Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2020:62
Faculty of Veterinary Medicine and Animal Science

Towards a sustainable tilapia breeding program in Tanzania

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SWEDISH UNIVERSITY
OF AGRICULTURAL
SCIENCES

DOCTORAL THESIS
Uppsala 2020

Acta Universitatis agriculturae Sueciae
2020:62

Cover: Aquaculture facilities at Kunduchi campus
(photo: Redempta Athanas Kajungiro)

ISSN 1652-6880
ISBN (print version) 978-91-7760-642-0
ISBN (electronic version) 978-91-7760-643-7

Uppsala
Print: SLU Service/Repro, Uppsala 2020

Towards a sustainable tilapia breeding program in Tanzania

Abstract

A structured breeding program is an important step towards sustainable tilapia aquaculture in Tanzania. The aim of the thesis was to generate baseline information for the establishment of tilapia breeding program in Tanzania.

In the first study of this thesis we determined the genetic diversity between and within local and exotic Nile tilapia (*Oreochromis niloticus*) and Rufiji strains (*Oreochromis urolepis urolepis*) in Tanzania. Strains of FETA, Victoria, Igunga and TAFIRI had low genetic variation ranging from 0.057 to 0.1 while Kunduchi, Karanga and Ruhila showed highest genetic diversity from 0.214 to 0.212. Strong genetic differentiation was revealed between Karanga and the closely related strains of FETA, Lake Victoria, and Igunga with values from 0.533 to 0.548. STRUCTURE analysis revealed highly admixture among Karanga, Kunduchi, and Ruhila strains while FETA, Victoria, Igunga and TAFIRI showed no admixture. Higher genetic variation was also revealed among Rufiji strains compared to exotic and local Nile tilapia strains. High F_{ST} values (0.6- 0.8) were observed between Rufiji strains and the local or exotic Nile tilapia strains.

The second part of this thesis based on a common garden experiment where the different strains of tilapia were compared in two environments with differing salinity. Differences in growth performance for all body traits were significant among Nile tilapia strains ($P< 0.001$) and strains ranked differently across the two environments. In freshwater environment, Karanga strain ranked first while TAFIRI strain ranked last. Regarding brackish water environment, Igunga strain was ranked first and the Victoria strain ranked last. Heritability estimates for harvest weight were low in both freshwater (0.10) and brackish water (0.09) environments compared to weight at tagging. Genetic correlations were low (0.35) for harvest weight and families ranked differently across the two environments indicating the existence of substantial GxE. Our results suggest that some strains are better suited than others to form the basis of a selective breeding program of Nile tilapia in Tanzania. Base population for the breeding program needs to strike the right balance between picking the best performing strains and having a broad genetic basis. Caution though is needed due to the high GxE across the tested environments.

Keywords: Aquaculture, genetic diversity, Nile tilapia, ddRADseq, common garden, genetic parameters, brackish environment, freshwater environment

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Towards a sustainable tilapia breeding program in Tanzania

Abstract

Ett välstrukturerat avelsprogram är ett viktigt steg mot en ökad och hållbar odling av tilapia i Tanzania. Syftet med avhandlingen var att ta fram grundläggande information för att kunna upprätta ett avelsprogram för tilapia i Tanzania.

I den första delen av avhandlingen undersökte vi den genetiska variationen mellan och inom lokala och exotiska stammar av niltilapia (*Oreochromis niloticus*) och rufijitilapia (*Oreochromis urolepis urolepis*). Bland de lokala niltilapia-stammarna hade FETA, Victoria, Igunga och TAFIRI låg genetisk variation (från 0,057 till 0,1) medan stammarna Kunduchi, Karanga och Ruhila hade störst genetisk variation (från 0,214 till 0,212). Stor genetisk differentiering sågs mellan Karanga och de närliggande stammarna FETA, Lake Victoria och Igunga med värden från 0,533 till 0,548. STRUCTURE-analys visade på stor genetisk inblandning i stammarna Karanga, Kunduchi och Ruhila, medan FETA, Victoria, Igunga och TAFIRI inte hade någon inblandning. Större genetisk variation sågs bland rufiji-stammarna jämfört med de exotiska och lokala stammarna av niltilapia. Höga F_{ST}-värden, från 0,6 to 0,8, observerades mellan Rufiji-stammarna och de lokala eller exotiska niltilapia-stammarna.

Den andra delen av avhandlingen baserades på ett common-garden-försök i vilket de olika tilapia-stammarna jämfördes i två miljöer med olika salthalt. Signifikanta skillnader mellan niltilapia-stammarna avseende tillväxt sågs för alla studerade kroppsegenskaper ($P< 0.001$) och stammarna rankades olika i de två studerade miljöerna. I sötvattensmiljön rankades Karanga-stammen högst medan TAFIRI-stammen rankades lägst. I brackvattenmiljön rankades Igunga-stammen högst och Victoria-stammen rankades lägst. Heritabiliteten för skördevikt var låg i både sötvatten- (0,10) och brackvattenmiljön (0,09) jämfört med vikt vid PiT-märkning. Den genetiska korrelationen var låg (0,35) för skördevikt och familjerna rankades olika i de två miljöerna, vilket indikerar förekomst av betydande GxE. Våra resultat tyder på att vissa stammar är mer lämpliga som basgeneration i ett avelsprogram än andra. Vid val av basgeneration för avelsprogrammet krävs rätt balans mellan hög tillväxt och stor genetisk variation. Försiktighet krävs dock på grund av hög GxE i de båda testade miljöerna.

Keywords: Akvakultur, genetisk variation, niltilapia, ddRADseq, common garden, genetiska parametrar, brackvattenmiljö, sötvattenmiljö

Dedication

To my lovely husband *Emmanuel Mrisho Sungwa*, thank you for your support, encouragement, motivation, prayers, patient and for always being there for me. This journey could have not been easy without your endless love. God bless you always.

To my lovely mother *Grace Simon Rugaimukamu*, thank you for encouragement. I remember you asked me if I will write that big book (Thesis). Yes, mom the book is ready

To all my family and friends in Tanzania and Sweden for their love and support. God bless you abundantly.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Kajungiro, R. A., Palaiokostas, C., Pinto, F. A. L., Mmochi, A. J., Mtolera, M., Houston, R. D., and De Koning, D. J. (2019). Population Structure and Genetic Diversity of Nile Tilapia *(Oreochromis niloticus*) Strains Cultured in Tanzania*. Frontiers in Genetics*, 10, pp. 1269.

II. Kajungiro*, R. A., Nyinondi* C., Mbiru*, M., Palaiokostas, C., Pinto, F. A. L., Norman-Haldén, A., Mtolera, M., Houston, R. D., and de Koning, D. J. Using ddRAD sequencing to assess genetic differentiation within and between exotic and local strains of Nile tilapia (*Oreochromis niloticus)* and Rufiji tilapia (*Oreochromis urolepis urolepis)* (*manuscript)*

III. Kajungiro, R. A., Palaiokostas, C., Norman Haldén, A., Mwita, C., Mtolera, M. and De Koning, D. J. Common garden comparison of native Nile tilapia (*Oreochromis niloticus*) strains in brackish and freshwater environments. *Aquaculture international (Submitted*).

IV. Kajungiro, R. A., Palaiokostas, C., Norman Haldén, A., Mwita, C., Mtolera, M. and De Koning, D. J. Genetic Parameter Estimates and Genotype by Environment Interaction in Nile Tilapia (*Oreochromis niloticus)* strains Cultured in Tanzania *(manuscript*).

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***** These authors contributed equally.

The contribution of Redempta Athanas Kajungiro to the papers included in this thesis was as follows:

I. Took part in planning the study, performed field work by collecting fish from local government hatcheries, performed data analyses with the help of co-authors, performed lab work, interpreted the results together with co-authors. Drafted and wrote the manuscript with inputs and comments from the co-authors and contributed to the correspondence with the journal

II. Performed bioinformatics and statistical analyses with the help of co-authors, performed lab work, wrote methodology and results part of the manuscript with inputs and comments from the co-authors

III. Planned and designed the common garden study with co-authors, performed field work by collecting fish from local government hatcheries, took part in fish breeding and tagging, performed data analyses, interpreted the results together with co-authors. Drafted and wrote the manuscript with inputs and comments from the co-authors. Writing the final version of the manuscript with contributions from the co-authors and the corresponding author.

IV. Designed the common garden study with co-authors, performed data analyses, interpreted the results together with co-authors. had the responsibility for drafting and writing the manuscript with inputs and comments from the co-authors. Writing the final version of the manuscript with contribution from co-authors and the corresponding author.

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Abbreviations

BLUP	Best Linear Unbiased Prediction
DAPC	Discriminant Analysis of Principal Components
ddRADseq	Double digesting Restriction site associated DNA sequencing
DO	Dissolved oxygen
EBV	Estimated Breeding Value
FETA	Fisheries Education and Training Agency
F_{ST}	Fixation Index Statistics
GIFT	Genetically Improved Farmed Tilapia
GxE	Genotype by Environment Interaction
IMC-MC	Institute of Marine Sciences Mariculture Centre
MT	Metric tonnes
PCA	Principal Component Analysis
PIT	Passive Integrated Transponders
RADseq	Restriction-site associated DNA sequencing
SNP	Single Nucleotide polymorphism
TAFIRI	Tanzania Fisheries Research Institute

1. General Introduction

1.1 Global aquaculture

Aquaculture is the fastest growing food production sector. In 2018, aquaculture production reached 82.1 million tonnes of the total global fish production (179 million tonnes), (Figure 1) mostly dominated by finfish culture of which 47 million tonnes originated from inland aquaculture and 7.3 million tonnes from marine and coastal aquaculture. The production accounted for 52% of fish used for human consumption (FAO, 2020).

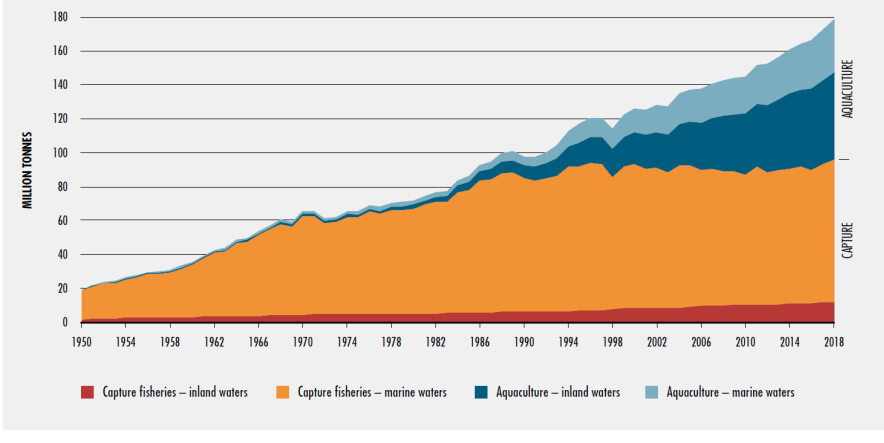

Figure 1 World capture fisheries and aquaculture production, 1950-2018. (Source: FAO, 2018)

The world aquaculture production of farmed fish is dominated by Asia. In the last 20 years, Asia has contributed about 88.69% of the global total aquaculture production (FAO, 2020). China is the main producer in Asia

contributing about 57.9% of the total aquaculture production, followed by India (8.61%), Indonesia (6.61%), Vietnam (5.04%) and Bangladesh (2.93%) (FAO, 2020).

