Genetic variation in wild and farmed tilapia and catfish in Nigeria

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Abstract

Aquacultural production is increasing rapidly in sub-Saharan Africa, often through the use of imported strains of fish species that are either native to the area or are so closely related to native species that they can successfully hybridise. This leads to a significant risk that escaped farmed fish will have significant deleterious genetic impacts on wild fish populations (as has been seen elsewhere in the world, notably in the context of salmon farming). The extent of this threat is evaluated in the first main chapter of this thesis, which reviews the state of freshwater aquaculture in sub-Saharan Africa, with a special emphasis on the legal and regulatory frameworks present in each of the main countries involved in aquaculture. It concludes that most lack the necessary means to monitor and control the impact of farming practices on wild fish populations. The remainder of the thesis concentrates on the situation prevailing in Nigeria, one of the main fish producing nations in sub-Saharan Africa.

Species of Tilapia and the African catfish *Clarias gariepinus* are important native freshwater fish species in Nigeria that have contributed immensely to both capture fisheries and aquaculture. They are widely distributed across the country in rivers, lakes, and reservoirs. The tilapia species used for aquaculture are selectively bred, mostly being genetically improved farmed strains that have been introduced from Thailand and Egypt. Escapes of these farmed fish can potentially interbreed with wild populations, leading to genetic introgression and a loss of genetic diversity in native species, as well as posing significant challenges for both conservation efforts and aquaculture management. However, studies on the genetic diversity and differentiation between introduced strains of tilapia (such as the Genetically Improved Farmed Tilapia, GIFT) and native tilapia species in Nigeria are scarce. Furthermore, recent attempts to investigate the population structure and genetic diversity of *C. gariepinus* have been based only on mitochondrial DNA and microsatellite markers. While these studies might have laid the foundation for the investigation of species diversity, a genomic perspective has been lacking.

This study therefore investigated the genetic diversity and population structure of farmed and wild tilapia and *C. gariepinus* populations in Nigeria. It utilised analysis of haplotypes of mitochondrial DNA cytochrome c oxidase subunit I (COI) and double-digest restriction site-associated nuclear DNA sequencing (ddRADseq) to study the tilapia group. Meanwhile a triple restriction site-associated DNA sequencing (3RAD) approach, in parallel with analysis of COI mtDNA haplotypes, was used to assess the genetic diversity and differentiation in *C. gariepinus* populations.

The phylogenetic tree of tilapia species based on analysis of COI haplotypes was unresolved, but differentiated the mouthbrooders and the substrate spawners. Although there were discrepancies between morphological identification keys and genetic species identification based on the mtDNA analyses, tilapia were found to be highly admixed based on the ddRADseq data, with evidence of hybridisation between named species in the wild. Extensive ongoing hybridisation was also observed among the farmed populations and between farmed and wild samples, highlighting the indiscriminate breeding practices among Nigerian farmers. Population structure analysis was unable to differentiate between farmed and wild *Oreochromis* spp. including *O. niloticus, O. aureus, O. urolepis,* and *O. mossambicus*.

In the *C. gariepinus* mtDNA analysis, samples of farmed and wild fish appeared to be genetically distinct, with low genetic diversity within the wild population

compared to previous studies but higher than in the farmed populations. The analysis of the COI gene identified two distinct haplotypes specific to the farmed populations of *C. gariepinus*. Notably, samples of an albino population captured from the wild was found to be associated with these unique farmed haplotypes. This finding was supported by the results of the 3RAD analysis, which showed that the albino population exhibited clustering with farmed rather than wild populations, further confirming their origin as escaped individuals from aquaculture facilities. The 3RAD analyses suggested higher genetic diversity in wild compared to farmed populations but also higher levels of inbreeding in the former, which could be a warning sign that wild populations have suffered from bottlenecks that could compromise their long-term resilience. The 3RAD analyses also suggested high admixture among farmed populations, suggesting that hybridisation has been used extensively in the production of broodstocks. This study has therefore revealed insights into the genetic interactions between farmed and wild fish populations in Nigeria, highlighting genetic mixing of farmed and wild tilapia and providing genetic evidence of escaped farmed catfish strains living in the wild. It highlights the need for conservation management strategies such as aquaculture zoning and genetic monitoring in order to mitigate the genetic impacts of aquaculture on wild fish populations.

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Author's Declaration

I declare that this thesis is a record of my own research work. No part of this work has been submitted for any other degree or qualification, either at the University of Glasgow or elsewhere. I carried out all the lab and bioinformatic analyses myself, and these have been conducted in accordance with the ethical guidelines of the MVLS Graduate School.

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Definitions/Abbreviations

Code	Meaning
CZ	Coptodon zillii
f_CMC	CMC farm
f_EGLA	Black GIFT from Egypt collected in Lagos
f_LAC	LAC farm
f_LAT	Black GIFT from Thailand collected in Lagos
f_NTB	Black GIFT from Thailand collected from Nasarawa
f_NTR	Red GIFT from Thailand collected from Nasarawa
f_ODC	ODC farm
f_OGEB	Black GIFT from Egypt collected in Ogun
f_OGER	Red GIFT from Egypt collected in Ogun
f_OGTR	Red GIFT from Thailand collected in Ogun
f_SAC	SAC farm
f_SB	Black GIFT from Thailand collected in Lagos
GIFT	Genetically Improved Farmed Tilapia
HF	Hemichromis fasciatus
OA	Oreochromis aureus
OM	O. mossambicus
ON	O. niloticus
OU	O. urolepis
PM	Pelmatolapia mariae
SG	Sarotherodon galilaeus
SM	S. melanotheron
w_AST	Asejire dam tilapia from Oyo
w_BDT	Badagry creek tilapia from Lagos

w_BNT	River Benue tilapia from Numan, Adamawa
w_BYC	River Benue Yola
w_BYT	River Benue tilapia from Yola, Adamawa
w_DKAL	Albino Dadin Kowa dam
w_DKC	Dadin Kowa dam
w_DKT	Dadin Kowa dam tilapia from Gombe
w_ETT	Etele River tilapia from Ogun
w_KDC	Kiri dam
w_KDT	Kiri dam tilapia from Adamawa
w_LGC	Lake Geriyo
w_LGT	Lake Geriyo tilapia from Adamawa
w_ODT	Odo Idimu tilapia from Ogun
w_OYT	Oyan dam tilapia from Ogun
w_SL	Lagos lagoon tilapia from Lagos

Chapter 1 General introduction

1.1 Impacts of climate change on fish

Climate change affects the survival, growth, reproduction, and distribution of individuals within a species (Brander, 2007). Aquatic ectotherms are especially vulnerable to climate change due to rising temperatures causing an increase in their oxygen requirements (since metabolism is temperature-dependent) but also causing a reduction in the oxygen content of the water (Belton et al., 2020). Recent studies have provided information on the impact of climate change on biodiversity, including local extinctions. For example, Wiens (2016) in an analysis of climate data reported that climate-related local extinctions have occurred in hundreds of species but that the rate of extinctions was higher in freshwater environments compared to marine environments (74% versus 51%). Pinsky et al. (2019) suggested that marine species, such as fish and other aquatic organisms, are more susceptible to the impacts of warming temperatures and are facing greater challenges in adapting to rising ocean temperatures compared to their counterparts on land (Pinsky et al., 2019). The warming temperatures can lead to a shift in species distribution and loss of suitable habitat for some fish species, particularly those that are highly specialised or restricted to specific temperature ranges (Cheung et al., 2009).

Climate change has also been found to have an impact on fish species and genetic diversity. For example, a study conducted on the subtropical coast of the Gulf of Mexico suggested that climate-related factors are driving changes in the diversity of fish and invertebrates, leading to shifts in species composition and potentially altering the structure and function of these ecosystems (Fujiwara et al., 2019). In most cases, these changes will reduce genetic diversity in populations and species, to the point where loss of genetic variation could lead to reduced population viability and extinction (Pauls et al. 2013). Despite these negative impacts of climate change on fish, knowledge gaps still exist in the monitoring and prediction of adaptive genetic responses to environmental change. Understanding the impacts of climate change thus remains critical to the conservation of biodiversity (Pearman et al., 2024). For example, genomics can be applied to generate more DNA sequencing data that can be used to design a robust scientific evidence-based fish conservation and

management approach. By analysing sequencing data, it is possible to identify genetic markers that are linked with traits related to adaptation to climate change (Aguirre-Liguori et al., 2021). These traits include temperature tolerance, disease resistance, and physiological adaptations. Genetic markers can be used to develop strategies for selective breeding to enhance the resilience of fish populations to changing environmental conditions (Boudry et al., 2021).

1.2 Convention on Biological Diversity (CBD)

In 1992, the Convention on Biological Diversity (CBD) was adopted during the Earth Summit in Rio de Janeiro, Brazil, to protect all forms of life by conserving biological diversity while ensuring their sustainable utilisation including equitable access to all its benefits derived from the management of genetic resources (Convention on Biological Diversity, 2001). Three decades later, the countries that signed the CBD treaty are still mandated to commit to conserving and promoting the sustainable use of biodiversity and to share the benefits of genetic resources in a fair and equitable way at the national level (Maney et al., 2024). In 2019, the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) published the Global Assessment Report on Biodiversity and Ecosystem Services, stating the key drivers of biodiversity loss including habitat fragmentation, climate change, pollution, invasive species, and overexploitation (Hald-Mortensen, 2023). The issue of biodiversity deteriorating at unprecedented rates, with around 1 million species (animals and plants) already facing extinction as highlighted in the IPBES report, was addressed during the Kunming-Montreal Global Biodiversity Framework (IPBES, 2019b). In addition, habitat degradation and stock depletion, along with other anthropogenic activities, are resulting in a general decline in genetic diversity (Hoban et al., 2023a).

Previous studies using DNA-based approaches have documented the high rate at which genetic diversity is deteriorating. For example, over the past 50 to 100 years, there has been a loss of about 28% of island species and 14% loss of harvested species due to overfishing (Pinsky and Palumbi, 2014). Since genetic diversity is important for a species' ability to adapt to changing environmental conditions (Reed and Frankham, 2003), loss of genetic diversity will pose a

serious threat to fish adaptations. As part of ongoing efforts to conserve species, the CBD Kunming-Montreal Global Biodiversity Framework (GBF), which was approved in December 2022 at COP15 (15th Conference of the Parties), has been taking critical steps to address the importance of conserving genetic diversity (Hoban et al., 2023b). Some of the important decisions adopted by COP15 include compiling the scientific and technical evidence base for the Kunming-Montreal Global Biodiversity Framework, reviewing progress in the implementation of the Convention and the Strategic Plan for Biodiversity 2011-2020 and the achievement of the Aichi Biodiversity Targets.

1.3 Monitoring genetic diversity for conservation

Conservation programmes should be based on, among other factors, the preservation of genetic diversity (Eknath et al., 2023). Conserving the local genetic resources, along with understanding the role of genes, is crucial for maintenance of biodiversity and food security for the future (de Almeida Cançado, 2011). Genetic diversity is the quantifiable measure of genetic variation within a population (Hughes et al., 2008). This genetic diversity is important for both individuals and populations, since it affects how species adapt and evolve to different environmental pressures (Mukhopadhyay and Bhattacharjee, 2016). Advances in molecular genetics and the emergence of next-generation sequencing has made it possible to study the genome of organisms at a lesser cost compared to previous decades (Mable, 2019). Molecular genetic tools have successfully been applied to identify and delineate species (Chan et al., 2016; Zhai et al., 2019), identifying cryptic species (Mat Jaafar et al., 2012), including an investigation of how selective pressures have influenced the genetic diversity and composition of farmed *O. niloticus* (Cádiz et al., 2020; Hong Xia et al., 2015). Genetic analysis has also been applied to test ecological and evolutionary hypotheses. For example, genetic analysis can reveal a great deal of information about the life-history (Roff, 2007), ecology, and behavioural characteristics of species, critical for their long-term management and forecast of the impact of future environmental and climate changes on their adaption (Bylemans et al., 2016). Without a proper understanding of the genetic diversity of fish populations, it will be difficult to formulate policies that would address the issue of species conservation and monitor biologically relevant processes such as changes in species composition, genetic diversity and

population structure that are needed for management actions (Reiss et al., 2009). A detailed understanding of genetic diversity is crucial when planning conservation programmes to ensure the long-term utilisation of genetic resources and combat abiotic stress (Kanaka et al., 2023).
The conservation of biodiversity at all levels from genes to ecosystems represents a global concern (Dudu et al., 2015) and advancement in sequencing technology has led to the discovery of molecular markers that are now being applied to various areas of population genetics including population structure, stock identification, detecting hybridisation, authentication of fish products and in genetic improvement. Thus, genomic data will provide greater utility than what is currently known to identify differentiation in a population, detect inbreeding and genetic drift, and allow genetic monitoring of species (Coates et al., 2018).

1.4 Application of molecular markers for population genetics studies

The precise detection of genetic variation/diversity has greatly enhanced the study of evolutionary processes (Mukhopadhyay and Bhattacharjee, 2016). Overall, the history of molecular markers for studying genetic diversity reflects continual advances in technology and methodology, leading to increasingly precise and comprehensive analyses of genetic variation within and between populations. Molecular markers have transformed the study of genetic diversity by providing researchers with tools to understand the underlying genetic variation within and between populations (Primmer, 2009). The application of molecular markers in studying genetic diversity has evolved from the use of protein markers to high-throughput Next-Generation Sequencing (Mable, 2019); the next sections describe the history and limitations of these different approaches.

1.4.1 Protein markers (allozymes)

Allozymes were the first true molecular markers to be established for population genetics. Allozyme electrophoresis is a procedure for separating proteins of different molecular sizes and electrical charges to detect levels of genetic variation within and between populations (Berta et al., 2015). The allelic variants are visualised by chemical staining of the electrophoretic gel after

migration (Schlötterer, 2004). Allozyme techniques can be used directly with any fresh tissue sample of animal, plant or microbe because all such tissues contain enzymes (Allendorf, 2017). The approach was first adopted in the 1960s and within a decade, genetic variation at multiple loci had been described in 125 animals and eight plant species (Nevo, 1978). Allozymes have been utilised in aquaculture to track levels of inbreeding, identify stock, and perform parentage analysis (Liu and Cordes, 2004). Although these protein markers, particularly those detected through enzyme electrophoresis, were groundbreaking in their time and contributed significantly to our understanding of genetic diversity, they have several disadvantages compared to modern DNA-based markers.
Some of the limitations associated with protein markers include complexities associated with the Interpretation of protein banding patterns on electrophoresis gels; moreover, different isoforms of a protein may have similar mobilities on gels, leading to difficulties in distinguishing between them (Pandian and Koteeswaran, 1998). Critics argued that allozyme markers, which rely on variations in protein electrophoretic mobility encoded by DNA, are indirect and less sensitive method for detecting variation in DNA compared to DNA-based markers that allow the number of mutations between different alleles to be quantified (Schlötterer, 2004).

Despite these limitations, allozymes have been useful in solving taxonomic problems, especially in the delimitation of species (Chung and Chung, 2012). This includes work on species used for aquaculture, including various species of tilapia. For example, allozyme electrophoresis was useful in detecting hybridisation between sympatric autochthonous species of red belly tilapia (*Coptodon zillii*) and Guinean tilapia (*C. guineensis*) (Agnese et al., 1997). Allozymes have been used to investigate the genetic diversity in nine populations of blackchin tilapia (*Sarotherodon melanotheron*) and *S. melanotheron heudelotti* in the coastal ecosystems of Senegal, Gambia, and Ivory Coast (Yoboué et al., 2012). The method has also been applied to differentiate *S. melanotheron* populations from West African hydrographic basins (Agnèse et al., 1998a). However, allozymes can be poor at detecting polymorphic sites. For example, Appleyard et al. (2001) in a study investigating relative individual heterozygosity at microsatellite and allozyme loci in cultured *O. niloticus,* reported that several allozyme loci were not polymorphic. Display of occasional heterozygote deficiencies due to null alleles caused by inactive enzymes and

scoring bias were reported in marine bivalves (Gaffney, 1994). It is therefore important, when studying highly evolving species like the cichlids known for their adaptive radiation, to use markers that can detect sufficient polymorphic sites across the genome.

1.4.2 Mitochondrial DNA (mtDNA)

The use of mitochondrial DNA analysis in population and quantitative genetic studies first appeared in the late 1970s (Avise et al., 1979). mtDNA provided a different perspective of the genetic structure of natural populations because of its maternal inheritance and general lack of recombination between mtDNA molecules (Allendorf, 2017). Typically, there are about 1000-2000 mitochondria per cell, occupying about one-fifth of the cell volume (Alberts et al., 2007). The mtDNA molecule contains 37 genes including 13 mitochondrial protein-coding genes (MPCGs), two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and a noncoding region (referred to as the D-loop or control region, CR) which controls its replication and transcription (Boore, 1999; Cameron, 2014). mtDNA has been used extensively to study different freshwater and marine species including in the field of molecular taxonomy (Iyiola et al., 2018; Nwani et al., 2011b; Ward et al., 2005), detection of seafood mislabelling (Carvalho et al., 2015; Cawthorn et al., 2012; Clark, 2015), and genetic diversity and population structure (April et al., 2011; Chan et al., 2016; Nwafili and Gao, 2016). Universal primers have been developed for the most frequently used mtDNA genes, for example, cytochrome c oxidase subunit I (COI), ATPases 6 and 8, cytochrome b (cytb), and NADH dehydrogenase subunits 4 and 5, for use across many species (Ramya and Behera, 2023).

The COI gene has been suggested as a common marker for identifying species based on the principle of sequencing a short segment of DNA from a uniform region of the mitochondrial genome of the target specimen and comparing these unknown barcodes to an existing barcode database (Hebert et al., 2003). This ‘DNA barcoding’ has been used to identify fish species (Ward et al., 2005) including processed products (Shokralla et al., 2015) and has been applied in the discrimination of Nigerian cichlids (Iyiola et al., 2018). However, a problem with using mtDNA genes, such as the COI gene, for DNA barcoding is that they contain limited information compared with the nuclear genome which contains more genes and is inherited through both parents, while mtDNA is only inherited

through females (Dasmahapatra and Mallet, 2006). DNA barcoding has received criticism mainly in response to the view that single-gene sequences should be the primary identifier for species (Moritz and Cicero, 2004). Also, the entire mitochondrial genome acts as a single locus because there is no recombination (Allendorf, 2017).

1.4.3 Microsatellites (Simple Sequence Repeats, SSRs)

Microsatellites are short, tandemly repeated DNA sequences that exhibit high levels of polymorphism and are abundant in the genomes of higher organisms (Ellegren, 2004). These microsatellites, also known as Simple Sequence Repeats (SSRs), became widely used markers in the 1990s since they offer more power than mtDNA (which has been criticised for ancestral polymorphism and male-biased gene flow (Moritz and Cicero, 2004)) for describing population structure, detecting population bottlenecks, and estimating effective population size (Allendorf, 2017). Microsatellites have played a vital role in fisheries and aquaculture research, providing valuable insights into population genetics, conservation biology, selective breeding, and sustainable management practices (Olagunju, 2019; Seth et al., 2021). Microsatellite markers have been applied, for example, in salmonid fish to provide insights into inter-specific hybrids, assess genetic diversity, population structure and intra-specific variation, and identify individual organisms (Zhivotovsky et al., 2013). Despite the broad application of microsatellites in population genetic studies, they are not, however, without some challenges. Microsatellites are believed to primarily mutate by strand slippage during DNA replication, which manifests as the gain or loss of repeats (Putman and Carbone, 2014). There is also the problem of null alleles caused by poor primer annealing due to mutations or indels in one or both flanking primers (Dakin and Avise, 2004).

1.4.4 Single Nucleotide Polymorphisms (SNPs)

With the completion of the Human Genome Project in 2003 and advances in DNA sequencing technologies, Single Nucleotide Polymorphisms (SNPs) gained prominence as molecular markers (Shastry, 2007). SNPs represent single nucleotide variations in the DNA sequence and have become the marker of choice due to their abundance throughout the genome and ease of detection using high-throughput sequencing methods (Pradeep et al., 2012). The advances

in sequencing technology have greatly enhanced the discovery and genotyping of SNPs in fish genetic research and have been useful for instance in specifying genetic differences between farmed and wild fishes (Rasal et al., 2017). SNPs have become commonplace in genetics and genomics, due to advances in next-generation sequencing (NGS) technologies (Sato et al., 2019). These technologies, developed in the 2000s, allowed for the simultaneous sequencing of millions of DNA fragments (Hu et al., 2021). SNPs have been applied as a diagnostic tool for population and parentage assignment, identification and tracing of captive-bred fish (Bylemans et al., 2016). Several methods are commonly used for SNP discovery, as described in the following sections.

1.4.4.1 Whole Genome Sequencing (WGS)

WGS involves sequencing the entire genome of an organism, providing comprehensive coverage of genetic variation, including SNPs (Ng and Kirkness, 2010). The Japanese pufferfish (*Fugu rubripes*) was the first fish to have its whole genome sequenced (Aparicio et al., 2002). Major progress in sequencing technology has led to the sequencing of economically important fish species like Atlantic cod (*Gadus morhua*) (Star et al., 2011), *O. niloticus* (Tao et al., 2021), *C. gariepinus* (Nguinkal et al., 2023), and Atlantic salmon (*Salmo salar*) (Lien et al., 2016). Sequencing the entire genome of important fish species has helped to resolve population structure and facilitate the analysis of genetic diversity (Vij et al., 2016), including increased understanding of fish biodiversity, speciation, adaptation, conservation and sustainable utilisation (Fan et al., 2020). WGS is highly accurate but can be costly (Schwarze et al., 2020) and computationally intensive (He et al., 2019).

1.4.4.2 Reduced-representation sequencing

Although the cost of sequencing has decreased over time, many facilities are still unable to afford whole-genome sequencing for numerous individuals. However, instead of sequencing the complete genome, high-throughput sequencing of a subset of the genome can be used to obtain SNPs for the studied species (Luca et al., 2011). This approach has been widely applied in molecular marker development, population genetic analysis, genetic map construction, quantitative trait locus (QTL) mapping, and genome-wide association analysis (Lee et al., 2019; Yang et al., 2016). Some of the common reduced-

representation sequencing methods include genotyping-by-sequencing, restriction site-associated DNA sequencing (Baird et al., 2008; Hohenlohe et al., 2012), double digest restriction site-associated DNA sequencing (Peterson et al., 2012), and triple enzyme restriction site-associated DNA sequencing (Bayona-Vásquez et al., 2019). Each of the methods relies on digesting the DNA (starting material) with restriction enzyme(s) prior to sequencing but the specific approaches differ as follows:

1.4.4.3 Genotyping-by-sequencing (GBS)

GBS involves digesting genomic DNA with a restriction enzyme and then sequencing a reduced representation of the genome using high-throughput sequencing (Elshire et al., 2011). This is a cost-effective approach that allows for the discovery of SNPs by sequencing specific genomic regions across multiple individuals (Wang et al., 2020). GBS was originally developed for crops (Elshire et al., 2011; Huang et al., 2009) but has now been applied to multiple fisheries and aquaculture research. For example, Li and Wang (2017) reviewed the application of GBS in the field of fisheries and aquaculture, including stock identification, estimating the effective population size, migration rates, understanding the roles of evolutionary processes that influence variation across genomes, and identifying mutations associated with disease. GBS was applied in the assembly of a comprehensive, open-access baseline of 45 SNPs from 172 Chinook salmon (*Oncorhynchus tshawytscha*) populations ranging from Russia to California for genetic stock identification (Templin et al., 2011). The GBS approach is similar to restriction site-associated DNA (RAD) sequencing (see next section) but the procedure is less complicated with fewer steps than RAD with a single digestion of genomic DNA. There are fewer purification steps and fragments are not size-selected (Elshire et al., 2011). The lack of size-selection in GBS presents more complex sequencing data than the RAD which contains smaller fragments (Wickland et al., 2017).

1.4.4.4 Restriction site-associated DNA sequencing (RADseq)

RADseq is a reduced-representation sequencing method that is used to sequence short fragments of DNA adjacent to each instance of a particular restriction enzyme recognition site (Baird et al., 2008). The RADseq protocol was created to generate thousands of SNPs from across multiple individuals at a low cost and

offers researchers the flexibility to modify the protocol to suit a diversity of evolutionary genetic questions (Hohenlohe et al., 2012). For example, RADseq has been applied to reveal evolutionary histories for different regions of the genome and identified intergeneric hybridization events among five sympatric cichlid species of two genera, *Pundamilia* and *Mbipia* at Makobe Island, an offshore island in southern Lake Victoria, Tanzania (Keller et al., 2013). RADseq data has also been used to resolve species-level phylogenetic relationships of 16 species of Lake Victoria cichlid fish (Wagner et al., 2013). In a similar study, RADseq was used to determine the genetic basis underlying male colour dimorphism in the Lake Tanganyika cichlid fish *Cyprichromis leptosome* (Takahashi et al., 2013). This approach uses a single restriction enzyme of choice to digest the DNA and these fragments are then ligated to adapters that will bind to an Illumina flow cell during sequencing (Baird et al., 2008; Davey and Blaxter, 2011). It is suitable for organisms that do not have a reference genome because loci can be mapped using a *de novo* approach to build a catalogue of loci and deliver huge numbers of SNPs for analysis (Davey and Blaxter, 2011; Rivera-Colón and Catchen, 2022). RADseq is feasible for genomes of any size enabling studies of non-model organisms and wild populations (Davey and Blaxter, 2011).

1.4.4.5 Double-digest restriction site-associated DNA sequencing (ddRADseq)

This approach uses two restriction enzymes (one rare and one frequent-cutting enzyme) to digest the DNA followed by a precise size selection that excludes regions flanked by the enzymes (Peterson et al., 2012). Typically, the rare cutter determines the number of fragments sequenced and the frequent cutter determines the average length of these fragments (Lajmi et al., 2023). Just like the RADseq approach, ddRADseq is widely used to generate genomic data for non-model organisms in evolutionary and ecological studies (Peterson et al., 2012). This approach is time-consuming and can be more expensive than RADseq, but the double digestion of DNA fragments allows for the discovery and genotyping of more polymorphic loci (Lajmi et al., 2023).

1.4.4.6 Triple enzyme restriction site-associated DNA sequencing (3RAD)

3RAD, like RADseq and ddRADseq, involves digesting the DNA with restriction enzymes; however, as indicated in the name, this method uses three restriction enzymes, with digestion and ligation happening simultaneously (Bayona-Vásquez et al., 2019). Bayona-Vásquez et al. (2019) highlight common problems associated with RADseq that are solved using this approach, including adapter dimer formation, inability to reduce chimera formation, requirements of a high concentration of DNA, limited ability to multiplex high numbers of libraries due to few primer combination options for tagging libraries, and thus, high sequencing costs, and workflows of varying complexity. The 3RAD offers multiple primers combination that was developed in *Adapterama I* to make fully active quadruple-indexed Illumina libraries that can be highly-multiplexed (Bayona-Vásquez et al., 2019). This makes the 3RAD method more cost-effective, especially when studying large numbers of samples, compared to other reduced-representation sequencing methods. The value of the approach has been demonstrated in several fish species, including *Eurycea bislineata*, *Wisteria floribunda x Wisteria sinensis hybrid population, Rhodnius pallescens, Gambusia affinis, Sphyrna tiburo*, and *Sphyrna lewini* (Bayona-Vásquez et al., 2019); it has also been applied in a phylogenetic study of 12 Nearctic-Neotropical lubber grasshopper species (De Jesús-Bonilla et al., 2019).

1.4.4.7 Microarray-based genotyping

Microarray technology can be used to genotype SNPs by hybridising DNA samples to SNP-specific probes immobilised on a solid support (Ramya and Behera, 2023). For example, medium- to high-density SNP microarrays have been developed for aquaculture species such as Atlantic salmon (Houston et al., 2014), common carp (*Cyprinus carpio*) (Xu et al., 2014), Nile tilapia (*O. niloticus*)) (Joshi et al., 2018), and rainbow trout (*Oncorhynchus mykiss*) (Palti et al., 2015). While microarrays are efficient for genotyping known SNPs in large numbers of individuals, they have relatively low accuracy, precision and specificity, and are not suitable for discovering novel SNPs; there is also the disadvantage of the high initial cost of developing the microarray (Draghici et al., 2006; Jaksik et al., 2015). DNA arrays only detect sequences designed on them. This means that if

the hybridised solution lacks complementary sequences on the array, those sequences will not be detected (Bumgarner, 2013).

1.4.4.8 RNA-Sequencing (RNA-seq)

RNA-seq can be utilised for SNP discovery by sequencing transcripts from RNA samples and identifying differences in nucleotide sequences among individuals (Zhao et al., 2019). SNPs detected in transcribed regions (exons) using RNA-seq can provide insights into functional genetic variation associated with gene expression and phenotypes (Podnar et al., 2014). RNA-seq technology has been instrumental in identifying and categorizing fish transcriptomes, which includes gene expression, metabolic pathways, gene regulatory networks, and protein-protein interaction networks as well as providing valuable insights into various biological processes such as adaptive evolution, stress responses, development, and host immune responses (Qian et al., 2014). The development of RNA-seq is evident in the discovery of large numbers of transcripts, many of which were novel, in model and non-model aquaculture species including channel catfish (*Ictalurus punctatus*) (Liu et al., 2016; Sun et al., 2016), zebrafish (*Danio rerio*) (Collins et al., 2012), European sea bass (*Dicentrarchus labrax*) (Sarropoulou et al., 2019), and rainbow trout (Palstra et al., 2013). RNA-seq is revolutionizing the field of transcriptomics by improving our understanding of genome expression and regulation (Qian et al., 2014), however, it produces large and complex data sets, whose interpretation is not straightforward (Finotello and Di Camillo, 2014; Oshlack et al., 2010).

1.5 Fish production in Nigeria

The specific focus of this thesis is the assessment of genetic diversity in farmed and wild fish in Nigeria, which represents an under-studied geographic region where reliance on aquaculture is increasing. Fish are a vital source of animal protein in the Nigerian diet and have been identified as a crucial nutritional component that can help reduce food and nourishment insecurity (Olaifa et al., 2022). Artisanal fisheries contribute over 85% of the total fish output in Nigeria (Sylvanus and Gao, 2007) and have continued to dominate the fisheries sector; however, inland water capture fisheries production has declined from about 370,000 tonnes, live weight in 2019 to 350,000 tonnes in 2022 (FAO, 2024b). In Nigeria, fish biodiversity, distribution, status, and biology are understudied

(Emmanuel et al., 2021). The last comprehensive survey of Nigerian inland water fisheries was conducted in 1993 when 230 species were reported (Ita, 1993). However, recent regional studies have reported Cichlidae, Cyprinidae, Mormyridae, and Clariidae among the dominant families in the Northcentral (Iyiola et al., 2018; Oladipo et al., 2021; Popoola et al., 2022), Southeast (Nwani et al., 2011a; Ude et al., 2020), and Southwest Oyo (Ajagbe et al., 2021). However, only species from the Cichlidae and Clariidae families have been successfully domesticated in Nigeria for aquaculture purposes, with the primary species being African catfish (*Clarias gariepinus* and *Heterobranchus bidorsalis*) and Nile Tilapia (Kaleem and Bio Singou Sabi, 2021).

1.5.1 Fisheries production in Nigeria

Data on fish production in Nigeria indicate that over 5 million tonnes of fish were harvested between 2010 and 2015, with the highest annual production being 1.1 million tonnes in 2014 (National Bureau of Statistics, 2017). Clariids, cichlids, cyprinids, mormyrids, including species like Nile perch (*Lates niloticus*), and bony tongue (*Heterotis niloticus*) were among the top produced species as revealed by the National Bureau of Statistics Nigeria. Recent information on the state of world fisheries and aquaculture production ranked Nigeria among the top 25 producers of inland waters capture fisheries production, with an estimated total production of 350,000 tonnes live weight production in 2022 constituting 3% of total inland water capture production (FAO, 2024b). Despite the positives from growing production, there is a large production-consumption deficit, and demand has outweighed production since 2014 (Emmanuel et al., 2014). The FAO has warned that the data collection systems for many inland waters remain unreliable or non-existent for many nations including for several major producers (FAO, 2022). Consequently, it is probable that the data pertaining to Nigeria is speculative, given that the last comprehensive assessment of Nigerian inland fish was conducted in 1993 (Ita, 1993).

In addition to the unreliable data reporting on catch assessment, Nigerian fisheries also face severe threats affecting fish production including climate change (Olutumise, 2023), oil spillages in the Niger Delta region (Olaifa et al., 2022), overfishing, habitat fragmentation, poor management, and conservation

decisions (Emmanuel et al., 2021). These factors play important roles in defining species diversity and distribution (Makki et al., 2023).

1.5.1.1 Impacts of climate change in Nigeria

Rising temperatures have necessitated immediate initiatives to enhance and expedite climate mitigation and adaptation efforts needed for fisheries and aquaculture sectors to adjust to the impacts of climate change(FAO, 2020; FAO, 2022). Nigeria, currently facing the impact of climate change ranging from desert encroachment in the northern Sahara region, reduction in rainfall distribution (Umar and Ismaila, 2017), increasing temperatures (Buba and Ibrahim, 2017), and receding of lakes and rivers (Ikusemoran et al., 2018), has been identified in an FAO report in 2018, as the second most vulnerable national fishing industry to climate change in Africa after Uganda (Soto et al., 2019). This prediction is due to elevated temperatures in the tropical region that is contributing to the reduction of fishery productivity (FAO, 2020). The impact of climate change on Nigerian fisheries resources is now imminent (Isa et al., 2023; Olutumise, 2023), with important lakes losing a significant portion of their surface area to drought, increasing seasonality in rainfall distribution, and increase in temperature (Oyebola et al., 2020). Flooding is an increasing issue in large parts of the country, and fish farms around the flood risk zones will be greatly impacted during the peak of the rainy season (Oyebola et al., 2020).

1.5.1.2 Oil spillage in the Niger Delta

A further challenge facing Nigerian fish production is the damage posed by pollution. The Niger Delta, located by the Atlantic Coast where the River Niger divides into numerous tributaries, directly at the tip of the Gulf of Guinea, is Nigeria's major oil-producing region (Osuagwu and Olaifa, 2018). The fishes of the Niger Delta ecosystems of Nigeria are facing threats as a result of environmental pollution from oil exploration, drilling, refining and transportation (Emmanuel et al., 2021). Both the exploration activities of oil companies and the vandalism of pipelines by the residents of host communities have resulted in oil pollution, including damage to fisheries resources and pollution of water bodies that are now unsuitable for aquaculture activities (Ikhumetse et al., 2022; Osuagwu and Olaifa, 2018). The Nigerian Department of Petroleum Resources (DPR) has reported that approximately 31 million barrels of

oil were spilled in the region in over 9,000 events between 1976 and 2005 (Adesipo et al., 2020; Anejionu et al., 2015). Damage from oil spillage can vary from being negligible to the destruction of aquatic ecosystems, leading to loss of species diversity (Zerebecki et al., 2022). Most agricultural land and water bodies in the oil-rich Delta state of Nigeria have been degraded and are no longer suitable for agriculture or fishing because of oil exploration in the region that contaminates the water bodies due to the chemical content of crude oil (Elum et al., 2016; Olaifa et al., 2022).

1.5.1.3 Overfishing and habitat degradation in Nigeria

The increasing demand for fish as a dietary protein source has led to overfishing, the use of banned fishing devices and other activities that are inimical to fisheries and represent threats to productivity in aquatic systems in general (Adeosun, 2019). Overfishing has led to alteration in species composition within Nigerian aquatic ecosystems, so causing a decline in yield. Exploitation is posing a severe threat to fish stocks including population depletion because of the ineffectiveness and inadequacy of existing regulations (Olopade et al., 2017). Part 3, Section 9(j) of the Fisheries Act 2014 highlights the need for sustainable fishing practices including fish trade while emphasizing the need to enforce compliance with laws, guidelines, conservation, and management by adopting appropriate monitoring of fishing activities and extent of overfishing (Nigeria Fisheries Act, 2014). However, compliance with this law of fisheries regulation can only be achieved when there is effective enforcement in place. Otherwise, more fish beyond the maximum sustainable yield will continue to be harvested in the wild until the populations become depleted. Important inland waters like the River Niger and River Benue have been exploited by artisanal fishers and have received less attention from the government despite their contributions to food security (Olopade et al., 2017). The widespread overfishing of Nigerian inland fish has been well-documented by Emmanuel et al. (2021), who outlined how overfishing and habitat degradation from dam construction, loss of catchments, habitat disruption and destruction are resulting in loss of species diversity.

In northern Nigeria, agricultural areas are facing exacerbated damage due to the notable shift in climate patterns, characterised by a shortened rainy season and an extended dry season (Sunmonu et al., 2022). This climatic shift has prompted

farmers to transition from traditional rain-fed crop production to irrigation farming, particularly in riverine areas. Consequently, there is a concentration of agricultural activities around receding water bodies, resulting in increased disturbance to fish species and significant habitat destruction. Overfishing and habitat degradation emerge as two prominent threats to fish diversity in Nigeria and both threats can potentially lead to the local extinction of fish species (Kenchington, 2003; Yan et al., 2021).

1.5.2 Aquaculture production in Nigeria

The major aquaculture species in Nigeria include tilapia and catfish (Emmanuel et al., 2014; Omitoyin, 2007). Catfish production in Nigeria plays an important role in the country's aquaculture industry with *C. gariepinus* and *H. bidorsalis* as the main production species. The aquaculture systems being practised are predominantly at the subsistence level, with little contribution to national economic development (Kaleem and Bio Singou Sabi, 2021). The common culture system employed for fish culture includes the use of concrete tanks with reinforced walls to reduce water seepage and improve the pond's water retention capacity and earthen ponds (Omitoyin, 2007).

The aquaculture sector has also faced problems, notably inadequate infrastructure, inadequate supply of fish feed, irregular electricity supply, poor finance, disease, poaching, and poor extension services (Kaleem and Bio Singou Sabi, 2021). Despite Nigerian dominance in the catfish aquaculture industry, farmers must constantly deal with the high cost of fish feed, limited access to good quality fish eggs, water, and electricity to keep the water running (FAO, 2022). With the Nigerian human population currently growing at an exponential rate, the fisheries and aquaculture industries are currently unable to sustain the demand for fish, and therefore the country has to rely on the importation of fish to close the demand and supply gap (Liverpool-Tasie et al., 2021). There is therefore a clear need to increase fish production, almost certainly from aquaculture rather than wild-caught fish. Recently, attention has been shifting from capital-intensive catfish farming to tilapia aquaculture.

Tilapia are cheaper to rear because they have a lesser demand for pelleted feed compared to catfish, and farmers can supplement the pellets with plant source feed such as duckweed that can be accessed freely in the wild and cultured on

the farm (Cipriani et al., 2021). However, the prolific breeding behaviour of tilapia is a problem for farmers engaged in mixed-sex tilapia aquaculture because they can breed naturally in captivity and become overcrowded within the aquaculture system. This overcrowding can lead to competition for resources such as food, space and dissolved oxygen, and result in stunted growth. To address the challenge, farmers are introducing non-native, selectively bred, tilapia and reversing the sex of female fry through hormone treatment so as to have an all-male monosex population (Abucay and Mair, 1997). The government has been supporting the tilapia industry and is driving the transition from subsistence concrete and earthen pond systems to a more intensive culture approach by installing fish cages in major fishing waters (Ogunji and Wuertz, 2023). The growing interest in cage aquaculture following the ongoing introduction of non-native tilapia strains, some of which have undergone intensive domestication (e.g. the Genetically Improved Farmed Tilapia (GIFT) strain of *O. niloticus* that is now widely farmed in Nigeria (Kaleem and Bio Singou Sabi, 2021)). A recent assessment of the prospects for *C. gariepinus* and Nile tilapia revealed promising potential for intensive tilapia cage systems within vast water bodies (Ribeiro, 2021). This suggests that significant aquaculture opportunities remain untapped, particularly in the southwest, central, and northern regions of the country (FAO, 2022). This expansion of aquaculture activities and the introduction of domesticated strains calls for increased monitoring and management of genetic diversity in Nigeria.

1.6 Conservation of fish genetic diversity in Nigeria

There is a need for the implementation of conservation measures to minimise threats to the diversity of fish populations in Nigeria and their vulnerability to overexploitation and extinction. The Nigeria Fisheries Act (2014) covers the rules and regulations guiding the exploitation of wild and aquaculture in the country. Under the Act, the Federal Government of Nigeria has sole responsibility for controlling, managing, and regulating fishing activities in the sea, and over inland water bodies shared by two or more States, while the separate State governments exercise authority over water bodies within a single State. These State governments typically implement a co-management system that entrusts residents around inland waters with management and conservation responsibilities, including overseeing fishing operations and implementing

fisheries management plans. However, a paucity of data on catch composition, species diversity and fish yield currently make it impossible to implement the conservation policies outlined in the Act.

