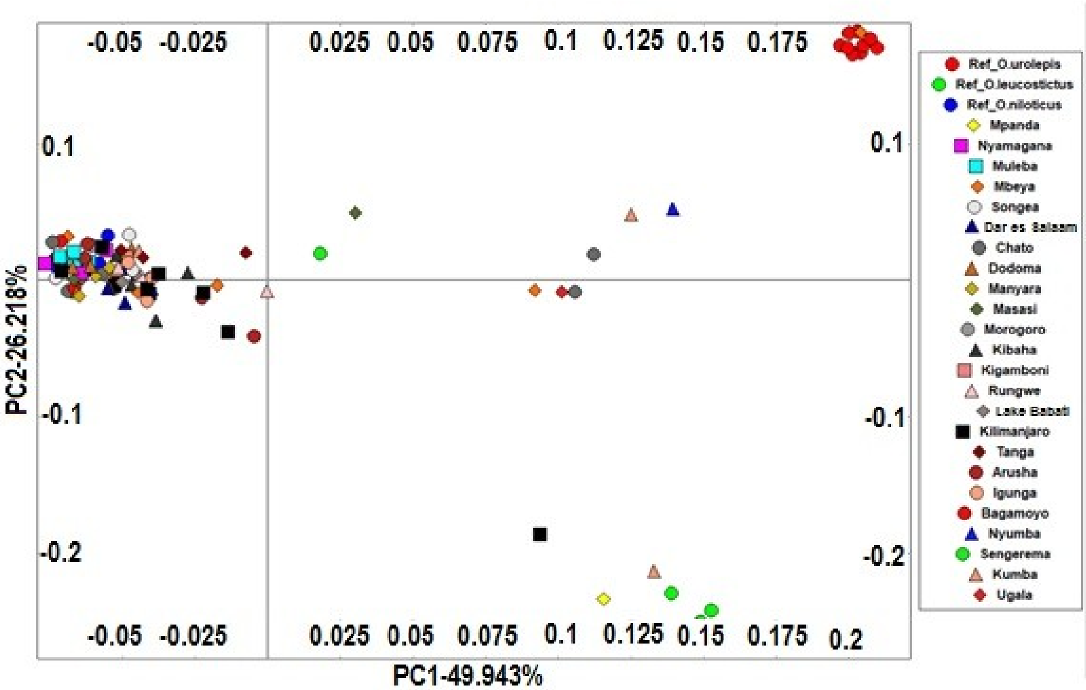

Figure 3.4: Principal component analysis (PCA) for 94 tilapia samples from farms (after removing 17 *Coptodons* and 79 samples from hatcheries) with three reference of *Orechromis* species (*O. niloticus, O. leucostictus and O. urolepsi*).

3.4.4 Tilapia species identification in fish farms using admixture proportions

Admixture proportions using a threshold of 80% cluster membership was used to classify samples as pure species. Individuals with cluster membership of less than 80% were considered to be potential hybrids. Based on this analysis, it was estimated that more than half of the sampled fish farms were culturing hybrids of tilapia species (Appendix 4). Only pure *O. niloticus* (indicated in brackets) were observed at J&B-Muleba–Kagera (2), Nyegezi–Nyamagana (1), Mitawa–Songea (2), SUA–Morogoro (2) and Kasagara-Chato (2) (Appendix 4). A mixture of tilapia species were observed at Buzirayombo-Chato, Shamwengo-Mbeya, Bujinga–Rwungwe, Miswe-Kibaha, Pongwe-Tanga, Mshikamano-

Masasi, Babati (J)-Manyara, Hai (MFF)-Kilimanjaro, Hai (MF)-Kilimanjaro, Karanga – Moshi, Meru–Arusha, Lake Babati, Kumba dam. Only two specimen of *Oreochromis leucostictus* and one specimen of *Oreochromis jipe* were identified at Mpanda-Katavi and Nyumba ya Mungu farm, respectively (Appendix 4).

3.4.5 Tilapia species and hybrids cultured in fish farms

Principal component analysis (PCA) and Admixture were used to classify the tilapia samples collected from farms in different agro-ecological zones in Tanzania. The first and second principal components identified three species based on the three reference species (*O. niloticus, O. urolepis* and *O. leucostictus*). Individuals that were not clearly identified by PCA, were clearly identified by admixture proportions whereby individuals were assigned to pure or hybrid status using the cluster membership likelihood values from admixture, with greater than 80% membership assignment indicating pure species, while those with less than 80% indicating hybrid. Based on admixture proportions values, four tilapia species (i.e; *O. niloticus, O. urolepis, O. leucostictus* and *Coptodon* species), together with hybrids of other species were identified in the collected tilapia samples. It was found that 5 (8.48%) out of 59 fish farms were culturing pure *O. niloticus* while most (91.52%) of the sampled fish farms were not culturing pure *O. niloticus,* instead they stocked either other species of tilapia, hybrids of different tilapia species or a mixture of *O. niloticus* and hybrids of other tilapia species (Table 3.3). Likewise it was found that 50.86% of sampled fish farms were culturing only hybrids of different tilapia species (Table 3.3).

Table 3.2: **Number of fish farms culturing the different tilapia species and hybrids in Tanzania mainland**

Tilapia species/Hybrid	**Number of fish farms**	**Percentage of fish farms**
O. niloticus	5	8.48
O. niloticus+ Hybrids	9	15.25
O. niloticus+ O. urolepis	1	1.69
O. jipe	1	1.69
O. leucostictus	1	1.69
O. leucostictus +Hybrids	2	3.39
Coptodon species	5	8.48
Coptodon +Hybrids	2	3.39
Hybrids	30	50.86
Hybrids + Unknown	2	3.39
Unknown	1	1.69
Total	**59**	**100**

3.4.7 Population genetic structure of tilapia species in farms

Population structure analysis suggested that K = 4 was the most probable number of separating clusters for the studied tilapia species. Admixture results indicate that individuals from Mpanda were *O. leucostictus* as they clustered together with the reference *O. leucostictus*. For Dodoma, Kigamboni, Kilimanjaro, Arusha, Rungwe, Mbeya, Lake Babati, Kumba and Ugala river the samples were not pure *O. niloticus*, instead they were hybrids while few individuals of pure *O. niloticus* were observed in Nyamagana, Songea, Chato, Manyara, Morogoro, Masasi, Kilimanjaro, Tanga and Bagamoyo (Figure 3.5). Furthermore, high numbers of hybrids between *O. niloticus* (blue in color) and unknown tilapia species (red in color) were observed in most of the sampled fish farms (Figure 3.5). This unique genetic clusters (red in color) did not cluster with any of the reference *Oreochromis* species, but appeared in most of the fish farms (Figure 3.5), suggesting that it could be another native *Oreochromis* species which was not included in the reference samples.

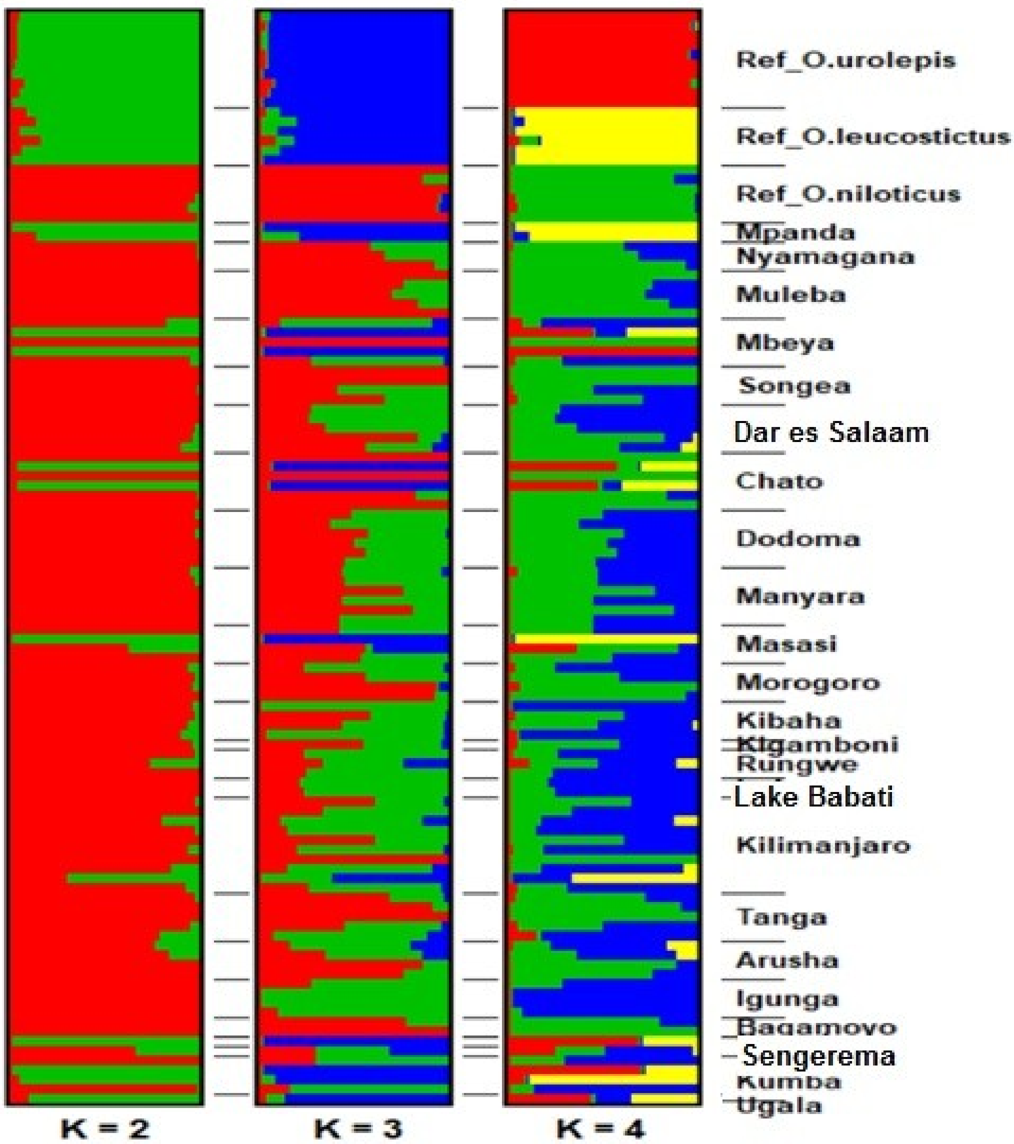

Figure 3.5: Structure analysis bar plots for K=2, 3 and 4 (admixture model) showing population structure of different tilapia species in sampled fish farms located in different part of Tanzania. Note: Each horizontal stripe represents an individual. Each color represents the proportion of membership with regard to each assigned to different genetic clusters. Same color in different individual fish indicates that they belong to the same cluster.

3.4.8 Identified tilapia species and hybrids in fish hatcheries

Based on this analysis, it was estimated that more than half of the hatcheries were not stocking pure *O. niloticus*. Hybrids of tilapia species were found in seven out of ten hatcheries surveyed (Table 3.4). Only few pure *O. niloticus* (indicated in brackets) were observed at Ruvu fish farm (12.90%), Safina bigfish (12.90%) and 821KJ-Bulamba (51.61%) (Table 3.4). A mixture of tilapia species were observed at Ruhila, Mwamapuli, Kwamngumi Prison, Eden Agri-Aqua Limited, Faiza fish farm and only hybrids individuals were identified at SAMMEKI Limited (Table 3.4).

Table 3.3: **Number of pure and hybrid individuals for each tilapia species identified in hatcheries**

Fish hatchery	*O. niloticus*	*O. leucostics*	Hybrid	Coptodon	Unknown	Total number of fish Sampled
Ruhila	-	-	4(10.81%)	2 (100%)	-	6
Mwamapuli	3 (9.68%)	-	18(48.65%)	-	9 (90%)	30
Kingolwira	-	-	3 (8.11%)	-	1 (10%)	4
821KJ	16 (51.61%)	-	-	-	-	16
Bulamba						
Kwamngumi Prison	-	-	3 (8.11%)	-	-	3
EdenAgri-Aqua Limited	1 (3.23%)	1 (100%)	4 (10.81%)	-	-	6
SAMMEKI Limited	-	-	4 (10.81%)	-	-	4
Ruvu fish farm	4 (12.90%)	-	-	-	-	4
Faiza fish farm	3 (9.68%)	-	1(2.70%)	-	-	4
Safina bigfish	4 (12.90%)	-	-	-	-	4
Total	**31 (100%)**	**1 (100%)**	**37(100%)**	**2 (100%)**	**10 (100%)**	**81**

3.4.9 Tilapia fish species and hybrids in the sampled fish hatcheries

It was found that 3 (30%) out of 10 fish hatcheries were culturing pure *O. niloticus* while most (70%) of the sampled hatcheries were not culturing pure *O. niloticus*, instead they were culturing either other species of tilapia, hybrids of different tilapia species or a mixture of *O. niloticus* and hybrids of other tilapia species (Table 3.5). Likewise it was found that 20% of sampled fish hatcheries were culturing a mixture of *O. niloticus* and hybrids of different tilapia species (Table 3.5).

Table 3.4: **Tilapia fish species identified in ten hatcheries**

Tilapia species/Hybrid	Number of fish hatcheries	Percentage of fish hatcheries
O. niloticus	3	30
O. niloticus+hybrid	2	20
O. niloticus+Unknown+hybrid	1	10
O.niloticus+*O.leucostictus*+hybrid	1	10
Coptodon	1	10
Hybrid+Unknown	1	10
Hybrid	1	10
Total	**10**	**100**

3.4.10 Population genetic structure of tilapia species in hatcheries

Population structure analysis suggested that K = 4 was the most probable number of clusters for the studied tilapia species and their hybrids. Admixture results indicate that individuals from 821KJ-Bulamba, Ruvu fish farm and Safina bigfish farm appeared to be pure *O. niloticus* as they shared the same cluster with the reference *O. niloticus* (in blue), and were assigned to the cluster with the probability greater than 80% (Figure 3.6). Samples from the Ruhila, Kingolwira, Sammeki farm Ltd, Eden IAGRI-AQUA-Ltd and Kwamngumi Prison exhibited evidence of admixture. In addition, there were unique genetic clusters (red in color) which did not cluster with any of the reference *Oreochromis* species and this cluster was comprised by individuals from Mwamapuli (Figure 3.6). This cluster seemed to be made of other native *Oreochromis* species not included in the reference samples.

