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Developing the basis of a breeding program for sustainable Rufiji tilapia aquaculture in Tanzania

Christer Simon Nyinondi

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Christer Simon Nyinondi
Faculty of Veterinary Medicine and Animal Science
Department of Animal Breeding and Genetics
Uppsala

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Cover: Male Rufiji tilapia
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Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, Uppsala, Sweden
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Abstract

Tanzania has a rich natural fauna of tilapiine fish. However, a proper management of tilapia farming is lacking. Use of inappropriate technology to produce seed and inadequate extension efforts resulted in a poor production output from aquaculture. This aquaculture gap has led the society to rely on wild captures which are depleting and cannot be easily accessed by poor communities and hence increased the rate of child and infant malnutrition. Developing a breeding program for Rufiji tilapia will ensure the production of high-quality egg and larvae in culture systems and at the same time preserve the natural biodiversity of these species in Tanzania. This thesis aims to generate information that will be used for the development of a Rufiji tilapia strain that will be used as the base for a selective breeding program in Tanzania. This study was divided into two experiments. A pilot study survey was conducted beforehand on the status of fish farming in Tanzania. The survey provided key information that facilitated better understanding of the status and availability of infrastructure for effective dissemination of a structured breeding program. Poor infrastructure, poor fingerlings quality, and lack of health farming management practices were found.

Thereafter, the genetic diversity and population structure of both wild and farmed Rufiji tilapia populations and their relation with exotic and local Nile tilapia (*Oreochromis niloticus*) were studied using high-throughput sequencing. Double-digest restriction-site-associated DNA (ddRAD) libraries were constructed from 195 animals originating from 8 wild and 2 farmed populations. Genetic distance estimates (F_{ST}) were low among populations from neighbouring locations, with the exception of Utete and Chemchem populations (F_{ST} = 0.34). Bayesian and multivariate statistical approaches indicated the existence of three distinct genetic clusters. The former analysis also revealed high admixture among Mindu and Wami populations and low admixture in Mansi and Utete populations. When compared to exotic and local Nile tilapia, Rufiji tilapia showed high genetic variation. High F_{ST}

values (0.6 - 0.8) were observed between Rufiji strains and the local or exotic Nile tilapia strains. Interestingly, the aforementioned two highly admixed population from Rufiji tilapia were closely related to Nile tilapia but genetically distant to other Rufiji tilapia populations.

The second part of this thesis was based on a common garden experiment where the existence of genotype by environment interaction was investigated by rearing Rufiji tilapia populations in two sites (Pangani and Kunduchi) of differing salinity and temperature levels. Nine populations were set-up for individual mating and 35 full-sib families were produced resulting in a pedigree that consisted of 1,392 animals. The best performing populations in terms of the recorded growth-related traits were Wami and Mindu that were reared in Pangani, while the lowest growth performance was recorded in the case of Ruaha population reared at Kunduchi. Moderate to high heritabilities (0.39 – 0.74) and genetic correlations (0.73 - 0.74) between these growth traits indicated the possibility of moderate reranking of the best performing animals. Overall, the mean family estimated breeding value (EBV) was higher in animals reared in Pangani compared to their full-sibs that were reared at Kunduchi. Furthermore, selecting a Rufiji tilapia as a base population for a selective breeding program needs to balance between best phenotypic performance and broad genetic variation. Notably, if rearing is to take place on sites of varying salinity and temperature levels selection should not only be based on using data from the breeding nucleus.

Keywords: Aquaculture, ddRAD-seq, genetic diversity, common garden, selective breeding, genotype by environment interaction

Author's address: Christer Simon Nyinondi, SLU, Department of Animal Breeding and Genetics, P.O. Box 7023, 750 07 Uppsala, Sweden

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Abstract

Tanzania har en rik naturlig fauna av tilapia-arter, men saknar tillfredställande förvaltning av odling av tilapia. Användning av undermålig teknologi för att producera ägg och larver för utsäde och otillräcklig rådgivning till fiskodlare har resulterat i låg produktion. Den låga vattenbruksproduktionen har lett till att samhället förlitar sig på vilda fångster som håller på att utarmas och som är svårtillgängliga för fattiga samhällen, vilket har bidragit till ökad grad av undernäring hos barn och spädbarn. Att utveckla ett avelsprogram för rufijitilapia (*Oreochromis urolepis urolepis*) kommer att säkerställa produktionen av högkvalitativa ägg och larver för användning i fiskodling och samtidigt bevara den naturliga biologiska mångfalden för dessa arter i Tanzania. Denna avhandling syftar till att generera information som kan användas för utvecklingen av en rufijitilapia-stam som i sin tur kan användas som bas för ett selektivt avelsprogram i Tanzania. Studien som ligger till grund för avhandlingen är uppdelad i två försök, vilka föregicks av en bakgrundsstudie om fiskodlingens status i Tanzania. Bakgrundsstudien gav nyckelinformation som ökade förståelsen för vilken infrastruktur som finns tillgänglig för effektiv spridning av ett strukturerat avelsprogram. Dålig infrastruktur, låg utsädeskvalitet och bristande rutiner för fiskhälsovård noterades.

Därefter studerades den genetiska mångfalden och populationsstrukturen hos både vilda och odlade populationer av rufijitilapia och deras relation med exotiska och lokala populationer av niltilapia (*Oreochromis niloticus*) med hjälp av så kallade Next Generation Sequencing (NGS). Med ddRAD-sekvensering (double digest restrictionsite associated DNA sequencing) konstruerades DNA-bibliotek utifrån 195 fiskar som härstammade från 8 vilda och 2 odlade populationer. Den genetiska skillnaden (F_{ST}) var låg bland populationer från närliggande platser, med undantag för Utete- och Chemchem-populationerna (F_{ST}= 0,34). Bayesianska och multivariata statistiska metoder indikerade förekomsten av tre distinkta genetiska kluster. Den

förra analysen avslöjade också hög genetisk inblandning (admixture) bland Minduoch Wami-populationerna och låg inblandning i Mansi- och Utete-populationerna. Jämfört med exotiska och lokala niltilapia visade rufijitilapia stor genetisk variation. Höga F_{ST}-värden (0,6 - 0,8) observerades mellan Rufiji-stammar och de lokala eller exotiska Nile tilapia-stammarna. Intressant nog var de ovannämnda två rufijitilapiapopulationerna med hög genetisk inblandning nära besläktade med niltilapia men genetiskt avlägsna från andra rufijitilapia-populationer.

Den andra delen av avhandlingen baseras på ett common-garden-försök där förekomsten av genotyp-miljö-interaktion undersöktes genom att odla rufijitilapiapopulationer på två platser (Pangani och Kunduchi) med olika salthalt och temperaturnivåer. Nio populationer sattes upp för individuell parning och 35 helsyskonfamiljer producerades vilket resulterade i en stamtavla som bestod av 1 392 fiskar. De bäst presterande populationerna när det gäller de registrerade tillväxtrelaterade egenskaperna var Wami och Mindu som hölls i Pangani, medan den lägsta tillväxtprestationen registrerades i fallet med Ruaha-populationen som hölls vid Kunduchi. Måttlig till hög ärftlighet (0,39 – 0,74) och genetisk korrelation (0,73 – 0,74) mellan dessa tillväxtegenskaper indikerade möjligheten till måttlig omrankning av de bäst presterande individerna. Sammantaget var medelvärdet för familje-avelsvärdet högre hos individer som hölls i Pangani jämfört med deras helsyskon som hölls vid Kunduchi. Valet av en rufijitilapia som baspopulation för ett selektivt avelsprogram måste balansera mellan bästa fenotypiska prestanda och bred genetisk variation. Särskilt om uppfödning ska äga rum på platser med varierande salthalt och temperaturnivåer bör valet inte bara baseras på data från avelspopulation.

Keywords: Akvakultur, ddRAD-sekvensering, genetisk variation, common garden, selektiv avel, genotyp-miljö-interaktion

Dedication

To Jeanelle,
My lovely daughter, my hope, my light even in my darkest days. You are the strength I need to keep going.

To Dr. Philbert Nyinondi,
My amazing brother, you became my shield and never allowed obstacles to stop me from moving forward. Your intense caring and support has made me who I am today. I wholeheartedly thank you brother for every aspect of my life. *Mungu akubariki sana kaka yangu.*

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, Mapenzi L.L, Nyinondi C.S, Haldén A.N, Mmochi A.J, Chacha M, et al. (2019). The Need of a Structured Tilapia Breeding Program in Tanzania to Enhance Aquaculture Production: A Review. Tanzania Journal of Science, 45(3): 355–71. Available from: www.ajol.info/index.php/tjs/

II. Nyinondi C. S, Mtolera, M. S. P, Mmochi, A. J, Lopes Pinto, F. A, Houston, R. D., de Koning, D. J., & Palaiokostas, C[§]. (2020). Assessing the genetic diversity of farmed and wild Rufiji tilapia (*Oreochromis urolepis urolepis*) populations using ddRAD sequencing. Ecology and Evolution, 10(18), 10044–10056. https://doi.org/10.1002/ece3.6664

III. Nyinondi, C.S*., Kajungiro, R.A *., Moses M.M *., Mtolera, M.S.P., Mmochi, A.J., Chauka, L.J., Chacha, M., Houston, R.D., Lopes Pinto, F.A., Norman Haldén, A., de Koning, D.J. Palaiokostas C[§]. (2022). Diversity and differentiation between local and exotic strains of two farmed Tilapia species in Tanzania using ddRAD sequencing (manuscript)

IV. Nyinondi C. S, Mtolera, M. S. P, Mmochi, A. J, Lopes Pinto, F. A, Houston, R. D., de Koning, D. J., & Palaiokostas, C[§]. (2022). Selective breeding for growth increase in monosex Rufiji tilapia (*Oreochromis urolepis urolepis*). Journal of Applied Aquaculture (Submitted)

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§Corresponding author

* These authors contributed equally.

The contribution of Christer Simon Nyinondi to the papers included in this thesis was as follows:

I. Conceptualisation of the research question, designed and collected data for the study together with other co-authors. Drafted, wrote and review the manuscript in collaboration with all co-authors

II. Planned and designed the study with other co-authors, collected and analysed data. Drafted, wrote and review the manuscript in collaboration with all co-authors

III. Planned and designed the study with other co-authors, collected and analysed data. Drafted, wrote and review the manuscript in collaboration with all co-authors

IV. Planned and designed the common garden study with other co-authors, collected and analysed data. Drafted, wrote and review the manuscript in collaboration with all co-authors

List of tables

List of figures

Abbreviations

DAPC	Discriminant Analysis of Principal Components
ddRAD-seq	Double digesting Restriction site associated DNA sequencing
DO	Dissolved oxygen
DNA	Deoxyribonucleic acid
EBV	Estimated Breeding Value
FIS	Inbreeding coefficient
FST	Fixation Index Statistics
GBS	Genotype by sequencing
GIFT	Genetically Improved Farmed Tilapia
G × E	Genotype by Environment Interaction
HE	Expected heterozygosity
HO	Observed Heterozygosity
IMC-MC	Institute of Marine Sciences Mari-culture Centre
SNP	Single Nucleotide polymorphism
PCA	Principal Component Analysis
PIT	Passive Integrated Transponders
RADseq	Restriction-site associated DNA sequencing

URT-MLF	United Republic of Tanzania, Ministry of Livestock and Fisheries

1. Introduction

The global human population has grown tremendously from 1 billion in 1800 to 7.9 billion in 2020 with an annual increase of 1.1%. The population is predicted to keep growing to reach 9.7 billion by 2050 (United Nations, 2022). As the global population continues to grow, demand for food is also expected to increase. Africa has the fastest growth among all regions with an expected population growth rate of 2.6% annually. It should be noted that the highest population growth in Africa is reported in the least developed countries (LDCs) making it more difficult for the governments to sustain the basic needs of people and running the risk of famine and malnutrition. As such, the increasing demand for food in these countries has inspired their governments to develop livestock farms and fish farming sectors to sustain the rising demand for animal protein. However, their policies to promote and attract private and public investments in the industry are still uncertain (FAO, 2022).

Fish is one of the best sources of protein in developing countries. It contains several unique and critical nutritional components such as essential micronutrients – vitamins A, B, D and minerals i.e. calcium, iodine, phosphorus and zinc, essential amino acids such as methionine, lysine and long-chain, polyunsaturated fatty acids (LC-PUFA) that are of importance for a healthy diet (Adeniyi et al., 2012; Islam et al., 2021; Karapanagiotidis, 2017). Fish provides about 17% of the average per capita intake of animal protein and 6.5% of all protein consumed by more than 4.5 billion people worldwide (Chenyambuga, 2018). Moreover, fish is a major source of income, employing more than 158 million people in the world in different fish-related activities like fishing, farming, processing and transportation (FAO, 2022). In this regard, a significant increase in fish production all around the globe has been observed in a few decades. Until four decades ago

fish farming contributed about 5% of the total aquaculture production, nowadays contributes about 47.4% (54.3 million tonnes) (FAO, 2020). Tanzania, like many other developing countries, aims to develop the aquaculture sector and improve fish protein consumption rate from its current 8kg/person/year (URT, 2020) to at least 20kg consumption /person/year as recommended by Food and Agriculture Organization (FAO, 2022). Despite both local and central governmental efforts, the country is far behind in reaching the target goals.

Tanzania has over 630 freshwater fish species, most found in Lakes Victoria, Malawi and Tanganyika. However, only a few species have shown a significant potential for commercial aquaculture practices. In Tanzania, especially *Oreochromis* species are farmed of which Nile tilapia (*Oreochromis niloticus*) is the most farmed fish (Shechonge et al., 2018). Additionally, the country has been experiencing a decline of wild fisheries catches due to overfishing and illegal fishing practices (URT, 2020). Meanwhile, the demand for fish and fishery products is expected to increase even the coming years due to population growth. In order to boost the national production, introduction of fish species in non-native water bodies has taken place (Kajungiro et al., 2019a; Moses et al., 2019; Shechonge et al., 2018). Despite the advantages of introducing Nile tilapia in non-native habitat to boost fish production, its negative impact to the tilapia biodiversity should be considered as well as Nile tilapia can cross breed with other closely related endemic Oreochromis species (Nyinondi et al., 2020; Shechonge et al., 2018).

Rufiji tilapia (*O. urolepis urolepis*) is a cichlid with potential for aquaculture. This species is endemic to Tanzania and distributed mainly in the Rufiji river basin (Nyinondi et al., 2020). Like most cichlids, Rufiji tilapia have the ability to grow fast, tolerate environmental changes and are easy to raise. Unlike most *Oreochromis* species, it has the capacity to tolerate high water salinities that could be of advantage for costal aquaculture due to the scarcity of fresh water.

In general, male tilapias are preferred for aquaculture due to their ability to invest all their energy into growth and hence achieve a larger size compared to females that start to reproduce at small body sizes before the animals reach the harvest size. Male Rufiji tilapia have the ability to cross breed with female Nile tilapia and produce all-male hybrids, which is economically important (Mapenzi & Mmochi, 2016). Since Rufiji tilapia is

native to Tanzania, its promotion in aquaculture could reduce the impact of introducing non-native species that could jeopardize the cichlid biodiversity (Kajungiro et al., 2019a; Nyinondi et al., 2020).

In order to improve fish production, investigating the molecular genetic diversity of wild and farmed Rufiji tilapia is important. Understanding the genetic structure and diversity of these species can assist in the conservation of genetic resources of wild populations and set the basis for the establishment of a breeding program, which requires background information about the genetic status of the founder population in order to minimize inbreeding accumulation (Kajungiro et al., 2019a). Through the establishment of a breeding program the sustainability of fish seed can be achieved resulting in a more efficient and sustainable production.

2. Background

2.1 Global aquaculture production

Aquaculture is currently the fastest growing food-producing sector with an average growth rate of 5.3% per year over the last two decades. Global aquaculture production is mainly dominated by aquatic animals where in the year 2020 a total of 122.6 million tonnes in live weight was attained with a total value of USD 281.5 billion. Aquatic production in 2020 consisted of 87.5 million tonnes of aquatic animals and 35.1 million tonnes of aquatic algae (Figure 1) (FAO, 2022).

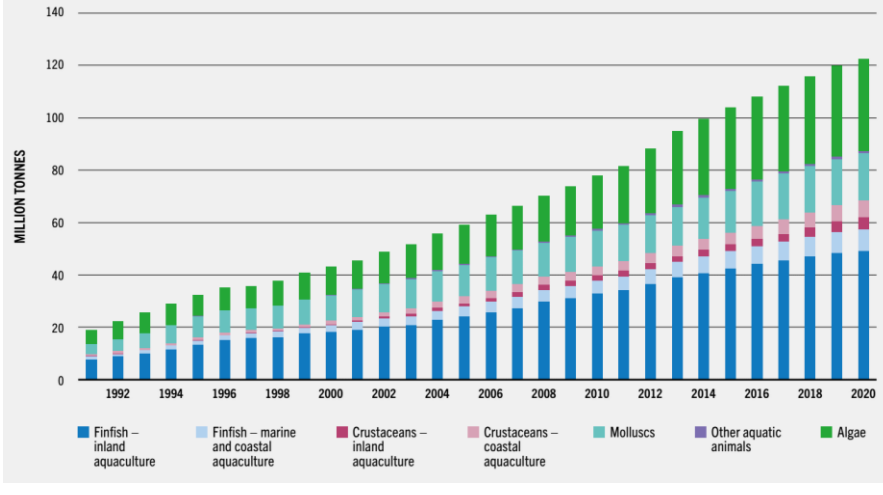

Figure 1. World aquaculture production of aquatic animals and algae (Data expressed in live weight equivalent from 1991 - 2020). source: FAO, 2022

World aquaculture fish production has grown progressively over continents accounting 46% of the total fishery production, and supplying over 52% of the world's fish for human consumption. Asia has remained the leading producer contributing more than 88% of the total aquaculture production over the past two decades, with China as the largest producer with a significant share of over 57% in 2018. However, there is an increase on aquaculture production in other regions even though their contribution is still low. The Americas contribute around 5%, Europe contributes around 4% and lastly Africa contributing less than 3% of the total aquaculture production for the past two decades (FAO, 2020). This large uneven distribution pattern of aquaculture production across the continents and countries has remained unchanged over decades despite high aspirations for strong aquaculture development around the globe to sustain their fast-growing populations, especially in developing countries (FAO, 2022). Similar to global food fish production, its consumption has increased tremendously at an average annual rate of 3.1% from 1961 to 2017, a rate higher than that of all other animal protein foods such as meat, dairy, milk, etc. which increased by 2.1% per year for the same period (FAO, 2022).

African aquaculture production still has a long way to go to surpass that of capture fisheries. At region level Africa's share is still low about 2.7% of the total world aquaculture production. Within the region, aquaculture accounted for 16 –18% of total fish production. Although the contribution of aquaculture production in Africa is still very low, a notably fast growth rate of 2.5% has been observed (FAO, 2020). Aquaculture in Africa is dominated by Egypt accounting for 1.9% of the total word fish production and about 71% of African aquaculture fish production. In addition to Egypt, Nigeria has also shown a remarkable increase in its aquaculture production to become the second major producer in Africa and leading in sub-Saharan Africa (Figure 2).

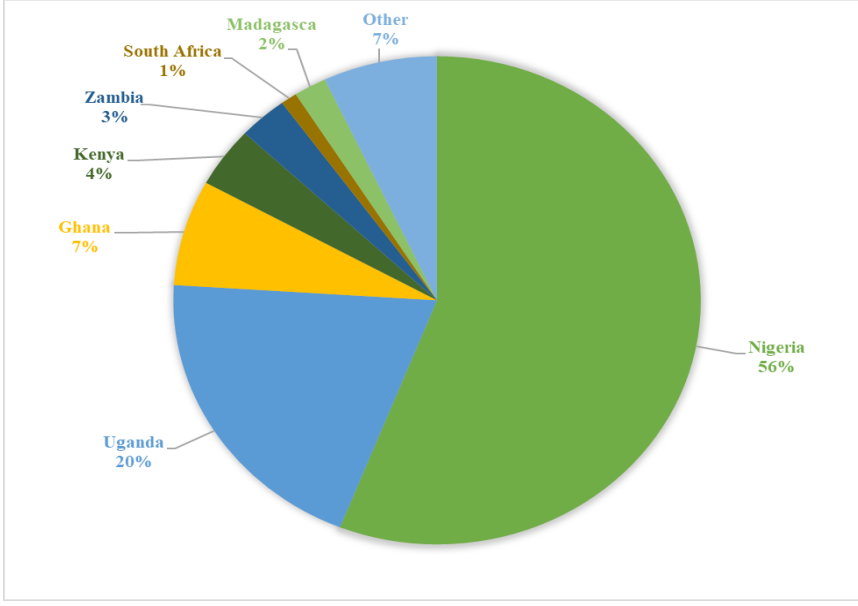

Figure 2. Main seven aquaculture producers in Sub-Sahara Africa in 2018 (quantity in Percentages). *Source*: FAO, 2020.

2.2 Tanzanian aquaculture

Tanzania is a coastal state on the Western Indian Ocean situated in the Eastern part of Africa. It is well endowed with natural resources in terms of fisheries and aquaculture potential such as Ocean, lakes, dams, and rivers. The total water coverage is 346,337 Km^2 which is equivalent to 36.7% of the total land area. Tanzania has varying climatic condition from the temperature ranging from 10°C in the North-western and southwest highlands to 30°C in the coastal regions where the weather is hot and humid. This enables the country to culture both temperate and tropical aquatic animals (URT, 2020).

In Tanzania fish farming is thought to have started in 1927 when rainbow trout (*Onchorynchus mykiss*) was released into water streams in the northern and southern highland regions of Kilimanjaro and Mbeya by Scottish missionaries (Balarin, 1984; Shoko et al., 2011). However, aquaculture property started by the earthen pond experimental culture of Tilapia in 1950's at Korogwe in Tanga and Malya in Mwanza regions (Shoko et al., 2011). The government and Non-Government Organizations (NGOs) helped the fish farming communities by providing fingerlings, financial and

technical support in the "Eat more fish campaign" (Rukanda & Sigurgeirsson, 2016). By 1960, the country had about 10,000 earthen ponds stocked with tilapia fingerings introduced from Lake Victoria, river Pangani and river Congo (Shoko et al., 2011).

Other species that were cultured for food after the first introduction of Nile tilapia (*Oreochromis niloticus*) were Singida tilapia (*O. esculentus*), longfin tilapia (*O. Macrochir*), Victoria tilapia (*O. Variabilis*), Three spotted tilapia (*O. Andersonii*), Mozambique tilapia (*O. Mossambicus*), redbreast tilapia (*Coptodon rendalli* & *Coptodon zillii*), Nile perch (*Lates niloticus*), African catfish (*Clarias gariepinus*), Bagrus docmac, Ningu (*Labeo victorianus*), *Citharinus spp*, Rabbitfish (*Siganus canaliculatus*) (Dadzie, 1992). In recent years other tilapia species like Rufiji/Wami/Zanzibar tilapia (*Oreochromis urolepis urolepis*), Shile tilapia (*Oreochromis shiranus*) and Karonga tilapia (*Oreochromis Karongae*) native of lake Malawi are also getting attention for their use in aquaculture (Genner et al., 2018). By1990's, many aquaculture projects in this country failed to reach their full potential due to lack of suitable technology and withdrawal of financial support from donor (Shoko et al., 2011).

Inland aquaculture is currently picking up in the country with the annual production of 18,081.6 tonnes in 2018. Nile tilapia and catfish (*Clarius gariepinus*) contribute a major share of national finfish production with the contribution of 16,288 tonnes in 2018. Overall, inland aquaculture contributes less than 5% of the total fish production of which Nile tilapia accounts about 95% of the total production (URT-MLF, 2019). On the other hand, marine aquaculture is dominated by seaweeds (*Eucheuma denticulatum* and *Kappaphycus cottonii*), milkfish (*Chanos chanos*) and prawns (URT-MLF, 2019). Tanzania is among the top ten major producers of seaweed where in 2018, its production was 103.2 thousand tonnes, live weight contributing about 3.2% of global aquaculture production of aquatic algae (FAO, 2020). Despite immense potential for fish aquaculture development, this sector still mainly relies in small earthen ponds. Tanzania has about 26400 earthen ponds, 408 cages and 1 recirculating aquaculture system (RAS) for shrimps (Figure 3). Overall, the aquaculture sector contribution to the national economy in gross domestic products (GDP) is still below 2% with the growth rate of 9.2% by 2018 (URT, 2020).

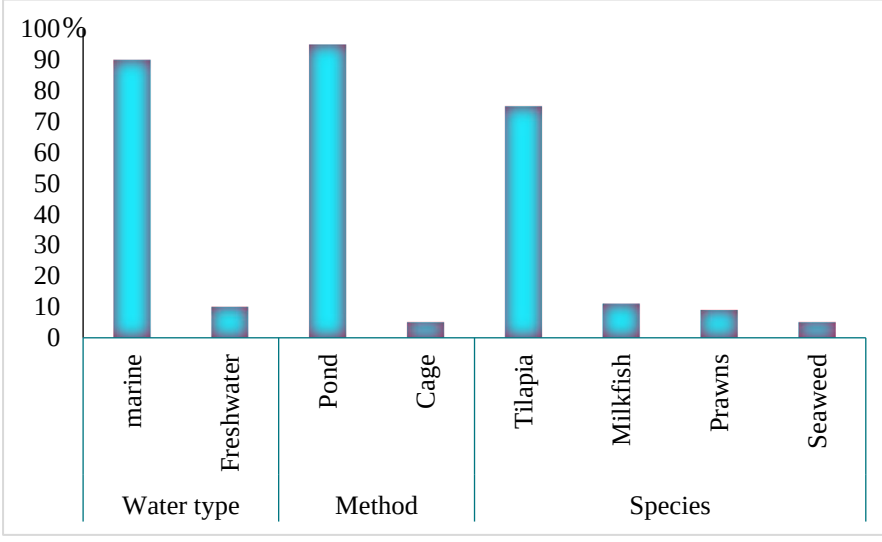

Figure 3. Aquaculture Production in Tanzania by type of water, farming method and main farmed aquatic species. *Source*: URT-MLF, 2019

2.3 Tilapia farming

The genus Tilapia (Smith, 1940) comprises of mainly four sub-genera (*Alcolapia, Coptodon*, *Oreochromis* and *Tilapia*) which are taxonomically classified by both feeding and reproductive characteristics. *Oreochromis* and *Alcolapia* are mouth brooders where the female mouth brood fertilized eggs for several weeks before releasing the fry. *Tilapia* and *Coptodon* are substrate spawners where both males and females guard the eggs and fry until they are old enough to leave the nest. They are primarily herbivores, feeding mainly on phytoplankton (Genner et al., 2018).

Fish farming started in China with the farming of common carp (*Cyprinus carpio*) in earthen ponds about 4-5000 years ago (Gjedrem & Baranski, 2009). Tilapia farming have been ongoing for more than 1000 years and Nile tilapia is among the earliest aquatic animals to be farmed under captivity (Houston et al., 2020). At present, tilapias of family Cichlidae are cultured in more than 140 countries worldwide in both tropical and temperate regions with the production volume exceeding 5 million tonnes (FAO, 2022; Shechonge et al., 2019). This family is very diverse, with over 100 species and sub species (Ansah et al., 2014). However, few species are commercially cultured, with Nile tilapia being the most cultured species (FAO, 2022).

Tilapias are preferred for aquaculture purposes due to their fast growth capacity on both manufactured and natural feeds, easy propagation, high fecundity, tolerance to handling in different farming systems, and resistance to stress and diseases (Ansah et al., 2014). Globally, tilapias are the second most cultured species contributing more than 10% of total finfish aquaculture production whereas carp species lead by contributing above 70% of total finfish aquaculture production (FAO, 2022).

In order to increase fisheries production, economically important species were introduced in different natural water bodies such as lakes, rivers dams outside their geographical range. For instance, in the 20th century more than 90 countries reported the introduction of tilapias for various economical purposes (Ansah et al., 2014; Shechonge et al., 2018). Tilapia introduction has been reported to have adverse impact in the ecosystem in case of escapees since tilapias have invasive tendencies and can interbreed with other cichlids when introduced to natural water bodies (Bradbeer et al., 2018; Shechonge et al., 2018; Shechonge et al., 2019). Presence of hybrids and reduction of native species have been observed in local waters and hence disturb natural genetic diversity (Champneys et al., 2021; Shechonge et al., 2018). Apart from hybridizing and compensation with native species, the introduced species may carry disease which can have adverse consequences for native species especially virus caused diseases (Shechonge et al., 2018). Alternatively, breeding programs for tilapia have the potential to increase production and conserve the wild genetic diversity.