Previous studies investigating genetic diversity of wild and native species in Nigeria have been limited but there are a few examples. DNA barcoding using COI has been used to identify freshwater fish species in the Southeast (Nwani et al., 2011b) and Northcentral (Iyiola et al., 2018) regions of Nigeria. Microsatellites have been applied to some species used in aquaculture that originated from Africa. Ukenye and Megbowon (2023) compared the genetic diversity in farmed *O. niloticus* and unidentified wild tilapia in Nigeria using microsatellite markers. In a similar study, Ukenye et al. (2016) assessed the genetic diversity of *Tilapia guineensis* in 12 coastal populations in the Niger Delta region using microsatellite markers. Awodiran et al. (2019) studied the genetic diversity of two wild *C. gariepinus* populations in Nigeria. These studies have established crucial foundations for genetic assessments of native species. However, there remains a gap in research concerning introduced species and their comparison to native species, including the genetic impacts of fish introductions. ddRADseq has been used in a number of studies on cichlid fish, for example, to assess the genetic structure of introduced *O. niloticus* strains in Tanzania (Kajungiro et al., 2019b; Moses et al., 2020). To my knowledge, the 3RAD approach has not yet been used on catfish.

1.7 Conclusions

Technological breakthroughs have led to a dramatic increase in recent years in the feasibility of using molecular markers to measure genetic variation in natural and domesticated populations. This is very relevant in the context of fisheries management and aquaculture of non-native species, since these tools provide the potential for gaining insights into population dynamics, genetic diversity, and potential risks associated with fish introductions or escapes. These markers allow for accurate identification of species and cultivated strains, gene flow monitoring, and assessment of hybridisation, which can be used to develop effective conservation strategies and sustainable aquaculture practices. The utilisation of these molecular techniques will be beneficial in offering insights into the species identification, genetic variability, population dynamics, and

genetic divergence that is lacking in economically important fish species in sub-Saharan Africa like tilapias and catfish.

1.8 Thesis aims

The overall aim of this thesis is to investigate the genetic diversity and population structure of two economically important tilapia species including the genetically improved farmed tilapia introduced from Egypt and Thailand for aquaculture purposes, their native wild counterparts, as well as farmed and wild African catfish (*C. gariepinus*).

In Chapter Two, I present a review on the potential impact of aquaculture on the genetic diversity and conservation of wild fish in sub-Saharan Africa. I critically evaluate the fisheries and aquaculture policies in Ghana, Kenya, Malawi, Nigeria, South Africa, Tanzania, Uganda, and Zambia, specifically looking at how they are addressing the potential risks of fish escapes including introgressive hybridisation between farmed and wild species, risk assessment and risk management measures when introducing aquaculture species, and provided a regulatory framework that could be adopted to minimise these risks. This chapter has been published in *Aquatic Conservation: Marine and Freshwater Ecoystems* (Sanda et al., 2024).

In Chapter Three, I use mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) marker to identify several introduced farmed and wild tilapia species that exhibit comparable physical characteristics, including body shape, colouration, fin structure, and scale patterns, which can result in misidentification when relying solely on external features. Additionally, there is a lack of knowledge regarding the haplotype diversity and phylogenetic relationships among these tilapia species. This study aims to explore the genetic diversity and phylogenetic relationships among introduced Genetically Improved Farmed Tilapia (GIFT) from Egypt and Thailand and wild native tilapia populations in Nigeria using COI. The specific aims of this study are to:

i. Confirm the taxonomic identity of each sample using the BOLD database, to identify which species occur in wild and farmed populations across Nigeria
ii. Determine the diversity and distribution of mtDNA haplotypes of farmed and wild tilapia sampled

iii. Investigate the phylogenetic relationships between introduced genetically improved aquaculture fish and native wild tilapia species in Nigeria, compared to the global distribution identified from the BOLD database.

In Chapter Four, I address concerns about the widespread introduction of non-native selectively-bred tilapia which is usually unregulated in Nigeria. Using double-digest restriction site-associated DNA sequencing (ddRADseq), I explore the genetic diversity, population structure and genetic interactions of introduced farmed and native tilapia species in Nigeria. The overall goal is to comprehensively understand the genetic makeup of introduced Genetically Improved Farmed Tilapia (GIFT) and native tilapia populations in Nigeria. The specific objectives are to:

i. Compare patterns of genetic diversity and relative levels of inbreeding of introduced farmed GIFT compared to wild native tilapia populations across different regions of Nigeria
ii. Assess whether patterns of differentiation are consistent with the species classifications of farmed and wild species based on mitochondrial DNA sequencing
iii. Investigate whether there is evidence of introgression between farmed GIFT and wild native populations

By addressing these objectives, I aim to provide valuable insights into the genetic dynamics of tilapia populations in Nigeria, which can inform conservation efforts, aquaculture management strategies, and breeding programs.

In Chapter Five, I use both mitochondrial marker (cytochrome c oxidase subunit I) and genomic perspectives using triple double-digest restriction site-associated DNA sequencing to investigate the genetic diversity and population structure of Nigeria's most important aquaculture species, African catfish (*Clarias gariepinus*). Specifically, this study aims to examine farmed and wild *C. gariepinus* in Nigeria by employing both a DNA barcoding approach (using the mitochondrial cytochrome oxidase I (COI) gene) and 3RAD high-throughput sequencing approach to:

i. Assess whether farmed and wild *C. gariepinus* can be differentiated using mtDNA COI haplotypes

ii. Investigate the mtDNA haplotype diversity and genetic differentiation between farmed and wild *C. gariepinus* as well as their geographical distributions

iii. Assess the genetic diversity within populations of farmed and wild *C. gariepinus* using 3RAD

iv. Investigate the genetic differentiation between farmed and wild populations using 3RAD

In Chapter Six, I provide a general discussion, conclusions, and recommendations for future studies.

Chapter 2 [1]The potential impact of aquaculture on the genetic diversity and conservation of wild fish in sub-Saharan Africa

Abstract

An increasing focus on aquaculture using introduced strains or species poses a serious threat to native wild species in sub-Saharan Africa, yet almost no policies have been enacted, or regulations put in place, to address this environmental challenge. Aquaculture in these regions has traditionally been conducted on a relatively small scale, but it is currently expanding rapidly and is projected to continue increasing in the coming decades, with increasing use of genetically improved strains. This expansion is occurring in a region known for its high biodiversity, creating challenges for increasing fish production without damaging wild fish populations. However, few studies have yet assessed the impacts of changes in aquaculture practice on the genetic composition and diversity of wild populations. The use of non-native improved strains for aquaculture could cause competition, gene introgression when there is interbreeding with native populations or species, displacement of species and possible extinction of the native wild populations. After providing historical context on African aquaculture, this review describes the current methods used for fish breeding and genetic improvement programmes for the main species of cultured fishes, focusing on the potential conservation impacts of the use of introduced (and selectively bred) farmed species. Existing aquaculture policies, legislation, and regulations regarding the import and farming of fish are then compared across the main fish-producing countries. We recommend a regional policy framework which considers how fish introduction, risk analysis and risk management, human resources development and genetic monitoring that could be drafted into the existing policies to strengthen conservation efforts. We conclude by making recommendations for refining existing regulations and for future research aimed at minimising the impacts of aquaculture on wild fish populations in sub-Saharan Africa. Aquaculture in this region needs implementation of responsible

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guidelines to avoid genetic impacts on native populations of high conservation value.

2.1 Review approach

This study reviewed how aquaculture, including the culture of introduced non-native farmed fish, could genetically impact native wild populations through gene introgression, competition, displacement, and extirpation. We conducted a literature search on fisheries and aquaculture policies with respect to: i) the history of fish introduction and their impacts in sub-Saharan Africa; ii) aquaculture practices for commonly cultured species, including breeding programmes implemented; and iii) escape events and preventive measures. Inferences from well-developed aquaculture countries like Norway, the United Kingdom and the United States were drawn, citing escapes of rainbow trout and Atlantic salmon as guidelines for addressing these problems in sub-Saharan Africa. Reviewed documents, including grey literature on fisheries and aquaculture policy in sub-Saharan Africa, were obtained from the respective governments, fisheries and aquaculture websites, but also included policy documents not available online that were obtained via email communications with relevant organisations. Online searches on Google Scholar, Web of Science, and the University of Glasgow online library used the following keywords: “fish escape”, “fish introduction”, “fisheries and aquaculture policies in sub-Saharan Africa”, “impact of aquaculture on the genetic diversity of native fish”, “fish breeding programmes in Africa”.

2.2 Global aquaculture production: History and status

Aquaculture is believed to have started earlier than 1000 BCE in China, with the common carp (*Cyprinus carpio*) being the first species to be held in captivity for food (Rabanal, 1988). However, aquaculture remained a low-density non-intensive means of rearing fish for many centuries, until the late 20th century, when it started a transition into a modernised intensive form of food production as a result of advances in technology and global information-sharing (Jones, 1987). Progress continues regarding technological developments in culture systems, genetic improvement of species through selective breeding, and feed production (Naylor et al., 2021). The most intensive rearing regimes include recirculating aquaculture systems that allow effective economies of scale and

result in the highest production of fish per unit area (Ebeling and Timmons, 2012). Recent decades have seen steady increases in the proportion of farmed fish that gain their nutrition from manufactured feeds, rather than from food generated within the water body in which they are living (Naylor et al., 2009). The associated feed industry is witnessing a drastic change, including new technologies such as the biofloc system, which converts nitrogenous waste from feed into microbial biomass that can be immediately used by fish or shellfish harvested and processed into feed ingredients (Avnimelech, 2009; Bossier and Ekasari, 2017; Kuhn et al., 2010). There have also been more gradual changes away from a reliance on marine fishmeal and fish oils and towards plant-based feeds (Naylor et al. 2009, 2021).

The past four decades have been significant for global aquaculture development, with the sector recording an average annual growth rate of about 8.6% from 1980 to 2012 (FAO, 2014). Fish are an important source of food security and contribute 15% of the total animal protein in human diets globally (Casal, 2006). The increasing human population exerts high fishing pressures on wild fisheries, and there has been a shift to reliance on farmed fish production as an alternative (Ahmed et al., 2019; Naylor et al., 2000). Aquaculture is not, however, without its inherent challenges, which unfortunately are more evident in lower and middle income (LMIC) countries where there has been less investment. Moreover, these countries tend to have greater focus on freshwater aquaculture, which is projected to expand more than cultivation in marine environments (Belton et al., 2020; Zhang et al., 2022). Since many of the problems associated with the expansion of aquaculture relate to its environmental impacts, there is clearly a risk that LMIC countries will experience disproportionate environmental pressures in the drive to increase their production of farmed fish. In this review, we summarise the current state of freshwater aquaculture in sub-Saharan Africa, including its regulation, and provide an assessment of potential environmental risks (with an emphasis on the genetic impacts) and the approaches that could be taken to minimise them. These issues are particularly pertinent to this region given its rich endemic fish diversity and the rapid expansion of aquacultural activities (Lind et al., 2012).

2.3 Aquaculture development in sub-Saharan Africa

Fish farming first started in colonial areas of sub-Saharan Africa in the 1940s and 1950s, with the establishment of aquaculture research stations in the Republic of the Congo, Democratic Republic (DR) of the Congo, Central African Republic, Cameroon, Côte d'Ivoire, Kenya, Madagascar, Uganda, Zambia, and Zimbabwe (Brummett et al., 2008). The initial aim was to produce fish for sport and food fishes to supplement the diets of plantation workers (Pouomogne and Brummett, 2004). It was also accompanied by substantial investment to support its development (Adeleke et al., 2021; Brummett et al., 2008). The focus was primarily on subsistence-level, pond-based systems (Blow and Leonard, 2007), since few local people could afford the investment needed for intensive large-scale production. Fish production fell and remained low for several decades after these countries became independent from colonial rule, due to the new governments' failure to maintain investment in aquaculture (Pouomogne and Brummett, 2004). In 1975, the Food and Agriculture Organisation of the United Nations (FAO) organised the First Africa Regional Workshop on Aquaculture, to gauge the extent of aquaculture sustainability in the region and to assess the level of support given to the sector by African governments (Coche et al., 1994). Commercial aquaculture was initially slow to develop, with over half of the African countries, including the top producers in the region, like Nigeria, Madagascar, and Zambia, reportedly producing less than 100 tonnes of fish annually by 1975 (Moehl and Machena, 2000). African countries south of the Sahara have contributed less than 1% to total global aquaculture output over the last decade (Mapfumo, 2022).

The slow growth of sub-Saharan aquaculture has been linked to the lack of a market-driven agenda and governance limitations (Satia, 2011). Production has also been hampered by fish diseases triggered by poor water quality and suboptimal farm management practices (Ragasa et al., 2022). There are very few hatcheries producing fingerlings for other farmers (Anetekhai et al., 2004), and prices of imported, high-quality feed have risen steeply in recent years, with few options for alternative cheaper locally produced feeds. Some countries have been receiving financial aid from the international community to support the development of small-scale commercial aquaculture. For example, the International Fund for Agricultural Development (IFAD) announced in 2020 a US$

49 million project in Mozambique aimed at moving the aquaculture sector from a subsistence to a commercial level (Moyo and Rapatsa, 2021). Possibly as a result of such initiatives, production in sub-Saharan Africa has increased markedly in recent decades, with the total production reaching 550,000 tonnes by 2014, mostly consisting of freshwater fishes such as catfishes and tilapias (Subasinghe, 2017). While production in Africa is still at a relatively low level overall, it has increased by 9.8% per annum during 2000–2017, faster than the 5.8% world average (FishStatJ, 2019) and faster than on any other continent (Garlock et al., 2020). In particular, aquaculture growth in sub-Saharan Africa has been on the rise since 2000, with average production increasing by 11% p.a., more than twice the world's average (Ragasa et al., 2022).

Production in terms of quantity of fish produced and financial value is currently dominated by Nigeria, Uganda, Tanzania, Ghana, Zambia, Madagascar, Kenya, and Malawi (FAO, 2020). These countries have built aquaculture infrastructures through interventionist programmes such as the National Institute for Freshwater Fisheries Research (NIFFR) in Nigeria, the National Aquaculture Centre in Malawi, and the National Aquaculture Research Development and Training Centre in Kenya. These centres serve as research institutes for providing high-quality fish fry and broodstock to local farmers, and so have proven pivotal to regional aquaculture development (Jamu et al., 2012).

Successful and profitable aquaculture production relies on the supply of good-quality broodstock and fingerlings (Nadarajah and Flaaten, 2017). There is thus a demand for genetically improved strains that are tolerant to a wide range of environmental conditions, have a good feed conversion ratio, are disease-resistant, and are capable of attaining marketable size within the stipulated production period. However, improved strains are in short supply, since the fisheries and aquaculture research institutes responsible for providing good fish eggs and fry have been overwhelmed by farmers' requests and are unable to meet all of their demands (Munguti et al., 2014; Shikuku et al., 2021). Broodstock are rarely sourced from the wild, and developing alternative sources of broodstock from wild-harvested stocks is threatened by unsustainable fishing practices. Moreover, seasonality in most rivers and lakes that rely on rain-fed water makes it difficult to find ready-to-breed adults in the wild (Charo-Karisa et al., 2012; Muringai et al., 2022; Ponzoni and Nguyen, 2008).

2.4 Fish breeding

The African catfish (*Clarias gariepinus;* Family Clariidae) and the Nile tilapia are among the most important freshwater fisheries and aquaculture species in Africa (El-Sayed and Fitzsimmons, 2023; Munguti and Iteba, 2022; Munguti et al., 2022; Nankinga et al., 2022). The two species have been cultured together under mixed-species (polyculture) farming (Mandal et al., 2014; Shoko et al., 2015). *C. gariepinus* and *O. niloticus* are native to Africa but are now being bred for mass fingerling production following their successful domestication (Ponzoni and Nguyen, 2008). Some of the advantages of *Clarias gariepinus* over other freshwater fish species include rapid growth rates that result in attainment of marketable size within six months (Trofymchuk et al., 2021). Other reasons for their culture include tolerance to a wide range of environmental conditions and good feed conversion rates (Abraham et al., 2018).

The farmed cichlids referred to as tilapias are actually comprised of multiple species, notably including: *O. niloticus*, *O. aureus, Coptodon zillii,* and *Sarotherodon galilaeus. O. niloticus* is the most successfully cultured of these species due to its fast-growth, tolerance of harsh environmental conditions and ease of breeding in captivity (Galemoni de Graaf and Huisman, 1999). The WorldFish selective breeding programme for this species has further increased farmers' interests in its culture (El-Sayed and Fitzsimmons, 2023; Henriksson et al., 2017; McAndrew, 2000).

Every successful breeding programme depends on the farmers' ability to select the right broodstock and apply the appropriate techniques to induce reproductive activity and spawning (Moorhead and Zeng, 2010). Since the aquaculture sector in sub-Saharan Africa is dominated by small-scale farmers, they must rely on low-level hatchery technology for fish breeding (Adeleke et al., 2021; Kajungiro et al., 2019a). However, minimal or absent regulatory frameworks to control indiscriminate breeding and require confinement pose significant threats to wild populations and can compromise one of the crucial aspects of the breeding objectives, which is to preserve the genetic resources within the species/breed (Farstad, 2018). Farmed tilapias and catfishes are produced in sub-Saharan Africa by very different methods in terms of the techniques involved, hatchery facilities required, and levels of investment

needed (Chaube, 2023). If these processes are unregulated or unsupervised, fish breeders who lack basic genetic knowledge are at risk of making poor breeding decisions that may harm their production stock, as well as native populations and species if the cultured fish escape to the wild.

2.5 Catfish breeding

Breeding of catfish species (principally *Clarias gariepinus*, but also *C. anguillaris* and *Heterobranchus bidorsalis*) is induced by hormone treatment (Madu and Offor, 2005). Gravid females, usually at least nine months old, are obtained from hatcheries and transferred to a holding facility (tanks or ponds), where they are held before breeding. Male *Clarias* become mature when about a year old, but male *Heterobranchus bidorsalis* take longer to attain maturity (Legendre et al., 1992). The chosen broodstock (*Clarias* spp. or *Heterobranchus* spp.) are starved for 24 hours before breeding, after which the female is injected intramuscularly with 0.5ml/kg ovaprim (Syndel, USA), to facilitate ovulation (Marimuthu, 2019). Injected females are held in a separate pond from the breeding males. At an optimum temperature of 27-30 °C, the female will be ready for egg stripping in about 12 hours. Eggs are collected into a sterilised bowl; the male is then sacrificed and dissected to collect the milt, which is subsequently used to fertilise the eggs. Fertilised eggs are spread on a fine-mesh net placed on the surface of the breeding pond, which is kept aerated by a continuous flow of water from the inlet until hatching is complete.
Eggs hatch within 24 hours, but the free-swimming larvae are sustained by their yolk sac for three days, after which they are fed with shell-free *Artemia* for about 2-3 weeks (Munguti and Iteba, 2022). Catfish are then fed a formulated diet, which comes in various-sized pellets ranging from 0.1 mm to 9 mm in size. Catfishes can then be hatched using locally available resources such as bowls, and jerrycans cut in halves and placed outdoors under shade, so that hatcheries can be built with limited resources without a dedicated building. These methods are commonly used by farmers that cannot afford to build a hatchery with modern facilities, such as sophisticated recirculating aquaculture systems.

Producing hybrid catfish is not an uncommon practice among sub-Saharan Africa fish farmers. Hybrids have positive heterosis for growth rate and are potentially able to interbreed with parental species (Senanan et al., 2004).The most

commonly farmed hybrids are dubbed “heteroclarias”, an inter-specific hybrid of either *Heterobranchus bidorsalis* or *H. longifilis* and *C. gariepinus* (Bartley et al., 2000). Introgressive hybridization of the genus *Clarias* into the native populations could result in (unrecognized) introgressed individuals (Senanan et al., 2004). This process of introgressive hybridization can result in the loss of genetic diversity or coadapted gene complexes for a species, subspecies, or population (Allendorf et al., 2001).

2.6 Tilapia breeding

Captive breeding of tilapia requires more investment in technology and skills than catfish breeding. Selected male and female broodstock are placed in pairs in the breeding nets (Figure 2.1). The male will fertilise the eggs laid by the female; but being mouthbrooders, the female then normally collects the fertilised eggs back in her mouth to start the incubation process (Popma and Masser, 1999). This process is altered by the farmer, who collects the eggs and transfers them to a special incubating system in the hatchery, where eggs are commonly held in a jar or column held over a tray, with water flowing into the jar directly from an inlet at a regulated speed. Once hatched, the fry swim from the jar into the tray and remain there until they are sorted and moved to the nursery tanks, where they receive their first meal, which is usually a fine, powder-like formulated feed. Most farmers choose to keep only male tilapia because the growth of females is reduced once they become sexually mature; males thus produce a faster return on the initial investment (Fuentes-Silva et al., 2013). Therefore, all-male populations have been developed using a sex-reversal process (Chen et al., 2018), which involves feeding fry with feed treated with the hormone 17 α-methyltestosterone (MT) (Abucay and Mair, 1997). This provides control of reproduction and prevents the unwanted breeding that leads to overcrowding. For example, the nonsteroidal aromatase inhibitor Fadrozole incorporated into the Nile tilapia feed at 50, 75 and 100 mg/kg dosages produced a population of between 67-100% males (Afonso et al., 2001). Similarly, methyltestosterone treatment at a dose of 50 µg/g diet from 8-30 days after hatching resulted in 100% male Nile tilapia (Bhandari et al., 2006). Sex-reversal has been successfully carried out on several species of mouth-brooding tilapias, including *O. aureus, O. mossambicus, O. hornorum* and the red

tilapia, which is a diploid interspecific hybrid between *O. mossambicus* and *O.*

Figure 2.1 Tilapia broodstock unit in Abeokuta, Nigeria, made with blue mesh netting and installed in an earthen pond in which the fish lay and fertilise their eggs. The female tilapia starts the incubation by carrying the fertilised eggs in her mouth before eggs are collected and transferred to the hatchery where the incubation process is completed.

niloticus (Popma and Green, 1990). The male tilapias grow faster than the females and are desired by farmers; however, sex-reversal does not guarantee to induce sterility and males are still capable of breeding with any remaining females to produce viable embryos (Mair et al., 1997). It is also possible to achieve monosex tilapia population by crossing genetically modified super males with YY sex chromosome and normal females (XX) or genetically feminised males (XY) following oestrogen treatment (Fuentes-Silva et al., 2013).

2.7 Producing triploid farmed fish as control measure for genetic contamination

Many fish farms in sub-Saharan Africa are vulnerable to fish escapes, often because they lack barriers or screens (an especial problem with earthen ponds – see Figure 2.2). In this situation the production of sterile farmed fish would be advantageous for the conservation of wild fish and their gene pool (Chen et al., 2023). Partial or complete sterility can be achieved in farmed fish through the induction of triploidy. This is a chromosomal manipulation process that involves impairing or suppressing the second meiotic division through use of chemicals, heat, pressure, or electric shock of the fertilised eggs; the process produces infertile fish that would avoid any genetic impact on the wild population if they escaped (Arai and Fujimoto, 2018; Marx and Sukumaran, 2007; Okomoda et al.,

2020; Pradeep et al., 2012). Ploidy manipulations also can be applied to farmed

Figure 2.2 An earthen pond used for the culture of introduced tilapia with low embankment and poor screen netting material.

fish to improve their growth and survival (Pandian and Koteeswaran, 1998). The use of triploid fish in aquaculture would negate the problem associated with early sexual maturation and minimise the main genetic concerns potential escapees might pose to wild populations (Farstad, 2018; Iversen et al., 2016). This method has been demonstrated to be effective in *C. gariepinus* using both cold shock and heat shock on fertilised eggs (Marx and Sukumaran, 2007) and also in red hybrid tilapia *O. niloticus* x *O. mossambicus* (Pradeep et al., 2012). Triploidy has long been recommended as one of the best possible solutions for controlling the problem of early sexual maturity and unwanted reproduction in cultured tilapia (Mair, 1993). However, even after decades of inducing triploidy for practical applications in aquaculture, the approach is yet to be utilised at commercial scales in sub-Saharan Africa (Chen et al., 2023). It appears that there is a gap in implementing new and innovative ideas that could potentially benefit the farmers while also protecting the diversity of native species. Most farmers might not be aware of or understand how the triploid technology works and this lack of awareness may be attributed to ineffective communication, knowledge-sharing, and training by fisheries research institutions to local breeders.

2.8 Introductions of non-native species or improved strains

The first introductions of non-native fish species to sub-Saharan Africa occurred between the late 1890s to early 1900s, when brown trout *(Salmo trutta*) were introduced from the United Kingdom and France into South Africa, Kenya, Malawi and Zimbabwe (Weyl et al., 2017). The motive surrounding the initial introduction into sub-Saharan Africa of fishes from outside the region was to promote sport fishing, alongside increasing fish production for human consumption (Ogutu-Ohwayo and Hecky, 1991; Weyl et al., 2017). Following their successful breeding and establishment in South Africa, brown trout were distributed to neighbouring Swaziland (1915), Lesotho (between 1907 and 1914), Zimbabwe (1907), and Tanzania (1934) (Welcomme, 1988). The period from 1940 to 1950 was an era characterised by unsuccessful rainbow trout (*Oncorhynchus mykiss*) introductions to Congo, Sudan, and Zambia (Crawford and Muir, 2008), with its success being linked to unsuitable temperatures, acidic waters, lack of breeding grounds, seasonal droughts, and predation (Crawford and Muir, 2008; De Moor and Bruton, 1988).The unsuccessful introduction hindered the establishment of rainbow trout as one of the main aquaculture fishes in sub-Saharan Africa, although South Africa and Kenya have continued to farm both brown and rainbow trout (Stander et al., 2011) but in Kenya, it is still done on only a small scale, constituting one percent of total aquaculture production in 2009 (Munguti et al., 2014). Although both rainbow and brown trout are currently farmed in a number of African countries (Bjørndal and Tusvik, 2019; du Preez and Lee, 2010; Munguti et al., 2014), their culture in most parts of Africa is yet to become widespread and continues to remain secondary as farmed species relative to *O. niloticus* and *C. gariepinus* (Kaleem and Bio Singou Sabi, 2021; Munguti et al., 2022).

The period between the mid-1950s and late 1970s witnessed the introduction of mainly freshwater farmed fishes both from within and outside the continent of Africa (Brummett et al., 2008; Welcomme, 1986), but cichlids and cyprinids dominated the list of introduced species. For example, Welcomme (1988) documented intentional introductions both within and outside the native range of multiple species (Table 2.1). Cyprinids (e.g., *Carassius auratus, Catla catla, Cirrhinus mrigala, Ctenopharyngodon Idella, Cyprinus carpio, Labeo rohita*) also

were translocated from India, Indonesia and Israel to Cameroon, Central African Republic, Egypt, Ethiopia, Ivory Coast, Kenya, Mauritius, Nigeria, Rwanda, South Africa, Sudan and Tanzania. The introduction of cichlids was more successful than that of salmonids, a fact that was attributed to their tolerance to variable water quality and ability to survive in both freshwater and marine environments (Canonico et al., 2005).

Table 2.1 Early introduction of freshwater fish in sub-Saharan Africa

Period	Event
1956	Introduction of *Oreochromis niloticus* to Madagascar from Egypt.
1962	Introduction of *O. aureus* from Israel to Uganda.
1968	*O. andersoni* was introduced to Tanzania from Zambia.
1972 – 1973	African catfish (*Clarias gariepinus*) was introduced from the Central African Republic to Cameroon, Congo, Gabon, Ivory Coast, and Zaire.
1976	Introduction of *O. aureus* and *O. niloticus* to South Africa from Israel.

The most significant recent introduction was selectively-bred Genetically Improved Farmed Tilapia (GIFT) strain of Nile tilapia. The GIFT tilapia strain was developed from pure native Nile tilapia stocks from Egypt, Ghana, Kenya, and Senegal together with commercial experimental strains from Israel, Singapore, Taiwan, and Thailand (Pullin et al., 1991). They were first introduced to Africa as a result of the WorldFish Center's official distribution of the strain to the Water Research Institute, Ghana in 2012, solely for research purposes (https://worldfishcenter.org/pages/gift/). GIFT is a strain of *O. niloticus* developed from selective breeding programmes initiated by the International Center for Living Aquatic Resources Management (ICLARM, later re-named WorldFish) in what started as a ten-year (1988-1997) collaborative project with the Institute of Aquaculture Research in Norway (also known as AKVAFORSK), the Philippines National Freshwater Fisheries Technology Research Center of the Bureau of Fisheries and Aquatic Resources, the Freshwater Aquaculture Center of the Central Luzon State University, and the Marine Science Institute of the University of the Philippines (Puttaraksar, 2004). For its founding populations, the project used Nile tilapia (*O. niloticus*) sourced from wild populations in Egypt, Ghana, Kenya and Senegal, and farmed populations from Israel, Singapore, Taiwan, and Thailand (Eknath and Acosta, 1998; Yáñez et al., 2020)

WorldFish adopted a selective breeding method similar to the Atlantic salmon (*Salmo salar*) and rainbow trout breeding programmes developed in Norway in the 1970s (Subasinghe et al., 2021). The approach produced improved GIFT with sustained increases in weight-at-age of 10–15% per generation over more than six generations (Dey et al., 2000; Ponzoni et al., 2011). Coupled with the high survival consistently observed in the GIFT strain, the high potential for growth has made it a very attractive genetic resource for aquaculture (Ponzoni et al., 2011). This WorldFish GIFT strain, now in its 20th generation after about 30 years of selective breeding, is transforming aquaculture in sub-Saharan Africa (Trinh et al., 2021).

However, private farms have been importing and breeding the GIFT strain outside of the official dissemination programme run by WorldFish. For example, GIFT farming is currently illegal in Ghana, but was detected in a Ghanaian farm following an unauthorised introduction (Anane-Taabeah et al., 2019). In Nigeria, it was only in 2022 that the first official agreement was signed between Premium Aquaculture Limited and WorldFish to disseminate GIFT to the country in 2023, but the strain was already present in a number of farms (MKS, pers. obs.). It is a similar situation in Malawi, Zambia, Tanzania, and Kenya, where GIFT are becoming an important farmed strain of tilapia (Akongyuure et al., 2015). GIFT is also now widespread in most Southern African countries despite legislation that prohibits their introduction or culture (Moyo and Rapatsa, 2021). This GIFT strain is currently undergoing mass artificial propagation in commercial hatcheries that supply local farmers; these have undoubtedly played an important role in the expansion and transformation of Nile tilapia farming to a more intensive farming system in Ghana, Kenya, Nigeria, Malawi, Zambia, Zimbabwe, Cote d'Ivoire and Uganda (El-Sayed and Fitzsimmons, 2023).

Clarias gariepinus is another important aquaculture species that is endemic to Africa and found in almost all freshwater systems across the continent (Hecht et al., 1996; Van Steenberge et al., 2020). In the 1970s, Dutch researchers developed an improved strain of *C. gariepinus* that was derived from the native populations from Cote d'Ivoire, Central African Republic, Cameroon and Israel (Holčík, 1991). This strain, known as "Dutch *Clarias*", was selected for fast growth, body size and fillet quality (Cambray and van der Waal, 2006) and was subsequently re-introduced into Africa (Holčík, 1991; Huisman and Richter, 1987;

Richter et al., 1987; Welcomme, 1988). There is no record of when the first reintroduction into sub-Saharan Africa was made, but the Dutch *Clarias* is now farmed widely in West Africa (e.g. Cameroon, Ghana, Nigeria), East Africa (e.g. Kenya) and South Africa (Cambray and van der Waal, 2006; Iswanto et al., 2015; Williams et al., 2008).

2.9 Local genetic improvement programmes

Several attempts at selective breeding have been made in the past to diversify aquaculture production using native species, based on the recommendation that diversification of species will boost aquaculture (Oboh, 2022) and minimise negative impacts from the introductions of exotic species (Ross et al., 2008). However, most of these efforts are still at an experimental stage. So far, the most successful genetic improvement programme in sub-Saharan Africa has involved Nile tilapia in Ghana, employing WorldFish GIFT methodology. The selective breeding programme was conducted by Ghana's Aquaculture Research and Development Centre of the Water Research Institute to improve the native "Akosombo" strain of tilapia for farming purposes, and achieved a 30% increase in growth performance by the 10th generation (Anane-Taabeah et al., 2019; Trinh et al., 2021). Kenya, Malawi and Zambia (https://www.worldfishcenter.org/pages/gift/) have also carried out successful selective breeding programmes using the GIFT technology (Ansah et al., 2014; Ragasa et al., 2022). For example, the technology was applied to *O. shiranus* in Malawi, *O. niloticus* in Kenya and the three-spotted tilapia (*O. andersonii*) in Zambia (Trinh et al., 2021).

2.10 Fish escape: Impact of fish introductions

Fish escapes from aquaculture can result from equipment failure, handling and transport operations, predator intrusion, storm damage, flooding (in freshwater systems) and other mechanisms (Hine et al., 2010). Reported cases of cultured fish (including *O. niloticus*) regularly escaping from suspended cages and from bankside ponds are a threat to fish biodiversity and the environment (Moyo and Rapatsa, 2021). These escapes have been linked to poor management, leading to dire consequences such as hybridisation with indigenous species (Gupta et al., 2004). The threats posed by escapes from fish farms include loss of species

diversity, disease and parasites, loss of local adaptive gene variation, displacement of native fish, and challenges to conservation efforts; fish escapes are considered to be a significant factor contributing to the global extinction of endemic species (Gupta, 2002; Latini and Petrere Jr., 2004; Lind et al., 2012; Olden et al., 2007). While not all introduced or escaped fish have an adverse effect on their new environments, many exert ecological, evolutionary, and economic impacts (Cucherousset and Olden, 2011). The introduction of farmed fish into the wild thus can be considered a potential ecological catastrophe (Lévêque, 1996). The relative risks posed by farming non-native or selectively bred species are a function of the chances of escape into the wild, and the magnitude of each escape event is determined by the outcomes of interactions with native species (Naylor et al., 2005).

Nile tilapia have been described as an "aquaculture pest" due to their invasive and aggressive nature, which could negatively impact native populations through dominance in interference competition following an escape (Champneys et al., 2021; Vitule et al., 2009). For example, the introduction of Nile tilapia into important lakes in Brazil led to unpredictably negative consequences, as there was a noticeable decline in native fish production and changes in native population structure (Vitule et al., 2009). In addition, since farmed fishes are usually to some extent genetically-altered through inbreeding, hybridisation and selective breeding, any escape event could compromise the population structure of the wild fish with which they interbreed, including a reduction of their genetic diversity over several generations (Atalah and Sanchez-Jerez, 2020; Bolstad et al., 2017; Bourret et al., 2011; Glover et al., 2010; Hindar et al., 1991; Miralles et al., 2016). Not only are Nile tilapia, the basis for the GIFT strain, more aggressive than most native cichlid species, they have been known to interbreed with closely related species (Gregg et al., 1998).

Introgressive hybridisation of selectively-bred escapees with wild individuals may result in offspring with low fitness, posing the risk of outbreeding depression and loss of genetic diversity among wild populations (Ansah et al., 2014). Examples of this type of negative interaction between different tilapia species are provided by cases of interbreeding between introduced Nile tilapia and both native *Oreochromis jipe (*listed by the IUCN in 2006 as critically endangered; Ref. No. 125652) and *O. leucostictus* in Tanzania (Bradbeer et al., 2019; IUCN,

2022). Repeated hybridisation and gene flow between the cultured and wild species (IUCN, 2022) has led to irreversible loss of genetic diversity, reduced environmental adaptability, fitness reduction and potential local extirpation of wild populations (Atalah and Sanchez-Jerez, 2020; Bourret et al., 2011; Wringe et al., 2018). Another example of negative effects of escapes has been the displacement of South African native *O. mossambicus* from its habitat following hybridisation with introduced *O. niloticus* (Bradbeer et al., 2019; D'Amato et al., 2007; Diedericks et al., 2021). Hybridisation between introduced and native species can lead to reduced fitness that may arise from break-up of co-adapted gene complexes, i.e., disruption of local adaptations that have evolved within the native species over many generations (Muhlfeld et al., 2009).

WorldFish reported that GIFT strains could have escaped and formed feral populations in the wild or contributed genes to wild tilapia populations in Nigeria, although evidence of the adverse effects of hybridisation with native species is yet to be established (Bartley, 2021). The impact of introduced strains on native species is usually difficult to detect at the initial stages of introduction into the wild or escape from farms, and might take a while to become apparent (Vitule et al., 2009). However, depending on the extent of the invasion and the vulnerability of the ecosystem being invaded, the loss of diversity at genetic, population, species and community levels can become evident over time (Erarto and Getahun, 2020). Most of these negative impacts are driven by escapes from farms to the wild. For example, a study conducted in Volta Lake, Ghana using mitochondrial and microsatellite DNA markers found admixed individuals from non-native Nile tilapia from two local farms, indicative of interbreeding between the farmed and wild tilapia populations (Anane-Taabeah et al., 2019). In Zambia, introduced farmed Nile tilapia were identified phenotypically around the Itezhi-tezhi Dam and Kafue River, close to the point of introduction. Further genetic analysis confirmed a high degree of introgression involving the introduced Nile tilapia and two native species, *O. andersonii* and *O. macrochir* (Deines et al., 2014). The Limpopo River of southern Africa serves as an example of the negative effects of the introduction of non-native fish species; pure native *O. mossambicus* has been replaced with red hybrid populations (*O. niloticus* x *O. mossambicus*) throughout the natural range of the native tilapia, and there has been a subsequent loss of genetic integrity since the introduction of *O. niloticus* in reservoirs (Van der Waal and Bills, 2000).

The risk and impact of fish farm escapes on aquatic ecosystems depends on the farming system employed. With intensive aquaculture now occupying a strategic position within the fisheries sector, cage systems are now being employed in important fishing lakes such as the great East African lakes, where the negative impact of *O. niloticus* escapes on native *O. variabilis* and *O. esculentus* has been realised (Wasonga et al., 2017). In 2006, both native species were declared critically endangered by the IUCN (2022) as a result of their hybridisation with *O. niloticus*. Important freshwater fish habitats, such as Volta Lake in Ghana and both Badagry Creek and Lagos Lagoon in Nigeria, are witnessing an expansion in cage aquaculture primarily used for rearing Nile tilapia (Asmah et al., 2016). Cage systems have been associated with fish escapes due to multiple causes, including structural failures (e.g., leaky nets), operational errors, damage due to biological causes (e.g. net-biting), and flooding and storms (Jackson et al., 2015). Employing floating net-pen systems in the farming of improved tilapia strains increases the impacts on native populations because fish releases are almost inevitable from such systems, given that cages have direct contact with the external environment, often are not properly maintained, and are prone to damage (Azevedo-Santos et al., 2011).

Despite clear evidence of harmful outcomes, current policies for aquaculture management often do not include an assessment of the impacts of introducing non-native species for aquaculture purposes. There are often conflicts of interest between the fish producers, who are the proponents of species introduction, and environmentalists, who are more concerned about biodiversity conservation and sustainability – a problem that is not unique to Africa (Vitule et al., 2009). As with other geographic regions (Allendorf, 1991), in sub-Saharan Africa these impacts are blurred by the immediate socioeconomic benefits related to food security and poverty reduction (Kassam 2014), which require improved aquaculture productivity (Anane-Taabeah et al., 2019; Ansah et al., 2014). It is often either the case that the impact has not been measured/assessed or the relevant authority does not envision fish escape as a threat to conservation. There is a risk that the absence of evidence for ecological effects could be substituted in management and policy decisions for evidence of the absence of ecological effects (Ansah et al., 2014; Lövei et al., 2012). Holistic policies would call for specific strategies for risk management

and clearer communication about potential risks (Arthur et al., 2009; Campbell, 2006; Hallerman, 2008).

2.11 Risk assessment and risk management

With growing interest in sub-Saharan Africa related to the use of non-native species for aquaculture as a means to ensure food security and as a source of livelihood (Ansah et al., 2014), it is important to measure the risk factors associated with potential threats to native species. A good risk assessment model assists in decision-making when considering the introduction of new species, and provides a means of assessing the ecological risk posed by the further spread of those introduced fish that are already present (Rowe and Wilding, 2012). The introductory stage of a risk assessment in this context should take into consideration whether the species: is highly domesticated or cultivated for commercial use; can become naturalised where introduced; has invasive relatives; can reproduce across a wide environmental range; has a history of introduction outside its native range in other places; has the potential to out-compete native species; and/or hybridizes naturally with native species (Copp et al., 2005). Lind et al. (2015) provide an in-depth analysis of different risk assessment methodologies in their study of risk analysis in aquaculture based on the outcomes of the Workshop on Risk Assessment Methodologies and Tools for Aquaculture in sub-Saharan Africa. The risk assessment should be designed to include risks associated with changing the genetic composition and genetic diversity of wild populations, such as can arise from introgression, but also through competition with introduced species or strains (Hallerman, 2008). Although there will be inherent uncertainty related to risks associated with incomplete information on species distributions (Copp, Garthwaite, & Gozlan, 2005), important considerations for risk management include consistency in methodology, use of stakeholder consultation, and application of high levels of stringency (Arthur, 2008). Risk analysis, therefore, makes available to stakeholders detailed information on potential risk factors and their causes (Andersen et al., 2022). Applying such precautionary approaches and involving the relevant stakeholders is always a good starting point before deciding to introduce a new species outside its native range (Bartley, 2021).