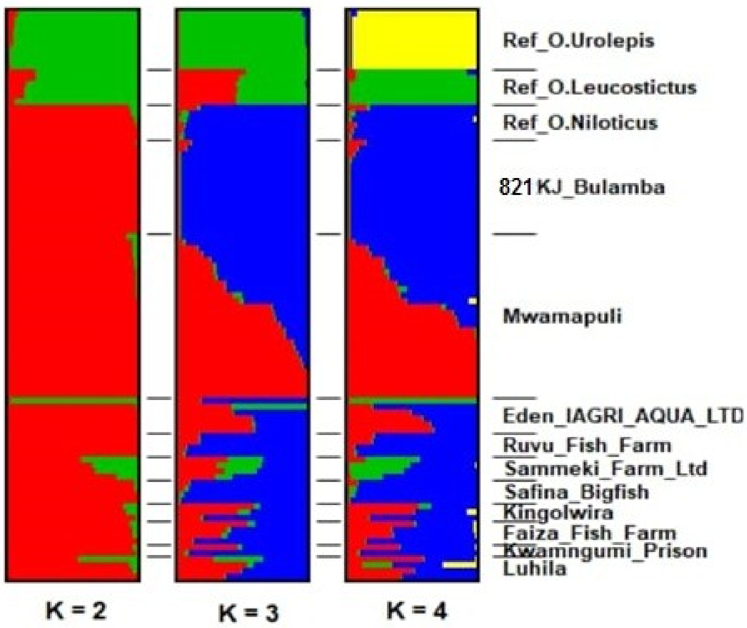

Figure 3.6: **Structure analysis bar plots for K=2, 3 and 4 (admixture model) showing population structure in different hatcheries.** Note: Each horizontal stripe represents an individual. Each color represents the proportion of membership with regard to each assigned to ten genetic clusters. Same color in different individual fish indicates that they belong to the same cluster.

3.5 Discussion

Identification of farmed tilapia species and their hybrids in fish farming subsector is essential for both efficient aquaculture production and wild population management. This study was intended to identify the tilapia species and hybrids cultured in fish farms and hatcheries in Tanzania. The results in this study revealed the existence of *O. niloticus, O. urolepis, O. leucostictus* and *Coptodon* and hybrid individuals in some of the sampled hatcheries and farms in the country. This is probably due to a tendency of collecting both fingerlings and broodstocks from different sources and mixing them into one pond during the same production cycle (Shabani *et al.*, 2020 unpublished data). This observation is in agreement with the report by Shechonge *et al.* (2018b) who mentioned that most cultured species in Tanzania are a mixture of various tilapia species and their hybrids rather than a pure single species.

Availability and distribution of *Coptodons* in different farms in Southern highland zone and Ruhila fish hatchery, indicates that nowadays this species is unknowingly farmed by most fish farms in these two regions of Mbeya and Ruvuma. This is due to proximity to Lake Nyasa which borders Ruvuma and Mbeya regions. The findings in this study indicate that fish farmers in Rungwe district do not culture pure *O. niloticus*, instead they culture *Coptodons* and hybrids of other tilapia species. Therefore, *Coptodon*, especially *Coptodon rendalli* is available locally in Tanzania, perhaps from Lake Nyasa. The results of the present study agree with the report by Shechonge *et al*. (2018a) who found, *Coptodons* and four hybrids at Ruhila fish hatchery. Also, availability of *Coptodon* individual at Mara River in Lake Victoria catchment suggests the existence of *Coptodons* in Lake Victoria. *Coptodon* species has not previously been identified from any Tanzanian habitat outside the Lake Victoria basin, except one occasion in the Lake Malawi catchment where it was reported in a survey in 2011 (Genner *et al*., 2018).

The present study has revealed that the proportion of farms which do not contain pure *O.niloticus* is high across all seven agro-ecological zones of Tanzania. This is different to the belief of fish farmers that they culture pure *O. niloticus*. This may be due to collection of fingerlings from either hatchery with a mixture of tilapia species or wild sources containing different tilapia species. For example, it has been reported that the Ruhila Aquaculture Development Center located in the Southern part of Tanzania, stocked fish from Kingolwira Aquaculture Center in Morogoro in 2011 (Kajungiro *et al.,* 2019b). The Kingolwira Aquaculture Center obtained broodstock from Lake Victoria which contains various tilapia species. The native species in Lake Victoria are *O. esculentus* and *O. variabilis* while *O. leucostictus* and *O. niloticus* were introduced in the Lake in 1950s (Bradbeer *et al*., 2019).

Moreover, Shechonge *et al.* (2019b) found evidence of introduced *O. leucostictus* males from Ruhila government pond in Songea and also, reported that fish farmers misidentified *O. leucostictus* as *O. niloticus*. The proportion of fish farms which do not culture pure *O. niloticus* in the country will continue to increase if there is no planned breeding, conservation program and biosecurity practices coupled with little knowledge on tilapia fish species identification. The co-existence of *O. niloticus* and *O. urolepis* in Shamwengo farm in Mbeya region, suggests unintentional mixing of these two species. This is because the farmers claimed to stock pure *O. niloticus* which were obtained only from ponds of fellow fish farmers (Personal communication with fish farmers, 2020). This observation suggests production of hybrids of the identified tilapia species in the mentioned farm. It has been reported that hybrids between non- native tilapia and *O. urolepis* exists in Mindu dam (Shechonge *et al.,* 2018a).

The observation that both *O. niloticus* and *O. leucostictus* are present at Eden IAGRI-AQUA-LTD hatchery is in agreement with the report by Shechonge *et al*. (2018a), who found a mixture of the two species (*O. niloticus* and *O. leucostictus*) at two hatcheries that supplies fingerlings (labelled as *O. niloticus*) to many fish farmers. Actually, this co-occurence of *O. leucostictus* with *O. niloticus* in hatcheries and farms in the country suggests that *O. leucostictus* stock is misidentified as the most preferred *O. niloticus*. It seems that, there is high level of hybridization in some of these hatcheries, probably due to the practice of culturing a mixture of tilapia species unknowingly. This practice is caused by misidentification of tilapia species during stocking or production and collection of fingerlings from sources with mixed tilapia species such as Lakes and Rivers (Shechonge *et al*., 2018b). Existence of all pure *O. niloticus* samples, at 821KJ-Bulamba, Ruvu Fish and Safina bigfish farms suggests good management of broodstocks in these hatcheries (Rukanda, 2018), and probably good ability for tilapia fish species identifications which is contributed by having good experience in tilapia fish identification among the hatchery managers in the mentioned hatcheries (Shabani *et al*., 2020 unpublished data). On the other hand, the occurrence of hybrids or tilapia of other species in seven out of ten sampled hatcheries, may be caused by the practice of collecting broodstocks from different sources including different hatcheries such as hatcheries in Kenya, Uganda and Thailand, and wild sources and rearing them together in production ponds (Rukanda, 2018; Shabani *et al.,* 2020 unpublished and data). For example, it has been reported that Eden IAGRI-AQUA-LTD imported tilapia species from Thailand in 2016 (Mbiru *et al.,* 2020). On the other hand, farm managers at Kingolwira, Kwamugumi Prison and Ruhila mentioned that sometime they collect broodstocks from wild sources, without taking into account that, natural water bodies in the country such as lakes, dams and rivers have a high diversity of tilapia species which can interbreed and produce fertile hybrids (Shechonge *et al.,* 2018b).

Likewise, the present study identified *O. jipe* in Nyumba ya Mungu, *Coptodon* in Kumba dam. This suggests a continuous existence of native *O. jipe* in the Pangani system and a new report of *Coptodon* in Kumba dam which is near to the Pangani system. The findings in this study agree with the report by Shechonge *et al.* (2018a) who found native *O. jipe* population and other native *Oreochromis* species in Pangani basin including Lake Jipe. Other *Oreochromis* species including *O. pangani*, introduced *O. niloticus* and *O.*

esculentus have been observed in the Pangani ecosystem (Shechonge *et al.,* 2018a). Also, the results of the present study at Kumba dam agrees with Bradbeer *et al*. (2019) who found no evidence of hybrid individuals within Lake Malimbe and Kumba. Also, existence of *O. niloticus* and hybrid in Lake Babati in the Northern part of the country and hybrid in Ugala River in the Western zone of Tanzania is suggesting the existence of more than one tilapia species in these ecosystems. The existence of many tilapia species in one ecosystem poses a risk of having high possibility of losing genetic diversity through hybridization between the species in these natural water bodies. High number of hybrid individuals in the samples from fish farms and hatcheries as well as the reference samples from natural water bodies in different parts of the country is indicating high level of hybridization between farmed and wild tilapia species, not only in hatcheries and farms but also in the wild populations. This observation is in line with the report by Shechonge *et al*. (2018b) that most cultured species in Tanzania are a mixture of various tilapia species and their hybrids. Actually in the field of biology, hybridization is generally considered as a negative process for biodiversity as it leads to erosion of genetic purity or uniqueness of a species (Todesco *et al.,* 2016). It has been reported that hybrids may possess novel traits that enhance their potential to outcompete the indigenous populations (Gaskin and Schaal, 2002; Facon *et al.,* 2005). Also in freshwaters, genetic or demographic swamping during hybridization is considered as a major driver of biodiversity loss, alongside with habitat loss and pollution (Scribner *et al.,* 2000).

3.6 Conclusions

The current study has revealed that *O. niloticus* is the most preferred tilapia species for farming in the country, but interestingly most of the fish farms and hatcheries in the country are not culturing pure *O. niloticus*, instead they culture a mixture of *O. niloticus*, *O. leucostictus, O. urolepsi* and high number of hybrids between tilapia species. For hatcheries only Ruvu fish farm, Safina bigfish and 821KJ-Bulamba culture pure *O. niloticus*. In addition, the study has found that most of the fish farms in Rungwe district in the Southern highlands zone are culturing *Coptodons* unknowingly. In general, more tilapia hybrids are cultured in farms and hatcheries compared to pure *O. niloticus*. Overall, the present study has demonstrated that there are different species of tilapia that are cultured in fish farms and hatcheries, including *O. niloticus, O. leucostictus, O. urolepsi, Coptodons* as well as unknown tilapia species and their hybrids.

3.7 Recommendations

From the results of this study it is recommended to:-

i. Establish certified hatcheries that can produce tilapia fingerlings of pure single species.
ii. Establish a practical tilapia breeding program for maintaining the purity of tilapia strains, ensure active dissemination of good quality fish seeds and guarantee permanent genetic gain in farmed fish.
iii. Train fish farmers and hatchery managers on how to handle and manage broodstock and fingerlings to avoid unintentional mixing of tilapia species from different sources.
iv. Rigorously control importation of both broodstock and fingerlings in order to avoid mixing of different species in the established hatcheries and farms.

3.8 Acknowledgements

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CHAPTER FOUR

4.0 MANUSCRIPTS III

Assessment of population structure and genetic diversity of Nile tilapia (*O. niloticus*) cultured in Tanzania using single nucleotide polymorphisms

Status of the Manuscript: In Preparation

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Abstract

Assessment of population structure and genetic diversity within and between *O. niloticus* cultured in Tanzania is important for sustainable tilapia farming and conservation of wild population. This study assessed the genetic structure and diversity of *O. niloticus* cultured in five hatcheries (Mwamapuli, 821KJ-Bulamba, Ruvu fish farm, Faiza fish farm and Safina bigfish) and 13 fish farms. A total of 50 Nile tilapia samples were purposively selected from a total of 190 tilapia fish samples. In addition, three *Oreochromis niloticus* reference samples were included. One hundred and twenty (120) SNPs were selected for tilapia species genotyping. Population structure was assessed using STRUCTURE analysis and principal component analysis (PCA). Within population, the genetic diversity indicated that expected heterozygosity ranged from 0.18778 in the 821KJ-Bulamba population to 0.47475 in the Faiza fish farm population, while observed heterozygosity ranged from 0.39706 in Mwamapuli to 0.49495 for both 821KJ- Bulamba and Faiza fish farm. Inbreeding coefficient (F_{is}) values ranged from low values in Safina bigfish (-0.23750), Ruvu fish farm (-0.08791), 821KJ-Bulamba (-0.06979) and Faiza fish farm (-0.05376) to relatively high values in Mwamapuli (0.04706). Also the results indicate that pairwise F_{ST} values varied from 0.072 to 0.359. The lowest F_{ST} values were observed between 821KJ-Bulamba and populations from Mwamapuli, Ruvu fish farm, and Safina bigfish. The highest F_{ST} value was observed between 821KJ-Bulamba and Faiza fish farm. Admixture was detected at Mwamapuli, Faiza fish farm, Ruvu fish farm and Safina bigfish, but was not detected at 821KJ-Bulamba. In fish farms admixture was detected in SUA-Morogoro and Babati populations. Analysis of molecular variance (AMOVA) showed that 17.71% of genetic variation was found among populations while -7.69% of the genetic variation was observed among individuals within populations and 89.98% of the variation was found within individuals. Principal component analysis indicated one genetic group of *O. niloticus* which clustered together with *O. niloticus* reference samples

and no any individual clustered together with either *O. urolepsi* reference or *O. leucostictus* reference samples. Therefore, this study concluded that there is a greater genetic diversity within populations than among populations. Individuals from fish farms as well as in hatcheries at 821KJ-Bulamba, Ruvu fish farm, Safina bigfish and Mwamapuli populations are closely related, with different genetic structure at Faiza fish farm populations.

Keywords: aquaculture, hatcheries, fish farms, genetic diversity, population

4.1 Introduction

Tanzania is a diversity hotspot of tilapias ranging from *Oreochromini, Tilapiini* to the tribe *Coptodini* (Genner *at el*., 2018; Shechonge *et al*., 2019). Nile tilapia is the most farmed tilapia in the country (Chenyambuga *et al*., 2014; Shabani *et al*., 2020 unpublished). In the past five years, inland aquaculture of tilapia has grown substantially, whereby the number of earthen fish ponds increased from 27 979 in 2019 producing 1231 tonnes per year to 30 032 fish ponds in 2020 producing 4118 tonnes per year (URT, 2020). About 90% of the technologies used to culture tilapia are earthen ponds of average size of 200 m^2 and a few farmers culture tilapia in cages in Lake Victoria (URT, 2019). The recent development in tilapia farming in the country has included the establishment of more than ten (10) tilapia hatcheries scattered in different parts of the country. Nevertheless, the capacities of the hatcheries are inadequate to meet the increasing demand of fingerlings due to low level of investment and limited electric power supply (Rukanda, 2018). The estimated country demand is 40 000 000 tilapia fingerlings, but the current supply is 21 173 226 fingerlings per year (URT, 2019). To counterbalance the huge demand of tilapia fingerlings in the country, some fish farmers collect both broodstocks and fingerlings from natural water bodies such as lakes, rivers and natural dams for stocking in their farms (Shoko and Kamugisha, 2018; Shabani *et al*., 2020, unpublished data). It has been reported that most fish farms in Tanzania culture a mixture of various tilapia species and their hybrids (Shechonge *et al*., 2018), rather than a pure single species. Under this situation, it is difficult to obtain higher production returns from aquaculture because the broodstock and fingerlings are not pure, hence, their management is difficult and practical genetic improvement is impossible (Kajungiro *et al.*, 2019a).

Genetic diversity is important for a population's adaptation capacity in the face of changing environmental conditions (Fischer *et al.*, 2017). The current common hatchery practices of mixing different tilapia species into one pond could result in rapid reduction of the genetic diversity of the cultured species (Kajungiro *et al*., 2019a). Genetic differentiation between populations is further affected by mutation, migration, drift, selection and habitat destruction (Holsinger and Weir, 2009).