The major three fish species produced in the world aquaculture are Grass carp, *Ctenopharyngodon idellus* (10.5%), Silver carp, *Hypophthalmichthys molitrix* (8.8%) and Nile tilapia, *Oreochromis niloticus* (8.3%) (FAO, 2020).

Fish farming in Africa has increased in the last 20 years, from contributing 0.45% of the world aquaculture production in 1995 to 2.67% in 2018 (Halwart, 2020). Currently Egypt is the main producer in Africa followed by Nigeria, contributing 1.90% and 0.35% respectively to the world aquaculture production. Countries of sub-Saharan Africa excluding Nigeria contributed only about 0.37% of the total aquaculture production in Africa in 2018 (FAO, 2020).

The sub-Saharan region has a notable increase in terms of aquaculture production from 110,200 tonnes in 1995 to 2,196,000 tonnes in 2018 with annual increase of 15.55% (FAO, 2020; Halwart 2020). Most of the production comes from inland freshwater aquaculture, which accounts for about 99% of the total production while marine aquaculture contributes only about 1%. The main cultured species are African catfish (*Clarias gariepinus*) and tilapia (FAO, 2016, 2018). Top aquaculture producers in Africa are Egypt, Nigeria, Uganda, Ghana, Tunisia, Kenya, Zambia, Madagascar, Malawi and South Africa (Satia, 2011). Among them the leading producers in 2018 were Egypt, Nigeria and Uganda which contribute about 90% of the total aquaculture production in Africa (Adeleke et al., 2020) (Figure 2).

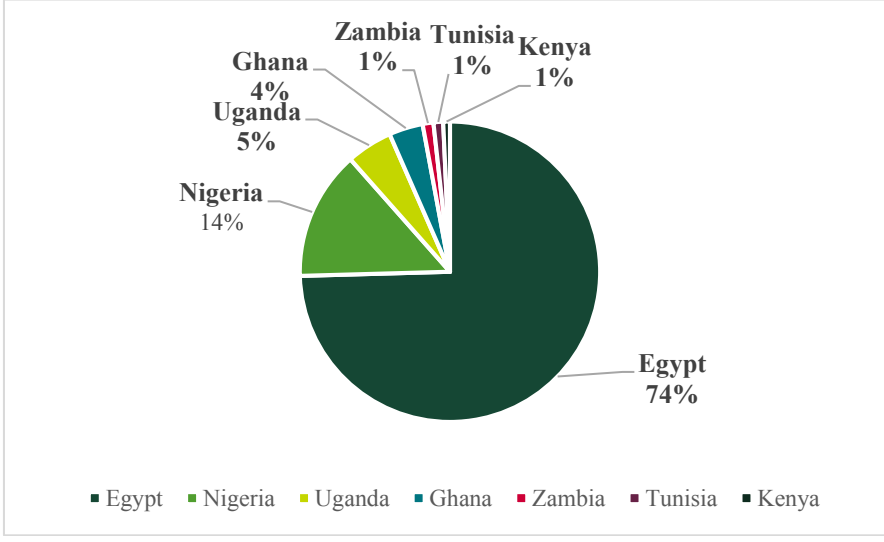

Figure 2 Top seven aquaculture producers in Africa in 2018 by production (metric tonnes). (Source: Adeleke, 2020)

This notable growth and development of aquaculture in these leading countries is a result of many factors such as capacity building in aquaculture, good governance, research and development, access to credit facilities, promotion of private sector (Satia, 2011), interventions from the government and aids from development partners (Cai et al., 2017). Despite the fact that aquaculture industry continues to grow in these countries, the sector still faces many challenges such as capital, inadequate quantities and quality of seed and feeds and land, and water and feed competition (Adeleke et al., 2020).

1.2 Aquaculture in Tanzania

Aquaculture in Tanzania started in the late 1920s, with the culturing of trout introduced from Scotland in the regions of Kilimanjaro and Mbeya (Balarin, 1985). Since in 1950s, aquaculture was practiced at experimental level in ponds at Korogwe (in the Tanga Region) and Malya (in the Mwanza Region) with tilapia fingerlings sourced from Lake Victoria, Congo and Pangani Rivers (FAO, 2012; Rothuis et al., 2014). Later government stations started distributing fingerlings supplied by Hombolo Fish farm center to fish farms and public water reservoirs (Coche et al., 1994 ; Madalla, 2008). However,

the production was not promising and lied dormant due to poor management, inadequate good quality fish feeds and seeds (Coche et al., 1994). From the 1950s onwards, aquaculture in Tanzania has been practiced at small scale in earthen ponds (Shoko et al., 2011), largely in extensive or semi-intensive farming systems and more recently in tanks, hapas and cages (Chenyambuga et al., 2014). Furthermore, in recent years aquaculture production has increased because of rise in awareness to people about aquaculture. Fish farming has been enlarged and the number of ponds in the country have increased, from 19,860 ponds, producing 2,979 MT of fish in 2012 (Figure. 3) to 26,445 fishponds producing 18,082 MT of fish in 2018 (URT, 2019). Tanzania is among the top 10 producers of aquatic algae worldwide producing 103,200 MT of seaweed in 2018 (FAO, 2020). The most common finfish species farmed in the country are the African catfish *(Clarias gariepinus)* and Nile tilapia (*O. niloticus*). Farming of Nile tilapia has increased for the past five years contributing largely to aquaculture production (Figure. 3).

Nevertheless, tilapia farming in the country is still considerably low in comparison with the country's potential. The main challenges for the industry are poor quality and inadequate supply of seeds, poor management of broodfish, insufficient investment capital and government support and scarcity of experts in fish genetic and breeding (Kajungiro et al., 2019a).

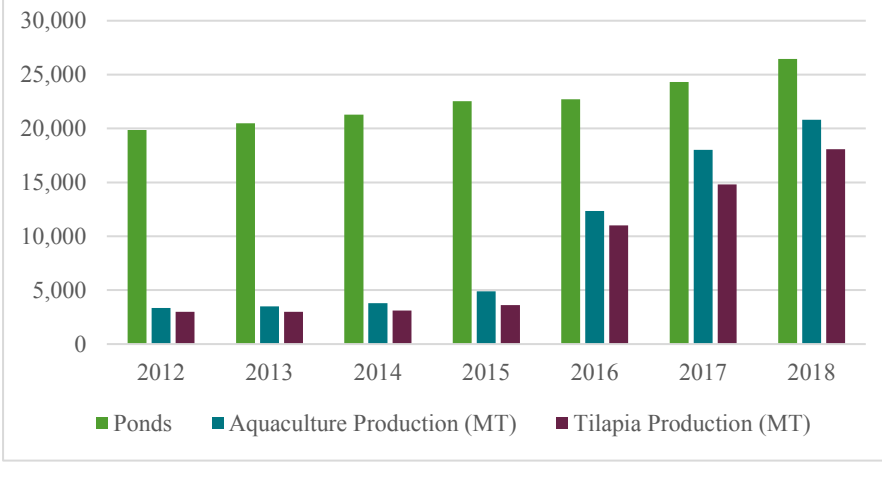

Figure 3 Trends of Aquaculture production, number of ponds and tilapia Production, 2012-2018. (Source: URT, 2019)

1.3 Breeding Program

Genetic improvement in aquaculture started in the mid-1970s with the initiation of selective breeding program in Norwegian salmon (Gjedrem, 2000). Since then, selective breeding has been one of the most common traditional approaches used in tilapia genetic improvement programs in most countries (Uraiwan, 1988). Selective breeding is based on the underlying principle that genetic variations present in desirable traits within a population are directly heritable from parent to offspring (Ansah et al., 2014**).**

Selective breeding has a very high potential for improving the genetic makeup of fish usually focuses on the improvement of economically important traits (Gjedrem et al., 2012). Selective breeding of growth related traits are usually among the first trait considered for improvement (Tave**,** 199**5**). Preferred traits for selection should have moderate to high levels of heritability (Gjedrem, 2005). Lind et al., 2012**,** reported the advantages of selective breeding over other genetic approaches (hybridization, crossbreeding, chromosome manipulation, sex control and transgenesis) such as continuous genetic gain which can be transmitted from one generation to the next. Therefore, genetic improvement is an important option for increasing the productivity and profitability of aquaculture production (Gjedrem et al., 2012).

Several selective breeding programs for Nile tilapia have been established, mainly focused on growth rate and body traits (Ambali & Malekano, 2004), starting with the GIFT (Genetically Improved Farmed Tilapia) (Eknath & Hulata, 2009) and SEAFDEC-selected GET-EXCEL, GenoMar Supreme Tilapia (GST) and Progift in Hainan, China (Zak et al., 2014).

The strain from the GIFT program set an example of the most successful tilapia selective breeding program in aquaculture. The GIFT strain is the most widely farmed tilapia variety across the Asia because of its fast growth and high yield (Hamzah et al., 2014). GIFT was developed by ICLARM now World fish Center in from 1988 to 1997 in the Philippines (Eknath et al., 1993). In addition, the GIFT strain was developed under genetic improvement program through selective breeding from wild Nile tilapia populations brought from Ghana, Egypt, Kenya and Senegal and four strains of tilapia from Israel, Singapore, Taiwan and Thailand (Eknath et al., 1993).

More recently, two genetically improved strains of Nile tilapia that grow 30% faster than non-improved strains have been established in West Africa

and Egypt. A collaboration between the WorldFish with partners from Ghana and Egypt resulted in two breeding programs in the aforementioned countries. In particular, the Abbassa (Ibrahim et al., 2013) and the Akosombo strains of Nile tilapia (*Oreochromis niloticus*) (Ansah et al., 2014).

1.4 Tilapia

Tilapia is the common name for the species belonging to the Tilapiine group of the family cichlidae, native to Africa and Middle East and the most cultured species worldwide after Carp species (FAO, 2020; Watanabe et al., 2002**)**. Several tilapia species such as Nile tilapia (*O niloticus*), blue tilapia (*O. aureus*) and Mozambique tilapia (*O. mossambicus*) are cultured in more than 90 countries (Lim & Webster, 2006). Tilapia species are suitable for aquaculture because of their tolerance to handling, fast growth, tolerance of wide range of environmental conditions such as pH, temperature and salinity and high marketability (El-Sayed et al., 2005; Klett & Meyer, 2002). *O. aureus* and *O. mossambicus* have higher range of salinity tolerance than *O. niloticus* species which is confined to reproduction, survival and growth in saline conditions (Suresh & Lin, 1992). Tilapia species can be cultured in a broad range of aquaculture production systems from extensive, semi-intensive (earthen ponds) to intensive (cages, tanks, raceways and recirculation systems) (El-Sayed, 2006).

Tanzania is a hotspot for diversity of *Oreochromis* including more than 30 *Oreochromis* species and 11 of which are only found in the country (Shechonge et al., 2019). Nile tilapia is the most cultured species in Tanzania and locally known as *sato* in the regions around Lake Victoria or *perege* in other regions. In Tanzania, Nile tilapia is believed to be native from Lake Tanganyika catchment (Trewavas, 1983) and in 1950s the species was introduced in Lake Victoria (Goudswaard et al., 2002). Since then the Nile tilapia population from Lake Victoria is widely farmed and distributed across the country (Shechonge et al., 2018).