2.4 Rufiji tilapia

Oreochromis urolepis is a species of tilapia, cichlid fish, endemic to Tanzania that has a broad distribution inhabiting from River Rufiji basin, Wami and Ruvu rivers, Kilombero river, Mindu dam, Lake Mansi and Zanzibar. Rufiji tilapia can be found primarily in freshwater habitats, such as rivers, streams, lakes and Oxbow lakes, swamps, dams, and ponds and estuaries. The species is herbivore with some omnivorous traits. They feed on phytoplankton and algae and sometimes zooplanktons. However, in farming systems they can feed on manufactured feed. Like other tilapias, males of Rufiji tilapia are bigger and grow faster than females. Similar to other *Oreochromis*, Rufiji tilapia are also maternal mouthbrooders and the care of the fry is carried out by a female. Rufiji tilapia has two sub-species

namely *O. urolepis urolepis* and *O. urolepis hornorum*. These species are sometime referred as the same one species (Genner et al., 2018; Shechonge et al., 2018) despite their differences. Based on the pigmentation of mature fish, the males of *O.u.urolepis* are dark olive grey with pinkish upper lips and red fin margins (Figure 4) while males of *O.u. hornorum* are entirely black even in non-stressful environment, their lips are pale or black. Breeding female of *O.u.urolepis* are silvery grey with the narrow pinks edge on the dorsal fins (figure 5) but females of *O.u. hornorum* have no pink edges (Trewavas, 1983). Rufiji tilapia is not only an endemic species; it is also the second largest species in size after Nile tilapia with potential for aquaculture in Tanzania (Genner et al., 2018). This species has high potential for both aquaculture and simultaneous conservation of native species due to their fast growth and high tolerance of water salinity making it the best candidate for coastal aquaculture.

Figure 4. Male Rufiji tilapia (*O. urolepis urolepis*). *Source*: Nyinondi

Figure 5. Female Rufiji tilapia (*O. urolepis urolepis*). *Source*: Nyinondi

2.5 Tilapia breeding program

Selective breeding for genetic improvement has been done for years in livestock farming and agriculture and has increased the sustainability of both animal and plant production systems. Unlike plants and farm animals, selection for genetic improvement of aquatic animals in breeding programs started a few decades ago (Gjerde & Rye, 1998; Houston et al., 2020). Historically, fish were selected for farming depending on their observed traits (i.e. fast growth, fillet, high fecundity, disease resistance etc.). Today, even with traditional selective breeding of some fish species, fish farming still depends mainly on wild counterparts for seed supply, making them genetically closer to wild state. Therefore, there is a great gap between increasing aquaculture production and sustainably maintaining genetic quality of the farmed species.

Several approaches have been used to genetically improve fish production in farming systems. These include sex control, cross breeding, hybridization, transgenesis, chromosome manipulation and selective breeding. However, for sustainable genetic gain through generation only selective breeding approaches have reported to offer continuous gain in the production sector (Ponzoni et al., 2011). Selective breeding through breeding program have been reported to sustainably increase fish production while maintaining the genetic quality by improving phenotypes at the same time conserving the genetic biodiversity of native species (FAO, 2018). This

improvement in aquaculture sector has led to the development of several finfish breeding programs for commercially important species like salmon (Symonds et al., 2019; Thodesen & Gjedrem, 2006), trout (Lhorente et al., 2019; Sae-Lim et al., 2013), carps (Bakos et al., 2006; Dong et al., 2015) and tilapia (Ansah et al., 2014; Hong Nguyen et al., 2014; Ponzoni et al., 2011).

Tilapia breeding program started with Nile tilapia in 1988 with Genetic Improvement of Farmed Tilapias (GIFT) project. This was a collaborative research project between the International Center for Living Aquatic Resources Management (ICLARM, currently called WorldFish) and AKVAFORSK in Norway which was later co-financed by the Asian Development Bank (ADB) (Eknath et al., 1993). The significant success of GIFT project led to development of other Nile tilapia breeding programs using GIFT technology such as GenoMar Supreme Tilapia (GST), Genetically Enhanced Tilapias for Excellence (GET-EXCEL), FAC Selected Tilapia (FaST), Abassa, and Akosombo (Ponzoni et al. 2008, Ansah et al. 2014). Tilapia that has been genetically improved from these breeding programs grow about 18 to 58% faster per generation compared to unselected lines when reared in ponds (Ansah et al., 2014; Gjedrem & Robinson, 2014). Most of these tilapia breeding programs are located in Asia except for Abassa, and Akosombo that are located in Egypt and Ghana, respectively. The countries where these successful breeding programs are located became the major producers of tilapia globally.

The use of selective breeding programs in aquaculture varies with continents, with European aquaculture production deriving more than 80% from selective breeding programs while Africa derives less than two percent (FAO, 2022; Houston et al., 2020). Naturally, tilapia populations are primarily restricted to Africa, but Asia is the main global producer. To improve tilapia production, several selective breeding trials have been done in Africa using GIFT technology. Still, only the Abassa tilapia breeding program has shown a significant achievement over years (Ansah et al., 2014; FAO, 2022). Therefore, there is a pressing opportunity to use these genetic diverse tilapias in Africa to harness the untapped genetic potential and improve desirable traits in selective breeding programs.

2.6 Towards a structured breeding program for Rufiji tilapia.

In this thesis we want to study the building blocks for a successful breeding program for Rufiji tilapia. In order to establish a brood stock that is the starting point for a structured breeding program we need to 1. identify the genetic variation within and between different wild and farmed populations of Rufiji tilapia, 2. compare the performance of these populations in different environments and 3. estimate genetic parameters for the traits we want to improve as well as any gene by environment interaction (G × E).

2.6.1 Genetic diversity

Genetic diversity refers to the range of different inherited traits within a species. This explains the ability of a species to adopt to the surrounding environmental conditions in terms of phenotypic changes induced by environment over organism's lifetime, behaviour and phenotype plasticity, and canalized phenotype (Houston et al., 2020; Svanbäck & Eklöv, 2006). In many aquaculture systems, breeding candidates are selected based on their phenotypic performance through either individual or mass selection. This type of fish selection is mainly applied for live-fish recorded traits such as body weight and fecundity. However, this method has negative impact on fish such as high inbreeding rates (Houston et al., 2020). Inbreeding in aquaculture has been reported to result in poor survival rates and growth of farmed fish. It's important to note that the population size influences the genetic diversity of that population (Gjedrem, 2005). Meaning that if the population is big there is the large chance that that population has high variation in genetic traits hence high chance for containing alleles that contribute to new environment adaptation (Keller & Waller, 2002). Also, high genetic diversity of a population provides broad range of trait improvement in selective breeding without losing genetic diversity (Gjedrem, 2005).

A key factor in successful selective breeding program is determining the genetic diversity of the base population, that is whether the trait of interest has sufficient genetic variation for sustainable breeding (Gjerde & Rye, 1998). This will give valuable information for understanding the potential of certain traits in fish adaptability to various environmental changes in aquaculture system. Therefore, to avoid genetic bottle-necks and preserve fish genetic variation in fish production, obtaining information on the genetic

diversity of the candidate selected fish is crucial. Some existing fish breeding programs developed approaches to secure the genetic diversity of the base population. For instance, salmon, rainbow trout and Nile tilapia breeding programs secure base population genetic diversity by forming a synthetic population that is composed of several genetically diverse populations or stocks (Gjedrem, 2005; Gjerde & Rye, 1998).

2.6.2 Genetic markers

Understanding fish genetic diversity is very crucial for the establishment of a selective breeding program. There are several genomic tools that provide information on fish diversity for sustainable genetic improvement. These tools provide valuable information on how to minimize inbreeding and maximize genetic gain in a selective breeding. DNA genomic markers is one of the tools that measures genetic diversity directly across the entire genome. DNA markers that can be applied to investigate the genetic variation of fish for the improvement of the interested trait includes; allozymes, mitochondrial DNA (mtDNA) that were popular in the past aquaculture genetic researches, but recent most used DNA molecular markers are restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD, amplified fragment length polymorphism (AFLP), expressed sequence tags (EST), microsatellite and single-nucleotide polymorphism arrays (SNP) markers, (Liu & Cordes, 2004).

Direct genotype by sequencing (GBS) techniques have reinforced advances in aquaculture genetic development. This is due to the fact that these GBS techniques do not necessarily require a reference genome for detecting genetic markers (Robledo et al., 2018). For instance, restriction-site associated DNA sequencing (RAD-Seq) have been used to generate SNP data of fish populations, and its beneficial for fish species that received less research interest. RAD-Seq techniques have been applied in generating linkage maps, improvement of reference genome and selection for traits of interests in aquaculture (Houston et al., 2020; Nyinondi et al., 2020; Palaiokostas et al., 2021; Robledo et al., 2018).

2.6.3 Common garden experiments in tilapia

Common garden experiments aim to determine the relationship between different fish genotypes and phenotype with their surrounding environment. In fish, common garden experiments can be indoors or outdoors where fish

are reared in a shared water condition. Common garden experiments can be conducted in multiple distinct geographical locations to explore gene by environment interactions (Nguyen et al., 2017; Sae-Lim et al., 2013, 2016). Fish adaptation to changing environments depend on their genetic disposition (Gjerde & Rye, 1998). Therefore, studying these genetic adaptation traits and controlling to a certain extent their effect in fish phenotypic performance and genotype-by-environment interaction is through a well-designed common garden (de Villemereuil et al., 2016). Environmental location of wild fish can create isolation within fish species due to their adaptation ability, this in turn can divide species into subspecies. Therefore, common garden experiments have been used to unravel species genetic source on complex phenotypic traits, traits of interests, adaptation traits for both aquaculture and conservation purposes (de Villemereuil et al., 2016).

In aquaculture, genetically improved species are preferred by phenotypically performing better in wide range of geographical location, meaning that an aquatic species that has low phenotypic plasticity is preferred (Ansah et al., 2014). Therefore, designed common garden experiments have been used to study several farmed fish to evaluate the relationship between genetic variations and phenotypic performance in different geographical locations or environment. Areas of interest in aquaculture for these experiments include; growth-related traits performance, diseases resistance, fecundity, genotype-by-environment interaction and heritability (Abou et al., 2007; Megahed, 2019; Mengistu et al., 2021; Sae-Lim et al., 2013).

3. Aim of the thesis

3.1 General Objective

The overall aim of this thesis is to generate information that can be used for the development of a Rufiji tilapia population that will be the base of a selective breeding program in Tanzania.

The specific objectives of the study (papers I – IV) were to:

I. Determine the status of tilapia farming in Tanzania

II. Assess the genetic diversity and population structure of different wild and farmed Rufiji tilapia populations in Tanzania

III. Determine the genetic diversity and population structure of native, exotic, and wild populations of tilapia in Tanzania.

IV. Compare growth performance of Rufiji tilapia in two environments through a common garden experiment.

V. Evaluate the effect of Genotype –by – Environment interaction on phenotypic performance of Rufiji tilapia (i.e. growth performance and survival) and estimate the heritability for relevant traits.

3.2 Structure of the thesis

This thesis is based on paper I-IV (Figure 6). Paper I analyses the status of tilapia farming in Tanzania. Paper II and III examine the genetic diversity and population structure of native, exotic, and wild populations of Tilapia in Tanzania. Paper IV evaluates the growth performance of Rufiji tilapia in two environments through common garden experiment and the effect of Genotype –by – Environment interaction on phenotypic performance and the heritability estimate for relevant traits.

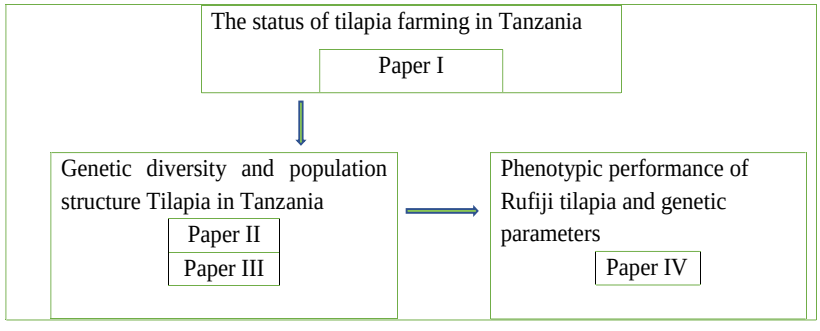

Figure 6. Structure of the research on Rufiji tilapia (*O.u. urolepis*) for the development of a breeding program involving the status of tilapia in Tanzania (paper I), genetic diversity and structure (paper II and III) and phenotypic performance as well as estimate for genetic parameters (paper IV).

4. Material and Methods

4.1 Tilapia farming field survey for Paper I

A pilot study of this thesis was conducted to identify and determine the status of tilapia farming in mainland Tanzania. Stakeholders interviews and field observations were carried out in mainland Tanzania. The field sites were chosen in a systematic manner trying to include all areas with fish farming activities, private and government hatcheries, and policy makers. Areas with multiple aquaculture activities were preferred. Ten tilapia fish farming sites were selected from Coast (Pwani), Dar es Salaam, Kagera, Kilimanjaro, Mbeya and Mwanza region. Also, seven hatcheries owners, five aquaculture experts from research and training institutions and three policy makers from the ministry of Livestock and Fisheries development were interviewed. The data were collected using a semi-structured interview with both close and open-ended Swahili questionnaire and later translated to English. Questions regarding the sources of fingerlings, farming system (pond, cage or other), stocking densities, growth performance, market and challenges they are facing were given to farmers. Government and private hatcheries were also asked the source of their broodstock, broodstock reproduction period, price of fingerlings, market, growth performance of their fingerling, challenges and government intervention. While government officials were asked the government strategies for improving fish farming in the country.

4.2 Genetic diversity studies for paper II and III

4.2.1 Sample collection

Rufiji tilapia used in this study were collected from both farmed and wild environments in mainland Tanzania. For Nile tilapia (paper III), sample were collected from the wild, fish farms and hatcheries. The sample locations were selected based on the available information of their distribution around the country (Figure 7). Identification of the species was based on their morphological characteristics as detailed in paper II and III. Fish weighing 30 g and above were carefully chosen for the experiment.

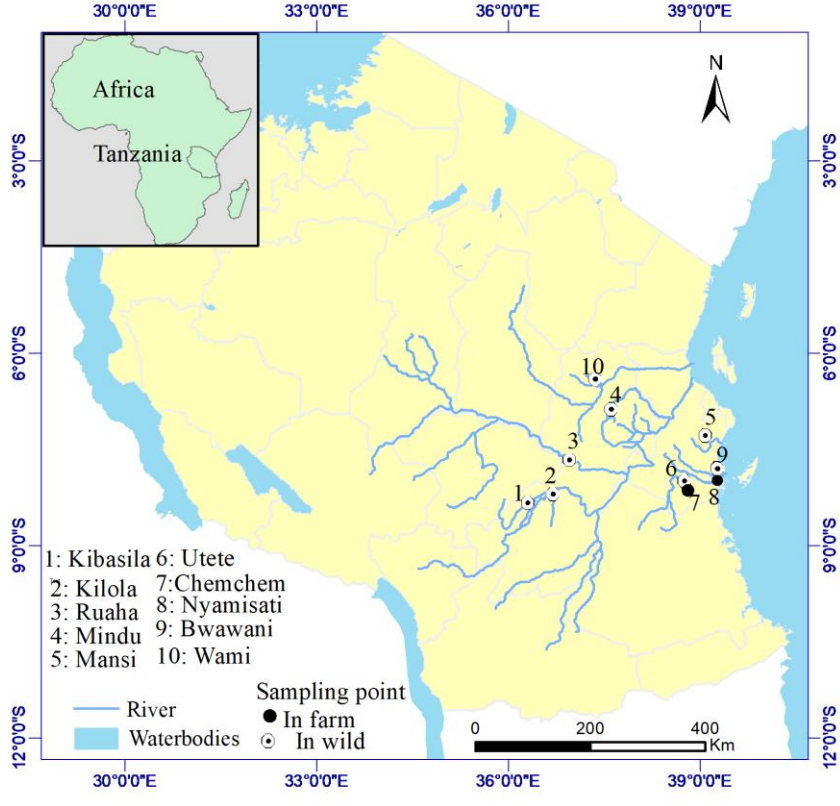

Figure 7. Sampling sites for Rufiji tilapia (*O.u. urolepis*) collected from both farm and wild in mainland Tanzania.

4.2.2 DNA extraction, ddRAD library preparation and sequencing

A total of 550 fish were used with 15 - 20 animals from each population. From each fish, 0.05g of fin clips were collected and preserved in 95% ethanol for the extraction of genomic DNA. DNA extracted from the collected fin clips was used for the construction of Double-digest restriction-site-associated DNA (ddRAD) libraries. ddRAD libraries were prepared according to Peterson et al. (2012), with minor modifications described in Palaiokostas et al. (2015). The sequencing of ddRAD libraries was done at Edinburgh Genomics Facility, University of Edinburgh using an Illumina HiSeq 4000 instrument.

4.2.3 Sequence Data Analysis and SNP Genotyping

Reads of low quality (Q < 20) and missing the expected restriction sites were discarded. The retained reads were aligned along Nile tilapia reference genome assembly [GenBank accession number GCA_001858045.1 (Conte, Gammerdinger, Bartie, Penman, & Kocher, 2017)] using bowtie2 (Langmead & Salzberg, 2012) in paper II and assembled de novo using the denovo pipeline (denovo_map.pl) of Stacks v2.5 (Catchen et al., 2011; Rochette et al., 2019) in paper III. Stacks of loci were formed for each individual sample supported by at least three reads. Thereafter, a catalogue of putative loci across all samples was formed allowing a maximum number of three mismatches amongst the individual sample loci. Finally, SNPs were identified using gstacks (settings: --var-alpha 0.001 –gt-alpha 0.001 –min-mapq 40). Only a single SNP from each locus was considered for downstream analysis in order to minimize the possibility of genotypic errors and reduce computation time. SNPs with minor allele frequency (MAF) < 0.05 across all tested samples were discarded. Finally, only SNPs found in at least 75% of the samples in each population were retained for downstream analysis. The aligned reads in the format of bam files were deposited in the National Centre for Biotechnology Information (NCBI) Bioproject repository under project ID PRJNA518067 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA518067).

4.2.4 Population Structure and Relationships

To determine genetic clusters among the tilapia populations we used both multivariate and Bayesian methods. Bayesian clustering methods implemented in the program Structure v2.3.4 (Pritchard et al., 2000) were

applied with 100,000 burn-in and 200,000 Markov chain Monte Carlo repetitions for each K-value. Three replicates were performed for each number of underlying populations (k) from 2 to 20. The most probable K-value was determined using Evanno method (Evanno et al., 2005) implemented in Structure Harvester (Earl & vonHoldt, 2012). Furthermore, Q-matrices of three replicates from STRUCTURE were used in CLUMPAK (Kopelman et al., 2015) to visualize the population structure.

Multivariate approach Discriminant Analysis of Principal Components (DAPC) (Jombart et al., 2010), was further used to identify genetically distinct clusters of tilapia species. DAPC was performed using the R package ADEGENET version 2.1.1 (Jombart et al., 2018). DAPC transformed the SNP data using a prior PCA step and subsequently applied a discriminant analysis step (Jombart & Collins, 2015). DAPC uses find.clusters() function of adegenet to implement a clustering algorithm k-means and Bayesian Information Criterion (BIC) for each value of K to identify the optimal value of k (Jombart et al., 2010). To determine genetic similarities and relationships among tilapia populations, Principal component analysis (PCA) was carried out using the R package ADEGENET version 2.1.1 (Jombart et al., 2018).

4.2.5 Population differentiation and Genetic diversity

Genetic diversity indices; mean observed (H_O), expected (H_E) heterozygosity and inbreeding coefficients (F_{IS}) among tilapia populations in Tanzania were estimated using Stacks v2 (Rochette et al., 2019). For population genetic differentiation, pairwise F_{ST} values were obtained using the *stamppFst* function (Pembleton et al., 2013) according to Cockerham and Weir (1984).

4.3 Common garden study (Paper IV)

A common garden experiment was performed with the aim to compare the growth performance of Rufiji tilapia in terms of body weight, length and girth in two environments based on water salinity, and evaluate their Genotype –by – Environment interaction and estimate heritability for related traits in those environments. The common garden experiment for Rufiji Tilapia was performed jointly with Nile tilapia native and exotic lines but those results are presented elsewhere.

4.3.1 Study area

The common garden experiment was conducted in two different locations. First, the Institute of Marine Sciences Mariculture Centre (IMS-MC) at Bweni village, Pangani, Tanga (05° 26′ 0″ South, 38° 58′ 0″ East). This place is characterized by precipitation ranging from 33 mm to 278 mm during the wettest month. It receives about 99 mm (3.91 inches) of precipitation and has about 178 rainy days annually. The district temperature varying between 25 and 33 °C. Second, the School of Aquatic Science and Fisheries Technology, University of Dar es Salaam at Kunduchi in Kinondoni, Dar es Salaam (06° 66′ 0″ South, 39° 21′ 0″ East). The Kinondoni district is characterized by precipitation ranging from 27 mm to 269 mm during the wettest month. Kinondoni typically receives about 146 mm (5.76 inches) of precipitation and has about 230 rainy days annually. The temperature varies between 25 °C and 31 °C. Both districts are allocated along the coastline of the Indian ocean and have small variations of climatic conditions throughout the year. The choice of the study locations was based on the climatic conditions that favors tilapia farming and the limited environmental difference between two stations. Water used in this study had different salinities with brackish water (>2‰) at Pangani and freshwater (< 0.05‰) at Kunduchi.

4.3.2 Broodstock population

Rufiji tilapia broodstock used in this study were collected from nine different geographical locations as described in paper II. Brooders with an average weight between 200g to 300g were selected for natural spawning. Thirty-six hapas measuring 1m x 1m x 1.5m were used for spawning. From each population, four males and four females were stocked at a sex ratio of one male: one female per hapa. All selected female brooders were inspected for eggs before stocking to ensure that all eggs were spawned after pairing. Broodstock synchronization to stimulate spawning was done by starving brooders for one day after providing them enough food for nine days simultaneously. To ensure good quality of eggs and hence quality of fingerlings, brooders were fed twice per day with formulated diet from Koudijs Animal Nutrition-Netherlands containing 35% crude protein. After a period of two to three weeks, eggs were collected from the mouth of the female brooder and placed into separate labelled incubation jars for hatching. Newly hatched fry were collected into a 10 L incubation basin with a flow-through system and were kept there until the absorption of the egg yolk.

4.3.3 Production of mono-sex fingerling by hormone treatment

All fingerlings were transferred into separate 80 L labelled aerated plastic basins after the absorption of egg yolk. 17-alpha methyl testosterone hormone (17α-MT, Sigma Aldrich, China) hormone was administered through hormone treated feed prepared as described by Killian and Kohler (1991) to produce all male fingerlings. Hatchlings were fed the diet twice daily with a formulated starter feed from Koudijs Animal Nutrition-Netherlands containing 45% crude protein for 30 days at a rate of 15% to 20% by body weight. Water drainage, siphoning and refill was done daily to ensure overall good water quality. Moreover, water was aerated throughout the experiment to ensure consistent supply of oxygen using aquarium bubblers connected to an air compressor electric motor (Single phase, volts 220 V–50 Hz/60 Hz, model YL90L-2, Zhejiang, China).

4.3.4 Rearing of hormone treated fingerlings

After 28 days of hormone treatment, fingerlings from each female were divided into two equal groups. One group of fingerlings stayed at Pangani while the other group was transported to Kunduchi. Since all fingerling were hatched in Pangani (brackish water), the group of fingerlings that was transported to Kunduchi (fresh water) was acclimatized before stocking into the ponds. Acclimatization was done by gradually adding small amount of fresh water into the plastic bags that were used to transport fingerlings and removing saline water for about 45 minutes. Dead fingerlings were counted and discarded. At both locations, fingerlings from each female were stocked randomly into labelled separate hapas (1m x 1m x 1.5m) within a $20m^2$ pond for further growth before tagging.

4.3.5 Tagging and grow out stocking

Fingerling from 35 families of 9 populations weighing 20g and above were tagged using passive integrated transponders (PIT) at both Kunduchi and Pangani. Before tagging fish were anesthetized in a mixture of 0.1ml clove oil per litter of water. Anesthetized fish were tagged with a R5M-pro PIT microchip (12mm long and 2.1mm in diameter), in the muscle tissue between the pelvic fins. Tagged fish were scanned by R5M-Pro PIT tag reader and then their tag numbers were recorded. Fish were then stocked into seven different grow-out hapas each measuring 12m x 8.5m x 2m aligned into a $20m^2$ pond. At Kunduchi two hapas were placed into each of the two

concreate tanks, while at Pangani two ponds were also used where two hapas were placed in one pond and the remaining hapa was placed in another pond. Approximately equal number of fishes from each family were distributed in each grow-out hapa in a completely randomized block design. The individual fish growth data were collected by scanning the tag number throughout the experiment period.

4.3.6 Water quality monitoring and feeding regime

Water quality parameters (Temperature, pH and Dissolved oxygen) were monitored and recoded on daily basis using a portable oxygen meter (Hanna, model HI 98193, Hanna Instruments Inc, Woonsocket, Rhode Island, USA). The water exchange was done on a biweekly basis by 20% of the water in the pond to maintain the quality.

The experimental fish were fed to satiation twice daily with pellet formulated diet from Koudijs Animal Nutrition-Netherlands containing 35% crude protein content. Feeding regime was split into morning session between 0900 and 1000 hrs. and evening session between 1500 and 1700 hrs. The grow-out duration for each population of experimental fish is provided in table 2.

4.3.7 Traits recordings

Growth performance of fish including body weight and length were measured using a sensitive weighing balance (Boeco, model 43, Germany) and a flat ruler, respectively. Fish were initially anesthetized with clove oil (Zanzibar, Tanzania) 0.1m/L to reduce handling stress before taking growth parameter measurements. Moreover, fish mortalities were recorded on a daily basis.

5. Main results

Below is a brief description of the main findings of papers I – IV. For detailed description of the findings, please refer to the individual papers.

5.1 Status of Tilapia farming in Tanzania (Paper I)

This study showed that fingerlings were collected from either government or private hatcheries depending primarily on the location of the hatchery. The stocking densities ranged from 5-10 fingerling/m^2 with minimum water exchange for earthen ponds and 20 fingerling/m^2 in cages. Fish were harvested from 8-12 months with weight ranging from 30 - 500g during harvest with the vast majority of fish sold locally. The main challenges fish farmers faces were lack of seed quality and feed, high price of fingerling from private hatcheries, poor performance fingerling from government hatcheries. For government hatcheries tilapia broodstock were collected from Lake Victoria and were used for several generations until they showed poor performance. The main challenges for government hatcheries were poor infrastructure and low financial support. Broodstock in 4 out of 5 interviewed private hatcheries were imported in the country. The challenge private hatcheries were facing is the production expenses especially high electricity costs and strict government importation policies. Both government and private hatcheries sell fingerling to local farmers.

5.2 Genetic diversity (Paper II – III)

5.2.1 Genetic diversity within and amongst populations

The expected heterozygosity (H_E) and observed heterozygosity (H_O) estimates were indistinguishable with values ranging from 0.08 to 0.32 and 0.08 to 0.22 respectively. The highest values were observed in samples from Mindu (H_E = 0.23; H_O = 0.23) Wami (H_E = 0.33; H_O = 0.19) and Mansi populations (H_E = 0.17; H_O = 0.17). Lower values were observed in samples from Bwawani and Kibasira (H_E = 0.08; H_O = 0.08). On the other hand, Chemchem and Mansi populations showed negative F_{IS} of -0.001 ± 0.033 and -0.012 ± 0.028 respectively while Wami had the F_{IS} values (0.337 ± 0.033) (Table 1).

Table 1. Genetic diversity parameters for local Rufiji tilapia (*O. urolepis urolepis*) local and exotic Nile tilapia (*O. niloticus*). H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient

Species	H_O (mean ± SE)	H_E (mean ± SE)	F_{IS} (mean ± SE
Rufiji tilapia populations			
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
Kibasira	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 populations			
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 populations			
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 estimated genetic distances among the tested Rufiji tilapia populations varied extensively according to the F_{ST} metric. The highest genetic distance was observed between Mindu and the populations from Bwawani and Kibasira. While, the lowest genetic distance was observed between the Kibasira and Kilola populations and Bwawani and Nyamisati populations (Figure 8). Rufiji populations showed higher genetic distance when compared to both exotic and native Nile tilapia except for Mindu and Wami populations. The highest differentiation was observed between the Kibasira and FETA, Pangani Rufiji and FETA and Chifive-C (F_{ST} > 0.8) (Figure 8).

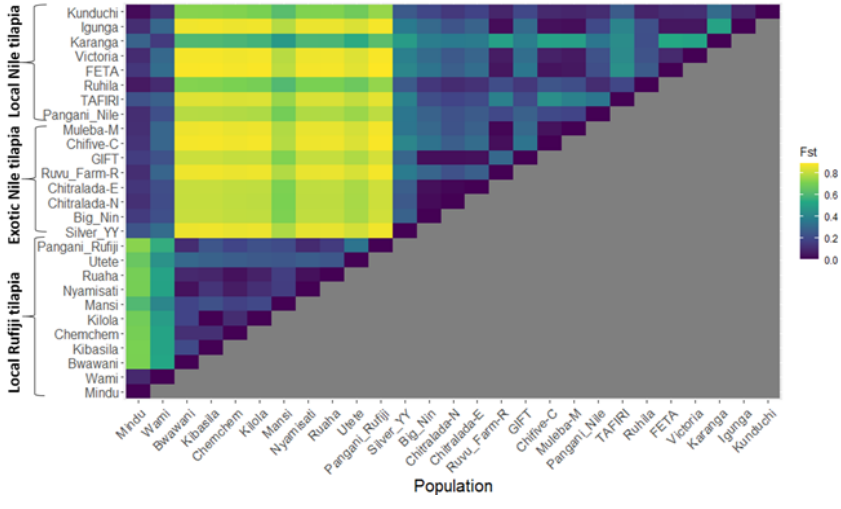

Figure 8. Genetic diversity among populations based on estimated F_{ST} values of 27 population from three tilapia strains

5.2.2 Population structure and admixture

A principal component analysis (PCA) and further discriminant analysis of principal components (DAPC) were used to study individual relationships within and between populations. DAPC indicated the existence of three clusters among populations. The first genetic cluster included Mindu and Wami populations, the second cluster was comprised of Utete population and the third cluster comprised of the Kibasira, Kilola, Mansi, Bwawani, Ruaha, Nyamisati, and Chemchem populations (Figure 9).