Conducting risk analysis before any introduction is made will be beneficial to the conservation goal of maintaining the genetic integrity of populations and minimise the transfer of different genetic stocks (Reantaso, 2001). Risk management weighs policy alternatives, in consultation with all interested parties, by considering the risk assessment and seeks the means to reduce either the likelihood of the exposure to hazard or the consequences of harm being realised following exposure (Sumner et al., 2004). It is conducted in two stages: risk identification, where the risks are measured and analysed, and risk treatment, where decisions are made on the next course of action (Sethi, 2010).

2.12 Current aquaculture policies and legislation in sub-Saharan Africa

Global aquaculture has been associated with controversial issues regarding resource management, policy and regulations (Anderson et al., 2019). It is therefore the responsibility of individual governments to adopt, a framework that follows the Food and Agriculture Organization of the United Nations Guidelines for Sustainable Aquaculture (UNFAO, 2022). These guidelines apply principles of genetic management to domesticated aquatic resources, so as to facilitate the implementation of policies, laws and regulations that will promote environmentally friendly, technically feasible, and socially responsible aquaculture. A policy that works and drives sustainable fish production, preventing or regulating activities that could pose threats to species conservation, needs to be based on realistic expectations (Brummett et al., 2008). A good effective policy, for example, would manage the negative impact of escaped GIFT tilapia on other tilapia populations realised through hybridization and genetic introgression (Lind et al., 2015). But such policies are currently not in force in most sub-Saharan countries, which are yet to promulgate and implement policies to address genetic concerns associated with aquaculture activities. Current fisheries and aquaculture policies in most of sub-Saharan Africa were either enacted or reviewed between 2000 and 2015, to address the present-day challenges associated with fish farming while fostering the growth of the aquaculture industry; these are described below for each major country and are summarised in Table 2.2. Aquaculture has been recognised in the sub-Saharan fisheries policies as a viable means towards achieving food security means; however, these legislative documents have not

clearly addressed the impact of introducing non-native fishes as farmed species, particularly as regards the risk of escapes and the potential impact of aquaculture fish in the event of an escape.

2.12.1 South Africa

An exception to policies failing to address the problems of aquaculture discussed above is South Africa, which has one of the best-developed environmental policies and implemented legislation in sub-Saharan Africa. The South African Department of Environmental Affairs (DEA) National Environmental Management: Biodiversity Act 2004 (https://www.gov.za/sites/default/files/gcis_document/201409/a10-04.pdf) mandates guidelines on the introduction of species, and considers the potential genetic impact and adverse effects of such species on wild populations (DEA, 2014). This Act also provides a framework for deciding the aquaculture sites where such introductions can occur. South Africa's Alien and Invasive Species Regulations 2014 provide the general rules on monitoring, control and eradication plans for invasive species, while the National Aquaculture Policy Framework 2013 addresses general aquaculture issues, including decision-making, management and regulation. The Act promotes the management and conservation of both non-native and translocated native species. Likewise, a permit for the introduction of species considered to be alien or invasive can be issued only upon fulfilling the requirements that adequate risk assessment and risk management measures have been taken by the applicant to prevent escape. The policy addresses prohibitive measures by ensuring that any planned introduction must have been found to have negligible or no invasive potential. The section of the National Aquaculture Policy Framework on norms and standards for sustainable aquaculture takes into consideration area-wide planning and zoning, including risk assessments as well as the requirement to obtain permits before farmed fish can be sold. The South African government has identified protected areas considered unsuitable for aquaculture, so as to prevent the introduction of invasive alien species either from farms, conservation projects or angling (Ellender and Weyl, 2014).

Table 2.2 Summary of fisheries and aquaculture policies in those sub-Saharan African countries that are the biggest producers of farmed fish, together with details of introduced tilapia strains, tilapia breeding programmes and native species that are known to be impacted by these activities.

Country	Legislation	Conservation strategy on fish introduction	Introduced strains	Fish escape	Breeding programme	Native species under threat
Ghana	a. The Fisheries Act of 2002 b. Fisheries Regulations of 2010	Prohibition of non-native genetically improved fish culture in cages.	GIFT	Not addressed	Akosombo tilapia genetic improvement programme	*Oreochromis mossambicus, O. niloticus* (Anane-Taabeah et al., 2019)
Kenya	Fisheries Act 2012	i. Farmers need Fish Mover's License for live fish movement ii. Stocking of fish species that were hitherto not present in a water body is prohibited by law	GIFT and *O. leucostictus*	Not available	Selective breeding programme of tilapia	*O. variabilis, O. esculentus* (Wasonga et al., 2017).
Malawi	National Fisheries and Aquaculture Policy 2016	Use of native species and improved strains of the indigenous species for biodiversity conservation	GIFT	Not addressed	Selective breeding programme of tilapia	*O. karongae, O. mossambicus* (Nzohabonayo et al., 2017)
Nigeria	Fisheries Act 2014	Written permission from the Minister is required to import into Nigeria any live fish introduced into any inland water system	GIFT from Thailand, Egypt, the Netherlands,	License is required from the Minister or Commissioner to establish	None	Potential genetic impact on *O. aureus, Sarotherodon melanotheron,*

			and Ghana; Dutch *Clarias*	farms with a surface area of more than one hectare where the escape of farmed fish into the fisheries waters is likely to occur		and *S. galilaeus* (Bartley, 2021)
South Africa	Alien and Invasive Species Regulations, 2014 National Aquaculture Policy Framework 2013 National Environmental Management: Biodiversity Act, 2004	Provides guidelines on the genetic management of domesticated stocks including genetically improved organisms in terms of protecting the natural biodiversity	Tilapias and other cichlids	Not addressed	None	*O. mossambicus* (D'Amato et al., 2007)
Tanzania	National Fisheries Policy of 2015	Not available	*O. niloticus*	The Government conducts surveillance to monitor and control fish escapees	None	*O. esculentus*, *O. jipe* and *O. korogwe* (Bradbeer et al., 2019)

Uganda	National Fisheries Policy 2004	Regulate import and export of fish and fisheries product	GIFT	Not addressed	None	*O. niloticus* and *O. leucostictus* (Diedericks et al., 2021)
Zambia	National Fisheries and Aquaculture Policy 2023	provide for restrictions of the importation of fish	GIFT	Not addressed	Selective breeding of tilapia	Introgression with native *O. andersonii* (Brummett et al., 2008)

2.12.2 Nigeria

The Nigeria Fisheries Act, 2014, is the policy document regulating fisheries and aquaculture activities in the country. It provides the framework for the conservation, management and development of marine fisheries, inland fisheries, aquaculture, and related matters. The Act requires that an individual importing live fish into the country or introducing species into any inland water must obtain written permission from the Minister of Agriculture, under which the Federal Department of Fisheries operates (Nigeria Fisheries Act, 2014). The culture of non-native genetically improved strains is allowed under such licenses. The Minister or Commissioner could issue a license to an individual intending to establish a fish farm with a surface area greater than 1 hectare and at sites close to natural waters where fish escape is likely to occur. Any business or experimental operation involving aquaculture activities, including the processing of aquaculture products, requires the written permission of the relevant authority (Nigeria Fisheries Act, 2014). However, even with recurring cases of fish farm escapes in Nigeria, the country is yet to consider reporting escape events, a monitoring measure that would help the authority build a database of the causes and measures that can be taken to prevent future reoccurrences. Given that this danger to native fish species may not always be clear to the farmers, sensitisation exercises to explain the section of the Fisheries Act on escapes would be beneficial over the long run in support of the management of wild fish populations. Likewise, reviewing the policy to include the conservation genetics of wild fish species could support actions to reduce the risk of population depletion, introgression of non-native gene pools and species extinctions.

2.12.3 Tanzania

In Tanzania, the National Fisheries Policy of 2015 was implemented to address major concerns with aquaculture, including management and control of aquatic resources, knowledge of the fisheries resource base, processing and marketing, research development, extension services, manpower and aquaculture development (http://faolex.fao.org/docs/pdf/tan168881.pdf). This policy is an update on the previous National Fisheries Sector Policy and Strategy Statement of

1997 and identifies challenges within the sector that should be addressed through policy reform (Mwaijande and Lugendo, 2015). The document highlights the potential seriousness of fish escapes, including the surveillance, monitoring and control of fish escapees (The United Republic of Tanzania, 2015). Given that the introduction of Nile tilapia in Tanzania led to the displacement of indigenous tilapia species now considered critically endangered, as highlighted above, stringent measures to prevent future reoccurrence starting with risk assessment and risk management measures before introducing non-native species and setting up a fish farm should be a focal point of the country's policy on aquaculture. However, there is no obligation to report any suspected escape to the relevant authority, a measure employed in countries such as Norway and Scotland that have well-developed fisheries and aquaculture industries, so as to learn and take actions to prevent future escape (Jackson et al., 2015; Thorvaldsen et al., 2015). Mandatory reporting would help assess of the scale of overall escape events and likely causes, and would inform development of guidelines to monitor the risks of escapes in the future (Jensen et al., 2010).

2.12.4 Kenya

Fisheries and aquaculture activities in Kenya are regulated by the Kenyan Fisheries Services. One of their mandates is to regulate and promote the genetic improvement of farmed fish. The Fisheries Act 2012 guides overall fisheries activities, including aquaculture (https://infotradekenya.go.ke/media/Fisheries%20Act%20CAP%20378.pdf). The Act prohibits the import, export and movement of fish from one water body to another unless the person possesses a permit. A special license is required for collection of broodstock for breeding purposes. However, priority is given to researchers who might want to collect fish for scientific research, breeding, or educational purposes. The current law is in place to regulate the introduction and movement of live fish within the country, but there are no measures to address questions related to fish escape and how it should be reported. The Act fails to provide remedial measures like tracing fish escapes, and regulation of facilities like cages installed in natural lakes to raise farmed fish by ensuring that they meet standard requirements to minimise escapes. The Act is also not specific on how to deal with

the impact of farmed fish on wild populations, a highly relevant topic since GIFT are now being cultured in Kenya (Munguti et al., 2022) and could be a threat to native species in the event of an escape.

2.12.5 Ghana

The Fisheries Act of 2002 (Act 625) is the main legislative instrument that governs the practice of aquaculture in Ghana. Section 60 of the Act stipulates that a licence obtainable from the Fisheries Commission is required before setting up any aquaculture project (Awity, 2005; Ghana, 2002). In 2008, Ghana enacted new regulations to augment research capacity to bridge the gap between national fish demand and supply over the medium term (MoFAD, 2015). They subsequently drafted a Fisheries and Aquaculture Sector Development Plan (2011–2016), which outlined the steps taken to implement the Policy, followed by a Marine Fisheries Management Plan (Ameyaw et al., 2021). The Ghanaian authorities prohibit the culture of GIFT in Lake Volta and also mandate that an environmental risk assessment be conducted before installing cages in the lake (Blow and Leonard, 2007). However, there is evidence of farmers producing non-native GIFT in the lake and other aquaculture sites (Anane-Taabeah et al., 2019), which defeats the purpose of the law that prohibits such activities. Good policy and legislation on managing fish escape must be accompanied by enforcement, while ensuring that penalties are enforced on offenders. Only then will proper actions be taken to manage risk, including the reporting of escape events to the relevant authority.

2.12.6 Uganda

While Uganda is one of the leading fish producers in sub-Saharan Africa, its fisheries and aquaculture industries have been beset with problems due to weak legal, institutional and policy frameworks. To address these challenges, the Ugandan authority implemented the National Fisheries Policy 2004 to increase sustainable fish production through properly managing capture fisheries, promoting aquaculture and reducing post-harvest losses (Mugambwa et al., 2021). This policy also provides regulations for the import and export of fish and fisheries products (http://extwprlegs1.fao.org/docs/pdf/uga201565.pdf). The guidelines only highlight how to regulate the introduction of live fish, but since non-native farmed

strains have found their way into Ugandan fish farms, there is a need for the authorities to introduce guidelines and also punitive measures to ensure that where it is prohibited, no such strains are being cultured. Likewise, addressing technical standards for aquaculture facilities and reporting escapes would help prevent future escapes, and enable the government to take swift action to minimise the impacts.

2.12.7 Zambia

Zambia's current principal legal framework for regulating fishing-related activities and aquaculture is the National Fisheries and Aquaculture Policy 2023. This new policy is an update of the previous Fisheries Act No. 22 of 2021 and the Fisheries Regulation No. 24 of 2012 (Shula and Mofya-Mukuka, 2015; Zambia, 2012). The National Fisheries and Aquaculture Policy addresses the issue of illegal introductions of non-native species, poor management of fish breeding areas, and measures to prevent translocation of non-native species to the wild environment; the species that it covers include *O. niloticus, Cherax quadricarinatus* (redclaw crayfish) and *Cyprinus carpio* (common carp). The major challenge of the policy is low compliance due to inadequate personnel to enforce the law (https://www.mfl.gov.zm/wp-content/uploads/2023/06/NATIONAL-FISHERIES-AND-AQUACULTURE-POLICY.pdf). With the thriving *O. niloticus* production industry in Zambia posing a possible threat to native *O. andersonii* (Basiita et al., 2022), there is a need for the implementation of import and movement controls (Ellender et al., 2014).

2.12.8 Malawi

In Malawi, the policy document guiding the management of the fisheries resources prior to 2016 was the National Fisheries and Aquaculture Policy of 2001 (Malawi, 2001). The need for more inclusive management and conservation to promote sustainable utilisation of aquatic resources and income generation led to the development of a new National Fisheries and Aquaculture Policy (https://faolex.fao.org/docs/pdf/mlw190922.pdf) in 2016. This policy addresses more aquaculture-related activities, with an emphasis on the use of native species and improved strains derived only from indigenous species (National Fisheries and

Aquaculture Policy, 2016). However, with the GIFT now available in Malawi, provisions need to be made in the law or implementing regulations made to strengthen the National Fisheries and Aquaculture Policy of 2006 to address cases of fish escapes. Conducting risk assessments and requiring risk management measures before establishing a farm intended for the culture of non-native species such as GIFT (Lind et al., 2015) would minimise the future impact of escapes. It is also important to integrate mandatory reporting of fish escapes to enable early tracking by the relevant authority. Farmers producing non-native species need proper orientation to the dangers of fish escapes and the roles they can play to minimise the risks that escapes pose to fish conservation.

2.12.9 Summary of legislative deficiencies

It is clear that the existing legislation and regulations relating to aquaculture in the relevant countries of sub-Saharan Africa do not effectively safeguard the conservation of wild fish species. In addition to a general failure to consider the risks of fish introductions, there are few regulations on the import of non-native species and genetically improved strains. There is also a lack of policies on facilities monitoring, technical assessment of cages and nets holding the fish, educating farmers on the risk of escapes, reporting every escape as soon as it occurs and implementing consequences for violating existing regulations. Fish escape is taken seriously in countries with long histories of intensive aquaculture such as Norway and Scotland, where escaped farmed Atlantic salmon have been reported to negatively impact wild salmon populations (Thorstad et al., 2008). Lessons can be learned from both countries, where reporting fish escapes as soon as they occur is mandatory (https://lovdata.no/dokument/LTI/forskrift/2022-08-22-1484). Introducing technical assessments of facilities and ensuring that individual farmers have the professional competence to help prevent escapes of fish from aquaculture facilities, as applied in the new Norwegian policy review (NYTEK23, 2022), would be a major boost to the genetic conservation of native wild fish populations in sub-Saharan Africa. Aquaculture policies across sub-Saharan Africa must be designed to take into account aquatic genetic resource conservation in order to protect the declining wild fish populations already threatened by unsustainable fishing practices and environmental change.

2.13 Recommendations for regional policy frameworks

Having highlighted some of the threats that fish introduction can cause to native wild populations, it is therefore important that the relevant authorities enact policies that follow guidelines such as those that the Commission on Genetic Resources for Food and Agriculture mandates for promoting the sustainable use of resources for food security and human well-being (FAO, 2019b). In order to minimise future risks from the introduction of non-native farmed fish, we suggest that a holistic policy centred on the conservation of aquatic genetic resources should incorporate the following recommendations:

i. Risk assessments should be conducted as a precautionary approach to estimate the likelihood of contamination of the genetic pool of wild fish following exposure to farmed fish, and risk management measures should be in place to minimise the impacts in the event of escapes (Hallerman, 2008).
ii. Genetic diversity indicators, including numbers of species, geographical distributions of native species and DNA-based monitoring, should be combined with information on the locations of fish farms sites when considering the risks posed by farming introduced species (FAO, 2019b; Hoban et al., 2020).
iii. The farming of non-viable monosex or sterile triploid stocks should be considered in sites where escapes are likely (e.g. outdoor ponds with no barriers) (Mair et al., 1997).
iv. Aquaculture sites should be regularly monitored to ensure the compliance and implementation of the enacted policies.
v. Farmers and fishers should receive training in genetic resource management and conservation, provided by Government fisheries and aquaculture research institutions (FAO, 2019b).
vi. There should be a greater emphasis on capacity building and investment in research and development, so as to monitor genetic resources and anticipate the effects of any proposed introduction of non-native fish species or strains (Allendorf, 1991).

2.14 Recommendations for future research and policy development

Aquaculture involving production of non-native species is a potential threat to wild fish conservation. The impacts of farmed fish on wild populations can therefore only be minimised if relevant authorities adopt risk assessment and management strategies that will reduce the number and impact of escapees. Thorstad et al. (2008) recommended that the first management action is to understand the causes, circumstances, and sources of fish escape to identify relationships between particular culture technologies, techniques and site locations and escapes. We provide a summary of the common features of aquaculture activities that lead to fish escapes in Table 2.3, along with suggestions for how either the probability of escapes or their impact can be reduced. Dealing with uncertainties posed by fish farms that could threaten the genetic diversity of native species requires adopting best management practices, which includes setting up minimum standards in terms of policy, choice of cultured species quality of rearing facilities and implementation of effective confinement. Ensuring the conservation of native wild fish genetic diversity in this era of rapid aquaculture development should be a priority. Hybridisation between farmed and wild species can be controlled only if hybrids involving genetically improved strains stocked in the farms are non-viable.

Currently there is limited information on the genetic diversity of native fish species in sub-Saharan Africa. Efforts aimed at conserving fisheries genetic resources must address this knowledge gap and place greater emphasis on research development in applied population genetics in the various country's fisheries and aquaculture policy documents. There is a need to investigate species distributions and conduct more research on genetic monitoring of the population structure of native species, as this is the best approach to detect interbreeding between farmed and native species (D'Ambrosio et al., 2019).

As a supporting approach, Lind et al. (2012) recommended a combination of geographical zoning, environmental risk analysis and molecular characterization approaches as the best overall strategy to minimise potential genetic contamination from farmed fish to wild populations. WorldFish has developed

detailed guidelines for using GIFT; these were presented at the Workshop on Risk Assessment Methodologies and Tools for Aquaculture in Sub-Saharan Africa, and adopt the principle of responsible introduction before transferring the strain to any country (Lind et al., 2015). While implementation of these guidelines and regulating the introduction of farmed fish is the responsibility of the individual countries, which must ensure that effective policies are in place to address the potential threats of fish introduction to the conservation of their native wild populations, there also should be consideration of cross-border regulations for countries that share water bodies.

2.15 Conclusions

In conclusion, most of the top fish-producing sub-Saharan African countries have policies and legislation that clearly outline the importance of the conservation of fish species so as to increase fish production and provide households with an inexpensive source of protein (Table 2.2). However, these policies omit key details, and alone do not ensure effective outcomes, since government competence is judged by the effective implementation of these policies (Mugambwa et al., 2021). If responsible aquaculture practices are to be adopted across sub-Saharan Africa, there is a need for the implementation of policies enshrined in the fisheries and aquaculture legislation. Some sub-Saharan countries, like Nigeria, Ghana, Tanzania and Zambia, have updated their fisheries and aquaculture legislation to address the present-day challenges while increasing fish production, and Nigeria and Tanzania have recognised the need to address fish escapes. However, more effort needs to be placed on implementation and surveillance, as well as a more specific focus on understanding genetic impacts on wild populations.

Table 2.3 Features of aquaculture activities that influence the likelihood of fish escape, and the mitigation strategies that could be put in place to regulate these events, including potential minimum requirements a farm must have to comply with these strategies.

Aquaculture feature	Mitigation strategy	Minimum requirement
Introduction of non-native fish species	a. Establishing biosecurity measures and ethical guidelines on fish introduction and preventing illegal introduction through regulation. b. Establishment of protected areas where farmed fish must not be cultured, so as to prevent escapes from farms into native waters of conservation importance.	i. Conduct a risk assessment and management analysis to investigate the genetic and ecological impact of the prospective species. The goal is to reduce the introduction risk to the bare minimum. ii. An Act or decree regulating aquaculture fish introductions, covering both translocations within a country from one water body to another and introductions that transcend political borders. iii. Up-to-date database for introduced fish and stocking destinations.
Culture facilities	a. Farms must have effective screens or physical barriers to prevent escapes. b. Cages must be made from reliable materials to withstand attacks	i. Facilities must be certified suitable for the culture of non-native species, so as to minimise negative interactions with native species. ii. The freshwater Fish Invasiveness Screening Kit (FISK) risk

	from fish predators and heavy storms without degradation. c. The use of recirculating aquaculture systems (RAS) to minimise fish escape. d. Public education and farm monitoring programmes.	assessment method to be used to evaluate invasion risks and classify fish under threats of invasion (Almeida et al., 2013; Marr et al., 2017).
Species/Strains	Use of sterile populations such as triploid fish that lack the ability to reproduce, so eliminating the risk that escaped farmed fish can hybridise with native species (Muir and Howard, 1999).	Establishing and stocking in farm exclusion zones and implementing higher biosecurity measures (Thorstad et al., 2008; Xu et al., 2023).
Escape reporting	Documentation of the nature, scale and timing of any farm escapes	Provide information to farmers on the appropriate channel to report escape events for early detection (Wasonga et al., 2017). Requirement for immediate reporting of the situation, with any remedial actions taken.

Chapter 3 Genetic diversity of farmed and wild tilapia in Nigeria based on analysis of the mitochondrial Cytochrome c Oxidase subunit I (COI) gene

Abstract

The tilapia aquaculture industry in Nigeria relies heavily on the introduction of Genetically Improved Farmed Tilapia (GIFT) strains to enhance production efficiency and meet the increasing demand for fish as a cheap protein source. If not properly regulated this ongoing introduction of non-native GIFT could pose a potential threat to native species including *Oreochromis niloticus, O. aureus, Sarotherodon galilaeus, S. melanotheron, Coptodon zillii* and *C. guineensis*, due to hybridisation and genetic introgression. Many tilapia species share similar morphological traits, such as body shape, colouration, fin morphology, and scale patterns, which can lead to misidentification when relying on external features alone. Also, an understanding of the haplotype diversity and ancestral relationships of these tilapia species is lacking. In this study, I investigated the genetic diversity and phylogenetic relationships of introduced GIFT from Egypt and Thailand and wild native tilapia populations in Nigeria, using the mitochondrial DNA (mtDNA) marker cytochrome c oxidase subunit I (COI). The analysis revealed significant differences in haplotype diversity between farmed and wild populations, with wild populations having higher levels of genetic diversity compared to farmed populations. Using the Barcode of Life Database (BOLD), all haplotypes were assigned to named species, including those that could not be identified morphologically, with similarity scores ranging from 99.62 to 100%. Phylogenetic reconstruction showed a separation of tilapia species based on their breeding habits, with mouthbrooders (*Oreochromis* spp. and *Sarotherodon* spp.) forming a separate cluster from the substrate spawners (*Coptodon* spp., *H. fasciatus,* and *P. mariae*). Although bootstrap resolution was limited, as might be expected for adaptively radiating cichlids, some of the named species were not monophyletic and could represent misidentifications in BOLD or cryptic diversity. In conclusion, species identification using DNA barcoding is important, especially in cases of taxonomic uncertainty or cryptic species complexes, as a first point for conservation of wild species and

understanding the origin of farmed strains. A further investigation on the genetic diversity and population structure of farmed and wild species is needed to understand the interactions between the two and detect possible escapes or hybridisation in the wild.

3.1 Introduction

Tilapia represent a large number of freshwater fish species within the family Cichlidae that are used for aquaculture purposes (El-Sayed, 2019; Trewavas, 1982; Trewavas, 1983). They are native to Africa, Jordan, and Israel, with more than 70 identified species (Macintosh and Little, 1995; McAndrew, 2000; Philippart and Ruwet, 1982) and have been classified based on their reproduction, feeding habits and biogeography as belonging to the genera *Oreochromis*, *Sarotherodon,* and *Tilapia* (Popma and Masser, 1999; Viki et al., 2016). *Oreochromis* are maternal mouthbrooders, *Sarotherodon* exhibit biparental and paternal mouthbrooding behaviour, while *Tilapia* are substrate spawners (Trewavas, 1981; Trewavas, 1982). However, they share similar morphological characteristics and have a rapid rate of evolution that has made their taxonomic classification difficult (El-Sayed, 2019). Commercially important species include *Tilapia rendalli, Sarotherodon galilaeus*, *S. melanotheron*, *Oreochromis mossambicus*, *O. urolepis hornorum*, *O. niloticus*, *O. aureus*, red tilapia hybrids obtained from crossbreeding between either *O. mossambicus* or *O. hornorum* with *O. niloticus* or *O. aureus*, and hybrids between *O. niloticus* x *O. aureus* (Webster and Lim, 2006).

Tilapia are important species for fisheries, aquaculture and studies of evolutionary biology (Rometsch et al., 2020) and are mainly found in freshwater but some species such as *O. mossambicus S. galilaeus, C. zillii* and some red hybrids like the *O. urolepis hornorum* can thrive in saltwater (Stickney, 2017; Yue et al., 2023). They are highly adaptable to different culture systems such as low-density ponds, cage culture, raceways, and super-intensive culture units (Gutierre et al., 2014; Prabu et al., 2019). One of the most successful members of the tilapia complex is the Nile tilapia (*O. niloticus*), which is prioritised for aquaculture: in 2007 it accounted for almost 280,000 t (33%) of the total wild tilapia production (El-Sayed, 2019). It is among the world's top three aquaculture species, contributing 9% of the total fish production in 2020 (FAO, 2022).

There is a growing interest in tilapia aquaculture in Africa, with successful selective breeding programmes being conducted in Egypt on the native Abbassa strain of Nile tilapia (*O. niloticus*) (Rezk et al., 2009), the Akosombo breeding programmed in Ghana, selective breeding of *O. shiranus* in Malawi, *O. niloticus* improved breeding in Kenya, and the three-spotted tilapia (*O. andersonii*) project in Zambia (Trinh et al., 2021). The Nigerian tilapia aquaculture industry is still in its infancy and relies on the introduction of selectively bred Genetically Improved Farmed Tilapia (GIFT), primarily sourced from Egypt and Thailand. The GIFT is revolutionising the aquaculture industry due to the improved performance in growth rate and survival since the initiation of the project by WorldFish in 1988 (Eknath and Acosta, 1998; Ponzoni et al., 2011). Non-native introduced tilapia could, however, pose serious threats to Nigerian native species, including *C. zillii, H. fasciatus, O. niloticus, P. mariae, S. galilaeus, C. guineensis,* and *T. mariae* (Adaka et al., 2014; Ataguba et al., 2014; Oladipo et al., 2018; Olufeagba et al., 2015). These species have been predicted to be potential candidates for hybridisation when farmed tilapia escape to the wild because of lack of reproductive barriers between closely related species in the group (D'Amato et al., 2007). However, the limited understanding of tilapia species diversity in Nigeria, particularly in regions with high species richness or in aquaculture settings where multiple species may coexist, has made it impossible to gain insights into the true extent of species diversity within native tilapia populations. There is also poor understanding of the haplotype diversity of famed and wild tilapia species across the country.

While some studies have investigated the genetic diversity of farmed and wild fish populations separately using mitochondrial markers (Ejikeme Odo et al., 2009; Iyiola et al., 2018; Nwani et al., 2011b), there is a lack of comparative studies that directly assess the genetic diversity and ancestral relationships between farmed and wild tilapia populations as well as their global haplotype distribution. In particular, the genetic impacts of introducing these improved strains into new environments, particularly those containing closely related native wild fish populations, remain poorly understood. Furthermore, the difficulties associated with the morphological identification of cryptic tilapia species, coupled with the introduction of new farmed strains, calls for more in-depth molecular characterisation of both the introduced and the wild populations (Trewavas, 1983; Van Der Bank, 1994). Taxonomic research in Lake

Victoria has also shown that the identification has become further confounded by the extensive introduction of alien species and transfer of native forms outside of their natural ranges (Welcomme, 1967). This unresolved taxonomic conflict regularly hinders the assessment, conservation and management of fish biodiversity (Ward et al., 2009). It is therefore important to accurately identify species to understand their diversity and ancestral relationships (Kürzel et al., 2022). The problems of unresolved phylogeny in the tilapia group has been documented to be associated with their rapid radiation rates (Irisarri et al., 2018), which has made it difficult to infer their phylogenetic lineages with both morphological (Fryer and Iles, 1972) and molecular approaches (Henning and Meyer, 2014). A close relationship within the mouthbrooding tilapia has been reported but the exact relationships remain unresolved (Franck et al., 1994).

Since the early 2000s, significant advancements have been made in taxonomic methodologies, including molecular-based taxonomy (Bingpeng et al., 2018; Clark, 2015; Mwita and Chuhila, 2023; Ward et al., 2005). The use of mitochondrial DNA (mtDNA) sequences together with other molecular markers, and the development of affordable sequencing services, has transformed species conservation across multiple taxa (Mable, 2019). DNA sequences from various parts of the mitochondrial genome have been used for studying genetic diversity (Galtier et al., 2009), molecular taxonomy, and species identification (Ratnasingham and Hebert, 2007), population genetic structure and phylogeography (Avise et al., 1986; Heist and Gold, 1999; Ward and Grewe, 1994), investigating the origin of naturalised populations (Colihueque et al., 2019), and authentication of seafood (Cawthorn et al., 2012). Hebert et al. (2003) proposed that the cytochrome c oxidase subunit I (COI) of the mtDNA could be used as a common basis for animal identification across the tree of life, leading to the concept of DNA barcoding (Hebert et al., 2003). This molecular approach is based on the principle of sequencing a short segment of DNA from the target specimen and comparing these unknown barcodes to an existing barcode database to identify species (Hebert et al., 2003). A major principle of the Barcode of Life Database (BOLD) is that sequences submitted must have verified species identifications, including photographs of voucher specimens, geographic location reference points and other meta-data, making it more reliable than less curated databases such as GenBank (Ratnasingham and Hebert, 2007). A wide range of fish species have accurately been identified at the

species level using this method (Bingpeng et al., 2018; Guo et al., 2004; Iyiola et al., 2018; Nwani et al., 2011b; Ward et al., 2005). An advantage of making inferences based on variation in mitochondrial genes is that mtDNA is maternally inherited and does not normally recombine; it therefore stores information about the sequence of events that led to the emergence of a particular species (Artamonova et al., 2018). However, current species identification methods in Nigeria rely mostly on morphological features including body and head shapes, pigmentation, and fin ray counts, that may lack the resolution needed to accurately differentiate between closely related tilapia species. This gap highlights the need for genetic tools, such as DNA barcoding to provide a more reliable and precise means of species identification (Hebert et al., 2003).

The application of DNA barcoding in to study Nigerian freshwater species will be beneficial at this time when tilapia aquaculture development in the country is driven by the unregulated and unsupervised introduction of non-native selectively bred strains it is important to identify both the farmed and wild tilapias and investigate their phylogenetic relationships. The aims of this study are therefore to:

i. confirm the taxonomic identity of each sample using the BOLD database, to identify which species occur in wild and farmed sites across Nigeria
ii. determine the diversity and distribution of mtDNA haplotypes of farmed and wild tilapia sampled
iii. investigate the phylogenetic relationships between introduced genetically improved aquaculture fish and native wild tilapia species in Nigeria, compared to the global distribution identified from the BOLD database.

3.2 Materials and methods

3.2.1 Description of the study area

Sampling was carried out in six Nigerian states from three geographic regions that are termed Northcentral (Nasarawa state), Northeast (Adamawa and Gombe collected from five sampling locations in Lagos (three farms and two wild sites), six from Ogun state (three farms and three wild), one from Oyo state (wild), four from Adamawa state (wild), one from Gombe state (wild), and two farmed strains collected at the same site from Nasarawa state. The wild sampling locations consist of four man-made dams (Asejire, Dadin Kowa, Kiri, and Oyan),

three rivers (Etele, Odo-Idimu, and the River Benue, which was sampled at two points - Yola and Numan), one natural lake (Lake Geriyo), one creek (Badagry creek), and one lagoon (Lagos lagoon). The dams sampled in this study were initially constructed to serve as sources of public water supply (Asejire and Oyan dams), irrigation farming (Dadin Kowa dam) and hydroelectricity power generation (Kiri dam) but are now used as important fishing waters), and Southwest (Lagos, Ogun, and Oyo states; Figure 3.1).

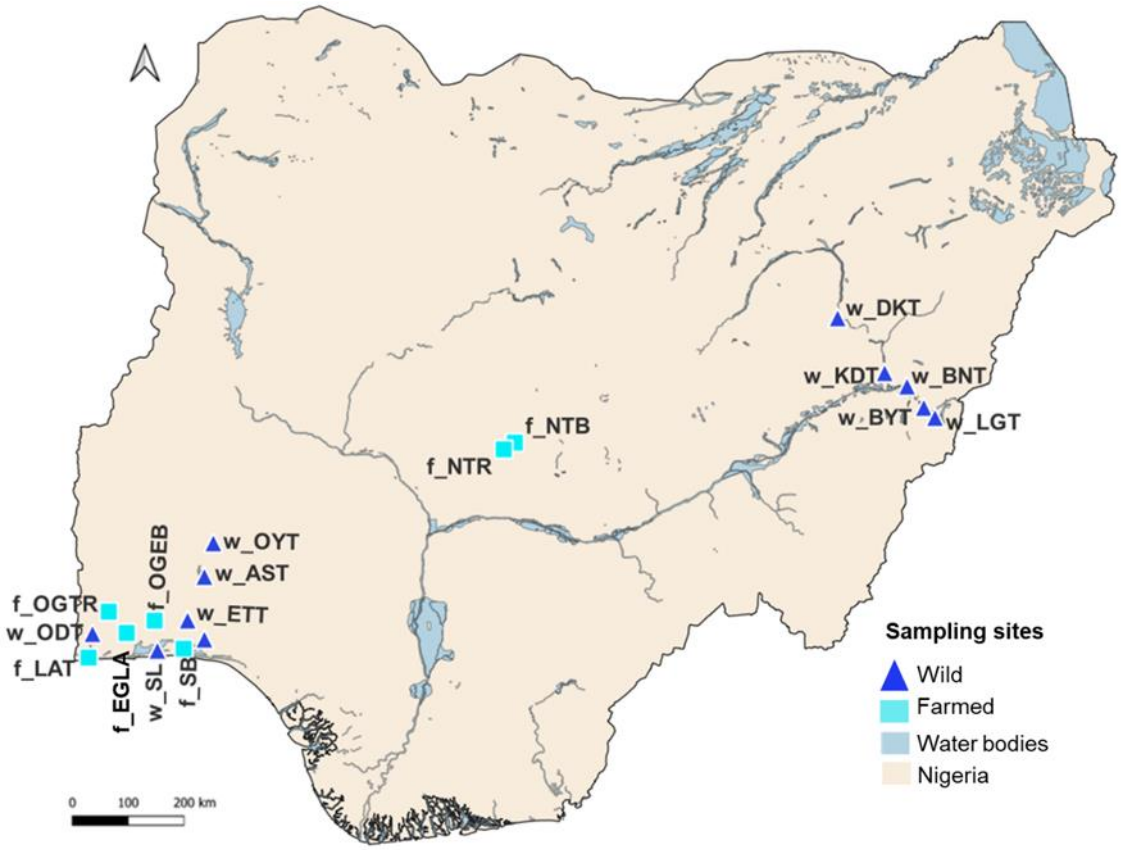

Figure 3.1 Map of Nigeria illustrating the spatial distribution of water bodies and sampling locations within the study area, categorised based on the origin of the sampled tilapia (farmed and wild). The code names are interpreted as follows: f_EGLA=black GIFT from Egypt collected in Lagos, f_LAT=black GIFT from Thailand collected in Lagos, f_NTB=black GIFT from Thailand collected from Nasarawa, f_NTR=red GIFT from Thailand collected from Nasarawa, f_OGEB=black GIFT from Egypt collected in Ogun, f_OGER=red GIFT from Egypt collected in Ogun, f_OGTR=red GIFT from Thailand collected in Ogun, f_SB=black GIFT from Thailand collected in Lagos, w_BNT=River Benue tilapia from Numan, Adamawa, w_BYT=River Benue tilapia from Yola, Adamawa, w_KDT=Kiri dam tilapia from Adamawa, w_LGT=Lake Geriyo tilapia from Adamawa, w_DKT=Dadin Kowa dam tilapia from Gombe, w_ETT=Etele River tilapia from Ogun, w_ODT=Odo Idimu tilapia from Ogun, w_OYT=Oyan dam tilapia from Ogun, w_SL=Lagos lagoon tilapia from Lagos, w_BDT=Badagry creek tilapia from Lagos, w_AST=Asejire dam tilapia from Oyo.

These water bodies have been reported to contain different cichlids including *C. zillii, H. fasciatus, S. galilaeus*, *O. niloticus*, *O. aureus*, and *T. mariae* (Nazeef and Abubakar, 2013; Olopade and Rufai, 2014; Zira et al., 2017).

One of the sampling locations, Lake Geriyo, started as a small gully and is now usually flooded by rain-fed waters and influx of waters from the River Benue (Bawuro et al., 2018). It is used for all year-round fishing and irrigation during the dry season for rice farming and vegetable cultivation around its flood plain. Badagry creek in Lagos state runs across two national boundaries, Nigeria and the Republic of Benin, and connects with Nigeria's 960 km of coastline bordering the Atlantic Ocean in the Gulf of Guinea (Agboola et al., 2008). There are over 30 species of freshwater fish in this water body, including *C. zillii, Hemichromis fasciatus*, and *S. melanotheron* (Agboola and Anetekhai, 2008). Lagos lagoon is primarily used for fishing, cage aquaculture, sand mining, and inland waterways transportation. It has a rich diversity of marine and freshwater fish species including species from the families Mugilidae, Clupeidae, and Cichlidae (Ajagbe et al., 2012). Both Etele and Odo-Idimu rivers are small fishing waters in Ogun state that were included due to presence of aquaculture activities around the areas.

The farmed fish sampling sites include earthen ponds, indoor and outdoor concrete tanks, as well as fish cages installed in lagoons. The ponds receive water from underground borehole water with the help of submersible pumps powered with electricity. Some of the ponds are situated in open ground without protective barriers, making them susceptible to flooding, which could result in fish escaping and the introduction of species or strains from other farms.

3.2.2 Sample collection

The original target of the sampling was to collect thirty fish samples per site. However, some of the wild sites were depleted so that fewer than this could be collected (Asejire dam, n=20, Badagry creek, n=29, Lagos lagoon, n=29), while in the cage aquaculture site (lagoon cage, n=23) fewer samples than the intended sample size were obtained due to the prior sale of fish. Furthermore, samples collected from the Kiri dam exhibited significant degradation in DNA quality, resulting in a reduced sample size of less than 15 individuals. To ensure a more even distribution of samples across sites, a revised threshold was set to sequence between 10 to 12 individuals per site. Despite this adjustment, only six high-quality sequences were obtained from the Kiri dam samples. These challenges prompted a modification of the original sampling plan to account for

varying sample sizes and DNA quality, thereby ensuring representative sampling across sites.