It has been reported that, establishment of well-managed tilapia breeding program would enable cumulative genetic improvement for a specific trait and concurrently minimizing inbreeding as well as loss of genetic diversity. Despite the interest in tilapia farming and its potential to contribute to local food production, at the moment no genetic improvement program exists in Tanzania. According to a study by Shabani *et al.* (2020, unpublished data), the most cultured tilapia species in Tanzania is *O. niloticus*. However, movement of fish from one part of the country to another may have caused hybridization of these tilapia species and consequently affected the genetic diversity and population structure of the cultured fish species (Basiita *et al*., 2018). Because of species misidentification, mismanagement and multiple sources of fingerlings and broodstock in fish farms and hatcheries in Tanzania, there is a high chance that the genetic purity of the farmed fish has been disrupted. Therefore, there is a need to assess the genetic structure and diversity of

O. niloticus in order to determine the level of genetic diversity and relationship of farmed *O. niloticus* using single nucleotide polymorphisms (SNPs).

High polymorphism, abundance and reproducibility of results of single nucleotide polymorphisms (SNPs) markers allow easy investigation of genetic diversity for different tilapia species. A panel of 96 targeted SNPs can consistently discriminate several native *Oreochromis* species in Tanzania (Ciezarek *et al*., 2021). The SNP markers have been used successfully for identification of broodstocks and strains used in aquaculture, as well as identifying candidate genes for economically important traits and quantitative trait loci (QTL) useful in aquaculture (Oyarzún, *et al*., 2013; Yáñez *et al*., 2015). Double-digest restriction site-associated DNA (ddRAD) has been used to study the population structure and genetic diversity of Nile tilapia strains cultured in Tanzania (Kajungiro *et al*., 2019b). Also, characterization of the genetic structure of introduced tilapia strains has been done using double digest RAD sequencing (Mbiru *et al*., 2020). However, there is no information about the genetic structure and diversity of farmed *O. niloticus* generated using SNPs in Tanzanian fish farms and hatcheries. The current study intended to assess the genetic structure of most cultured *O. niloticus* in hatcheries and farms in the country by using SNPs. The generated data are useful in designing management practices and genetic improvement programs that would make the aquaculture sector a profitable venture. The use of SNP data to infer the population structure and diversity is of great importance, both for estimating genetic diversity of farmed Nile tilapia and wild population and designing conservation practices.

From the tilapia farming perspective, assessment of the genetic diversity among and within populations is very important in order to ensure that the most diverse fish are chosen for selective breeding programs. The knowledge on genetic structure and diversity will be useful in appropriate management of farmed Nile tilapia populations.

4.2 Materials and Methods

4.2.1 Ethics statement

This study was carried out in accordance with the law on the protection of animals against cruelty (Act no.12/1974 of the United Republic Tanzania) and was approved by the research ethics committee at Sokoine University of Agriculture (Appendix 5). All the permits required to sample farmed tilapia species were obtained and the sampling protocol adhered. Research clearance was obtained from SUA on behalf of the Tanzania Commission for Science and Technology (COSTECH) (Appendix 6) and district authorities in the localities where sample collection was done.

4.2.2 Fish sample collection and preparation

The study was carried out between October 2019 and April 2020. A total of 336 and 200 tilapia pectroral fins were collected from fish farms and hatcheries, respectively, making a total of 536 fish samples, but due to budget limitation only 109 tilapia samples were selected for genotyping from 59 fish farms located in seven agro-ecological zones of Tanzania; namely Eastern, Northern, Southern, Central, Southern highland, Western and Lake zones (Appendix 3). In addition, due to budget limitation only 81 pectoral fins samples were selected for genotyping out of 200 collected samples from ten fish hatcheries (five Public and five Private) found in different parts of the country.

Both farms and hatcheries were selected purposively based on the criterion that the farm or hatchery cultures tilapia. Following euthanasia, clips of the pectoral fins, approximately 1.5 cm long, were obtained and placed into a labelled vial containing 95% ethanol and the vials were put in a cool box packed with ice. The fish fin samples were transported to Sokoine University of Agriculture within 24 – 72 hours, and kept at 4°C until DNA extraction. A photograph of each sampled fish was taken then uploaded into the

TilapiaMap app for species identification. Photographs of sampled tilapia fish in the tilapiamap app were identified based on morphological characteristics using tilapia fish species identification guide by Trewavas (1983) and Genner *et al*. (2018). In particular, *O. niloticus* were distinguished from other tilapia species by having a large deep-bodied size with relatively small head and presence of regular vertical stripes throughout the depth of caudal fin.

Based on the assessment of the photographs in the tilapiamap app it was observed that a mixture of different tilapia species and their hybrids are cultured in the farms and hatcheries. Out of 190 fish samples collected, only 50 individualds were identified as pure *Oreochromis niloticus* fish species (Shabani *et al.*, 2020 unpublished data). As the numbers of fish which were pure for the other species were small (one to seventeen individuals), assessment of the genetic structure and diversity of farmed tilapia species was done only on *O. niloticus*. Only individuals that were identified to be pure species of *O. niloticus* were selected for genetic diversity and structure analysis. Out of 190 tilapia fish collected *O. niloticus* were the most (26.316%) cultured species after hybrids that ranked first in numbers of fish that were cultured (53. 684%); The other species i.e; *Coptodon* (8.947%), *O. urolepsi* (0.526%), *O. leucostictus* (2.632%), unknown tilapia species (7.368%) and *O. jipe* (0.526%) were found in small numbers (Shabani *et al.*, 2020 unpublished data). The SNP chip (Ciezarek *et al.*, 2021) used has reference samples which consisted of six specimens of *Oreochromis niloticus* and *Oreochromis leucostictus* from Lake Albert in Uganda and 10 specimens of *Oreochromis urolepis* from Wami River in Tanzania.

4.2.3 DNA extraction and sample processing

DNA extraction for SNP genotyping was done using the BioArk extraction kit, following manufacturer’s instructions. The genotyping of SNP was performed by LGC Genomics

GmbH (*https://www.lgcgroup.com/about-us/locations/*). Genotyping used next generation sequencing based SeqSNP genotyping (NextSeq 500/550 v2), 1 x 75 bp read length, at a depth of 200 X. Initial data filtering and quality control was performed by the LGC Genomics GmbH in Berlin. Demultiplexing of libraries was performed using bcl2fastq v2.20 software (Illumina). Sequence reads were clipped for adapter remnants and quality trimmed at the 3'-end to get a minimum average Phred quality score of 30 over a window of ten bases. Reads with Ns and those with a final length of less than 65 bases after quality trimming were discarded. The quality trimmed reads were aligned against the *O. niloticus* reference genome NCBI Orenil1.1 genome version GCA_000188235.2 (Brawand *et al*., 2014) using Bowtie2 v2.2.3 software (Langmead and Salzberg 2012). Genotyping at the target SNP sites was then performed using Freebayes v1.2.0 (Garrison and Marth, 2012) with ploidy set to two and genotypes filtered for a minimum coverage of eight reads.

4.2.4 SNP genotyping

The NextSeq sequencing generated a total 10 081 178 reads, with a mean of 53 058 reads per sample. A total of 8 064 943 (80%) of the reads were used in the downstream genotyping, with each target SNP having an average effective coverage of 357x. Of the 120 target SNPs, 106 passed quality threshold having greater than 85% of samples covered by a minimum of 8 reads. When the genotyped dataset was combined with reference samples from the previously published genotype array (Ciezarek *et al.*, 2021), a total of 73 SNPs were overlapping, giving a dataset of 195 samples (173 in the present study that passed quality control, and 22 reference samples of *Oreochromis* species (*O. niloticus, O.urolepis and O. leucostictus*). A total of 73 SNPs were used for further analysis.

4.2.5 Identification of tilapia fish species

Ancestry analysis using ADMIXTURE v1.3.0 software (Alexander *et al.*, 2009) was performed to determine the proportion of ancestral admixture for assigning appropriately the different species and their hybrid present in both fish hatcheries and farms to their respective tilapia species of origin. A threshold of 80% cluster membership was used to classify samples as pure species. Individuals with cluster membership of less than 80% were considered to be potential hybrids. Based on cluster membership criterion, we identified a total of 50 *O. niloticus* out of 190 collected and genotyped samples. Of the 50 samples of *O. niloticus*, 31 and 19 samples were from fish hatcheries and farms, respectively, which were then selected as samples for further analysis in this study. Table 4.1 shows the number and percentage of Nile tilapia (*O. niloticus*) identified by Shabani *et al*. (2020 unpublished data) and used in the genetic structure and diversity analysis in the current study.

Table 4.1: **Identified tilapia species and hybrids in sampled fish farms and hatcheries**

S/N	Tilapia species	Number of individuals	Percentage (%)
1	*O. niloticus*	50	26.316
2	*Hybrid*	102	53.684
3	*Coptodon*	17	8.947
4	*Unknown*	14	7.368
5	*O. leucostictus*	5	2.632
6	*O. jipe*	1	0.526
7	*O. urolepsi*	1	0.526
	Total	**190**	**100.00**

4.2.6 Population genetic structure analyses in fish hatcheries and farms

The population structure of the fish samples was analyzed using STRUCTURE v.2.3.4 Software (Pritchard *et al*., 2000; Falush *et al*., 2003; Hubisz *et al*., 2009) with K values ranging from 2 to 5. Markov chain Monte Carlo (MCMC) of 100 000 iterations with a burn-in period of 10 000 was carried out for each K value. For each tested K value, three independent MCMC samplings were performed. The obtained posterior probability values (Pritchard *et al*., 2000) were used to determine the optimal number of clusters. Structure results were interpreted using a program called Structure Harvester program (Earl, 2012) and CLUMPAK (Kopelman *et al.*, 2015) for identifying the most probable number of clusters. Furthermore, p-values for significant deviations from zero were estimated using 1 000 bootstraps.

4.2.7 Genetic similarity and relationship among populations of *O. niloticus*

Within population, the genetic diversity was assessed using observed (Ho) and expected (He) heterozygosity for the hatchery populations. Similarly, average individual inbreeding coefficient (F_{is}) for *O. niloticus* identified in fish hatcheries were estimated using stacks v2 (Rochette *et al*., 2019). The R package StAMPP (Pembleton *et al*., 2013) was used to perform an Analysis of Molecular Variance (AMOVA) using 1 000 permutations. Also, the principal component analysis (PCA) was carried out using the R package ADEGENET version 2.1.1 (Jombart *et al.,* 2018).

4.3 Results

4.3.1 Within population genetic diversity

Results on within population genetic diversity indicate that expected heterozygosity ranged from 0.18778 in 821KJ-Bulamba population to 0.47475 in Faiza fish farm population, while observed heterozygosity ranged from 0.375 in Ruvu fish farm and 0.39706 in Mwamapuli population to 0.49495 for both 821KJ- Bulamba and Faiza fish farm populations (Table 4.2). In breeding coefficient (F_{is}) values were very low in Safina bigfish (-0.23750), Ruvu fish farm (-0.08791), 821KJ-Bulamba (-0.06979) and Faiza fish farm (-0.05376) populations and relatively high values in Mwamapuli population (0.04706) (Table 4.2).

Table 4.2: **Within population genetic diversity and inbreeding coefficient parameters for the five *O. niloticus* populations**

Population	n	He	Ho	F_{is}
821KJ-Bulamba	16	0.18778	0.49495	-0.06979
Faiza fish farm	3	0.47475	0.49495	-0.05376
Mwamapuli	4	0.41387	0.39706	0.04706
Ruvu fish farm	4	0.34903	0.375	-0.08791
Safina bigfish	4	0.38294	0.45833	-0.23750

4.3.2 Genetic differentiation and structure

The population pairwise F_{ST} values varied from 0.072 to 0.359 (Table 4.3). The lowest F_{ST} values were observed between 821KJ-Bulamba and populations from Mwamapuli, Ruvu fish farm and Safina bigfish. On the other hand, the highest F_{ST} values were found between 821KJ-Bulamba and Faiza fish farm. The analysis of molecular variance (AMOVA) was used to determine within and among population genetic variations. The AMOVA results showed that 17.71% of the variation was found among populations, -7.69% of the variation was observed among individuals within population and 89.98% of the variation was observed within individuals (Table 4.4).

Table 4.3: **Pairwise F_{ST} values among the five fish hatchery populations: 821KJ-Bulamba, Faiza Fish Farm, Ruvu Fish, Safina bigfish and Mwamapuli**

Population 1	Population 2	F_{ST}	*P* value
821KJ-Bulamba	Faiza Fish Farm	0.35886	0.00293
821KJ-Bulamba	Mwamapuli	0.07200	0.00098
821KJ-Bulamba	Ruvu Fish Farm	0.07616	0.00293
821KJ-Bulamba	Safina bigfish	0.12792	0.00000
Faiza Fish Farm	Mwamapuli	0.23697	0.02734
Faiza Fish Farm	Ruvu Fish Farm	0.18806	0.02051

Faiza Fish Farm	Safina bigfish	0.12792	0.02930
Mwamapuli	Ruvu Fish Farm	0.09101	0.03613
Mwamapuli	Safina bigfish	0.15584	0.02148
Ruvu Fish Farm	Safina bigfish	0.14911	0.02832

Table 4.4: **The variation within and among *O. niloticus* populations from fish hatcheries computed by analysis of molecular variance (AMOVA)**

Source of variation	**Percentage variation**	***P*** **value**
Among populations	17.71	0.00000
Among individuals within populations	-7.69	0.90909
Within individuals	89.98	0.14467

4.3.3 Population genetic structure

The Structure analysis of *O. niloticus* from both hatcheries and farms indicated that K=3 was the most probable number of clusters (Figure 4.1 and 4.2). Admixture between *O. niloticus* and *O. leucostictus* individuals were observed at Mwamapuli, Ruvu fish farm and Safina bigfish, while admixture between *O. niloticus*, *O. urolepsi* and *O. leucostictus* was observed at Faiza fish farm (Figure 4.1). Also, admixture between *O. niloticus*, and *O. urolepsi* was observed at SUA-Morogoro and admixture between *O. niloticus* and *O. leucostictus* was observed at Babati fish farm (Figure 4.2).