In comparison to exotic and native Nile tilapia, PCA revealed a clear distinction between Rufiji tilapia species and Nile tilapia with some overlaps between with native Nile tilapia populations. No overlaps were observed between Rufiji tilapia and the exotic Nile tilapia populations (Figure 10).

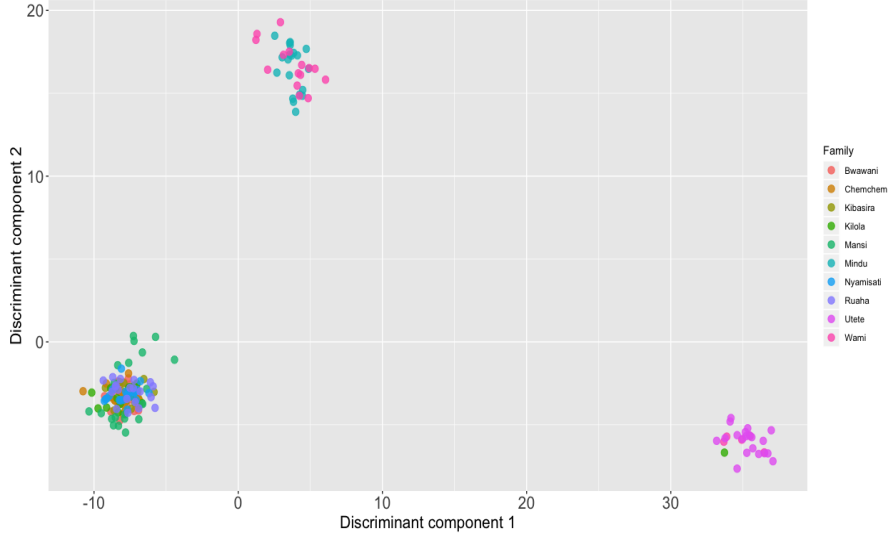

Figure 9. Discriminant analysis of principal component (DAPC) for Rufiji tilapia populations

Moreover, DAPC demonstrated the existence of two separate groups corresponding to Nile and Rufiji tilapia (Figure 11)

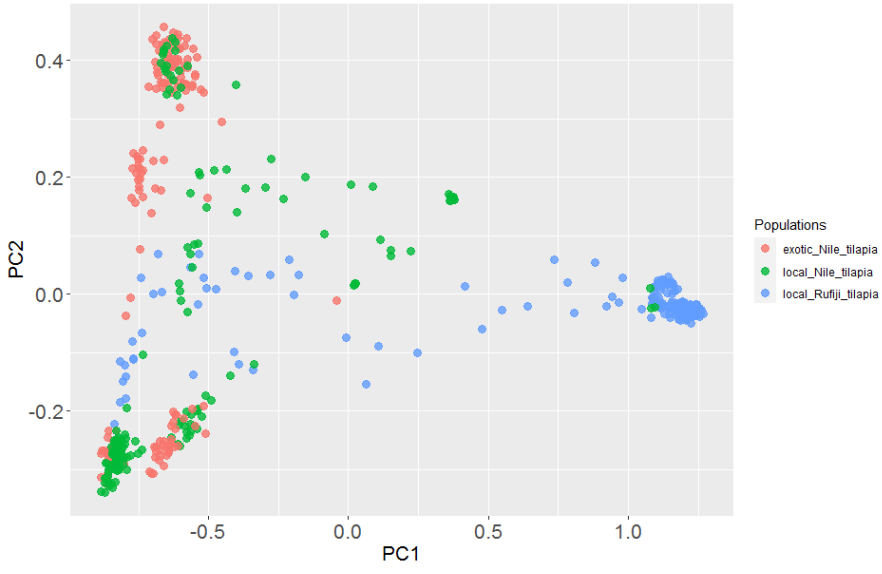

Figure 10. Principal component analysis (PCA) showing genetic relationships among exotic Nile tilapia, local Nile tilapia and Rufiji tilapia species. Individual fish are represented by one dot, with its symbol colour corresponding to the assigned population

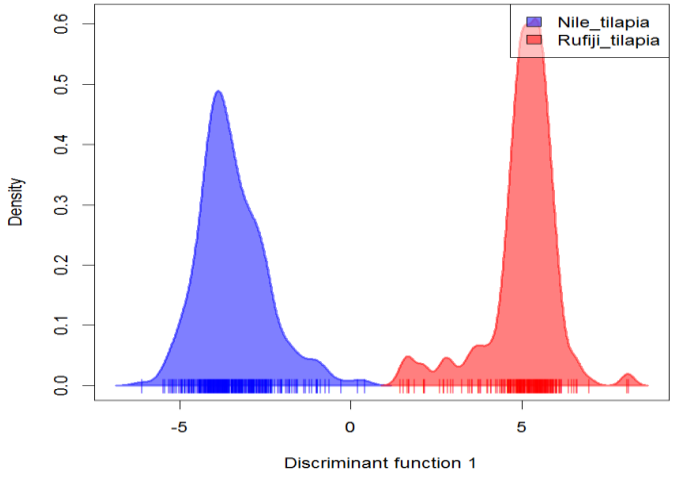

Figure 11. Discrimination between Rufiji tilapia and local Nile tilapia found in Tanzania using Discriminant analysis of principal component (DAPC)

The STRUCTURE analysis provided further evidence regarding the existence of clusters and admixture potential among tested populations. Evidence for admixture was found for the Mindu, Wami and Utete populations. Mindu and Wami show evidence of hybridization with Nile tilapia while Utete shows admixture with a line not represented in any of the other populations. Comparing with Nile tilapia, most Rufiji tilapia populations were quite distinct and more homogeneous than the Nile tilapia (Figure 12).

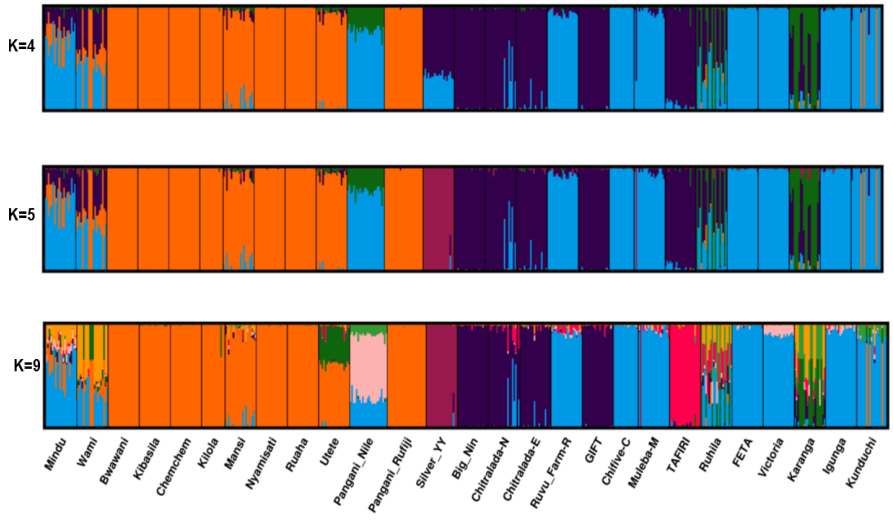

Figure 12. STRUCTURE admixture plots for K = 4, 5 and 9 showing population structure of different tilapia strains

5.3 Common garden experiment (Paper IV)

5.3.1 Growth performance

Growth-related traits were recorded including harvest weight, weight gain, AGR and TGC. The results showed that all recorded growth traits had higher mean values in the fish reared in Pangani. The best performing population in terms of the recorded growth traits was the Wami that was reared in Pangani, while the lowest growth performance was observed in Ruaha population reared in Kunduchi (Table 2).

Table 2. Mean harvest weight, weight gain, absolute growth rate (AGR) and thermal-unit growth coefficient (TGC) for each strain of Rufiji tilapia after 8 weeks of rearing in a freshwater (Kunduchi) and brackish water (Pangani) environment

Location	Strain	N	Age (weeks)	$WF^{(SE)}$	$WG^{(SE)}$	$AGR^{(SE)}$	$TGC^{(SE)}$
Kunduchi	Bwawani	78	28	$42.7^{4.06}$	$86.6^{5.18}$	$0.75^{0.06}$	$0.008^{0.0006}$
	Chemchem	88	29	$63.2^{3.90}$	$116.7^{4.98}$	$1.17^{0.05}$	$0.010^{0.0005}$
	Kibasira	45	29	$51.3^{5.30}$	$93.6^{6.80}$	$0.91^{0.06}$	$0.010^{0.0006}$
	Kilola	84	27	$58.6^{4.10}$	$101.0^{5.26}$	$0.94^{0.05}$	$0.010^{0.0004}$
	Mindu	87	28	$76.1^{3.54}$	$134.5^{4.54}$	$1.30^{0.06}$	$0.010^{0.0005}$
	Nyamisati	85	27	$56.9^{3.93}$	$102.9^{5.05}$	$0.94^{0.05}$	$0.010^{0.0006}$
	Ruaha	87	28	$31.2^{3.20}$	$54.2^{6.33}$	$0.53^{0.03}$	$0.006^{0.0003}$
	Utete	91	30	$42.2^{4.00}$	$80.8^{5.14}$	$0.85^{0.04}$	$0.009^{0.0004}$
	Wami	52	27	$86.2^{3.82}$	$147.4^{4.91}$	$1.55^{0.07}$	$0.017^{0.0007}$
Pangani	Bwawani	91	28	$63.3^{3.77}$	$107.7^{4.84}$	$1.14^{0.07}$	$0.010^{0.0007}$
	Chemchem	85	29	$84.4^{3.91}$	$120.4^{5.02}$	$1.53^{0.08}$	$0.015^{0.0008}$
	Kibasira	41	29	$54.5^{5.53}$	$96.2^{5.29}$	$0.96^{0.09}$	$0.010^{0.0009}$
	Kilola	82	27	$70.9^{4.12}$	$120.5^{7.10}$	$1.16^{0.06}$	$0.010^{0.0007}$
	Mindu	43	28	$108.1^{5.44}$	$163.6^{6.68}$	$1.87^{0.15}$	$0.019^{0.002}$
	Nyamisati	96	27	$85.6^{3.72}$	$142.1^{4.78}$	$1.46^{0.09}$	$0.011^{0.0008}$
	Ruaha	93	28	$41.4^{4.51}$	$73.1^{5.79}$	$0.71^{0.06}$	$0.007^{0.0006}$
	Utete	89	30	$57.7^{4.04}$	$106.1^{5.19}$	$1.13^{0.08}$	$0.010^{0.0007}$
	Wami	62	28	$119.9^{3.71}$	$177.7^{4.76}$	$2.14^{0.10}$	$0.021^{0.001}$

Note: N= number of fish, WF=final weight, WG= weight gain, AGR= absolute growth rate, TGC= thermal-unit growth coefficient

5.3.2 Heritability estimates - Genetic correlations among rearing environments

Heritability estimates for the growth-related traits ranged between 0.39 – 0.74. The results showed that the highest heritability in Kunduchi was obtained for weight gain (0.42), while in Pangani the corresponding heritability was 0.74. In case of AGR and TGC the estimated heritabilities for Kunduchi and Pangani were 0.39 (0.11) and 0.69 (0.14) respectively. Furthermore, the genetic correlations regarding fish reared in the two environments were 0.74 for estimated growth-related traits.

5.3.3 Family ranking based on estimated breeding values (EBVs)

Generally, fish reared in Pangani showed higher mean family EBV compared to their full-sibs that were reared at Kunduchi (Figure 13). However, 10 out of 35 full-sib families reared in Kunduchi clearly outperformed their full-sibs in Pangani (Figure 14). These families originated from Chemchem

(n=2), Mindu (n=1), Utete (n=2), Kibasira (n=2), Bwawani (n=1), Kilola (n=1) and Ruaha (n=1).

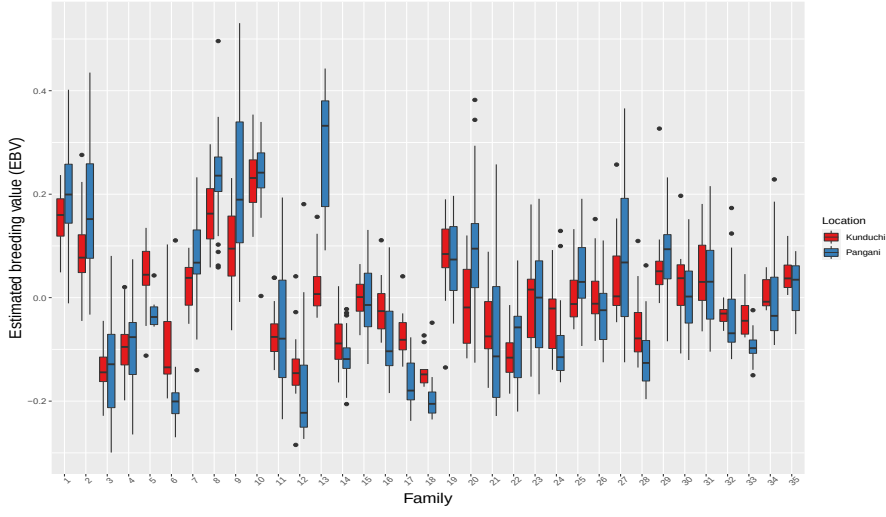

Figure 13. Mean estimated breeding value for 35 families of Rufiji tilapia population reared in two locations (Kunduchi and Pangani)

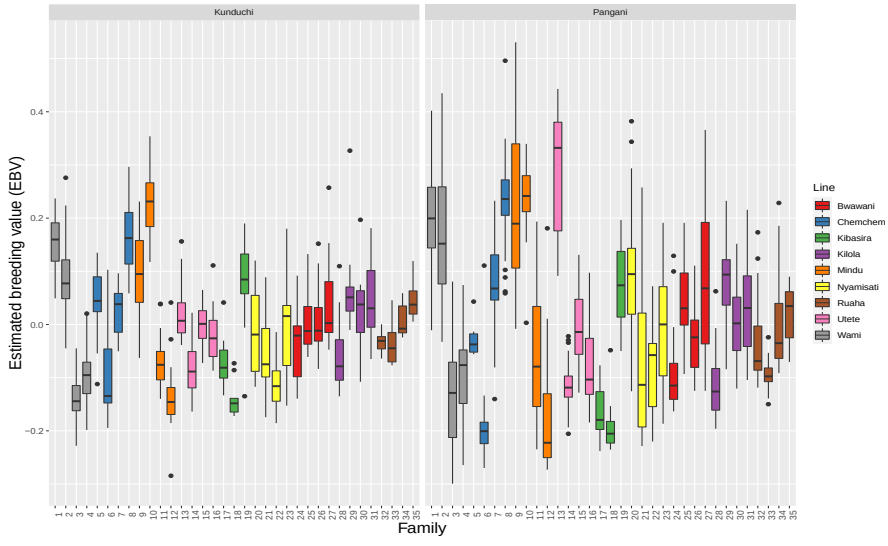

Figure 14. Mean estimated breeding value for 35 families of the nine Rufiji tilapia population reared in two locations (Kunduchi and Pangani).

6. General discussion

Rufiji tilapia is an endemic cichlid in Tanzania with the potential for diversifying the countries' aquaculture industry. The inherent ability to grow fast under rearing conditions, produce of all-males when crossed with Nile tilapia and tolerate salinities up to 35ppt make this species appealing for farming, especially in coastal areas with high saline water (Mapenzi & Mochi, 2016). Moreover, the potentially negative effect on the wild stock in case of escapees is considerably lower compared to Nile tilapia since Rufiji tilapia is endemic to Tanzania. However, to increase its production and ensure the quality of seed for better performance, a selective breeding program is essential.

Developing a selective breeding program requires knowledge on the genetic diversity of the founding population and its inherent genetic potential for improving traits important for production (Gjerde & Rye, 1998). In this thesis, we initially performed a preliminary survey around the country to obtain basic information on the status of tilapia farming in Tanzania. This survey provided key information that facilitated a better understanding of the status and availability of infrastructure for setting up a structured breeding program. Secondly, we analysed the genetic diversity of Rufiji tilapia populations of both farm and wild origin. Furthermore, we compared their genetic diversity status to that of Nile tilapia strains. Finally, we conducted a common garden experiment in two different water environments (fresh water and brackish water) to test growth performance and estimate genetic parameters for growth related traits. The overall study highlighted the basic information required to establish a Rufiji tilapia selective breeding program.

6.1 The status of tilapia farming in Tanzania

Tanzania's contribution to global fish aquaculture production is currently very minimal (URT, 2020; URT-MLF, 2019). Despite harbouring more than 30 different species of tilapia and having about 30% land potential for fish farming. In comparison with some African counties like Egypt where the only source of water for fish farming is river Nile but contribute more than 70% of fish farmed in African (FAO, 2022; URT, 2020), developing this sector in Tanzania could solve the problem of malnutrition in a country.

This pilot study revealed that fish farming industry in Tanzania at farmer and government level is underprivileged. The major reason behind the poor performance of farmed fish is lack of quality feed, expensive quality seed and feeds which the majority of farmers cannot afford. This study also found that out of 17 existing hatcheries in the country only 12 are active but not performing well. Financial limitation and lack of good aquaculture policies or poor implementation of existing aquaculture policies lead to poor performance of most government hatcheries and some private hatcheries.

6.2 Genetic diversity

Genetic diversity and structure amongst Rufiji tilapia populations in Tanzania were analysed using ddRAD-seq aiming to gaining insights about the availability-distribution of Rufiji tilapia genetic resources upon which a tilapia breeding program could be established (Kajungiro et al., 2019a), to boost fish production. The estimated genetic diversity metrics of the studied populations differed widely. More specifically, the observed heterozygosity ranged from 0.10 to 0.21, the expected heterozygosity ranged from 0.01 to 0.36, nucleotide diversity ranged from 0.10 to 0.37 and ranged from -0.03 to 0.05 with the exception of the Wami population where of 0.42 was obtained. High inbreeding is usually associated with population subdivision and lack of random mating due to small size due or migration (Neaves et al., 2015; Nichols, 2017; Nyinondi et al., 2020). Since the Wami population is from the wild, high F_{IS} values could be due sampling fish subset that do not represent the whole population or due to strain subdivision (Holsinger & Weir, 2009; Neaves et al., 2015; Shechonge et al., 2018). Notably, in comparison to Nile populations, the Rufiji tilapia ones showed higher genetic variation.

The estimated genetic distances according to the F_{ST} metric varied widely between 0.001 – 0.75 amongst the tested populations (Figure 8). The highest genetic distance was observed between Mindu and the populations from Bwawani and Kibasira (F_{ST} = 0.73) and the lowest genetic distance was observed between Kibasira and Kilola populations (F_{ST} = 0.001). The geographical location possibly contributed to the low genetic distance among the tested populations. For instance, Kibasira and Kilola populations are from neighbouring location and may share water during rainy season. However, Utete and Chemchem populations that are from neighbouring sites had a moderate high genetic distance (F_{ST} = 0.34). Overall, the genetic distances obtained in our study were within the range reported by Lind et al. (2019) in their genetic diversity study of West African Nile tilapia populations, but in contrast with studies reported by Hassanien and Gilbey (2005), Simbine et al. (2014), Mireku et al. (2017) and Sherman et al. (2020), which reported higher genetic distances. Sample size and methods used for analyses could be the reason for the differences with aforementioned studies. Additionally, the use of the Nile tilapia reference genome and de novo approach for detecting SNPs showed slight genetic variation for Rufiji tilapia.

Multivariate and Bayesian approaches were applied to test genetic differentiation among different populations of Rufiji tilapia. Both approaches showed the existence of three major genetic clusters. When compared to exotic and native Nile tilapia some of Rufiji tilapia fish overlapped with native Nile tilapia. This overlap could be due to hybridization reported by Shechonge et al. (2018) on sampled sites. Furthermore, the presence of admixture among studied populations was suggested, with higher levels of admixture found in the case of the Wami and Mindu populations. The presence of hybrids from these sites has been previously reported by Shechonge et al. (2018). Nevertheless, in comparison to the Nile tilapia populations, the Rufiji tilapia populations were more homogenous.

6.3 Growth performance of Rufiji tilapia in two environments

The main aim of selective breeding programs is to identify the individuals with the highest genetic potential for improving desirable traits. The growth performance of Rufiji tilapia used in this study varied within and between

the tested environments. In general, fish reared in at Pangani performed better than the ones at Kunduchi. As aforementioned the water salinity and temperature in these locations differed where Kunduchi had average temperature of 30℃ and salinity of <0.05‰ while Pangani had average temperature of 32℃ and salinity of >5‰. Previous studies showed that Rufiji tilapia performed better in medium levels of salinity (Mapenzi & Mochi, 2016; Ulotu et al., 2016). Similar finding was reported in the study of blue tilapia (*O. aureus*) reared in different water salinities (Küçük et al., 2013). Even though low levels of salinity were used in our study compare to previous studies on tilapia, the ability of Rufiji tilapia to tolerate wide range of salinities could be the reason behind better growth performance in brackish water (Genner et al., 2018; Nehemia et al., 2013). In other hand, Rufiji tilapia fingerlings were initially reared at Pangani for 30 days before being transferred to Kunduchi where rearing water conditions were different. Temperature variation is diversely reported to affect fish growth rate, survival and other physiological traits by triggering physiological mechanism changes (Boltaña et al., 2017). Therefore, poor performance of animals reared at Kunduchi could also be associated with transportation stress and changing of rearing environment.

6.4 Genetic parameters of growth-related traits

Growth is one of the most economically important traits in aquaculture. Heritability estimates of growth-related traits can provide significant insights about the potential improvement of these traits through selective breeding. Heritability indicates the proportion of total phenotypic variation that can be attributed to genomic variation. Therefore, a high heritability shows that a large part of phenotypic variance is due to additive genetic variance (Gjedrem, 2005). In this study, we observed moderate to high heritability for growth-related traits (h^2: 0.39 – 0.74). Even though no previous study has been performed in Rufiji tilapia, several studies documented heritability estimates in other tilapia strains. In Nile tilapia, heritability for growth-related traits seem to range from 0.10 to 0.68 (Charo-Karisa, 2006; Khaw et al., 2009; Mengistu et al., 2021; Nguyen et al., 2017). This indicate that Rufiji tilapia has the potential for further genetic improvement. Apart from harvesting weight (HW), other growth traits like weight gain (WG), average

growth rate (AGR) and thermal growth coefficient (TGC) are noteworthy analysing for fish selection for a breeding program.

6.5 Genotype by environment (G × E) interaction

The performance of a farmed population to different rearing environments can be described by the genotype by environment (G × E) interaction (Nguyen et al., 2017). It is of paramount importance to obtain information of G × E interaction, since fish rearing conditions are usually diverse from that on the breeding nucleus. Breeding nucleus conditions are commonly monitored and/or controlled to ensure best performance of reared fish (Mengistu et al., 2020; Sae-Lim et al., 2013). The distribution of fish from these breeding nuclei to the diverse rearing conditions can led to lower than expected performance if G × E exists (Sae-Lim et al., 2013). Moreover, Tanzania is located in area with different altitudes, photoperiod, temperatures and feed sources which can result in stronger G × E interaction. Therefore, understanding this interaction between fish genotype and the environment will assist in the selection of rearing conditions and predict beforehand the phenotypic performance of farmed fish.

The genetic correlations for the growth-related traits in our study ranged between 0.73 - 0.74 indicating that a moderate re-ranking of the tested strains/families is possible amongst the two environments that were studied in this thesis. Since there are no prior studies on Rufiji tilapia regarding G × E interaction, Nile tilapia as a closely related species could be used for comparison. Studies on Nile tilapia indicate that genetic correlation can range from -0.19 to above 0.80 (Mengistu et al., 2020, 2021; Nguyen et al., 2017). The genetic correlation of less than 0.8 is reported to have a biological importance for G × E interactions for farmed Nile tilapia. However, genetic correlation between 0.8 and above for G × E interactions is not considered strong (Mengistu, 2022). Meaning that the genetic gain of more than 80% can be achieved in the rearing environment similar to the breeding nucleus.

Furthermore, water salinity was below 6‰ in both environments in our study, but Rufiji tilapia tolerates wide ranges of salinity level (Mapenzi & Mochi, 2016; Nehemia et al., 2013; Ulotu et al., 2016). Hence, it is expected for this species to be farmed in different salinities, which can result in different G × E interactions from one in this study. Therefore, in case of high G × E interaction, having two separate breeding programs would probably

not be economically viable. Additionally, for the development of a Rufiji tilapia structured breeding program in Tanzania, it is necessary to consider trait recordings from related individuals such as full-sibs and/or half-sibs for rearing in both brackish and freshwater.

6.6 Limitation of the Rufiji tilapia study for genetic improvement

Our study had a shallow pedigree spanning only one generation, while the cross design included only unique mating pairs. The latter resulted in fully confounding the common environmental effect with the animal effect. Nevertheless, the aforementioned breeding design allowed us to set up a more diverse panel of families that could be of interest in future breeding schemes. Also, we performed only one common garden experiment in two locations. Multiple replicates of the experiment are required to better understand what contributes to the G × E besides salinity. Rearing condition in our study is relatively similar to ones for other fish species e.g. feeding to satiation, use of monosex and weekly water exchange. However, these conditions probably do not represent the typical rearing conditions encountered in local fish farms in Tanzania. Since fish feed costs more than 50% of the production costs (Iversen et al., 2020), feeding to satiation for small scale fish farmers is not practical. Therefore, future studies could concentrate on both low and high input environments. For instance, the use of aerated and non-aerated ponds, feeding in limited amount, several salinity levels and monosex versus mixed sex.

6.7 Towards a selective breeding program

Developing a structured selective breeding program requires as a first step setting up a breeding goal, then study the genetic variance of target traits in desirable species, determine their heritability and genetic correlations among traits or amongst rearing environments (Gjedrem, 2005). From the derived information of this study the best Rufiji tilapia populations were Wami and Mindu populations. These populations outperformed other Rufiji populations in growth related traits in both rearing environments. They also had higher genetic diversity required for a breeding program founder population. However, these two populations are highly admixed and hence

not preferred for a pure strain breeding program for conservation purpose. However, if the pure strain of Rufiji tilapia were to be used as the base of a breeding program, Nyamisati, Chemchem and Bwawani populations would have been better choice. Unfortunately, these populations have low genetic diversity (H_O, H_E and $F_{ST} < 0.11$), even though their growth rate was higher than the rest of the tested non-admixed populations. Pure breeding is the mating of initially unrelated individuals within a population or a strain (Gjedrem, 2005). Application of selective breeding in purebred provide good possibilities for success especially when one strain performs better than the alternative strain. However, it's impossible to keep crossing pure unrelated individuals for several number generations especially with low genetic diversity (Gjedrem, 2005). Consequently, purebred breeding in a closed population increases inbreeding levels every generation. To keep inbreeding levels down in pure breeding system, mating of close relative (full, half-sibs and parents) should be avoided (Gjedrem, 2005). Breeding pure strain from the wild conserves the natural biodiversity. In case of escapees, no new alleles from purebred will be introduced to the wild stock, instead the escapees can gain genes that were eliminated during selection. However, escapees of selectively purebred fish into the wild may introduce weak allele to their offspring in case of interbreeding with wild population. Interbreeding may also homogenize wild population resulting in to outbreeding depression and low wild population fitness (Ansah et al., 2014).

Crossbreeding is an alternative of keeping inbreeding levels down and increasing genetic variation in a closed production system. In fish crossbreeding involve the mating of different strains, inbred lines and different species, henceforth increasing levels of heterozygosity (Gjedrem, 2005). This method in fish breeding aims to produce offspring with superior phenotypic performance compared to their parents (Gjedrem & Robinson, 2014). Through crossbreeding non-additive genes genetic variance is exploited leading to heterosis where hybrid surpass their parent average performance (Gjedrem, 2005). Therefore, this is another area of interest for a potential Rufiji tilapia and Nile tilapia crossbreeding program. Rufiji tilapia is reported to produce all male offspring when a Rufiji tilapia male is crossbred with a female Nile tilapia (Mapenzi & Mochi, 2016). In tilapia males are preferred due to their fast growth compare to females (Abo-Al-Ela, 2018). Hybrids of Nile and Rufiji tilapia have the advantage of caring favourable traits from both species, they grow faster than Rufiji tilapia and

tolerate a wider range of salinity than Nile tilapia (Mapenzi & Mochi, 2016). However, these hybrids are fertile and can backcross and produce mixed-sex offspring. Notably, the admixed populations in our study could in fact be hybrids between Nile and Rufiji tilapia. Farming such populations in net pens could have a negative effect to the native biodiversity in case of escapees.