Wild samples were obtained from harvested fish caught by commercial fishers with either bamboo fish traps or cast or gill nets, while samples of farmed fish were caught with drag nets or a hand scoop net. Fin clips were collected from a total of 551 fish from the different sampling locations across the farmed (n=233) and wild (n=318) sites (Table 3.1). I used fish identification guides and keys that are specific to Nigerian freshwater fish (Olaosebikan and Raji, 1998), as well as cichlids morphological identification guidelines from FishBase (Froese and Pauly, 2010), to identify the farmed strains and wild species. During the sampling process, I relied on visual inspection of the fish's overall shape, size, colour, pigmentation, and banding patterns to identify the species. I compared the morphological information gathered for each sample with the field guide, which was not very helpful for the coloured strains due to being in black and white print, and used supportive information from FishBase. A lateral view photograph was taken for each sample before fin clips collection. The morphological field guide used to identify species presented some challenges because it did not have colour photographs, making it difficult to differentiate based on colour. Furthermore, the guide did not provide options for identifying introduced species, which made it necessary to rely solely on DNA barcoding for identification within these groups. All the samples of farmed tilapia came from the selectively bred GIFT strains introduced from Egypt and Thailand. Collected fin clips were stored in individual 1.5 ml microcentrifuge tubes containing RNAlater solution (Thermo Fisher Scientific, Waltham, MA, United States) to prevent nucleic acid degradation.

3.2.3 Genomic DNA extraction

Genomic DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen Inc, Paisley, UK) following overnight digestion at 56°C after the lysis step, as outlined in the manufacturer’s protocol. A negative treatment control was included with each set of extractions to monitor and detect contamination from external sources such as reagents, equipment, or environmental DNA; it was obtained by subjecting a blank sample (water) to the same extraction process as the experimental samples. DNA was eluted in a 100 µL volume elution buffer

Table 3.1 Collection site and source of introduced Genetically Improved Farmed Tilapia (GIFT) and wild tilapia samples collected from various states in Nigeria, indicating region, source of the introduction, whether the site is GIFT or wild, the colour of the GIFT strain, the total sample size collected per site (N), the number of barcoded samples (#COI), and the geographical coordinates. The f prefix indicates farmed and w indicates wild samples.

Collection site	Region	Code	Source	TYPE	STRAIN	Sample size	#COI	Latitude	Longitude
Lagos	southwest	f_EGLA	Egypt	GIFT	Black	30	10	7.259	3.256
Lagos	southwest	f_LAT	Thailand	GIFT	Black	30	11	6.416	2.876
Nasarawa	northcentral	f_NTB	Thailand	GIFT	Black	30	10	8.880	7.763
Nasarawa	northcentral	f_NTR	Thailand	GIFT	Red	30	11	8.880	7.763
Ogun	southwest	f_OGEB	Egypt	GIFT	Black	30	11	9.457	12.038
Ogun	southwest	f_OGER	Egypt	GIFT	Red	30	8	9.457	12.038
Ogun	southwest	f_OGTR	Thailand	GIFT	Red	30	9	6.642	3.203
Lagos	southwest	f_SB	Thailand	GIFT	Black	23	8	6.428	2.848
Adamawa	northeast	w_BNT	R. Benue Numan	Wild	Wild	30	9	9.475	12.039
Adamawa	northeast	w_BYT	R. Benue Yola	Wild	Wild	30	8	9.284	12.467
Adamawa	northeast	w_KDT	Kiri Dam	Wild	Wild	30	6	9.681	12.009
Adamawa	northeast	w_LGT	Lake Geriyo	Wild	Wild	30	9	9.293	12.434
Gombe	northeast	w_DKT	Dadin Kowa Dam	Wild	Wild	30	9	10.319	11.477
Ogun	southwest	w_ETT	Etele River	Wild	Wild	30	10	6.586	3.162
Ogun	southwest	w_ODT	Odo Idimu River	Wild	Wild	30	10	6.580	3.182
Ogun	southwest	w_OYT	Oyan Dam	Wild	Wild	30	11	7.363	4.136
Lagos	southwest	w_SL	Lagos lagoon	Wild	Wild	29	12	6.428	2.848
Lagos	southwest	w_BDT	Badagry Creek	Wild	Wild	29	11	6.416	2.876
Oyo	southwest	w_AST	Asejire Dam	Wild	Wild	20	9	7.363	4.136
Total		-	-	-		551	181		

supplied with the kits and stored at 4°C. The nucleic acid concentration was estimated at an absorbance of 260nm with a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, United States) to confirm the presence of DNA in the sample and the ratio of 260/230 absorbances to check quality. The DNA integrity was verified on 2% agarose gel electrophoresis.

3.2.4 PCR amplification

The COI gene was amplified for 192 samples using the primer pair FISH-BCL 5'-TCAACYAATCAYAAAGATATYGGCAC-3' and FISH-BCH 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Baldwin et al., 2019). A 20 µL PCR master mix was prepared containing 15 µL ddH2O, 2 µL 10X buffer, 1 µL 50mM MgCl, 0.4 µL 10mM dNTPs, 0.2 µL 10mM primer F, 0.2 µL 10mM primer R, 0.2 µL of 5,000 units/ml Taq (New England Biolabs Inc), and 1 µL DNA. PCR was performed under the following conditions: initial denaturing at 94°C for 4 min, 52°C for 50 sec, 72°C for 1 min; followed by 35 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C for 1 min; and final extension at 72°C for 6 min and held at 10°C. The amplification was verified in a 2% agarose gel electrophoresis alongside a 1kb ladder (Promega, Madison, WI USA) run at 100 volts for 45 minutes and visualised under a UV transilluminator with Gel Doc Imaging System 1708195 (Bio-Rad Laboratories, Inc.).

3.2.5 Sequencing of PCR products

PCR products of 10-40 ng were diluted with ddH2O, to a total volume of 30 µL. Products with a final concentration of 0.3 ng/µL to 1.3 ng/µL per reaction were sequenced using both forward and reverse primers, on an ABI 3730 automated sequencer at the University of Dundee Sequencing Service.

3.2.6 Sequence analysis and species identification

Sequences were edited in Sequencher 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) after assembling automatically into contigs of similar sequences based on the alignment algorithm, using minimum thresholds of 80% similarity and 20 bp overlap. Consensus sequences built from the contigs for the forward and reverse sequences for each sample were exported as a fasta file for further population genetics analysis. The consensus fasta files were aligned using the

Muscle algorithm in Aliview v. 1.28 (Larsson, 2014) and grouped into unique haplotypes with DnaSP v. 6 (Rozas et al., 2017). For each haplotype, the Barcode of Life Database (BOLD SYSTEMS) (https://boldsystems.org/index.php) was queried to determine closest similarity to the CO1 gene from named species.

3.2.7 Haplotype distribution and phylogenetic relationships

All sequences belonging to species that matched the haplotype queries above were downloaded from the BOLD system and aligned with the new sequences generated in this study, in order to determine the global distribution of variation within the group. The downloaded sequences were filtered to retain only those with ≥500 bp, based on the BOLD animal identification standard (Ratnasingham and Hebert, 2007). In addition, to more specifically testing how the haplotypes identified in this study mapped onto genetically improved farmed strains, COI GenBank sequences from improved tilapia strains were collected from a study conducted on farmed tilapia strains (*Oreochromis* spp.) in the Philippines using COI gene (Ordoñez et al., 2017). The source of the samples used in the Ordoñez et al. (2017) study include the Freshwater Aquaculture Center – Central Luzon State University (FAC-CLSU), City of Muñoz, Nueva Ecija selectively bred tilapia strains: 1) FAST (FAC-selectively bred tilapia) created from four stains of *O. niloticus*: Taiwan, Singapore, Thailand, and Israel (Bolivar and Newkirk, 2002); 2) GIFT, a product of eight strains of *O. niloticus*: Israel, Singapore, Taiwan, Thailand, Ghana, Egypt, Kenya and Senegal (Eknath et al., 1993); 3) Taiwan Red suspected to be either *O. niloticus or O. mossambicus* (Ordoñez et al., 2017); GMT (genetically male tilapia) derived from YY-males between FAST and *O. niloticus* Egypt strain (Mair et al., 1995) and 4) National Freshwater Fisheries Technological Center (NFFTC) City of Muñoz, Nueva Ecija *O. aureus* (Ordoñez et al., 2017). FAC Red (Freshwater Aquaculture Center – Red tilapia), a hybrid from *O. mossambicus x O. Hornorum* (F) *and O. niloticus* cross collected from the Southeast Fisheries Development Center – Aquaculture Division (SEAFDEC-AQD), Binangonan, Rizal. FAC-CLSU was one of the national research institutions in the Philippines that collaborated with WorldFish in the GIFT strain selective breeding project conducted between 1977 to 1988 (Eknath et al., 1993; Puttaraksar, 2004). Sequences downloaded included *O. aureus* (KU565844, KU565851), FAC Red (KU565850, KU565854, KU565865, KU565835, KU565857), GIFT (KU565827, KU565864), GMT (KU565824, KU565826, and Taiwan Red

(KU565855, KU565809, KU565823) (Ordoñez et al., 2017). Aligned sequences were collapsed into unique haplotypes using DNAsp v. 6 (Rozas et al., 2017) and the frequency of each recorded, along with the country of origin reported by the authors submitting each sequence. From the full set of unique haplotypes, Arlequin v. 3.5.2.2 (Excoffier and Lischer, 2010) was used to calculate the number of segregating sites (S), haplotype diversity, and nucleotide diversity (Pi). A minimum spanning network was constructed in PopART (Bandelt et al., 1999) to visualise relationships among haplotypes. A maximum likelihood tree was constructed using MEGA X, after selecting the best substitution model based on the lowest Bayesian Information Criterion score (Kumar et al., 2018). Confidence in branching relationships was assessed using bootstrap resampling, using 1000 pseudoreplications. Nodes with bootstrap values ≤70% were considered as unresolved (Hillis and Bull, 1993). The tree was customised and annotated using the Evolview v2 online visualisation and management tool for phylogenetic trees (He et al., 2016), so as to plot the maximum likelihood tree with a midpoint rooting.

3.3 Results

3.3.1 Species identification of Nigerian samples

Out of the 192 samples that were sequenced, 10 samples were unreadable from the chromatogram files after about 280 – 360 bp and one failed the sequencing and as such were dropped from the analysis. The 526 bp fragments from the 181 samples (farmed = 78, wild =103) that were retained formed 25 haplotypes (Table 3.2). Sequence queries on BOLD for species identification returned matches with similarity percentages ranging from 98.10 – 100% (Table 3.2). While 13 of the haplotypes had exact matches to named species, the remainder had 1-3 mutations compared to published haplotypes, but were resolved to species (Table 3.2). The following species were identified: *C. guineensis, C. zillii, H. fasciatus, O. aureus, O. mossambicus, O. niloticus, O. urolepis, Pelmatolapia mariae, S. galilaeus,* and *S. melanotheron*. All except two sites (f_SB; w_KDT) contained more than one species (Table 3.3). The farmed samples were comprised mainly of one of the three *Oreochromis* spp. identified, except for the Egypt GIFT strain (f_EGLA), which included a haplotype identified as *S. melanotheron* (H20) and shared with five samples from the Badagry creek

samples; and the Thailand red GIFT (f_OGTR) which included a haplotype identified as *P. mariae* (H9) shared with one individual from the Lagos lagoon site. *Oreochromis* spp. were consistently the most abundant and widely distributed species in both farmed and wild samples, but two of the wild sites (w_BDT, w_AST) only contained genera (*Coptodon*, *Hemichromis)* or species (S*arotherodon galilaeus*) that were not identified from farmed samples (Table 3.3). Furthermore, I identified several sites with diverse species compositions, including multiple genera and haplotypes. For instance, the Lagos lagoon (w_SL) had seven haplotypes, representing a mixture of *C. guineensis*, *P. mariae*, *O. urolepis*, and *O. niloticus*. Similarly, the Badagry creek site (w_BDT) included six different haplotypes, with three each identified as *C. guineensis* and *S. melanotheron*. The Asejire dam site (w_AST) had five haplotypes, identified as *H. fasciatus*, *C. zillii*, and *C. guineensis*.

3.3.2 Comparison between morphological and DNA barcoding species identification

Investigation into the genetic diversity of farmed and wild tilapia sites revealed a notable discrepancy between morphological identification and DNA barcoding (Table 3.4). The varying morphological features exhibited by the tilapia did not match most of the tilapia images on the field guide, which made it difficult to identify some individuals to species level. For individuals with haplotype 1, the closest match on BOLD was identified as 99.62% similar to *H. fasciatus,* whereas the field guide would have suggested *Coptodon* spp. Individuals with haplotypes 2, 3, and 4 could only be resolved at the genus level as *Coptodon* spp. using morphological identification but the BOLD search confirmed their identity as haplotypes within C. zillii, with a perfect match of 100% to each. Despite initial morphological identification suggesting haplotypes 5 and 6 as belonging to *S. melanotheron*, the BOLD search indicated a closer genetic match with *C. guineensis* for both haplotypes, with a similarity of 99.62% and 99.81%, respectively. For haplotype 7, initial morphological identification suggested *Coptodon* spp., but the sequence showed a 98.10% match with *C. guineensis*. Morphological identification suggested haplotype 8 as *S. melanotheron*; however, the BOLD database revealed contradicting results, with a 99.81% best match with *C. guineensis*.

Table 3.2 Frequency of different haplotypes observed in farmed Genetically Improved Farmed Tilapia (GIFT) and wild tilapia samples, along with sampling sites where the haplotypes were found, Barcode of Life Data Systems (BOLD) species identification and their respective similarity percentages.

Haplotype	Farmed	Wild	N	Sites	BOLD ID	Similarity (%)
H1	-	1	1	w_AST_HF	*Hemichromis fasciatus*	99.62
H2	-	3	3	w_AST_CZ	*Coptodon zillii*	100
H3	-	1	1	w_AST_CZ	*C. zillii*	100
H4	-	1	1	w_AST_CZ	*C. zillii*	100
H5	-	1	1	w_BDT_CG	*C. guineensis*	99.62
H6	-	1	1	w_BDT_CG	*C. guineensis*	99.81
H7	-	2	2	w_AST_CG, w_SL_CG	*C. guineensis*	98.10
H8	-	1	1	w_BDT_CG	*C. guineensis*	99.81
H9	1	1	1	f_OGTR_PM, w_SL_PM	*Pelmatolapia mariae*	99.12
H10		2	2	w_SL_PM	*Pelmatolapia mariae*	99.81
H11	-	3	3	w_BYT_ON, w_LGT_ON	*Oreochromis niloticus*	100
H12	-	1	1	w_LGT_OA	*O. aureus*	99.81
H13	-	1	1	w_LGT_OA	*O. aureus*	99.81
H14	-	2	2	w_LGT_OA	*O. aureus*	99.81
H15	10	49	59	w_BNT_OA, w_BYT_OA, w_DKT_OA, w_ETT_OA, w_KDT_OA, w_LGT_OA, w_ODT_OA, w_OYT_OA,	*O. aureus*	100

				f_LAT_OA, f_NTB_OA, f_NTR_OA, f_OGER_OA		
H16	-	8	8	w_ETT_SG, w_ODT_SG, w_OYT_SG	*Sarotherodon galilaeus*	100
H17	-	1	1	w_BNT_SG	*S. galilaeus*	100
H18	-	3	3	w_OYT_SG	*S. galilaeus*	99.81
H19	-	2	2	w_BDT_SM	*S. melanotheron*	100
H20	1	5	6	w_BDT_SM, f_EGLA_SM	*S. melanotheron*	100
H21	-	1	1	w_BDT_SM	*S. melanotheron*	99.81
H22	17	-	17	f_NTR_OU, f_OGER_OU, w_SL_OU	*O. urolepis*	100
H23	6	1	7	F_EGLA_OM, f_LAT_OM, f_OGEB_OM, f_OGER_OM	*O. mossambicus*	100
H24	42	9	51	w_BYT_ON, w_DKT_ON, w_ETT_ON, w_SL_ON, w_OYT_ON, f_ETT_ON, f_EGLA_ON, f_LAT_ON, f_NTB_ON, f_OGEB_ON, f_OGTR_ON, f_SB_ON	*O. niloticus*	100
H25	1	-	1	f_NTB_ON	*O. niloticus*	100

There was a lack of feasible morphological resolution for haplotype 9, which consisted of a sample each from the red Thailand GIFT strain (OGTR) and Lagos lagoon (SL), but the molecular identification revealed a match of 99.12% for *P. mariae*. Within the all-Lagos lagoon haplotype 10, morphological identification at the genus level suggested *Coptodon* spp., yet molecular analysis revealed a high match of 99.81% to *P. mariae*. Haplotype 11, initially identified morphologically as *O. niloticus*, also demonstrated a perfect match of 100% with *O. niloticus* in BOLD. Conversely, haplotype 12, also identified morphologically as *O. niloticus*, exhibited a match of 99.81% with *O. aureus*. Both haplotypes 13 and 14, morphologically identified as *O. niloticus,* returned a 99.81% best match for *O. aureus*. Haplotype 15, the most widespread among the 25 haplotypes, with 49 wild and 10 farmed individuals distributed across 11 sites, revealed complicated morphological diversity both within and between sites. While some individuals were morphologically identified as *O. niloticus* and *S. galilaeus,* a few samples from Lagos lagoon and the red Thailand strain from the Northcentral could not be identified with the field guide. However, results from BOLD revealed the samples in this haplotype as 99.81% *O. aureus*. For the all-wild Southwest haplotype 16, which returned a 100% match for *S. galilaeus,* one individual from Odo-Idimu river was morphologically identified as *O. niloticus* while one each from Etele river and Oyan dam were identified as *S. galilaeus*. Haplotype 17 showed 100% to *S. galilaeus* in the BOLD database but included one individual each from River Benue Numan and Oyan dam that were identified morphologically as *O. niloticus* and *S. galilaeus*, respectively but both samples morphological identification for haplotypes 18 and 19, identified as *S. galilaeus* and *S. melanotheron*, respectively, returned a corresponding 99.81% and 100% match in BOLD with the same species identified in both cases. Haplotype 20, which showed a 100% match to *S. melanotheron*, on BOLD, contained one wild sample from Badagry creek that was assigned to the same species but also one farmed sample from the Egypt strain obtained from Lagos that could not be identified morphologically. Individuals with haplotype 21 were morphologically identified as *S. melanotheron*, which corresponded with BOLD identification, with a best match of 99.81%.

Table 3.3 Summary of species diversity based on COI gene of the mtDNA BOLD identification from farmed and wild tilapia sites in Nigeria, showing the sample size (N, number of sequences), the number of haplotypes (N haps, with the acronyms referring to the named species from the BOLD analysis). CG = *Coptodon guineensis* CZ = *C. zillii*; HF = *Hemichromis fasciatus*; OA = *Oreochromis aureus*; OM = *O. mossambicus*; ON = *O. niloticus*; OU = *O. urolepis*; PM = *Pelmatolapia mariae*; SG = *Sarotherodon galilaeus*; SM = *S. melanotheron*

Code	Source	N	N haps	Species diversity (hap)
f_EGLA	Egypt	10	3	SM (H20), OM (H23), ON (24)
f_LAT	Thailand	11	3	OA (H15), OM (H23), ON(24)
f_NTB	Thailand	11	3	OA (H15), ON (H24, H25)
f_NTR	Thailand	11	2	OA (H15), OU (H22)
f_OGEB	Egypt	11	2	OM (H23), ON (H24)
f_OGER	Egypt	8	3	OA (H15), OU (H22), OM (H23)
f_OGTR	Thailand	8	2	PM (H9), ON (H24)
f_SB	Thailand	8	1	ON (H24)
w_BNT	R. Benue Numan	8	2	OA (H15), SG (H17)
w_BYT	R. Benue Yola	8	3	**ON (H11, H24), OA (H15)
w_KDT	Kiri Dam	5	1	OA (H15)
w_LGT	Lake Geriyo	10	5	**ON (H11), OA (H12, H13, H14, H15)
w_DKT	Dadin Kowa Dam	9	2	OA (H15), ON (H24)
w_ETT	Etele River	10	3	OA (H15), SG (H16), ON (H24)
w_ODT	Odo Idimu River	10	3	OA (H15), SG (H16), ON(H24)
w_OYT	Oyan Dam	11	4	OA (H15), SG (H16, H18), ON (H24)
w_SL	Lagos lagoon	12	7	CG (H7), PM (H9, H10), OU (H22), ON (H24)
w_BDT	Badagry Creek	11	6	CG (H5, H6, H8), SM (H19, H20, H21)
w_AST	Asejire Dam	9	5	HF (H1), CZ (H2, H3, H4), CG (H7)

**Identified in BOLD SYSTEMS as O. aureus

Table 3.4 Table showing comparison between morphologically identified tilapia specimens from both farmed and wild sites and their corresponding identification through DNA barcoding using the Barcode of Life Database (BOLD). Each row represents an individual tilapia specimen, with columns displaying the photographs of the morphologically identified tilapia specimens captured during field sampling arranged based on their haplotypes; expected species is showing identified species identification based on morphological characteristics; BOLD species are the BOLD Identification through DNA barcoding; and the percentage similarity between queried sequences and the top match returned by BOLD is given in the last column

Haplotype	Specimen	Expected species	BOLD species	Similarity (%)
1	 Source: Asejire dam (w_AST: Southwest)	*Coptodon* spp.	*H. fasciatus*	99.62
2	  	*Coptodon spp.*	*C. zillii*	100

	Source: Asejire dam (w_AST: Southwest)			
3	 Source: Asejire dam (w_AST: Southwest)	*Coptodon spp.*	*C. zillii*	100
4	 Source: Asejire dam (w_AST: Southwest)	*Coptodon spp.*	*C. zillii*	100
5		*S. melanotheron*	*C. guineensis*	99.62

	Source: Badagry creek (w_BDT: Southwest)			
6	 Source: Badagry creek (w_BDT: Southwest)	*S. melanotheron*	*C. guineensis*	99.81
7	 Source: Asejire dam (w_AST: Southwest)  Source: Lagos lagoon (w_SL: Southwest)	*Coptodon spp.*	*C. guineensis*	98.10

8	 Source: Badagry creek (w_BDT: Southwest)	*S. melanotheron*	*C. guineensis*	99.81
9	 Source: Thailand strain (f_OGTR: Southwest) ϕ  Source: Lagos lagoon (w_SL: Southwest) ϕ	ϕ Unknown	*P. mariae*	99.12

10	  Source: Lagos lagoon (w_SL: Southwest)	*Coptodon* spp.	*P. mariae*	99.81
11	 	*O. niloticus*	**O. niloticus*	100

	Source: River Benue Yola (w_BYT: Northeast)  Source: Lake Geriyo (w_LGT: Northeast)			
12	 Source: Lake Geriyo (w_LGT: Northeast)	*O. niloticus*	*O. aureus*	99.81
13	 Source: Lake Geriyo (w_LGT: Northeast)	*O. niloticus*	*O. aureus*	99.81

14	 Source: Lake Geriyo (w_LGT: Northeast)	*O. niloticus*	*O. aureus*	99.81
15	 [a] Source: River Benue Numan (w_BNT: Northeast)  	[a] *O. niloticus* [ϕ] *Unknown* [b] *S. galilaeus*	*O. aureus*	100

	[a] Source: Thailand strain (f_NTB: Northcentral)  [ϕ] Source: Red Thailand strain (f_NTR: Northcentral)  [a] Source: Odo-Idimu river (w_ODT: Southwest)  [b] Oyan dam (w_OYT: Southwest)			

	 [a] Source: River Benue Yola (w_BYT: Northeast)  [a] Source: Dadin Kowa dam (w_DKT: Northeast)  [a] Source: Etele river (w_ETT: Southwest) 			

	[a] Source: Thailand strain (f_LAT: Southwest)  [ϕ] Source: Lagos lagoon (w_SL: Southwest)			
16	 [a] Source: Odo-Idimu river (w_ODT: Southwest)  [b] Source: Etele river (w_ETT: Southwest)	*O. niloticus* [a] *S. galilaeus* [b]	*S. galilaeus*	100

	 [b] Source: Oyan dam (w_OYT: Southwest)			
17	 [a] Source: River Benue Numan (w_BNT: Northeast)	[a] *O. niloticus*	*S. galilaeus*	100
18	 [b] Source: Oyan dam (w_OYT: Southwest)	*S. galilaeus*	*S. galilaeus*	99.81

19	 Source: Badagry creek (w_BDT: Southwest)	*S. melanotheron*	*S. melanotheron*	100
20	 Source: Badagry creek (w_BDT: Southwest)  ϕ Source: Egypt strain (f_EGLA: Southwest)	*S. melanotheron* ϕ Uknown	*S. melanotheron*	100
21	 Source: Badagry creek (w_BDT: Southwest)	*S. melanotheron*	*S. melanotheron*	99.81

22	  ϕ Source: Thailand strain (f_NTR: Northcentral)  [a] Source: Lagos lagoon (w_SL: Southwest)	ϕ Unknown [a] *O. niloticus*	*O. urolepis*	100
23	 ϕ Source: Egypt strain (f_EGLA: Southwest)	ϕ Unknown [a] *O. niloticus*	*O. mossambicus*	100

	 [a] Source: Lagos lagoon (w_SL: Southwest)  [a] Source: Thailand strain (f_LAT: Southwest)			
24	 	*O. niloticus* [ϕ] *Unknown*	*O. niloticus*	100

	 ϕ Source: Egypt strain (f_EGLA: Southwest)  Source: Dadin Kowa dam (w_KDT: Northeast)  ϕ Source: Thailand strain (f_OGTR: Southwest)			

	  Source: Thailand strain (f_SB: Southwest)   Source: Thailand strain (f_LAT: Southwest)			

	 Source: Oyan dam (w_OYT: Southwest)  Source: Etele river (w_ETT: Southwest)  			

	Source: Thailand strain (f_NTB: Northcentral)   ϕ Source: Lagos lagoon (w_SL: Southwest)			
25	 Source: Thailand strain (f_NTB: Northcentral)	*O. niloticus*	*O. niloticus*	100

For haplotype 22, which showed a 100% match to *O. urolepis* on BOLD, one individual from Lagos lagoon was identified morphologically as *O. niloticus* whereas two individuals from the red farmed Thailand strain collected from the Northcentral region could not be identified based on the field guides. Haplotype 23, which showed a 100% match to *O. mossambicus* on BOLD, was made up of two farmed individuals from Egypt and Thailand and a wild sample from Lagos lagoon. The Lagos lagoon and the Thailand samples were identified morphologically as *O. niloticus,* but the Egypt strain could not be identified morphologically The predominant haplotype 24 displayed notable morphological diversity across the sampled sites, particularly among the farmed samples from Egypt and the wild samples from Lagos lagoon. Due to the extensive variations observed, it was challenging to definitively assign these individuals to specific species based solely on morphological characteristics. However, the majority of the samples within haplotype 24 were morphologically identified as *O. niloticus*. Consistent with this identification, the results from BOLD confirmed a 100% similarity match for *O. niloticus*. Haplotype 25, represented by a single individual from the Thailand strain in the Northcentral region, was morphologically characterised as *O. niloticus*, a designation consistent with the BOLD identification, which yielded a 100% match for *O. niloticus*.

3.3.3 Distribution of mtDNA haplotypes and genetic diversity analysis

The mtDNA COI analysis of genetic diversity showed varying levels of segregating sites, haplotype diversity, and nucleotide diversity across species from different locations. All the *O. niloticus* samples within the different study locations had a single haplotype except for f_NTB which had two haplotypes, moderate haplotype diversity (Hd = 0.667±0.314) but low nucleotide diversity, Pi = 0.001±0.002 (Table 3.5). Within *O. aureus* across all the sampling locations, only w_BYT (Hd = 0.429±0.169, Pi = 0.001±0.001) and w_LGT (Hd = 0.756±0.130, Pi = 0.002±0.002) showed some level of genetic variation (Table 3.5). Three haplotypes were found in *O. urolepis* with one each in f_NTR, f_OGER, and w_SL (Table 3.6). Also, one haplotype each was found in the locations that had *O. mossambicus* (Table 3.6). All three *C. zillii* haplotypes were found in the w_AST site (

Table 3.7) which had high haplotype diversity (Pi = 0.700± 0.218) but low nucleotide diversity (Hd = 0.003± 0.003). *C. guineensis* had five haplotypes distributed among three sampling sites (w_AST = 1, w_BDT = 3, w_SL = 1). The w_BDT location showed some level of genetic variation with lower haplotype diversity (Hd = 0.074 ± 0.027) but higher nucleotide diversity than for the other species (Pi = 0.043 ± 0.033) with the haplotypes differing by 34 segregating sites (

Table 3.7). *S. galilaeus* haplotypes were distributed among w_BNT, w_ETT, w_ODT, and w_OYT (Table 3.8). Only samples from w_OYT demonstrated some genetic variation, with two haplotypes, high haplotype diversity (Hd = 0.536 ± 0.123) and low nucleotide diversity (Pi = 0.002 ± 0.002). For *S. melanotheron*, w_BDT samples exhibited moderate genetic diversity, characterised by seven segregating sites and three haplotypes (Table 3.8). This is reflected in the relatively high haplotype diversity (Hd = 0.607 ± 0.164) and nucleotide diversity (Pi = 0.005 ± 0.004). The other population, which was farmed, only had a single haplotype.

Table 3.5 Summary of genetic diversity parameters within *Oreochromis niloticus* and *O. aureus,* showing the sampling site ID (Code), source of the sample (Source), the sample size (N), the number of segregating sites (S), the number of haplotypes (N haps), haplotype diversity (Hd) including the standard error (SE) and pairwise nucleotide diversity (pi) which is a measure of the average number of nucleotide differences per site between two DNA sequences in all possible pairs in the sample population. Columns represented with "-" indicates that these parameters were not calculated due to a lack of genetic variation (only one haplotype observed).

		Oreochromis niloticus				
Code	Source	N	S	N haps	Hd±SE	Pi± SE
w_BYT	River Benue Yola	1	0	1	-	-
f_EGLA	Egypt	8	0	1	-	-
w_DKT	Dadin Kowa	1	0	1	-	-
w_ETT	Etele River	1	0	1	-	-
f_LAT	Thailand	7	0	1	-	-
f_NTB	Thailand	3	1	2	0.667±0.314	0.001±0.002
f_OGEB	Egypt	10	0	1	-	-
f_OGTR	Thailand	7	0	1	-	-
f_SB	Thailand	8	0	1	-	-
w_SL	Lagos lagoon	4	0	1	-	-
w_OYT	Oyan Dam	1	0	1	-	-
w_ODT	Odo-Idimu River	1	0	1	-	-
		O. aureus				
Code	Source	N	S	N haps	Hd±SE	Pi± SE
w_BNT	River Benue Numan	7	0	1	-	-
w_BYT	River Benue Yola	8	0	2	0.429±0.169	0.001±0.001
w_DKT	Dadin Kowa	8	0	1	-	-

w_ETT	Etele River	8	0	1	-	-
w_KDT	Kiri Dam	5	0	1	-	-
w_LGT	Lake Geriyo	9	3	4	0.756±0.130	0.002±0.002
f_NTB	Thailand	8	0	1	-	-
f_NTR	Thailand	1	0	1	-	-
w_ODT	Odo-Idimu River	7	0	1	-	-
f_OGER	Egypt	1	0	1	-	-
w_OYT	Oyan Dam	1	0	1	-	-
f_LAT	Thailand	1	0	1	-	-
w_SL	Lagos lagoon	1	0	1	-	-

Table 3.6 Summary of genetic diversity parameters within O. urolepis and O. mossambicus

				O. urolepis		
Code	Source	N	S	N haps	Hd±SE	Pi± SE
f_NTR	Thailand	10	0	1	-	-
f_OGER	Egypt	6	0	1		
w_SL	Lagos lagoon	1	0	1	-	-
				O. massambicus		
Code	Source	N	S	N haps	Hd±SE	Pi± SE
f_EGLA	Egypt	1	0	1	-	-
f_LAT	Thailand	3	0	1	-	-
f_OGEB	Egypt	1	0	1	-	-
f_OGER	Thailand	1	0	1	-	-
w_SL	Lagos lagoon	1	0	1	-	-

Table 3.7 Summary of genetic diversity parameters within the genus *Coptodon*

Coptodon zillii						
Code	Source	N	S	N haps	Hd±SE	Pi± SE
w_AST	Asejire Dam	5	3	3	0.700±0.218	0.003±0.003
C. guineensis						
Code	Source	N	S	N haps	Hd±SE	Pi± SE
w_AST	Asejire Dam	1	0	1	-	-
w_BDT	Badagry Creek	3	34	3	0.074±0.027	0.043±0.033
w_SL	Lagos lagoon	1	0	1	-	-

Table 3.8 Summary of genetic diversity parameters within the genus *Sarotherodon*

S. galilaeus						
Code	Source	N	S	N haps	Hd±SE	Pi± SE
w_BNT	River Benue Numan	1	0	1	-	-
w_ETT	Etele River	1	0	1	-	-
w_ODT	Odo-Idimu River	2	0	1	-	-
w_OYT	Oyan Dam	8	2	2	0.536±0.123	0.002±0.002
S. melanothron						
Code	Source	N	S	N haps	Hd±SE	Pi± SE
f_EGLA	Egypt	5	0	1	-	-
w_BDT	Badagry Creek	8	7	3	0.607±0.164	0.005±0.004

as demonstrated by the presence of only one haplotype and the absence of calculated diversity parameters.

3.3.4 Species-level haplotype diversity within Nigeria

The species-level haplotype network analysis conducted for farmed and wild tilapia samples revealed interesting patterns, particularly within *O. niloticus*, *O. aureus, S. galilaeus, S. melanotheron, C. guineensis,* and *C. zillii* (Figure 3.2). For *O. niloticus* two haplotypes were identified (H24 and H25) that were separated by a single mutation (Figure 3.2a). The most frequent haplotype (H24) was shared by both farmed and wild sites but dominated by farmed sites including Egypt strains (f_OGEB_ON and f_EGLA_ON) and Thailand strains (f_LAT_ON, f_OGTR_ON, and f_SB_ON). The Lagos lagoon (w_SL_ON) is the wild sampling sites with the highest frequency (5) of the *O. niloticus* haplotypes. Other wild samples with frequencies between one to two are Oyan dam (w_OYT_ON), Etele river (w_ETT_ON), Odo-Idimu river (w_ODT_ON), Dadin Kowa

dam (w_KDT_ON), River Benue Numan and Yola (w_BNT_ON and w_BYT_ON), and Lake Geriyo (w_LGT_ON). Another haplotype (H11) that had the top match in the BOLD database to *O. niloticus* showed extensive variation from the other haplotypes, being separated from haplotype 24 (H24) by 43 mutations; it was found in two samples from River Benue Yola and one from Lake Geriyo. It was also identified as *O. aureus* on some BOLD entries, and it was only separated from the most frequent *O. aureus* haplotype (H15) by a single mutation, just like the other three haplotypes (H12, H13, H14); so, it was classified as this species for all subsequent analyses (Figure 3.2b). The most common haplotype (H15) was at higher frequency in the wild (49) than in the farmed sampling sites (10). It was found to be present in nine wild sampling sites: Lake Geriyo (w_LGT_OA = 5), River Benue Numan (w_BNT_OA = 7), River Benue Yola (w_BYT_OA = 5), Dadin Kowa dam (w_DKT_OA = 8), Etele river (w_ETT_OA = 8), Kiri dam (w_KDT_OA = 5), Odo-Idimu river (w_ODT_OA = 7), Oyan dam (w_OYT_OA = 1), and Lagos lagoon (w_SL_OA = 1). This haplotype was also found in farmed sites: Thailand strains (f_NTB_OA = 8, f_NTR_OA = 1, f_LAT_OA = 1) and red Egypt strain (f_OGER_OA = 1). Most of the farmed and wild sites included only H15, except the most diverse Lake Geriyo samples, which had H15, along with three private haplotypes (H12, H13, H14) separated by a single mutation from H15, along with the H11 haplotype that had misidentified as *O. niloticus,* which was also shared with River Benue Numan (which also had H15). The *S. galilaeus* haplotype network (Figure 3.2 Haplotype networks of tilapia samples showing genetic relationships within six distinct tilapia species

with distinct colour *coding* for each sampling location or *group while* the lines between haplotypes represents *the number* of mutation: (a) *Oreochromis niloticus* (ON); (b) O. *aureus (*OA); (c*) Sarotherodon* galilaeus (SG); (d) S. melanotheron (SM); (e) C. guineensis and (f) C. zillii. Sequences found *on BOLD* that had been sampled from Nigeria are also indicated (BOLD hap Nigeria). In the network for O. aureus, haplotype 15 was shared between all of the farmed and wild *sites sampled,* with three distinct haplotypes (H12, H13, H14) *observed in* the wild Lake Geriyo (w-LGT) samples. S. galilaeus was only identified in the wild. For S. melanotheron one haplotype was shared between wild *and farmed* samples (H20), and two others were identified only in the wild samples. The haplotype network for C. guineensis revealed extensive variation between haplotypes 5 and 7. Haplotype 5 is distinguished from haplotype *6 by* two

mutations, whereas only one mutation separates haplotype 7 from haplotype 8. In the haplotype network of C. zillii, which has also been documented in previous studies from Nigeria, haplotype was the most frequent and is separated by two mutations from haplotype 2 and one mutation from haplotype 3. showed three distinct haplotypes separated by two mutations each and made up of only wild sampling sites, with samples from Odo-Idimu river (w_ODT_SG; H16= 2), Etele river (w_ETT_SG; H16= 1), Oyan dam(w_OYT_SG; H16 = 5, H18 =3), River Benue Numan (w_BNT_SG; H17 = 1), and previously sequenced samples from Nigeria registered on BOLD sharing a single haplotype (H16 = 2). Three distinct haplotypes were identified for *S. melanotheron*Figure 3.2d). The *S. melanotheron* haplotypes were dominant in Badagry creek samples (w_BDT_SM) with three haplotypes (H19 = 2, H20 = 5, and H21 = 1) while the farmed Egypt strain (f_EGLA_SM) had just one haplotype (H20 = 1). The three *S. melanotheron* haplotypes identified (H19, H20, H21) have not been previously reported in Nigeria.

The haplotype network for *C. guineensis* showed two sets of distinct haplotypes connected in the centre by haplotypes 5 (H5) and 7 (H7) with 27 mutations (Figure 3.2e). Haplotypes 5 (H5) and 6 (H6) are separated by two mutations while H7 and H8 are separated by just one mutation. These haplotypes are made up of individuals from Badagry creek (w_BDT_CG; H5 = 1, H6 = 1, H8 = 1), Asejire dam (wAST_CG; H7 = 1), and Lagos lagoon (w_SL_CG; H7 = 1). The only shared haplotype within this group is H7 shared between the Asejire dam and Lagos lagoon samples. These haplotypes were only present among the wild samples and have not been previously reported in Nigeria. The *C. zillii* haplotype network (Figure 3.2f) had three haplotypes from the same site (Asejire dam; H2 = 3, H3 = 3, H4 = 1). Among the three haplotypes, H3 and H4 have been reported in Nigeria based on BOLD records, with a frequency of 1 and 18, respectively. H3 and H4 are separated with a single mutation while H2 and H4 are separated with two mutations.