Figure 4 Nile tilapia (*Oreochromis niloticus*) at Kunduchi campus, University of Dar es Salaam, Tanzania. (Source: Redempta)

1.5 Genetic Diversity

Genetic diversity is an essential feature for the robustness and the viability of animal populations (Mukhopadhyay & Bhattacharjee, 2014). Genetic diversity between populations explains the ability of a population to adapt to a certain environmental condition (Markert et al., 2010). The success of natural selection or artificial selection depends on the amount of genetic diversity present in the population (García-Ballesteros et al., 2017). For a breeding program, the genetic diversity between and within breeds or populations should be known as this will influence the response to selection

(Oldenbroek, 2017). Knowledge of genetic diversity in fish species is important for understanding their potential adaptation to various aquaculture techniques (Houston et al., 2020). High genetic diversity allows populations to adapt to new conditions in changing environments and inbreeding results in the reduction of genetic diversity by increasing homozygosity in the genomes of individual (D'Ambrosio et al., 2019). Establishing a good base population determines the success of any aquaculture breeding program (Fernández et al., 2014). Genetic variation within breeds can decrease as a result of selection for economically important traits which can lead to an increase of inbreeding (Howard et al., 2017). Starting a breeding program with sufficient genetic variation between and within breeds and monitoring and managing the levels of inbreeding (per generation) are important as raw materials for genetic improvement (D'Ambrosio et al., 2019). Therefore it is very important to assess the genetic variation within and between populations and detect similarities as well as differences between individuals/ populations (Dudu et al., 2015).

1.5.1 Genetic Markers

The genetic diversity of cultured tilapia strains must be identified for better understanding of genetic structure of the selected strains to design effective breeding program and conserve their diversity. DNA-based genetic markers allow the characterization of the genetic variation and divergence between and within populations (Ditta et al., 2018; Romana-Eguia et al., 2004). Molecular markers have been used to determine genetic diversity in Nile tilapia, these include mitochondrial DNA, restriction fragment length polymorphisms (mtDNA-RFLPs) (Agnèse et al., 1997; Romana-Eguia et al., 2004), random amplified polymorphic DNA (RAPD (Hassanien et al., 2004; Mahboob et al., 2019), microsatellite markers, simple sequence repeats (SSRs) (Dias et al., 2016; Hassanien & Gilbey, 2005; Romana-Eguia et al., 2004; Tibihika et al., 2020) and single nucleotide polymorphisms (SNPs) (Delomas et al., 2019; Kajungiro et al., 2019b; Lind et al., 2019).

For genetic diversity studies more SNPs need to be genotyped compared to microsatellites. However, SNPs have several advantages, which have led to a rapid increase in their popularity over recent years. Some attributes that make SNPs to be mostly used are; simplicity in detection and cost-effective genotyping per locus (Houston et al., 2014), highly abundant in the genome (Van Bers et al., 2012), simple to use for scoring in large numbers in the form

of SNP arrays and their existence in both coding and non-coding regions (Yáñez et al., 2014).

1.5.2 Restriction-site associated DNA sequencing (RADseq) and double digesting RAD sequencing (ddRADseq)

Next-generation sequencing technologies (NGS) are making a substantial impact on many areas of biology, including the analysis of genetic diversity in populations. NGS combined with restriction enzymes (REs) have proven most valuable for genotyping purposes (Guo et al., 2014). Restriction-site associated DNA sequencing (RADseq) technique sequence the regions around RE recognition sites and produce a reduced representation of the genome (Wang et al., 2013). RADseq is performed mainly using Illumina sequencing and can identify large numbers of SNP markers for quantitative trait locus (QTL), linkage mapping (Kai et al., 2014) and population genetics analysis (Kakioka et al., 2013). A main advantage of RADseq is that it can be applied in species that lack an available reference genome (Pfender et al., 2011). Conventional RADseq generates random sheared fragments from genomic DNA and this creates high DNA loss steps that allow little control over the fragments that are sequenced. A lot of RADseq data has to be discarded due to variable site sequencing coverage (Peterson et al., 2012). ddRADseq uses two restriction enzymes which consist of a rare-cutting one and a frequently-cutting one and this avoids random shearing of the DNA (Peterson et al., 2012). The resulting fragments undergo adaptor ligation and precise size selection before sequencing. RADseq and ddRADseq have been applied in several studies of aquaculture species. For example, to generate high-density linkage maps *e.g*. Japanese eel (Kai et al., 2014), Cichlid fishes Amphilophus spp (Recknagel et al., 2013), cutthroat and rainbow trout (Amish et al., 2012). SNPs detection and identification *e.g* marine snail (Kess et al., 2016) and perform genome wide association studies e.g Coho salmon *Oncorhynchus kisutch* (Barría et al., 2018), genetic diversity e.g salmon) (Antoniou et al., 2017; Houston et al., 2012), tilapia (Kajungiro et al., 2019b; Moses et al., 2020; Nyinondi et al., 2020).

1.6 Genomic work in Nile tilapia

Following the recent evolution in next generation sequencing (NGS), SNPs markers have been identified in large numbers. Currently, SNPs covering the

entire genome and SNP chips are available and have been identified in Nile tilapia (Joshi et al., 2018; Peñaloza et al., 2020; J. M. Yáñez, Joshi, & Yoshida, 2020). The application of dense SNPs distributed across the entire genome have allowed the advance from traditional breeding program of using phenotypic and pedigree information to genomic selection (Meuwissen et al., 2001). The use of SNP panels has accelerated the discovery of QTL (quantitative trait locus) for harvest weight and fillet yield traits and speeded up the genetic progress in breeding program of commercial Nile tilapia from Costa Rica (Yoshida et al., 2019). Dense SNP genotypes have also been used for characterizing regions associated with signatures of selection in tilapia species, for example Hong Xia et al., (2015) reported the identification of 100 putative selective sweep regions in Nile tilapia from South Africa, China and Singapore. Several studies describing regions involved in sex determination has been reported in Nile tilapia using SNP markers, for example Palaiokostas et al., (2015) described significant QTLs in regions LG20 and LG1 in sex determination in Nile tilapia.

1.7 Common Garden

Common garden refers to a variety of experimental designs where organisms from different locations or strains are grown and exposed to a common environment of the same conditions (Berend et al., 2019; Moloney et al., 2009). Common garden studies are conducted so as to reduce the influence of environmental conditions on trait variation and provide a way to compare the performance of different strains for a given environment (Hutchings, 2011). Therefore common garden experiments can help to distinguish the effects and contributions of genetic and environmental factors to the physical appearance of the trait of interest (Guèye, 2016). Common garden experiments can be designed to explore phenotypic plasticity (Liu & El-Kassaby, 2019; Rajkov et al., 2018), local adaptation (Gradil et al., 2016), compare population's growth performance (Guèye, 2016; Harvey et al., 2016) and genotype x environment interaction (GxE) (Klápště et al., 2020). Common garden experiments have been broadly used in a range of organisms including fish to evaluate genetic differences and performance comparison among strains, and individuals have been communally cultured in different production systems and environments for example in tilapia

(Guèye, 2016; Mcginty, 1983) and salmon (Ljungfeldt et al., 2014; Solberg et al., 2013).

Traits such as growth which are controlled by many genes are strongly influenced by the environment (Vasemägi et al., 2016). The phenotype of an individual is the function of its genotype, the environment and the interaction between them (GxE). GxE exist if the individual's genotype performance changes due to the changing environment, that's when its higher performance in one environment might be lower in another environment (Falconer and Mackay, 1996; Li et al., 2017).

Common garden experiment can estimate the effect of genotype by environment interaction (GxE) by rearing individual from different strains in two environments It resolves the genetic basis of quantitative physical expression of DNA of the populations in the absence of confounding effects of the analogous environment (De Villemereuil et al., 2016). There are two types of GxE interaction: re-ranking or scaling effects. Re-ranking effects is when the individuals of given strain or genotype, rank differently in different environments, based on their performance. The scaling effect occurs when the magnitude differences between genotypes varies across environment without changing their rank (Falconer and Mackay 1996; Wakchaure et al., 2016). For the efficiency of a breeding program, the existence of GxE should be determined as it can affect the genetic gain. Scaling interaction is of a less importance compared to re-ranking effects because the best performing individuals in one environment can still perform the best in other environments (Wakchaure et al., 2016). Re-ranking interaction is a critical component for breeding programs because there is a chance that higher ranking genotypes in one environment will not be higher ranking genotypes in another environment (Li et al., 2019).

For two different environments the interaction between genotype and environment can be evaluated by measuring the same traits in two environments and consider them as different traits and estimate the genetic correlation between these traits (Falconer and Mackay 1996). Genetic correlation for a target trait in two environments is the measure of genotypes' performance in two environments and a key factor to consider in breeding programs (Falconer, 1952). GxE interaction is of great importance and should be contemplated when genetic correlation is lower than 0.8 (Robertson, 1959). In fish breeding a separate breeding program was suggested when genetic correlation is lower than 0.7 (Sae-Lim et al., 2013).

2. Aims of the thesis

A rich variety of tilapia species is encountered in Tanzania (Genner et al., 2018) with Nile tilapia (*Oreochromis niloticus)* being the most cultured species in the country. Most of the Nile tilapia strains that are farmed in Tanzania are either pure local, exotics from other countries or hybrids between the above categories. The high morphological similarities between tilapia strains and species causes difficulties in their identification by the fish farmers. As most people in the country depend on aquaculture for their livelihoods, there is a great need for a faster-growing and better performing strain of Nile tilapia, which can be cultured successfully in great varieties of production environments. To improve aquaculture production and livelihood of fish farmers in Tanzania, there is a need to identify pure strains and know which strains of local Nile tilapia are available in the country. Furthermore, the performance of these strains in different culture environments needs to be assessed.

The general aim of this thesis was to produce information for establishing a future breeding program of Nile tilapia in Tanzania.

Specific Objectives were to:

- Evaluate the population structure and genetic diversity of local Nile tilapia (*O. niloticus)* strains using ddRAD sequencing.
- Assess population differentiation of local and exotic Nile tilapia and local Rufiji tilapia strains in Tanzania.
- Conduct a common garden experiment and compare the growth performance of local Nile tilapia strains (*O. niloticus*) at two environments.
- Estimate genetic parameters for growth in local Nile tilapia strains in two environments and estimate gene by environment interaction.

3. Summary of studies and main results

This thesis is comprised of the following papers:
Paper I focused on comprehending the population structure and genetic diversity within and between seven local Nile tilapia strains in Tanzania (Karanga, Igunga, Ruhila, FETA, TAFIRI, Kunduchi, and Lake Victoria). This study was followed by paper II that evaluated genetic diversity of both introduced and local strains of Nile tilapia and Rufiji tilapia. Paper III was based on a common garden experiment, which evaluated the performance of the Nile tilapia strains in two environments (Pangani and Kunduchi). This investigation was followed by the estimation of genetic parameters and the magnitude of the genotype by environment interaction for growth traits of local Nile tilapia strains reared in two environments (Pangani and Kunduchi).