6.8 Possibilities and constraints of using admixed tilapia populations in a structured breeding program

Through tilapia selective breeding program, we will be able to lessen the gap between fish production and the quality of needed stock to sustain the fish demand. This can be achieved by selecting a base population with most desirable economically important traits and meet aforementioned genetic parameters required for selection. In this study, Wami and Mindu populations met most of the requirements for a base population of a selective breeding program. However, these populations are highly admixed and therefore both possibilities and constraints of using them should be analysed. Since the main focus of establishing tilapia breeding program is to boost tilapia production in a sustainable manner, the use of Wami and Mindu population in a selective breeding program is very appealing. Arguably, these populations showed admixture because they are Nile and Rufiji tilapia hybrids due to the introduction of Nile tilapia in their catchments (Bradbeer et al., 2018; Shechonge et al., 2018; Shechonge et al., 2019). Therefore, If Mindu and Wami population are to be used as the base of the breeding program, the breeding program should be allocated in areas with existing mixed population to avoid introduction of new allele into areas with reported pure strains of tilapias.

In contrast, although it is appealing to use the Wami and Mindu population for the development of a breeding program, preventing escapees from farms could be challenging. Non-native species and hybrids existence in natural water bodies is the results of intentional introduction and poor fish farming management (Shechonge et al., 2019). Escapees can also have adverse impact to the wild biodiversity such as carrying new diseases and introduction of weak new allele through hybridization (Kajungiro, et al., 2019a). Therefore, we need to evaluate impacts of using these populations as a base of a breeding program beforehand.

7. Conclusion

This research has generated basic information for the establishment of a Rufiji tilapia breeding program. A future breeding program would benefit from background knowledge regarding the genetic diversity and phenotypic performance information of the various Rufiji tilapia populations in Tanzania.

The genetic diversity analysis revealed a wide range of genetic variation among the studied Rufiji tilapia populations. Mindu and Wami population showed the highest genetic variation which was associated with mixing with another tilapia strain as reflected in admixture analysis. Understanding the genetic structure of this species, will enhance effective conservation practices of wild fish, while the genetic diversity will assist in the most suitable aquaculture management and future planning of Rufiji tilapia aquaculture breeding practices especially towards establishing the base population.

In addition, the phenotypic performance of the Rufiji tilapia populations at the two environments, provided fundamental information their growth potential for aquaculture purposes. Tilapia is farmed in different geographical location mostly in tropical and subtropical environment. Apart from different environments, tilapia is farmed in different culture systems both controlled and uncontrolled for instance pond, cages, RAS and others. Therefore, the population that perform better in different water condition and culture systems is the most desirable for sustainable aquaculture. Our study revealed moderate to high G × E interaction of Rufiji tilapia reared in two environments. Based on our results, substantial benefits in terms of improving both harvest weight and growth rate are to be expected through selective breeding. A moderate reranking of the best performing families was observed among the two rearing sites that differed in salinity. Therefore,

future breeding practices, selection of Rufiji tilapia should take into consideration both data from the breeding nucleus and rearing environment especially if it is to take place on sites of varying salinity levels.

References

Abo-Al-Ela, H. G. (2018). Hormones and fish monosex farming: A spotlight on immunity. In *Fish and Shellfish Immunology* (Vol. 72, pp. 23–30). Academic Press. https://doi.org/10.1016/j.fsi.2017.10.038

Abou, Y., Fiogbé, E. D., & Micha, J.-C. (2007). Effects of stocking density on growth, yield and profitability of farming Nile tilapia, *Oreochromis niloticus L.*, fed Azolla diet, in earthen ponds. *Aquaculture Research*, *38*(6), 595–604. https://doi.org/10.1111/j.1365-2109.2007.01700.x

Adeniyi, S. A., Orjiekwe, C. L., Ehiagbonare, J. E., & Josiah, S. J. (2012). Nutritional composition of three different fishes (*Clarias gariepinus, Malapterurus electricus* and *Tilapia guineensis*). *Pakistan Journal of Nutrition*, *11*(9), 793–797. https://doi.org/10.3923/pjn.2012.891.895

Ansah, Y. B., Frimpong, E. A., & Hallerman, E. M. (2014). Genetically-improved tilapia strains in Africa: Potential benefits and negative impacts. *Sustainability*, *6*, 3697–3721. https://doi.org/10.3390/su6063697

Bakos, J., Varadi, L., Gordas, S., & Jeney, Z. (2006). Lessons from the breeding program on common carp in Hungary. In *Worldfish Center* (pp. 27–33). WorldFish Center. http://hdl.handle.net/1834/20391

Balarin, J. D. (1984). *National reviews for aquaculture development in Africa. 11. Tanzania. FAO Fisheries Circular No. 770.11. FIRI/C770.11. Rome, Italy*. https://www.fao.org/3/w7387e/W7387E06.htm

Boltaña, S., Sanhueza, N., Aguilar, A., Gallardo-Escarate, C., Arriagada, G., Valdes, J. A., Soto, D., & Quiñones, R. A. (2017). Influences of thermal environment on fish growth. *Ecology and Evolution*, *7*(17), 6814–6825. https://doi.org/10.1002/ece3.3239

Bradbeer, S. J., Harrington, J., Watson, H., Warraich, A., Shechonge, A., Smith, A., Tamatamah, R., Ngatunga, B. P., Turner, G. F., & Genner, M. J. (2018). Limited hybridization between introduced and critically endangered indigenous tilapia fishes in Northern Tanzania. University of Bristol. *Hydrobiologia*. https://doi.org/10.1007/s10750-018-3572-5

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: Building and Genotyping Loci De Novo from Short-Read Sequences. *G3 Genes|Genomes|Genetics*, *1*(3), 171–182. https://doi.org/10.1534/g3.111.000240

Champneys, T., Genner, M. J., & Ioannou, C. C. (2021). Invasive Nile tilapia dominates a threatened indigenous tilapia in competition over shelter. *Hydrobiologia*, *848*(16), 3747–3762. https://doi.org/10.1007/s10750-020-04341-8

Charo-Karisa, H. (2006). *Selection for growth of Nile tilapia (Oreochromis niloticus L.) in low-input environments*. Wageningen University.

Chenyambuga, S. W. (2018). Development of sustainable Nile tilapia (Oreochromis niloticus) culture for improved food security and poverty reduction. In *Sokoine University*. Cambridge University Press.

Dadzie, S. (1992). An overview of aquaculture in eastern Africa. *Hydrobiologia*, *232*, 99–110. https://link.springer.com/content/pdf/10.1007/BF00014618.pdf

de Villemereuil, P., Gaggiotti, O. E., Mouterde, M., & Till-Bottraud, I. (2016). Common garden experiments in the genomic era: new perspectives and opportunities. *Heredity*, *116*(3), 249–254. https://doi.org/10.1038/hdy.2015.93

Dong, Z., Nguyen, N. H., & Zhu, W. (2015). Genetic evaluation of a selective breeding program for common carp Cyprinus carpio conducted from 2004 to 2014. *BMC Genetics*, *16*(1). https://doi.org/10.1186/s12863-015-0256-2

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361. https://doi.org/10.1007/s12686-011-9548-7

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

FAO. (2018). Aquaculture Development 9. Development of aquatic genetic resources: A framework of essential criteria.

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. In *INFORM* (Vol. 32, Issue 6). American Oil Chemists Society. https://doi.org/10.4060/ca9229en

FAO. (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. *Rome, Italy*. https://doi.org/10.4060/cc0461en

Genner, M. J., Turner, G. F., & Ngatunga, B. P. (2018). A guide to the tilapia fishes of tanzania. 36.

Genner, M., Ngatunga, B., Tamatama, R., Shechonge Asilatu, & Turner, G. (2018). Sustainable fisheries, food security: lessons from tilapia fish in Tanzania. In *Policy Report* (Vol. 34).

Gjedrem, T. (2005). Selection and Breeding Programs in Aquaculture. Selection and Breeding Programs in Aquaculture Netherlands: *Springer*. https://doi.org/10.1007/1-4020-3342-7

Gjedrem, T., & Robinson, N. (2014). Advances by Selective Breeding for Aquatic Species: A Review. *Agricultural Sciences*, *05*(12), 1152–1158. https://doi.org/10.4236/as.2014.512125

Gjerde, B., & Rye, M. (1998). *Design of breeding programmes in aquaculture species: Possibilities and constraints*. http://om.ciheam.org/article.php?IDPDF=98606203

Hassanien, H. A., & Gilbey, J. (2005). Genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus*) revealed by DNA microsatellites. *Aquaculture Research*, *36*(14), 1450–1457. https://doi.org/10.1111/j.1365-2109.2005.01368.x

Holsinger, K. E., & Weir, B. S. (2009). Genetics in geographically structured populations: defining, estimating and interpreting FST. *Nature Reviews Genetics*, *10*(9), 639–650. https://doi.org/10.1038/nrg2611

Hong Nguyen, N., Hamzah, A., Ponzoni, R. W., Ling Khaw, H., Yip Yee, H., & Azizah Mohd Nor, S. (2014). Performance of the Genetically Improved Farmed Tilapia (GIFT) Strain Over Ten Generations of Selection in Malaysia. *Pertanika J. Trop. Agric. Sci*, *37*(4), 411–429. http://www.pertanika.upm.edu.my/

Houston, R. D., Bean, T. P., Macqueen, D. J., Gundappa, M. K., Jin, Y. H., Jenkins, T. L., Selly, S. L. C., Martin, S. A. M., Stevens, J. R., Santos, E. M., Davie, A., & Robledo, D. (2020). Harnessing genomics to fast-track genetic improvement in aquaculture. *Nature Reviews Genetics*, *21*(7), 389–409. https://doi.org/10.1038/s41576-020-0227-y

Islam, S., Bhowmik, S., Majumdar, P. R., Srzednicki, G., Rahman, M., & Hossain, M. A. (2021). Nutritional profile of wild, pond-, gher- and cage-cultured tilapia in Bangladesh. *Heliyon*, *7*(5). https://doi.org/10.1016/j.heliyon.2021.e06968

Iversen, A., Asche, F., Hermansen, Ø., & Nystøyl, R. (2020). Production cost and competitiveness in major salmon farming countries 2003–2018. *Aquaculture*, *522*. https://doi.org/10.1016/j.aquaculture.2020.735089

Jombart, T., & Collins, C. (2015). A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 2.0.0. In *Imperial College London*. https://adegenet.r-forge.r-project.org/files/tutorial-dapc.pdf

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(1), 94. https://doi.org/10.1186/1471-2156-11-94

Jombart, T., Kamvar, Z. N., Collins, C., Lustrik, R., Beugin, M.P., Knaus, B. J., … And Morkovsky, L., (2018). Exploratory Analysis of Genetic and Genomic Data. https://github.com/thibautjombart/adegenet

Kajungiro, R. A., Mapenzi, L. L., Nyinondi, C. S., Haldén, A. N., Mmochi, A. J., Chacha, M., Mtolera, M. S., Lamtane, H. A., & Jan De Koning, D. (2019). The Need of a Structured Tilapia Breeding Program in Tanzania to Enhance Aquaculture Production: A Review. *Tanzania Journal of Science*, *45*(3), 355–371. www.ajol.info/index.php/tjs/

Kajungiro, R. A., Palaiokostas, C., Pinto, F. A. L., Mmochi, A. J., Mtolera, M., Houston, R. D., & de Koning, D. J. (2019). Population Structure and Genetic Diversity of Nile Tilapia (Oreochromis niloticus) Strains Cultured in Tanzania. *Frontiers in Genetics*, *10*. https://doi.org/10.3389/fgene.2019.01269

Karapanagiotidis, I. T. (2017). Nutrient profiles of tilapia. In *Tilapia in Intensive Co-culture* (pp. 261–305). wiley. https://doi.org/10.1002/9781118970652.ch16

Keller, L. F., & Waller, D. M. (2002). Inbreeding effect in wild populations. *TRENDS in Ecology & Evolution*, *17*(5), 293–301. http://tree.trends.com0169-5347/02/$-seefrontmatter

Khaw, H. L., Bovenhuis, H., Ponzoni, R. W., Rezk, M. A., Charo-Karisa, H., & Komen, H. (2009). Genetic analysis of Nile tilapia (Oreochromis niloticus) selection line reared in two input environments. *Aquaculture*, *294*(1–2), 37–42. https://doi.org/10.1016/j.aquaculture.2009.05.025

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology*

Resources, *15*(5), 1179–1191. https://doi.org/10.1111/1755-0998.12387

Küçük, S., Karul, A., Yildirim, Ş., & Gamsiz, K. (2013). Effects of salinity on growth and metabolism in blue tilapia (*Oreochromis aureus*). *African Journal of Biotechnology*, *12*(19), 2715–2721. https://doi.org/10.5897/AJB12.1296

Lhorente, J. P., Araneda, M., Neira, R., & Yáñez, J. M. (2019). Advances in genetic improvement for salmon and trout aquaculture: the Chilean situation and prospects. *Reviews in Aquaculture*, *11*, 340–353. https://doi.org/10.1111/raq.12335

Lind, C. E., Agyakwah, S. K., Attipoe, F. Y., Nugent, C., Crooijmans, R. P. M. A., & Toguyeni, A. (2019). Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-53295-y

Liu, Z. J., & Cordes, J. F. (2004). DNA marker technologies and their applications in aquaculture genetics. *Aquaculture*, *238*(1–4), 1–37. https://doi.org/10.1016/j.aquaculture.2004.05.027

Mapenzi, L. L., & Mmochi, A. J. (2016). Effect of Stocking Density on Growth Performance of Hybrids of *Oreochromis niloticus*♀ and *Oreochromis urolepis urolepis*♂ in Saline Water. *West Indian Ocean Journal of Marine Science*, *15*(2), 67–74. http://www.ajol.info/index.php/wiojms/about/submissions

Mapenzi, L. L., & Mochi, A. J. (2016). Role of Salinity on Growth Performance of *Oreochromis niloticus*♀ and *Oreochromis urolepis urolepis*♂ Hybrids. *Journal of Aquaculture Research & Development*, *7*(6). https://doi.org/10.4172/2155-9546.1000431

Megahed, M. E. (2019). Genetic selection for improved disease resistance in aquaculture with special reference to shrimp and tilapia breeding programs in Egypt. *Journal of Applied Aquaculture*. https://doi.org/10.1080/10454438.2019.1697783

Mengistu, S. B. (2022). Closing the yield gap: improving production efficiency in smallholder farms of Nile tilapia through selective breeding. PhD thesis, Wageningen University.

Mengistu, S. B., Mulder, H. A., Benzie, J. A. H., & Komen, H. (2020). A systematic literature review of the major factors causing yield gap by affecting growth, feed conversion ratio and survival in Nile tilapia

(*Oreochromis niloticus*). In *Reviews in Aquaculture* (Vol. 12, Issue 2, pp. 524–541). Wiley-Blackwell. https://doi.org/10.1111/raq.12331

Mengistu, S. B., Palstra, A. P., Mulder, H. A., Benzie, J. A. H., Trinh, T. Q., Roozeboom, C., & Komen, H. (2021). Heritable variation in swimming performance in Nile tilapia (*Oreochromis niloticus*) and negative genetic correlations with growth and harvest weight. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-90418-w

Mireku, K. K., Kassam, D., Changadeya, W., Attipoe, F. Y. K., & Adinortey, C. A. (2017). Assessment of genetic variations of Nile Tilapia (*Oreochromis niloticus L.*) in the Volta Lake of Ghana using microsatellite markers. *African Journal of Biotechnology*, *16*(7), 312–321. https://doi.org/10.5897/AJB2016.15796

Moses, M., Mtolera, M. S. P., Chauka, L. J., Lopes, F. A., de Koning, D. J., Houston, R. D., & Palaiokostas, C. (2019). Characterizing the genetic structure of introduced Nile tilapia (*Oreochromis niloticus*) strains in Tanzania using double digest RAD sequencing. *Aquaculture International*, *28*(2), 477–492. https://doi.org/10.1007/s10499-019-00472-5

Neaves, L. E., Eales, J., Whitlock, R., Hollingsworth, P. M., Burke, T., & Pullin, A. S. (2015). The fitness consequences of inbreeding in natural populations and their implications for species conservation – a systematic map. *Environmental Evidence*, *4*(1), 5. https://doi.org/10.1186/s13750-015-0031-x

Nehemia, A., Mmochi, A., & Mtolera, M. (2013). Survival and Growth of *Tilapia zillii* and *Oreochromis urolepis urolepis* (Order Perciformes; Family Cichlidae) in Seawater. *Western Indian Ocean J. Mar. Sci*, *12*(1), 37–45.

Nguyen, N. H., Hamzah, A., & Thoa, N. P. (2017). Effects of Genotype by Environment Interaction on Genetic Gain and Genetic Parameter Estimates in Red Tilapia (*Oreochromis spp.*). *Frontiers in Genetics*, *8*(JUN). https://doi.org/10.3389/fgene.2017.00082

Nichols, H. J. (2017). The causes and consequences of inbreeding avoidance and tolerance in cooperatively breeding vertebrates. *Journal of Zoology*, *303*(1), 1–14. https://doi.org/10.1111/jzo.12466

Nyinondi, C. S., Mtolera, M. S. P., Mmochi, A. J., Lopes Pinto, F. A., Houston, R. D., de Koning, D. J., & Palaiokostas, C. (2020). Assessing the genetic diversity of farmed and wild Rufiji tilapia (*Oreochromis*

urolepis urolepis) populations using ddRAD sequencing. *Ecology and Evolution*, *10*(18), 10044–10056. https://doi.org/10.1002/ece3.6664

Palaiokostas, C., Anjum, A., Jeuthe, H., Kurta, K., Lopes Pinto, F., & de Koning, D. J. (2021). A genomic-based vision on the genetic diversity and key performance traits in selectively bred Arctic charr (*Salvelinus alpinus*). *Evolutionary Applications*, *15*(4), 565–577. https://doi.org/10.1111/eva.13261

Palaiokostas, C., Bekaert, M., Khan, M. G. Q., Taggart, J. B., Gharbi, K., McAndrew, B. J., & Penman, D. J. (2015). A novel sex-determining QTL in Nile tilapia (*Oreochromis niloticus*). *BMC Genomics*, *16:171*. https://doi.org/10.1186/s12864-015-1383-x

Pembleton, L. W., Cogan, N. O. I., & Forster, J. W. (2013). StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Molecular Ecology Resources*, *13*, 946–952. https://doi.org/10.1111/1755-0998.12129

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLoS ONE*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Ponzoni, R. W., Nguyen, N. H., Khaw, H. L., Hamzah, A., Bakar, K. R. A., & Yee, H. Y. (2011). Genetic improvement of Nile tilapia (*Oreochromis niloticus*) with special reference to the work conducted by the World Fish Center with the GIFT strain. *Reviews in Aquaculture*, *3*(1), 27–41. https://doi.org/10.1111/j.1753-5131.2010.01041.x

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, *155*, 945–959. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1461096/pdf/10835412.pdf

Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P., & Houston, R. (2018). Applications of genotyping by sequencing in aquaculture breeding and genetics. In *Reviews in Aquaculture* (Vol. 10, Issue 3, pp. 670–682). Wiley-Blackwell. https://doi.org/10.1111/raq.12193

Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based

population genomics. *Molecular Ecology*, *28*(21), 4737–4754. https://doi.org/10.1111/mec.15253

Rukanda, J. J., & Sigurgeirsson, O. (2016). *Evaluation of aquaculture development in Tanzania*. http://www.unuftp.is/static/fellows/document/janeth16aprf.pdf

Sae-Lim, P., Gjerde, B., Nielsen, H. M., Mulder, H., & Kause, A. (2016). A review of genotype-by-environment interaction and micro-environmental sensitivity in aquaculture species. *Reviews in Aquaculture*, *8*(4), 369–393. https://doi.org/10.1111/raq.12098

Sae-Lim, P., Kause, A., Mulder, H. A., Martin, K. E., Barfoot, A. J., Parsons, J. E., Davidson, J., Rexroad Iii, C. E., van Arendonk, J. A. M., & Komen, H. (2013). Genotype-by-environment interaction of growth traits in rainbow trout (*Oncorhynchus mykiss*): A continental scale study 1. *J. Anim. Sci*, *91*, 5572–5581. https://doi.org/10.2527/jas2012-5949

Shechonge, A., Ngatunga, B. P., Bradbeer, S. J., Day, J. J., Freer, J. J., Ford, A. G. P., Kihedu, J., Richmond, T., Mzighani, S., Smith, A. M., Sweke, E. A., Tamatamah, R., Tyers, A. M., Turner, G. F., & Genner, M. J. (2019). Widespread colonisation of Tanzanian catchments by introduced Oreochromis tilapia fishes: the legacy from decades of deliberate introduction. *Hydrobiologia*, *832*(1), 235–253. https://doi.org/10.1007/s10750-018-3597-9

Shechonge, A., Ngatunga, B. P., Tamatamah, R., Bradbeer, S. J., Harrington, J., Ford, A. G. P., Turner, G. F., & Genner, M. J. (2018). Losing cichlid fish biodiversity: genetic and morphological homogenization of tilapia following colonization by introduced species. *Conservation Genetics*, *19*(5), 1199–1209. https://doi.org/10.1007/s10592-018-1088-1

Shechonge, A., Ngatunga, B. P., Tamatamah, R., Bradbeer, S. J., Sweke, E., Smith, A., Turner, G. F., & Genner, M. J. (2019). Population genetic evidence for a unique resource of Nile tilapia in Lake Tanganyika, East Africa. *Environmental Biology of Fishes*, *102*(8), 1107–1117. https://doi.org/10.1007/s10641-019-00895-2

Sherman, K. D., Paris, J. R., King, R. A., Moore, K. A., Dahlgren, C. P., Knowles, L. C., Stump, K., Tyler, C. R., & Stevens, J. R. (2020). RAD-Seq Analysis and in situ Monitoring of Nassau Grouper Reveal Fine-Scale Population Structure and Origins of Aggregating Fish. *Frontiers in Marine Science*. https://doi.org/10.3389/fmars.2020.00157

Shoko, A. P., Lamtane, H. A., Wetengere, K., Kajitanus, O. O., Msuya, F. E., Mmochi, A. J., & Mgaya, Y. D. (2011). The status and development of aquaculture in Tanzania, East Africa. *In: Proceedings of the International Conference on Ecosystem Conservation and Sustainable Development (ECOCASD 2011)*, 85–97. https://www.researchgate.net/publication/257145458

Simbine, L., Viana da Silva, J., & Hilsdorf, A. W. S. (2014). The genetic diversity of wild *Oreochromis mossambicus* populations from the Mozambique southern watersheds as evaluated by microsatellites. *Journal of Applied Ichthyology*. https://doi.org/10.1111/jai.12390

Svanbäck, R., & Eklöv, P. (2006). Genetic variation and phenotypic plasticity: Causes of morphological and dietary variation in Eurasian perch. *Evolutionary Ecology Research*, *8*, 37–49. https://www.researchgate.net/publication/228664624

Symonds, J. E., Clarke, S. M., King, N., Walker, S. P., Blanchard, B., Sutherland, D., Roberts, R., Preece, M. A., Tate, M., Buxton, P., & Dodds, K. G. (2019). Developing successful breeding programs for New Zealand aquaculture: A perspective on progress and future genomic opportunities. *Frontiers in Genetics*, *10*(FEB). https://doi.org/10.3389/fgene.2019.00027

Thodesen, J., & Gjedrem, T. (2006). *Breeding programs on Atlantic salmon in Norway: lessons learned*. https://www.researchgate.net/publication/227642728

Ulotu, E. E., Mmochi, A. J., & Lamtane, H. A. (2016). Effect of Salinity on the Survival and Growth of Rufiji Tilapia (*Oreochromis urolepis urolepis*) Fry. *West Indian Ocean Journal of Marine Science*, *15*(2), 31–37. http://www.ajol.info/index.php/wiojms/about/submissions

United Nations. (2022). *World Population Prospects 2022*. https://www.un.org/development/desa/pd/

URT. (2020). Annual Fisheries Statistics Report 2020. In Ministry of Livestock and Fisheries, Aquaculture development division, Dodoma, Tanzania.

URT-MLF. (2019). Livestock and Fisheries Commodity Value Chain Briefs*: Aquaculture policy brief*.

Popular science summary

Fish farming has come a long way, since 475 BC when Fan Lai wrote a book teaching how to grow fish for food called The Classic of Fish Culture. Over time, various advancements were made resulting in increasing the sustainability of fish farming. Tilapia is one of the most preferred fish for farming due to their ease of propagation, tolerance to handling, fast growth on both natural and manufactured feeds, tolerance of a wide range of environmental conditions, high palatability, marketability and nutritional content. Fish farming, especially tilapia, is important in Tanzania where it is expected to contribute towards both monetary and food requirements of the poor communities. However, production from fish farming is very low despite the abundance of more than 30 different types of tilapia. The lack of high-quality fingerlings and unclear origin of broodstock is presumed to be the main reason behind the poor production of farmed fishes in the country. This problem has led to many fish farmers to import fish from outside the country which endanger native tilapia populations due to disease transmission and pollution of the unique genetic diversity of the endemic populations. This project aimed to generate information that will be used for the development of a tilapia selective breeding program in Tanzania. The project was divided into two main studies, the genetic diversity and common garden studies. Moreover, a prerequisite study on the status of fish farming in Tanzania was performed beforehand. This survey provided key information that led towards a better understanding of the status and availability of infrastructure for effective dissemination of a structured breeding program.

Thereafter, the genetic diversity and structure of Rufiji tilapia was analysed for both wild and farm populations using modern sequencing technologies. Overall, 195 animals originating from eight wild (Nyamisati,

Utete, Mansi, Mindu, Wami, Ruaha, Kibasira, and Kilola) and two farmed (Bwawani and Chemchem) populations were used in this study. The genetic diversity results showed low genetic variations among populations from neighbouring locations, with the exception of Utete and Chemchem populations. Moreover, our study, revealed the presence of four admixed populations namely; Wami, Mindu, Mansi and Utete. The shared ancestry between the remaining six populations was in agreement with the low genetic distance between them, hence believed to be pure Rufiji tilapia. Additionally, the genetic diversity of Rufiji tilapia was compared with that of local and exotic Nile tilapia. Interestingly, two of the Rufiji populations that were admixed (Mindu and Wami) were closely related to Nile tilapia suggesting the presence of hybrids amongst the two species. The presence of Nile tilapia in Mindu reservoir and Wami river could explain the admixture since tilapia has the tendencies of crossbreeding.

A common garden experiment was conducted to investigate the growth performance of Rufiji tilapia in two environments (fresh and brackish water). The results showed that the fish from Mindu and Wami performed best in both freshwater and brackish water environment. Overall results showed that Rufiji tilapia performed better in brackish water than in fresh water. Growth-related traits were measured on 1392 animals from 35 families followed by estimation of genetic parameters. The results showed moderate to high heritability and genetic correlations. Therefore, a moderate re-ranking of the best performing animals is to be expected amongst the two environments.

To meet the increasing demand for aquatic food, tilapia is farmed in diverse rearing environments and culture systems. The selection of fish strain or population that would perform best in all conditions is very difficult. However, fish improvement through a structured breeding program can improve fish production. Therefore, if Rufiji tilapia rearing is to take place on sites of varying salinity and temperature levels, a breeding program should not base selection only on data from the breeding nucleus where all rearing conditions are either monitored and/or controlled, but instead fish performance on production sites should be included as well.

Populärvetenskaplig sammanfattning

Odling av fisk har kommit långt sedan 475 f.Kr. då Fan Lai skrev boken The Classic of Fish Culture som lärde ut hur man odlar fisk för mat. Med tiden har olika framsteg gjorts som resulterat i att fiskodlingens hållbarhet har ökat. Tilapia är en av de mest uppskattade fiskarna för odling på grund av att de är lätta att få att föröka sig, tolererar hantering, har snabb tillväxt på både naturligt och tillverkat foder, tolererar ett brett spektrum av miljöförhållanden, samt har hög smaklighet, säljbarhet och näringsinnehåll. Fiskodlingen, särskilt odlingen av tilapia, är viktig i Tanzania där den förväntas bidra till att möta behov av både inkomster och livsmedel i fattiga samhällen. Produktionen från fiskodling i Tanzania är dock mycket låg trots att det finns mer än 30 olika typer av tilapia-arter i landet. Bristen på högkvalitativa yngel för odling och oklart ursprung hos stamfisken antas vara huvudorsaken till den låga produktionen av odlad fisk i landet. Detta problem har lett till att många fiskodlare importerar fisk från andra länder, vilket äventyrar inhemska tilapia-populationer på grund av sjukdomsöverföring och förorening av den unika genetiska mångfalden.

Denna avhandling syftade till att ta fram information som kan användas för utvecklingen av ett selektivt avelsprogram för rufijitilapia (Oreochromis urolepis urolepis) i Tanzania. Studien som ligger till grund för avhandlingen är uppdelad i två försök, en studie av genetisk mångfald och ett common-garden-försök. Innan dessa försök gjordes en bakgrundsstudie om fiskodlingens status i Tanzania. Bakgrundsstudien gav nyckelinformation som ökade förståelsen för vilken infrastruktur som finns tillgänglig för effektiv spridning av ett strukturerat avelsprogram. Den genetiska mångfalden och populationsstrukturen studerades hos både vilda och odlade populationer av rufijitilapia med hjälp av moderna sekvenseringsmetoder. Totalt i studien användes 195 individer från 8 vilda (Nyamisati, Utete,

Mansi, Mindu, Wami, Ruaha, Kibasira och Kilola) och 2 odlade (Bwawani och Chemchem) populationer. Resultaten visade låga genetiska variationer bland populationer från närliggande platser, med undantag för Utete- och Chemchem-populationerna. Vidare visade vår studie förekomsten av fyra genetiskt blandade populationer: Wami, Mindu, Mansi och Utete. Det delade ursprunget mellan de återstående sex populationerna överensstämde med litet genetiskt avstånd dem emellan, och de antas därför vara rena rufijitilapia. Dessutom jämfördes den genetiska mångfalden hos rufijitilapia med den hos lokal och exotisk niltilapia (Oreochromis niloticus). Intressant nog var två av de rufiji-populationerna som var blandade (Mindu och Wami) nära besläktade med niltilapia, vilket tyder på hybrider mellan de två arterna. Närvaron av niltilapia i Mindu-reservoaren och Wami-floden kan förklara inblandningen eftersom tilapia har tendenser till korsning (hybridisering) mellan arter.