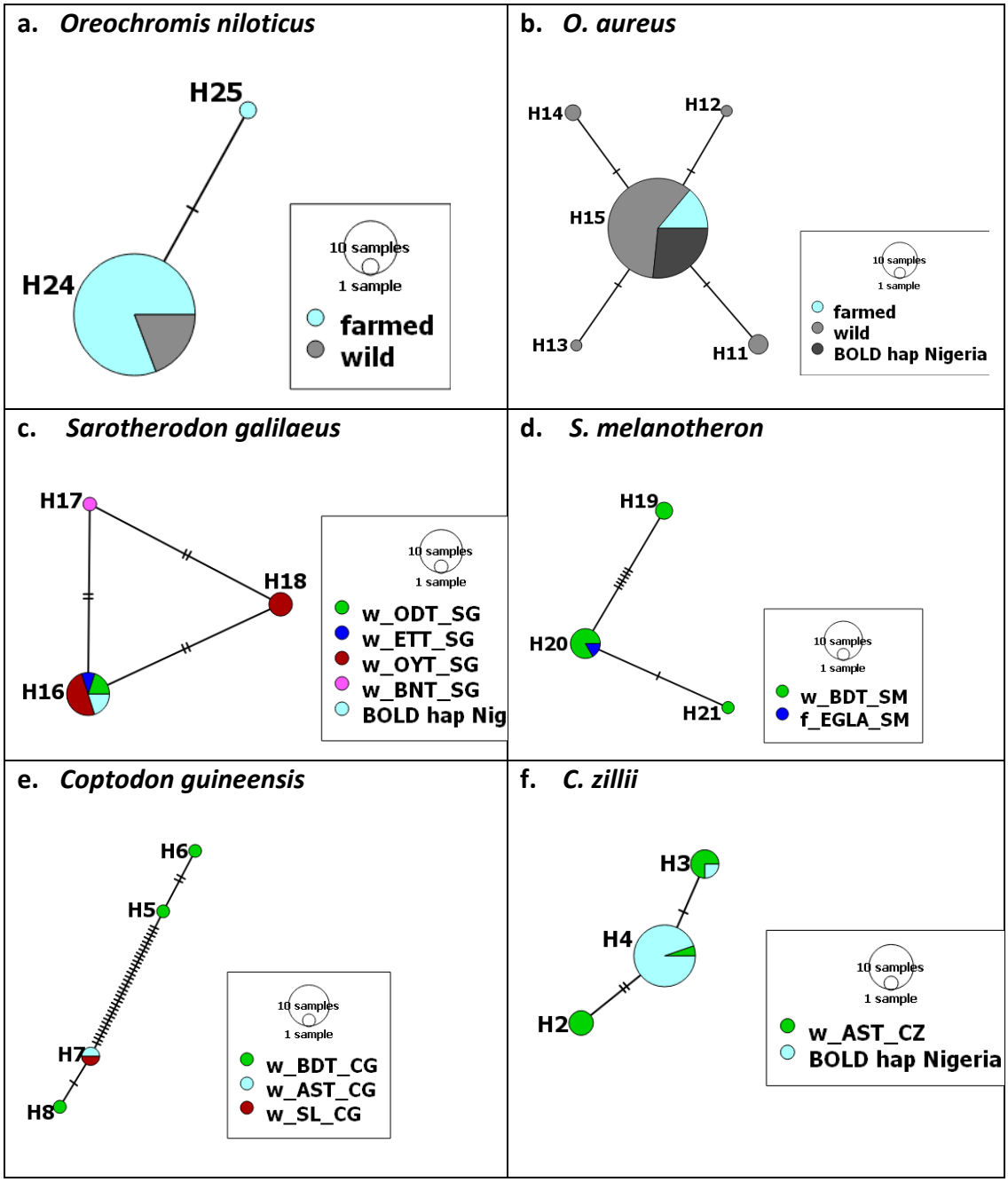

Figure 3.2 Haplotype networks of tilapia samples showing genetic relationships within six distinct tilapia species with distinct colour coding for each sampling location or group while the lines between haplotypes represents the number of mutation: (a) *Oreochromis niloticus* (ON); (b) *O. aureus* (OA); (c) *Sarotherodon galilaeus* (SG); (d) *S. melanotheron* (SM); (e) *C. guineensis* and (f) *C. zillii*. Sequences found on BOLD that had been sampled from Nigeria are also indicated (BOLD hap Nigeria). In the network for *O. aureus*, haplotype 15 was shared between all of the farmed and wild sites sampled, with three distinct haplotypes (H12, H13, H14) observed in the wild Lake Geriyo (w-LGT) samples. *S. galilaeus* was only identified in the wild. For *S. melanotheron* one haplotype was shared between wild and farmed samples (H20), and two others were identified only in the wild samples. The haplotype network for *C. guineensis* revealed extensive variation between haplotypes 5 and 7. Haplotype 5 is distinguished from haplotype 6 by two mutations, whereas only one mutation separates haplotype 7 from haplotype 8. In the haplotype network of *C. zillii*, which has also been documented in previous studies from Nigeria, haplotype was the most frequent and is separated by two mutations from haplotype 2 and one mutation from haplotype 3.

3.3.5 Phylogenetic relationships and global haplotype distribution

A total of 985 tilapia sequences were downloaded from the BOLD database including 14 sequences from the FAC-CLSU, (SEAFDEC-AQD), and NFFTC, from the Philippines. These were collapsed into 121 haplotypes but only those that showed 100% similarity to haplotypes identified in this study were included in the phylogenetic analysis. The midpoint-rooted phylogenetic tree is drawn from the perspective of *H. fasciatus* as the basal lineage (Figure 3.3). Overall, there was little bootstrap support (grey circles indicate nodes $\geqslant$70%) for relationships between genera but there appeared to be division into two major lineages of substrate spawners (node A; *Coptodon*) and a lineage (node B) consisting of both mouthbrooders (*Oreochromis* spp. and *Sarotherodon* spp.) and substrate spawners (*P. mariae*). Although the bootstrap support at node A was low, there was high bootstrap support for the two species of substrate spawners *C. zillii* and *C. guineensis*. At node B, there was a further division (still unresolved) of the clade into two lineages that completely separated the mouthbrooders (node C) from the substrate-spawning *P. mariae* (H9 and H10). Bootstrap support was below 70% for these divisions; however, there was high support for shared ancestry among *O. mossambicus, O. urolepis,* and *O. niloticus* (Node D). The placement of *S. melanotheron* was unresolved within clade E but there was high support for shared ancestry between *O. aureus* and *S. galilaeus* (Node F). *O. aureus* also appears to be paraphyletic, with one of the haplotypes (H12) differentiated from the others with high bootstrap support but the placement of the other four haplotypes not resolved in relation to each other. Although bootstrap support is low for the relative placement of *S. galilaeus* and the *Oreochromis* spp., the topology shown would make them paraphyletic in relation to the common ancestor (Clade F).

In terms of the global distribution of haplotypes, for *S. melanotheron* (H19, H20, H21), only H19 and H20 had 100% match in BOLD and but only sequences from the Philippines were reported, at a frequency of 12 and 4, respectively. Two sequences corresponding to *S. galilaeus* (H16) were reported from Nigeria. The added sequences from FAC-CLSU, and NFFTC did not change the orientation of the phylogenetic tree but mapped with the known haplotypes. The NFFTC *O.*

aureus sequence mapped with the predominant *O. aureus* haplotype (H15) that had a wide global distribution with reports in the following regions: Egypt (8), India (2), Israel (32), Nigeria (21), the Philippines (5), Uganda (1), Africa (Congo = 10, Liberia = 2, and South Africa = 11), America (Brazil = 2 and Panama = 17), Asia (Pakistan = 1) and Europe (Russia = 4). Other selectively bred strains from FAC-CLSU and NIFTDC including GIFT, GMT, and FAC Red mapped with the predominant GIFT H24. The predominant *O. niloticus* (H24) was reported in Egypt (119), Madagascar (64), Uganda (44), the Philippines (38), Kenya (26), China (9), Thailand (8) India 6), other parts of Africa (Mozambique = 2, South Africa = 5, Sudan = 1), America (Mexico = 1 and USA =12), Asia (Bangladesh = 1, Indonesia = 10, Malaysia = 2, Myanmar = 1, Pakistan = 1, and Singapore = 1), and Europe (Italy = 2). This haplotype showed more abundance in Africa than in Asia and Europe. Taiwan Red and other GMT and FAC Red aligned with *O. mossambicus* (H23). *O. mossambicus* (H23) was reported in China (9), India (16), Madagascar (11), the Philippines (6), Thailand (1), other parts of Africa (Mozambique = 1, South Africa = 3, Swaziland = 2, and Zimbabwe = 2), America (Canada = 1, Columbia = 4, Mexico = 1, USA = 10), and Asia (Australia = 5, Indonesia = 8, Malaysia = 4, Myanmar = 17, Pakistan = 2, Syria = 1). This haplotype is more abundant in Asia than in any other region. Additionally, some FAC Red sequences aligned with *O. urolepis* (H22). *O. urolepis* (H22) was found to be present in Madagascar (14), Uganda (2), America (Brazil = 6, Colombia = 1), and Asia (Indonesia = 1). The BOLD search conducted for *C. zillii* Haplotype 4 (H4) showed reports of this haplotype in Israel (17), China (11), Egypt (5), Thailand (2), other parts of Africa (Mauritania = 10 and Algeria = 1) and Asia (Jordan = 1 and Syria = 2). Haplotype 3 (H3), also belonging to *C. zillii,* was only reported in Uganda (4), Madagascar (2), and Nigeria (1).

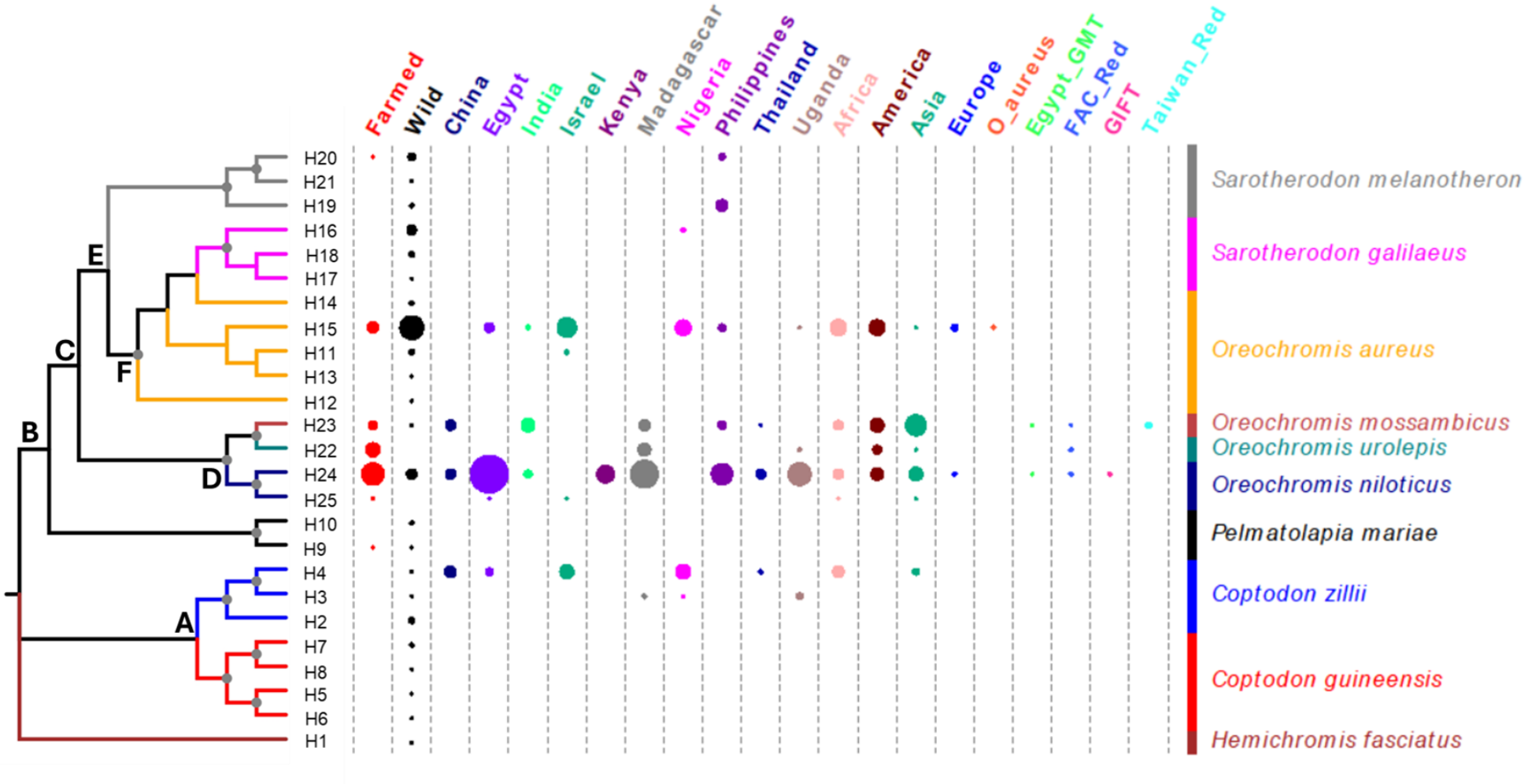

Figure 3.3 Phylogenetic analysis of the cytochrome c oxidase subunit I (COI) gene of the mitochondrial DNA depicting relationships between introduced Genetically Improved Farmed Tilapia (Farmed; red column), wild tilapia species (Wild; black column) sampled here, along with countries and regions

representing the global distribution of haplotypes associated with each farmed and wild species from the BOLD database. Countries with few haplotypes including Algeria, DR Congo, Liberia, Mauritania, Mozambique, South Africa, Sudan, Swaziland, and Zimbabwe were grouped as Africa; Brazil, Canada, Colombia, Mexico, Panama, and USA as America; Bangladesh, Jordan, Myanmar, Pakistan, and Singapore), and Europe (Italy and Russia as Asia. Other sequences were added from the Freshwater Aquaculture Center – Central Luzon State University (FAC-CLSU), City of Muñoz, Nueva Ecija (GIFT, GMT, and Taiwan Red); the National Freshwater Fisheries Technological Center (NFFTC), City of Muñoz, Nueva Ecija (*O. aureus*); and the National Integrated Fisheries Technology Development Center (NIFTDC), Dagupan City, Southeast Fisheries Development Center – Aquaculture Division (SEAFDEC-AQD), Binangonan, Rizal (FAC Red). Given the expected star-like radiation expected for cichlids, the tree was rooted at the midpoint with H. fasciatus. Nodes with bootstrap support ⩾70% are indicated with grey circles. Although there was low bootstrap support, distinct lineages for mouthbrooding and substrate spawners were identified (node a). Node B separates mouthbrooders (node C: *Oreochromis* spp. and *Sarotherodon* spp.) and substrate spawners (Node A: *Coptodon* spp.; and *Pelmatolapia mariae*: H9 and H10).

3.4 Discussion

This study aimed to identify, investigate the genetic diversity, and assess the phylogenetic relationships of introduced farmed tilapia strains and native wild samples through a combination of morphological assessment and DNA barcoding methods. Based on barcoding of the COI gene, samples from both farmed and wild samples were identified at species level including farmed samples with complex traits that could not be resolved morphologically. Overall, 25 haplotypes were found in both farmed and wild samples, with more diversity in the wild samples. Although not well resolved, the phylogenetic tree separated the tilapia according to their breeding habits as mouth brooders (*Oreochromis* spp. and *Sarotherodon* spp.) and substrate spawners (*Coptodon* spp., *H. fasciatus,* and *P. mariae*). The haplotypes found in this study were distributed mostly in Egypt for the farmed *O. niloticus* and Israel for the wild *O. aureus*. These two countries have contributed most to the global distribution of tilapia and donated some of the founding stock for the WorldFish Center's GIFT project (Eknath and Acosta, 1998; Welcomme, 1988).

3.4.1 Efficacy of morphological and DNA barcoding species identification techniques

In an analysis of the efficacy of these two identification approaches, the morphological technique proved to be broadly useful in the characterisation of tilapia genera in Nigeria. However, morphological methods for identifying species most especially cryptic tilapias are usually compounded with some challenges and limitations. For example, the farmed strains in this study exhibited morphological divergence from body and head shapes and colour. These features, not captured in the freshwater fish identification field guide for Nigerian fishes, made their identification impossible in the field. Also, some wild samples in Lake Geriyo were misidentified as *O. niloticus* instead of *O. aureus,* while several samples in Lagos lagoon could not be identified to species level. Previous studies have acknowledged that morphological identification of tilapia species can be prone to inaccuracies and inconsistencies. For example, it was suggested that phenotypic plasticity in tilapia, the ability of an organism to alter its phenotype in response to environmental cues, is increasingly recognised as a significant factor in evolutionary processes that can contribute to the origin of

novel phenotypes, facilitating divergence among sampling sites and species (Machado-Schiaffino et al., 2014). Difficulty in distinguishing among the *Oreochromis* spp. has also been linked to the relative ease of hybridization among members of the genus (Nagl et al., 2001). Furthermore, morphological identification challenges within the tilapias as a result of subtle morphological variations both within and between species will be difficult to resolve due to their explosive radiation rates that has made it impossible to develop a standardised identification protocol.

Given these challenges, DNA barcoding has proven to be an important complementary technique for the identification of species like the tilapias that have overlapping morphological traits (Ward et al., 2009). Using this method, I was able to identify all the introduced farmed sampling sites to species, including native wild samples that could not be identified morphologically. The integration of morphological and DNA barcoding techniques would therefore be useful in approaches to tilapia biodiversity assessment and identification of species. Despite the positives of this molecular approach, databases for species identification using the molecular approach can still be prone to flaws if previously deposited sequences have been wrongfully identified. For example, there was some discrepancies in the BOLD database with samples from haplotype 11 (H11). The best top three matches from the database were 100% Nile tilapia (*O. niloticus*). However, placing this haplotype in the *O. niloticus* haplotype network revealed extensive variation with the other two haplotypes (H24 and H25). Further investigating down into the list of the possible target species in BOLD revealed two sequences from Israel with 100% match for *O. aureus*. Placing the haplotype together with the other *O. aureus* haplotypes showed better resolution, with just one mutation separating it from the most frequent *O. aureus* haplotype (H15). Also, the placement of the haplotype in the phylogenetic tree is a further proof the species in BOLD corresponding to *O. niloticus* were misidentified. Errors in species identification databases like BOLD and GenBank can be minimised if species-level taxonomy is available for many groups (Pentinsaari et al., 2020). Integrating morphological and molecular taxonomy approaches will provide a systematic framework for accurately identifying and categorising tilapia species based on their morphological, genetic, and ecological characteristics. This will help in ensuring reliable species identification in genetic databases. However, a note of caution is that mtDNA

only reflects maternal ancestry, and so identification of "species" could include hybrids (see Chapter 4).

3.4.2 Species diversity of farmed and wild tilapias within Nigeria

Comparison of the introduced farmed and native wild tilapia samples revealed interesting patterns of species diversity. Wild samples had higher species richness, including different arrays of tilapia species, such as the mouthbrooders (*O. niloticus, O. aureus, O. urolepis, S. galilaeus,* and *S. melanotheron*) and substrate spawners (*C. guineensis, C. zillii, H. fasciatus,* and *P. mariae*), than the farmed samples that had mostly the mouthbrooders (*O. niloticus, O. aureus, O. mossambicus, O. urolepis,* and *S. melanotheron*) and one substrate spawner (*P. mariae*). The abundance of *O. niloticus* in the farmed sampling site only confirmed their use as the primary tilapia aquaculture species and the originating species of the GIFT strain (Eknath et al., 1993). Likewise, the other *Oreochromis* spp. found among the farmed sites have been documented as important aquaculture species. For example, *O. niloticus*, *O. aureus,* and *O. mossambicus* have been described as excellent aquaculture species because they are easily bred and are tolerant to salty and alkaline environments (Nagl et al., 2001; Wu and Yang, 2012).

The presence of *S. galilaeus, C. guineensis,* redbelly tilapia *C. zillii, and H. fasciatus* only in the wild means they have not been utilised or extensively bred in aquaculture settings, thereby maintaining their genetic integrity within wild habitats in Nigeria. Redbelly tilapia was identified as a potential candidate for aquaculture but *O. niloticus* remains the species of interest following its successful selective breeding programme, rapid growth, high fecundity, and market acceptability (Eknath and Acosta, 1998; Ponzoni et al., 2011; Pullin et al., 1991; Trinh et al., 2021). The co-occurrence of multiple species in specific site, such as *C. guineensis, P. mariae*, *O. urolepis*, and *O. niloticus* in one site, presumably reflects habitat heterogeneity that allows the coexistence of species with different ecological requirements. The presence of non-native cichlids (*Amatitlania nigrofasciata*), tilapia (*Oreochromis* sp.), and the first discovery of spotted tilapia (*P. mariae*) in Europe has been documented in the Gillbach, marking the initial instance of a reproducing population in this location (Lukas et al., 2017). Wild samples characterised by a diverse assemblage of species may

exhibit higher functional diversity and ecosystem resilience compared to monoculture-dominated farmed samples (Bolger, 2001). The presence of multiple species within wild sampling location will facilitate niche partitioning, resource utilisation, and trophic interactions, thereby enhancing ecosystem stability and functioning (Galvez et al., 2022). However, it is worth investigating if the contributing species in the wild are not escapees from farmed sites. Having escapees in the wild could lead to hybridisation between farmed and wild species and subsequently alter the genetic diversity of the native species and the local adaptation they have accumulated over generations could be loss. Since this cannot be determined only by assessing mtDNA, this issue is addressed in chapter 4.

3.4.3 mtDNA haplotype diversity in farmed and wild tilapia within Nigeria

The distribution of mtDNA haplotypes in both farmed GIFT and wild tilapia samples shows different levels of genetic variation and evolutionary dynamics within each site. Although farmed sites displayed some degree of genetic variation, wild sites tended to possess greater genetic diversity. High haplotype diversity was found across the farmed and wild sites, except in the cage aquaculture and Kiri dam samples which only included single haplotypes. The low nucleotide diversity observed within *Oreochromis* species in both farmed and wild sampling sites is similar to the results obtained among farmed *O. niloticus* including the GIFT strain in Madagascar (Hubert et al., 2021). However, using the same marker (CO1), Hubert et al. (2021) reported a higher number of haplotypes (ranging from two to eight) and haplotype diversity (0.2 to 0.8) against one or two haplotypes reported in this study. In a study of wild tilapia species in Japan, using the highly variable control region of the mtDNA, Fatsi et al. (2020), observed low haplotype diversity and nucleotide diversity among *O. niloticus, O. aureus, O urolepis, O. mossambicus,* and *C. zillii.* Low nucleotide diversity among cichlid fishes has been linked to hybridisation and large amounts of variation shared among species (Svardal et al., 2021). Single haplotypes reported in wild Kiri dam and farmed cage samples could be linked to habitat fragmentation and inbreeding in aquaculture, respectively. Kiri dam experiences massive amounts of water reduction during the long dry season, which leads to receding of the lake and fragmentation of the water body, disconnecting it from

the River Benue. Other studies have reported the impact of habitat fragmentation on genetic diversity. For example, Pavlova et al. (2017) in their study of endangered Australian freshwater Macquarie perch (*Macquaria australasica*) using the mtDNA control region and 19 microsatellite markers, found low genetic diversity, and effective population sizes below the threshold required to retain adaptive potential due to habitat fragmentation. Pinto et al. (2024) using SLiM modelling, predicted that isolated populations and those experiencing a decline in effective population size will face increasing genetic drift and inbreeding.

Lower genetic diversity in farmed compared to wild saples is consistent with their different histories: selective breeding for aquaculture species while their wild counterparts deal with complex interactions between natural selection, genetic drift, and human-mediated selective pressures that could influence their genetic diversity and the accumulation of unique haplotypes over time (Allendorf, 2017). Selective breeding programmes targeted at enhancing desirable traits such as growth rate and disease resistance may reduce genetic variation within farmed samples, resulting in fewer observed haplotypes compared to their wild counterparts. The use of fewer breeding populations has been reported as one of the factors that leads to reduction of genetic diversity among farmed sampling sites. For example, indiscriminate breeding in aquaculture practices may reduce the genetic diversity in farmed strains due to the inbreeding effects of small broodstock population size (Beardmore et al., 2001; Brummett et al., 2004; Wu and Yang, 2012). In my study, within *Oreochromis* spp. comparison revealed five haplotypes for *O. aureus,* two for *O. niloticus,* and one each *O. mossambicus* and *O. urolepis*. The predominant sites that constituted the *O. aureus* haplotypes were from the wild. Conversely, *O. niloticus* that has been bred extensively was dominated by the farmed samples. This further confirms that extensive breeding can lead to reduced genetic diversity in the farmed sites.

Assessment of shared haplotypes between farmed and wild sampling sites showed that the wild samples collected in the Lagos lagoon (w_SL), a water body used for intensive cage aquaculture, had the greatest number of haplotypes (7) of any of the studied samples and included four different species among the 12 individuals sampled. The single haplotype (H24) of *O. niloticus* found in the cage

farmed site installed in the same water body (f_SB) was shared with the wild part of the site, which could suggest that farmed fish have escaped. However, this same haplotype was also found in other wild and farmed sites, both in Nigeria and globally, so further research would be required to determine whether this haplotype was used for the production of the GIFT strains but was originally native to Nigeria. Similarly, the *O. urolepis* haplotype (H22) found in w_SL was also shared with farmed sites from different regions in Nigeria, as well as globally. *O. urolepis* is known to be a farmed species and has not previously been reported in the wild in Nigeria so my findings could also be a case of fish escape from one the farms in Nigeria. Identification of unique haplotypes in farmed and wild sampling sites will be an important indicator for tracing escapees since the complex morphological traits exhibited by both farmed and wild tilapia has made morphological identification difficult. Hatchery practices which are still unregulated in Nigeria, which could lead to reduced fitness in farmed samples, caused by inbreeding. In the future, these practices will be detrimental to the long-term viability of the selective improvement programmes and defeat the overall goal of the farms, which is solely for economic gains and achieving food security (Frost et al., 2006).

3.4.4 Phylogenetic relationships between farmed and wild tilapia in Nigeria and their global haplotype distribution

The COI gene tree did not resolve all of the nodes of the tree but showed a close relationship between the maternal mouthbrooding *O. niloticus* and the biparental and paternal mouthbrooding *Sarotherodon* while separating them from the substrate spawning *Coptodon* spp. Although not resolved with high confidence, the biparental and paternal mouthbrooding *S. galilaeus* showed a more recent ancestral relationship with *Oreochromis aureus* than with *S. melanotheron*. Before 1973 both *Sarotherodon* and *Oreochromis* used to be classified under the same genus until the first separation happened during which *S. melanotheron* became the first member of the *Sarotherodon* to be given a separate name (Trewavas, 1982; Trewavas, 1983). The genetic variation that led to their separation is noticeable in the phylogenetic tree with the assignment of a separate clade for *S. melanotheron* while *S. galilaeus* shared a common recent ancestry with *O. aureus*. The clustering pattern highlighting common recent ancestry between *O. urolepis* and *O. niloticus* aligns with the results of Wu and

Yang (2012), which also showed separation between the mouthbrooders and the substrate spawners using the mtDNA control region. The unresolved phylogenetic relationships observed in this study have also been reported previously. For example, Nagl et al. (2001) in a phylogenetic relationships study of African Tilapiine fishes using the mtDNA control region showed unresolved but a similar clustering pattern in the *Oreochromis, Sarotherodon,* and *Tilapia,* now revised *as Coptodon.* Klett and Meyer (2002), demonstrated this in their mtDNA NADH dehydrogenase subunit 2 (ND2) gene phylogeny in African cichlids. They reported that tilapiines do not form a monophyletic group in a phylogenetic tree with low resolution. This is consistent with the adaptive radiation for which cichlids are famous (Rometsch et al., 2020).

However, contrary to an earlier report (Syaifudin et al., 2019), my phylogenetic analysis of the cytochrome c oxidase subunit I (COI) gene revealed a clear separation between mouthbrooding tilapia and substrate spawners. Specifically, distinct clades were observed, indicating genetic divergence between mouthbrooding species. The closer similarity of *S. galilaeus* and *O. aureus* confirms their shared common ancestry (McAndrew and Majumdar, 1984; Pouyaud and Agnèse, 1995; Trewavas, 1982; Trewavas, 1983). Based on sequences deposited to the BOLD database, the predominantly farmed sites haplotype of *O. niloticus* (H24) have a major global distribution in Egypt, Madagascar, Uganda, Kenya, and the Philippines where the GIFT was developed (Ordoñez et al., 2017). The mapping of the Philippines sequences from the Freshwater Aquaculture Center – Central Luzon State University (FAC-CLSU), City of Muñoz, Nueva Ecija Specifically, to the GIFT sampled from Nigeria, suggests that the introduced improved farmed from Nigeria to have originated from the Philippines. The mapping of the other sequences to O. urolepis and O. mossambicus indicates that introduction must have come from multiple sources. Egypt and the Philippines were among the countries that contributed to early introduction of Tilapia including *O. niloticus* into countries in Asia and the Pacific (Welcomme, 1988). The GIFT project was also developed using native species from Egypt and farmed strains from the Philippines. Interestingly, most of the GIFT supplies come from these countries and by extension Thailand and China, where this haplotype was also found in my study. Madagascar was among the countries with the highest frequency of this haplotype. Madagascar has also benefited from early introduction of *O. niloticus* in 1956 and it has since then

become an important aquaculture species in the country (FAO, 2024a). Other countries like Kenya and Uganda where this haplotype was also reported might have been due to the recent GIFT introduction in sub-Saharan Africa (Sanda et al., 2024).

The most frequent *O. aureus* haplotype (H15), which included both farmed and wild sites, was predominantly found in Israel, Egypt, and the National Freshwater Fisheries Technological Center, City of Muñoz, Nueva Ecija, the Philippines (Ordoñez et al., 2017). In the global account of tilapia introduction of Welcomme (1988), Israel and Egypt were identified as being among the top donors of *O. aureus*. The high frequencies of matches in BOLD to sequences in Egypt, Israel, Madagascar and the Philippines can be credited to the extensive research on tilapia species identification in an effort to conserve native species and provide better aquaculture management plan that will not impact the wild sites negatively. Species identification serves as the cornerstone of biodiversity assessment and conservation efforts. The ability to differentiate between native tilapia species and introduced farmed strains is crucial for regulatory authorities, facilitating a comprehensive understanding of tilapia diversity within a country and enabling targeted conservation measures (Fischer, 2014).

3.5 Conclusions

In conclusion, the analysis of haplotype diversity, species identification and phylogenetic relationship of farmed and wild tilapia samples highlights valuable insights into the factors shaping tilapia genetic diversity, which could be used to investigate the environmental factors shaping the evolutionary relationships of these species. By integrating genetic data with updated morphological records of farmed and wild tilapia species, we can better safeguard the genetic integrity of tilapia populations, support sustainable aquaculture practices, and contribute to the preservation of freshwater biodiversity that will be beneficial for addressing the food security challenges.

Chapter 4 Population structure and genetic differentiation of farmed and wild tilapia using double digest restriction-site associated DNA (ddRADseq)

Abstract

The interest in tilapia aquaculture has resulted in the widespread introduction of GIFT by private fish farmers across different regions in Nigeria. However, since there have been unauthorised introductions by some these farmers from multiple sources that are not carried out by WorldFish, the genetic sources of the introduced farmed strains remain largely unknown. There is a significant dearth of information regarding the genetic diversity and population structure of farmed and native tilapia species in Nigeria. This study compared the genetic diversity and genetic differentiation between farmed and wild across species in Nigeria using double-digest restriction site-associated DNA sequencing (ddRADseq). In both *Oreochromis aureus* and *O. niloticus*, genetic diversity revealed that expected heterozygosity (*He*) and nucleotide diversity (*pi*) are lower in farmed compared to their wild counterparts. *He* and *pi* for *Pelmatolapia mariae* were found in two wild sites between the Asejire Dam (*He* = 0.276, *pi* = 0.311) and Lagos Lagoon (*He* = 0.224, *pi* = 0.256). Similarly, in *Sarotherodon galilaeus*, genetic diversity in River Benue Numan (*He* = 0.121, *pi* = 0.135) was lower compared to Lagos Lagoon samples (*He* = 0.334, *pi* = 0.353). Principal component analysis (PCA) did not reveal a clear genetic structure between the introduced farmed tilapia of the GIFT strain and the wild Oreochromis spp. and population. Species PCA only show a clear genetic cluster for *S. melanotheron*. Admixture analysis showed extensive hybridisation across multiple species including in *Oreochromis* spp., and what looks like an F1 hybrid between *P. mariae* and *S. melanotheron* in *Coptodon zillii* samples. Admixture analysis across sampling sites, revealed some samples from the cage site and Lagos Lagoon sharing similar genetic compositions. Previous mitochondrial DNA results confirmed these individuals in both the cage and Lagos Lagoon to share the same haplotype including GIFT samples previously identified in the Philippines, suggesting that they could be escapees from the installed aquaculture cages in

Lagos Lagoon. The results of this study provide an important genomic perspective on the genetic diversity and population structure of tilapia that that will be useful in designing a conservation programme for farmed strains and wild tilapia species.

4.1 Introduction

Fish are an essential source of livelihood and cheap animal protein alternative to millions of people around the world. It has been projected that about 90% (181 Mt) of fish produced by the year 2030 will be consumed as food, with the remaining 10% used for other essential services like fishmeal and fish oil (OECD/FAO, 2021). There has been a 14% rise in global capture fisheries production and a geometric rise of 527% in global aquaculture production from 1990 to 2018 (FAO, 2020). However, Africa, the continent with the fastest growing human population, has been predicted to outpace growth in its food fish supply, which will lead to a reduction in per capita fish consumption (OECD/FAO, 2021). When the human population outpaces the fish that is being produced, more pressure will be exerted on the fisheries resources. To ensure the continuous utilisation of fish and its byproducts for the benefit of humans, sustainable aquaculture development and effective management of fisheries must be implemented (FAO, 2020). The absence of fisheries management or its lack of enforcement has led to the depletion of fish stocks (FAO, 2020). The Conference of the Parties of the Convention on Biological Diversity (CBD) and the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) have set up global targets for biodiversity conservation and ecosystem services (Díaz et al., 2019). To support long-term human well-being, sustainable development, and the conservation and sustainable use of biodiversity, IPBES seeks to improve the science-policy interface for biodiversity and ecosystem services (IPBES, 2019b). IPBES produces assessments, reports, and recommendations—including Global Assessment Reports, Thematic Assessments, Methodological Assessments, and Policy Support—that help shape global biodiversity targets and policies (IPBES, 2019a). IPBES (2019b) have expressed concerns over the rate at which species are declining including the loss of genetic diversity in native species. IPBES is evaluating how humans affect ecosystem services and biodiversity; compiling current data on these topics to produce fresh policy-relevant insights; fostering an ongoing conversation

between researchers, policymakers, and knowledge holders; and identifying and addressing gaps in the body of global knowledge about ecosystem services and biodiversity (Bridgewater, 2017). At the 15th Conference of the Parties (COP15) in Montreal, Quebec, four goals and twenty-three targets were adopted in the UN biodiversity pact for 2030 (Li et al., 2023). The CBD adopted the Kunming-Montreal Global Biodiversity Framework (GBF) that outlined ambitious goals and targets for biodiversity conservation to be achieved by 2030, with a vision for 2050. This framework sets out the roadmap for how the nations of the world will attempt to halt and then reverse biodiversity loss through 2030 (Moss et al., 2023). Together, the CBD and IPBES hope to stop biodiversity loss, support ecosystem restoration, and guarantee the sustainable use of natural resources—all of which will help achieve the overarching objective of coexisting peacefully with the environment by the year 2050 (IPBES, 2019a).

Nigeria, being a member country of the CBD and IPBES will have to play a crucial role in addressing biodiversity loss through policy development and implementation that aligns with CBD's targets. Nigeria as a nation must play its roles in ensuring the conservation and sustainable utilisation of important habitats and species through monitoring and reporting of progress on biodiversity targets to the CBD Secretariat (IPBES, 2019b). However, when it comes to promoting sustainable aquaculture development and effective fisheries management including conservation of genetic resources, there is currently little data and the data that exist are mostly based on rough estimates (FAO, 2022).

Freshwater fish production has significantly expanded in the past seven decades, increasing from 19 million tonnes (live weight equivalent) in 1950 to an all-time high of approximately 179 million tonnes in 2018 (FAO, 2022). As of 2009, over 400 million Africans rely on fish as an important source of protein, minerals and micronutrients and demands are predicted to increase to 2.6 million tons a year by 2030 (WorldFish, 2009). In 2018, fisheries and aquaculture have provided employment to over five million people in Africa (FAO, 2020). With the African population growing at an exponential rate, the demand for fish will likely increase and more fish will be harvested from the depleting wild populations. Inland/freshwater fishes, which are the major source in Africa, face threats from overfishing, climate change, dams and water diversions, extensive wetland drainage, groundwater depletion, aquatic habitat loss and fragmentation, and

establishment of introduced non-native species (Dudgeon et al., 2006; IPBES, 2019a; Muringai et al., 2021; Osathanunkul and Suwannapoom, 2023). Also, the lack of implementation or absence of effective fish conservation policies in most sub-Saharan African countries (Sanda et al., 2024) is resulting in poor conservation and management efforts as well as a predicted decline in the genetic diversity of native species. These threats are now a global phenomenon that will likely have adverse effects ranging from the collapse of freshwater fish populations to species extinction in regions where proper fish conservation and management guidelines are lacking. However, despite the ongoing introduction of non-native aquaculture species into new environments, there is a notable lack of genomics studies assessing the genetic diversity of both farmed and wild stocks of these species in Nigeria. Furthermore, the potential consequences of fish escape from aquaculture facilities have not been adequately addressed. This knowledge gap represents a critical area of research that requires attention.

Aquaculture has been identified as an alternative source of fish production and a way to ease fishing pressure in the wild. This industry has become increasingly important in the global economy (FAO, 2022). In sub-Saharan Africa, for example, it has been gaining attention to address issues related to food security and economic development (Olu, 2023). However, fish farming in this region is mostly done at a subsistence level, with low levels of technology being employed. The challenge has been, therefore, accessibility of quality seed (fingerling), a key determinant for a successful and fast-growing fish stock (Moyo and Rapatsa, 2021). Farmers have resorted to introducing breeding populations and ready-to-stock fingerlings from other countries. The most farmed species in sub-Sahara Africa is the Nile tilapia (*Oreochromis niloticus*), a hardy species with a high tolerance to a wide range of environmental conditions (Gracida-Juárez et al., 2022). This species is native to Africa, but most countries in sub-Saharan Africa are yet to harness its full potential and therefore must rely on countries like Egypt, the Netherlands and Thailand for the supply of fast-growing Genetically Improved Farmed Tilapia (GIFT) (Ragasa et al., 2022). The GIFT is a product of a Nile tilapia selective breeding programme that started in 1988 in Malaysia when the International Center for Living Aquatic Resources Management (ICLARM), now WorldFish Center, collaborated with the Institute of Aquaculture Research in Norway (also known as AKVAFORSK), the Philippines National Freshwater Fisheries Technology Research Center of the Bureau of Fisheries and

Aquatic Resources, the Freshwater Aquaculture Center of the Central Luzon State University, and the Marine Science Institute of the University of the Philippines (Puttaraksar, 2004). Now past their 20th generation of selective breeding, the GIFT strain is one of the major turning points for the tilapia aquaculture industry (Henriksson et al., 2017). Following the success of the project, strict guidelines were implemented for its dissemination to avoid potential negative genetic impacts on native tilapia species in the case of wrongful introduction (Walter, 2005). However, this approach has not prevented the unlawful distribution of GIFT in countries that do not have a dissemination agreement with WorldFish or have not regulated its introduction (Ansah et al., 2014).

Addressing the negative impacts of species introductions is essential for maintaining a healthy aquatic ecosystem. To minimise the negative impact of the introduction of non-native species, the Food and Agriculture of the United Nations (FAO) recommend that it is important to have a comprehensive understanding of the species and the specific environmental conditions (UNFAO, 2022). Understanding a species and its environment before introduction is crucial for several reasons, including the risk of disrupting existing ecological balances, outcompeting native species, and genetic introgression or hybridization with native species (Atalah and Sanchez-Jerez, 2020; Hoban et al., 2023a). Past events have linked these threats of invasiveness and introgression to tilapia; for example, evidence of *O. niloticus* introgressive hybridisation into other native tilapias has been established in Ghana, Kenya, Malawi, South Africa, Tanzania, Uganda and Zambia (Anane-Taabeah et al., 2019; Bartley, 2021; Bradbeer et al., 2019; Brummett et al., 2008; D'Amato et al., 2007; Diedericks et al., 2021; Nzohabonayo et al., 2017; Wasonga et al., 2017). Other negative genetic impacts recorded from the introduction of non-native *O. niloticus* include: (i) hybridisation with critically endangered *O. jipe* in Tanzania (Bradbeer et al., 2019); (ii) hybridisation with South African native *O. mossambicus* (D'Amato et al., 2007); and (iii) the disappearance of *Oreochromis variabilis* from Lake Victoria, Kenya, following its hybridisation with *O. niloticus* (Wasonga et al., 2017). However, despite these harmful effects of tilapia introduction in sub-Saharan Africa, appropriate measures including the right policy framework are still not in place to address these problems.

The tilapia aquaculture sector in Nigeria is in its infancy and is the second most farmed species after the African catfish (*Clarias gariepinus*) (FAO, 2022). The GIFT is the choice species for the Nigerian tilapia aquaculture industry and private farms are introducing this strain from Thailand, Egypt, and the Netherlands without the government's support. It is only recently, in 2022, that the Nigerian government signed an agreement with WorldFish through Premium Aquaculture Limited to officially transfer GIFT from Malaysia to assist in developing the seed stocks in Nigeria (Bartley, 2021). However, the Ministry of Agriculture and Rural Development in Nigeria, which is responsible for the regulation and management of fisheries and aquaculture activities and who through the Minister of Agriculture and Rural Development made the request for the GIFT introduction, have not put measures in place to control any negative impacts that might arise from fish escape; neither has the Fisheries Act of 2014 addressed the potential genetic impacts of aquaculture species on wild fish (Nigeria Fisheries Act, 2014). Such unplanned introductions will predispose native species (including *Coptodon zillii, O. niloticus, O. aureus, Sarotherodon melanotheron, S. galilaeus,* and *Tilapia mariae*) to risks of getting displaced or local extinction (Champneys et al., 2021). The lack of risk assessment and risk management measures before planning the introduction of species that might be considered invasive contradicts CBD targets aimed at reducing the rate of introduction and establishment of invasive non-native species (Hoban et al., 2020).