3.1 Genetic diversity study

Population Structure and Genetic Diversity of Nile Tilapia (Oreochromis niloticus) Strains Cultured in Tanzania
In this study, fish samples were collected from seven sites: TAFIRI, FETA, Karanga, Igunga, Kunduchi, Ruhila and Lake Victoria. A total of 140 fish, twenty fish from each strain, were sampled and fin clips were collected for genomic analysis.

ddRAD Library Preparation, Sequencing and SNP Genotyping
DNA was extracted from fin clip using a spin column (QIAsymphony DSP DNA Mini Kit; Qiagen, Hilden, Germany). ddRAD library preparation was performed according to Peterson et al. (2012), with minor modifications described in Palaiokostas et al. (2015). The libraries were sequenced at Edinburgh Genomics Facility, University of Edinburgh on an Illumina HiSeq

4000 instrument. Reads were aligned to the *O. niloticus* reference genome assembly [Genbank accession number GCA_001858045.2 (Conte et al., 2017)] using bowtie2 (Langmead & Salzberg, 2012). Stacks v2 (Catchen et al., 2011; Rochette et al., 2019) was used to identify and extract single nucleotide polymorphisms (SNPs) using gstacks (settings: –var-alpha 0.001 –gt-alpha 0.001 –min-mapq 40).

Results revealed a total of 2,180 SNPs with a MAF above 0.05 across all samples.

Genetic diversity and Structure among strains

Genetic diversity parameters were estimated using Stacks v2 (Rochette et al., 2019). The R package StAMPP (Pembleton et al., 2013) was used to calculate pairwise F_{ST} values using the stamppFst function according to Cockerham and Weir (1984). Principal component analysis (PCA) was carried out using the R package ADEGENET version 2.1.1 (Jombart et al., 2018). Discriminant analysis of principal components (DAPC) and Bayesian-model-based approaches implemented in the program Structure v2.3.4 (Pritchard et al., 2000), were used determine genetic structure and admixture of seven *O. niloticus* strains.

Results showed that the Kunduchi strain had a high expected heterozygosity value (0.214) and high inbreeding coefficient (Fis) value (0.557). FETA strain had the lowest value of expected heterozygosity (0.057) and lowest Fis value (0.006). Principal component analysis (PCA) grouped together individual fish from FETA, Lake Victoria, Igunga and most individuals from Kunduchi strains. TAFIRI strain formed a distinct cluster while Karanga and Ruhila showed evidence of mixed individuals (Figure. 5). Lowest F_{ST} values were observed between Igunga, Lake Victoria and FETA. The highest F_{ST} values were between Karanga and FETA, Lake Victoria and Igunga (F_{ST} = 0.548, 0.538, and 0.533 respectively). Admixture analysis suggested K=7 as the most probable number of genetically distinct strains. The strains of FETA, Lake Victoria, Igunga and most of individual fish from Kunduchi shared the same genetic cluster, while the TAFIRI strain formed a unique group. Finally, the strains, Karanga and Ruhila provided evidence of admixture.

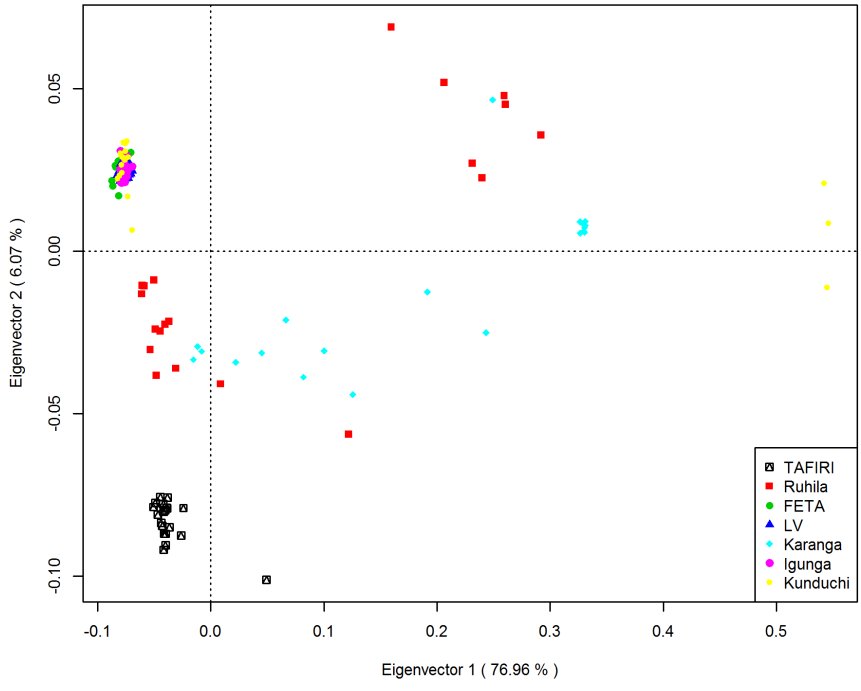

Figure 5 Principal components analysis (PCA) of the strains for 139 individual fish based on 2,180 single-nucleotide polymorphisms (SNPs). The genetic relationships among individual fish as seen when plotting the first and second principal components (PCA1 and PCA2). Each individual is represented by one dot, with its symbol color corresponding to the assigned strain.

Using ddRAD sequencing to assess genetic differentiation within and between introduced and local strains of Nile tilapia and Rufiji tilapia

In this study, a total number of 550 animals and twenty seven farmed and wild strains of exotic and local Nile tilapia and Rufiji tilapia were sampled across Tanzania in 2017 from privately owned hatcheries, government Aquaculture Development Centre hatcheries, lake, dams, river basins, swamp, and estuary. Fish were kept separate for 4 months in hapas (2 m × 2 m) within an earthen pond and plastic tanks (1.5 m × 1.5m × 1.5 m) at Kunduchi Campus in Dar es Salaam and at the Institute of Marine Sciences Mariculture Centre (IMS-MC), Pangani Tanga, respectively. A total of 550 fish weighing from 50 to 200 g were fin clipped. A total of 1583 SNPs from

a de novo assembly with a MAF above 0.05 across all samples and found in more than 75% of the genotyped fish in each strain were retained for downstream analysis.

Genetic Diversity and relationship among tilapia strains

Rufiji tilapia strains appeared to be more diverse than the exotic and local tilapia strains with the average genetic diversity (He) ranging from 0.078 to 0.326. The local Nile tilapia revealed moderate diversity, while the exotic Nile tilapia strains showed low to average genetic diversity (He) compared to local Rufiji tilapia (Table 1).

Table 1. Genetic diversity parameters for local Rufiji tilapia (*O. urolepis urolepis*), local and exotic Nile tilapia (*O. niloticus*). Ho, observed heterozygosity; He, expected heterozygosity; Fis, inbreeding coefficient

Species	Ho (mean ± SE)	He (mean ± SE)	Fis (mean ± SE
Rufiji tilapia strains			
Mindu	0.228 ± 0.004	0.233 ± 0.004	0.034 ± 0.028
Wami	0.188 ± 0.004	0.326 ± 0.005	0.337 ± 0.033
Bwawani	0.084 ± 0.004	0.084 ± 0.004	0.010 ± 0.030
Kibasila	0.075 ± 0.004	0.080 ± 0.004	0.017 ± 0.035
Chemchem	0.092 ± 0.005	0.090 ± 0.004	-0.001 ± 0.033
Kilola	0.078 ± 0.004	0.083 ± 0.004	0.028 ± 0.022
Mansi	0.174 ± 0.004	0.169 ± 0.003	-0.012 ± 0.028

Nyamisati	0.095 ± 0.005	0.096 ± 0.004	0.010 ± 0.027
Ruaha	0.091 ± 0.004	0.093 ± 0.004	0.010 ± 0.028
Utete	0.117 ± 0.004	0.115 ± 0.004	0.006 ± 0.026
Pangani_Rufiji	0.080 ± 0.004	0.078 ± 0.004	0.002 ± 0.043
Exotic Nile tilapia strains			
Silver-YY	0.125 ± 0.007	0.088 ± 0.004	-0.074 ± 0.029
Big-Nin	0.132 ± 0.005	0.141 ± 0.005	0.044 ± 0.038
Chitralada-N	0.137 ± 0.004	0.149 ± 0.004	0.047 ± 0.033
Chitralada-E	0.140 ± 0.005	0.145 ± 0.004	0.029 ± 0.033
Ruvu Farm-R	0.086 ± 0.004	0.086 ± 0.004	0.006 ± 0.022
GIFT	0.135 ± 0.005	0.137 ± 0.005	0.018 ± 0.044
Chifive-C	0.073 ± 0.005	0.068 ± 0.004	-0.003 ± 0.037
Muleba-M	0.082 ± 0.005	0.086 ± 0.004	0.034 ± 0.046
Local Nile tilapia strains			
Pangani_Nile	0.190 ± 0.007	0.153 ± 0.005	-0.077 ± 0.039

TAFIRI	0.106 ± 0.005	0.108 ± 0.004	0.015 ± 0.035
Ruhila	0.130 ± 0.004	0.216 ± 0.005	0.242 ± 0.037
FETA	0.067 ± 0.004	0.067 ± 0.004	0.005 ± 0.032
Lake Victoria	0.075 ± 0.004	0.075 ± 0.004	0.004 ± 0.035
Karanga	0.111 ± 0.003	0.218 ± 0.006	0.259 ± 0.038
Igunga	0.079 ± 0.004	0.079 ± 0.004	0.005 ± 0.026
Kunduchi	0.083 ± 0.004	0.215 ± 0.004	0.505 ± 0.026

The PCA analysis revealed a clear distinction between local Nile tilapia and exotic Nile tilapia. However, there is a distinction between Nile and local Rufiji tilapia species but with partial overlap. There are some animals though from Rufiji strains that appear as potential hybrids. The results also showed that local Nile tilapia are not genetically divergent from exotic Nile tilapia (Figure.6). Local Nile tilapia from Tanzania seemed to overlap with exotic strains from Thailand and Uganda.

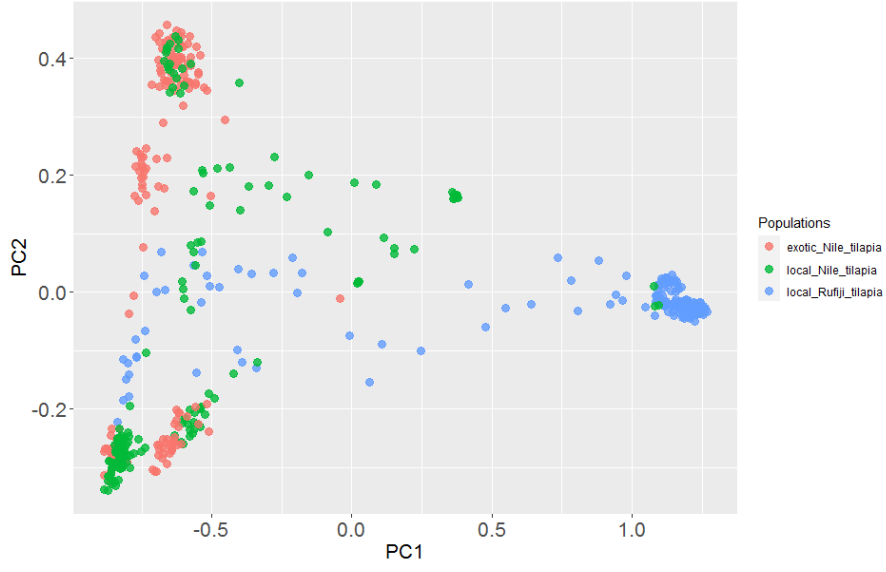

Figure 6 Principal components analysis (PCA) showing genetic relationships among exotic Nile tilapia, local Nile tilapia and Rufiji tilapia species. Individual fish are represented by one dot, with its symbol colour corresponding to the assigned strain

Population Structure and Differentiation

The highest F_{ST} values (F_{ST} = 0.6- 0.8) were found between Rufiji strains and the local or exotic Nile tilapia strains. Low F_{ST} values were observed between strains of the same geographic location, for example between Kibasila and Kilola (F_{ST} < 0.001) of local Rufiji tilapia and Chitralada-N and Chitralada-E (F_{ST} =0.010) of exotic Nile tilapia. The Ruvu farm-R exotic Nile tilapia strains showed lowest F_{ST} values with all local Nile tilapia strains except TAFIRI, Ruhila and Karanga. Furthermore, local Nile tilapia and exotic Nile tilapia showed moderate F_{ST} values amongst them except between Karanga and Silver YY (F_{ST} =0.485), Ruvu Farm- R (0.503) Chifive-C (F_{ST} =0.505) and Muleba-M (F_{ST} =0.498)

STRUCTURE analysis suggested K = 9 as the most probable number of clusters for the tilapia strains. The analysis showed that Rufiji tilapia strains were more distinct and homogeneous than the Nile tilapia. YY silver strain from exotic Nile tilapia and TAFIRI strain from local Nile tilapia each formed a unique cluster. Strains of Mindu, Wami, Ruhila, Karanga and Kunduchi were highly admixed. Local Nile tilapia strains (Victoria, Igunga and FETA shared the same cluster with exotic Nile tilapia strains (Chifive-

C, Muleba-M and Ruvu Farm-R). Nile tilapia Strains from Tanzania provided evidence of admixture with individuals from Uganda. Discriminant analysis demonstrated the existence of two separate groups of Nile and Rufiji tilapia.