Ett common-garden-försök genomfördes för att studera tillväxten hos rufijitilapia i två olika miljöer (söt- och brackvatten). Resultaten visade att fisken från Mindu och Wami presterade bäst i både sötvattens- och bräckvattenmiljön. Överlag visade resultaten att rufijitilapia presterade bättre i bräckt vatten än i sötvatten. Tillväxtrelaterade egenskaper mättes på 1392 individer från 35 familjer följt av uppskattning av genetiska parametrar. Resultaten visade på måttlig till hög ärftlighet och genetiska korrelationer. Därför kan en måttlig omrankning av de bäst presterande individerna förväntas mellan de två miljöerna. För att möta den ökande efterfrågan på sjömat i Tanzania odlas tilapia i olika miljöer och odlingssystem. Valet av fiskstam eller population som skulle fungera bäst under alla förhållanden är mycket svårt. Ett strukturerat avelsprogram kan dock förbättra fiskproduktionen. Om odling av rufijitilapia ska äga rum på platser med varierande salthalt och temperaturnivåer, bör ett avelsprogram därför inte basera urval enbart på data från avelskärnan där alla uppfödningsförhållanden antingen övervakas och/eller kontrolleras, utan fiskprestandan vid produktionsanläggningar bör också ingå.

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I

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The Need of a Structured Tilapia Breeding Program in Tanzania to Enhance Aquaculture Production: A Review

Redempta A Kajungiro [1,2], Levinus L Mapenzi[3,4], Christer S Nyinondi[1,3], Anna Norman Haldén[6], Aviti J Mmochi[3], Mwita Chacha [2], Matern SP Mtolera[3], Hieromin A Lamtane[5], and Dirk Jan de Koning[1*]

[1]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, P. O. Box 7023, Uppsala, Sweden.*

[2]*Department of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, P. O. Box 60091, Dar es Salaam, Tanzania.*

[3]*Institute of Marine Sciences, University of Dar es Salaam, P. O. Box 668, Zanzibar, Tanzania.*

[4]*Department of Biology, University of Dodoma, P. O. Box 338, Dodoma, Tanzania.*

[5]*Department of Animal, Aquaculture and Range Sciences, Sokoine University of Agriculture, P. O. Box 3004, Morogoro, Tanzania.*

[6]*Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, P. O. Box 7084, Uppsala, Sweden.*

**Corresponding author; e-mail address: dj.de-koning@slu.se*

E-mail addresses of co-authors: srahmy40@yahoo.co.uk (Kajungiro); mwitachacha@udsm.ac.tz (Chacha); avitimmochi@gmail.com (Mmochi); mtolera@ims.udsm.ac.tz (Mtolera); christer.simon@yahoo.com (Nyinondi); levinus@yahoo.com (Mapenzi); hlamtan@suanet.ac.tz (Lamtane)and anna.norman-halden@slu.se (Haldén)

Abstract

Breeding programs are crucial for boosting productivity and increase sustainability of aquaculture. Over years, Tanzania has witnessed fluctuation in its capture fisheries production from 320,900 to 375, 535 and back to 362,595 metric tonnes in the years 2000, 2005 and 2016, respectively (URT 2016). The declining trend in fish production has made fish supply in the country unstable and conversely, increased the demand for fishes to about 730,000 metric tonnes in 2017. However, the local aquaculture production has not increased accordingly. Tanzania is importing fish mainly from Asia to meet its increased demand. In 2017, a total of 2,055,721 kg of frozen tilapia were imported from China and Mozambique (URT 2017). The introduction of exotic fish species in Tanzania should be carefully managed because introduced species have many negative impacts on the indigenous species. Tanzania should have a moderate scale tilapia breeding program that will produce good quality fingerlings at affordable prices for smallholder fish farmers. The availability of reliable good quality fingerlings is key to improve aquaculture production in the country. Among 17 existing hatcheries, only 12 hatcheries are active; however these hatcheries are not performing well due to low investment and technology, leading to the production of low quantity and quality fingerlings. The need for a structured sustainable Tilapia breeding program with bio secured and reliable hatcheries to enhance aquaculture production in Tanzania is put forward in this review.

Keywords: Aquaculture, Breeding programs, Nile tilapia, Local strains.

Introduction

Aquaculture is an important sector which contributes to food security and income generation (FAO 2018, Rothuis et al. 2014), poverty reduction and provide nutritional benefits in developing countries (Allison 2011). Since the late 1980s, capture fishery production has not changed much (Figure 1) while aquaculture continues at an increasing trend contributing 47% of total global fish

production (FAO 2018). Globally, aquaculture has become the major food production responsible for supplying fish for human consumption (FAO 2018). Aquaculture in Tanzania is mainly practiced at small scale in earthen ponds (Shoko et al. 2011), largely in extensive and semi-intensive farming systems. Inland fresh water aquaculture, dominated by mainly tilapia species such as Nile tilapia (*Oreochromis niloticus*) (Figure 2) while other common fish species cultured include African catfish (*Clarias gariapinus*). The number of earthen fish ponds for catfish and tilapia have increased from 24,302 in 2017/2018 producing 14,800 tonnes to 26,445 fish ponds in 2018/2019 producing 18,081.6 tonnes with the addition of fish production from 408 fish cages in lakes (L. Victoria and L. Tanganyika) and ponds (Malambo) (URT 2019). Despite the increase in production but still the supply is low to meet the current demand of 750,000 tonnes of fish in the country (URT 2019).

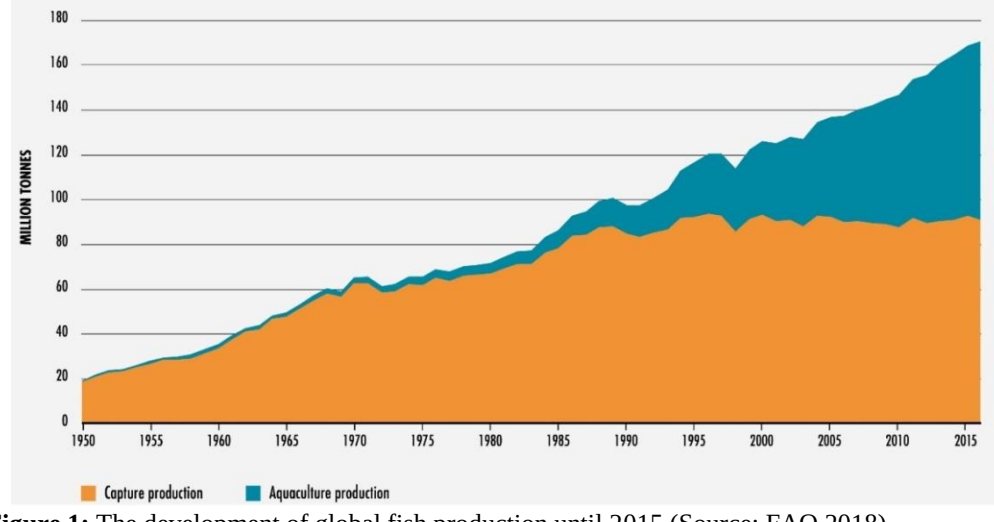

Figure 1: The development of global fish production until 2015 (Source: FAO 2018).

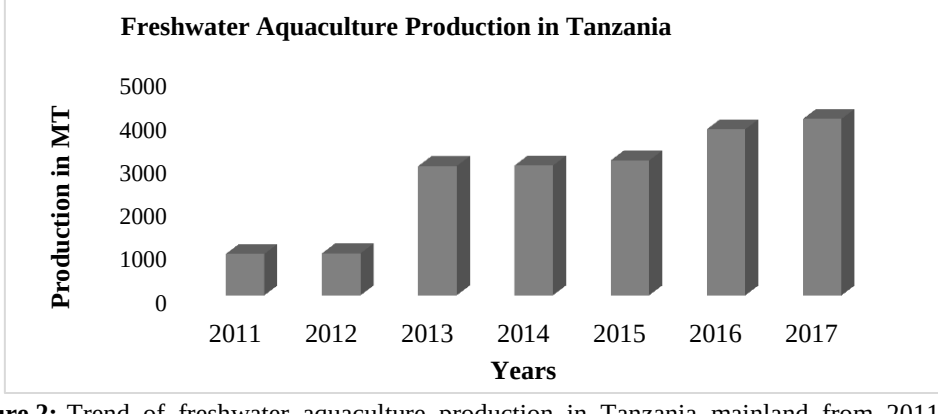

Figure 2: Trend of freshwater aquaculture production in Tanzania mainland from 2011-2017 (Source: URT 2017).

With the development of aquaculture in Tanzania, a total of 17 hatcheries have been established, seven public and 10 private owned hatcheries for tilapia, catfish and mariculture production (URT 2019). Catfish and tilapia fingerlings production for both private and public hatcheries have increased from 3,039,775 in 2017/2018 to 6,221,076 fingerlings in 2018/2019 (URT 2019). Mariculture is dominated by mainly seaweeds from Zanzibar islands (Msuya et al. 2016) and the production has increased from 1,197.5 tonnes in 2016/2017 to 1,329.9 tonnes in 2017/2018 (URT 2018). Nile tilapia (*O. niloticus*) is the most important cultured species in Tanzania (Shechonge et al. 2018b). This is because the species has a short generation time, fast growth, tolerance to a wide range of environmental conditions, resistance to stress and disease, ability to reproduce in captivity, and acceptance of artificial feeds right after yolk-sac absorption (Costa-Pierce 2003, Vicente and Fonseca-Al 2013, Ansah et al. 2014). Based on these attributes, the species has been used for breeding programs in other countries and is important for low-input aquaculture production (Ansah et al. 2014). Although Nile tilapia has characteristics which are well-suited for culturing in developing countries, they tend to mature early especially when cultured in ponds and spawn before they reach market sizes (Nkhoma and Musuka 2014). Because of early sexual maturity and high fecundity, they produce large number of small fry that leads to overcrowding and "stunting" where adult fish can sometimes mistakenly be stocked as fingerlings (Shoko et al. 2016). Early sexual maturation has disappointed many fish farmers in Tanzania leading to some farmers, government and private owned hatcheries importing Nile tilapia fingerlings and brood stock from neighbouring countries, i.e., Kenya, Uganda, and Zambia or even further afield from Thailand, believing that they would perform better than native species (Rukanda 2018, Shechonge et al. 2018a). Most of these imports are illegal and put the country at the risk of genetic pollution and introducing diseases.

Aquaculture in Tanzania is currently developing in good pace but still cannot cope with the increase in demand for fish and fish products. Consumers want a good flavoured strain and it has been reported that many consumers prefer red tilapia over Nile tilapia because of fewer problems with off-flavour (Lovshin 2000). Tanzanian consumers are diverse in their preference for all the tilapia features. They prefer fresh, wild, medium (400-600 g) and large (> 600 g) sized tilapia over smoked, farmed, and small (150-400 g) sized tilapia, respectively (Darko et al. 2016). Middle and high-income consumers (and hotels and restaurants) can afford the large sized fish while low-income households cannot (Rothuis et al. 2014). Consumers' preferences for large sized tilapia can be met by improving the quality of farmed strains of tilapia through genetic improvement.

Currently, there are 12 active tilapia hatcheries in the country that are either government (3) or privately (9) owned (Table 1) which produce fingerlings for distribution to the local farmers. For all the visited hatcheries, there were no biosecurity restriction rules and practices to minimize the risk of contamination. Moreover, the capacities of those hatcheries are still inadequate (Table 1) due to low level of investment and limited electric power supply (Rukanda 2018). Additionally, the number of fingerlings produced is lower compared to the demand. The current estimated demand is 40,000,000 fingerlings, yet the supply is still low; about 21,173,226 fingerlings per year (URT 2019). Also, the training and research institutions such as Tanzania Fisheries Research Institute (TAFIRI), Sokoine University of Agriculture (SUA), and Fisheries Education and Training Agency (FETA) serve as fingerling producers and distributers to fish farmers. These centres still face many challenges including the production of poor quality fingerlings due to

mixed species and lack of reliable hatcheries, poor government support, and lack of experts in feed formulation and breeding. Feed experts need to know the feed materials, developmental stage of the fish and the nature of the pond (fertilized or unfertilized) before the feed can be formulated. Parent stock and fingerlings have different nutrient requirement, for example fingerlings need higher protein (40-45%) than older fish (Hänninen 2014). Nevertheless, sustainable development of the aquaculture sector requires all potential players to be pro-active and collaborate. These include feed manufacturers, fish farms entrepreneurs, aquaculture experts, and government agencies (Rothuis et al. 2014). Many attempts to improve aquaculture production in Tanzania failed due to poor husbandry, low technology and insufficient long-term funding.

Despite the availability of reliable water from lakes, rivers and 30% of land valued potential for aquaculture (Shoko et al. 2011), Tanzanian aquaculture production is far from optimal. Therefore, a structured breeding program is required to increase food production without further negative impacts to the native germline. Tanzania being a hotspot of biodiversity of about 30 species of tilapia including *O. niloticus* (Di Palma 2017), the importance of having a sustainable aquaculture production as a solution for conserving this diversity cannot be underestimated .

Table 1: Number of hatcheries and production capacity per year 2018/2019 in Tanzania

Owner	Fish species	Hatchery name	Location (District, Region)	Capacity
Public	Tilapia	Kingolwira	Morogoro	1,200,000
		Ruhila	Songea, Ruvuma	840,000
		Mwamapuli	Igunga, Tabora	120,000
			Total	**2,160,000**
Private	Tilapia	Ruvu Fish Farm	Bagamoyo, Pwani	2,400,000
		Big Fish	Dar es salaam	2,400,000
		Eden Agri Aqua	Dar es Salaam	2,400,000
		Indian ocean	Kibiti, Pwani	1,440,000
		Jans Aqua	Dar es salaam	960,000
		JUDASA	Dar es Salaam	960,000
		Mpanju Farm	Ilemela, Mwanza	1,440,000
		Shazein	Arusha	1,440,000
		Rofacol	Kyela, Mbeya	1,440,000
			Total	**14,880,000**

(Source URT 2019).

Methodology

This review was based on literature, field visits and interviews. For the literature, we used published journal articles and reports, government documents such as reports and speech budget from the Ministry of Livestock and Fisheries Tanzania, a desk review, workshops and 'Google Scholar' with the search terms breeding programs, hatchery, tilapia, etc. For the field study, we visited seven hatcheries and 10 fish farms located in Kagera, Mwanza, Kilimanjaro, Mbeya, Dar es Salaam, Morogoro and Pwani regions. During the visits we asked about species cultured, sources of fingerlings, farm productivity, and techniques used to get farmed seeds and farm management practices. For the interview, we interviewed 10 fish farmers, seven hatchery owners, five scientists from training and research institutions and three policy makers from the Ministry of Livestock and Fisheries. Here we wanted to understand the role of the

government in fish farming, the knowledge gaps and the challenges encountered.

Why a Structured Tilapia Breeding Program in Tanzania?

Tanzania has a high diversity of tilapia species with great ability to interbreed leading to fertile hybrids (Shechonge et al. 2018b). Culturing mixed sex of these species has resulted into slow growth because of early maturity and overcrowding, leading the fish to spawn before reaching market size and harvest weight (Shoko et al. 2016). Some fish farmers depend on the available hatcheries in the country (Table 1) as source of fingerlings while most of them are still collecting fingerlings from the wild, which are of poor quality because they often collect mixed species. This is considered unsustainable for aquaculture development because poor quality fingerlings result into poor harvests. Furthermore, the similarity between different tilapia species at the fingerling stage increases the probability of mixed stocks in production ponds, which may not result into expected profitable production.

Male tilapias are the desired sex for culture because they grow faster (Ferdous et al. 2014), since they divert less energy to reproduction (Phelps and Popma 2000). Pressure from consumers has compelled some producers to practice hormonal sex reversal using 17-α methyl testosterone to produce all-male tilapias. However, hormonal sex reversal is expensive (Shoko et al. 2016) and this has been a challenge to the farmers as they cannot afford to purchase hormones. Moreover, using hormonal treatment requires experts and well established hatcheries to ensure right quantities are applied to fish and to avoid possible impacts on humans and the environment. Also, fish farmers are concerned about fish treated with hormones and consumers' preference (Dergal et al. 2016). Other techniques for controlling mixed sex tilapia have been applied in aquaculture production, such as: polyculture, manual sorting (Forgako 2018) and hybridization (Bartley et al. 2001, Beardmore et al. 2001). Experiments for producing all-male hybrid tilapia production have been done in Tanzania. Mapenzi and Mmochi (2016) reported that hybridization between *O. niloticus* and *O. urolepis hornorum* showed better growth results producing 100% all males. This finding gives the basis for a prospective sustainable tilapia breeding program in Tanzania.

Currently, most cultured species in Tanzania are a mixture of tilapia species and their hybrids (Shechonge et al. 2018b), rather than a pure single species. Therefore, it is difficult to obtain higher production returns from aquaculture because species in practice are unknown so their management is difficult and genetic improvement is impossible. For those reasons, there is a need for developing a sustainable and well-maintained breeding program for aquaculture improvement in the country. A selective breeding program for production of better performing tilapia in Tanzania is important for providing good quality fingerlings and brood stock, aquaculture enhancement, nutritional supply, food security, employment, poverty eradication, and adaptation to impacts of climate change. A breeding program is expected to improve aquaculture production in the whole country by 1) providing a clear understanding of loci affecting the trait of the cultured species using molecular techniques, 2) producing good quality seed from domesticated brood stock and not depending on wild collected seed and brood stock, and 3) good management and proper record keeping for brood stock.

Consideration for Introductions of Improved Tilapia Strains in Tanzania

Introduced species, exotic species, alien species, non-native species, and non-indigenous species have the same biological significance (Simberloff 2013). Introduced species mean any species carried and arrived with human assistance out of their natural environments on purpose or accidently

(Vicente and Fonseca-Al 2013). In Tanzania, this is an old practice since Nile tilapia was introduced to the Lake Victoria in the 1950s from Lake Edward for sport fishing and to enhance the declining fisheries (Njiru et al. 2005, Shechonge et al. 2018a). The Nile tilapia, *O. niloticus* is an African cichlid native to the Nile delta, coastal rivers of Israel, and the Niger, Benue, Volta, and Senegal rivers, Chad basin, as well as lakes Tanganyika, Albert, Edward, and Kivu (Trewavas 1983). In Tanzania, Nile Tilapia (*O. niloticus)* is native to Lake Tanganyika (Shechonge et al. 2018a). The introduced *O. niloticus* strain from Lake Victoria has been a species of choice for aquaculture across the country.

Introduced strains can escape in the natural environment and compete for space and food with native species and lead to the extinction and endangerment of native species population (Canonico et al. 2005). Genetically improved Tilapia strains like Genetically improved Farmed Tilapia (GIFT) have been selectively bred for disease resistance (Acosta and Eknath 1998), and are highly resistant to certain diseases. However, GIFT may still carry the diseases (Jansen et al. 2018) when moving from their breeding environment to another area during the dissemination process. Introduction of genetically improved Nile tilapia strains in Tanzania can result into the introduction of new alleles through hybridization. There is evidence of hybridization between native and introduced *Oreochromis* species in different catchments of Tanzania. Examples of such hybridization are found in Lake Victoria catchment where introduced species *O. niloticus* and *O. esculentus* hybridized with native *O. esculentus*, *O. variabilis* and *O. urolepis* (Turner et al. 2017)**.** The situation can result into decline of population size of indigenous species and decrease genetic diversity.

To meet the increased fish demand, fish farmers are considering the introductions of genetically improved tilapia strains as an alternative for providing good quality fingerlings. Private hatcheries owners such as Eden, Big Fish and Mbarali farms stated that wild collected brood stocks and seeds are obstacles to aquaculture development because of mixed tilapia species in the wild, hence preferred to use already improved strains from abroad. Already genetically improved strains such as GIFT from Worldfish Centre in Malaysia, Akosombo strain from Ghana, and Abbasa strain from Egypt have proven to perform better by growing faster than local African tilapia strains (Ansah et al. 2014). Medium to large-scale fish producers are introducing other strains of tilapia fingerlings in Tanzania from neighbouring countries. Surveyed fish farms in Tanzania confirmed importation of tilapia strains such Chitralada strain of *O. niloticus* from Asian Institute of Technology (AIT) in Thailand (Shechonge et al. 2018a), while others imported unknown strains of tilapia from Uganda and Nam Sai Farms in Thailand (Pers. Comm). One farm imported YY-Male Silver (wild type) and red strain of tilapia from Til-Aqua International in Netherlands (Pers. Comm). Whether these introductions are legal with all the required permits and certificates or illegal, they still pose a threat to native species since it is not certain if the introduced strains are pure *O. niloticus*, hybrids, genetically improved strains of Nile tilapia, or other tilapia species.

There is a debate whether a tilapia breeding program in Tanzania should use local species or improved strains. In 2011, Kenya initiated a selective breeding program for Nile tilapia at the National Aquaculture Research Development and Training Centre (NARDTC) in Sagana. The program started with a base population formed from locally available strains (Omasaki 2017). The Nile tilapia breeding program which aimed at improving growth and survival was successful and currently they are at the F7 generation (Nyonje et al. 2018). In Egypt and Ghana, Abbassa and Akosombo strains, respectively were improved from local strains through selective breeding programs (Worldfish Center 2012). Breeding

programs for Nile Tilapia in those countries have been successful and can be used as a model for Tanzania to establish a Nile tilapia breeding program.

Before opting for introductions of improved strains in Tanzania to increase the aquaculture production, conservation of indigenous species should be taken into consideration. The use of improved strains from other countries could be a threat to native tilapia populations in Tanzania and can result in reduction of alleles which have great importance for future selective breeding programs (Brummett 2013). At present, there is inadequate information whether pure or nearly pure populations of all native species still exist. Furthermore, it is not known if farmed tilapia species are exotic *O. niloticus*, native tilapia species, hybrids or genetically improved strains. An on-going study in Tanzania on tilapia ecology, genetic diversity and conservation has not yet provided enough information to allow or consider the introductions of new genetically improved Nile tilapia strains in the country.

It should be understood that moving genetically improved strains from their optimal environment for aquaculture to other places can result into negative effects on natural ecosystems and on the growth performance of the strain (Devlin et al. 2015). Environmental changes regulate genes. Differences in the environmental parameters such as photoperiod, temperature and production systems can influence the growth performance of fish and may create the situation known as genotype by environment interaction (G × E) (Bangera et al. 2015). G x E occurs as a result of differences in the responsiveness of individuals to the production environments (Mulder and Bijma 2005). G x E can be exhibited in two forms: re-ranking of individuals and heterogeneity of variances make phenotypic performance in one production environment to differ from other environments (Sae-Lim et al. 2013). Therefore, due to G x E interaction, the introduced genetically improved strains may not perform well in some environments. Luan et al. (2008) reported strong G × E interaction for harvest weight and survival in Nile tilapia GIFT strain cultured in the fresh and brackish water ponds. In the presence of G x E interaction, the breeding program should be optimised for the production environment. If there are different production environments, it may be economically infeasible to have lines specifically targeted for each environment. The best option then is to select the most robust fish that show the lowest G x E. Omasaki et al. (2016) suggested that breeding programs for Nile tilapia must include more sib information from production environments when culturing hormone mediated mono-sex fish for accurate estimation of breeding values.

Breeding Program

The concept

The science of applied selective breeding and genetics has contributed greatly to the gradually increase in productivity in animal and plants husbandry (Gjerde and Rye 2010). While in agriculture the high yields are almost entirely based on genetically improved breeds, in aquaculture supply is mainly based on wild population (Subasinghe et al. 2009). Genetic variability of fish held in African hatcheries is reported to be 40-70% less and growth rates 12-40% less than wild stocks (Morissens et al. 1996, Pouyaud and Agnèse 1996, Ambali et al. 1999). Therefore, increased aquaculture production is linked to the genetic quality of the brood stocks available to meet that increasing demand. There are several fish breeding programs that have been successful such as GIFT, Genetically Enhanced Tilapias for Excellence (GET-EXCEL), FAC Selected Tilapia (FaST), GenoMar Supreme Tilapia (GST), Abassa, and Akosombo strain for Nile tilapia (Ponzoni et al. 2008, Ansah et al. 2014), Atlantic salmon (Jonsson and Jonsson 2017); and rainbow trout (Janssen et al. 2015, Sae-Lim and Mulder 2016). However, these programs are yet to sustain the global demand of fish and fish products. Therefore, more breeding programs should be developed,

limitations and challenges such as financial resources and human capacity (Ponzoni et al. 2009) facing the successful ones should be explored.

At present, aquaculture is mainly small scale in most African countries and facing many challenges. Developing a tilapia strain like GIFT or one of several other similar lines that grow 40-60% faster than the typical farm populations can make the difference. Currently in Tanzanian fish markets, just like in many other African countries, there are large numbers of imported fish from Asia, mostly China (Olingo 2018, Okai 2019). These imported frozen tilapia outcompete the local tilapia due to their low prices.

In Tanzania, hatcheries are operating without a properly planned breeding program. Poorly managed hatcheries produce poor quality and "stunted" fingerlings as a result of high levels of inbreeding. Most of the hatcheries in Tanzania are privately owned while the government owned hatcheries are not performing well due inadequate infrastructures and limited financial resources from the government.

Before starting up a breeding program, the institutions developing a program in Tanzania should consider the target beneficiaries and the strain(s) they want to improve. A tilapia breeding program in Tanzania should aim at helping fish farmers to develop a fast growing strain with high resistance to environmental stressors and diseases.

The steps necessary to establish a breeding program

For any genetic improvement program, the following are some prerequisites:

Description of the production system

The production system should be defined, whether it is polyculture, intensive, semi-intensive or recirculating. The breeding program should be tailored to the farming systems being practiced. In Tanzania the production system is mainly semi-intensive mostly done in earthen ponds and currently there are no intensive systems in place.

Choice of the species or strain

Species or strains to be used in the breeding program should be known. The local strains can be used to form a base population for the breeding program, for example, *O. niloticus* strain has proved to grow up to 250-350 g in six months when they are not improved (Meiludie 2013). Once they are genetically improved, they can grow even faster. It is therefore recommended to use locally available strains and compare their performance with already improved strains like GIFT. The most important precaution is to use local strains available in the region. The local strains of tilapia or other improved strains with high genetic variations for the traits of interest can be used to form a breeding population.

Formulating the breeding objectives

It is important to know the objective of the breeding program since it defines the traits of interest to farmers. Surveyed fish farmers in Tanzania mentioned growth rate to be the most important trait because a faster growing fish will reach harvest weight earlier at lower feeding costs (Gjedrem 2005). Tanzanian fish farmers can choose to start with growth, which contribute to profit and is economically valuable. In the future, growth may be combined with other attributes that affect profit. Growth rate is more correlated with other traits, so that selecting for growth rate can lead to gains in other traits. In many species, growth rate is positively correlated to increase feed conversion efficiency (Ponzoni et al. 2008, Trọng et al. 2013). The other important traits are harvest weight, fecundity and survival rate. It is advised not to include more than six traits in the breeding goal in order to ensure sufficient genetic progress.

Selection criteria

Selection traits used need to be checked to ensure that they are in line with the breeding

goal. Not all traits in the breeding objective can be measured directly, but correlated traits can be measured instead. For instance, farmers prefer growth rate as an important trait to improve, but in practice we can select for body weight at a given age (at harvest). Furthermore, indicator traits can be selected instead of the traits in the breeding objective. For example length in fish can be used as indicator of weight. Traits used as selection criteria are associated with the traits in breeding objective through genetic co-variances and can be used in estimation of breeding values (Ponzoni et al. 2006).

Designation of genetic evaluation

Genetic evaluation system, depending on heritability of the breeding goal traits, can vary from least costly and most rapid response like mass selection and between family selection to more complex and more costly approaches such as within family selection, combined selection or genomic selection. Mass selection is based on individual phenotypic performance and can be for one or few traits. Within family selection require individual identification and is based on an individual's performance and its relationship with other relatives in the pedigree. Families can be reared in tanks or hapas where inbreeding can be easily controlled. Within family selection is very effective but needs more infrastructures. Combined selection is the best method; more efficient but more expensive (Farias et al. 2017). Selection method which is efficient, with reduced inbreeding and less costly could be applied in the proposed tilapia breeding program in Tanzania.

There can be family based breeding programs at a central location in Tanzania where fish are individually identified, measured, and selected. The breeding generations from this nucleus can be distributed to hatcheries where the genetic variation is maintained, and additional genetic progress can be obtained, using a cohort mating system. Such an approach means that the breeding nucleus can aim for a new breeding generation every few years but it not concerned with the supply of fingerlings to the industry. Likewise, the use of a cohort mating approach at the hatcheries means that their broodstock can act as a back-up for the breeding nucleus. This becomes highly relevant when the nucleus suffers a disease outbreak or other catastrophe that leads to loss of the fish in the nucleus. Also in a scenario where exotic lines are used a brood stock, local hatcheries could maintain a cohort mating system to reduce inbreeding and to reduce the need for import of new improved strains over time.