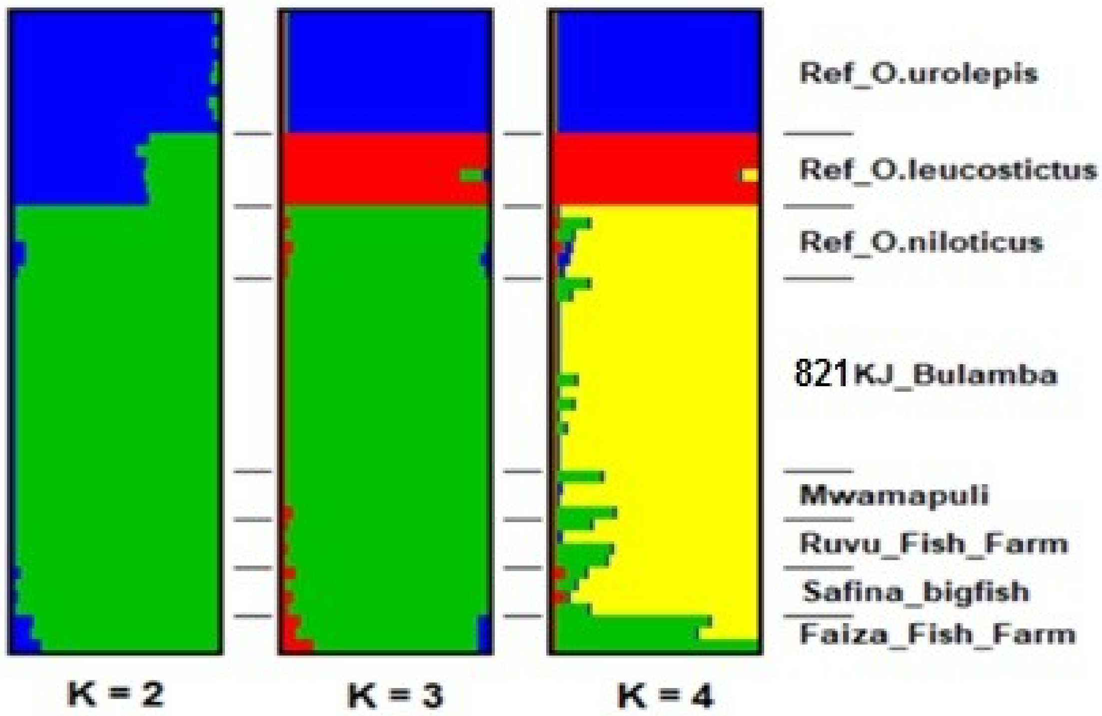

Figure 4.1: Structure analysis bar plots for K=2, 3 and 4 (admixture model) showing population structure of *O. niloticus* cultured in five tilapia hatcheries with large number of *O. niloticus* among ten hatcheries. Each horizontal stripe represents an individual. Each color represents the proportion of membership with regard to individual fish assigned to five genetic clusters. Same color in different individual fish indicates that they belong to the same cluster.

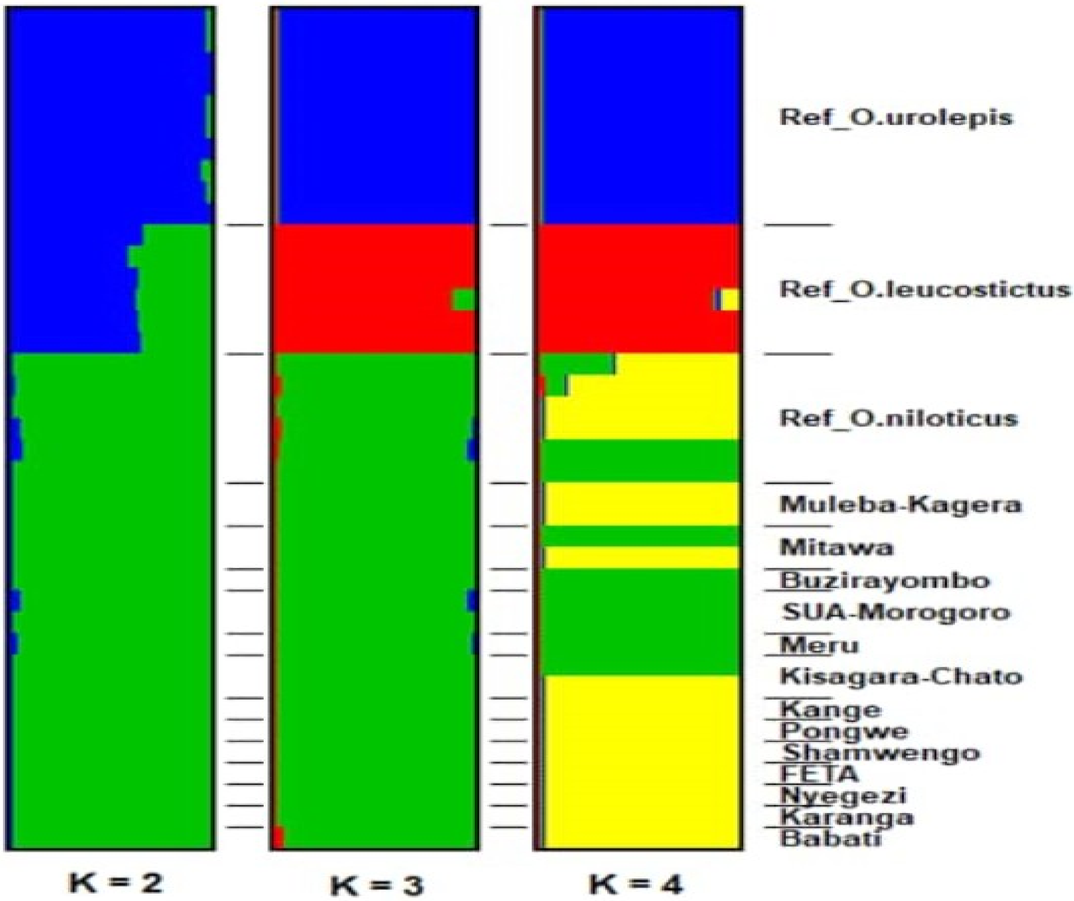

Figure 4.2: Structure analysis bar plots for K=2, 3 and 4 (admixture model) showing population structure of *O. niloticus* cultured in 13 farms. Each horizontal stripe represents an individual. Each color represents the proportion of membership with regard to individual fish assigned to 13 genetic clusters. Same color in different individual fish indicates that they belong to the same cluster.

4.3.4 Genetic relationship of *O. niloticus*

The results of Principal component analysis (PCA), which was used to visualize individual relationships within and between farms/hatcheries, indicate that the first and second principal components accounted for 40.21% and 17.52% of the total genetic variation, respectively. Overall, the PCA revealed the existence of one genetic group of *O. niloticus* which clustered together with *O. niloticus* reference samples and no any individual fish clustered with either *O. urolepsi* reference or *O. leucostictus* reference samples (Figure 4.3). All *O. niloticus* shared a PC1 score of - 0.05 or less whereas the *O. urolepis* and *O. leucostictus* reference samples formed two different genetic groups.

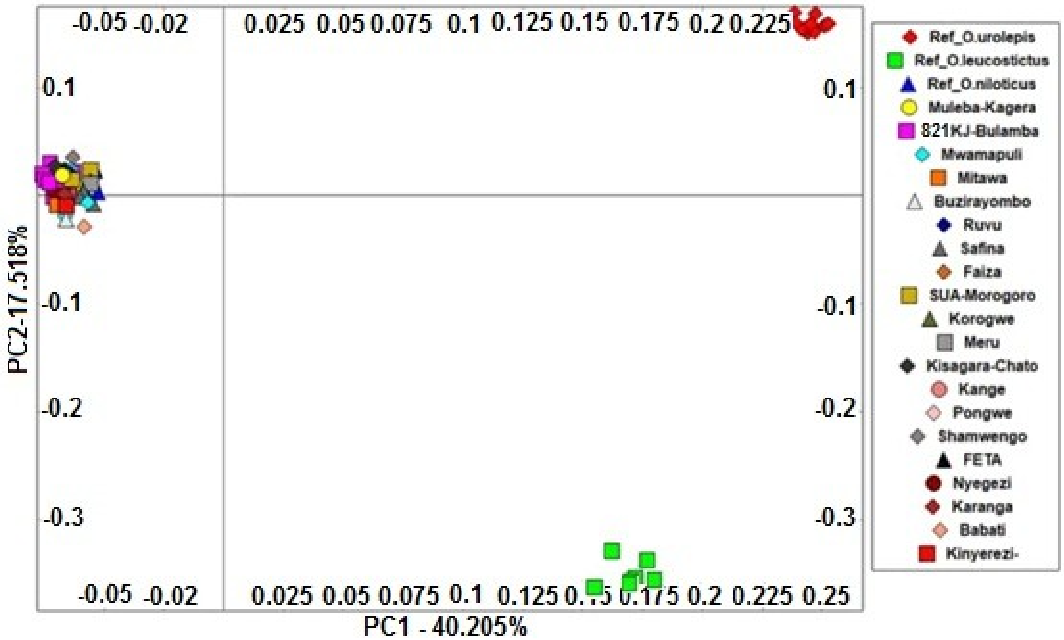

Figure 4.3: Principal component analysis (PCA) for 50 *O. niloticus* samples from farms and hatcheries, together with three reference samples of *Orechromis* species (*O. niloticus, O. leucostictus and O. urolepsi*).

4.3.5 Genetic relationship among hatchery populations

Principal component analysis (PCA) was used to visualize individual relationships between populations from different hatcheries. The first and second principal components accounted for 29.910% and 14.627% of the total genetic variation, respectively. All individuals from 821KJ-Bulamba, Ruvu fish farm, Safina bigfish and Mwamapuli formed a group of genetically similar *O. niloticus* (Figure 4.4). All fish from Faiza fish farm formed a relatively different genetic group with one individual showing divergence from other individuals. Generally, all individuals from this farm were slightly distinct from the populations in other hatcheries.

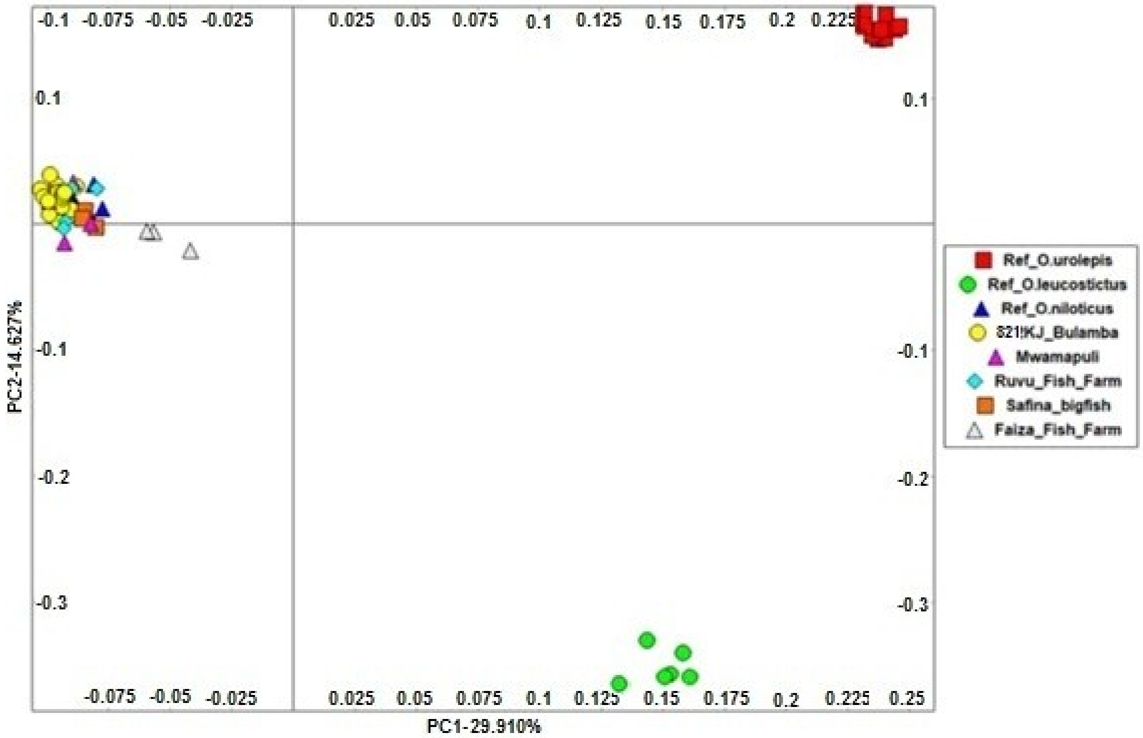

Figure 4.4: Principal components analysis (PC1 and PC2) for 31 identified *O. niloticus* from hatcheries, together with three reference samples of *Orechromis* species (*O. niloticus, O. leucostictus and O. urolepsi*). Each individual is represented by one figure, with its symbol color corresponding to the assigned population.

4.4 Discussion

The overall objective of this study was to investigate the genetic diversity and structure among Nile tilapia populations from five hatcheries (821KJ-Bulamba, Faiza, Ruvu, Mwamapuli and Safina bigfish) and 13 fish farms in Tanzania. Heterozygosity is a commonly used measure of the amount of genetic variation within populations (Templeton and Read, 1994; Gu *et al.*, 2014). The results of this study revealed that populations from 821KJ–Bulamba, Faiza fish farm, Ruvu fish farm and Safina bigfish had higher level of observed heterozygosity than expected heterozygosity, suggesting that random mating potentially occurs in these populations (Templeton and Read, 1994). This is supported by the low values (negative values) of inbreeding coefficients (F_{IS}) in the mentioned populations. The findings in this study concurs with the results obtained by Mireku *et al.* (2017) who observed heterozygosity of nine populations of *O. niloticus* in Lake Volta of Ghana is slightly higher than the expected heterozygosity. On the other hand, the Mwamapuli population showed high level of expected heterozygosity than observed heterozygosity, together with high positive F_{IS} value compared to other populations in this study. The high positive F_{IS} values indicate the existence of non-random mating or population subdivision. The higher diversity in Mwamapuli, Faiza fish farm, Ruvu fish farm and Safina bigfish populations may be due to both the existence of non-random mating and a higher degree of population admixture as revealed by the STRUCTURE analysis. The observation that the expected herozygisy is higher than observed heterozygosy agrees with Kajungiro *et al*. (2019b) who found that overall observed heterozygosity is lower than the expected heterozygosity for most cultured Nile tilapia populations in Tanzania. Also, Gu *et al*. (2014) found that observed heterozygosity in six *Oreochromis* populations in the primary rivers of Guangdong province are lower than the expected heterozygosity.

In the present study, it was observed that both observed and expected heterozygosity differed among Nile tilapia populations. Differences of observed and expected heterozygosities among different Nile tilapia populations also have been reported by Maruki and Lynch (2017) who observed that the average expected and observed heterozygosity are higher in *O. niloticus* populations from river Nile than from Delta Lake populations. However, it should be noted that the present study used SNPs markers as opposed to the aforementioned studies which used microsatellites. Generally, in this study the levels of heterozygosity in all populations were low. The low heterozygosity levels could be attributed to the Wahlund effect (Saenz-Agudelo *et al.*, 2015) whereby observed heterozygosity is reduced as populations diverge. Furthermore, this study demonstrated the potential influence of the relatively small to moderate sample size for each population (3 -16 fish per population). It has been shown that the estimates of heterozygosity from empirical data are relatively sensitive to sample size (Allendorf and Luikart, 2007).