The native tilapia species are morphologically diverse in terms of body shape and colour and their distribution ranges from lakes, rivers, creeks, and dams across the country. These species have been characterised morphologically (Idodo-Umeh, 2003), but with only a few studies on molecular identification at the species-level using DNA barcoding approaches to discriminate freshwater fish species including cichlids in Nigeria (Iyiola et al., 2018; Nwani et al., 2011b). Moreover, little is known about the levels of inter- and intra-specific levels of genetic diversity that exist in African tilapia species.

Furthermore, most aquaculture sites in the country fall within the extreme flood zones (Nkwunonwo et al., 2016) and face threats of fish escape from farms to the wild. Bartley (2021) predicted that it is possible that GIFT will hybridise with native *O. niloticus* and recommended that special attention should be given to

the depleted *S. galilaeus* that could potentially be impacted genetically following the GIFT introduction in the event of an escape from farms to the wild. Furthermore, the pre-agreed actions for the GIFT introduction required WorldFish to verify the genetic diversity of the strain before introduction while the government of Nigeria is expected to protect the native genetic diversity. Other responsibilities that the Nigerian government must implement include reducing unauthorised movement of GIFT and following best practices in grow-out of these fish (Bartley, 2021). However, Nigerian aquaculturists have not been following best breeding practice since the time of unauthorised introduction of GIFT to the current era of official introductions. There has also been uncontrolled movement of GIFT across the country without any hindrance. This unethical practice could alter the genetic diversity and population structure of wild tilapia and inbreeding in the farmed GIFT populations.

Genetic population structure is important for understanding species evolution, and discriminating distinct populations based on the evolutionary trajectories or how they are connected by gene flow (Jérôme Duminil et al., 2007). The accessibility of whole-genome-based perspectives enables more detailed assessment of possible impacts of introduced species on wild populations than individual gene-based approaches, including detection of hybridisation and gene flow between species and populations, which could be important indicators for the conservation, management and design of breeding programmes (Lind et al., 2019). The absence of these genetic perspectives in Nigeria's fish conservation at a time when the country is carrying out massive fish introduction without designated aquaculture zones, is a threat to other native tilapia species. The integration of genetic approaches into fisheries management and aquaculture is thus important for preserving genetic diversity of wild stock and managing genetic impacts of aquaculture (Laikre et al., 2005).

Advances in sequencing technologies have led to several genomic approaches for studying large numbers of individuals for large numbers of populations, including cost effective reduced-representation sequencing approaches that do not require sequencing the whole genome but sections of the genome targeted with the aid of restriction enzymes (Gu et al., 2011). One of the reduced representation methods that has been applied in fisheries and aquaculture research is restriction site-associated DNA sequencing (RADseq), which is based

on the sequencing of DNA fragments following digestion of the genome with restriction enzyme(s) (Baird et al., 2008; Bayona-Vásquez et al., 2019; Hohenlohe et al., 2011; Hohenlohe et al., 2012; Moses et al., 2020; Peterson et al., 2012). RADseq has been used to investigate population structure, admixture, and phylogenomic (Moses et al., 2020). In this study, I used double digest restriction site-associated DNA sequencing (ddRADseq), a variant of RADseq that involves digesting genomic DNA with two restriction enzymes (usually combining a frequent with a rare cutter) followed by ligation with unique barcodes, size selection of a specific range to be sequenced, PCR amplification and sequencing to discover single-nucleotide polymorphisms (Peterson et al., 2012).
The overall aim was to use this approach to study the genetic diversity and population structure of introduced GIFT and native tilapia species in Nigeria. The specific objectives were to:

i. Compare patterns of genetic diversity and relative levels of inbreeding of introduced farmed GIFT compared to wild native tilapia populations across different regions of Nigeria
ii. Assess whether patterns of differentiation are consistent with the species classifications of farmed and wild species based on mitochondrial DNA sequencing
iii. Investigate whether there is evidence of introgression between farmed GIFT and wild native populations

4.2 Methods

4.2.1 Sampling

Farmed (n=203) and wild tilapia (n=318) were collected from 19 locations (11 wild and 8 farmed) in three regions (North-Central, North-East and South-West) across Nigeria (Figure 4.1), targeting 30 samples per location (Table 4.1). Tilapia samples were identified using a taxonomic field guide for Nigerian freshwater fishes (Olaosebikan and Raji, 1998) and lateral view photograph images of the samples were taken for both wild (Figure 4.2) and non-native farmed (Figure 4.3) species and strains. Wild fish were sampled after harvest at the respective fish landing sites. Most of the tilapias were caught with cast nets, lines, and traps. For all tilapia samples, we collected a fin clip and preserved it in RNA*later* solution, preceding genomic DNA extractions.

4.2.2 Genomic DNA isolation and sample selection

As described in Chapter 3, genomic DNA was extracted from all 521 fish fin clips (≤25 mg) using DNeasy Blood & Tissue Kits (Qiagen Inc, Paisley, UK), following the manufacturer's instructions and eluted into 100 µl of AE buffer from the Qiagen kit. The integrity of the extracted DNA was verified in 2% agarose gel electrophoresis and the concentrations (ng/µl) were measured using a Qubit 2.0 Fluorometer, using the broad-range kit (Invitrogen, MA, USA). Species identification was confirmed for each individual was based on DNA barcoding using the cytochrome oxidase I gene, as described in Chapter 3. Samples for the library preparation were selected based on three criteria: 1) firstly, samples that were used in the mtDNA analysis were selected, ensuring representation of individuals from the 25 identified haplotypes (see Chapter 3); 2) secondly, degraded DNA samples used for mtDNA analysis were dropped from the library selection and replaced with other samples from the same site with the degraded samples; 3) RADseq library samples were screened for clear band on a gel and having a concentration of ≥ 20 ng/µl. The aim of including duplicates is to assess the technical reproducibility of the library preparation process by monitoring the distribution or reads between duplicate samples to track variation introduced during library preparation and sequencing steps. This resulted in 193 samples, separated into two libraries, including two negative controls (one per library) and on average 10 individuals per sampling site (Table 4.1).

4.2.3 Library preparation

4.2.3.1 Digestion

To enable pooling of samples and demultiplexing of reads during downstream bioinformatic analysis, unique pairs of molecular barcodes (i.e. short nucleotides sequences used to tag both ends of fragments from a particular individual) were assigned to each sample. To minimise bias in the distribution of samples in terms of barcode allocations, the samples were randomised in a spreadsheet before assigning to positions in two 96 well plates (Table 4.2).

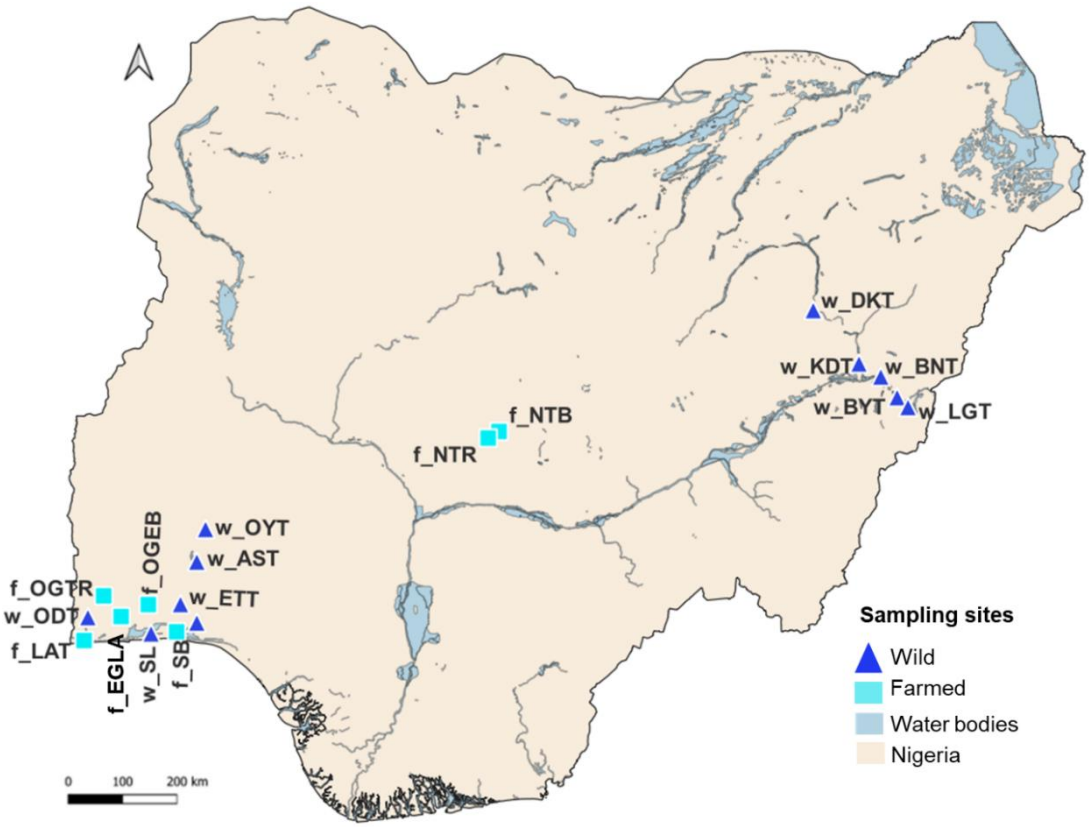

Figure 4.1 Map of Nigeria illustrating the spatial distribution of water bodies and sampling locations within the study area, categorised based on the origin of the sampled Tilapia sampling site (farmed = squares, prefix "f_"; wild = triangles, prefix "w_"). The code names are interpreted as follows: EGLA=black GIFT from Egypt collected in Lagos, LAT=black GIFT from Thailand collected in Lagos, NTB=black GIFT from Thailand collected from Nasarawa, NTR=red GIFT from Thailand collected from Nasarawa, OGEB=black GIFT from Egypt collected in Ogun, OGER=red GIFT from Egypt collected in Ogun, OGTR=red GIFT from Thailand collected in Ogun, SB=black GIFT from Thailand collected in Lagos, BNT=River Benue tilapia from Numan, Adamawa, BYT=River Benue tilapia from Yola, Adamawa, KDT=Kiri dam tilapia from Adamawa, LGT=Lake Geriyo tilapia from Adamawa, DKT=Dadin Kowa dam tilapia from Gombe, ETT=Etele River tilapia from Ogun, ODT=Odo Idimu tilapia from Ogun, OYT=Oyan dam tilapia from Ogun, SL=Lagos lagoon tilapia from Lagos, BDT=Badagry creek tilapia from Lagos, AST=Asejire dam tilapia from Oyo.

Table 4.1 Collection site and source of introduced Genetically Improved Farmed Tilapia (GIFT) and wild tilapia samples collected from various states in Nigeria, indicating region, source of the introduction, whether the sample is GIFT or wild, the colour of the GIFT strain, the total sample size collected per site (N), the number of samples included for ddRAD, and the geographical coordinates.

Collection site	Region	Code	Source	GIFT	STRAIN	Sample size	#ddRAD	Latitude	Longitude
Lagos	southwest	f_EGLA	Egypt	Yes	Black	30	10	7.259	3.256
Lagos	southwest	f_LAT	Thailand	Yes	Black	30	11	6.416	2.876
Nasarawa	northcentral	f_NTB	Thailand	Yes	Black	30	11	8.880	7.763
Nasarawa	northcentral	f_NTR	Thailand	Yes	Red	30	11	8.880	7.763
Ogun	southwest	f_OGEB	Egypt	Yes	Black	30	10	9.457	12.038
Ogun	southwest	f_OGER	Egypt	Yes	Red	30	10	9.457	12.038
Ogun	southwest	f_OGTR	Thailand	Yes	Red	30	12	6.642	3.203
Lagos	Southwest	f_SB	Thailand	Yes	Black	23	11	6.428	2.848
Adamawa	northeast	w_BNT	R. Benue Numan	Wild	Wild	30	9	9.475	12.039
Adamawa	northeast	w_BYT	R. Benue Yola	Wild	Wild	30	10	9.284	12.467
Adamawa	northeast	w_KDT	Kiri Dam	Wild	Wild	30	8	9.681	12.009
Adamawa	northeast	w_LGT	Lake Geriyo	Wild	Wild	30	10	9.293	12.434
Gombe	northeast	w_DKT	Dadin Kowa Dam	Wild	Wild	30	9	10.319	11.477
Ogun	southwest	w_ETT	Etele River	Wild	Wild	30	11	6.586	3.162
Ogun	southwest	w_ODT	Odo Idimu River	Wild	Wild	30	11	6.580	3.182
Ogun	southwest	w_OYT	Oyan Dam	Wild	Wild	30	10	7.363	4.136
Lagos	southwest	w_SL	Lagos lagoon	Wild	Wild	29	10	6.428	2.848

Lagos	southwest	w_BDT	Badagry Creek	Wild	Wild	29	10	6.416	2.876
Oyo	southwest	w_AST	Asejire Dam	Wild	Wild	20	9	7.363	4.136
Total		-	-	-		551	193		

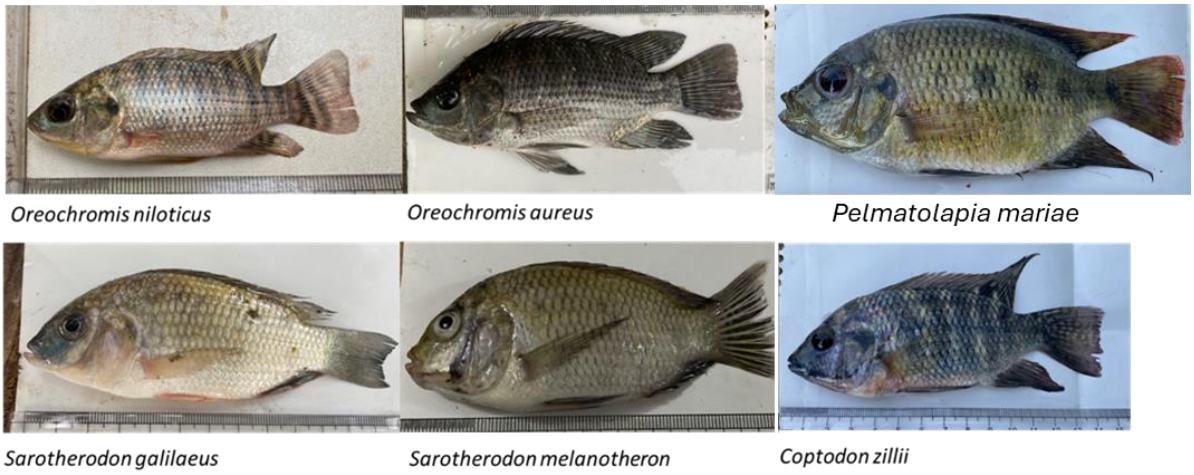

Figure 4.2 Wild tilapia species collected across three geographic locations (North-Central, North-East and South-West) in Nigeria

Figure 4.3 Non-native farmed tilapia strains introduced from Egypt and Thailand collected in commercial farms in North-Central and South-Western Nigeria

Equimolar concentrations of DNA from each sample were digested at 37°C for 3 hours with two restriction enzymes (1 µl each) -*MspI* (frequent cutter) and *PstI-HF* (rare cutter), 5 µl of cutsmart buffer (New England Biolabs, UK) and water was added to a final reaction volume of 50 µl. Digested DNA was cleaned with a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) to remove the residual restriction enzymes; final elution was in 23 µl of elution buffer. To verify that the digestion was successful, 5 µl of the sample was checked by running on a 2% gel electrophoresis and the final concentrations were measured using the Qubit broad-range protocol (Invitrogen, MA, USA).

4.2.3.2 Adapter ligation

Equimolar concentrations of the cleaned digested products were ligated to the unique molecular barcodes assigned to each sample in a 40 µl reaction volume that included 0.5 µl of T4 ligase (2,000 U/µl), 4 µl of 10X T4 ligation buffer, 0.5 µl of 10 µM barcodes P1 and P2. Each DNA fragment in the library has P1 adapter sequences ligated to one end and P2 adapter sequences ligated to the other end. The ligation mix was incubated at 25°C for 30 minutes, 65°C for 10 min, then slowly cooled down to room temperature at 2°C per 90 sec. The two libraries were pooled separately, cleaned using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany) as described above in the digestion section and the concentrations measured using Qubit 2.0 Fluorometer (Invitrogen, MA, USA).

4.2.3.3 Size selection

Size selection in the range of 300-450 bp was performed using a Pippin Prep (Sage Science, Beverly, MA) with a 2% agarose gel cassette following the manufacturer's protocol and eluted in 40 µl Tris-TAPS buffer. The concentration of the size-selected multiplexed libraries was measured with a Qubit high sensitivity assay.

4.2.3.4 RAD tag enrichment, purification, quantification, and sequencing of libraries

PCR amplifications were set up using 2.5 – 10 ng of RAD library template for each library to enrich the loci of interest (300-450 bp) in a 20 µl PCR reaction volume using the Phusion High-Fidelity DNA Polymerase kit (Thermo Scientific, USA). The reaction master mix included 10 µl of 2X Phusion High Fidelity MasterMix, 1.0 µl

each of RAD F and RAD R primers (10 µM), 2.5-10 ng (max. 8µl) of RAD library template filled up to 20 µl with nuclease-free water. The 10 cycle PCR reaction was performed in a thermal cycler using the program set up of: 98˚C at 30sec; 9 cycles of 98˚C for 10 seconds, 65˚C for 30 seconds, and 72˚C for 30 seconds.

Table 4.2 DNA combinatorial barcodes denoted by their unique names, P1 and P2, with corresponding barcode adapters for double digest restriction site-associated DNA sequencing (ddRADseq) library preparation. Together, P1 and P2 adapters allow for multiplexing of multiple samples in a single sequencing run and subsequent demultiplexing of sequencing data.

P1	Barcode P1	P2	Barcode P2
sP_1	CTCC	P2_1	TAG
sP_2	TGCA	P2_2	CCT
sP_3	ACTA	P2_3	ATCG
sP_12	TGCGA	P2_4	GAGC
sP_13	CGCTT	P2_5	CTAA
sP_14	TCACC	P2_6	TTGC
sP_34	GGTTGT	P2_7	GCAT
sP_35	CCAGCT	P2_8	ACTG
sP_36	TTCAGA		
sP_57	CTTGCTT		
sP_58	ATGAAAC		
sP_60	GAATTCA		
sP_78	ACGACTAC		

This was followed by one final cycle of 72˚C for 5 min, and held at 4˚C. The final PCR products containing the libraries were visualised on a 1.5% agarose gel electrophoresis using 1X Orange Loading Dye alongside a 100 bp DNA marker to verify the size selection result (300-450 bp). PCR reactions from each library were pooled separately and loaded in a single well on a 1.25% agarose gel and run for 45 minutes with a 100 bp DNA Marker. The product was excised from the gel with a razor blade and purified with the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany), following the gel clean-up protocol and eluted twice with 10 µl elution buffer to make a final elution volume of 20 µl. Libraries were quantified using a Qubit 2.0 Fluorometer broad range assay. A further verification of the size-selection range for each library was carried out (Figure 4.4) on a TapeStation D1000 ScreenTape (Agilent Technologies, Inc.,

Santa Clara CA). Each library was sequenced using 100bp paired-end reads on an Illumina Nextseq 2000 (Illumina Inc., San Diego, CA, USA) at the University of Glasgow Polyomics facility.

4.2.3.5 Sequence analysis

ddRADseq libraries were demultiplexed using the *process_radtags* module in Stacks v2.65 (Rivera-Colón and Catchen, 2022) to assign sequencing reads to their respective samples based on the unique barcode sequences incorporated during library preparation. This searches for the cut sites of the restriction enzymes used during the digestion step and then finds the pair of barcodes for each sample before distributing the reads to the respective samples. Default minimum base quality (10) and Phred scores (33) were applied as filtering parameters during the demultiplexing stage. Further filtering parameters that were applied include the removal of reads with uncalled bases and rescuing barcodes and RAD-tag cut sites to assign sequencing reads to their respective samples. The quality of the reads was summarised using FastQC v0.12.0, a program designed to provide quality control checks on raw sequence data from high throughput sequencing pipelines (Andrews, 2010). Further quality control steps were carried out to filter out low coverage data (typically less than 1 million reads).

4.2.4 Data analysis

Twelve and seven individuals were removed from libraries and 1 and 2, respectively. The remaining reads were aligned to the Nile tilapia (*Oreochromis niloticus*) reference genome (GCF_001858045.2) using the Burrow-Wheeler Aligner (BWA-MEM) v0.7.17 for short read alignments (Li and Durbin, 2009). This operation is necessary to identify reads belonging to a specific locus and for placing genotyped loci in the context of chromosomes, as opposed to clustering based on sequencing similarity used by the *de novo* approach (Rivera-Colón and Catchen, 2022). Stacks *ref_map.pl* pipeline module was executed to construct a catalog of loci (genomic regions) and alleles present in the dataset, that serves as a reference for subsequent analyses. Duplicate samples were excluded from further analysis because some of them have low coverage which can lead to false SNP discovery.

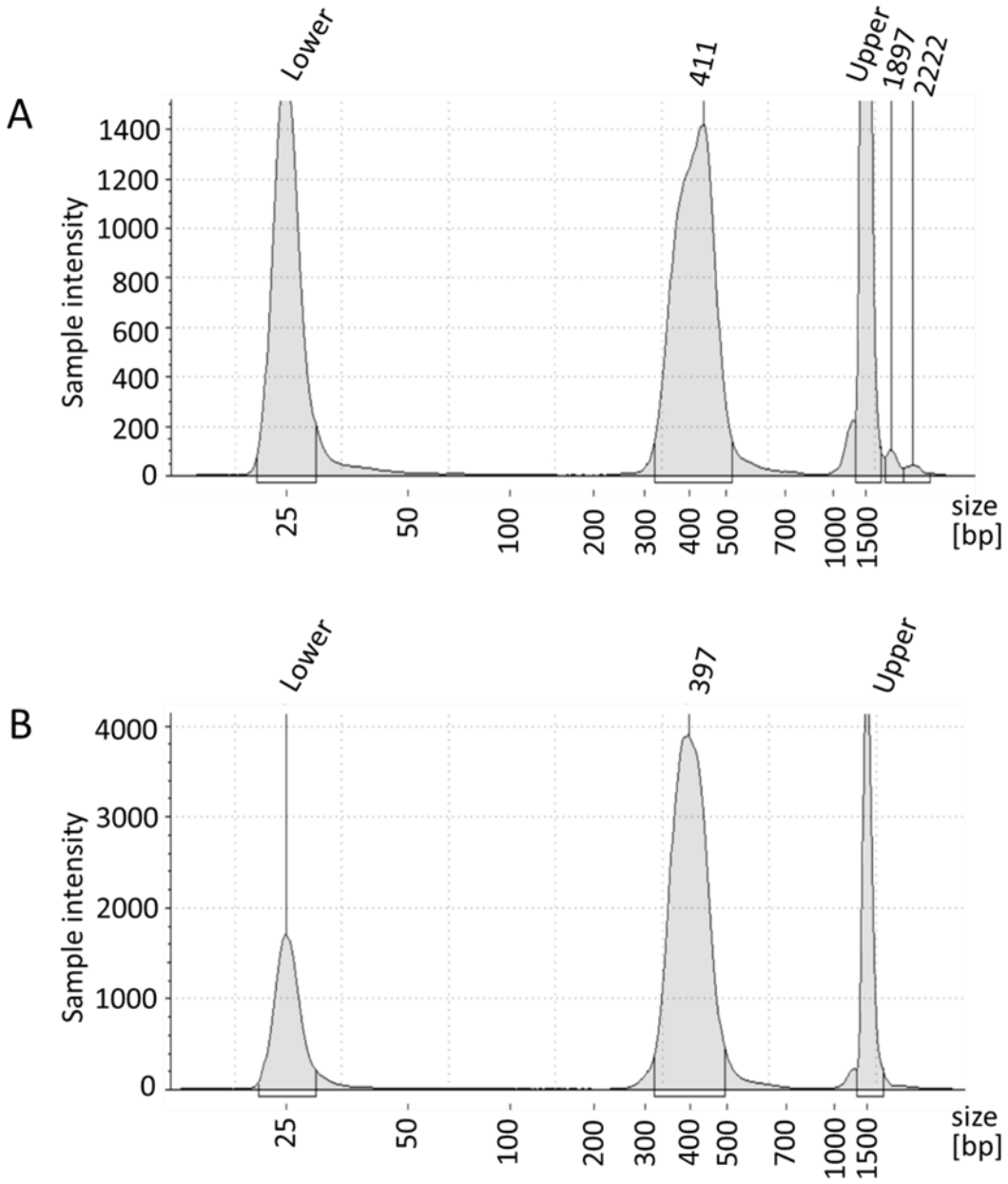

Figure 4.4 Analysis of sample size, quantity, and integrity of ddRAD libraries 1 (A) and 2 (B). The middle peaks between the lower and upper range show the sizes of the libraries.

4.2.4.1 Genetic diversity and inbreeding

The *populations* module in Stacks was executed to calculate summary statistics to compare species in farmed and wild sampling sites. Observed heterozygosity (*Ho*) was calculated based on the proportion of heterozygous individuals in a sampling site, and the expected heterozygosity (*He*, gene diversity) was estimated based on allele frequencies predicted under Hardy-Weinberg equilibrium (HWE) using Stacks (Rivera-Colón and Catchen, 2022). The inbreeding coefficient (*Fis*) was calculated from the observed and expected heterozygosity data to quantify whether there was an excess or deficit of heterozygotes within sampling site. Nucleotide diversity, also known as pi (π),

was calculated to show the average nucleotide difference per site and the variability within sampling site or species. To avoid issues with linkage among SNPs within a locus, all the sampling site genetic analyses were restricted to only the first SNP per locus (Rivera-Colón and Catchen, 2022). To test whether there were significant differences in the summary statistics between farmed and wild samples, paired t-tests were conducted.

4.2.4.2 Principle Components Analysis

A Principal Components Analysis (PCA) was conducted based on allele frequencies from data contained in the Genepop file generated from Stacks populations analysis. The Genepop file was loaded into R using the Adegenet package and converted into GenInd object (Jombart, 2008). The GenInd object was used to generate a population sample table with individual samples and population ID and converted into dataframe. To visualise genetic variation and relatedness among individuals within and differentiation across sampling sites and species through patterns of clustering, the scale function scaleGen and dudi.pca were executed. PCA eigenvalues were added to the dataframe generated earlier and a scatterplot function was executed using ggplot2 package in R (Wickham, 2016) to visualise principal components 1 and 2, which explained most of the genetic variation in the data. Colour were added manually using ggplot2 scale_color_manual function to identify different sampling cluster.

4.2.4.3 Genetic differentiation and admixture analysis

Pairwise *Fst* was calculated between each pair of populations using Genepop output from the Stacks populations analysis (Rivera-Colón and Catchen, 2022). To assess genetic differentiation among sampling sites, *Fst* values were calculated using the hierfstat (Goudet, 2005) and adegenet (Jombart et al., 2020) packages in R (R Core Team, 2018). The Genepop file was read into R and converted to a genind object to compute pairwise *Fst* values using the genet.dist function with the "WC84" method for implementation of Weir and Cockerham (1984) *Fst*. Clustered heatmaps were generated based on the *Fst* values using the Euclidean distance function from the pheatmap package in R (Kolde, 2019). Using the boot.ppfst function, a permutation test with 1,000 bootstrap replicates was performed to assess the significance of the reported *Fst* values (Goudet, 2005). Genetic variation within and between sampling sites was

visualised using principal components analysis (PCA), as implemented in R using the Adegenet (Jombart et al., 2020) and ggplot2 (Wickham, 2016) packages. To infer and quantify the ancestry proportions of individuals, sampling sites and species, the SNPs from the populations analysis in Stacks (Rivera-Colón and Catchen, 2022) in VCF format was filtered using PLINK v1.9 (Chang et al., 2015) to remove minor allele frequency (MAF) that are less than 0.05 from the genetic data. The ADMIXTURE program (Alexander et al., 2009), which is based on maximum likelihood and a fast numerical optimization algorithm, was used to estimate the proportion of ancestry contributed by individuals across the 19 tilapia sampling sites, with K values ranging from 1 to 20 using 1000 bootstrap replicates and 3 iterations (Alexander et al., 2009) and visualised in R (R Core Team, 2018). A separate analysis was conducted to test patterns of admixture at the species level, using K values ranging from 1 to 9.

4.3 Results

4.3.1 Sequencing reads and depth of coverage

After demultiplexing and cleaning the reads with default parameters, 689,738,734 (78.9%) and 796,667,910 (94.6%) reads were retained in libraries 1 and 2, respectively. Overall, retained reads across the 19 sampling sites was over 90%, except for the w_KDT samples in library 1 which had individuals with reads ranging from about 80 – 95%. Library 2 had a better proportion of retained reads than library 1, with most individuals in each sampling site having over 90% retained reads. In terms of read numbers, Library 1 had a retained reads distribution from 55,058 – 49,231,001 while library 2 had reads ranging from 52,515 – 47,334, 744. Samples with small amount of reads (less than 1 million reads) were filtered out to avoid introducing genotyping bias in the downstream analyses. The mean coverage after filtering out samples with less than 1 million reads and combing reads from both libraries was 17.1X (Figure 4.5), with 2317083 assembled loci in total (Figure 4.6).

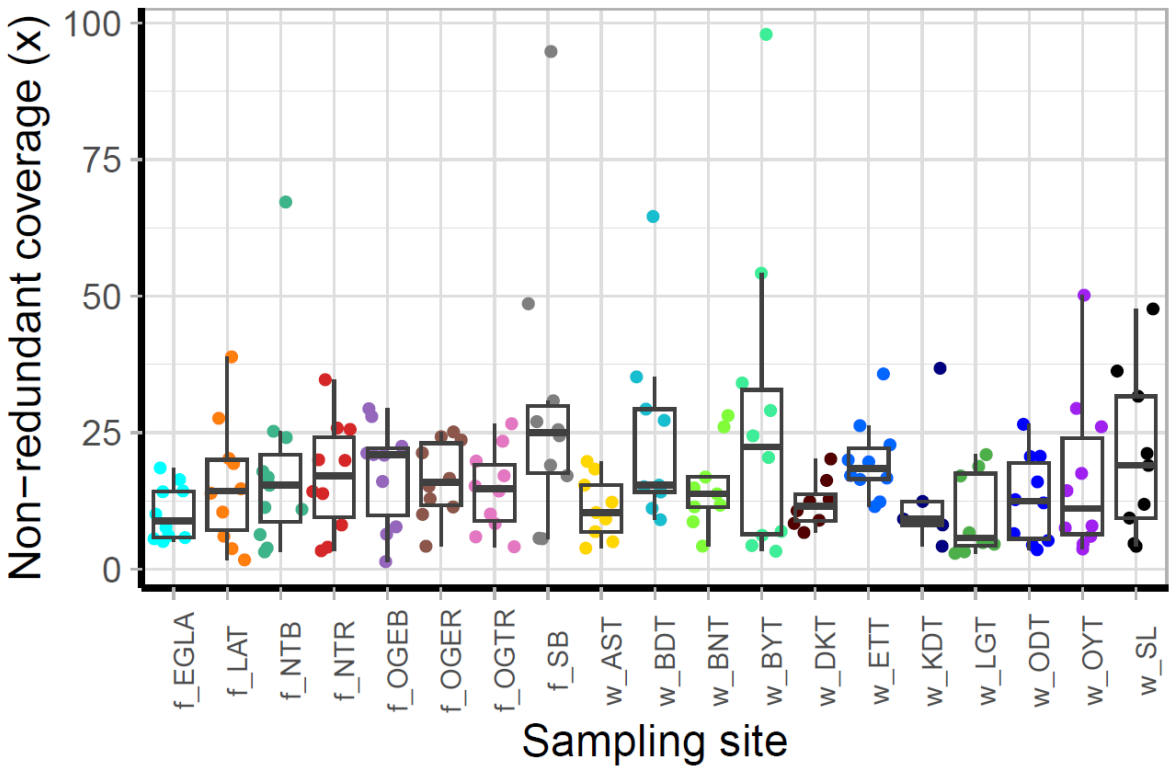

Figure 4.5 Boxplots showing the average coverage and distribution of reads, after pooling libraries 1 and 2 together. Each point represents a sample in each sampling site. The x axis represents the 19-sampling sites and the y axis the non-redundant read coverage (i.e. the number of unique reads that cover a specific locus, providing information about the depth of sequencing coverage for that region without counting duplicate reads). Samling sites with the prefix "f_" represents farmed tilapia while those with "w_" are the wild native tilapia samples.

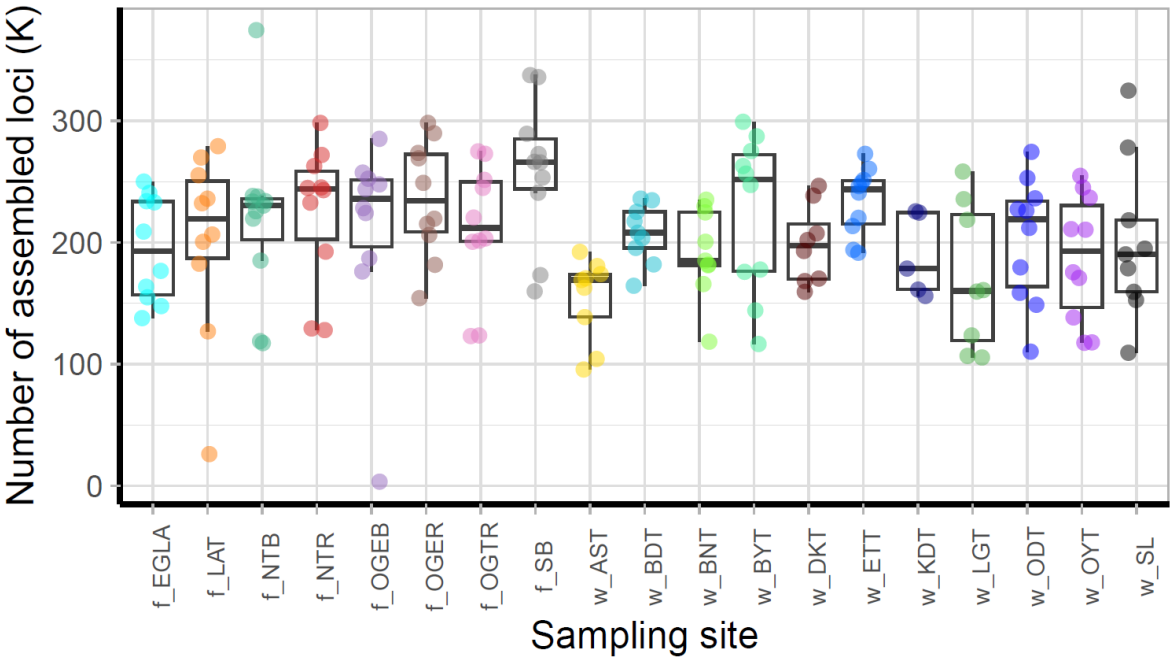

Figure 4.6 Boxplot showing the number of assembled RAD loci (K = x1000) in each sampling sites from sequencing of the pooled libraries.

4.3.2 Comparing genetic diversity and inbreeding between farmed and wild tilapia

The genetic diversity summary statistics and inbreeding coefficients were similar across the farmed and wild sampling sites for the *O. aureus* samples. *O. aureus* was found in seven wild but only one farmed (f_NTB) site (Table 4.3). Observed heterozygosity (*Ho*) in f_NTB (0.214) was higher than for the wild sampling sites except for w_ETT where it was observed to be slightly higher (0.247). Expected heterozygosity was slightly higher in f_NTB (*He* = 0.228) and w_DKT (*He* = 0.207) compared to the other sampling sites. ranging from *He* = 0.087 (w_ODT) to *He* = 0.193 (w_ETT). Similarly, except for w_ETT (0.257), nucleotide diversity (*pi*) was higher in the farmed f_NTB compared to the wild samples, which ranged from 0.116 (w_ODT) to 0.222 (w_DKT). Except for w_DKT (*Fis* = 0.312), inbreeding coefficients were low for all of the farmed and wild sampling sites, ranging from 0.008 (w_BNT) to 0.068 (f_BNT).

Within *O. niloticus,* observed heterozygosity was significantly higher (*t* = 3.650, *df* = 7.712, *p* = 0.007) in the six farmed (mean = 0.175, range = 0.093 – 0.205) compared to the four wild sampling sites (mean = 0.102, range = 0.075 – 0.125) (Table 4.4). Expected heterozygosity was also higher in farmed (mean = 0.187, range = 0.182 – 0.193) compared to wild (0.114, range = 0.056 – 0.214) but their mean difference was not statistically significant (*t* = 2.119, *df* = 3.016, *p* = 0.124). Pairwise nucleotide diversity in farmed *O. niloticus* was lowest in f_SB (*pi* = 0.195) and highest in f_NTB (pi = 0.220). The wild sites had nucleotide diversity ranging from 0.075 (w_KDT) to 0.245 (w_SL). However, comparison of nucleotide diversity between farmed (mean = 0.203) and wild (mean = 0.131) was not statistically significant (*p* = 0.156). The highest positive inbreeding coefficients (*Fis*) were in farmed f_NTB (*Fis* = 0.234) and wild w_SL (*Fis* = 0.245). The remaining farmed and wild sites had low *Fis* or weakly negative *Fis* and an excess of heterozygotes (*Ho* > *He*) in farmed f_LAT and f_OGEB as well as the wild w_ETT, w_KDT, and w_ODT. The difference between farmed and wild sampling sites was not statistically significant (p = 0.990).
P. mariae were only found in the wild Asejire dam (w_AST) and Lagos Lagoon (w_SL) sites. Observed heterozygosity in w_SL (*Ho* = 0.237) was higher than w_AST (*Ho* = 0.142) (Table 4.5). However, expected heterozygosity and

nucleotide diversity were slightly higher in w_AST (He = 0.276, pi = 0.311) compared to w_SL (He = 0.224, pi = 0.256). The inbreeding coefficient was found to be high in w_AST (*Fis* = 0.383) in comparison with w_SL (*Fis* = 0.042).

Observed heterozygosity in w_BNT (0.116) was slightly higher than w_OYT (0.077), however, genetic diversity parameters estimated in w_BNT(*He* = 0.121, *pi* = 0.135) were lower than the ones from w_OYT (*He* = 0.353, pi = 0.353) (Table 4.6). Inbreeding coefficient was very high in w_OYT (*Fis* = 0.715) compared to w_BNT (*Fis* = 0.042).

Table 4.3 Genetic diversity and inbreeding coefficients of farmed and wild *Oreochromis aureus* samples across Nigeria. Site: sampling site; N: sample size; *Ho*: observed heterozygosity; *He*: expected heterozygosity; *pi*: pairwise nucleotide diversity; *Fis*: inbreeding coefficient.

Site	N	*Ho*	*He*	*pi(π)*	*Fis*
f_NTB	7	0.214	0.228	0.245	0.068
w_BNT	3	0.155	0.133	0.159	0.008
w_BYT	9	0.149	0.149	0.158	0.024
w_DKT	8	0.141	0.207	0.222	0.312
w_ETT	2	0.247	0.193	0.257	0.015
w_KDT	3	0.170	0.146	0.175	0.010
w_LGT	7	0.138	0.139	0.151	0.031
w_ODT	2	0.109	0.087	0.116	0.011

Table 4.4 Genetic diversity parameters and inbreeding coefficient across farmed and wild *Oreochromis niloticus* in Nigeria.

Site	N	*Ho*	*He*	*pi(π)*	*Fis*
f_EGLA	9	0.179	0.188	0.199	0.057
f_LAT	9	0.205	0.191	0.204	-0.001
f_NTB	3	0.093	0.183	0.220	0.234
f_OGEB	10	0.204	0.193	0.204	0.000
f_OGTR	8	0.183	0.182	0.195	0.033
f_SB	10	0.183	0.187	0.197	0.042
w_ETT	6	0.110	0.099	0.108	-0.004
w_KDT	2	0.075	0.056	0.074	-0.001
w_ODT	7	0.098	0.088	0.095	-0.006
w_SL	4	0.125	0.214	0.245	0.251

Table 4.5 Genetic diversity parameters and inbreeding coefficient in wild *Pelmatolapia mariae* in Asejire dam (w_AST) and Lagos Lagoon (w_SL) in Nigeria.