Breeding population and mating scheme

Starting a breeding program with a small population results into uncertain response to selection and potentially a high inbreeding level. With a large population response to selection is high and inbreeding is lower. A base population with large number of fish (more than 200 families) is better for a breeding program in the longer term. Logistics for operation of large number of families is difficult and takes a longer time. With limited infrastructures available in Tanzania, starting a breeding program with 50 to 100 families will be more manageable. The number of families to begin within a breeding program will determine the effective population size. Effective population size is calculated as;

$$\mathbf{Ne = 4(M \times F) \div (M + F)}$$

where Ne = effective population size; M = number of males contributing to the next generation, and F = number of females contributing to the next generation.

Number of parents contributing to the next generation should be ½ Ne. Inbreeding should be avoided as much as possible so large population size will allow adequate number of brood stock to be spawned and decrease a chance of mating relatives.

Mating design depends on the infrastructures available for the breeding program and whether the identification is based

on strain, family or individual level. Pair mating scheme is very simple and more common in aquaculture but difficult to manage as many families will be produced and there is a possibility of mating between family members. In a strain comparison experiment, it is of interest to assess the performance of individual lines. Depending on the resources available mating at the ratios of 1:1, 1:2 or 1:3 can be adopted in a proposed tilapia breeding program in Tanzania.

Cohort matings can be used to control inbreeding and improve the efficiency of selection. The population is divided into cohorts depending on the spawning age and size of the fish and selection can be done separately in each cohort (Tave 1999). Fish in each cohort are tagged or can be kept separately in ponds/hapas or tanks. Rotational mating between cohorts can be applied in cohort selection to avoid inbreeding in such a way that females from one cohort mate with the males from another cohort and females and males from one cohort group cannot mate. For year 1 it will be 1->2, 2->3, 3->4 and 4->1 and for the next generation in year 2 it will be 1->3, 2->4, 3->1 and 4->2 with 8 cohorts (Figure 3).

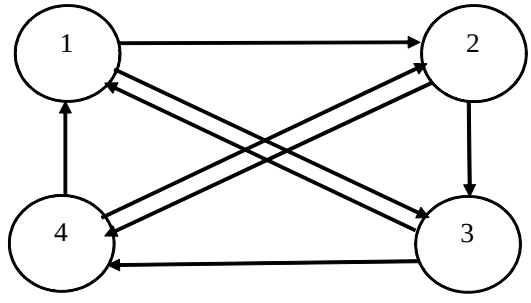

Figure 3: Cohort mating. Selected males from one group (cohort) are mated to females from another cohort. The next generation matings are made between different cohorts, thus avoiding inbreeding until all the unique combinations of cohorts have been exhausted.

Design system for production and dissemination

The improved strains or species should reach the targeted stakeholders who are fish farmers in Tanzania, hence involvement of farmers in the production system is very important (Eknath et al. 1991). Efforts need to be made to ensure fingerlings with genetic gain are disseminated to the farmers and managed in a way that utilizes their increased genetic potential. A well-organised production flow should be established from the breeding nucleus to reliable, bio-secure, hatcheries that are responsible for multiplication of improved fish strain and dissemination of fingerlings to the fish farmers (Figure 4). Bio-secure brood stock facilities with well-maintained and managed hatcheries must be established for the tilapia breeding program in Tanzania to avoid inbreeding (mating between closely related individuals).

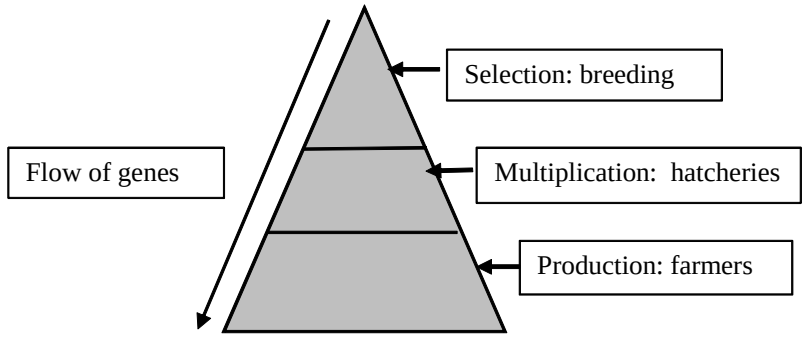

Figure 4: Flow of genes from the breeding to the farmers, modified from (Ponzoni 2008).

Economic and Funding Aspects of Fish Breeding Program

Fish breeding programs should be seen as investments for sustainable expansion of the aquaculture production and the potential to produce affordable food or other goods for the local community while maintaining their genetic diversity. In most developing countries, development of the breeding programs including livestock is initially made by the government in collaboration with other organizations to enable structure and investments be put in place and on time. Any breeding program must involve farmers at early stages to make sure their needs are taken into account and they provide the support needed for the breeding program to be successful (Philipsson et al. 2006). The size of investment in breeding program differs with species, location, availability of resources, the size of the breeding program and other factors. But all breeding programs require long-term investments with continuous support. This means that the support cannot be paused waiting for funds to be available. That is why it is very important for the governments or equivalent organizations to ensure that they have enough and continuous funds before they start aquaculture breeding programs. It must be noted that even in developed countries, many aquaculture breeding programs are supported by government funds either directly via national breeding programs (like those for rainbow trout and Arctic Char in Sweden) or indirectly via research and development grants to private companies, often in collaboration with research institutions.

Infrastructure for the Fish Breeding Program

Infrastructure is an important factor for the development of a fish breeding program. Lack of essential infrastructure is one of the most serious problems facing the development of indigenous breeds in tropical countries (Philipsson et al. 2006). Infrastructure includes a wide range of essential inputs that must be attained for the breeding program to succeed. Such infrastructure and inputs include skilled personnel or trained staff, facilities for breeding, hatching and rearing fish, method and means of recording, dissemination of improved genetic materials, handling and analysis of collected data and decision making bodies (Thien et al. 2001, Ponzoni et al. 2008, Ansah et al. 2014). The lack of an adequate number of people with appropriate training or incentives or institutions to successfully run a breeding program is another potential problem facing the development of indigenous breed in developing countries (Thien et al. 2001, Ojango et al. 2008). Development of a genetically improved fish strain requires highly skilled personnel with different expertise depending on the duties the person will be assigned. Duties may include the development of breeding strategies, designing system for genetic evaluation, reproduction methods, data

recording and processing, genetic analysis and estimation of breeding values, monitoring genetic progress, feed analysis and feeding, monitoring of other technical and operational framework including general daily fish management (Ponzoni et al. 2008). For example, to ensure development and success of GIFT projects, WorldFish Center established the International Network on Genetics in Aquaculture (INGA) in 1993 to train scientists in quantitative genetics applied to aquaculture and coordinate national breeding programs in the 13 member countries (Bangladesh, China, Cote d'Ivoire, Egypt, Fiji, Ghana, India, Indonesia, Malaysia, Malawi, Philippines, Thailand, and Vietnam) using the GIFT methodology to genetically improve their indigenous cultured species (Ansah et al. 2014). This has been proven by the success of breeding program developed in those countries such as Abbassa strain in Egypt. Tanzania can follow this example by using the available scientists and researchers or train other scientists in the sectors lacking expertise. Tanzania needs fish genetics experts to expand its knowledge base on fish genetics and breeding and meet aquaculture growing demand in the country. Currently, under the on-going SIDA sponsored project at the University of Dar es Salaam aiming at establishing a breeding program, four PhD students are involved in the project studying genetic purity and diversity of farmed Nile Tilapia strains from Tanzania for future tilapia breeding program. PhD students and supervisors under the project visited World Fish Malaysia for one week training on the GIFT breeding program. The training improved their knowledge on steps needed to establish a breeding program and the required infrastructures. More training and workshops with experts in fish genetics are needed in Tanzania to fill the gaps in quantitative genetics, genomics and selective breeding. In collaboration with Worldfish, the government of Tanzania through the Ministry of Livestock and Fisheries (MLF) are working to enhance aquaculture production in the country. The MLF and Worldfish can consider establishing the suggested breeding program as a means for improving aquaculture production Tanzania.

Government Policy, Legislation and Plan

The breeding program should be an important part of the National Fisheries Policy aiming at improving the food and income of a country, region or locality and of fish farmers (Philipsson et al. 2006). This should consider environmental, water and land use policies. Most successful top producers have strong policies, strategies and implementation plans. They have water and land rights, aquaculture mainstreamed into national development plan such as Poverty Reduction Strategic Plans and National Development Strategies (Thorpe et al. 2005). Tanzania can learn from successful breeding programs especially those from the developing countries such Abbassa in Egypt (Dickson et al. 2016) and take the opportunity to scale up aquaculture sector in Tanzania.

Conclusion and Recommendations

Sustainable tilapia breeding program and a well-managed hatchery in Tanzania are important for maintaining the purity of tilapia strains, ensure active dissemination of good quality fish seed, and guarantee permanent genetic gain in farmed fish. Establishing a structured tilapia breeding program in Tanzania to increase aquaculture production needs a number of facilities and materials. Therefore, the government should integrate breeding activities with existing farm infrastructures as much as possible. The institution managing a breeding nucleus can produce, multiply and distribute fish seed to the farmers or combined effort of private and government owned hatcheries both can be involved in multiplication and dissemination of fish seed to the farmers.

Clear policies governing the introductions and proper infrastructure need to be in place to avoid escapees to natural environment. Efforts should be made to reduce the import of exotic

species, genetically improved strains and other species in Tanzania. Much emphasis should be placed on improving native and locally available species and establishing a sustainable breeding program. Starting a tilapia breeding program with locally available tilapia species can minimize genetic and ecological effects brought by the introductions of exotic strains from other countries thereby protecting indigenous species diversity. Long-term genetic breeding programs should be developed to ensure better performing breeds to reduce the pressure on wild stocks while improving livelihood of fish farmers.

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References

Acosta B, Eknath A 1998 Manual on genetic improvement of farmed tilapia (GIFT) research methodologies.

Allison EH 2011 Aquaculture, fisheries, poverty and food security. *The WorldFish Center Working Papers*, Penang, Malaysia. 2011: 60-65.

Ambali AJD, Doyle RW and Cook DI 1999 Genetic changes in *Oreochromis shiranus* (Trewavas) associated with the early stages of national aquaculture development in Malawi. *Aquacult. Res.* 30(8) : 579–588.

Ansah YB, Frimpong EA and Hallerman EM 2014 Genetically-improved tilapia strains in Africa: Potential benefits and negative impacts. *Sustainability* 6(6) : 3697-3721.

Bangera R, Drangsholt TM, Nielsen HM, Sae-Lim P, Ødegård J, Puvanendran V, Hansen ØJ and Mortensen A 2015 Genotype by environment interaction for growth in Atlantic Cod (*Gadus morhua L.*) in four farms of Norway. *J. Mar. Sci. Eng.* 3(2): 412-427.

Bartley MD, Rana K and Immink JA 2001 The use of inter-specific hybrids in aquaculture and fisheries. *Rev. Fish Biol. Fisher.* 10(3): 325-337.

Beardmore JA, Mair GC and Lewis RI 2001 Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects. *Aquaculture* 197: 283-301.

Brummett RE 2013 Conservation Economic Growth and the use of Genetically Improved Tilapia in Africa. ISTA10 , *Special Issue* , Washington DC.

Canonico GC, Arthington A, Mccrary JK and Thieme ML 2005 The effects of introduced tilapias on native biodiversity. *Aquat. Conserv.* 15(5): 463–483.

Costa-Pierce BA 2003 Rapid evolution of an established feral tilapia (*Oreochromis* spp.): The need to incorporate invasion science into regulatory structures. *Biol. Invasions* 5(1-2): 71-84.

Darko FA, Quagrainie KK and Chenyambuga S 2016 Consumer preferences for farmed tilapia in Tanzania: A choice experiment analysis. *J. Appl. Aquacult*. 28(3): 131-143.

Dergal NB, Scippo ML, Degand G, Gennotte V, Mélard C and El-Amine AASM 2016 Monitoring of 17α-methyltestosterone residues in tilapia's (*Oreochromis niloticus*) flesh and experimental water after its sex reversal. *Int. J. Biosci.* 9(6): 101-113.

Devlin RH, Sundström LF and Leggatt RA 2015 Assessing ecological and evolutionary consequences of growth-accelerated genetically engineered fishes. *BioScience* 65(7): 685-700.

Di Palma F 2017 *Finding ways to double tilapia production in Tanzania without doing damage*, *THE CONVERSATION* . [Cited 29 January 2018.] Available from URL: http://theconversation.com/finding-ways-to-double-tilapia-production-in-tanzania-without-doing-damage-72099

Dickson M, Nasr-Allah AM, Kenawy D, Fathi

M, El-Naggar G and Ibrahim N 2016 *Improving employment and income through development of Egypt's Aquaculture sector (IEIDEAS) Project*.

Eknath AE, Bentsen HB, Gjerde BJ, Tayamen MM, Abella TA, Gjedrem TR and Pullin RS 1991 Approaches to national fish breeding programs: pointers from a tilapia pilot study. *Naga, the ICLARM Quarterly* 14(2)**:** 10-12.

FAO 2018 *The State of World Fisheries and Aquaculture 2018* - Meeting the sustainable development goals. Rome. www.fao.org/3/i9540en/I9540EN.pdf

Farias T, César J and Silva L 2017 Methods of selection using the quantitative genetics in aquaculture-a short review. *Insights Aquacult. Biotech.* 1: 1-8.

Ferdous Z , Nahar N, Hossen MS, Sumi KR and Ali MM 2014 Performance of different feeding frequency on growth indices and survival of monosex tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae) fry. *Int. J. Fish. Aquat. Stud*. 1(5): 80-83.

Forgako E 2018 Techniques of controlling mixed sex tilapia and the practicability of hormonal reversal in Cameroon. Nations University Fisheries Training Program, Iceland [final project]. http://www.unuftp.is/static/fellows/document/elizabeth16prf.pdf

Gjedrem T and Thodesen J 2005 Selection. In: Gjedrem T (Ed) *Selection and Breeding Programs in Aquaculture*. Springer, the Netherlands, pp. 255.

Gjerde B and Rye M 2010 Design of breeding programs in aquaculture species: Possibilities and constraints. *Aquac. Res.* 192: 181–192.

Hänninen A 2014 *Production and utilization of tilapia feed in Rural Zambia.* Bachelor's thesis, Turku University of Applied Sciences, Finland.

Jansen MD, Dong HT and Mohan CV 2018 Tilapia lake virus: a threat to the global tilapia industry? *Rev. Aquacult.* 11(3): 725-739.

Janssen K, Chavanne H, Berentsen P and Komen H 2015 Rainbow trout (*Oncorhynchus mykiss*)–Current status of selective breeding in Europe. *Aquaculture Europe*, pp.1-12.

Jonsson B and Jonsson N 2017 Maternal inheritance influences homing and growth of hybrid offspring between wild and farmed Atlantic salmon. *Aquac. Environ. Interact.* 9: 231-238.

Lovshin LL 2000 Criteria for selecting Nile tilapia and red tilapia for culture. *Tilapia Aquaculture in the 21st Century. American Tilapia Association and Departamento de Pesca e Aqüicultura*. Rio de Janeiro, Brazil.

Luan TD, Olesen I, Ødegård J, Kolstad K and Dan NC 2008 Genotype by environment interaction for harvest body weight and survival of Nile tilapia (*Oreochromis niloticus*) in brackish and fresh water ponds. *Proceedings from the Eighth International Symposium on Tilapia Aquaculture* 1: 231-240.

Mapenzi L and Mmochi AJ 2016 Role of Salinity on growth performance of *Oreochromis niloticus*♀ and *Oreochromis urolepis urolepis*♂ Hybrids. *J. Aquac. Res. Dev.* 7(6): 431

Meiludie EE 2013 *Comperative evaluation of growth performance and yield of four tilapia species under culture conditions*. PhD dissertation. Sokoine University of Agriculture, Tanzania.

Morissens P, Rognon X and Dembele I 1996 Comparison of growth performance and electrophoretic characteristics of three strains of *Oreochromis niloticus* present in Cote d'Ivoire. *In The Third International Symposium on Tilapia in Aquaculture* 41: 361-367.

Msuya FE, Muumin H and Hamed S 2016 Status of aquaculture in the Zanzibar Islands, Tanzania. *World Aquac.* 47: 25-29.

Mulder HA and Bijma P 2005 Effects of genotype x environment interaction on genetic gain in breeding programs. *J. Anim. Sci.* 83(1): 49-61.

Njiru M, Waithaka E, Muchiri M, Van Knaap M and Cowx IG 2005 Exotic introductions to the fishery of Lake Victoria: What are the management options? *Lake Reserv. Manage.* 10(3): 147-155.

Nkhoma J and Musuka CG 2014 Effect of commercial feed on sexual maturity of two tilapia species (*Oroechromis niloticus* and *Oreochromis tanganicae*). *Int. J. Aquac*. 4: 96-101.

Nyonje BM, Opiyo MA, Orina PS, Abwao J, Wainaina M and Charo-Karisa H 2018 Current status of freshwater fish hatcheries, broodstock management and fingerling production in the Kenya aquaculture sector. *Livest Res Rural Dev. 30, Article #6.* Retrieved 16/9/2018, from http://www.lrrd.org/lrrd30/1/mary30006.html

Ojango JM, Panandam JM, Bhuiyan AK, Khan MS, Kahi AK, Imbayarwo-Chikosi VE Halimani TE, Kosgey IS and Okeyo AM 2008 Higher Education In Animal Breeding In Developing Countries–Challenges and Opportunities. In *The 9th World Congress on Genetics Applied to Livestock Production*.

Okai KE 2019 *Africa's tilapia farmers rise to Chinese challenge.* Cited 21/7/2019 from https://thefishsite.com/articles/africas-tilapia-farmers-rise-to-chinese-challenge.

Olingo A 2018 *Fish imported into East Africa from China fails toxic minerals test.* Cited 21/7/2019 from URL: https://www.thecitizen.co.tz/news/Fish-imported-into-East-Africa-from-China-fails-toxic-/1840340-4899334-biyn7o/index.html

Omasaki SK 2017 *Optimization of breeding schemes for Nile tilapia (Oreochromis niloticus) in smallholder production systems in Kenya*. Doctoral dissertation, Wageningen University.

Omasaki SK, Charo-Karisa H, Kahi AK and Komen H 2016 Genotype by environment interaction for harvest weight, growth rate and shape between monosex and mixed sex Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 458: 75-81.

Phelps RP and Popma TJ 2000 Sex reversal of tilapia. In: Costa-Pierce BA and Rakocy JE (eds.) *Tilapia aquaculture in the Americas*. 2. The World Aquaculture Society, Baton Rouge, pp. 34-59.

Philipsson J, Rege JEO and Okeyo AM 2006 Sustainable breeding for tropical farming systems. In: Ojango JM, Malmfors B and Okeyo AM (Eds.), Animal Genetics Training Resource (version 2). International Livestock Research Institute, Nairobi, Kenya and Swedish University of Agricultural Sciences, Uppsala, Sweden, 1: 25.

Ponzoni RW, Nguyen NH and Khaw HL 2009 Genetic improvement programs for aquaculture species in developing countries: prospects and challenges. In *Proc. Assoc. Advmt. Anim. Breed. Genet* 18: 342-349.

Ponzoni RW, Nguyen NH, Khaw HL, Kamaruzzman N, Hmazah A, Bakar KR and Yee H 2008 Genetic Improvement of Nile Tilapia (*Oreochromis Niloticus*)–Present and Future. *8th International Symposium on Tilapia in Aquaculture*, pp. 33-52.

Ponzoni RW 2006. Genetic improvement and effective dissemination: keys to prosperous and sustainable aquaculture industries. In: Ponzoni RW, Acosta BO and Ponniah AG (Eds.) *Development of aquatic animal genetic improvement and dissemination programs*. WorldFish Center, Penang, Malaysia, pp. 1-6.

Pouyaud L and Agnèse J 1996 Genetic differentiation in several stocks of Sarotherodon melanotheron and Tilapia guineensis from Côte d'Ivoire, Senegal and Gambia. In *The Third International Symposium on Tilapia in Aquaculture* 41: 368.

Rothuis AJ, Turenhout MN, van Duijn A, Roem AJ, Rurangwa E, Katunzi EF, Shoko A and Kabagambe JB 2014 Aquaculture in

East Africa; A regional approach. *Wageningen UR (University and Research centre)*. LEI Report IMARES C153/14| LEI 14-120, pp. 54.

Rukanda JJ 2018 *Evaluation of aquaculture development in Tanzania.* Nations University Fisheries Training Program, Iceland [final project]. http://www.unuftp.is/static/fellows/document/janeth16aprf.pdf

Sae-Lim P and Mulder HA 2016 Selective breeding in aquaculture for future environments under climate change. *Internal project View project OrAqua View project*. In *Proceedings FAO International Symposium on the Role of Agricultural Biotechnologies in Sustainable Food Systems and Nutrition*, Rome.

Sae-Lim P, Kause A, Mulder HA, Martin KE, Barfoot AJ, Parsons JE, Davidson J, Rexroad Iii CE, Van Arendonk JAM and Komen H 2013 Genotype-by-environment interaction of growth traits in rainbow trout (*Oncorhynchus mykiss*): A continental scale study. *J. Anim. Sci.* 91(12): 5572-5581.

Shechonge A, Ngatunga BP, Bradbeer SJ, Day JJ, Freer JJ, Ford AG, Kihedu J, Richmond T, Mzighani S, Smith AM and Sweke EA 2018a Correction to: Widespread colonisation of Tanzanian catchments by introduced Oreochromis tilapia fishes: the legacy from decades of deliberate introduction. *Hydrobiologia* 832(1): 235-253.

Shechonge A, Ngatunga BP, Tamatamah R, Bradbeer SJ, Harrington J, Ford AG, Turner GF and Genner MJ 2018b Losing cichlid fish biodiversity: genetic and morphological homogenization of tilapia following colonization by introduced species. *Conservation Genetics* 19(5): 1199-1209.

Shoko AP, Lamtane HA, Wetengere K, Kajitanus OO, Msuya FE, Mmochi AJ and Mgaya YD 2011 The status and development of aquaculture in Tanzania, east Africa. *Proceedings of the international conference on ecosystem conservation and sustainable development*, Ambo University, Ambo, Ethiopia, pp. 85-97.

Shoko AP, Limbu SM, Mrosso HD, Mkenda AF and Mgaya YD 2016 Effect of stocking density on growth, production and economic benefits of mixed sex Nile tilapia (*Oreochromis niloticus*) and African sharptooth catfish (*Clarias gariepinus*) in polyculture and monoculture. *Aquac. Res.* 47(1): 6-50.

Simberloff D 2013 *Invasive species: what everyone needs to know*. Oxford University Press.

Subasinghe R, Soto D and Jia J 2009 Global aquaculture and its role in sustainable development. *Rev. Aquac.* 1: 2-9.

Tave D 1999 *Inbreeding and brood stock management* (No. 392). Food & Agriculture Organization.

Thien TM, Dan NC and Tuan PA 2001 Review of Fish Genetics Genetics and Breeding Research in Vietnam. *Review Literature and Arts of the Americas*, pp. 91-96.

Thorpe A, Reid C, van Anrooy R and Brugere C 2005 When fisheries influence national policy-making: an analysis of the national development strategies of major fish-producing nations in the developing world. *Marine Policy* 29(3): 211-222.

Trewavas E 1983 *Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia* (No. 878). London: British Museum (Natural History).

Trọng TQ, Mulder HA, van Arendonk JA, Komen H 2013 Heritability and genotype by environment interaction estimates for harvest weight, growth rate, and shape of Nile tilapia (*Oreochromis niloticus*) grown in river cage and VAC in Vietnam. *Aquaculture* 384: 119-127.

Turner G F, Ford A, Genner M J, Ngatunga B P, Shechonge A, Palma, F Di 2017 Edinburgh , Scotland. Genomic approaches to identification and preservation of wild tilapia genetic resources for Aquaculture.

Eur. Aquacult. Soc. - *Meeting Abs.* 3: 2016-2018.

United Republic of Tanzania (URT) 2016 The Tanzania Fisheries Sector: *Challenges and Opportunities, Ministry of Agriculture, Livestock and Fisheries*. Prepared with the support of the Royal Danish Embassy, Dar es Salaam, with technical assistance from MegapescaLda, Portugal, pp. 32.

United Republic of Tanzania (URT) 2017 *Tanzania Ministry of Livestock and Fisheries. Annual Fisheries Statistics Report, 2017*. Dodoma: Government of Tanzania.

United Republic of Tanzania (URT) 2018 *Tanzania Ministry of Livestock and Fisheries. Budget Speech 2018-2019.* Dodoma: Government of Tanzania, July 2018.

United Republic of Tanzania (URT) 2019 *Tanzania Ministry of Livestock and Fisheries. Budget Speech 2019-2020.* Dodoma: Government of Tanzania. [May 2019]

Vicente I and Fonseca-Al CE 2013 Impact of introduced Nile tilapia (*Oreochromis niloticus*) on Non-native Aquatic Ecosystems. *Pak. J. Biol. Sci.* 16: 121-126.

Worldfish Center 2012 *Improved breeding of Nile tilapia leads to productivity gains | WorldFish Press Release, Worldfish*. Cited 23/6/2018 from URL: https://www.worldfishcenter.org/content/improved-breeding-nile-tilapia-leads-productivity-gains.

II

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ORIGINAL RESEARCH

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Assessing the genetic diversity of farmed and wild Rufiji tilapia (*Oreochromis urolepis urolepis*) populations using ddRAD sequencing

Christer S. Nyinondi[1,2] | **Matern S. P. Mtolera**[2] | **Aviti J. Mmochi**[2] | **Fernando A. Lopes Pinto**[1] | **Ross D. Houston**[3] | **Dirk J. de Koning**[1] | **Christos Palaiokostas**[1,3]

[1]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

[2]Institute of Marine Sciences, University of Dar es Salaam, Zanzibar, Tanzania

[3]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK

Correspondence
Christos Palaiokostas, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden.
Email: christos.palaiokostas@slu.se

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Abstract

Rufiji tilapia (*Oreochromis urolepis urolepis*) is an endemic cichlid in Tanzania. In addition to its importance for biodiversity conservation, Rufiji tilapia is also attractive for farming due to its high growth rate, salinity tolerance, and the production of all-male hybrids when crossed with Nile tilapia (*Oreochromis niloticus*). The aim of the current study was to assess the genetic diversity and population structure of both wild and farmed Rufiji tilapia populations in order to inform conservation and aquaculture practices. Double-digest restriction-site-associated DNA (ddRAD) libraries were constructed from 195 animals originating from eight wild (Nyamisati, Utete, Mansi, Mindu, Wami, Ruaha, Kibasira, and Kilola) and two farmed (Bwawani and Chemchem) populations. The identified single nucleotide polymorphisms (SNPs; $n = 2{,}182$) were used to investigate the genetic variation within and among the studied populations. Genetic distance estimates (F_{st}) were low among populations from neighboring locations, with the exception of Utete and Chemchem populations ($F_{st} = 0.34$). Isolation-by-distance (IBD) analysis among the wild populations did not detect any significant correlation signal ($r = .05$; p-value $= .4$) between the genetic distance and the sampling (Euclidean distance) locations. Population structure and putative ancestry were further investigated using both Bayesian (Structure) and multivariate approaches (discriminant analysis of principal components). Both analysis indicated the existence of three distinct genetic clusters. Two cross-validation scenarios were conducted in order to test the efficiency of the SNP dataset for discriminating between farmed and wild animals or predicting the population of origin. Approximately 95% of the test dataset was correctly classified in the first scenario, while in the case of predicting for the population of origin 68% of the test dataset was correctly classified. Overall, our results provide novel insights regarding the population structure of Rufiji tilapia and a new database of informative SNP markers for both conservation management and aquaculture activities.

KEYWORDS
ddRAD-seq, genetic diversity, Rufiji tilapia

1 | INTRODUCTION

Tilapias (Cichlidae family) comprise a diverse group of over 70 species mostly encountered in tropical and subtropical regions (McAndrew & Majumdar, 1983; Trewavas, 1983). Native in a diverse range of habitats across Africa, they are particularly important in the biodiversity of freshwater ecosystems. Moreover, tilapias are of paramount value for the aquaculture industry, being cultured in over 120 countries with a global production volume exceeding 5 million tonnes (FAO, 2018). Overall, tilapia aquaculture production is dominated by Nile tilapia (*Oreochromis niloticus*) farming which has been introduced in a wide range of habitats worldwide. Nevertheless, the impact to the local fauna is in many cases poorly understood (Lima, Oliveira, Giacomini, & Lima-Junior, 2018) even though concerns have been raised (Canonico, Arthington, Mccrary, & Thieme, 2005). Furthermore, prior experience from several aquatic species suggests that introduced species can negatively affect biodiversity (Lovell, Stone, & Fernandez, 2006).