The assessment of genetic variations among populations indicates that the genetic differentiation among 821KJ-Bulamba, Mwamapuli in Igunga and Ruvu populations was very low. This indicates that individuals in these populations were almost genetically similar. The similarities among these three populations are probably due to their origin from the same region of Lake Victoria. According to Kajungiro *et al.* (2019b) the original stock of tilapia fish at Mwamapuli, Ruvu and FETA-Nyegezi were from Lake Victoria in the year 2016. Therefore, it is likely that these populations are genetically similar to each other and share the same genetic background. Likewise, Kajungiro *et al.* (2019b) recorded low levels of differentiation among FETA, Igunga and Lake Victoria Nile tilapia populations. However, in the case of Faiza fish farm a different trend was observed, despite the fact that the individuals at this farm originated from the same location (Lake Victoria). The high F_{ST} values between Faiza fish farm and other populations (821KJ-Bulamba, Mwamapuli, Ruvu fish farm, and Safina bigfish) indicated high isolation between them. Interestingly, the Faiza fish farm population was composed of fish which have been in captivity for 3 - 5th generations (Personal communication with the interviewed manager, October, 2019) and this could be a reason for its genetic uniqueness. Moreover, high level of genetic differentiation was observed between Safina bigfish population and the three closely related populations of Mwamapuli, Ruvu fish farm, and 821KJ-Bulamba. This could be the result of geographical isolation, which probably has acted as a barrier to gene flow between those populations, leading to the observed high level of genetic differentiation of *O. niloticus* in these hatcheries. On the other hand, gene flow is likely to have occurred among the admixed populations (Mwamapuli, Ruvu fish farm, Safina bigfish and Faiza fish farm) because there has been a transfer of broodstocks between these hatcheries. Existence of pure population at 821KJ-Bulamba may be due to the fact that fish at this hatchery have been in captivity for 2-3th generations (Personal communication with the interviewed manager, December, 2019).

Structure analysis revealed that *O. niloticus* populations in some hatcheries and farms were admixed with *O. urolepsi* and *O. leucostictus.* The existence of admixed individuals in some of the collected *O. niloticus* samples from farms suggests introgressive hybridization between *O. leucostictus* and *O. niloticus* at Babati, also between *O. urolepis* and *O. niloticus* at SUA-Morogoro. This observation could be due to the tendency of farming a mixture of different species in one fish pond. The problem is intensified by lack of controlled breeding. Similar results have been reported by other authors who found that hybridization and introgression are fairly common in tilapias, making challenges in management and conservation of both wild and farmed populations (Shirak *et al*., 2009; Wu and Yang, 2012). Studies have shown that the actual levels of differentiation is a balance between the homogenizing effects of gene flow and the disruptive effects of reproductive isolation and genetic drift (Allendorf and Luikart, 2007).

In this study, the results of F_{ST} and AMOVA indicate that much of the genetic variation was found within the populations and very little between the poptlations. This might be due to existence of frequent gene exchanges among the hatcheries and farms. Similar results have been obtained by Mireku *et al.* (2017) who found higher genetic variation within populations than among populations in Nile tilapia populations from Lake Volta in

Ghana. The Principal Component Analysis (PCA) showed that all Nile tilapia populations clustered together, indicating low level of genetic differentiation among the populations from different hatcheries and farms.

4.5 Conclusions and Recommendations

Generally, the results of the present study revealed greater genetic diversity within than among populations. The close clustering of all individuals from fish farms and also in hatcheries at 821KJ-Bulamba, Ruvu fish farm, Safina bigfish and Mwamapuli populations shows that the Nile tilapia in these populations are closely related. The distinct separation of the population at Faiza fish farm from other populations indicates that the Nile tilapias at this hatchery are pure populations without admixture with other populations. Therefore, this study recommended using 821KJ-Bulamba, Ruvu fish farm, Safina bigfish and Mwamapuli as well as Faiza fish farm populations as sources of Nile tilapia broodstock and fingerlings for stocking fish farms because they have demonstrated greater genetic diversity within populations than among populations.

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CHAPTER FIVE

5.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The results in the present study revealed that fingerlings production capacities of the surveyed fish hatcheries are considerably low compared to the actual demand. This might be due to low investment capacity among hatchery operators (Rukanda, 2018), and inadequate skills on fingerlings production has been reported in Sub-Saharan Africa by Bhujel (2014). Furthermore, the study has indicated that in Eastern zone of Tanzania more fingerlings are obtained from hatcheries than from the wild sources. This may be due to the availability of more hatcheries in this zone compared to other zones. Most of the respondents both in fish hatcheries and farms mentioned Eden Agri-Aqua limited, Ruvu fish farm, Sammeki Limited, Ruhila aquaculture development Centre, Safina big fish, Kingolwira aquaculture development center and Sokoine University of Agriculture which are located in the Eastern zone as the major producers and suppliers of tilapia fingerlings in the Eastern zone and beyond. According to Rukanda (2018), the main stations for fingerlings production in the country are Safina bigfish, Ruvu fish farm, Kingolwira, Ruhila and Mwamapuli aquaculture development center. These hatcheries have higher fingerlings production capacity and well-established facilities such as egg hatching jars, fry rearing facilities and water quality parameter kits.

On the other hand, the high contribution of wild sources to fingerlings production in Western zone may be due to lack of well-established hatcheries in the zone. Similar result has been observed by Shoko and Kamugisha (2018) who reported high utilization of wild sources as the major source of tilapia fingerlings in the Western zone of Tanzania. The comparatively high level of using of sources of fingerlings in the Lake zone may be due to proximity of most fish farms to Lake Victoria which acts as the major source of Nile tilapia broodstock as well as fingerlings in the country. According to Opiyo *et al.* (2017) obtaining broodstock from the wild is cheaper compared to fish hatchery sources, especially for small-scale fish farmers in Africa. The readily availability of fingerlings from wild sources encourages small-scale farmers to collect fingerlings from the wild. However, wild sources of fingerlings are considered unreliable source of fingerlings for tilapia fish farming development, and are of poor quality which result into poor yield at harvest (Shoko *et al.,* 2016).

Basically, it was found that the contribution of fish hatcheries to fingerlings production is higher compared to wild sources and recruits from other fish farms across all seven agro – ecological zones. This could be due to the perception of farmers that hatcheries are sources of better quality tilapia fingerlings compared to fellow farmers and wild sources. Wild-caught fingerlings are considered as inferior, as well as being an unreliable because the quality and quantity of fingerlings varies throw out the year (FAO, 2007). On the other hand, sourcing fingerlings from fellow farmers is easier, but it may lead to getting stunted mature fish which are mistaken as juvenile. This study has revealed that some hatchery managers imported broodstock from Uganda and Thailand, also some fish farmers imported tilapia fingerlings from Uganda, Zambia and Malawi. The results of this study agree with the report by Rukanda (2018) and Shechonge *et al.* (2018b) who reported that the sources of broodstock and fingerlings in Tanzania are both from the wild and fish hatcheries within and outside the country.

In addition, the results of this study discovered that most of the hatchery managers in Tanzania were intentionally mixing broodstock from different sources into one fish pond during the same cycle of seed production. This was done in order to improve growth performance of the fingerlings which were produced (Hatchery managers, Personal communication, 2019). Moreover, most of the farmers in fish farms were unintentionally mixing tilapia fingerlings from different sources into one fish pond during the same cycle of fish production. This might be due to shortage of fingerlings in those zones. Furthermore, the resemblance of different tilapia species at the fingerling stage increased the probability of having mixed stocks in fish ponds because of the difficulties in separating the fish belonging to different species (Charo-Karisa *et al.,* 2009). The rearing of mixed species into one pond may not result into expected profitable production (Kajungiro *et al.,* 2019a). This result indicates that, there is high level of intentional and unintentional hybridization of farmed tilapia species in both fish hatcheries and farms in the country. This may be attributed to the lack of controlled breeding programme, given the fact that Tanzania is a hotspot for tilapia biodiversity, hence, there is a high chance of tilapia species hybridization (Genner *et al.,* 2018). Moreover, in freshwaters, genetic or demographic swamping during hybridization is currently considered as the major driver of biodiversity loss, alongside habitat loss and pollution (Scribner *et al.,* 2000; Perry *et al.,* 2002). The results in this study discovered that high number of fish hatcheries and farms were not culturing pure, *Oreochromis niloticus* instead stocked hybrids or a mixture of tilapia species. Unfortunately the fish farmers believe to culture pure, *Oreochromis niloticus.* This may be due to the collection of fingerlings from either hatchery with a mixture of tilapia species or hatchery which obtained the original stock from wild sources (Kajungiro *et al*., 2019b).

The results of assessing within and between population genetic diversity of Nile tilapia broodstock in hatcheries and farms indicated that the level of genetic differentiation among 821- KJ Bulamba, Mwamapuli and Ruvu populations was low. This indicate that individuals in these populations are almost genetically similar. The similarities among these three populations are probably due to the fact that the fish cultured in these hatcheries have the same origin, which was Lake Victoria. According to Kajungiro *et al*. (2019b) tilapia stocked at Mwamapuli, Ruvu and FETA–Nyegezi were from Lake Victoria in the year 2016. Therefore, it is likely that these populations are genetically similar to each other as they share the same genetic background. The findings of this study concur with Kajungiro *et al.* (2019b) who found low levels of differentiation among FETA, Igunga and Lake Victoria Nile tilapia populations. However, in the case of Faiza fish farm a different trend was observed despite the individuals originating from the same location (Lake Victoria). The high F_{ST} values between Faiza fish farm and other populations (821KJ-Bulamba, Mwamapuli, Ruvu fish farm, and Safina bigfish) suggesting high isolation between them. Faiza fish farm population composed of fish stocks which have been in captivity for 3-5th generations (Hatchery manager, Personal communication, 2019) and this could be a reason for the genetic uniqueness of this population. Moreover, high level of genetic differentiation was observed between Safina bigfish population and the three closely related populations of Mwamapuli, Ruvu fish farm, and 821KJ-Bulamba. The differences could be due to geographical isolation, which probably has acted as a barrier to gene flow between these populations. On the other hand, gene flow is likely to have occurred among the admixed populations (Mwamapuli, Ruvu fish farm, Safina bigfish and Faiza fish farm), because there have been transfer of broodstock between these hatcheries. Occurrence of pure populations at 821KJ-Bulamba may be due to the fact that the fish stock at this hatchery has been in captivity for 2-3th generations (Hatchery managers, Personal communication, 2019), this could be a reason for its genetic purity. Existence of admixed individuals in some of the *O. niloticus* samples collected from farms suggests introgressive hybridization between *O. leucostictus* and *O. niloticus* at Babati, also between *O. urolepis* and *O. niloticus* at SUA-Morogoro. This observation could be due to the tendency of culturing a mixture of different species in one fish pond. The problem is intensified by lack of controlled breeding program in the country. In this study the results of F_{ST} and AMOVA indicated that much of the genetic variation is found within the populations and it is lower among the populations. This is in agreement with Mireku *et al.* (2017) who found higher genetic variation within populations than among populations in Nile tilapia populations from Lake Volta in Ghana. A principal component analysis of fish farms and hatcheries populations also showed a similar clustering trend. Based on these results, populations with abundant genetic variation should be conserved for future breeding programs in Tanzania mainland.

5.2 Conclusions

This study assessed the sources of tilapia broodstock and fingerlings in hatcheries and farms in Tanzania mainland, Also the study identified the cultured tilapia species and hybrids as well as assessed the within and between population genetic diversity of Nile tilapia broodstock in hatcheries and farms in seven agro-ecological zones of Tanzania mainland. From this study, it is concluded that;

i. The main sources of tilapia broodstock for fingerlings production in hatcheries are other hatcheries within Tanzania, followed by wild sources especially lakes and hatcheries outside Tanzania.

ii. The main sources of tilapia fingerlings for stocking fish farms in seven agro-ecological zones of Tanzania are hatcheries within Tanzania, wild sources and recruits from other fish farms.

iii. Most of the sampled fish farms and hatcheries do not culture pure *Oreochromis niloticus*, instead they culture hybrids and a mixture of either *Oreochromis niloticus* or other species of tilapia.

iv. High genetic impurity in farmed tilapia fish among hatcheries and farms is contributed by occurrence of *O. leucostictus*, *O. jipe*, *O. urolepsi* and

Coptodons species which are unintentionally farmed in the fish farms as well as sampled hatcheries in the country.

v. Inability to identify different species of tilapia among fish farmers and stocking fish pond with broodstock and fingerlings from multiple sources contributed on the observed genetic contamination of farmed tilapia species in the sampled farms as well as hatcheries.

vi. There is a greater genetic diversity within populations than among Nile tilapia populations (821KJ-Bulamba, Ruvu fish farm, Safina bigfish, and Mwamapuli and Faiza fish farm).

5.3 Recommendations

From this study it is recommended to;

i. Establish certified hatcheries in each agro-ecological zone to increase accessibility of quality seeds and minimize the dependence on wild sources as well as recruits from other fish farms.

ii. Establish specific guidelines and perform regular inspection for control of the quality of broodstock and fingerlings produced from fish hatcheries in order to ensure that the fingerlings produced are pure and of high quality.

iii. Establish a practical tilapia breeding program for maintaining the purity of tilapia strains, ensure active dissemination of good quality fish seeds and guarantee permanent genetic gain in farmed fish.

iv. Regularly train fish farmers and hatchery managers on how to handle and manage broodstock and fingerlings to avoid further genetic contamination in farmed tilapia fish species.

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APPENDICES

Appendix 1: Questionnare

Part I: Questionnare for fish farmers

SOKOINE UNIVERSITY OF AGRICULTURE

SCHOOL OF AGRICULTURAL ECONOMICS AND BUSINESS STUDIES

Research Title: 'ASSESSMENT OF SOURCES OF TILAPIA FINGERLINGS IN FARMS OF TANZANIA MAINLAND'

Phone: +255 756 634 485   E-mail: mashakashaban@yahoo.com

Preamble;

I am **MASHAKA SHABANI**; MSc. student from Sokoine University of Agriculture (SUA), Department of Veterinary Medicine and Public Health. The purpose of this study is to assess sources of tilapia fingerlings in farms in this area. This exercise is very important for the fulfilment of my studies and formulation of policies. Your household was randomly selected from the village list to participate in this research study. Taking part in this research study is entirely **VOLUNTARY**. Your responses will be kept **CONFIDENTIAL**. Your cooperation is highly appreciated.

General Instructions

1. For multiple choice questions, select one answer by putting an appropriate letter in the corresponding box in front of the multiple choices statements.
2. For open question respond by filling or mentioning the required answers according to the given question

3. Information obtained will be for the intended research only

QUESTIONNAIRE

1. Street /Sub-village …………………………………………………………….
2. Ward ………………………………………………………………. ………
3. Name of respondent…………………………….……………………… ……
4. Date of interview…………………………………………………………..
5. Questionnaire number ………………………………………………………….