Site	N	*Ho*	*He*	*pi(π)*	*Fis*
w_AST	5	0.142	0.276	0.311	0.383
w_SL	4	0.237	0.224	0.256	0.042

Table 4.6 Genetic diversity summary statistics and inbreeding coefficients in *Sarotherodon galilaeus* samples from River Benue Numan (w_BNT) and Oyan dam (w_OTY) in Nigeria

Site	N	*Ho*	*He*	*pi(π)*	*Fis*
w_BNT	5	0.116	0.121	0.135	0.042
w_OYT	10	0.077	0.334	0.353	0.715

4.3.3 Genetic differentiation among named tilapia species

The relative differences in diversity were also apparent in the population PCA. PC 1 and PC 2 explained 56.16% and 17.185, respectively, of the genetic variation in the dataset (Figure 4.7). There are noticeably two sub-populations within Asejire dam (w_AST) separated along both PC1 and PC2 (labelled A and B). Cluster A was shared with samples from Lagos Lagon (w_SL), while the second cluster (B) was shared with samples from both River Benue Numan (w_BNT) and Oyan dam (w_OYT) sampling sites. There was a noticeable single clustering (cluster C) of all the farmed samples with some native wild samples from Lake Geriyo (w_LGT), the two River Benue (w_BNT and w_BYT), Kiri dam (w_KDT), Odo- Idimu river (w_ODT), Etele river (w_ETT) and Lagos lagoon (w_SL). All samples from the wild brackish water Badagry creek (w_BDT) formed a cohesive group (cluster D). Meanwhile at cluster E is a grouping of predominantly samples from Oyan dam (w_OYT), and a few individuals from River Benue Numan (w_BYT) and Lake Geriyo (w_LGT). Overall, the PCA showed sub-populations within Oyan dam, River Benue Numan, Asejire dam, Etele river and the most variable Lagos lagoon site (w_SL) spread across multiple clusters; all of these contained multiple named species.

A PCA organised by species suggested more disconnect between mtDNA species names and nuclear clustering, with mixtures of species apparent within some of the clusters (Figure 4.8). The first (PC 1) and second (PC 2) principal components explained 53.79% and 16.99% of the observed genetic variation, respectively. Cluster A included mainly *Oreochromis* spp. but also some *S. galilaeus*

individuals. Cluster B separated *S. melanotheron* from the other species. Cluster C showed all the *C. zillii*, along with a sample each from *S. galilaeus* and *P. mariae*. Cluster D shows a group consisting of *P. mariae*, *O. niloticus*, and *S. galilaeus*. Cluster F shows a loose distribution of mainly *S. galilaeus*, two *O. aureus* and one *O. niloticus*.

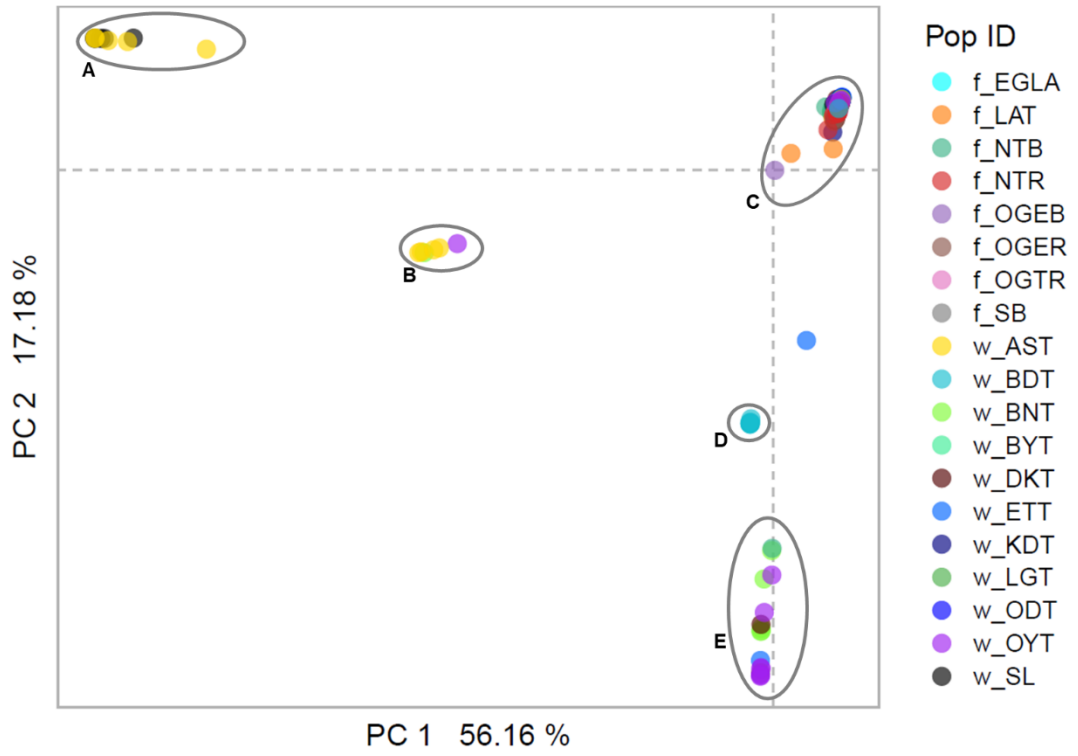

Figure 4.7 PCA plot visualising the genetic variation within and between Genetically Improved Farmed Tilapia (GIFT) and wild tilapia samples based on genotypic data. The x-axis and y-axis represent the first and second principal components, respectively, which capture the largest (56.16%) and second-largest (17.18%) sources of genetic variation in the dataset. Together, these axes explain 73.34% of the total genetic variation. Colours are used to distinguish between different sites, as indicated by the legend. Clusters A show sub-structure within the Asejire dam samples (w_AST). At cluster B is the farmed populations but including some native wild samples from Lake Geriyo (w_LGT), the two River Benue (w_BNT and w_BYT), Kiri dam (w_KDT), Odo-Idimu river (w_ODT), Etele river (w_ETT) and Lagos lagoon (w_SL). Samples obtained from the wild brackish water of Badagry creek (w_BDT) formed a tight cluster (D). A grouping at cluster E comprised samples from the Oyan dam (w_OYT), along with a few individuals from River Benue Numan (w_BYT) and Lake Geriyo (w_LGT).

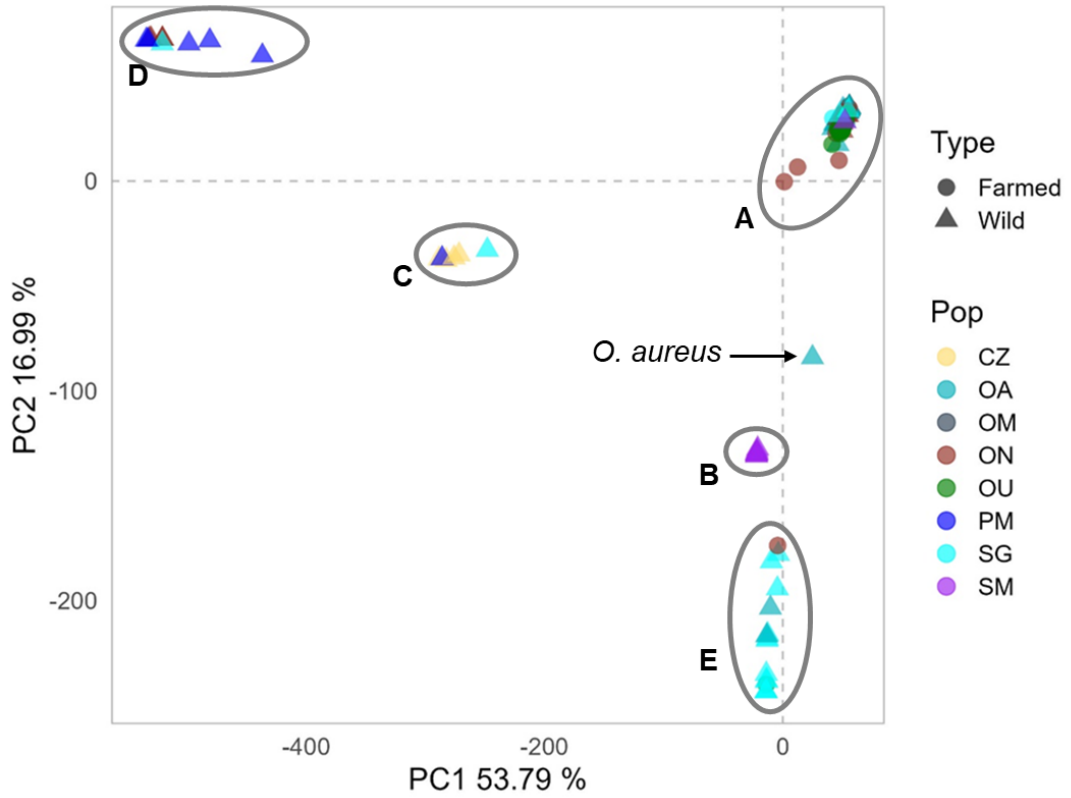

Figure 4.8 PCA plot visualising genetic variation within and between the eight tilapia species found in the study (CZ: *C. zillii*, OA: *O. aureus*, OM: *O. mossambicus*, ON: *O. niloticus*, OU: *O. urolepis*: PM: *P. mariae*, SG: *S. galilaeus*: SM: *S. melanotheron*) from 19 farmed and wild tilapia samples based on genotypic data in Genepop format. Each point on the plot represents an individual sample, with its position determined by its principal component (PC) scores. The x-axis and y-axis on the plot represent the first and second principal components, respectively. These components captured the largest (53.79%) and second-largest (16.99%) sources of genetic variation in the dataset. Different colours are used to distinguish between species, as indicated by the legend. The distance between points reflects the degree of genetic relatedness or differentiation between samples. Clusters or groupings of samples on the plot indicate species that are genetically similar. The point labelled A represents all the *Oreochromis* from both farmed and wild samples, along with a few *S. galilaeus* and *P. mariae* individuals. A distinctive sample from *O. aureus* indicated with an arrow. B is a cluster of only *S. melanotheron*, while cluster C shows a group of *C. zillii, P. mariae*, and *S. galilaeus*. Cluster D is a group including predominantly P. mariae, with a two O. niloticus and one *S. galilaeus* samples. Cluster E is a group of *S. galilaeus, O. aureus* and *O. niloticus*.

Patterns of genetic differentiation among the tilapia species (named based on the mtDNA haplotypes resolved in Chapter 3) based on *Fst* calculated using a single SNP per locus or haplotypes (i.e. considering all of the SNPs at a locus together) for *aureus* (Figure 4.9) and *O. niloticus* (Figure 4.10). Pairwise *Fst* among the eight *O. aureus* sampling samples (seven wild and one farmed) revealed very low to moderately high levels of genetic differentiation, ranging from 0.00 to 0.21 (Figure 4.9). Genetic differentiation between wild samples from the northeast (w_BNT, w_BYT, w_DKT, and w_KDT and w_LGT) showed relatively low *Fst* values (-0.03–0.04) and not statistically significant from zero. The *Fst* value between southwest *O. aureus* samples (w_ETT and w_ODT) was

not significantly different from zero (*Fst* [95% bootstrapped CI] = -0.04 [-0.047-(-0.031)]). However, comparison between both w_ETT and w_ODT and the wild northeast samples was significantly higher from zero. In the case of w_ETT, pairwise *Fst* comparison with northeast sampling sites ranged from (*Fst* [95% bootstrapped CI] = 0.05 [0.042-0.058]) between w_DKT to (*Fst* [95% bootstrapped CI] = 0.21 [0.169-0.186]) between w_BNT and (*Fst* [95% bootstrapped CI] = 0.21 [0.203-0.219]) between w_LGT. Pairwise *Fst* comparison w_ODT and northeast sampling sites ranged from (*Fst* [95% bootstrapped CI] = 0.08 [0.067-0.084]) between w_DKT to (*Fst* [95% bootstrapped CI] = 0.20 [0.189-0.211]) between w_BNT. Likewise, the *Fst* was statistically significant between the only farmed *O. aureus* (f_NTB) and the wild site while showing more close relationship with the southwest (*Fst* = 0.09 – 0.110) compared to the northeast samples (*Fst* = 0.10 – 0.15).

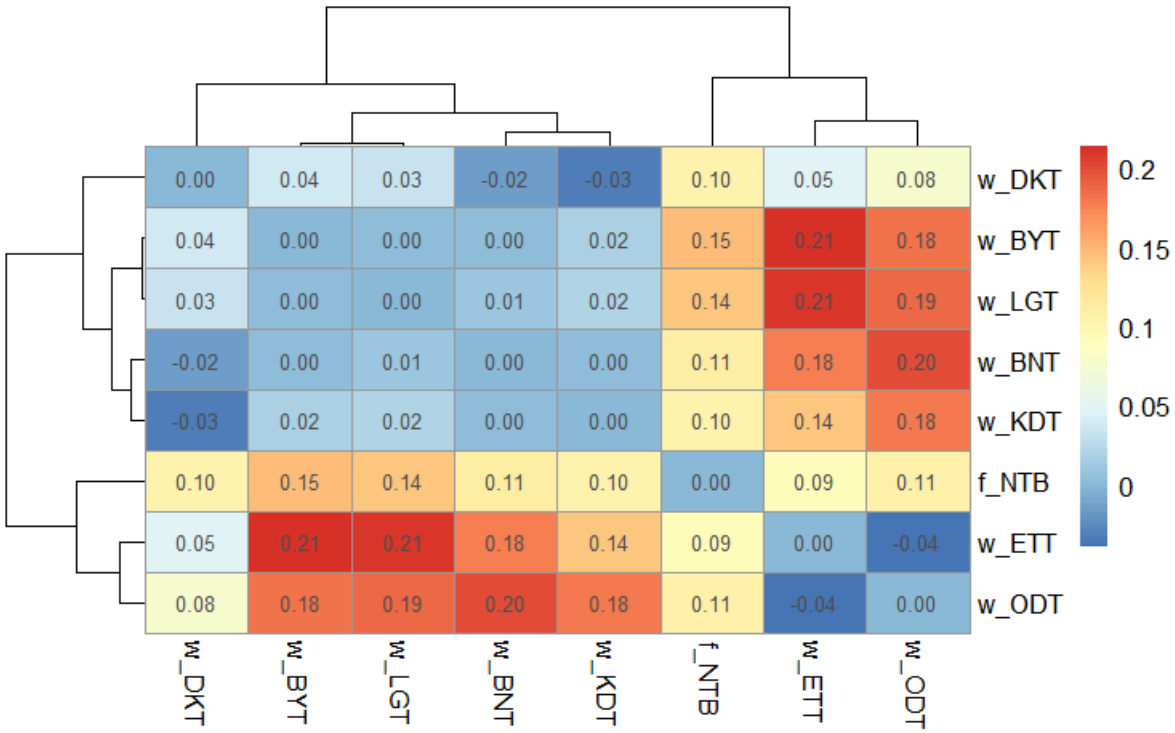

Figure 4.9 Pairwise *Fst* between *Oreochromis aureus* from farmed and wild sampling sites in the northcentral (f_NTB), southwest (w_ETT, w_ODT) and northeast (all other sites) regions of Nigeria

Pairwise *Fst* values among the ten *O. niloticus* sampling sites (6 farmed and 4 wild) revealed low to high genetic differentiation, ranging from -0.12 – 0.42 (Figure 4.10). Comparisons between farmed and wild sampling sites were statistically significant except for f_NTB and w_KDT (*Fst* [95% bootstrapped CI] = -0.01 [-0.022 – 0.001]) and f_NTB and w_SL (*Fst* [95% bootstrapped CI] = -0.12 [-0.128 – -0.113]). Among the farmed sites, f_OGTR (red tilapia) was found to be

significantly different from the others, with Fst values ranging from 0.21–0.23. *Fst* comparison between wild sites was significant except in the comparison between w_ETT and w_ODT (*Fst* [95% bootstrapped CI] = 0.00 [-0.001–0.008]).

4.3.4 Introgression between farmed and wild species

Cross validation error values suggested K = 8 to be the probable number of genetic clusters for the species level admixture analysis but lower values are also shown to specifically test whether there is genetic distinctiveness between farmed and wild species and/ or genera (Figure 4.11). Overall, the patterns

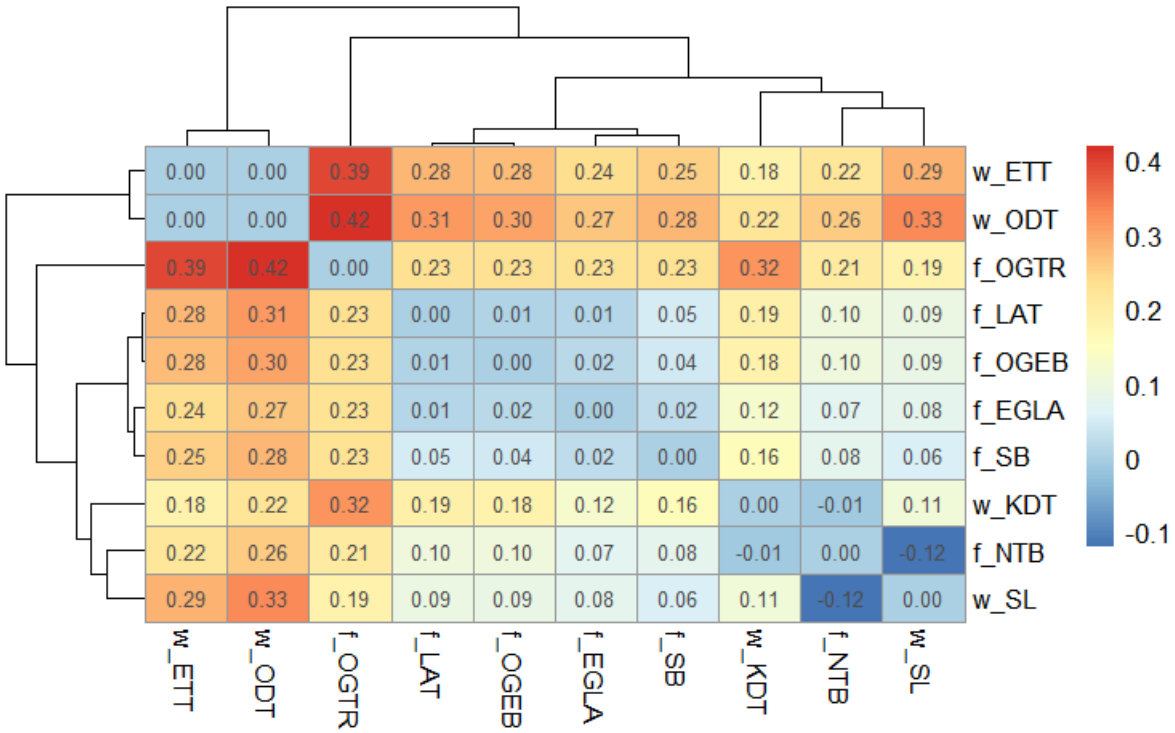

Figure 4.10 Pairwise *Fst* between *Oreochromis niloticus* from farmed and wild sampling sites in the northeast (w_KDT), northcentral (f_NTB) and southwest (f_EGLA, f_OGEB, f_OGTR, f_SB, w_ETT, f_LAT , w_ODT, w_SL) regions of Nigeria

suggest some mismatches between species level nuclear assignment of some individuals and their mtDNA lineage, as well as individuals showing admixture between more than one genetic cluster, both of which emphasise extensive hybridisation not only within but between genera. At K = 2, the admixture plot separated the species according to genera with the separation of *Oreochromis* from the others. However, there was evidence of admixture between *Oreochromis* and *Sarotherodon*, particularly in *S. galilaeus* and *S. melanotheron*. There was also a single individual of *P. mariae* (OGTR11) that was assigned to the *Oreochromis* cluster, despite sharing the same mtDNA haplotype as SL12

(Chapter 3). At K = 3, there was still no population structure within *Oreochromis* but, except for the OGTR11 individual noted, there was noticeable differentiation between *P. mariae* (predominantly green cluster) and both *S. galilaeus* and *S. melanotheron* (predominantly red cluster), while some of the *C. zillii* samples showed evidence of admixture, with equal assignment to both green and red clusters. There were also individuals of all genera except *Coptodon* that showed 100% assignment to a cluster other than their main species cluster. At K = 4, a pattern begins to appear, within the separation of the farmed and wild *O. niloticus* samples but extensive evidence of admixture in the farmed sites. K=5 separates *P. mariae, S. galilaeus, C. zillii,* and *S. melanotheron.* At K = 6, there was a noticeable separation of farmed f_OGTR and the rest of the farmed sites. There was also evidence of extensive admixture within the f_NTB (*O. aureus*) samples, with most individuals showing mixed assignment to clusters shared with f_NTR (*O. urolepis*) and *O. niloticus* from both GIFT and wild individuals. The K value with the lowest cross validation error (K = 8) showed distinct genetic signatures for farmed and wild samples of *O. niloticus*. Similarly, there was distinction between the only farmed *O. aureus* (f_NTB) and their wild counterparts. *O. mossambicus* and *O. urolepis* also appeared to be genetically distinct from *O. niloticus* and *O. aureus*.

The admixture analysis of farmed and wild tilapia samples revealed a mixture of pure and introgressed individuals across different sites (Figure 4.12). Among the tested K values (K = 2 to K = 20), the model with K = 13 yielded the lowest cross-validation error but there were also interesting patterns at lower values. At K = 2, there was a separation between Asejire dam (w_AST) and w_SL from the rest of the sites, but with admixture already apparent in three of the other wild sites (w_BNT, w_OYT and w_BDT). At K = 3, the population structure was similar to the K = 2 pattern but with admixture apparent in more of the samples. At the optimum K = 13, the Northeast sampling sites (w_BYT, w_DKT, w_KDT, and w_LGT) (pink cluster) were distinguishable from the rest of the samples. The patterns also confirm that the individuals from w_BYT and w_LGT that I suspected had been misidentified in BOLD (see Chapter 3) were indistinguishable from other individuals in those populations. However, the River Benue Numan (w_BNT) samples from the Northeast showed extensive admixture with the Oyan dam (w_OYT) samples from the Southwest, further confirming their shared population structure from the PCA analysis but w_BNT also including some

individuals with the northeastern cluster (pink). Southwest Etele river (w_ETT) and Odo-Idimu (w_ODT) clustered together but with evidence of admixture in both sites.

There was also extensive evidence of admixture within the Northcentral farmed sites, particularly for f_NTB and to a lesser extent f_NTR. The cage samples (f_SB) showed a unique genetic structure from the other farmed sites, with less admixture, except for a single individual that shared a genetic cluster with f_NTR and some evidence of admixture for individuals from the multi-species Lagos lagoon samples (w_SL) and some of the farmed samples (f_NTB, f_EGLA, f_LAT and f_OBEB). The presence of *O. urolepis* individuals in the w_SL sample, exhibiting a consistent yellow colouration similar to the cage samples (f_SB), suggests a potential escape event.

4.4 Discussion

This study presents invaluable insights into the genetic diversity, population structure, and potential genetic interactions between introduced GIFT and wild tilapia populations. Overall, the results suggest that while there is more diversity in the wild than the farmed sites, the complexity of patterns of differentiation and admixture suggest that there has been extensive hybridisation, both within farmed sites and between wild and farmed individuals. For example, the mismatch between species names based on maternal mtDNA lineages and nuclear patterns of clustering for some individuals suggests hybridisation, sometimes between genera. This was also reflected by extensive evidence for assignment to multiple clusters for many individuals in the admixture analysis for individuals sampled from both wild and farmed sources. Tilapia species are notorious for their ability to hybridise, especially in captive settings (Svardal et al., 2021). This phenomenon can occur in various scenarios: sometimes an introduced species hybridises with a native species, while in other cases, two introduced species hybridise in a novel environment (Agnèse et al., 1998b). Evidence of hybridisation between native and introduced species were found in Tanzania between the critically endangered *O. jipe* and two introduced species, *O. leucostictus* and *O. niloticus* (Bradbeer et al., 2019).

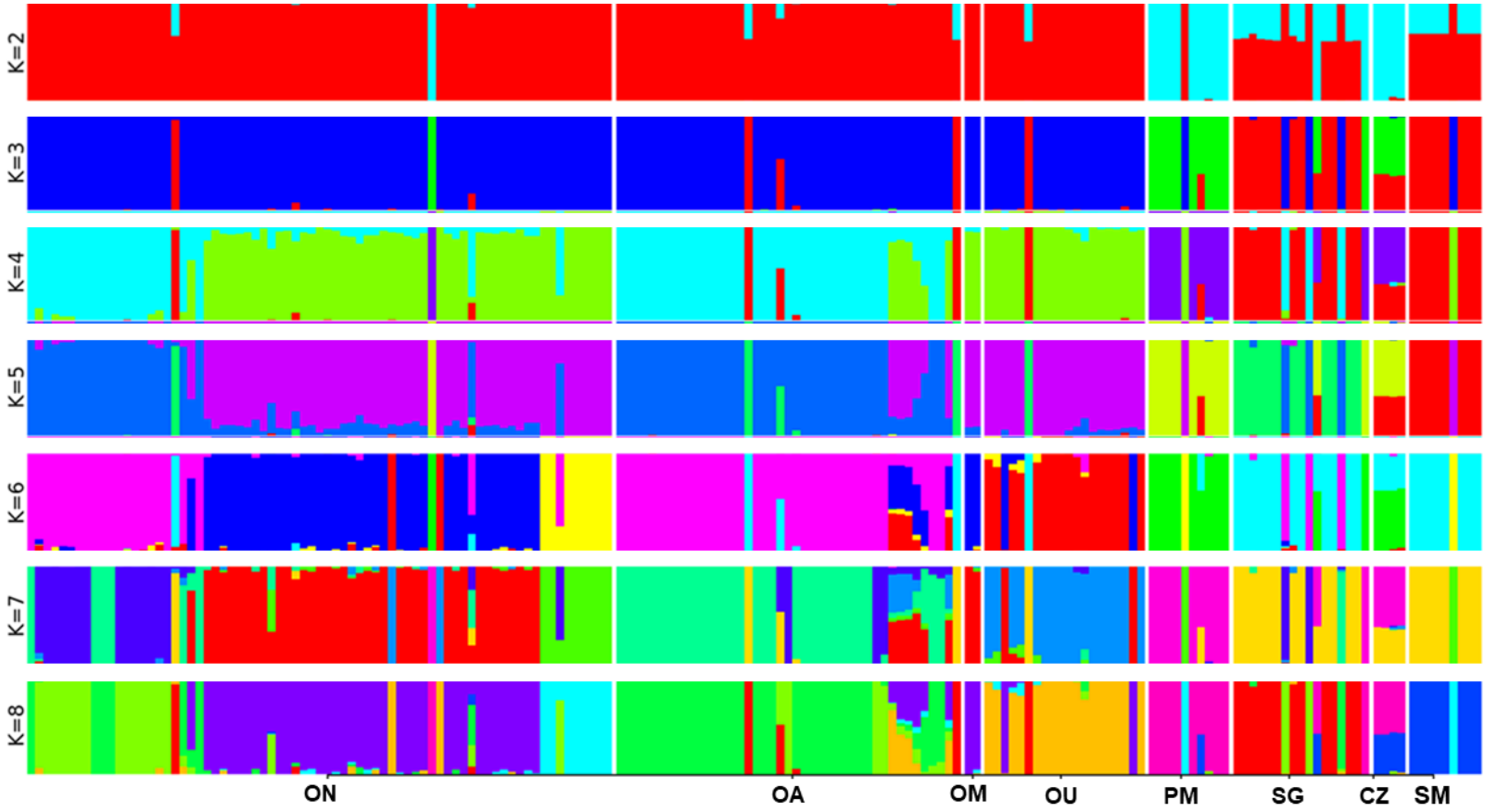

Figure 4.11 Admixture plot illustrating patterns of ancestry inferred from ddRAD SNPs across eight tilapia species: ON: *Oreochromis niloticus,* OA: *O. aureus,* OM: *O. mossambicus,* OU: *O. urolepis,* PM: *Pelmatolapia mariae,* SG: *Sarotherodon galilaeus,* CZ*: Coptodon zillii, and* SM: *S. melanotheron*. The plot depicts genetic clustering at varying K values from 2 to 9, with the optimal K value identified at 8. At K=2 in the admixture analysis, distinct genetically clusters were observed, separating *Oreochromis* spp. from *P. mariae, S. galilaeus, C. zillii*, and *S. melanotheron*. Remarkably, evidence of intergeneric hybridisation was detected in *C. zillii* and *P. mariae*. Moreover, extensive hybridisation among the *Oreochromis* spp. highlighted the indiscriminate breeding practices prevalent among fish farmers in Nigeria.

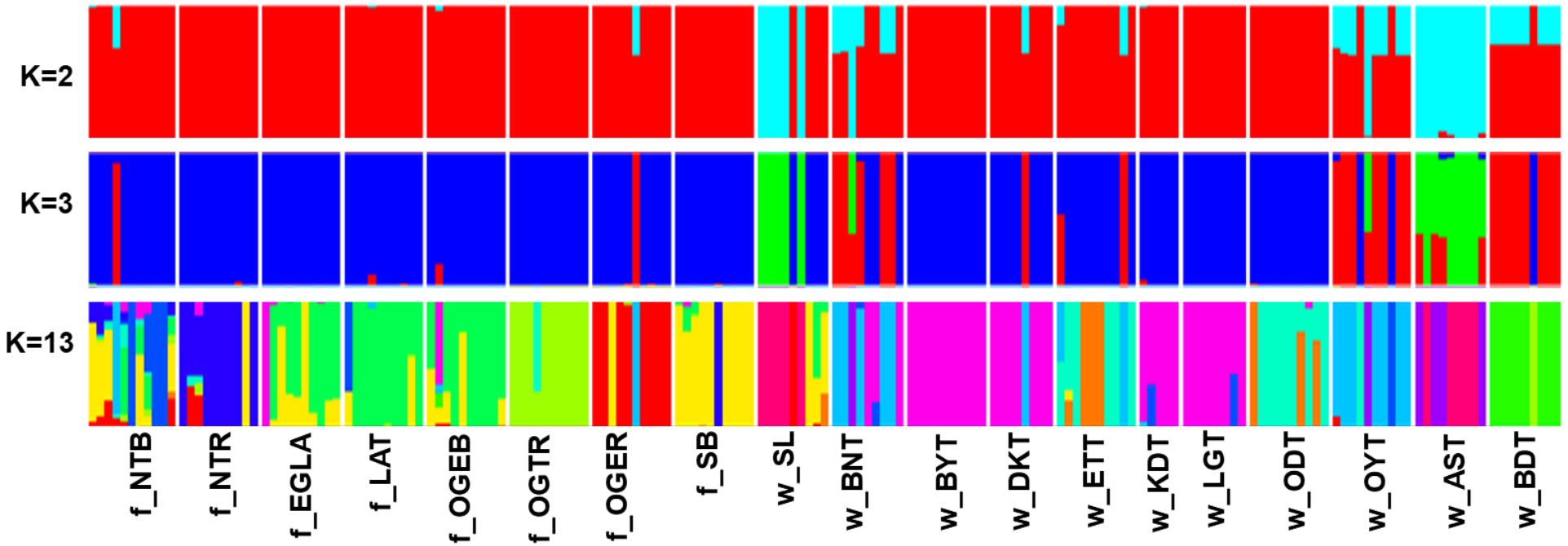

Figure 4.12 Admixture plot visualising the genetic ancestry of individual tilapia samples from multiple GIFT and wild sampling sites based on genotypes from a single SNP per locus. Each vertical bar represents an individual sample, while different coloured segments within each bar represent the proportion of genetic ancestry inferred from K genetic clusters. The x-axis lists the samples, grouped by site. Sites with the f_ and w_ prefixes represent the farmed GIFT and wild samples, respectively. The K values represent the tested the number of genetic clusters hypothesised in the study population (K= 1 – 20). K=2 indicates that the sampling sites can be divided into two genetic clusters. Likewise in K=3 for three genetic clusters. K=13 was chosen as the best genetic cluster that best describes the study sites based on the lowest cross-validation errors.

4.4.1 Genetic diversity across introduced farmed and native wild tilapia populations in Nigeria

Variation in genetic diversity between farmed and wild samples differed by species but there was also variation in the distribution of populations where wild and farmed fish were sampled. *O. aureus* was dominated by wild sampling sites with just one farmed site (f_NTB, from the northcentral region) and a bias towards populations from the northeast. Nevertheless, genetic diversity was higher at the farmed site than most of the wild sampling sites, and the farmed site did not have higher evidence of inbreeding suggesting that the breeding practices have enhanced or maintained the genetic diversity of the farmed f_NTB. This is contrary to the contrary to the situation where farmed populations for example in *O. niloticus* were found have reduced genetic diversity and high inbreeding compared to their wild counterpart (Ukenye and Megbowon, 2023). Maintaining high inbreeding among farmed tilapia has been linked to effective management strategies including the introduction of new broodstock and fingerling from multiples sources and/or the implementation of controlled breeding programs that maintain the genetic diversity and avoid inbreeding (Ravakarivelo et al., 2019). Additionally, reduced genetic diversity among the wild sites could be indicating that wild *O. aureus* populations have been subjected to selective pressures such as environmental changes, overfishing which can lead to a reduced genetic diversity (Ketchum et al., 2016; Zhao et al., 2023). In contrast, there were more sampling sites that included farmed *O. niloticus* but there was little difference in genetic diversity or levels of inbreeding between farmed and wild sampling sites. The genetic diversity statistics (*He* and *pi*) observed here across *Oreochromis* spp. were similar to results obtained in a study of Nile tilapia sampled across eight West African countries using SNP data, which found reduced levels of genetic diversity (*He* = 0.11 – 0.12) in the Gambia River (Kudang and Walekounda), the western Niger River (Lake Sélingué), and the upper Red Volta River (Kongoussi) populations (Lind et al., 2019). This was also similar to the results obtained in a study of population structure of Nile tilapia strains from Tanzania using a RADseq approach (Kajungiro et al., 2019b), which found low genetic diversity across the studied populations (*He* = 0.06 – 0.11). However, higher genetic diversity was

reported in a whole genome study of Nile tilapia GIFT strains from Brazil and Costa Rica, with *He* ranging from 0.2 to 0.4 (Cádiz et al., 2020). Higher genetic diversity in the farmed compared to the wild contradicts the assertion that low genetic diversity is expected in domesticated populations, compared to their wild conspecifics as these populations can lose genetic diversity due to selective breeding and the absence of gene flow with other populations (Baumung et al., 2004). However, the higher genetic diversity among the farmed group could be due to hybridisation from strains obtained from different sources such as Egypt and Thailand. Also, the overall low nucleotide diversity across the farmed and wild sites is consistent with the submission that within species nucleotide diversity is low in cichlids (Svardal et al., 2021). For both *O. niloticus* and *O. aureus,* nucleotide diversity (*pi*) was higher in the hatchery samples (f_NTB) than in the others and the high hybridisation observed at this site could be the reason for these high *pi* values. Wild species in the north that rely on rainfed water and overflow from larger rivers (for example, River Benue Numan and Kiri dam) might suffer from low genetic and species diversity due to continuous overfishing throughout the year. Both River Benue Numan and Kiri dam may become fragmented during the peak of the extended dry season, usually from October to May.

My sampling was conducted from November to March when the water levels have drastically declined. Other studies have shown that small and fragmented populations will suffer from low genetic diversity. For example, Kim (2019) in an extensive review on genetic diversity and the application of molecular markers in fish, stated that populations with fewer individuals (less than 100) will experience reduced genetic diversity due to genetic drift, inbreeding, and limited genetic flow. Likewise, the continuous overexploitation of declining fish population is likely to exert further pressure on genetic diversity (Sadler et al., 2023). For instance, this reduction in genetic diversity as a result of overexploitation of fish was demonstrated in a declining population of New Zealand snapper (*Pagrus auratus*) using microsatellite markers (Hauser et al., 2002). This unsustainable fishing practice can lead to severe consequences such as population decline and even local extinction (Hoarau et al., 2005; Saccheri et al., 1998).

S. galilaeus were only found in two sampling locations; genetic diversity was higher in w_OYT compared to w_BNT but the former had a very high inbreeding coefficient (*Fis* = 0.714), suggesting that mating between closely related individuals due to small population size. This was evident during sampling as only few *S. galilaeus* (less than 30) were landed by the fishers. The noticeable depletion of fish stocks in Nigeria has been associated with pollution, climate change, overfishing, and lack of updated scientific data (Adenike, 2023; Okafor-Yarwood, 2018). The high inbreeding coefficient in w_OYT despite the high genetic diversity could be indicative of a historical founder effect, where a small number of individuals with considerable genetic variation initially established the population as observed in *S. galilaeus* population in the Sea of Galilee (Borovski et al., 2018). The high genetic diversity in *P. mariae* compared to both *O. niloticus* and *O. aureus* in this study and high inbreeding coefficient (*Fis* = 0.383) but one of the *P. mariae* sites (w_AST), could be attributed to several factors, including mating patterns, population size, or genetic drift (Vitt et al., 2023). The depletion of the Asejire population during sample collection may have contributed to the observed high inbreeding coefficient, as reported from previous stickleback study which revealed that reduced population size can lead to an increase in inbreeding due to a limited number of breeding individuals (Fraimout et al., 2023). Some of the sites with the highest Inbreeding coefficients for different species, w_DKT (*O. aureus*), w_AST (*P. mariae*), and w_OYT (*S. galilaeus*), also were those with small numbers of individuals available to sample. Populations undergoing drastic reduction in size will likely suffer from inbreeding because of reduction in effective population size. Small inbred populations are at higher risk of extinction than a large population occupying a diverse landscape (Bercovitch, 2023). This raises concerns for the regulation of fishing practices in the capture fisheries as the situation can worsen with the increasing number of irrigation farming around major fishing rivers and lakes like River Benue Numan and Asejire dam.

4.4.2 Genetic differentiation and population structure of farmed and wild tilapia species

Population structure analysis using PCA could not differentiate between the different GIFT strains. This could be a reflection of the breeding practices employed in Nigeria or the overlapping genetic variations among tilapia species

(Svardal et al., 2021). The PCA plot did not show a clear genetic structure between the farmed GIFT and the wild *Oreochromis* spp. The difficulty in differentiating between GIFT strains and wild Nile tilapia using PCA analysis could be due to shared ancestry. This can be traced back to the GIFT founding population that consisted of eight strains, including four imported Nile tilapia from Egypt, Ghana, Kenya and Senegal and four established Asian farmed strains from Israel, Singapore, Taiwan, and Thailand strains (Eknath et al., 1993). The only clear genetic cluster based on sampling location is the *S. melanotheron* samples from the brackish water Badagry creek. Robledo et al. (2024) also reported a complex genetic clustering pattern of GIFT and native Nile tilapia in Uganda. However, the admixture analysis showed unique genetic structure for species in the wild sites. Notably, there was a unique genetic profile between the Northeast samples from River Benue Yola, Dadin Kowa dam, Kiri dam, and Lake Geriyo and their Southwest counterparts from Etele river and Odo-Idimu river.

There were also differences in patterns of differentiation between the two main farmed species. For *O. aureus*, there was very little differentiation between sites within the northeast geographic region, or between the two sites from the southwest but high differentiation between the populations within the two regions and compared to the farmed site (f_NTB), which was sampled from the northcentral region. It is possible to infer that the proximity of the northeast sampling sites Lake Geriyo (w_LGT), Kiri Dam (w_KDT), Upper Benue River (w_BNT and w_BYT), and Dadin Kowa Dam (w_KDT), may facilitate gene flow through migration of fish. This would maintain genetic similarity leading to low genetic differentiation among these sampling sites. Lake Geriyo is flooded by the upper Benue River at the peak of the rainy season (Eromosele et al., 1995), suggesting a geographical and hydrological link to the River Benue. Dadin Kowa Dam located in the Upper Benue River basin and under the management of the Upper Benue River Basin Development Authority (UBRBDA) could also have a connection to the Upper Benue River (Essien et al., 2019; Hassan et al., 2015) leading to exchange of genetic materials between the water bodies. In comparison to the southwest sampling sites which are geographically distant from the northeastern locations, the absence of possible connections between water bodies from these regions could limit migration and result in high genetic differentiation. This pattern of geographical genetic differentiation was

reported among Nile tilapia populations across West Africa, among major river basins including the Volta, Niger, Senegal, and Gambia where geographically distant populations were found to be more genetically distinct compared to populations with close distance (Lind et al., 2019). For *O. niloticus*, there was varying levels of differentiation within and between sites with farmed tilapia, which could suggest that different populations have had different histories of introduction of farmed fish or varying levels of introgression between farmed and wild fish. The lowest genetic differentiation observed among wild *O. niloticus* between w_ETT and w_ODT with an Fst of 0.00, suggests that there is no genetic differentiation between these two sampling sites. Both w_ODT and w_ETT are from the same state in the southwest region which could possibly be the reason for this low genetic differentiation, however, there is no record on possible interaction between the two water bodies. The high genetic differentiation observed between the red tilapia GIFT strain from Thailand (f_OGTR) and the other farmed strains could be attributed to selection for specific trait in the GIFT strain (Barría et al., 2023). Barría et al. (2023), using both SNP array data and Poolseq SNPs, found genomic regions with high *Fst* were between WorldFish GIFT strain and the other populations from Africa and Asia. Additionally, Romana-Eguia et al. (2004), reports that the high genetic differentiation observed between red tilapia (*O. mossambicus,* and *O. niloticus*) and *O. niloticus*, could be due to the hybrid nature of the red tilapia strains and the selective breeding methods employed. There was also variation in levels of differentiation between farmed and wild in the different geographic regions sampled but most of the sites with *O. niloticus* were from the southwest. Interestingly, *O. niloticus* from the farmed site (f_NTB) in the northcentral region, where *O. aureus* was also found, showed no differentiation from either a wild population from the southwest (w_SL) or the only wild site sampled from the northeast (w_KDT), suggesting recent admixture or shared genetic background. Based on mtDNA results (Chapter 3), the additional GIFT sequences from Freshwater Aquaculture Center – Central Luzon State University (FAC-CLSU), City of Muñoz, Nueva Ecija, mapped with samples from haplotypes 24 containing samples the cage site (f_SL) and Lagos lagoon (w_SL). From the admixture analysis, the observed genetic identity between samples from both sites suggests possible escapes from f_SB to w_SL where the cage is installed. The low genetic differentiation observed between farmed and wild *Oreochromis*

species have been reported from previous study. For example, Ukenye and Megbowon (2023) found low genetic differentiation(*Fst* = -0.628) between farmed *O. niloticus* and wild Wesafu ecotype. The *Fst* values obtained were generally lower compared to results obtained in a study using ddRADseq to investigate the population structure of Nile tilapia GIFT strains in Tanzania (*Fst* = 0.04 – 0.54) (Kajungiro et al., 2019b) and a microsatellite study Nile tilapia populations in Ethiopia (*Fst* = 0.04 – 0.56). While concerns must have been raised over the possible hybridization between farmed and wild tilapia species, these results revealing low genetic diversity but significant differentiation between farmed and wild sites is a positive sign and an important opportunity to further strengthen the conservation policy that will protect native species and promote sustainable aquaculture development.