3.2 Common Garden experiment study

A common garden experiment was conducted in two locations (Pangani and Kunduchi). Pangani has a brackish water environment (2-5 ppt) and is located at the Institute of Marine Sciences Mariculture Centre (IMS-MC) in Pangani, Tanga region. Kunduchi has a freshwater environment and is located at the University of Dar es Salaam, Kunduchi campus in Kunduchi, Dar es Salaam region. The study aimed to evaluate the growth performance of growth traits (body weight, total length, standard length, body depth and perimeter) in six Nile tilapia, *Oreochromis niloticus,* and strains, to estimate genetic parameters, and determine the magnitude of genotype by environment interaction in the two environments.

Origin of Strains

Nile tilapia from five farmed strains and one wild strain were used in the study. Fingerlings were collected in 2017 and kept separately in hapas (2 m x 2 m x 1.5m) within an earthen pond at Kunduchi campus. The founder strains were reared communally until they reached an average body weight of about 150–400 g where mating was initiated.

Production and rearing of fry

Single matings (one male × one female) and spawning were carried out separately in 1×1×1.5 m breeding hapas for four weeks from June to July 2019. After a week, fertilized eggs were collected from the mouth of fish and were artificially incubated until hatching. Fry hatched after about 5–7 days and were thereafter fed on a feed containing 44% with 60 mg of methyl testosterone (Sigma Aldrich, Germany) for one month. From each full-sib family, 50-70 fry were stocked separately in nursing hapas (1.5 x 1.5 x 1.5 m) in the same pond to reduce environmental differences between families. Families were kept separately until fish reached an average size of about 15-25 g, at which tagging took place. About 25-30 randomly sampled

fingerlings per full-sib family were tagged using Passive Integrated Transponders (PIT) (BTS-ID, Sweden).

A total of 1,368 fingerlings, 650 at Kunduchi and 718 at Pangani, were tagged. At tagging, the identification code, body weight (BW), total length (TL), standard length (SL), body depth (BD), and body perimeter (BP) were recorded. Tagging was conducted first at Kunduchi followed by Pangani.

Production environments

After conditioning for about 7 days all tagged fingerlings were pooled together and communally stocked in hapas (12m x 8.5m) installed in a pond (20m x 20m) in either fresh or brackish water. In the latter case, fish were stocked approximately two weeks later. Representatives of each full-sib family were assigned at random and communally grown in the test environments in hapas installed in the pond at a stocking density of 5 fish per square meter. Each pond contained two hapas and four hapas in total were used at each testing environment.

Stocking and Harvest

Fry were fed three times (8am, 12pm and 4pm) daily on a commercial feed starter (Aller Aqua) with a crude protein level of 44%. Juveniles and adults were fed two times (8am and 4pm) per day with 36% and 30% crude protein respectively. Fish were harvested after a grow-out period of about 94 days (84 to 90 days in freshwater and 99 to 102 days in brackish water), (October – December 2019) in the test environments. During harvest body measurements of each individual fish including body weight, total length, standard length, and body width and body depth were recorded.

Water Quality

The salinity concentration at Pangani's water ranged from 2 to 5ppt (brackish water). At Kunduchi, the average temperature during the growth period was about 31^{o}C with a range from 28^{o}C to 36^{o}C while at Pangani, the average temperature was about 33^{o}C with a range from 29^{o}C to 38^{o}C. During the experiment, ¼ of water in the pond was changed every 3 days to maintain water quality. Water quality parameters (dissolved oxygen (DO) and temperature) were measured twice a day, using a HI98193 Waterproof Portable Dissolved Oxygen and BOD Meter (Hanna Instruments, HannaNorden, Sweden).

Common garden comparison of native Nile tilapia (Oreochromis niloticus) strains in brackish and freshwater environments

Data analysis

Strain performance and environment comparison using a fixed effect model

After three months of grow-out (October- December 2019), body traits of all fish were recorded at both environments. All descriptive statistics for body traits were computed using R (R Core Team 2019).

A linear model was fitted to the data as shown below:

$$\mathbf{Y_{ijklmn} = \mu + E_i + S_j + A_k + P_l + (SE)_{ij} + H_{m(l)} + e_{ijklmn}}$$

where:

Y_{ijkn} is the recorded trait of the nth individual,

μ is the intercept,

E_i is the fixed effect of the ith test environment,

S_j is the fixed effect of the jth strain,

A_k is the post-hatching age covariate

P_l is the fixed effect of the lth pond

$(SE)_{ij}$ is the interaction effect between strain and test environment

$H_{m(l)}$ is the fixed effect of hapa nested within pond

e_{ijklmn} is the residual random error with mean 0 and variance σ^2

The results from this study showed that there were significant ($P< 0.001$) variations regarding growth performance for all the recorded body traits among the six Nile tilapia strains across the two environments. In the case of the freshwater environment across the six strains, the growth performance of Karanga strain (225g) ranked first followed by Igunga strain (206g), while TAFIRI strain (163g) ranked last. Moreover, the Ruhila, FETA and Victoria strains (188,187,187g) had a similar performance. In the case of the brackish water environment, Igunga strain (268g) was ranked first followed by TAFIRI strain (252g), while Ruhila and Karanga strain (231g) had a similar performance. Finally, the Victoria strain had the lowest performance (223g) (Figure 7).

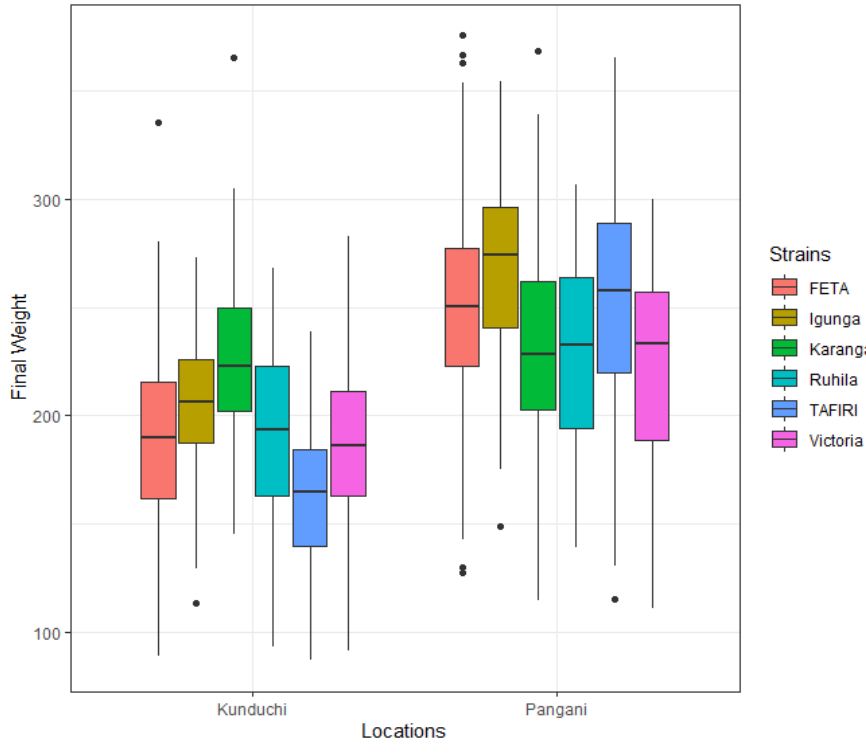

Figure 7 Comparison of growth performance in six strains of Nile tilapia in two environments: Kunduchi (freshwater) and Pangani (brackish water)

Genetic Parameter Estimates and Genotype by Environment Interaction in Nile Tilapia (Oreochromis niloticus) strains Cultured in Tanzania

Statistical Analysis

A univariate model was used to estimate heritability for traits across production environments, while bivariate model was used for estimating genetic correlation between body traits at different measurements points during the growth period. The models were fitted using the BLUPF90 (Misztal et al., 2018). The magnitude of the genotype by environment interaction was quantified either as the genetic correlation of harvest body weight between the freshwater (Kunduchi) and the brackish environment (Pangani) or by inspecting the ranking of families between the two environments.

The model was written as:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \boldsymbol{x}_1 & \mathbf{0} \\ \mathbf{0} & \boldsymbol{x}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \boldsymbol{b}_2 \end{bmatrix} + \begin{bmatrix} \boldsymbol{z}_1 & \mathbf{0} \\ \mathbf{0} & \boldsymbol{z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{u}_1 \\ \boldsymbol{u}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \boldsymbol{e}_2 \end{bmatrix}$$

Where, $\begin{bmatrix} \mathbf{y}_1 \\ \boldsymbol{y}_2 \end{bmatrix}$ is a vector of observations for body traits in the two production environments, $\begin{bmatrix} \mathbf{b}_1 \\ \boldsymbol{b}_2 \end{bmatrix}$ is a vector of fixed effects (site, strain, pond and hapa), $\begin{bmatrix} \mathbf{u}_1 \\ \boldsymbol{u}_2 \end{bmatrix}$ is a vector of random effects, $\begin{bmatrix} \mathbf{e}_1 \\ \boldsymbol{e}_2 \end{bmatrix}$ is a vector of residuals effects, $\begin{bmatrix} \boldsymbol{x}_1 & \mathbf{0} \\ \mathbf{0} & \boldsymbol{x}_2 \end{bmatrix}$ and $\begin{bmatrix} \boldsymbol{z}_1 & \mathbf{0} \\ \mathbf{0} & \boldsymbol{z}_2 \end{bmatrix}$ are the design matrix relating the observations in the two production environments to the fixed and additive effects , respectively.

In this study, lower heritability estimate for harvest weight was found for the animals reared in the freshwater (0.10 ± 0.06) and brackish water (0.09 ± 0.07) environments. Overall, an estimated heritability of 0.11 (SE 0.06) was found across production environments. The estimate of genetic correlation for harvest body weight between the two environments was low (0.35 ± 1.37), with large standard errors. Low genetic correlation implies high GxE between two environments. High GxE indicates a strong re-ranking of strains in terms of their growth performance between the two production environments. However, due to the very high standard errors we cannot make conclusions about the actual genetic correlations. Nevertheless, most families ranked differently across the two environments with only families 9 and 10 from the FETA strain having a similar performance between the two environments (Figure 8).