DATA FROM THE GROW OUT-FISH FARMS

1. Name of the farm …………………………………..
2. Year of Establishment……………………………….
3. Gender…………………………….......................
4. District…………Agro-ecological zone………….....
5. Is the farm owned by?
    a. Individual
    b. Group ( )
    c. Government
6. As a Manager/farm operator what age category do you have?
    d. 15 -19 Years
    e. 20 – 25 Years
    f. 26 – 30 Years
    g. 31 – 40 Years
    h. 41 – 50 Years
    i. 51 - 60 Years
    j. More than 60 Years
7. As a Manager what level of education do you have?
    a. Degree and above
    b. Diploma
    c. Certificate ( )
    d. Secondary school
    e. Primary school
    f. Non formal
8. Do you farm tilapia fish? Yes ( ) No ( )
9. If Yes in 8 above, what other fish species do you culture? (Name)………

10. Do you farm tilapia fish for which purpose? Choose one among the following options.
    a. Production and Marketing of tilapia fingerlings
    b. Growing and Marketing of table size fish ( )
    c. Production of fingerlings, Growing and Marketing of table size fish
11. How many fish ponds do you have?
    a. 1 to 5
    b. 6 to 10
    c. 11 to 15 ( )
    d. 16 to 21
    e. 22 and above
12. What is the average size of the fish pond (s) in meter square (M^2)
    ………………………………………………………………………………………
    ………………………………………………………………………………………
    ………………………………………………………………………………………
    ………………………………………………
13. What type of fish Feed do you use?
    a. Natural feeds
    b. Commercial (Formulated) feeds ( )
    c. Farm made (Locally made)
14. What is the source of fingerlings?
    a. Government (Name)………………………
    b. Private (Name)…………………………….
    c. Wild (Natural water bodies) (Name)……………..
    d. Recruits from other farmers (Name the farm)
15. As manager how many fingerlings you collected from the following sources of fingerlings in this cycle of production?
    a. Hatcheries inside Tanzania (Mention) ……..
    b. Natural water bodies (Mention) …….
    c. Hatcheries outside Tanzania (Mention) ……
16. What is the stocking density?
    a. 1- 4 fingerlings
    b. 5- 8fingerlings ( )
    c. 9 - 12fingerlings

d. 13 -15 fingerlings

e. 16 and above

17. Do you mix tilapia fish from two or more sources in one fish pond in the same production cycle? Yes ( ) No ( )
18. If the answer is **Yes** in Question 17 above. Give reason (s) ……………………
19. Can you distinguish different types (species) of tilapia at your farm? Yes ( ) No ( )
20. Do you import tilapia fingerlings from outside Tanzania? Yes ( ) No ( )
21. If the answer is **Yes** in Question 20 above, give reason(s) for importing fingerlings/broodstocks...

Part II: Questionnaire for fish hatchery managers

SOKOINE UNIVERSITY OF AGRICULTURE

SCHOOL OF AGRICULTURAL ECONOMICS AND BUSINESS STUDIES

Research Title: 'ASSESSMENT OF SOURCES OF TILAPIA BROODSTOCK AND FINGERLINGS IN HATCHERIES AND FARMS OF TANZANIA MAINLAND'

Phone: +255 756 634 485 E-mail: mashakashaban@yahoo.com

Preamble;

I am **MASHAKA SHABANI**; master's student from Sokoine University of Agriculture (SUA), Department of Veterinary Medicine and Public Health. The purpose of this study is to assess sources of tilapia brood stocks and fingerlings in hatcheries in this area. This exercise is very important for the fulfilment of my studies and formulation of policies. Your household was randomly selected from the village list to participate in this research study. Taking part in this research study is entirely **VOLUNTARY**. Your responses will be kept **CONFIDENTIAL**. Your cooperation will be highly appreciated.

QUESTIONNAIRE

1. Street /Sub-village…………………………………………………………………….
2. Ward ……………………………………………………………………………….
3. Name of respondent…………………………………….…………………… …………
4. Date of interview……………………………………………………………………..
5. Questionnaire number ………………………………………………………………….

DATA FROM HATCHERY

1. Name of the hatchery …………………………...
2. Year of hatchery establishment…………………

3. District …………………Agro-ecological zone……………

4. Gender of hatchery manager/operator……………………….

5. Is the hatchery owned by?

   a. Individual
   b. Government ( )
   c. Group

6 As a Manager what age category do you have?

   d. ≤ 20 Years
   e. 21 – 30 Years
   f. 31 – 40 Years
   g. 41 – 50 Years
   h. 51 – 60 Years
   i. More than 60 Years

7. As a Manager what level of aquaculture/fisheries knowledge do you have?

   e. Postgraduate studies
   f. Bachelor Degree
   g. Diploma
   h. Certificate
   i. Secondary school
   j. Primary school ( )
   k. Non formal

8. Do you farm tilapia fish? Yes ( ) No ( )

9. If Yes in 8 above, what other fish species do you culture? (Name)………

10. As manager how many years have been working in fish farming activities

   a. 0.5 – 1 Year
   b. 2 - 3 Years
   c. 4 – 5 Years
   d. 6 – 10 Years ( )
   e. 11 – 15 Years
   f. More than 15 Years

11. What is the stocking density of tilapia brood stock in hapas/tanks/ Ponds?

   a. 1-3/M^2
   b. 4-5/ M^2
   c. 6 -7/ M^2 ( )

d. 8-9/ M^2

e. Above 9/ M^2

12. How many fish fingerlings produced per month/Year?
…………………………………………………

13. How many fish fingerlings you're selling per month?
……………………………………………………..

14. Do you farm tilapia fish? Yes ( ) No ( )

15. Do you farm other fresh water fish species apart from tilapia? (Name)………

16. How many fish ponds do you have?

a. 1 to 5

b. 6 to 10

c. 11 to 15 ( )

d. 16 to 21

e. 22 and above

17. What is the average size of the fish pond (s) in meter square (M^2)

…………………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………
………………………………

18. What is the source of tilapia broodstocks?

i. Government hatchery (Name)………………………

ii. Private hatchery (Name)…………………………….

iii. Wild (Natural water bodies) (Name)……………..

iv. Recruits from other farmers (Name)…………

19. As manager how many fingerlings you collected from the following sources of fingerlings in this cycle of production?

a. Hatcheries inside Tanzania (Mention) ……..

b. Natural water bodies (Mention) …….

c. Hatcheries outside Tanzania (Mention) ……

20. Do you mix tilapia fish from two or more sources in one fish pond in the same production cycle? Yes ( ) No ( )

21. If the answer is **Yes** in Question 20 above. Give reason (s) ……………………

22. Can you distinguish different types (species) of tilapia at your farm? Yes ( ) No ( )
23. Do you import tilapia fingerlings from outside Tanzania? Yes ( ) No ( )
24. If the answer is **Yes** in Question 23 above, give reason(s) for importing fingerlings/broodstocks...

The end of questionnaire

Appendix 2: 120 SNPs Selected for genotyping in this study

S/N	Reference	Position	Identification (ID)	Reference allele	Alternate allele(s)
1	CM001444-1	1,097,041	CM001444_1097041	T	A
2	CM001444-1	9,397,073	CM001444_9397073	A	C
3	CM001444-1	11,724,351	CM001444_11724351	A	G
4	CM001444-1	20,922,817	CM001444_20922817	C	T
5	CM001444-1	26,863,205	CM001444_26863205	A	T
6	CM001444-1	30,797,066	CM001444_30797066	T	C
7	CM001445-1	3,414,893	CM001445_3414893	T	C
8	CM001445-1	7,641,145	CM001445_7641145	A	G
9	CM001445-1	16,281,719	CM001445_16281719	C	A;T
10	CM001445-1	21,300,950	CM001445_21300950	G	A
11	CM001446-1	360,333	CM001446_360333	G	A
12	CM001446-1	4,285,853	CM001446_4285853	A	.
13	CM001446-1	11,962,460	CM001446_11962460	G	T
14	CM001446-1	15,333,189	CM001446_15333189	G	T
15	CM001446-1	18,445,819	CM001446_18445819	C	T
16	CM001447-1	720,384	CM001447_720384	A	G
17	CM001447-1	5,058,328	CM001447_5058328	C	T
18	CM001447-1	19,386,468	CM001447_19386468	C	T
19	CM001447-1	24,314,703	CM001447_24314703	C	G
20	CM001447-1	26,637,101	CM001447_26637101	A	T
21	CM001448-1	1,609,623	CM001448_1609623	A	G
22	CM001448-1	6,707,910	CM001448_6707910	A	T
23	CM001448-1	13,253,954	CM001448_13253954	C	G;T
24	CM001448-1	19,107,635	CM001448_19107635	G	A
25	CM001448-1	25,876,913	CM001448_25876913	G	A
26	CM001448-1	33,979,761	CM001448_33979761	T	C
27	CM001449-1	184,937	CM001449_184937	C	T
28	CM001449-1	4,603,486	CM001449_4603486	C	G

29	CM001449-1	6,974,829	CM001449_6974829	G	A
30	CM001449-1	16,892,027	CM001449_16892027	G	A
31	CM001449-1	18,354,457	CM001449_18354457	A	G
32	CM001449-1	19,464,266	CM001449_19464266	T	A;C
33	CM001449-1	24,387,635	CM001449_24387635	A	T
34	CM001449-1	28,175,620	CM001449_28175620	G	A
35	CM001449-1	34,885,481	CM001449_34885481	A	.
36	CM001450-1	1,219,876	CM001450_1219876	C	T
37	CM001450-1	3,691,760	CM001450_3691760	C	A
38	CM001450-1	6,510,990	CM001450_6510990	G	A
39	CM001450-1	20,308,770	CM001450_20308770	G	C
40	CM001450-1	27,577,640	CM001450_27577640	G	A
41	CM001450-1	33,639,160	CM001450_33639160	G	A
42	CM001450-1	48,288,157	CM001450_48288157	T	A
43	CM001451-1	5,461,621	CM001451_5461621	T	C
44	CM001451-1	11,193,681	CM001451_11193681	C	T
45	CM001451-1	15,102,460	CM001451_15102460	G	A
46	CM001451-1	25,864,637	CM001451_25864637	C	T
47	CM001452-1	3,432,034	CM001452_3432034	C	T
48	CM001452-1	8,917,415	CM001452_8917415	A	T
49	CM001452-1	19,876,288	CM001452_19876288	G	T
50	CM001453-1	4,849,937	CM001453_4849937	C	T
51	CM001453-1	10,206,418	CM001453_10206418	C	T
52	CM001453-1	13,014,811	CM001453_13014811	A	C
53	CM001454-1	882,905	CM001454_882905	A	G
54	CM001454-1	8,412,420	CM001454_8412420	G	A
55	CM001454-1	9,445,114	CM001454_9445114	A	G
56	CM001454-1	13,197,547	CM001454_13197547	T	C
57	CM001454-1	15,678,656	CM001454_15678656	T	G
58	CM001454-1	23,640,878	CM001454_23640878	G	A

59	CM001454-1	29,832,850	CM001454_29832850	G	A
60	CM001455-1	2,685,883	CM001455_2685883	C	T
61	CM001455-1	10,622,533	CM001455_10622533	A	G
62	CM001455-1	31,326,118	CM001455_31326118	T	C
63	CM001455-1	32,684,059	CM001455_32684059	A	T
64	CM001456-1	2,618,669	CM001456_2618669	G	A
65	CM001456-1	13,152,873	CM001456_13152873	A	G
66	CM001456-1	22,320,417	CM001456_22320417	A	T
67	CM001456-1	30,478,945	CM001456_30478945	G	C
68	CM001456-1	31,685,286	CM001456_31685286	C	T
69	CM001457-1	4,704,416	CM001457_4704416	G	T
70	CM001457-1	6,660,838	CM001457_6660838	C	T
71	CM001457-1	11,145,801	CM001457_11145801	T	C
72	CM001457-1	16,112,115	CM001457_16112115	G	.
73	CM001457-1	24,907,166	CM001457_24907166	A	G
74	CM001457-1	31,603,132	CM001457_31603132	G	A
75	CM001458-1	309,328	CM001458_309328	T	C
76	CM001458-1	1,666,436	CM001458_1666436	T	C
77	CM001458-1	5,073,244	CM001458_5073244	G	C
78	CM001458-1	16,181,704	CM001458_16181704	C	T
79	CM001458-1	21,510,977	CM001458_21510977	A	G
80	CM001459-1	1,431,177	CM001459_1431177	T	C
81	CM001459-1	3,068,781	CM001459_3068781	A	G
82	CM001459-1	6,177,743	CM001459_6177743	C	A
83	CM001459-1	8,852,872	CM001459_8852872	T	.
84	CM001459-1	17,135,694	CM001459_17135694	A	C
85	CM001459-1	27,482,101	CM001459_27482101	T	C
86	CM001459-1	27,802,434	CM001459_27802434	G	T
87	CM001459-1	33,128,527	CM001459_33128527	T	C
88	CM001460-1	1,769,322	CM001460_1769322	C	T

89	CM001460-1	8,733,529	CM001460_8733529	C	G
90	CM001460-1	18,821,384	CM001460_18821384	C	A
91	CM001460-1	31,436,846	CM001460_31436846	T	C
92	CM001461-1	3,924,171	CM001461_3924171	C	A
93	CM001461-1	8,667,652	CM001461_8667652	C	.
94	CM001461-1	13,651,950	CM001461_13651950	C	T
95	CM001461-1	17,582,513	CM001461_17582513	C	G
96	CM001461-1	21,819,437	CM001461_21819437	G	T
97	CM001461-1	23,678,350	CM001461_23678350	C	T
98	CM001461-1	25,780,423	CM001461_25780423	T	G
99	CM001462-1	1,992,330	CM001462_1992330	T	C
100	CM001462-1	9,703,056	CM001462_9703056	A	G
101	CM001462-1	19,434,708	CM001462_19434708	G	A
102	CM001462-1	24,175,402	CM001462_24175402	T	A
103	CM001462-1	26,455,950	CM001462_26455950	C	T
104	CM001463-1	4,499,898	CM001463_4499898	A	T
105	CM001463-1	7,000,925	CM001463_7000925	G	A
106	CM001463-1	15,600,075	CM001463_15600075	G	.
107	CM001463-1	18,049,382	CM001463_18049382	T	C
108	CM001463-1	24,245,660	CM001463_24245660	T	A
109	CM001463-1	26,006,850	CM001463_26006850	G	.
110	CM001464-1	3,465,451	CM001464_3465451	A	G
111	CM001464-1	8,616,848	CM001464_8616848	C	T
112	CM001464-1	13,343,549	CM001464_13343549	T	.
113	CM001464-1	15,693,057	CM001464_15693057	A	G
114	CM001464-1	25,129,247	CM001464_25129247	T	C
115	CM001465-1	491,146	CM001465_491146	C	.
116	CM001465-1	3,022,972	CM001465_3022972	G	A
117	CM001465-1	5,551,677	CM001465_5551677	A	G
118	CM001465-1	11,568,956	CM001465_11568956	G	A