Tanzania is a hot spot for tilapias, with current knowledge suggesting that 10 Oreochromis species are endemic only to the country (Genner, Turner, & Ngatunga, 2018). In an attempt to boost the productivity of local fisheries and aquaculture farms, *Oreochromis* species like the Nile tilapia (endemic only to Lake Tanganyika) have been introduced to non-native habitats across the country often in an unregulated manner (Kajungiro, Mapenzi, et al., 2019). Recent studies posed concerns regarding the negative impact toward the local fish fauna due to the introduction of Nile tilapia to non-native habitats (Gu et al., 2017; Padial et al., 2017; Rico-Sánchez et al., 2020).

Furthermore, interspecific hybridization is common among *Oreochromis* species (Scribner, Page, & Bartron, 2000) with fertile hybrids occurring either spontaneously in the wild or due to aquaculture practices that aim to improve desirable traits in farmed stocks like growth and salinity tolerance (Kamal & Mair, 2005). Therefore, hybridization between introduced and native tilapia species can severely impact the unique genetic diversity of the latter affecting their adaptation capacity toward changing environmental conditions (Deines, Wittmann, Deines, & Lodge, 2016). Even though the exact consequences of introduced tilapia species in Tanzania to the local fauna are unknown, habitat loss and significant decline of population size have been recently documented for the endemic *Oreochromis hunter* in Lake Chala, in Kilimanjaro Tanzania due to introduced tilapia species (Moser, van Rijssel, Ngatunga, Mwaiko, & Seehausen, 2019).

Rufiji tilapia (*O. urolepis urolepis*) is an endemic species in Tanzania, distributed mainly across the south-eastern rivers, reservoirs, and oxbow lakes of Rufiji river basin (Ulotu, Mmochi, & Lamtane, 2016). Interestingly, according to Genner et al. (2018) the Wami, Zanzibar, and Rufiji tilapia all refer to the same species. Over the years, non-endemic species like the Nile tilapia and the blue-spotted tilapia (*Oreochromis leucostictus*) have been introduced in Rufiji tilapia habitats (Shechonge et al., 2019). Recently, a genetic diversity study based on microsatellites provided evidence of extensive hybridization between the native Wami tilapia (*Oreochromis urolepis hornorum*; as mentioned earlier, recent evidence suggests to be the same species with Rufiji tilapia) and the introduced tilapia species raising concerns regarding the impact of introgression into the native populations (Shechonge et al., 2018).

Apart from being a species of high ecological value for Tanzanian aquatic habitats, Rufiji tilapia is economically important for both local fisheries and aquaculture activities. Rufiji tilapia is an attractive species for farming due to its high growth capacity, its inherent high salinity tolerance that could assist toward the expansion of the coastal aquaculture production in the country (Kajungiro, Mapenzi, et al., 2019), and the production of all-male hybrids when crossed with female Nile tilapia (Mapenzi & Mmochi, 2016). Therefore, promoting Rufiji tilapia farming could result in the reduction of introduced non-native tilapia species for aquaculture purposes mitigating biodiversity related concerns.

Reduced-representation genotyping approaches constitute a powerful tool for conducting in-depth population genetics studies for any species of interest. Following the introduction of restriction-site-associated DNA sequencing (Baird et al., 2008), a wide range of related methodologies utilizing restriction enzymes have been introduced like genotyping by sequencing (Elshire et al., 2011), ddRAD-seq (Peterson, Weber, Kay, Fisher, & Hoekstra, 2012), 2b-RAD (Wang, Meyer, McKay, & Matz, 2012), ezRAD (Toonen et al., 2013), quaddRAD (Franchini, Monné Parera, Kautt, & Meyer, 2017), and 2RAD/3RAD (Bayona-Vásquez et al., 2019). The aforementioned platforms have been used extensively in studies on aquatic organisms focusing both in population genetic aspects (Andrews, Good, Miller, Luikart, & Hohenlohe, 2016) and in studying traits of interest for farming purposes (Houston et al., 2020; You, Shan, & Shi, 2020). ddRAD-seq is one of the most commonly utilized member of the reduced-representation family combining simplicity and cost efficiency during library construction (Peterson et al., 2012). Over the last years, ddRAD-seq has been successfully utilized in a plethora of tilapia focussed studies investigating the underlying genetic structure of traits of economic value (Jiang et al., 2019; Li, Zhu, Gu, Lin, & Xia, 2019; Li et al., 2017; Palaiokostas et al., 2015; Taslima et al., 2020), for species-specific SNPs (Syaifudin et al., 2019) and for deciphering the genetic diversity–population structure of wild and farmed populations (Kajungiro, Palaiokostas, et al., 2019; Moses et al., 2019).

The objective of the current study was to assess the genetic variation among 10 Rufiji tilapia populations of both wild (eight populations) and farmed (two populations) origin using ddRAD-seq. Single nucleotide polymorphisms (SNPs) were identified across 195 animals and were subsequently used to estimate standard genetic diversity

metrics both within and among populations, investigate for the existence of putative genetic clusters, test for the existence of isolation by distance, and assess the efficiency of predicting population of origin-based only on the genomic profile using cross-validation schemes. The aforementioned will facilitate both the conservation management of wild Rufiji tilapia populations and future breeding plans for aquaculture purposes where a broad genetic diversity is required for forming a base population.

2 | MATERIALS AND METHODS

2.1 | Sample collection and processing

Fish used in this study were collected from both wild and farmed environments in Tanzania mainland (Table 1). Sampling was performed using fishing nets (30 mm) with captured fish from 30 g and above being selected and conditioned for 24 hr at the sampling site or a nearby area before transportation. The sampled locations were selected based on prior available information regarding the *O. urolepis urolepis* distribution in Tanzania. In total, 10 different geographic locations were selected namely Nyamisati, Bwawani, Utete, Chemchem, Mansi, Mindu, Wami, Ruaha, Kibasira, and Kilola (Figure 1). The samples from Bwawani and Chemchem populations originated from fish farms located along the Rufiji River. In the case of the farmed population from Chemchem, available records suggest that the animals were in captivity for three consecutive generations, while in the case of Bwawani the sampled fish originated from the first generation in captivity. Species identification was performed using morphological criteria (Trewavas, 1983). In particular, coloration, size of jaws, and head shape were used to identify the Rufiji tilapia. More specifically, females and immature males had a light gray head, dark-brown body with dark patches along the lateral line. On the other hand, mature males had a gray head, reddish-pink fin margins and brownish-golden upper parts. Besides, mature males had enlarged jaws and a concave-shaped head. Regarding the population from the Wami river where *O.u. hornorum* is also endemic, identification of Rufiji tilapia was conducted based on skin pigmentation. In particular, males of *O.u.urolepis* are dark olive gray with pinkish upper lips and red fin margins, while *O.u. hornorum* males are entirely black with pale or black lips. In addition, *O.u. urolepis* females are silvery gray with a narrow pink edge on the dorsal fin, while the respective *O.u. hornorum* females have no pink edges. A total of 195 fish samples were collected and transported to the Institute of Marine Sciences Mariculture Centre (IMS-MC) in Pangani, Tanzania.

TABLE 1 Origin of Rufiji tilapia (*Oreochromis urolepis urolepis*) populations

Population	Origin	*N*	Latitude	Longitude
Mindu	Wild	20	−7.434444	38.01722
Wami	Wild	20	−6.652222	37.60139
Bwawani	Farmed	20	−8.3175	39.46667
Kibasira	Wild	20	−8.535556	36.51694
Chemchem	Farmed	20	−8.651389	39.26889
Kilola	Wild	15	−8.318056	37.1675
Mansi	Wild	20	−7.4525	39.13528
Nyamisati	Wild	20	−8.301944	39.45056
Ruaha	Wild	20	−8.085556	37.60139
Utete	Wild	20	−8.633889	39.26889

2.2 | DNA extraction and quantification

Fin clips of about 0.05 g were collected and preserved in 95% ethanol and stored at −20°C. Genomic DNA was extracted using QIAsymphony DSP DNA Mini Kit (Qiagen) and eluted into 100 µl of AE (EDTA) buffer (Qiagen) according to the manufacturer's tissue protocol and procedures. Quantification of DNA samples was done using a Qubit fluorimeter (Thermos Fisher Scientific, USA). Samples were diluted with TE buffer to 20 ng/µL followed by gel electrophoresis (1% agarose gel) to assess DNA quality.

2.3 | ddRAD library preparation and sequencing

Two ddRAD libraries comprised of 96 and 99 individuals, respectively, were prepared according to Peterson et al. (2012), with minor modifications described in Palaiokostas et al. (2015). Briefly, each sample (20 ng/µl DNA) was digested at 37°C for 60 min with SbfI (recognizing the CCTGCA|GG motif) and SphI (recognizing the GCATG|C motif) high fidelity restriction enzymes (New England Biolabs; NEB), using 6 U of each enzyme per microgram of genomic DNA in 1 × Reaction Buffer 4 (NEB). Reactions (6 µl final volume) were then heat inactivated at 65°C for 20 min. Individual-specific combinations of P1 and P2 adapters, each with a unique 5 or 7 bp barcode, were ligated to the digested DNA at 22°C for 120 min by adding 1 µl SbfI compatible P1 adapter (25 nM), 0.7 µl SphI compatible P2 adapter (100 nM), 0.06 µl 100 mmol/L rATP (Promega, UK), 0.95 µl 1 × Reaction Buffer 2 (NEB), 0.05 µl T4 ligase (NEB, 2 × 106 U/mL) with reaction volumes made up to 12 µl with nuclease-free water for each sample. Following heat inactivation at 65°C for 20 min, the ligation reactions were slowly cooled to room temperature (over 1 hr), combined in a single pool (for one sequencing lane) and purified. Size selection (300–600 bp) was performed by agarose gel separation followed by gel purification and PCR amplification. A total of 100 µl of the amplified libraries (13–14 PCR cycles) were purified using an equal volume of AMPure beads. The libraries were eluted into 20 µl EB buffer (MinElute Gel Purification Kit, Qiagen). The libraries were sequenced at Edinburgh Genomics Facility, University of Edinburgh on an Illumina HiSeq 4000 instrument.

2.4 | Sequence data analysis and SNP genotyping

Reads of low quality ($Q < 20$) and missing the expected restriction sites were discarded. The retained reads were aligned to the

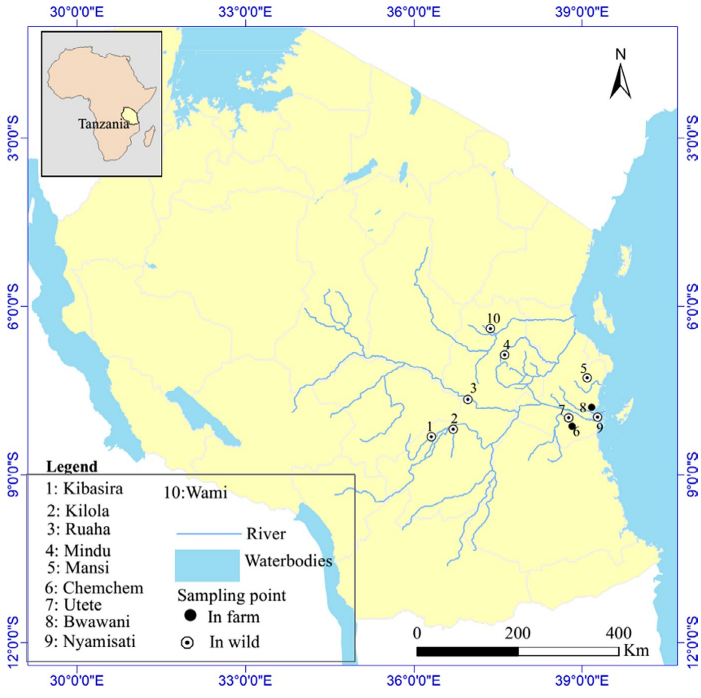


FIGURE 1 Sampling locations in Tanzania

Nile tilapia reference genome assembly [GenBank accession number GCA_001858045.1 (Conte, Gammerdinger, Bartie, Penman, & Kocher, 2017)] using bowtie2 (Langmead & Salzberg, 2012). Stacks v2.5 (Rochette, Rivera-Colón, & Catchen, 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). In the case where a single ddRAD locus had multiple SNPs, only the first encountered SNP was used for downstream analysis (--write-single-snp). SNPs with minor allele frequency (MAF) < 0.05 and maximum heterozygosity > 0.7 across the tested samples were discarded. Moreover, the genotypes obtained for each individual were interrogated for the number of reads supporting each allele. Genotypes supported by fewer than 20 reads or where the coverage of one of the alleles was more than three times higher than the other allele were substituted as missing. Finally, only SNPs found in at least 75% of the samples in each population were retained for downstream analysis.

2.5 | Genetic diversity within and among populations

General genetic variation metrics like mean observed (H_o) and expected (H_e) heterozygosity, nucleotide diversity (π), average individual inbreeding coefficients (F_{is}), and the corresponding standard errors (*SE*) were estimated using the Stacks software v2.5 (Rochette et al., 2019). Pairwise F_{st} values among all tested populations and their confidence intervals (using 1,000 bootstraps) were estimated using the R package StAMPP (Pembleton, Cogan, & Forster, 2013).

2.6 | Isolation-by-distance (IBD) analysis

The R package adegenet v2.1.1 (Jombart, Devillard, & Balloux, 2010) was used to evaluate the presence and magnitude of putative isolation by distance across the studied populations of wild origin (Table 1). The magnitude of the computed correlation between the estimated genetic distances (Edwards, 1971) among populations and their respective geographic locations (Euclidean distance) was assessed using the *mantel.randtest* function. Statistical significance was inferred through comparing the estimated correlations of the distance matrices through 100,000 random permutations under a scenario where spatial structuring is absent.

2.7 | Genetic clusters and ancestry

Principal component analysis (PCA) was conducted using the R package adegenet v2.1.1 for visualization purposes and for gaining insights regarding the existence of genetic clusters. The existence of putative genetic clusters was further investigated using the discriminant analysis of principal components (DAPC) (Jombart et al., 2010)

TABLE 2 Estimates of genetic diversity

Population	H_e (SE)	π (SE)	H_o (SE)	F_{is} (SE)
Mindu	0.22 ± 0.003	0.23 ± 0.003	0.21 ± 0.003	0.04 ± 0.04
Wami	0.36 ± 0.004	0.37 ± 0.004	0.18 ± 0.003	0.42 ± 0.03
Bwawani	0.10 ± 0.003	0.10 ± 0.004	0.10 ± 0.004	0.01 ± 0.03
Kibasira	0.10 ± 0.003	0.10 ± 0.004	0.10 ± 0.004	0.01 ± 0.04
Chemchem	0.11 ± 0.003	0.11 ± 0.004	0.11 ± 0.004	0.01 ± 0.02
Kilola	0.11 ± 0.003	0.11 ± 0.004	0.10 ± 0.004	0.05 ± 0.01
Mansi	0.18 ± 0.003	0.18 ± 0.003	0.19 ± 0.003	−0.03 ± 0.05
Nyamisati	0.11 ± 0.004	0.11 ± 0.004	0.11 ± 0.004	0.02 ± 0.02
Ruaha	0.11 ± 0.003	0.11 ± 0.004	0.11 ± 0.004	0.02 ± 0.02
Utete	0.15 ± 0.003	0.15 ± 0.004	0.15 ± 0.004	0.01 ± 0.02

Note: H_e refers to expected heterozygosity; H_o refers to observed heterozygosity; π refers to nucleotide diversity; and F_{is} refers to inbreeding coefficient.

with the same R package. More specifically, PCA was initially applied, followed by a cross-validation step using the *xvalDapc* function to select the optimal number of principal components (PCs). Thereafter, a discriminant analysis step was conducted using predetermined clusters from the PCs. The selection of the optimal number of clusters (*K*) was based on the elbow method (Jombart et al., 2010) in regard to the Bayesian information criterion (BIC) values for each tested value of *K*. Moreover, putative population admixture was investigated with Structure v.2.3.4 (Hubisz, Falush, Stephens, & Pritchard, 2009; Pritchard, Stephens, & Donnelly, 2000) using *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. Evidence for the optimal number of clusters was based on the obtained posterior probability values (Pritchard et al., 2000). In addition, for deciding regarding the optimal number of genetic clusters, we used the Structure Harvester (Earl & vonHoldt, 2012) and CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015) software.

2.8 | Prediction of population origin based on the genomic profile

Cross-validation schemes (fourfold) were performed using the R package adegenet v2.1.1 (Jombart et al., 2010) in order to test the utility of the SNP dataset for discriminating between (a) fish of farmed or wild origin and (b) fish originating from different geographic locations. Specifically, in the first cross-validation scheme, the origin of 25% animals from wild and farmed origin was masked and treated as a test set, while the rest of the dataset was used for model training purposes. Predictions regarding the population of origin on the aforementioned test set were performed using information obtained through DAPC (*predict.dapc*) on the remaining training data set. The same procedure was followed for the second cross-validation scheme where the origin of 25% of animals from each geographic population was masked and used as a test set. The entire procedure for both cross-validation schemes was repeated ten times in order to minimize potential bias due to the stochasticity of sample allocation in the training/test datasets.

3 | RESULTS

3.1 | SNP identification using ddRAD sequencing

More than 320 million paired-end reads were obtained. The sequenced reads were aligned to the Nile tilapia reference genome (GenBank accession GCA_001858045.2; Conte et al., 2017). Between 94% and 97% of the reads across the tested animals were aligned to the reference genome with approximately 16 million paired-end reads being removed as unmapped. Additionally, approximately 71 million paired-end reads were removed due to insufficient mapping quality (Phred-scale mapping quality < 40). In total, 28,712 putative ddRAD loci were identified, out of which 4,719 contained one or more SNPs. The mean sequence coverage of the identified loci was approximately 105× (*SD*, 44×). Overall, 2,182 SNPs passed all quality control steps and were retained for downstream analysis. Finally, all 195 samples had fewer than 25% missing genotypes and were utilized for the subsequent analysis.

3.2 | Genetic diversity within and among populations—isolation by distance

The expected heterozygosity (H_e) and nucleotide diversity (π) estimates were largely indistinguishable with values for both parameters ranging from 0.10 to 0.37 (Table 2). Highest values were observed in the samples from Mindu ($H_e = 0.22$; $\pi = 0.23$) and Wami populations ($H_e = 0.36$; $\pi = 0.37$). On the other hand, the lowest values were observed in samples from Bwawani and Kibasira ($H_e = 0.10$; $\pi = 0.10$). Observed heterozygosity (H_o) estimates ranged between 0.10 and 0.21 with the lowest values observed in samples from Bwawani and Kibasira and highest in samples from Mindu population. Moreover,

regarding the inbreeding coefficient (F_{is}), positive estimates were obtained for nine of the tested populations. After taking into account the corresponding standard error (*SE*) two populations showed suggestive evidence of putative loss of heterozygosity. The most striking difference was obtained in the Wami population (F_{is} = 0.42). An opposite trend was observed for the Mansi population (F_{is} = −0.03), suggesting a slight excess of heterozygotes. However, the corresponding *SE* was the highest among all tested populations (*SE* = 0.05).

The estimated genetic distances according to the F_{st} metric varied widely between 0.001 and 0.75 among the tested populations (Figure 2; Table S1). The highest genetic distance was observed between Mindu and the populations from Bwawani and Kibasira (F_{st} = 0.75). On the other hand, the lowest genetic distance was observed between the Kibasira and Kilola populations (F_{st} = 0.001).

The conducted isolation-by-distance analysis did not detect a statistically significant spatial pattern between the estimated genetic distances and the corresponding geographic locations on the studied wild populations. The correlation among the above was 0.05 with the corresponding p-value after 100,000 permutations being 0.39 (Figure 3).

3.3 | Population structure—admixture

Individual relationships within and between populations were visualized using PCA. The first and second principal components accounted for 58% and 6% of the observed variation, respectively. Overall, PCA indicated the existence of 3 groups among the sampled populations (Figure 4). Cross-validation suggested that the optimal number of principal components for clustering was 40. Thereafter, DAPC further deciphered the putative genetic structure suggesting *K* = 3 to be the most probable number of genetic clusters (Figure 5; Figure S1). The first genetic cluster included Mindu and Wami populations, while the second cluster was comprised of the Kibasira, Kilola, Mansi, Bwawani, Ruaha, Nyamisati, and Chemchem. Finally, the last suggested cluster included the Utete population, one individual from Kilola and five individuals from Wami populations.

Ancestry analysis provided further evidence regarding the existence of genetic clusters and potential admixture among the tested populations also indicating that *K* = 3 is the most probable number of clusters (Figure 6). Indication for admixture was observed between the Wami and Utete populations. Furthermore, admixture was suggested for the Mindu population and the genetic cluster comprised of Kibasira, Kilola, Mansi, Bwawani, Ruaha, Nyamisati, and Chemchem.

3.4 | Origin prediction using the genomic profile

The utility of the SNP dataset to predict farmed versus wild and population of origin was tested using DAPC. In the fourfold cross-validation, the mean successful assignment rate regarding farmed or wild origin on the test dataset was approximately 95% (Figure 7a). Regarding predictions for the population of origin, the overall successful classification was approximately 68% (Figure 7b). Classification success varied widely among populations with 100% for the Wami and only 10% for the Kilola population (Figure 7b).

4 | DISCUSSION

In the current study, we obtained an in-depth insight regarding the genetic variation within and among Rufiji tilapia populations in Tanzania using ddRAD-seq. It is worth mentioning that in both the Mindu and the Ruaha reservoirs, the Rufiji tilapia is the only indigenous *Oreochromis* species (Eccles, 1992). While IUCN Red List of Threatened Species assessments exist for populations of several Oreochromis species in Tanzania, limited information is available regarding the status of Rufiji tilapia populations (Shechonge et al., 2019). Information regarding the genetic diversity and structure of either farmed or wild populations can assist toward their most suitable management and increase the efficiency of conservation activities.

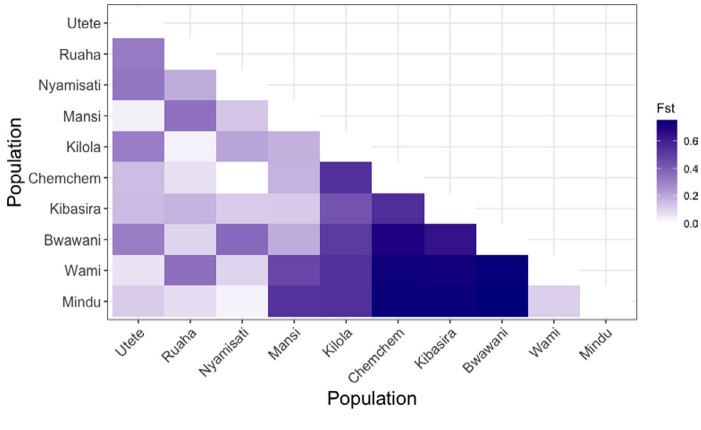


FIGURE 2 Genetic diversity among populations based on estimated F_{st} values. The Bwawani and Chemchem populations originated from fish farms located along the Rufiji River

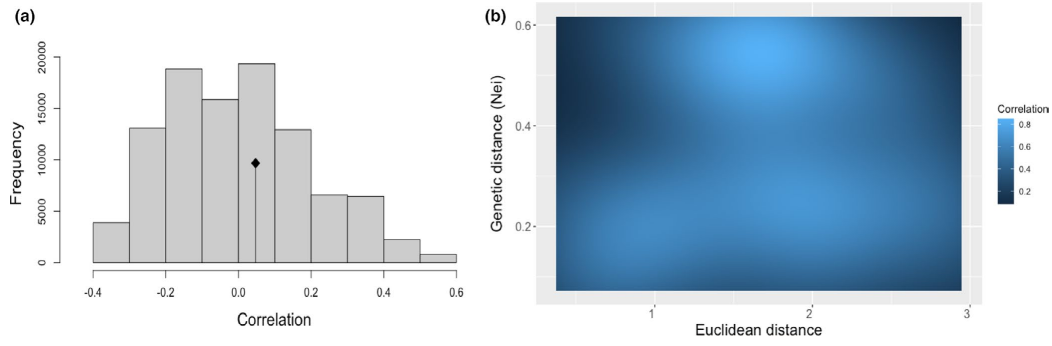
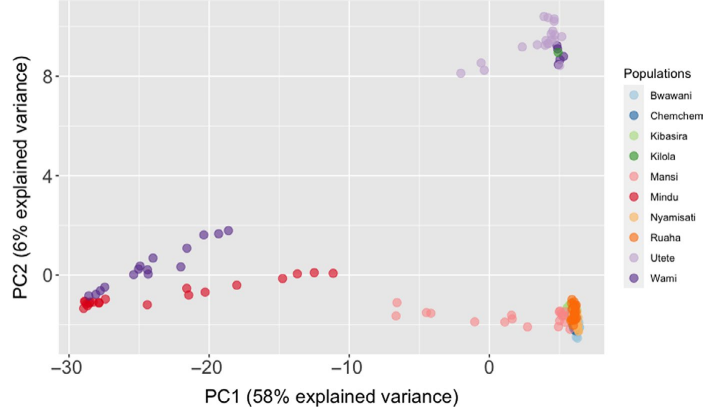


FIGURE 3 Isolation-by-distance analysis. (a) The original correlation among the distance matrices is represented by the dot. The histogram depicts the permuted correlation values under the absence of spatial structure. (b) Heatmap depicting the estimated correlation between the genetic and the Euclidean distance

FIGURE 4 Principal component analysis (PCA) of Rufiji tilapia populations



Reduced-representation sequencing platforms like ddRAD-seq are powerful tools for the aforementioned and have been widely applied in population genetic studies (McKinney, Larson, Seeb, & Seeb, 2017). The RAD-seq family allows for high-resolution studies of genetic diversity and relatedness at both population and individual levels (Lemopoulos et al., 2019; Palaiokostas et al., 2020). Moreover, reduced-representation sequencing platforms do not suffer from ascertainment bias opposed to other genotyping platforms where a priori identified genetic markers are utilized.

It has to be pointed out that the identified SNPs used in our study were detected after aligning the sequenced reads in the Nile tilapia reference genome (GenBank accession GCA_001858045.2) which could entail a certain level of bias during SNP detection. However, the fact that more than 94% of the sequenced reads were aligned to the reference genome indicates that the subsequent SNP detection is robust. Furthermore, even though our approach would not have been able to identify Rufiji tilapia specific loci, the high percentage of aligned reads indicates that the latter would have been most likely a very small percentage with limited effect on the downstream analysis. It would worth also to stress the fact that Nile and Rufiji tilapias can produce fully fertile hybrids (Ulotu et al., 2016) when crossed together therefore indicating the similarity among the two species.

4.1 | Genetic diversity within and among populations

According to the estimated genetic diversity metrics, the studied populations varied widely both in terms of H_e (0.10–0.36), π (0.10–0.37) and H_o (0.10–0.21). Compared to previous population genetics studies on aquatic organisms using RAD-family genotyping protocols (Drinan et al., 2018; Lemopoulos et al., 2019; Sherman et al., 2020; Vendramin et al., 2016), the obtained genetic diversity metrics for several of the populations in our study lie on the lower range of the reported values (Table 2). Nevertheless, in comparison to our previous studies on farmed Nile tilapia populations in Tanzania (Kajungiro, Palaiokostas, et al., 2019; Moses et al., 2019)

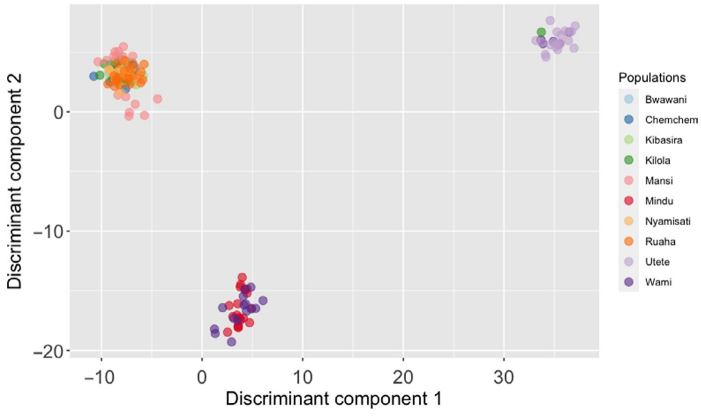


FIGURE 5 Discriminant analysis of principal components (DAPC) for Rufiji tilapia populations

where the same ddRAD library preparation protocol was used, the obtained genetic diversity metrics were in general higher in the current study. Additionally, it is worth to point out that low levels of heterozygosity were obtained for several tilapia populations in an extensive study across West Africa (Lind et al., 2019). Interestingly, the farmed populations of our study (Bwawani; Chemchem) ranked among the lowest in terms of heterozygosity values. However, four of the wild populations (Kibasira; Kilola; Nyamisati; and Ruaha) had indistinguishable genetic diversity estimates compared to the farmed ones suggesting that no clear inference could be drawn regarding a potential loss of genetic diversity due to farming practices. On the other hand, the inbreeding coefficient (F_{is}) indicated a potential loss of heterozygosity only for the wild population from Wami. In general, high F_{is} values indicate the existence of nonrandom mating or population subdivision (Allendorf & Luikart, 2007). Interestingly, concerns regarding the conservation of the unique genetic pool of endemic tilapias in the Wami water basin due to the introduction of nonendemic species have been documented recently (Shechonge et al., 2018). As documented also on previous occasions, introduced *Oreochromis* species can have a detrimental impact on endemic fish fauna (Angienda et al., 2011; Ndiwa, Nyingi, & Agnese, 2014) which could be the case for the Wami population of Rufiji tilapia.