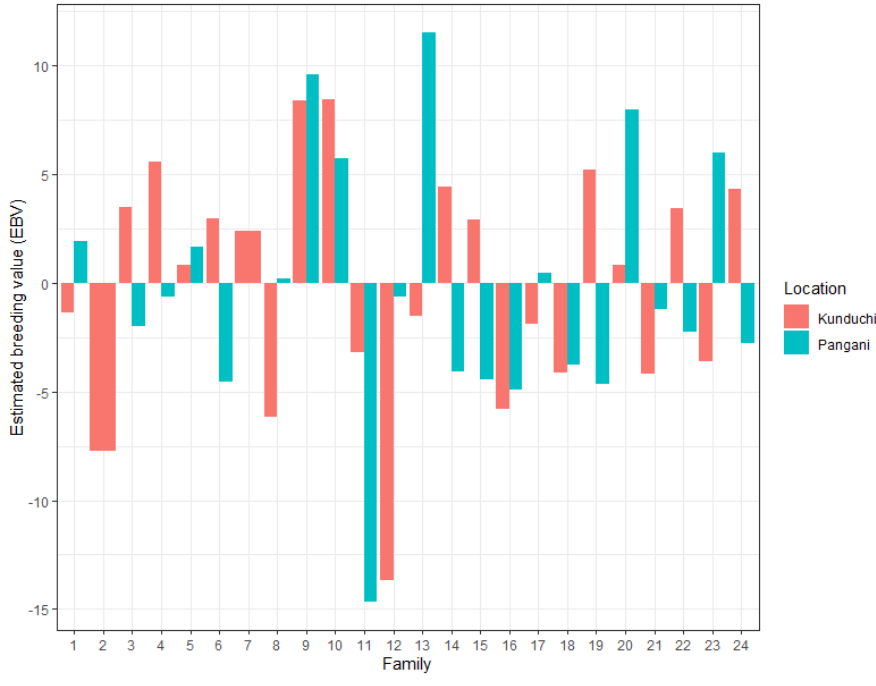

Figure 8 Barplot of average estimated breeding values showing families ranking for a trait in the two locations (Kunduchi and Pangani). Numbers 1-24 represent families in both environments

4. General discussion

Developing better fish strains with genetic supremacy for aquaculture require long term breeding programs (Bentsen and Gjerde, 1994). Selective breeding of Nile tilapia involves the selection of the best performing parents so as to build a base population with a broad genetic diversity upon which selection can be practiced (Eknath et al., 1998). Therefore, a good knowledge of the genetic diversity of available strains and their performance under prevailing production environments is a prerequisite for developing a tilapia breeding program in Tanzania. In this thesis i performed the groundwork to provide basic information for the establishment of a structured breeding program for Nile tilapia in Tanzania.

SNPs markers developed from ddRAD-seq were applied to study the genetic diversity of Nile tilapia strains (FETA, TAFIRI, Igunga, Kunduchi, Ruhila, Victoria and Karanga). Subsequently, a common garden experiment was conducted in brackish and freshwater environments to test their growth performance and estimate genetic parameters for growth related traits.

4.1 Genetic Diversity

Characterizations of genetic diversity of wild and stocked strains of tilapia in Tanzania provides valuable knowledge both for aquaculture and for conservation purposes of wild strains. Strains of Kunduchi, Karanga and Ruhila showed the highest levels of genetic diversity. This may be due to a higher degree of admixture as revealed by the STRUCTURE analysis and the existence of non-random mating (Huff et al., 2011).

Curiously, strong genetic differentiation was observed between highly admixed strains of Karanga, Ruhila and Kunduchi and three closely related strains of FETA, Igunga, and Lake Victoria. The differences could be

explained by geographical distance which has acted as a barrier to gene flow between those strains and resulted into genetic structure revealed by STRUCRURE analysis. However, the possibility of hybrids amongst the studied strains cannot be excluded.

Igunga, FETA, Lake Victoria and TAFIRI strains, showed no admixture and thus could be considered as pure strains. However, Igunga, FETA, Lake Victoria revealed relatively low level of genetic diversity, while TAFIRI showed moderate genetic variation. Therefore, despite the fact that Karanga, Ruhila, and Kunduchi strains were very diverse, the strains are also admixed making them unfit for the selective breeding. Although admixture has many benefits including increased genetic variation in isolated populations through segregation and recombination, formation of heterosis (hybrid vigour) (Verhoeven et al., 2011), admixture has been also reported to have negative effects (Allendorf et al., 2001). Hybridization resulted from anthropogenic activities causes hybrid swarms as explained by Allendorf et al. (2001). Hybrid swarms are characterized by extensive admixture which results into unique population of hybrids. This could explain the admixtures in strains of Kunduchi, Karanga and Ruhila. It is important to note that hybridization with introgression can contribute to outbreeding depression through dilution of alleles important for local adaptations (Roberts et al., 2010). For the case of GIFT tilapia, the base population was developed from known best performing pure bred of Nile tilapia from 8 strains, 4 wild strains from Africa and 4 domesticated strains from Asia. Thereafter a planned crossbreeding in full diallel cross design under controlled ratio of female and males was conducted. Crossbreeding programs allows to combine genetic advantages from multiple breeds that have dramatically different environmental adaptations and traits, this is different from just mixed strains of unknown origin, source and performance. Hence, strains with high genetic variation and high degree of admixture give some alerts supporting their avoidance for a future tilapia breeding program because their origin is unclear.

In this thesis, we also used ddRADseq to assess genetic differentiation within and between exotic and local strains of Nile tilapia and Rufiji tilapia. The results revealed higher genetic diversity among the Rufiji strains compared to exotic and local Nile tilapia. Wami, Mindu and Mansi strains of Rufiji tilapia species were more diverse, however these strains demonstrated evidence of admixture (Nyinondi et al., 2020). In addition, Ruhila, Karanga and Kunduchi strains of local Nile tilapia also revealed high value of

expected heterozygosity (0.216, 0.218 and 0.215 respectively). Admixture and possible hybridization with other tilapia species could have contributed to genetic divergence in those strains of Rufiji and Nile tilapia. This have further been confirmed by high F_{ST} values (F_{ST} = 0.6- 0.8) between Rufiji strains and the local or exotic Nile tilapia strains.

Weak genetic differentiation was revealed between strains of geographically similar strains, for example between Kibasila and Kilola of local Rufiji and Chitralada-N and Chitralada-E of exotic Nile tilapia. Furthermore, local and exotic Nile tilapia strains showed moderate genetic differentiation between them except some few strains that exhibited different trend. This was further explained by a PCA analysis, which showed that local Nile tilapia are not genetically divergent from exotic Nile tilapia. In addition, the main consequence could be introgression (Huff et al., 2011**)** which has resulted into admixture between cultured local and exotic strain of Nile tilapia.

Local strains (Victoria, Igunga and FETA) shared the same genetic cluster with exotic Nile tilapia strains (Chifive-C, Muleba-M and Ruvu Farm-R). This was confirmed by (Discriminant Analysis of Principal Components) DAPC admixture analysis, which revealed that Nile tilapia strains from Tanzania provided evidence of admixture with individuals from Uganda. Tanzania and Uganda being in the same region of East African could have contributed to admixture between strains due to nearby geographical location. Tibihika et al. (2020) reported that admixture and hybridization could have contributed to genetic structure of farmed Nile tilapia strains in Uganda.

In this thesis, ddRADseq a reasonably cheap molecular tool not only was able to differentiate between Nile tilapia and Rufiji tilapia but also between exotic and local strains of Nile tilapia in Tanzania. In addition, the tool enabled clear verification of local tilapia strains whether are pure or admixed. This implies that ddRADseq is a good tool to test the provenance of potential breeding strains.

4.2 Strains growth performance in two environments

Forming a base population with the best performing strains in different production environments is the main objective for the development of a future Nile tilapia breeding program in Tanzania. A common garden

experiment was conducted to compare the growth performance of six local Nile tilapia strains in brackish (2-5ppt) and freshwater environments.

The results indicated that there was significant variation in growth performance of local Nile tilapia strains across the production environments. Strains ranked differently in two environments. In particular, in the case of freshwater, the Karanga strain had the highest growth, whereas in the brackish water the top ranked strain was the Igunga strain. Nevertheless, in both fresh and brackish water, the growth of Karanga strain did not differ significantly. These findings were similar to the work reported by Pongthana et al. (2010) in Thailand who reported that the growth performance of red tilapia strain cultured in freshwater and saline water was significantly different. However, our results differ from a study by Eknath et al. (1993) which reported consistency in growth performance with small changes in the ranking of eight strains of Nile tilapia cultured in a range of environments.

Furthermore, our findings revealed that the performance of all strains was significantly higher in the brackish water than in the freshwater environment. Nevertheless, other environmental factors except of salinity (e.g. temperature) could have contributed to the growth performance between the tested strains across the two environments. Most interestingly, the interaction between strains and environments were significantly high resulting in strain re-ranking in the testing environments.

4.3 Genetic parameters

Knowledge regarding genetic parameters such as heritability, genetic correlation and estimated breeding value (EBV) are very important for the success of any breeding program. For highly heritable traits with heritability above 0.4, animals' phenotypic performance could be a good measure of their ability to produce best offspring compared when heritability is below 0.15 (Toghiani, 2012). Our results revealed low heritability estimates for harvest body weight in Nile tilapia across the freshwater (0.10) and brackish (0.09) environments

The estimates of heritability were higher for all tested traits at stocking and at the one-month time point of measurement in freshwater environment than brackish environments where zero heritability estimates were observed for total length and standard length. Heritability close to zero suggests that all the variations among individuals are due to environmental and non-

additive genetic effect of genes. For the case of freshwater environment, higher heritability suggests for the early selection of fish individual when the heritability is high with large response to selection at stocking than when heritability is low at harvest. The greater the superiority of the individuals selected for breeding purposes and the higher the heritability of the trait the more selection will be successful.

The genetic correlation is important in designing and developing breeding strategies. It can accelerate genetic gain for the correlated traits through indirect selection and can be used in estimating the magnitude of genotype-environment interaction. Our findings showed higher genetic correlation between the second and the final time point of measurements for body weight in the freshwater (0.64) than brackish water (0.12) environments. In addition, high positive genetic correlation was observed for body traits between first- and second-point measurements in brackish water (0.99) than freshwater (0.25) environments. The genetic correlation could be very large close to one with high standard error as in our case for growth traits between first- and second-point measurements in brackish water and between second and the final time point of measurements in freshwater environment. According to Falconer and Mackay (1996), the estimates of genetic correlations with small standard errors require large sample sizes because correlations represent the ratio of the covariance across environments over the additive genetic variances, all measured with error. In our study we acknowledge the small sample size (~1,007 animals) which might have contributed to the observed high standard errors in our analysis. On the other hand, the genetic correlation close to one implies that most genes are shared between the two traits with fewer genes that can be selected for independently, this means that selection on one trait will cause changes in the other.