119	CM001465-1	15,007,687	CM001465_15007687	C	A
120	CM001465-1	16,070,926	CM001465_16070926	C	T

Appendix 3: Sample collection sheet in fish hatcheries and farms

Part I: Tilapia samples from fish hatcheries

S/N	Sample ID	Place of Collection	Sex	Lat	Long	Species	Confirmed tilapiamap
1	P01-G10-LH3	Ruhila Aquaculture Development Centre	Female	-10.622	35.64	Rendalli	
2	P02-B12-LH8	Ruhila Aquaculture Development Centre	Male	-10.622	35.64	Niloticus	Possible hybrid
3	P02-D01-LH7	Ruhila Aquaculture Development Centre	Male	-10.622	35.64	Niloticus	Possible hybrid
4	P02-D10-LH6	Ruhila Aquaculture Development Centre	Male	-10.622	35.64	Niloticus	Possible hybrid
5	P02-F01-LH19	Ruhila Aquaculture Development Centre	Male	-10.622	35.64	leucostictus	Possible hybrid
6	P02-F11-LH5	Ruhila Aquaculture Development Centre	Male	-10.622	35.64	Rendalli	
7	P01-C05-SAFINA11	Big Fish Safina	Female	-6.8726	39.34		Hybrid
8	P01-C11-SAFINA14	Big Fish Safina	Male	-6.8726	39.34		Hybrid
9	P02-F03-SAFINA12	Big Fish Safina	Female	-6.8726	39.34		Hybrid
10	P02-F12-SAFINA15	Big Fish Safina	Male	-6.8726	39.34		Hybrid
11	P01-G05-F1	Faiza Fish Hatchery-Kiromo, Bagamoyo	Male	-6.4675	38.9	Hybrid	Possible hybrid
12	P01-H04-F13	Faiza Fish Hatchery-Kiromo, Bagamoyo	Male	-6.4675	38.9	Hybrid	Possible hybrid
13	P02-E07-F6	Faiza Fish Hatchery-Kiromo, Bagamoyo	Female	-6.4675	38.9	Hybrid	Possible hybrid
14	P01-E04-F4	Faiza Fish Hatchery-Kiromo, Bagamoyo	Female	-6.4675	38.9	Hybrid	Possible hybrid
15	P01-A06-822KJ16	Bulamba, Bunda- Musoma	Female	-2.1379	33.64		
16	P01-A12-822KJ14	Bulamba, Bunda- Musoma	Male	-2.1379	33.64		
17	P01-B04-822KJ12	Bulamba, Bunda- Musoma	Female	-2.1379	33.64		
18	P01-D04-822KJ10	Bulamba, Bunda- Musoma	Male	-2.1379	33.64	niloticus	
19	P01-E01-822KJ4	Bulamba, Bunda- Musoma	Male	-2.1379	33.64		
20	P01-F08-822KJ3	Bulamba, Bunda- Musoma	Female	-2.1379	33.64		
21	P01-F09-882KJ2	Bulamba, Bunda- Musoma	Male	-2.1379	33.64		
22	P01-G08-822KJ8	Bulamba, Bunda- Musoma	Male	-2.1379	33.64		
23	P01-G09-822KJ13	Bulamba, Bunda- Musoma	Male	-2.1379	33.64		
24	P01-H07-822KJ6	Bulamba, Bunda- Musoma	Female	-2.1379	33.64		
25	P02-C04-822KJ15	Bulamba, Bunda- Musoma	Female	-2.1379	33.64		
26	P02-C06-822KJ11	Bulamba, Bunda- Musoma	Female	-2.1379	33.64	niloticus	
27	P02-D03-822KJ9	Bulamba, Bunda- Musoma	Female	-2.1379	33.64		
28	P02-D05-822KJ1	Bulamba, Bunda- Musoma	Female	-2.1379	33.64	niloticus	
29	P02-D12-822KJ5	Bulamba, Bunda- Musoma	Female	-2.1379	33.64		
30	P02-E02-822KJ7	Bulamba, Bunda- Musoma	Female	-2.1379	33.64		
31	P01-B06-DSM25	Kinyerezi, Chanika, Ilala, Dar Es Salaam	Female	-6.9366	39.13	hybrid	
32	P01-H01-DSM7	Kinyerezi, Chanika, Ilala, Dar Es Salaam	Male	-6.9366	39.13	hybrid	
33	P02-A05-DSM29	Kinyerezi, Chanika, Ilala, Dar Es Salaam	Male	-6.9366	39.13	hybrid	
34	P02-B02-DSM4	Kinyerezi, Chanika, Ilala, Dar Es Salaam	Male	-6.9366	39.13	hybrid	

35	P02-E08-DSM2	Kinyerezi, Chanika, Ilala, Dar Es Salaam	Female	-6.9366	39.13	hybrid	
36	P02-H09-DSM8	Kinyerezi, Chanika, Ilala, Dar Es Salaam	Male	-6.9366	39.13	hybrid	
37	P01-C01-SAMEKI1	Kamanga- Mwanza	Male	2.5189	32.9	hybrid	
38	P01-E11-SAMEKI5	Kamanga- Mwanza	Male	2.5189	32.9	hybrid	
39	P01-H02-SAMEKI2	Kamanga- Mwanza	Male	2.5189	32.9	hybrid	
40	P02-B04-SAMEKI4	Kamanga- Mwanza	Male	2.5189	32.9	hybrid	
41	P01-D07-KINGOL26	Morogoro	Male	-6.77	37.76	hybrid	
42	P01-F01-KINGOL17	Morogoro	Male	-6.77	37.76	hybrid	
43	P02-C02-KINGOL10	Morogoro	Male	-6.77	37.76	hybrid	
44	P02-D04-KINGOL6	Morogoro	Female	-6.77	37.76	hybrid	
45	P01-B08-R24	Miswe-Mlandizi-Kibaha	Male	-6.8	39.28	hybrid	Possible hybrid
46	P01-C04-R21	Miswe-Mlandizi-Kibaha	Male	-6.8	39.28	hybrid	possible hybrid
47	P01-D08-R13	Miswe-Mlandizi-Kibaha	Female	-6.8	39.28	hybrid	possible hybrid
48	P01-G02-R5	Miswe-Mlandizi-Kibaha	Male	-6.8	39.28	hybrid	possible hybrid
49	P01-F12-KOROGWE15	Kwamngumi Prison	Male	-5.162	38.49	niloticus	very dark
50	P02-A01-KOROGWE1	Kwamngumi Prison	Male	-5.162	38.49	niloticus	weakly striped tail
51	P02-C01-KOROGWE7	Kwamngumi Prison	Male	-5.162	38.49	rendalli	
52	P01-A07-MAC21	Mwamapuli- Igunga	Female	-4.3647	33.88		
53	P01-A09-MAC7	Mwamapuli- Igunga	Male	-4.3647	33.88		
54	P01-A11-MAC24	Mwamapuli- Igunga	Female	-4.3647	33.88		
55	P01-B03-MAC4	Mwamapuli- Igunga	Female	-4.3647	33.88		
56	P01-B07-MAC30	Mwamapuli- Igunga	Female	-4.3647	33.88		
57	P01-C08-MAC22	Mwamapuli- Igunga	Female	-4.3647	33.88		
58	P01-D02-MAC9	Mwamapuli- Igunga	Female	-4.3647	33.88		
59	P01-D03-MAC29	Mwamapuli- Igunga	Female	-4.3647	33.88		
60	P01-D06-MAC20	Mwamapuli- Igunga	Female	-4.3647	33.88		
61	P01-E06-MAC17	Mwamapuli- Igunga	Male	-4.3647	33.88		
62	P01-E10-MAC25	Mwamapuli- Igunga	Male	-4.3647	33.88		
63	P01-F10-MAC23	Mwamapuli- Igunga	Female	-4.3647	33.88		
64	P01-G03-MAC8	Mwamapuli- Igunga	Male	-4.3647	33.88		
65	P01-G06-MAC15	Mwamapuli- Igunga	Female	-4.3647	33.88		
66	P01-H09-MAC26	Mwamapuli- Igunga	Female	-4.3647	33.88		
67	P01-H10-MAC1	Mwamapuli- Igunga	Male	-4.3647	33.88		
68	P02-A06-MAC16	Mwamapuli- Igunga	Male	-4.3647	33.88		
69	P02-A08-MAC6	Mwamapuli- Igunga	Male	-4.3647	33.88		
70	P02-A11-MAC28	Mwamapuli- Igunga	Female	-4.3647	33.88		
71	P02-B01-MAC13	Mwamapuli- Igunga	Female	-4.3647	33.88		
72	P02-B06-MAC14	Mwamapuli- Igunga	Male	-4.3647	33.88		
73	P02-E11-MAC18	Mwamapuli- Igunga	Male	-4.3647	33.88		

74	P02-F10-MAC12	Mwamapuli- Igunga	Male	- 4.3647	33.8 8		
75	P02-G01-MAC3	Mwamapuli- Igunga	Female	- 4.3647	33.8 8		
76	P02-G04-MAC2	Mwamapuli- Igunga	Male	- 4.3647	33.8 8		
77	P02-G06-MAC5	Mwamapuli- Igunga	Female	- 4.3647	33.8 8		
78	P02-G08-MAC10	Mwamapuli- Igunga	Female	- 4.3647	33.8 8		
79	P02-H07-MAC11	Mwamapuli- Igunga	Female	- 4.3647	33.8 8		
80	P02-H08-MAC27	Mwamapuli- Igunga	Female	- 4.3647	33.8 8		
81	P02-H10-MAC19	Mwamapuli- Igunga	Male	- 4.3647	33.8 8		

Part II: Tilapia samples from fish farms

Agroecological zone	No.	Sample ID	Place of Collection	Sex	Lat	Long	Species	State of sample
Western	1	**P01-A02-MP5**	Mpanda- Katavi	Male	-6.335	31.08	Leucostictus	
	2	**P02-D02-MP4**	Mpanda- Katavi	Female	-6.34	31.08	Leucostictus	
	3	**P01-E12-RM2**	Tabora	Male	4.21	33.54		SNP IDandNo photo
	4	**P02-B09-GM4**	Igunga- Tabora	Male	4.21	33.53		SNP IDandNo photo
	5	**P02-F07-GM5**	Igunga- Tabora	Male	4.21	33.53		SNP IDandNo photo
	6	**P02-B08-RM1**	Tabora	Male	4.21	33.54		SNP IDandNo photo
Lake	7	**P01-A03-LFF2**	Nyamagana- Mwanza	Female	2.36	32.53		SNP IDandNo photo
	8	**P01-H11-LFF1**	Nyamagana- Mwanza	Male	2.36	32.53		SNP IDandNo photo
	9	**P02-A10-KFF3**	Sengerema- Mwanza	Male	2.31	32.56		SNP IDandNo photo
	10	**P02-C07-NYEGEZI2**	Nyegezi- Mwanza	Male	2.35	32.54		SNP IDandNo photo
	11	**P01-B01-BZ4**	Chato- Geita	Female	-2.64	31.778		SNP IDandNo photo
	12	**P02-D02- BZS3**	Buzirayombo Site	Chato- Geita	-2.64	31.778		SNP IDandNo photo
	13	**P01-B01-BZ2**	Chato- Geita	Female	-2.64	31.778		SNP IDandNo photo
	14	**P01-C12-KADUDA3**	Chato- Geita	Male	-2.64	31.778		no tilapia map records
	15	**P01-G04-KSG1**	Chato- Geita	Female	-2.64	31.778	niloticus	PhotoandSNP ID
	16	**P02-A12-KADUDA2**	Chato- Geita	Male	-2.64	31.778		no tilapia map records
	17	**P02-C08-BZS1**	Chato- Geita	Male	-2.64	31.778		no tilapia map records
	18	**P02-D08-KSG2**	Chato- Geita	Male	-2.64	31.778	niloticus	PhotoandSNP
	19	**P01-A04-JB1**	Muleba- Kagera	Male	-1.562	31.711	niloticus	PhotoandSNP
	20	**P01-D11-JOSE1**	Muleba- Kagera	Male	1.05	31.5		SNP IDandNo photo
	21	**P02-E06-NF2**	Muleba- Kagera	Female	1.46	30.56		SNP IDandNo photo
	22	**P02-G09-JB2**	Muleba- Kagera	Female	-1.562	31.711	niloticus	photoandSNP
	23	**P01-C03-NF1**	Muleba- Kagera	Male	1.46	30.56		SNP IDandNo photo
Central	24	**P01-B02-UDOM5**	University Of Dodoma	Male	6.13	35.49		SNP IDandNo photo
	25	**P01-B09-UDOM6**	University Of Dodoma	Male	6.13	35.49		SNP IDandNo photo
	26	**P01-F05-CHAMWINO1**	Chamwino- Dodoma	Male	6.13	35.45		SNP IDandNo photo
	27	**P01-H12-CHAMWINO3**	Chamwino- Dodoma	Male	6.13	35.45		SNP IDandNo photo
	28	**P02-A09-JKT6**	Jkt- Dodoma	Male	6.08	35.5		SNP IDandNo photo
	29	**P02-H11-JKT5**	Jkt- Dodoma	Male	6.08	35.5		SNP IDandNo photo
Southern Highland	30	**P01-A05-DS1**	Mbeya	Male	8.87	33.54		SNP IDandNo photo
	31	**P01-H05-MM1**	Mbalizi- Mbeya	Male	8.561	33.2047		SNP IDandNo photo
	32	**P02-A02-SHE4**	Shamwengo- Mbeya	Female	-8.88	33.6	urolepis	photos code SH3,SH4 etc, 2 uncoded photos show ON/hybrids
	33	**P02-A04-SHE3**	Shamwengo- Mbeya	Male	-8.88	33.6	urolepis	photos code SH3,SH4 etc, 2 uncoded photos show ON/hybrids
	34	**P02-G10-IT-1**	Itimba- Mbeya	Male	8.47	33.43		SNP IDandNo photo