The SNP dataset provided indications regarding the genetic distance among the tested Rufiji tilapia populations. Populations sampled from neighboring locations were in general of low genetic distance (Figure 1; Figure 2) with most obvious the case of Kibasira and Kilola ($F_{st} = 0.001$). However, several exceptions were observed with the most striking exception being the one between Chemchem and Utete populations where a moderate-to-high genetic distance ($F_{st} = 0.34$) was estimated. In general, F_{st} values below 0.05 indicate minimal genetic differentiation, while values above 0.15 indicate the existence of substantial genetic differentiation (Wright, 1978). A followed up isolation-by-distance analysis conducted on the wild populations did not detect evidence for existing spatial structure patterns among the sampled populations. To the best of our knowledge, no prior study investigated for putative spatial structure patterns of *Oreochromis* species in Tanzania. However, prior studies reported the existence of significant spatial genetic structure among *Oreochromis* populations across Africa (Bezault et al., 2011; Lind et al., 2019). The suspected uncontrolled movement of tilapia stocks among different locations in Tanzania (Kajungiro, Mapenzi, et al., 2019) could be a possible explanation for the observed absence of any statistically significant spatial structure among the studied populations. Nevertheless, it would be of primary importance to further

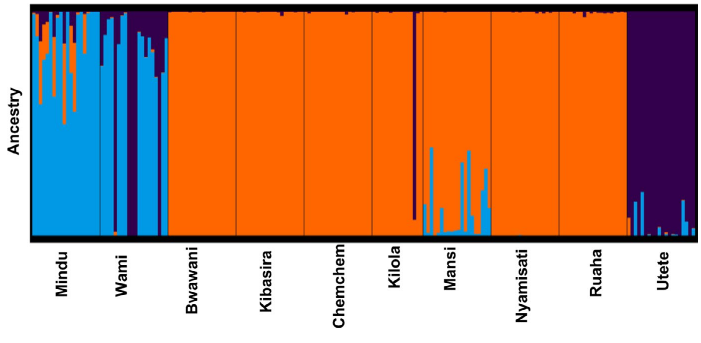


FIGURE 6 Ancestry analysis assigned individuals in clusters ($K = 3$). Each single vertical bar represents an individual and each color represents the probability that the individual is assigned to the respective gene pool. The Bwawani and Chemchem populations originated from fish farms located along the Rufiji River

verify the putative lack of spatial genetic structure we observed in future studies with larger number of samples per population.

4.2 | Genetic structure of the tested populations

Bayesian and multivariate approaches were used in the current study in order to decipher the genetic structure and putative admixture among the tested populations. Both approaches supported the hypothesis of three unique genetic clusters among the populations under study (Figures 4, 5). To the best of our knowledge, no prior study investigated the existence of genetic structure among Rufiji tilapia. Therefore, the above information could guide the management of the wild resources and inform breeding initiatives for aquaculture purposes. Regarding the latter, in order to maximize the genetic diversity for a founding breeding population (Gjedrem, Robinson, & Rye, 2012) obtaining broodfish originating from all three genetic clusters could be a valid strategy. More specifically, the majority of samples from seven tested populations including the farmed ones (Kibasira, Kilola, Mansi, Bwawani, Ruaha, Nyamisati, and Chemchem) formed a unique genetic cluster, while the Wami and Mindu populations formed a separate genetic cluster (substantially differentiated according to obtained F_{st} values). As previously mentioned, Rufiji tilapia is the only endemic *Oreochromis* species in the Mindu reservoir (Shechonge et al., 2018); therefore, appropriate conservation management appears as a necessity on the aforementioned genetic cluster. Interestingly, the Utete population formed an isolated cluster that included one animal from Kilola and five animals from Wami

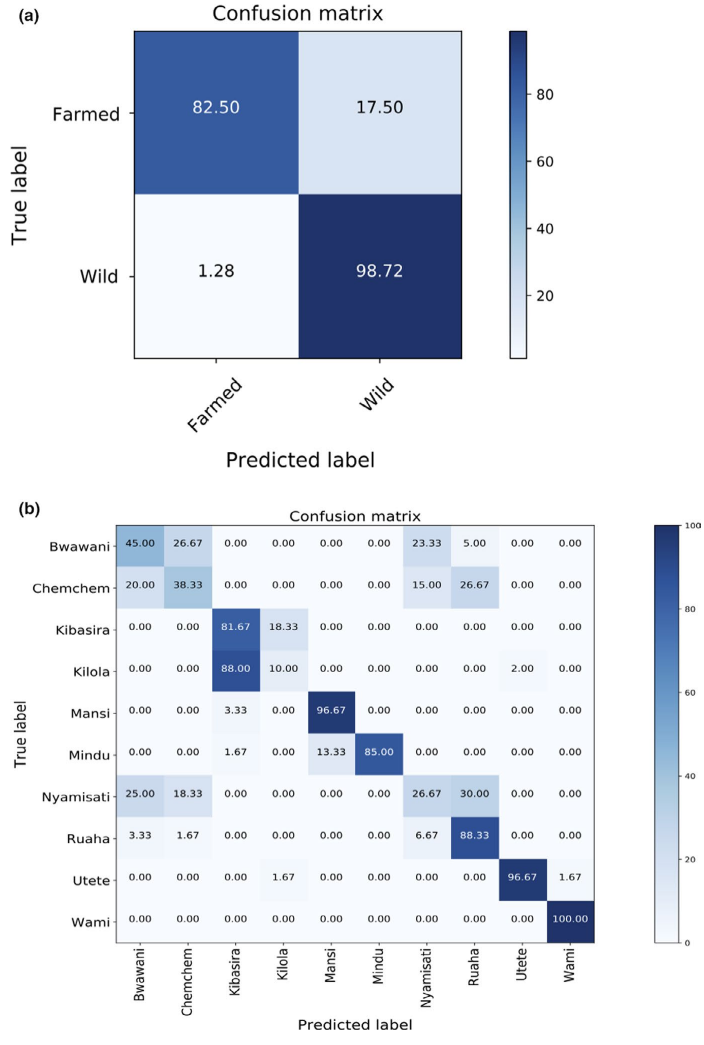


FIGURE 7 Confusion matrix for prediction efficiency (% of successful classification) of the SNP dataset using cross-validation. (a) Fourfold cross-validation to discriminate between farmed and wild origin. The origin of 25% randomly selected animals of wild and farmed origin was masked and used as a test set. Each population was considered of unknown origin. (b) Fourfold cross-validation to predict population of origin. The origin of 25% randomly selected animals from each population was masked and used as a test set. The entire procedure in both (a) and (b) was repeated 10 times in order to minimize potential bias due to the initial sample allocation in the training/ test dataset. The diagonal contains the mean % percentage of correct population assignments for the overall cross-validation scheme. Off-diagonals contain the mean % percentage of wrong population allocations for each particular case

populations. Moreover, ancestry analysis indicated the existence of admixture among the above populations. Nevertheless, taking into account the relatively small number of animals genotyped per population (n = 15–20) the possibility of sample mislabeling cannot be excluded especially in the case of the single animal from Kilola population that appeared genetically distant from its putative population of origin.

4.3 | Prediction of population origin using SNP derived information

Overall, our SNP dataset proved highly efficient in discriminating between farmed and wild populations with approximately 95% of "putative" unknown samples being classified correctly. Considerable evidence suggests that hatchery rearing in various fish species can negatively affect key phenotypic traits associated with adaptation in the wild (Fraser, 2008). It is likely that the above could be even more evident in tilapias due to their relatively small generation interval (6 months or less to be reproductive mature under optimal environmental conditions). Furthermore, considering the fact that Tanzania is a hot spot for wild cichlid populations, it is evident that introgression with farmed strains could jeopardize the local adaptivity of the wild populations (Shechonge et al., 2018). It is worth mentioning that a recent study detected introgression between introduced *Oreochromis* species in Tanzania oriented for aquaculture practices and the critically endangered *Oreochromis jipe* (Bradbeer et al., 2019). Nevertheless, we need to acknowledge the fact that only two farmed populations were used in our study which limits our ability to draw definite conclusions.

The efficiency of the SNP dataset dropped remarkably (68% successful classification) in the scenario of predicting for population of origin. The drop in the accuracy of successful classification appears to be in line with the obtained genetic distance of the respective populations. The above was more pronounced in the case of Kilola and Kibasira where the proportion of correctly classified fish dropped to only 10% indicating that the two populations were highly similar (also supported from their estimated genetic distance and population structure). Moreover, a similar pattern was observed in the case of the farmed populations (Bwawani and Chemchem) and the respective wild populations of most likely putative origin (Nyamisati and Ruaha) suggested by our data. Aiming to acquire deeper insights and confirm that the reduction of successful classification for predicting population of origin was due to the low genetic distance between some of the studied populations, we tested our dataset in a theoretical scenario aiming to predict for genetic cluster. In particular, since our analysis suggested the existence of three distinct genetic clusters, we followed the same cross-validation scheme as before for forming training and validation sets on each putative genetic cluster (fourfold cross-validation). The above allowed us to obtain close to 100% successful classification on the test dataset.

Moreover, a similar approach was followed in our prior studies on Nile tilapia strains (mainly of farmed origin) where the SNP information allowed for correctly classifying between 77% and 97% of the tested dataset to the respective population of origin (Kajungiro, Palaiokostas, et al., 2019; Moses et al., 2019). However, in the aforementioned studies we used mainly farmed populations of more pronounced genetic distance as opposed to the Rufiji populations of the current study which facilitated their discrimination in the followed cross-validation schemes. Therefore in this particular instance, the SNP dataset was less efficient on predicting for population of origin largely due to the fact that some of the tested populations proved to be less divergent than the aforementioned Nile tilapia populations. Nevertheless, we need to acknowledge the fact that a low-density genotyping approach was followed in our study which could limit our ability to discriminate between populations of low genetic distance. Therefore, high-density genotyping approaches through the application of either more frequent cutting restriction enzymes or the recently developed open access tilapia SNP array (Peñaloza et al., 2020) could be of value for predicting with higher accuracy the population of origin even among closely related samples.

5 | CONCLUSIONS

The current study is the first attempt of investigating the genetic diversity status of Rufiji tilapia populations using high-throughput sequencing-based platforms. Overall, the ddRAD-seq derived SNP dataset was applied in a wide range of analysis deciphering the underlying genetic diversity and structure among the studied populations. The identified genetic structure would be of value both for conservation purposes and for future aquaculture breeding practices aiming to establish base populations with the highest amount of genetic diversity. Finally, taking into consideration the desirable traits of Rufiji tilapia for farming purposes studies of common garden experiments between Rufiji and introduced Nile tilapia would be valuable for informing future breeding plans targeting the productivity increase of Tanzanian aquaculture.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Christer S. Nyinondi: Formal analysis (equal); investigation (equal); methodology (equal); software (equal); writing – original draft (equal). **Matern S. P. Mtolera:** Conceptualization (equal); funding

acquisition (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Aviti J. Mmochi:** Conceptualization (equal); methodology (equal); writing – review and editing (equal). **Fernando A. Lopes Pinto:** Investigation (equal); writing – review and editing (equal). **Ross Houston:** Conceptualization (equal); funding acquisition (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Dirk J. de Koning:** Conceptualization (equal); methodology (equal); project administration (equal); writing – review and editing (equal). **Christos Palaiokostas:** Conceptualization (equal); methodology (equal); supervision (equal); writing – review and editing (equal).

ETHICAL APPROVAL

The current study was carried out in accordance with the law on the protection of animals against cruelty (Act No. 12/1974. of the United Republic of Tanzania) upon its approval by the Department of Zoology and Wildlife Conservation, University of Dar es salaam. All the permits required to sample wild animals in Tanzania adhered to the Research clearance from Tanzania Commission for Science and Technology (COSTECH).

DATA AVAILABILITY STATEMENT

The aligned reads in the format of bam files were deposited in the National Centre for Biotechnology Information (NCBI) repository under project ID PRJNA518067. Supplementary information regarding all pairwise F_{st} values with their corresponding confidence intervals and the BIC values for each test number of clusters (*K*) were deposited in Dryad (https://doi.org/10.5061/dryad.x0k6djhgq).

ORCID

Christos Palaiokostas https://orcid.org/0000-0002-4480-4612

REFERENCES

Allendorf, F. W., & Luikart, G. (2007). *Conservation and the genetics of populations*. Oxford, UK: Blackwell. https://doi.org/10.1515/MAMM.2007.038

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, *17*, 81–92. https://doi.org/10.1038/nrg.2015.28

Angienda, P. O., Lee, H. J., Elmer, K. R., Abila, R., Waindi, E. N., & Meyer, A. (2011). Genetic structure and gene flow in an endangered native tilapia fish (*Oreochromis esculentus*) compared to invasive Nile tilapia (*Oreochromis niloticus*) in Yala swamp, East Africa. *Conservation Genetics*, *12*, 243–255. https://doi.org/10.1007/s10592-010-0136-2

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., … Johnson, E. A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One*, *3*, e3376. https://doi.org/10.1371/journal.pone.0003376

Bayona-Vásquez, N. J., Glenn, T. C., Kieran, T. J., Pierson, T. W., Hoffberg, S. L., Scott, P. A., … Faircloth, B. C. (2019). Adapterama III: Quadruple-indexed, double/triple-enzyme RADseq libraries (2RAD/3RAD). *PeerJ*, *7*, e7724. https://doi.org/10.7717/peerj.7724

Bezault, E., Balaresque, P., Toguyeni, A., Fermon, Y., Araki, H., Baroiller, J.-F., & Rognon, X. (2011). Spatial and temporal variation in population genetic structure of wild Nile tilapia (*Oreochromis niloticus*) across Africa. *BMC Genetics*, *12*, 102. https://doi.org/10.1186/1471-2156-12-102

Bradbeer, S. J., Harrington, J., Watson, H., Warraich, A., Shechonge, A., Smith, A., … Genner, M. J. (2019). Limited hybridization between introduced and Critically Endangered indigenous tilapia fishes in northern Tanzania. *Hydrobiologia*, *832*, 257–268. https://doi.org/10.1007/s10750-018-3572-5

Canonico, G. C., Arthington, A., Mccrary, J. K., & Thieme, M. L. (2005). The effects of introduced tilapias on native biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*, 463–483. https://doi.org/10.1002/aqc.699

Conte, M. A., Gammerdinger, W. J., Bartie, K. L., Penman, D. J., & Kocher, T. D. (2017). A high quality assembly of the Nile Tilapia (*Oreochromis niloticus*) genome reveals the structure of two sex determination regions. *BMC Genomics*, *18*(341), 1–19. https://doi.org/10.1186/s12864-017-3723-5

Deines, A. M., Wittmann, M. E., Deines, J. M., & Lodge, D. M. (2016). Tradeoffs among ecosystem services associated with global tilapia introductions. *Reviews in Fisheries Science & Aquaculture*, *24*, 178–191. https://doi.org/10.1080/23308249.2015.1115466

Drinan, D. P., Gruenthal, K. M., Canino, M. F., Lowry, D., Fisher, M. C., & Hauser, L. (2018). Population assignment and local adaptation along an isolation-by-distance gradient in Pacific cod (*Gadus macrocephalus*). *Evolutionary Applications*, *11*, 1448–1464. https://doi.org/10.1111/eva.12639

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361. https://doi.org/10.1007/s12686-011-9548-7

Eccles, D. H. (1992). *Field guide to the Freshwater fishes of Tanzania*, (1–145). Rome: FAO. FAO species identification guide for fishery purposes. https://doi.org/10.1038/303137a0

Edwards, A. W. F. (1971). Distances between populations on the basis of gene frequencies. *Biometrics*, *27*, 873. https://doi.org/10.2307/2528824

Elshire, R. J., Glaubitz, J. C., Sun, Q. I., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A Robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, *6*, e19379. https://doi.org/10.1371/journal.pone.0019379

FAO. (2018). *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals*. Rome.

Franchini, P., Monné Parera, D., Kautt, A. F., & Meyer, A. (2017). quad-ddRAD: A new high-multiplexing and PCR duplicate removal ddRAD protocol produces novel evolutionary insights in a nonradiating cichlid lineage. *Molecular Ecology*, *26*, 2783–2795. https://doi.org/10.1111/mec.14077

Fraser, D. J. (2008). How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications*, *1*, 535–586. https://doi.org/10.1111/j.1752-4571.2008.00036.x

Genner, M. J., Turner, G. F., & Ngatunga, B. P. (2018). *A guide to the tilapia fishes of Tanzania*. https://martingenner.weebly.com/uploads/1/6/2/5/16250078/tanzania_tilapia_guide_edition1_2018.pdf. Accessed April, 2020.

Gjedrem, T., Robinson, N., & Rye, M. (2012). The importance of selective breeding in aquaculture to meet future demands for animal protein: A review. *Aquaculture*, *350–353*, 117–129. https://doi.org/10.1016/j.aquaculture.2012.04.008

Gu, D., Hu, Y., Wei, H., Zhu, Y., Mu, X., Luo, D., … Yang, Y. (2017). Nile Tilapia *Oreochromis niloticus* (L.). In F. Wang, M. Jiang, & A. Zhan (Eds.), *Biological invasions and its management in China*. (pp. 77–89). Singapore City, Singapore: Springer Singapore. https://doi.org/10.1007/978-981-10-3427-5_5

Houston, R. D., Bean, T. P., Macqueen, D. J., Gundappa, M. K., Jin, Y. H., Jenkins, T. L., … Robledo, D. (2020). Harnessing genomics to fast-track

genetic improvement in aquaculture. *Nature Reviews Genetics*, *21*(7), 389–409. https://doi.org/10.1038/s41576-020-0227-y

Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, *9*, 1322–1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Jiang, D. L., Gu, X. H., Li, B. J., Zhu, Z. X., Qin, H., Meng, Z. N., … Xia, J. H. (2019). Identifying a long QTL cluster across chrLG18 associated with salt tolerance in tilapia using GWAS and QTL-seq. *Marine Biotechnology*, *21*, 250–261. https://doi.org/10.1007/s10126-019-09877-y

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94. https://doi.org/10.1186/1471-2156-11-94

Kajungiro, R. A., Mapenzi, L. L., Nyinondi, C. S., Haldén, A. N., Mmochi, A. J., Chacha, M., … de Koning, D. J. (2019). The need of a structured tilapia breeding program in Tanzania to enhance aquaculture production: A review. *Tanzania Journal of Science.45*(3), 355–371.

Kajungiro, R. A., Palaiokostas, C., Pinto, F. A. L., Mmochi, A. J., Mtolera, M., Houston, R. D., & de Koning, D. J. (2019). Population structure and genetic diversity of Nile tilapia (*Oreochromis niloticus*) strains cultured in Tanzania. *Frontiers in Genetics*, *10*(1269), 1–12. https://doi.org/10.3389/fgene.2019.01269

Kamal, A. H. M. M., & Mair, G. C. (2005). Salinity tolerance in superior genotypes of tilapia, *Oreochromis niloticus*, *Oreochromis mossambicus* and their hybrids. *Aquaculture*, *247*(1–4), 189–201. https://doi.org/10.1016/j.aquaculture.2005.02.008

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*, 1179–1191. https://doi.org/10.1111/1755-0998.12387

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*, 357–359. https://doi.org/10.1038/nmeth.1923

Lemopoulos, A., Prokkola, J. M., Uusi-Heikkilä, S., Vasemägi, A., Huusko, A., Hyvärinen, P., … Vainikka, A. (2019). Comparing RADseq and microsatellites for estimating genetic diversity and relatedness — Implications for brown trout conservation. *Ecology and Evolution*, *9*, 2106–2120. https://doi.org/10.1002/ece3.4905

Li, B. J., Zhu, Z. X., Gu, X. H., Lin, H. R., & Xia, J. H. (2019). QTL mapping for red blotches in Malaysia Red Tilapia (*Oreochromis* spp.). *Marine Biotechnology*, *21*, 384–395. https://doi.org/10.1007/s10126-019-09888-9

Li, H. L., Gu, X. H., Li, B. J., Chen, C. H., Lin, H. R., & Xia, J. H. (2017). Genome-wide QTL analysis identified significant associations between hypoxia tolerance and mutations in the GPR132 and ABCG4 genes in Nile Tilapia. *Marine Biotechnology*, *19*, 441–453. https://doi.org/10.1007/s10126-017-9762-8

Lima, L. B., Oliveira, F. J. M., Giacomini, H. C., & Lima-Junior, D. P. (2018). Expansion of aquaculture parks and the increasing risk of non-native species invasions in Brazil. *Reviews in Aquaculture*, *10*, 111–122. https://doi.org/10.1111/raq.12150

Lind, C. E., Agyakwah, S. K., Attipoe, F. Y., Nugent, C., Crooijmans, R. P. M. A., & Toguyeni, A. (2019). Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa. *Scientific Reports*, *9*, 1–12. https://doi.org/10.1038/s41598-019-53295-y

Lovell, S. J., Stone, S. F., & Fernandez, L. (2006). The economic impacts of aquatic invasive species: A review of the literature. *Agricultural and Resource Economics Review (Northeastern Agricultural and Resource Economics Association)*, *35*(1), 195–208. https://doi.org/10.1017/S1068280500010157

Mapenzi, L. L., & Mmochi, A. J. (2016). Effect of stocking density on growth performance of hybrids of *Oreochromis niloticus*♀ and *Oreochromis urolepis urolepis*♂ in saline water. *Western Indian Ocean Journal of Marine Science*, *15*, 67–74. https://doi.org/10.4314/wiojms.v15i2

McAndrew, B. J., & Majumdar, K. C. (1983). Tilapia stock identification using electrophoretic markers. *Aquaculture*, *30*, 249–261. https://doi.org/10.1016/0044-8486(83)90167-9

McKinney, G. J., Larson, W. A., Seeb, L. W., & Seeb, J. E. (2017). RADseq provides unprecedented insights into molecular ecology and evolutionary genetics: Comment on Breaking RAD by Lowry et al (2016). *Molecular Ecology Resources*, *17*, 356–361. https://doi.org/10.1111/1755-0998.12649

Moser, F. N., van Rijssel, J. C., Ngatunga, B., Mwaiko, S., & Seehausen, O. (2019). The origin and future of an endangered crater lake endemic; phylogeography and ecology of *Oreochromis hunteri* and its invasive relatives. *Hydrobiologia*, *832*, 283–296. https://doi.org/10.1007/s10750-018-3780-z

Moses, M., Mtolera, M. S. P., Chauka, L. J., Lopes, F. A., de Koning, D. J., Houston, R. D., & Palaiokostas, C. (2019). Characterizing the genetic structure of introduced Nile tilapia (*Oreochromis niloticus*) strains in Tanzania using double digest RAD sequencing. *Aquaculture International*, *28*, 477–492. https://doi.org/10.1007/s10499-019-00472-5

Ndiwa, T. C., Nyingi, D. W., & Agnese, J.-F. (2014). An Important Natural Genetic Resource of *Oreochromis niloticus* (Linnaeus, 1758) Threatened by Aquaculture Activities in Loboi Drainage. *Kenya. Plos One*, *9*, e106972. https://doi.org/10.1371/journal.pone.0106972

Padial, A. A., Agostinho, Â. A., Azevedo-Santos, V. M., Frehse, F. A., Lima-Junior, D. P., Magalhães, A. L. B., … Vitule, J. R. S. (2017). The '"Tilapia Law"' encouraging non-native fish threatens Amazonian River basins. *Biodiversity and Conservation*, *26*, 243–246. https://doi.org/10.1007/s10531-016-1229-0

Palaiokostas, C., Bekaert, M., Khan, M., Taggart, J. B., Gharbi, K., McAndrew, B. J., & Penman, D. J. (2015). A novel sex-determining QTL in Nile tilapia (*Oreochromis niloticus*). *BMC Genomics*, *16*, 171. https://doi.org/10.1186/s12864-015-1383-x

Palaiokostas, C., Clarke, S. M., Jeuthe, H., Brauning, R., Bilton, T. P., Dodds, K. G., … de Koning, D. J. (2020). Application of low coverage genotyping by sequencing in selectively bred Arctic Charr (*Salvelinus alpinus*). *G3: Genes|genomes|genetics*, *10*(6), 2069–2078. https://doi.org/10.1534/g3.120.401295

Pembleton, L. W., Cogan, N. O. I., & Forster, J. W. (2013). StAMPP: An R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Molecular Ecology Resources*, *13*, 946–952. https://doi.org/10.1111/1755-0998.12129

Peñaloza, C., Robledo, D., Barría, A., Trịnh, T. Q., Mahmuddin, M., Wiener, P., … Houston, R. D. (2020). Development and validation of an open access SNP array for Nile Tilapia (*Oreochromis niloticus*). *G3: Genes|genomes|genetics*, *10*(8), 2777–2785. https://doi.org/10.1534/g3.120.401343

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for De Novo SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*, e37135. https://doi.org/10.1371/journal.pone.0037135

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Rico-Sánchez, A. E., Sundermann, A., López-López, E., Torres-Olvera, M. J., Mueller, S. A., & Haubrock, P. J. (2020). Biological diversity in protected areas: Not yet known but already threatened. *Global Ecology and Conservation*, *22*, e01006. https://doi.org/10.1016/j.gecco.2020.e01006

Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology*, *28*, 4737–4754. https://doi.org/10.1111/mec.15253

Scribner, K. T., Page, K. S., & Bartron, M. L. (2000). Hybridization in freshwater fishes: A review of case studies and cytonuclear methods of biological inference. *Review in Fish Biology and Fisheries*, *10*, 293–323. https://doi.org/10.1023/A:1016642723238

Shechonge, A., Ngatunga, B. P., Bradbeer, S. J., Day, J. J., Freer, J. J., Ford, A. G. P., … Genner, M. J. (2019). Widespread colonisation of Tanzanian catchments by introduced *Oreochromis* tilapia fishes: The legacy from decades of deliberate introduction. *Hydrobiologia*, *832*, 235–253. https://doi.org/10.1007/s10750-018-3597-9

Shechonge, A., Ngatunga, B. P., Tamatamah, R., Bradbeer, S. J., Harrington, J., Ford, A. G. P., … Genner, M. J. (2018). Losing cichlid fish biodiversity: Genetic and morphological homogenization of tilapia following colonization by introduced species. *Conservation Genetics*, *19*, 1199–1209. https://doi.org/10.1007/s10592-018-1088-1

Sherman, K. D., Paris, J. R., King, R. A., Moore, K. A., Dahlgren, C. P., Knowles, L. C., … Stevens, J. R. (2020). RAD-Seq analysis and in situ monitoring of Nassau Grouper reveal fine-scale population structure and origins of aggregating fish. *Frontiers in Marine Science*, *7*, 157. https://doi.org/10.3389/fmars.2020.00157

Syaifudin, M., Bekaert, M., Taggart, J. B., Bartie, K. L., Wehner, S., Palaiokostas, C., … Penman, D. J. (2019). Species-specific marker discovery in Tilapia. *Scientific Reports*, *9*, 13001. https://doi.org/10.1038/s41598-019-48339-2

Taslima, K., Wehner, S., Taggart, J. B., de Verdal, H., Benzie, J. A. H., Bekaert, M., … Penman, D. J. (2020). Sex determination in the GIFT strain of tilapia is controlled by a locus in linkage group 23. *BMC Genetics*, *21*, 49. https://doi.org/10.1186/s12863-020-00853-3

Toonen, R. J., Puritz, J. B., Forsman, Z. H., Whitney, J. L., Fernandez-Silva, I., Andrews, K. R., & Bird, C. E. (2013). ezRAD: A simplified method for genomic genotyping in non-model organisms. *PeerJ*, *1*, e203. https://doi.org/10.7717/peerj.203

Trewavas, E. (1983). *Tilapiine fishes of the genera Sarotherodon, Oreochromis, and Danakilia*. London, UK: British Museum (Natural History).

Ulotu, E. E., Mmochi, A. J., & Lamtane, H. A. (2016). Effect of salinity on the survival and growth of Rufiji Tilapia (*Oreochromis urolepis urolepis*) fry. *Western Indian Ocean Journal of Marine Science*, *15*, 31–37. https://doi.org/10.4314/wiojms.v15i2

Vendramin, N., Zrncic, S., Padrós, F., Oraic, D., Le Breton, A., Zarza, C., & Olesen, N. J. (2016). Fish health in Mediterranean Aquaculture, past mistakes and future challenges. *Bulletin-European Association of Fish Pathologists*, *36*(1), 38–45.

Wang, S., Meyer, E., McKay, J. K., & Matz, M. V. (2012). 2b-RAD: A simple and flexible method for genome-wide genotyping. *Nature Methods*, *9*, 808–810. https://doi.org/10.1038/nmeth.2023

Wright, S. (1978). Variability within and among natural populations. In *Evolution and the genetics of populations*, *4*, Chicago: University of Chicago Press.

You, X., Shan, X., & Shi, Q. (2020). Research advances in the genomics and applications for molecular breeding of aquaculture animals. *Aquaculture*, *526*, 735357. https://doi.org/10.1016/j.aquaculture.2020.735357

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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The aim of this thesis was to generate basic information for the development of a sustainable tilapia breeding program. The genetic diversity and population structure of Rufiji tilapia were in comparison to local and exotic Nile tilapia. This was followed investigation of genotype by environment interaction in a common garden. Low to moderate genetic differentiation were found among population and high between strains. Growth traits indicated moderate to high heritabilities and genetic correlations hence the possibility of moderate reranking.

Christer Simon Nyinondi received her doctoral education at the Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences. She obtained her Master of Science degree from the University of Ghent, Belgium.

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