We also noticed change in strength and sign of genetic correlations across production environments over different time of measurements and at harvest the overall genetic correlation was low for body weight between two environments. In the case of the brackish water environment positive and high genetic correlation was observed between the first- and second-point measurements and negative correlation between second and the final time point of measurements. On the other hand in the freshwater, the results showed high genetic correlation between the second and the final time point of measurements, moderate between the between first and second time point of measurements and low genetic correlation between first and final time

point of measurements for body traits in the freshwater. A negative correlation means that as one-trait increases the other decreases while positive correlation means that the two traits tend to change in the same direction. When fish were moved from freshwater environment (Kunduchi) to brackish water environment (Pangani), there was slight change in environment which also affected gene expression of the trait as stated by de Jong (1995) that as environments differ, gene expression changes and the contribution of a new set of environment-specific genes, cause a change in gene effects on a trait which result changes in genetic correlation. Genetic correlations between environments change gradually by altering the covariance between environments and the additive genetic variances in each environment. The change depends on trait under selection, since most of growth related traits are controlled by genes whose effects are affected by environment, environmental factors such as extreme high or low temperatures as was observed in our study for the case of brackish water environments, may activate gene expression and affect genetic correlation (Schou et al., 2019). Therefore, the differences in genetic correlation for body traits between different time points of measurements in two environments, suggests the best time for the trait selection as genetic correlations evolve over time across the two environments.

4.4 Genotype by environment interaction (GxE)

GxE can affect the overall genetic gain of a breeding program. Understanding the consequences of GxE to the performance of Nile tilapia strains cultured at Pangani and Kunduchi can help in making appropriate decisions regarding the design of a future breeding program. The genetic correlation for harvest body weight between the two environments was low (0.35) with large standard errors. In general, genetic correlations below 0.8 suggest a significant genotype × environment interaction effects (Robertson, 1959). For aquaculture species, when genetic correlation between two environments is lower than 0.70 (Sae-Lim et al., 2013), separate breeding programs are suggested. Our findings were consistent with the values reported by Dinh Luan et al., (2008) were a genetic correlation of 0.45 for fresh water and brackish environments was found.

Our genetic correlations estimate (0.35 ± 1.37) was different to other estimates of GxE for harvest weight of Nile tilapia from previously studies:

0.81 between aerated and non-aerated ponds (Mengistu et al., 2020), 0.76–0.99 between different pond environments (Eknath et al., 2007), 0.86–0.94 between nucleus, cage and low input pond (Trong et al., 2013), 0.74 between mixed sex and mono sex Nile tilapia (Omasaki et al., 2016), and 0.74 between low input and high input pond environments (Khaw et al., 2009). Nevertheless, in our study we acknowledge the very high standard errors, therefore the evidence of a GxE interaction in our study might not be significant based on genetic correlation due to large standard errors. However, when the environment where animals were selected is different from where they will be tested then GxE is anticipated. Estimated breeding value (EBV) is a key parameter to a breeding program because it makes an estimate of the genes the parents convey to their offspring. The existence of GxE cause EBV re-ranking in families, this was confirmed by strong family re-raking observed in two cultured environments. Interestingly, families 9 and 10 both from FETA strain were less affected by GxE.

Based on genetic diversity study, pure strains (Igunga, FETA and Lake Victoria) exhibited low genetic variation while TAFIRI was moderate. Strains (Karanga, Ruhila and Kunduchi) showed highest level of diversity and provided evidence of admixture. Therefore, from the genetic analyses, the most promising strains are Igunga, FETA, Lake Victoria and TAFIRI.

During the common garden experiment, we observed clear ranking of strains but differences between Kunduchi and Pangani. The Igunga strain appeared to be the most robust, while the TAFIRI strain showed a high variation across the two environments. This may suggest the presence of GxE which could argue for a separate breeding program for each environment in Tanzania if we choose TAFIRI strain as a base population. However, the Igunga strain which has showed evidence of being vigorous and fast growing, provides evidence that the environmental differences did not have much influence on this strain.

Genetic variation in the population, heritability and genetic correlation (Bentsen and Gjerde, 1994) determine the final phenotypic performance in a breeding program. Nevertheless, higher heritability at one-month time point of measurement was observed at Kunduchi and lower heritability at Pangani. Therefore, the differences observed at Pangani could be non-genetic. In addition, lower heritability at Pangani suggest that environmental factors such salinity in brackish water could have restrained the fish from manifesting their full potential to utilize additive genetic for growth

performance. These restrictions most likely resulted in strong re-raking of strain and family as revealed by lower genetic correlation between two environments.

However, heritability estimates the genetic variation within population or strains, that's individual genetic differences within a strain and not genetic differences between strains. Therefore, the estimation of genetic parameters in this thesis have corrected away the genetic variation differences between strains.

5. General conclusions

In this thesis we have generated the basic information for the establishment of future sustainable breeding program for tilapia strains in Tanzania.

We have provided information about the genetic diversity of the strains, their performance in brackish and freshwater production environments, estimates of genetic parameters and the magnitude of GxE interaction between two environments. Genetic analysis revealed greater genetic diversity within than among strains. Genetically closed strains of Igunga, FETA and Lake Victoria TAFIRI, suggests that these could be pure strains without admixture. The gained information is useful for wild strains conservation practices and formation of a base population for the selective breeding purposes. In addition, the aforementioned information could help in making breeding decisions for the sustainable development of tilapia breeding programs in Tanzania. Our study presents the first estimate of genetic diversity of Nile tilapia strains in Tanzania based on SNP markers developed from ddRADseq, this provide the important baseline as SNP increasingly become the marker of choice for strain studies and for aquaculture breeding programs.

We communally reared Nile tilapia strains under common garden experiment and use pedigree and phenotypic information to estimate genetic parameters and GxE interactions. We observed lower heritability estimates for body weight between fish farmed brackish environments than those farmed in freshwater environments. Our results also revealed the occurrence of significant GxE interaction effects on traits based on weak genetic correlations and strong strain and family re-ranking between two environments. Nevertheless, in our analyses we observed genetic correlation with large high standard errors. Therefore, we cannot form conclusions about the actual genetic correlations.

Most of the strains ranked differently across two environments, however Igunga strain performed better in both environments. Since Tanzania is a developing country with inadequate resources, in this circumstance suggesting for having multiple breeding programs for Nile tilapia would be burdensome. Therefore, there is no need to for a separate breeding program as suggested by the existence of GxE interaction. Thus, the future breeding program can work with a robust strain.

6. Future perspectives

Nile tilapia culture in Tanzania is rapidly growing and increasing to meet the increased demand for farmed fish due to the growing population. The situation has escalated the need for high quality fish seed and brooders at reasonable and cost-effective prices. This thesis has provided information on suitable strains to be used in brackish and freshwater environments. Therefore, based on the findings of this thesis, the next step forward should superscribe the following issues related to Nile tilapia farming in Tanzania

Maintain available broodstock population which have known genetic diversity and have been tested for their performance in production environments, therefore the next step should focus on designing a selective breeding scheme based on the suggested strain (Igunga strain) and tested production environments. Because broodstock numbers may decline through attrition over time, leading to loss of both genetic diversity and pedigree information.

Develop a cost-benefit analysis for a tilapia breeding program including traits that are of value

Since in this thesis we have already developed genotype and phenotypic data of available strains in Tanzania, then the commencing and implementation of selective breeding program is now economically viable.

Avoid the direct transfer of improved strains from Asia such as GIFT tilapia because of possible potential harmful impact on native germplasm in Tanzania and undiscovered effects of gene-environment interactions.

Better to adapt the technology demonstrated in Asia for use in the genetic improvement of tilapia in Tanzania and study the potential environmental effects of improved stocks on local Nile tilapia stocks

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Popular science summary

The Nile tilapia (*Oreochromis niloticus*) is the most cultured fish species in Tanzania. The main challenge of Nile tilapia culture in Tanzania is the availability of better performing strain which can be reared in a wide range of production environments. The choice of suitable starting strains for the breeding program is important for a sustainable breeding program. Tanzania, as one of fortunate countries with several species of tilapia, needs to produce fast growing high yielding tilapia strains that will adapt wide range of local farming environments and that can be grown as low a cost as possible to benefit smallholders and to alleviate poverty and malnutrition. This thesis aimed at providing fundamental information for the establishment of a tilapia breeding program in Tanzania. The thesis consists of two main studies; genetic diversity study and common garden study presented in four papers.

In paper I, genetic diversity experiment was carried out using ddRADseq to study population structure and genetic diversity of local Nile tilapia strains cultured in Tanzania. Seven strains (TAFIRI, FETA, Kunduchi, Igunga, Karanga, Ruhila and Victoria) of local Nile tilapia were genotyped resulting into 2,180 informative SNPs. Strong genetic differentiation was revealed between closely related populations (FETA, Igunga and Victoria) and Karanga and TAFIRI. Karanga, Kunduchi and Ruhila strains showed genetic admixture while strains of FETA, Victoria, Igunga and TAFIRI showed no admixture. Admixed strains are not useful as the basis for a breeding program as segregation may make their performance less predictable.

In paper II, we studied the genetic differentiation within and between local and exotic Nile tilapia and Rufiji tilapia (*Oreochromis urolepis urolepis*) strains. The results showed high differentiation between Rufiji tilapia and exotic and local Nile tilapia but low differentiation between local and exotic Nile tilapia. Higher genetic variation was revealed among Rufiji

strains compared to exotic and local Nile tilapia. Wami, Mindu and Mansi strains of Rufiji tilapia species were most diverse but also highly admixed. The results also showed high genetic differentiation between Rufiji populations and the local or exotic Nile tilapia strains. Local strains (Victoria, Igunga and FETA) formed one genetic group with some exotic Nile tilapia strains (Chifive-C, Muleba-M and Ruvu Farm-R) from Uganda. The six strains of TAFIRI, FETA, Karanga, Igunga, Ruhila and Victoria were then compared for growth performance in two environments.

Therefore, in paper III, common garden experiment was conducted in Kunduchi (freshwater 0ppt) and Pangani (brackish water 2-5ppt). The animals were reared communally for a period of three months in hapas (12m x 8.5m) installed in a pond. The results showed that the differences in performance between the different strains were significant ($P< 0.001$) in the two environments and the strains ranked differently for growth between the two environments. In brackish water environment, the Igunga strain ranked first and lastly was Victoria strain while in freshwater Karanga strain ranked first in performance whereas TAFIRI was the lowest performing strain. However, the Igunga strain appeared to be the most robust across the two environments.

Paper IV was also based on the common garden experiment. We used pedigree and family information to estimate genetic parameters for the measured growth traits. Heritability estimates for harvest body weight were low in both the freshwater (0.10) and brackish water (0.09) environments compared to weight at tagging. Genetic correlations between the two environments were low (0.35) for body weight at harvest and families ranked differently across the two environments indicating the existence of substantial GxE.

In conclusion, genetic diversity study revealed that some of the tested local Nile tilapia strains are pure without admixture. Moreover, the local Nile tilapia and Rufiji tilapia are still genetically distinct while exotic and local Nile tilapia showed some degree of admixture in some strains. Common garden experiment showed that there are differences in growth performance among tested Nile tilapia strains and that strains ranked differently for growth between the two environments. The best performing strain in both environments, which was identified as Igunga strain, can be chosen for the planned tilapia breeding program in Tanzania. Overall, the obtained results from this thesis can be used as a guideline for selecting the strain to work

with as a base population for the future breeding program. Significant GxE interaction between freshwater and brackish water environments was found based on weak genetic correlations between two environments, strong family re-ranking and lower heritability in brackish water compared to freshwater environments. Therefore, the future tilapia breeding program in Tanzania should be based the statistical evidence on GxE, environmental conditions, and economic aspects of the country in relation to resources available for genetic improvement programs.