	35	**P01-A08-MIT1**	Mitawa- Songea	Male	-10.6	35.656	niloticus	PhotoandSNP
	36	**P02-C10-MIT2**	Mitawa- Songea	Female	-10.6	35.656	niloticus	PhotoandSNP
	37	**P02-F05-SOFFA2**	Msamala- Songea	Female	-10.6	35.64	ON hybrid	PhotoandSNP
	38	**P02-H03-SOFFA1**	Msamala- Songea	Female	-10.6	35.64	ON hybrid	PhotoandSNP
	39	**P01-C07-S12**	Ushirika - Rungwe	Female	9.22	30.41		SNP IDandNo photo
	40	**P01-C06-S3**	Ndubi - Rungwe	Female	9.19	33.41		SNP IDandNo photo
	41	**P01-D01-S14**	Lubigi - Rungwe	Female	9.13	33.35		SNP IDandNo photo
	42	**P01-D10-S2**	Segera - Rungwe	Male	9.13	33.35		SNP IDandNo photo
	43	**P01-E02-S7**	Ushirika - Rungwe	Female	9.13	33.35		SNP IDandNo photo
	44	**P01-E07-S8**	Bujinga - Rungwe	Male	9.1	33.32		SNP IDandNo photo
	45	**P02-A03-S13**	Lubigi - Rungwe	Female	9.1	33.32		SNP IDandNo photo
	46	**P02-B05-S9**	Bujinga - Rungwe	Female	9.08	33.32		SNP IDandNo photo
	47	**P02-C05-S15**	Ilundo - Rungwe	Female	9.08	33.32		SNP IDandNo photo
	48	**P02-F02-S4**	Ndubi - Rungwe	Female	9.08	33.32		SNP IDandNo photo
	49	**P02-G07-S10**	Bujinga - Rungwe	Male	9.12	33.35		SNP IDandNo photo
	50	**P02-G12-S1**	Bujela - Rungwe	Male	9.1	33.32		SNP IDandNo photo
	51	**P02-H01-S11**	Kyimo - Rungwe	Male	9.12	33.34		SNP IDandNo photo
	52	**P02-H06-S5**	Ndubi - Rungwe	Female	9.12	33.34		SNP IDandNo photo
	53	**P02-G05-S6**	Ushirika - Rungwe	Male	9.1	33.33		SNP IDandNo photo
Eastern	54	**P01-A10-KF2**	Kinondoni- Dar Es Salaam	Female	6.403	39.1234		SNP IDandNo photo
	55	**P01-C09-DZ1**	Kigamboni- Dar Es Salaam	Female	6.49	39.1058		SNP IDandNo photo
	56	**P01-F02-JUDASA2**	Ubungo- Dar Es Salaam	Female	6.49	39.1058		SNP IDandNo photo
	57	**P02-B03-JUDASA1**	Ubungo- Dar Es Salaam	Male	6.49	39.1058		SNP IDandNo photo
	58	**P02-F09-DZ2**	Kigamboni- Dar Es Salaam	Female	6.49	39.1058		SNP IDandNo photo
	59	**P02-E04-KF1**	Kinondoni- Dar Es Salaam	Female	6.403	39.1234		SNP IDandNo photo
	60	**P01-B11-KAPAGALA1**	Mvomero, Morogoro	Male	6.51	37.37		SNP IDandNo photo
	61	**P01-C02-RF4**	Bagamoyo- Coast Region	Male	6.353	38.415		SNP IDandNo photo
	62	**P01-F03-K13**	Bagamoyo- Coast Region	Male	-6.45	38.918	niloticus	few stripes
	63	**P02-B10-K11**	Bagamoyo- Coast Region	Male	-6.45	38.918	ON hybrid	pink tail, no stripes
	64	**P02-C09-JF3**	Kibaha-Coast Region	Male	6.43	38.4434		SNP IDandNo photo
	65	**P02-C12-JF1**	Kibaha-Coast Region	Female	6.43	38.4434		SNP IDandNo photo
	66	**P02-G03-RF1**	Bagamoyo- Coast Region	Male	6.353	38.415		SNP IDandNo photo
	67	**P01-B12-MV1**	Mvomero, Morogoro	Male	6.51	37.37		SNP IDandNo photo
	68	**P01-F11-SUA1**	Sua- Morogoro	Male	-6.85	37.60	ON Hybrid?	
	69	**P02-E10-SUA2**	Sua- Morogoro	Male	-6.85	37.60	ON Hybrid?	
	70	**P01-E08-KANGE1**	Kange- Tanga City	Male	-5.08	39.05	niloticus	
	71	**P01-H06-PONGWE1**	Pongwe-Tanga	Male	-5.13	38.975	niloticus	
	72	**P01-G12-KANGE5**	Kange- Tanga City	Female	-5.08	39.05	niloticus	possible hybrid
	73	**P02-E09-PONGWE4**	Pongwe-Tanga	Male	-5.13	38.975	niloticus	possible hybrid

	74	**P02-G11-CHONGOL2**	Chongoleani-Tanga	Male	-5	39.132	urolepis	possible hybrid
Southern	75	**P01-B10-MK3**	**Masasi-Mtwara**	**Male**	**-10.7**	**38.8**		
	76	**P01-C10-MS4**	Masasi-Mtwara	Male	-10.7	38.83		
	77	**P02-A07-MK2**	**Masasi-Mtwara**	**Male**	**-10.7**	**38.8**		
	78	**P02-H04-MS3**	Masasi-Mtwara	Male	-10.7	38.83		
Northern	79	**P01-B05-MJ1**	Babati-Manyara	Male	3.48	35.49	niloticus	
	80	**P01-D12-BABATI1**	Babati Manyara	Male	-4.23	35.74		
	81	**P01-E03-MJ2**	Babati-Manyara	Female	3.48	35.49		
	82	**P02-D07-BABATI4**	Babati Manyara	Male	-4.23	35.74		
	83	**P02-E03-JJ2**	Babati- Manyara	Male	3.26	35.41		
	84	**P02-E05-JJ1**	Babati- Manyara	Male	3.26	35.41		
	85	**P01-E05-MURO2**	Hai Kilimanjaro	Male	-3.33	37.26	ON hybrid	
	86	**P01-F04-KIRYA1**	Mwanga, Kilimanjaro	Male	3.55	37.28		SNP IDandNo photo
	87	**P01-F06-MASSAWE1**	Hai Kilimanjaro	Female	-3.29	37.22	ON hybrid	
	88	**P01-G07-KIRYA2**	Mwanga, Kilimanjaro	Female	3.55	37.28		SNP IDandNo photo
	89	**P01-G11-MURO1**	Hai Kilimanjaro	Female	-3.33	37.26		SNP IDandNo photo
	90	**P02-D06-ROMBO2**	Rombo Moshi	Male	-3	37.57	ON	
	91	**P02-D09-KARANGA-B3**	Moshi Mjini	Female	-3.34	37.3		SNP IDandNo photo
	92	**P02-D11-KARANGA-B2**	Moshi Mjini	Male	-3.34	37.3	ON	
	93	**P02-H02-MASSAWE2**	Hai Kilimanjaro	Male	-3.29	37.22		SNP IDandNo photo
	94	**P02-H05-ROMBO1**	Rombo Moshi	Male	-3	37.57	ON	
	95	**P01-E09-ARUSHA1**	Arusha	Male	-3.38	36.7	ON	
	96	**P01-F07-ARUSHA2**	Arusha	Male	-3.38	36.7	ON	
	97	**P01-G01-MERU2**	Meru- Arusha	Male	3.21	36.43	Leucostictus	
	98	**P02-F04-MERU1**	Meru- Arusha	Male	3.21	36.43	Leucostictus	
Natural water bodies	99	**P02-B07-KUMBA8**	Korogwe	Male	-4.806	38.62163	niloticus	
	100	**P02-B11-KUMBA-3**	**Korogwe**	Male	-4.806	38.62163	jipe	
	101	**P02-C03-Kumba**	Korogwe	Male	-4.806	38.62163	niloticus	
	102	**P02-C11-KUMBA7**	**Korogwe**	Female	-4.806	38.62163	niloticus	
	103	**P02-F06-KUMBA-4**	Korogwe	Male	-4.806	38.62163	coptodon	
	104	**P01-D09-LBABATI1**	**Manyara**	Male	-4.26	35.73	ON hybrid	
	105	**P02-E12-LBABATI-2**	**Manyara**	**Male**	-4.26	35.73	ON hybrid	resembles spilurus
	106	**P01-H03-NYUMBA-YA-MUNGU-2**	**Mwanga, Kilimanjaro**	Male	-3.67	37.41		
	107	**P02-E01-NYUMBA-YA-MUNGU-1**	**Mwanga, Kilimanjaro**	**Male**	-3.67	37.41	jipe	
	108	**P02-F08-UGALA-RIVER1**	**Uvinza -Kigoma**	Male	5.06	30.28		
	109	**P01-D05-MARA-RIVER1**	Mara	Male	1.31	33.58		

Appendix 4: Number of pure and hybrid fish identified for each tilapia species in different farms

Agroecological zone	Farm	*O.niloticus*	*O.lecostictus*	*O.urolepsi*	*Coptodon*	Hybrid	Unkown	Total number of fish genotyped
Western zone	Mpanda-Katavi	-	2	-	-	-	-	**2**
	Igunga (R)-Tabora	-	-	-	-	1	1	**2**
	Igunga(G)-Tabora	-	-	-	-	-	2	**2**
Lake zone	Luchelele-Nyegezi	-	-	-	-	2	-	**2**
	Kaduda-Chato	-	-	-	-	2	-	**2**
	Buzirayombo-Chato	1	-	-	-	1	-	**2**
	Kasagara-Chato	2	-	-	-	-	-	**2**
	Muleba (N)-Kagera	-	-	-	-	2	-	**2**
	Muleba (J&B)-Kagera	2	-	-	-	-	-	**2**
	Nyegezi-Nyamagana	1	-	-	-	-	-	**1**
	Kitana-Sengerema	-	-	-	-	1	-	**1**
	Muleba-Kagera	-	-	-	-	1	-	**1**
Central zone	University of Dodoma	-	-	-	-	2	-	**2**
	Chamwino-Dodoma	-	-	-	-	2	-	**2**
	JKT- Dodoma	-	-	-	-	2	-	**2**
Southern Highland zone	Shamwengo-Mbeya	1	-	1	-	-	-	**2**
	SOFFA-Songea	-	-	-	-	2	-	**2**
	Msamala-Songea	-	-	-	-	2	-	**2**
	Mitawa-Songea	2	-	-	-	-	-	**2**
	Ushirika-Rungwe	-	-	-	3	-	-	**3**
	Ndubi-Rungwe	-	-	-	3	-	-	**3**
	Lubigi-Rungwe	-	-	-	1	1	-	**2**
	Bujinga-Rungwe	-	-	-	2	1	-	**3**

	SISO-Mbeya	-	-	-	-	1	-	**1**
	Mbalizi-Mbeya	-	-	-	-	1	-	**1**
	Itimba-Mbeya	-	-	-	-	1	-	**1**
	Segera-Rungwe	-	-	-	1	-	-	**1**
	Ilundo-Rungwe	-	-	-	-	1	-	**1**
	Bujera-Rungwe	-	-	-	1	-	-	**1**
	Kyimo-Rungwe	-	-	-	-	1	-	**1**
Eastern zone	Kunduchi-Kinondoni	-	-	-	-	2	-	**2**
	Kigamboni- Dar es Salaam	-	-	-	-	2	-	**2**
	Kimara-Ubungo	-	-	-	-	2	0	**2**
	Miswe-Kibaha	-	-	-	-	1	1	**2**
	FETA-Bagamoyo	-	-	-	-	2	-	**2**
	Miswe (JF)-Kibaha	-	-	-	-	2	-	**2**
	Mvomero-Morogoro	-	-	-	-	2	-	**2**
	SUA-Morogoro	2	-	-	-	-	-	**2**
	Kange-Tanga	1	-	-	-	1	-	**2**
	Pongwe-Tanga	1	-	-	-	1	-	**2**
	Chongoleai-Tanga	-	-	-	-	1	-	**1**
Southern zone	Mkadaenda-Masasi	-	-	-	-	2	-	**2**
	Mshikamano-Masasi	-	1	-	-	1	-	**2**
Northern zone	Babati(M)-Manyara	-	-	-	-	2	-	**2**
	Babati (J)-Manyara	1	-	-	-	1	-	**2**
	Babati-Manyara	-	-	-	-	2	-	**2**
	Hai(MFF)-Kilimanjaro	1	-	-	-	1	-	**2**
	Hai(MF)-Kilimanjaro	1	-	-	-	1	-	**2**
	Kirya-Mwanga	-	-	-	-	2	-	**2**

	Rombo-Kilimanjaro	-	-	-	-	2	-	**2**
	Karanga -Moshi	1	-	-	-	1	-	**2**
	Arusha	-	-	-	-	2	-	**2**
	Meru - Arusha	1	-	-	-	1	-	**2**
	Babati -Manyara	-	-	-	-	2	-	**2**
Natural Water bodies	Mara River	-	-	-	1	-	-	**1**
	Lake Babati	1	-	-	-	1	-	**2**
	Kumba Dam	-	1	-	-	3	-	**4**
	Nyumba ya Mungu Dam	-	-	-	-	-	*O. jipe*	**1**
	Ugala River	-	-	-	-	1	-	**1**
	Total	**19**	**4**	**1**	**12**	**67**	**5**	**109**

Appendix 5: Approval of MSc research proposal

SOKOINE UNIVERSITY OF AGRICULTURE
DIRECTORATE OF POSTGRADUATE STUDIES, RESEARCH, TECHNOLOGY TRANSFER AND CONSULTANCY
P.O. Box 3151, MOROGORO, Tanzania,
Tel: +255 23 264 0013, 023 264006-9, E-mail Address: drpgs@sua.ac.tz

Our Ref:	MHA/D/2018/0004/12	**Our Date**	25th September, 2019

Mr. Mashaka Shabani,
Department of Veterinary Medicine and Public Health,
SUA – Morogoro.

U.f.s Head,
Department of Veterinary Medicine and Public Health,
SUA – Morogoro.

U.f.s Principal,
College of Veterinary Medicine and Biomedical Sciences,
SUA – Morogoro.

Forwarded 30/9/2019

Forwarded 27/9/2019

Dear Mr. Shabani,

RE: APPROVAL OF YOUR MSc. (AQUATIC ANIMAL RESOURCES) RESEARCH PROPOSAL

Please refer to the above captioned subject.

This is to inform you that, the Chairman of Senate Postgraduate Studies Committee (SPGSC) has noted the approval made by the Board of College of Veterinary Medicine and Biomedical Sciences for your MSc. research proposal. This means, you are now permitted to conduct research as per your approved research proposal.

In addition to the permission granted, please be reminded that, you are required to duly fill in progress report for the period ended June, 2019 and submit the same.

Wishing you all the best in your research work.

Yours sincerely,

P. L. Mresa,
For: DIRECTOR.

Director
Postgraduate studies, Research,
Technology Transfer and Consultancy
Sokoine University of Agriculture
P. O. Box 3151, Morogoro
TANZANIA

Appendix 6: Permission for conducting MSc research

CLEARANCE PERMIT FOR CONDUCTING RESEARCH IN TANZANIA

SOKOINE UNIVERSITY OF AGRICULTURE
OFFICE OF THE VICE-CHANCELLOR
P.O. Box 3000 CHUO KIKUU, MOROGORO, TANZANIA
Phone: 255-023-2640006/7/8/9, Direct VC: 2640015; Fax: 2640021;
Email: vc@sua.ac.tz;

Our Ref. SUA/ADM/R.1/8/453 **Date:** 10th October, 2019

The Regional Administrative Secretary,
Geita Region,
P.O. Box 315,
GEITA.

Re: UNIVERSITY STAFF, STUDENTS AND RESEARCHERS CLEARANCE

The Sokoine University of Agriculture was established by University Act No. 7 of 2005 and SUA Charter, 2007 which became operational on 1st January 2007 repealing Act No. 6 of 1984. One of the mission objectives of the University is to generate and apply knowledge through research. For this reason the staff and researchers undertake research activities from time to time.

To facilitate the research function, the Vice Chancellor of the Sokoine University of Agriculture (SUA) is empowered to issue research clearance to staff, students, research associate and researchers of SUA on behalf of the Tanzania Commission for Science and Technology.

The purpose of this letter is to introduce to you **Mr. Mashaka Shabani** a bonafide **MSc. (Health of Aquatic Animal Resources)** student with Registration number **MHA/D/2018/0004** of SUA. By this letter **Mr. Mashaka Shabani** has been granted clearance to conduct research in the country. The title of the research in question is **"Assessment of Genetic Diversity and Purity of Farmed Tilapia Fish in Tanzania".**

The period for which this permission has been granted is from **November, 2019** to **April, 2020**. The research will be conducted in **Chato District.**

Should some of these areas/institutions/offices be restricted, you are requested to kindly advice the researcher(s) on alternative areas/institutions/ offices which could be visited. In case you may require further information on the researcher please contact me.

We thank you in advance for your cooperation and facilitation of this research activity.

Yours sincerely,

Prof. Peter R. Gillah
FOR: VICE-CHANCELLOR

Copy to: Student – **Mr. Mashaka Shabani**

VICE CHANCELLOR
SOKOINE UNIVERSITY OF AGRICULTURE