Populärvetenskaplig sammanfattning

Niltilapia (*Oreochromis niloticus*) är den mest odlade fisken Tanzania. Den största utmaningen för odling av niltilapia i Tanzania är att hitta en stam som presterar bra och som kan odlas i många olika typer av miljöer. Valet av stam att utgå ifrån är avgörande för ett hållbart avelsprogram. Tanzania, som har turen att ha många olika tilapiaarter, behöver producera snabbväxande och högavkastande tilapia som kan anpassas till flera olika lokala odlingsmiljöer och som kan odlas till en så låg kostnad som möjligt för att gynna småskaliga fiskodlare och bidra till mindre fattigdom och undernäring.
Den här avhandlingen syftade till att ge grundläggande information för att kunna upprätta ett avelsprogram för tilapia i Tanzania. Avhandlingen består av två huvudstudier, en studie om genetisk diversitet och en common-garden-studie, som presenteras i fyra delstudier.

I studie I utfördes ett experiment med hjälp av ddRADseq för att undersöka populationsstruktur och genetisk diversitet hos lokala stammar av niltilapia som odlas i Tanzania. Sju lokala stammar av niltilapia (TAFIRI, FETA, Kunduchi, Igunga, Karanga, Ruhila och Victoria) genotypades, vilket resulterade i 2180 informativa SNP:ar. Stor genetisk differentiering sågs mellan närbesläktade populationer (FETA, Igunga och Victoria) och Karanga och TAFIRI. Karanga, Kunduchi och Ruhila visade sig ha genetisk inblandning (admixture) medan FETA, Victoria, Igunga och TAFIRI inte uppvisade någon genetisk inblandning. Genetiskt blandade stammar kan inte användas som bas för ett avelsprogram eftersom segregering kan göra deras prestanda mindre förutsägbar.

I studie II undersökte vi genetisk differentiering inom och mellan lokala och exotiska stammar av niltilapia och rufijitilapia (*Oreochromis urolepis urolepis*). Resultaten visade på stor genetisk differentiering mellan stammar av rufijitilapia och exotiska och lokala stammar av niltilapia men liten

genetisk differentiering mellan lokala och exotiska stammar av niltilapia. Större genetisk variation sågs hos rufijitilapia jämfört med exotiska och lokala stammar av niltilapia. De stammar bland rufijitilapia som hade störst genetisk variation men även stor genetisk inblandning var Wami, Mindu och Mansi. Resultaten visade också på stor genetisk differentiering mellan rufiji-stammarna och de lokala eller exotiska niltiapia-stammarna. Lokala stammar av niltilapia (Victoria, Igunga och FETA) visade sig tillhöra samma genetiska grupp som ett antal exotiska niltilapia-stammar (Chifive-C, Muleba-M och Ruvu Farm-R) från Uganda.

I studie III jämfördes de sex stammarna TAFIRI, FETA, Karanga, Igunga, Ruhila och Victoria med avseende på tillväxt i två olika odlingsmiljöer (common garden): i Kunduchi (sötvatten 0ppt) och Pangani (brackvatten 2-5ppt). Försöksupplägget var detsamma i de båda odlingsmiljöerna; fiskarna hölls och utfodrades i en gemensam damm men i olika nätkassar (hapas, 12m x 8,5m) under en period av tre månader. Resultaten visade att skillnaderna i tillväxt mellan de olika stammarna var signifikant ($P< 0,001$) i båda miljöerna samt att stammarna rankades olika i de två miljöerna vad gäller tillväxt. I brackvattenmiljön rankades Igunga högst och Victoria lägst, medan Karanga rankades högst och TAFIRI lägst i sötvattensmiljön. Igunga-stammen verkade dock vara den mest robusta i de två miljöerna sammantaget.

Även studie IV baserades på common-garden-försöket. Vi använde stamtavla och familjeinformation för att uppskatta genetiska parametrar för de uppmätta tillväxtegenskaperna. Uppskattad heritabilitet för skördevikt var låg i både sötvatten- (0,10) och brackvattenmiljön (0,09) jämfört med vikt vid PiT-märkning. Den genetiska korrelationen mellan de två miljöerna var låg (0,35) för skördevikt och familjerna rankades olika i de två miljöerna, vilket tyder på förekomsten av betydande GxE.

Sammanfattningsvis visade studien om genetisk diversitet att några av de testade lokala niltilapia-stammarna är rena utan genetisk inblandning. Studien visade också att de lokala stammarna av niltilapia och rufijitilapia fortfarande är genetiskt åtskilda, medan genetisk inblandning till viss del förekommer i några av de exotiska och lokala niltilapia-stammarna. Common-garden-försöket visade att det är skillnader i tillväxt bland de testade niltilapia-stammarna samt att stammarna rankades olika med avseende på tillväxt mellan de två odlingsmiljöerna. Den stam som presterade bäst i båda miljöerna, Igunga-stammen, kan väljas för det

planerade avelsprogrammet för tilapia i Tanzania. Sammantaget kan resultaten från denna avhandling användas som riktlinjer för val av stam att arbeta med som baspopulation för ett framtida avelsprogram. Signifikant GxE-interaktion mellan sötvatten- och brackvattenmiljön noterades, vilket beror på svag genetisk korrelation mellan de två miljöerna, stor familje-re-ranking och lägre heritabilitet i brackvatten- jämfört med sötvattenmiljön. Mot bakgrund av detta bör ett framtida avelsprogram för tilapia i Tanzania baseras på statistiskt bevis på GxE, miljöförhållanden samt ekonomiska aspekter för landet i relation till tillgängliga resurser för genetiska förbättringsprogram.

Acknowledgements

To the **almighty God** who has given me life and kept me alive until this time when I'm writing this thesis. **Glory** and **Praise**, you alone are worthy.

The work of this thesis was funded by Swedish International Development Cooperation Agency (Sida) through the bilateral program of Marine Science subprogramme between University of Dar es Salaam in Tanzania and Swedish University of Agricultural Sciences in Sweden. Thank you Sida and I am grateful for your financial support that has funded my four years of PhD education and the work presented in this thesis.

The studies described in this thesis were performed at the department of Aquatic Sciences and Fisheries Technology, Kunduchi campus, University of Dar es Salaam and at Pangani Mariculture centre, Institute of Marine Sciences, University of Dar es Salaam. Lab work for DNA extraction was performed at the department of Animal Breeding and Genetics, SLU and ddRADseq at Roslin Institute in Edinburg University, UK. This work would have not been possible without the assistance of technicians from those institutions. I would like to acknowledge the support from those institutions for providing their facilities and assistance during this work.

I would like to express my sincere gratitude to all the people who supported me during my PhD studies in Sweden and Tanzania and guiding me to become a better scientist. I'm very grateful for my supervising team in Sweden:

My sincere gratitude to my main supervisor, Professor **Dirk Jan de Koning**, thank you for your support, encouragement, motivation and guidance for the past four years. You were always there for me and bring solutions even when I was not capable in solving my own problems. You have been patient with me in this last year and your support has made a huge difference in my life.

To my co-supervisor**,** Dr**. Christos Palaiokostas**, thank you for your motivation and for helping me move forward when I stuck in data analysis. I really appreciate all the work and effort you put in assisting me with data analysis, interpretation and making sure all the analysis needed done for this project. Thank you for your guidance and assistance you gave me all the time I needed it, during night, weekends and holidays you were always available and there for me. Sometimes wondering if you ever leave your Mac Book (joke).

To my co-supervisor, Dr**. Anna Normal Haldén**, thank you for your support, encouragement and nice talks that gave me energy and pushed me forward no matter the difficulties I was going through.

I'm grateful for this supervising group in Sweden for your discussion meetings, suggestions, comments and inputs that were very valuable to this thesis and equipped me to a better expert in the field and animal breeding and genetics. Thank you for trusting me to do independent research and study and let me learn by myself and figure out things on my own.

Special thanks also goes to my co-supervisors in Tanzania Dr. **Matern Mtolera**, thank you for being a supervisor and PI for this project. Your support, encouragement and contributions during my PhD studies were highly appreciated. Thank you for your infinite patient, support and understanding especially during this last year of my study.

Professor **Chacha Mwita**, thank you for your support, constructive ideas, comments and supervision during my stay at Kunduchi campus. I remember you made sure that i get all the facilities for my experiment and accommodation at the campus, thank you.

I would like to acknowledge all the staff from the department of Animal Breeding and Genetics; **Lotta, Susanne**, **Anna J**, **Tomas B**, **Sofia M**, **Juliette**, **Tomas K**, **Martin** and **Fernando**. Thank you all for your knowledge, kindness and great moment we shared during fika time. **Cano**, thank you for your IT assistance and for giving me all the facilities; office, keyboard, big desktop screen so that I can comfortably finish this PhD, much appreciation.

Thanks to all the PhD students from Hgen and IMS, Berihu (former office mate), **Suv**i, **Christian**, **Julie**, **Patricia**, **Stanley**, **Moses**, **Justine**, **Francis**, **Sofia N** and **Maria**. Thank you all for your nice moments and support. I want to express my sincere gratitude to my Bolivian friend **Gabriela B**, thank you dear for the support in my difficult times. Even if you are back to Bolivia, I

can still feel your presence. I do remember and miss you so much. You were the first to show me the way out here in Uppsala during my first arrival in Sweden. Always available for helping and approachable. Thank you for your support, encouragement and friendship.

Christer, thank you for your support, encouragement and good moments we shared together in Pangani, Zanzibar, Malaysia, Finland, Uppsala and everywhere we had been together. Thank you for your patient and I have learned a lot from you the way you handle stress, thank you for the lesson. This PhD journey could have not been so easy without your advice and encouragement.

Levinus Mapenzi (Dr Mapenzi), my big brother thanks for your support, friendship and for treating me as your young sister. It was always funny when we were learning R together, I remember all the ups and downs but at last we were successfully.

My sincere thanks to all the farmers and both private and government owned hatcheries for their supporting when I was collecting fish from them. Thank you all, especially **Paul** from TAFIRI Mwanza and **Lucka** from Ruhila for their help with packing and transporting fish.

Special thanks to all the Laboratory technicians and assistance at Kunduchi and Pangani. **Selemani**, **Michael**, **Komba** and **Muhidin**, thank you for your support during feeding the fish, hapa installation and cleaning of fishpond. **Liberata**, thanks young sister for your kindness and sincere help during fish tagging at Kunduchi and Pangani

Finally, my deepest gratitude to my family who supported me throughout this journey, my husband, **Emmanuel Sungwa,** thank you for your love, support and letting me achieve this dream. My mother **Grace Simon**, brother **Rwekaza** and sister **Amina**, thank you all for your encouragement and strength you gave me.

"**I can do all things through Christ who strengthens me**" (Philippians 4:13)

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2020:62

The aim of this thesis was to explore the genetic diversity within and between local strains of Nile tilapia (*Oreochromis niloticus*) in Tanzania. Growth performance of these strains was then compared in a common garden experiment in two different environments. Genetic parameters for growth traits in the strains were estimated and significant genotype by environment interaction between strains in two environments was investigated. The results provide an important starting point for planning a structured breeding program for Nile tilapia in Tanzania.

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Online publication of thesis summary: http://pub.epsilon.slu.se/

ISSN 1652-6880
ISBN (print version) 978-91-7760-642-0
ISBN (electronic version) 978-91-7760-643-7