4.4.3 Introgressive hybridisation between farmed and wild tilapia populations

Admixture analyses revealed that in regions lacking farmed species, genetic structure remained predominantly pure, with minimal evidence of admixture between genetically distinct lineages. However, in areas with extensive non-native tilapia introductions, specific introgression events were observed, particularly in Lagos Lagoon (SL) originating from the cage site (f_SB). The extensive admixture in some farmed sites (notably in the Northcentral: f_NTB and f_NTR) could suggest hybridisation between introduced species, reflecting the indiscriminate fish breeding practices in Nigeria. High admixture among farmed tilapia from multiple ancestries also has been reported in Uganda (Robledo et al., 2024). Indiscriminate breeding, most especially in areas where there are minimal or no regulatory frameworks has been identified as serious threats to wild populations as they could compromise the crucial aspects of the breeding objectives, which is to preserve the genetic resources (Farstad, 2018). Also, fish breeding using broodstock from multiple ancestry by farmers who lack basic genetic knowledge may cause genetic deterioration of hatchery populations and compromise the genetic diversity of native species (Eknath, 1991; Sanda et al., 2024; Sonesson et al., 2023). It is therefore important to pay proper attention to the genetic makeup of broodstock to ensure the long-term viability of genetic resources.

Other studies have documented the genetic consequences of aquaculture activities on wild sites, highlighting the potential for genetic introgression and loss of genetic diversity. For example, Bradbeer et al. (2019) detected hybridisation between introduced *O. niloticus* and critically endangered native *O. jipe* in Pangani Falls, Tanzania. The phenomenon of introgressive hybridization, particularly between selectively bred escapees and wild individuals, poses significant risks to the genetic integrity and fitness of wild samples. The introgression of genes from selectively bred escapees may disrupt local adaptation within wild populations and compromise their ability to thrive in their natural environments (Ansah et al., 2014).

4.5 Conclusions

Exploring the genetic diversity and population structure of farmed and wild tilapia fish species at a genomic scale is necessary for their effective genetic enhancement, conservation, and management. This is most especially important at a time when the fisheries and aquaculture sector needs a reformed policy that will integrate a conservation genetics approach in the management of our aquatic biodiversity to ensure the effective management of native wild populations and regulation of aquaculture activities. Genomic information on genetic diversity, population structure, and admixture pattern provide insights that will be beneficial in preventing the contamination of genetic pool of native species arising from hybridisation with aquaculture species in the event of an escape. Tilapia aquaculture, particularly with the use of genetically improved strains, has the potential to develop the fish industry in Nigeria and to serve as an important source of economic empowerment. However, the introduction of non-native species for use as an aquaculture species requires careful planning that involves risk assessment and management to determine any potential risk such as introgressive hybridisation and competition with native species. Farmed species introduction must be conducted in a responsible way that will promote sustainable aquaculture practices and offer protection to native species. Fish escape has not been given proper attention by the Nigerian authority and with evidence of hybridisation between the cage and Lagos lagoon samples, there is the need for the relevant authority to redirect their attention to the conservation of genetic diversity of native species. Furthermore, the federal government must regulate the movement of species across national borders by

private individuals. Additionally, policies at both local and national levels must focus on strict screening of new aquaculture species to identify potential risks, address the implication of indiscriminate fish breeding, and implement sustainable breeding practices that will select for desirable traits while maintaining genetic diversity. Finally, public awareness and education about the importance of sustainable aquaculture practices can help promote responsible stewardship of aquatic resources.

Chapter 5 Genetic diversity, population structure and differentiation of farmed and wild African catfish (Clarias gariepinus) in Nigeria

Abstract

African catfish (*Clarias gariepinus*) is a commercially important species in Nigeria's fisheries and aquaculture industry. However, knowledge about genetic diversity and population structure of farmed and wild samples that is crucial for effective conservation and sustainable aquaculture management is scarce. I investigated the genetic diversity and population structure of farmed and wild *C. gariepinus* populations from northeast and southwestern Nigeria, including an albino group from the wild, using the mitochondrial DNA (mtDNA) cytochrome c oxidase 1 gene (COI) and triple restriction site-associated DNA sequencing (3RAD) approaches. Eleven COI haplotypes were identified including seven unique to wild, two shared between albino and farmed, and another two shared between farmed and wild samples. Albino did not share any haplotype with the wild samples. mtDNA diversity was similar across both farmed and wild sampling sites. 3RAD results from 14,410 variant sites genotyped from 20,126 revealed overlapping genetic diversity between farmed and wild. Wild sampling sites had slightly broader range and higher maximum values for observed heterozygosity (*Ho* = 0.109 – 0.165), expected heterozygosity (*He* = 0.111 – 0.216), and nucleotide diversity (*pi* = 0.125 – 0.225) compared to the farmed populations (*Ho* = 0.118 – 0.147, *He* = 0.112-0.144, *pi* = 0.117 – 0.151). There was a high genetic differentiation between the farmed and wild *C. gariepinus* sampling sites (*Fst* = 0.31 – 0.47). Albino "wild" sample showed more similarities to farmed (*Fst* = 0.16 – 0.28) samples in comparison to the wild (*Fst* = 0.33 – 0.42). Consistent with the *Fst* results, PCA and admixture analyses provided more evidence of the albino being escapees from fish farm based on the observed similarities between albino and farmed samples compared to the wild. Northeast wild samples showed evidence for geographical genetic differentiation between Adamawa and Gombe states sampling sites. Inbreeding coefficient was lower within the farmed (*Fis* = -0.34 – 0.052) compared to the wild (*Fis* = 0.038 – 0.196). These results showed strong genetic differentiation between farmed and wild with low genetic

diversity indices. This retention of genetic diversity suggests that catfish farming has not yet had a negative impact on the wild sampling sites examined in this study. Consequently, it is imperative to implement regulations governing aquaculture practices and fishing activities, as well as addressing other factors that pose threats to native fish genetic diversity.

5.1 Introduction

The African catfish, also known as the African sharptooth catfish or the North African catfish is a freshwater omnivorous species that belongs to the Claridae family and is found in tropical and subtropical climates (Omitoyin, 2007). Its native distribution range spans lakes, dams, and rivers in sub-Saharan Africa and it also has been introduced into South America, Southeast Asia, and Europe (Konings et al., 2019; Truter et al., 2023). It is an important commercial fish both as a capture and aquaculture species. *C. gariepinus* has drawn the attention of aquaculturists because of its biological attributes, which include a fast growth rate, resistance to diseases and the tolerance to high stocking density (Lal et al., 2003). The first *C. gariepinus* domestication trials started in 1950 and were adopted for aquaculture in the 1970s but it was not until the 1980s that artificial propagation protocols were developed (FAO, 2019a). Research on *C. gariepinus* has led to the development of a strain known as the "Dutch *Clarias*" through selective breeding conducted in Belgium and the Netherlands, following their introduction to those countries from Africa. The Dutch *Clarias* have since been introduced to different African countries including the Central African Republic, South Africa, Côte d'Ivoire, and Nigeria where they are cultivated as farmed fish for food (Holčík, 1991; Huisman and Richter, 1987; Roodt-Wilding et al., 2010).

The conservation of *C. gariepinus* in the wild while ensuring its continuous propagation for aquaculture, is essential to maintain a balance between exploiting its economic potential and preserving its genetic diversity. Studies have revealed that *C. gariepinus* populations exhibit high genetic variation, which is an important parameter for the species' adaptability and resilience to changing environmental conditions in the wild (Barasa et al., 2014; Barasa et al., 2017). Studies have revealed that *C. gariepinus* populations exhibit a high genetic variation, which is an important parameter for the species' adaptability and resilience to changing environmental conditions in the wild (Barasa et al.,

2014; Barasa et al., 2017). Given the importance of genetic diversity as a valuable resource for fish species, it is therefore the responsibility of the policy makers and the relevant fisheries department to define a programme that will ensure the sustainable utilisation of economically important species like *C. gariepinus*. To conserve the genetic diversity of *C. gariepinus,* conservation officers must learn to deal with factors posing threats to genetic conservation of natural resources including loss of genetic diversity due to hybridisation with other species, as observed in Bangladesh with *C. gariepinus* and *C. batrachus* (Parvez et al., 2022), habitat fragmentation, overfishing, and pollution. Additionally, the introduction of Dutch Clarias outside their native range can negatively impact biodiversity in the event of an escape, as observed in Brazil and Turkey (Dumith and Santos, 2022; Turan and Turan, 2016).

The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) and the Convention on Biological Diversity (CBD) have both been playing important roles in the conservation of biodiversity including the genetic conservation of wild and farmed fish species such as *C. gariepinus* (Hoban et al., 2020; Hoban et al., 2023a; Hoban et al., 2023b). The CBD proposed 21 targets as part of the New Global Framework for Managing Nature Through 2030, that will ensure that at least 30 percent of land areas and of sea areas around the world are conserved through effective and equitable management, as well as reduce by 50%, the rate of introduction and establishment of invasive non-native species (Hoban et al., 2023a; Hoban et al., 2023b). IPBES on the other hand provide guidance for policy-making by synthesising scientific knowledge on biodiversity and ecosystem services (Baste et al., 2024). IPBES work with government authorities of member nations to provide scientific knowledge on biodiversity conservation. Both CBD and IPBES have made giant stride towards ensuring the conservation of biodiversity, however, they are yet to fully achieve the goals in the international agreements targeted at slowing the rate of species decline (Baste et al., 2024).

In Nigeria, *C. gariepinus* is one of the most important aquaculture species and by far the most farmed fish in the country (FAO, 2022). Both wild-caught *C. gariepinus* and the Dutch *Clarias* are widely bred artificially across Nigeria by induced breeding through hormone treatment with Ovaprim (Ataguba et al., 2009). Aquaculture production of important species like *C. gariepinus* has been

identified as a cheap animal protein source and means to help prevent food insecurity, as well as job creation (Folorunso et al., 2021). However, despite this prospect of aquaculture as an important economic tool, the sector dominated by *C. gariepinus* face challenges, including poor management skills, limited access to quality seed, lack of capital, high cost of feed, faulty data collection, lack of environmental impact consideration, and marketing of products (Emmanuel et al., 2014). Also in the wild, fish production decline was said to have worsened in 2020 with a sharp decrease of about 10% (FAO, 2022). Other challenges affecting the fisheries and aquaculture sector are: extended dry season, leading to habitat fragmentation and flooding during the peak of the rainy season; increasing interest in irrigation farming, which is leading to more activities around fishing rivers and lakes; and overfishing (Kenchington, 2003; Yan et al., 2021). Habitat degradation can lead to small and isolated populations that can cause loss of genetic diversity (Coleman et al., 2018). Genetic diversity is crucial for the long-term survival, adaptation, and resilience of individuals, populations, species, and ecosystems, as it forms the foundation of biodiversity (Hvilsom et al., 2022). However, little is known about the impacts of these forces in Nigeria. Conservation genetic approaches to identify species and assess patterns of genetic diversity and gene flow are important at a time like this when unsupervised and unregulated aquaculture activities, climate change, and lack of enforcement of the Fisheries Act are threatening both wild and aquaculture species. Past studies in Nigeria have attempted to identify and assess the genetic diversity of *C. gariepinus* species using DNA markers. For example, Suleiman et al. (2020) observed high genetic diversity within farmed and wild *C. gariepinus* populations in Nigeria using random amplified polymorphic markers (RAPD). However, RAPDs have low reproducibility. Popoola (2022) observed high genetic differentiation among three wild *C. gariepinus* populations from Southwestern Nigeria using the mitochondrial cytochrome b (*cytb*) gene. This molecular approach has been extended to identify wild freshwater fish in Southeastern Nigeria using DNA barcoding (Nwani et al., 2011b). That study offered information on species diversity in the Southeast region but did not address genetic diversity within the species or populations studied. Using microsatellite markers, Awodiran et al. (2019) were able to differentiate between wild *C. gariepinus* samples from Northcentral and Southwestern Nigeria. However, while microsatellite markers can provide valuable insights into

population structure and genetic diversity, they may lack the resolution to distinguish closely related populations or identify subtle genetic differences within populations and repeatability across studies is challenging. Although these previous studies have contributed to background information on the genetic diversity of *C. gariepinus* in Nigeria, what is lacking is assessment of fine-scale population structure between farmed and wild samples, levels of hybridisation, or detection of possible escapes from farmed to the wild. Different population genomics approaches have been employed to study the genetic variation and structure within and between populations of a range of fish species. High-throughput sequencing technologies and genomic approaches offer greater resolution and precision in population genetic analyses but detailed study on the use of genomics to understand the population structure and ancestral relationships of *C. gariepinus* across different geographical regions, particularly in areas where both wild and farmed populations coexist, is lacking. The use of genomics in fisheries and aquaculture allows the collection of powerful data, that can be used to inform fisheries management, identify escapees from fish farms, and for biosecurity applications (Bernatchez et al., 2017). The advancement in next-generation sequencing has led to the assembly of over 270 fish genomes to promote studies on comparative genomics, evolution, and systematics and more importantly for its application in aquaculture and fisheries (Bian et al., 2019; Crollius and Weissenbach, 2005; Hughes et al., 2018; MacKenzie and Jentoft, 2016). Whole genome sequencing provides comprehensive information on genetic variation, including single nucleotide polymorphisms (SNPs), insertions, deletions, and structural variants (Ng and Kirkness, 2010). Due to the high cost of sequencing the entire genome of an organism, alternative approaches like reduced representation sequencing technologies, based on high-throughput SNP genotyping from DNA fragmented with restriction enzyme(s), have made it possible to study a fraction of genomes (Peterson et al., 2012). The most employed technique for reduced-representation sequencing is known as restriction site-associated DNA sequencing (RADseq). Several RADseq methods have evolved over the years, including RADseq based on one restriction enzyme (Baird et al., 2008), double digest restriction site-associated DNA (ddRAD) sequencing (Peterson et al., 2012) and triple-enzyme restriction site-associated DNA sequencing (3RAD) (Bayona-Vásquez et al., 2019). The 3RAD approach provides a low-cost, highly robust and

simple method for the construction of dual-digest RADseq libraries (using 96 pairs of Illumina compatible iTru5 and iTru7 primers), with simultaneous digestion and ligation of DNA, enabling multiplexing of more samples and pooling of more libraries than other RADseq approaches (Bayona-Vásquez et al., 2019). Integrating the mtDNA COI marker with the cost-effective 3RAD genomic approach offers a promising strategy to improve the accuracy and robustness of population genetic analyses in *C. gariepinus*. This combined approach will provide a more comprehensive understanding of not only the genetic diversity and population structure but also allow for the assessment of the impact of aquaculture on native *C. gariepinus* populations.

This study aimed at examining farmed and wild *Clarias gariepinus* in Nigeria employing a DNA barcoding approach using the mitochondrial cytochrome oxidase I gene (COI) and 3RAD high-throughput sequencing approach to:
Assess whether farmed and wild *C. gariepinus* can be differentiated using mtDNA COI haplotypes

i. Investigate the mtDNA haplotype diversity and genetic differentiation between farmed and wild *C. gariepinus* as well as their geographical distributions
ii. Assess genome-wide genetic diversity within sampling sites of farmed and wild *C. gariepinus* using 3RAD
iii. Investigate patterns of genetic differentiation and introgression among farmed and wild sampling sites using 3RAD

5.2 Materials and Methods

5.2.1 Sampling

A total of 222 samples of *C. gariepinus* were collected from northeastern and southwestern regions in Nigeria during a four-month period between November 2021 and March 2022 (Figure 5.1, Table 5.1). In the southern region, I collected a total of 90 samples from three farmed locations (30 samples per location), including one selectively bred Dutch *Clarias* (f_ODC) samples. The other two sites were coded as (f_LAC and f_CMC). In the northeast, samples were collected from two states (Adamawa and Gombe). Three wild sites were in Adamawa state from River Benue Yola (w_BYC), Kiri dam (w_KDC), and Lake Geriyo (w_LGC) and one farmed site SAC farm (f_SAC). A detailed description of the wild sampling

locations was provided in Chapter 3. In Gombe state, samples from Dadin Kowa dam were divided into 30 wild-caught *C. gariepinus* (w_DKC) and 5 albino individuals (w_DKAL). The albino samples were caught in the dam by fisheries officers and kept in a holding facility. These two were treated as separate groups. Fishes were caught using gill and cast nets in the wild and with drag or hand scoop nets for the farmed samples. Caudal fin clips were collected and individually stored in absolute RNAlater and stored in a refrigerator. Upon completion of the fieldwork, samples were shipped on icepack from Nigeria to the School of Biodiversity, One Health and Veterinary Medicine (SBOHVM), University of Glasgow, UK for genetic analyses.

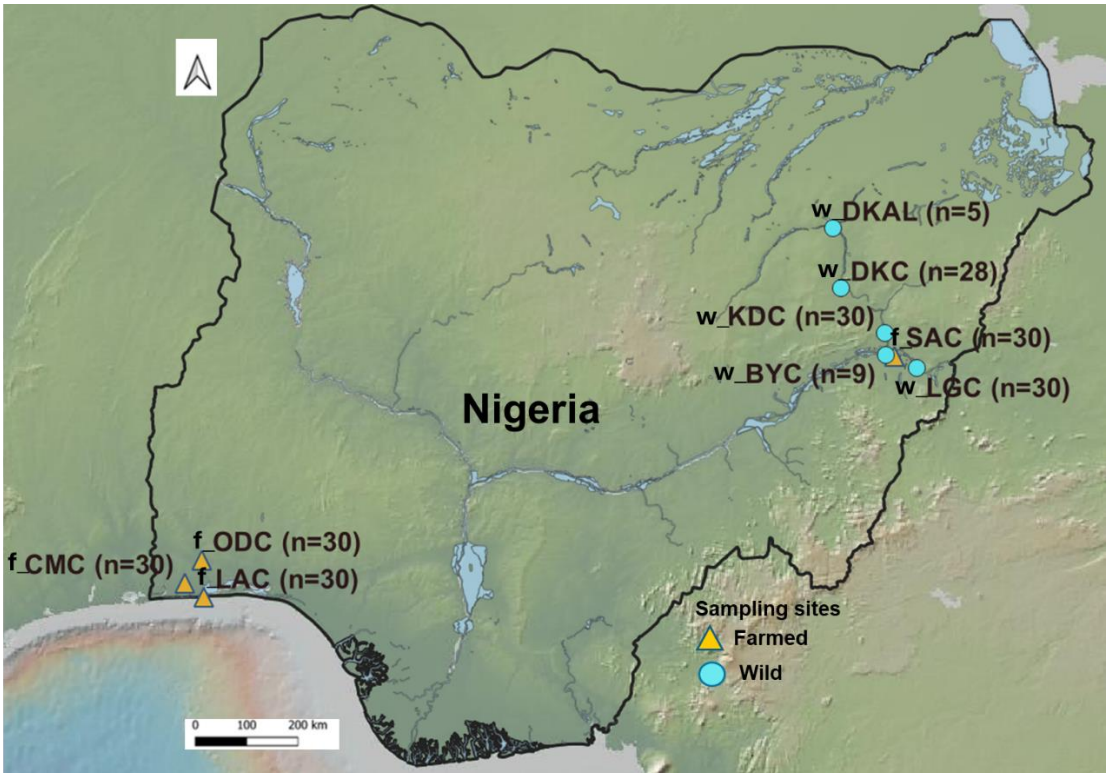

Figure 5.1 Map of Nigeria illustrating the spatial distribution of water bodies and sampling locations within the study area, categorised based on the origin of the sampled *Clarias gariepinus* samples (farmed and wild), with the sample size in parenthesis. The code names are interpreted as follows: f_CMC=CMC farm, f_LAC=LAC farm, f_ODC=ODC farm, f_SAC=SAC farm, w_KDC=Kiri dam, w_LGC=Lake Geriyo, w_BYC=River Benue Yola, w_DKC=Dadin Kowa dam, w_DKAL=Albino Dadin Kowa dam

Table 5.1 Origin of farmed and wild *Clarias gariepinus* collected from various states in Nigeria, indicating the sampling locations, region, state, population code, geographical coordinates, whether the population is farmed or wild, the total sample size collected per population (N), and sample sizes for mitochondria DNA barcoding (#COI) and 3RAD (#3RAD) analyses. The Dadin Kowa dam with an asterisk are albino samples, which were considered separately from the other samples in the population.

Sampling location	Region	State	Code	Latitude	Longitude	Source	N	#COI	#3RAD
CMC farm	Southwest	Ogun	f_CMC	6.583	3.158	Farmed	30	11	11
LAC farm	Southwest	Lagos	f_LAC	6.398	3.401	Farmed	30	11	12
Dutch *Clarias*	Southwest	Ogun	f_ODC	6.877	3.368	Farmed	30	14	12
SAC farm	Northeast	Adamawa	f_SAC	9.456	12.153	Farmed	30	12	12
Kiri dam	Northeast	Adamawa	w_KDC	9.681	12.001	Wild	30	14	10
Lake Geriyo	Northeast	Adamawa	w_LGC	9.293	12.434	Wild	30	15	12
River Benue Yola	Northeast	Adamawa	w_BYC	9.681	12.009	Wild	9	9	8
Dadin Kowa dam	Northeast	Gombe	w_DKC	10.319	11.477	Wild	28	15	13
Dadin Kowa dam**	Northeast	Gombe	w_DKAL	10.319	11.477	Wild	5	5	5

**Albino samples

Samples for genetic analysis were chosen by aiming for a subset of 15 samples from each population in this study. This subset was chosen to ensure sufficient representation of genetic diversity within each population. The selection process involved randomly sampling individuals from each population. However, due to DNA degradation issues observed in some populations, the actual sample sizes varied. For populations with sample sizes less than 15, such as the albino population (n = 5) and River Benue Yola population (n = 9), I adjusted the subset to include all available samples to maximise the representation of genetic diversity within these populations. This approach allowed me to adapt to the observed sample sizes and DNA quality constraints while ensuring adequate representation for downstream genetic analysis.

5.2.2 DNA isolation and PCR amplification

As described in Chapter 3, genomic DNA was extracted from all 222 fish fin clips (≤25 mg) using DNeasy Blood & Tissue Kits (Qiagen Inc, Paisley, UK), following the manufacturer's instructions and eluted into 100 µl of AE buffer from the Qiagen kit. The integrity of the extracted DNA was verified in 2% agarose gel electrophoresis and the concentrations (ng/µl) were measured using a Qubit 2.0 Fluorometer, using the broad-range kit (Invitrogen, MA, USA).

5.2.3 Mitochondrial COI analysis

Polymerase chain reaction amplifications and sequencing of the COI gene are as described for Tilapia samples, in Chapter 3. Sequences were edited in Sequencher 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned using the Muscle algorithm in Aliview v. 1.28 (Larsson, 2014) and grouped into unique haplotypes with DnaSP v. 6 (Rozas et al., 2017) as described in Chapter 3. Species identification was carried out using the Barcode of Life Database (BOLD SYSTEMS) (https://boldsystems.org/index.php). Haplotype frequencies were calculated and used to generate minimum spanning haplotype networks in Popart version 1.7 (Bandelt et al., 1999), both to visualise comparisons between farmed and wild populations in my study and to set the Nigerian samples into a global context by comparing the geographic distributions of haplotypes with 100% match with in sequences in BOLD. Arlequin v. 3.5.2.2 (Excoffier and Lischer, 2010) was used to calculate summary statistics per population (number of haplotypes, *Na*; number of segregating sites, *S*; haplotype diversity, *Hd*;

pairwise nucleotide diversity, *pi*), along with pairwise patterns of maternal genetic differentiation (*Fst*) and hierarchical Analysis of Molecular Variance (AMOVA). Summary statistics was compared between farmed and wild using t-test implemented in R. For the AMOVA analyses, comparisons were originally made considering groups (farmed vs wild), populations within groups and variation within groups.

5.2.4 3RAD library preparation

5.2.4.1 Library optimisation

The 3RAD sequencing offers 72 possible restriction enzyme combinations, with two restriction enzymes used to digest the DNA and a third referred to as the dimer-cutting enzyme digests adapter dimers during the simultaneous digestion and ligation steps, creating the desired library with fewer dimers and chimeras (Bayona-Vásquez et al., 2019). The two recommended combinations are: i) XbaI, EcoRI-HF, and NheI; and ii) MspI, BamHI-HF, and ClaI. Each of the combinations has unique double-stranded iTru adapters (hereafter referred to as ds iTru) pairs compatible with the Illumina sequencing platform and designed to bind to the ends of fragmented DNA molecules during library preparation for sequencing (Graham et al., 2015; Hoffberg et al., 2016), with eight and twelve distinct adapters, respectively for P1 and P2. The adapters are given the same name corresponding to the last two digestion enzymes. For example, combination 1 adapters are named Nhe and Eco while combination 2 are named as Cla and Bam. Optimisation for the best restriction enzymes combination was carried out using 24 (at least 2 samples per population) pure, high molecular weight extracted DNA samples with at least 30 ng/µl concentration. This was done as part of the RADCAMP2023 workshop designed to guide participants through a full RADseq pilot study using the 3RAD approach. The concentration for each sample was normalised to 20 ng/µl by diluting with Qiagen DNA elution buffer (Qiagen Inc, Paisley, UK), the same buffer used to elute the DNA during extraction. The optimised DNA samples were visualised on a 2% agarose gel electrophoresis. For each sample, 20 µl volume was transferred into labelled 0.2 mL PCR tubes and shipped on ice packs to Daren Eaton's lab at Columbia University, New York, for library optimisation. Results from test libraries conducted using the two restriction enzyme combinations mentioned above were compared to obtain the best pair. Both combinations worked for the samples but combination 2 (MSPI,

BamHI-HF, and ClaI) showed better PCR amplification results after the library preparation and was adopted as the ideal restriction enzymes of choice.

5.2.4.2 Digestion and ligation

The DNA was digested for 1 hr at 37 °C in a reaction mix that consisted of: 1.5 µL 10x CutSmart Buffer (New England Biolabs, Inc., UK), 5.0 µL ddH_2O, 0.5 µL of MspI at 20 U/µL (New England Biolabs, Inc., UK), 0.5 µL of BamHI-HF at 20 U/µL (New England Biolabs, Inc., UK), 0.5 µL of ClaI at 20 U/µL (New England Biolabs, Inc., UK), 1 µL 5 µM double-stranded iTru read 1 adapter, 1 µL 5 µM double-stranded iTru read 2 adapter (BadDNA, The University of Georgia, USA), and 5 µL DNA. After incubation at 37°C for 1 hour, 2.0 µL dH_2O, 1.5 µL ATP (10 µM), 0.5 µL 10x Ligase Buffer, and 1.0 µL T4 DNA Ligase (100 units/µL, NEB M0202L buffer diluted 1:3 in NEB B8001S enzyme dilution buffer) were added to each reaction, before running the digested/adapter-ligated mixtures in a thermocycler with the following conditions: 22°C for 20 min, 37°C for 10 min, 22°C for 20 min, 37°C for 10 min, 80°C for 20 min, then hold at 10°C. The ligated products were pooled by taking 10 µL from each ligation into new 1.5 mL centrifuge tubes. Thus, when using a 96-well plate, each tube in the strip had 120 µL of pooled ligation (i.e. 120 µL x 8 tubes). From each of the 8 tubes, 60 µL of ligation product was transferred into a single 1.5 mL tube to yield 480 µL of ligation product. The remaining 60 µL from each strip were kept in the freezer for potential future use.

5.2.4.3 Pre-PCR clean up

I performed two bead clean-ups splitting the 480 µL into equal halves (i.e. 240 µL). The two pre-PCR magnetic bead clean-ups were performed using NEBNext Ultra II DNA Library Prep with Sample Purification Beads (New England Biolabs Inc., UK). The clean-up was performed at a dilution of 0.9x, followed by resuspension in 30 µL of dH2O. Subsequently, the cleaned products from both rounds were combined into a single tube, resulting in a total volume of 60 µL.

5.2.4.4 PCR set-up

The 3RAD protocol uses 96 pairs of iTru5 and iTru7 primers that can also be used to tag libraries in case multiple libraries need to be pooled. For example, two libraries that use the same adapter combinations can be tagged during PCR with

unique iTru5 and iTru7 primers and pooled together before sending for sequencing. After sequencing, libraries are demultiplexed using either iTru5 or iTru7 before demultiplexing individual libraries using the adapters used during preparation. The PCR protocol was designed to use 20 µL cleaned ligated DNA fragments in a 50 µL total reaction volume (BadDNA, The University of Georgia, USA). Therefore, three 50 µL PCR were set up to generate full-length library constructs using 20 µL each from the 60 µL of the cleaned ligated DNA fragments using 10.0 µL Kapa HiFi Buffer (Roche, Basel, Switzerland), 1.5 µL dNTPs (10 mM), 7.5 µL ddH_2O, 1.5 µL Kapa HiFi DNA Polymerase (1 unit/µL), 5.0 µL iTru5 primer (5 µM), and 5.0 µL iTru7 primer (5 µM). In a thermocycler, the PCR master mix was amplified using the set-up: 98°C for 1 min.; then, 12 cycles of 98°C for 20 sec, 60°C for 15 sec, 72°C for 30 sec; 72°C for 5 min; hold at 15°C. To validate that the library preparation process was successful, I ran 5 µL of the PCR product with 2 µL loading dye on a 2.0% agarose gel for 45 minutes at 100 volts along with a 1kb DNA Marker (Promega, Madison, Wisconsin, USA). A smear of evenly distributed and bright DNA around ~300-800 bp, without noticeable bands in this target size zone, indicated successful library preparation.

5.2.4.5 Post-PCR clean-up and size selection

PCR products were purified with NEBNext Ultra II DNA Library Prep Sample Purification Beads in a 1:1.5 (DNA:Beads) ratio and cleaned DNA was eluted in 44 µL of ddH_20. The purified product was quantified using a Qubit Fluorimeter broad-range assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the pooled library size-selected using a Pippin Prep (Sage Science, Beverly, MA, USA) with a 2% dye-free Marker L agarose gel cassette (CDF2010), set to capture fragments of 300-450bp, eluted in 40 µL of Tris-TAPS (N-[tris (hydroxymethyl) methyl]-3-amino propane sulfonic acid) buffer. The eluted library was quantified with the Qubit high-sensitivity assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Sequencing (150bp paired-end) was conducted using a single lane on a Novaseq X (Illumina, San Diego, California, USA) at Novogene Co., Ltd (Cambridge, UK).

5.2.4.6 3RAD sequencing analysis

Raw reads were demultiplexed into individual samples using the process_radtags module in Stacks V2.65 (Rivera-Colón and Catchen, 2022). Filtering parameters

were set to drop reads with a Phred quality score of 20 or less and to remove any reads with uncalled bases. After demultiplexing of the reads, the quality of each sample was assessed using FastQC to provide a summary of base quality scores, per-based sequence content, per-sequence quality scores, and sequence length distribution (Andrews, 2010). Reads were mapped to the *C. gariepinus* reference GCF_024256425.1 using BWA-MEM (Li and Durbin, 2009). Population genetics analysis was performed in Stacks V2.65 (Rivera-Colón and Catchen, 2022) to generate SNPs for: calculation of summary statistics for each population (observed heterozygosity, *Ho*; expected heterozygosity, *He*; pairwise nucleotide diversity, *pi*; inbreeding coefficient, *Fis*); quantification of genetic differentiation (*Fst*) between populations; principal component analysis; and admixture analyses.

5.2.4.7 Genetic diversity within farmed and wild C. gariepinus

The analyses of genetic diversity were estimated based on a single SNP per locus specified during the population analysis in Stacks (Rivera-Colón and Catchen, 2022). Summary statistics (*Ho*, *He*, *pi*) and mean inbreeding coefficient based on the difference between *Ho* and *He* (*Fis*) were computed for each population and compared between farmed and wild populations.

5.2.4.8 Genetic differentiation and population structure

Pairwise *Fst* was calculated between each pair of populations using Genepop output from the Stacks populations analysis (Rivera-Colón and Catchen, 2022). To assess genetic differentiation among populations, *Fst* values were calculated using the hierfstat (Goudet, 2005) and adegenet (Jombart et al., 2020) packages in R (R Core Team, 2018). The Genepop file was read into R and converted to a genind object to compute pairwise *Fst* values using the genet.dist function with the "WC84" method for implementation of Weir and Cockerham (1984) *Fst*. Clustered heatmaps based on the *Fst* values using Euclidean distance function from the pheatmap package in R (Kolde, 2019). Using the boot.ppfst function, a permutation test with 1,000 bootstrap confidence interval resampling was performed to assess the significance of the reported *Fst* values (Goudet, 2005). Genetic variation within and between populations was visualised using PCA, as implemented in R using the Adegenet (Jombart et al., 2020) and ggplot2 (Wickham, 2016) packages. Genetic variation within and between populations

was visualised using PCA, as implemented in R using the Adegenet (Jombart et al., 2020) and ggplot2 (Wickham, 2016) packages.

Admixture analysis was conducted using ADMIXTURE software(Alexander et al., 2009). To infer the optimal number of ancestral populations (K) contributing to the genetic structure of the *C. gariepinus* populations, cross-validation was performed for K values ranging from 2 to 10, using 1000 bootstrap replicates and 3 iterations. The K value associated with the lowest cross-validation error was selected as the best estimate of population structure. To test the hypothesis that farmed individuals can be differentiated from wild ones, variation at K = 2 was also assessed.

5.3 Results

5.3.1 mtDNA haplotype distribution and diversity in farmed and wild C. gariepinus populations

Overall, 106 COI sequences of approximately 700 bp were resolved from the X samples amplified. The alignment was pruned to 613bp equal length for all sequences to facilitate the application of statistical analysis in DnaSP that require aligned sequences to be of the same length. This requirement for equal-length sequences is essential for certain analyses within DnaSP, ensuring that all sequences are directly comparable and eliminating potential biases that may arise from variations in sequence length. The pruned alignment had a mean nucleotide composition of C=26.19%, T=28.67%, A=27.45%, and G=17.69%. Overall, eleven unique haplotypes were resolved; identification on the BOLD Systems search engine returned a match for *C. gariepinus* for all, with similarity scores ranging from 99.68 to 100% (Table 5.2). The wild sampling sites had all eleven haplotypes while in the farmed group, only four haplotypes were found. Two farmed haplotypes (10 and 11) were shared only with the albino samples (w_DKAL). In contrast, the remaining two were shared with one wild individual from Kiri dam (haplotype 5) and wild individuals from River Benue, Kiri dam and Lake Geriyo (haplotype 7). Haplotype 10 was found in most of the farmed samples (31 out of 43) whereas among the wild sampling sites, haplotype 8 was most common (n = 27), with the remaining samples distributed across different haplotypes. Haplotypes 1 – 4, 6, 7 – 9 were unique to the wild sites. The haplotype network comparing farmed and wild *C. gariepinus* populations

Table 5.2 . Haplotype identification table based on the Barcode of Life Database (BOLD) showing haplotype ID, sample sizes (N), species similarity percentage, and source classification (farmed/wild).

Haplotype ID	N	Farmed	Wild	Similarity	Source
1	1	-	w_KDC (1)	99.83	wild
2	4	-	w_DKC (4)	99.68	wild
3	5	-	w_BYC (1), w_LGC (4)	99.84	wild
4	1	-	w_KDC (1)	99.82	wild
5	15	f_CMC (4), f_LAC (9)	w_KDC (1)	100	farmed/wild
6	1	-	w_LGC (1)	99.84	wild
7	8	f_LAC (1)	w_BYC (2), w_KDC (3), w_LGC (2)	100	farmed/wild
8	27	-	w_BYC (6), w_DKC (5), w_KDC (8), w_LGC (8)	100	wild
9	6	-	w_DKC (5), w_KDC (1)	100	wild
10	34	f_CMC (6), f_LAC (1), f_ODC (14), f_SAC (10)	w_DKAL (3)	100	farmed/wild
11	4	f_CMC (1), f_SAC (1)	w_DKAL (2)	100	farmed/wild

revealed patterns of shared maternal ancestral relationship (Figure 5.2). Haplotype 8 appeared to be an ancestral haplotype shared among the wild sampling sites with numerous connections within and between wild and farmed sites. Interestingly, the albino samples (w_DKAL) stands out as the only wild individuals associated with haplotypes 10 and 11, which are predominantly found in farmed sites. The main farmed (H10) and wild (H8) haplotypes are separated with 11 mutations. Within the wild haplotypes, the mutation separating the ancestral haplotype from the connecting haplotypes ranged from one in Haplotypes 3, 5, and 9 to two in haplotype 7. Only one haplotype (H11) shares connection with haplotype 10 and there are separated by six mutations. The BOLD Systems search results for haplotypes that had a 100% match (5, 7 – 11) revealed the global distributions across Africa, Asia, and the Middle East (Table 5.3). Haplotypes 5, 7, and 8 were found in Nigeria based on previous studies conducted in the country. Haplotype 8, which was predominant in the wild, was also found in Israel Thailand, Bangladesh, Syria, and India. In contrast, the predominantly farmed haplotype 10 was found in DR Congo and Brazil.

5.3.2 mtDNA population genetic diversity

Based on the COI gene, wild sampling sites tended to have more haplotypes (ranging from 2 – 5; average = 3.6) than farmed sites (ranging from 1 – 3; average = 2.5), with the highest number in w_KDC (n = 5) and only a single haplotype in the Dutch *Clarias* samples (f_ODC) (Table 5.4). There was no significant difference between the farmed and the wild groups (including w_DKAL, whose source was ambiguous) (t = -1.540, p = 0.168, 95% CI = -2.794, 0.594). A similar analysis between the farmed (mean = 2.5) and wild excluding, the w_DKAL (4.0) was also not significant (t = -2.324, p = 0.061, 95% CI = -3.095 – 0.949). In contrast, farmed sampling sites exhibited more segregating sites (14 – 18; average = 16.7) compared to wild sites (3 – 7; average = 4.8), suggesting more divergence among the farmed haplotypes. However, independent t-tests including (t = 1.776, p = 0.169, 95% CI = -5.668, 21.068) and excluding w_DKAL (t = 1.835, p = 0.157, 95% CI = -5.295, 21.295) were not statistically significant. Haplotype diversity including w_DKAL tended to be higher (t = -2.496, p = 0.078, 95% CI = -0.715, 0.063) in wild sampling sites (average = 0.65) compared to farmed (mean = 0.32) sites, except for f_CMC, which had higher *Hd* than some of the farmed sampling sites. When w_DKAL was excluded from the analysis, the

results between the farmed (average = 0.32) and wild (0.66) was still not significant (t = -2.548, p = 0.070, 95% CI = -0.723, 0.047). The highest haplotype diversity was observed in w_DKC. Pairwise nucleotide diversity (*pi*) was highest in f_CMC and tended to be higher (t = 0.861, p = 0.443, 95% CI = -0.005, 0.010) in the farmed (mean = 0.005) than wild (mean = 0.003) sampling sites, except for the albino samples (w_DKAL). The nucleotide diversity between farmed (mean = 0.005) and wild, excluding w_DKAL (mean = 0.002) was not significant (t = 1.196, p = 0.316, 95% CI = -0.005, 0.011).

5.3.3 mtDNA COI genetic differentiation among nine *C. gariepinus* populations

A hierarchical molecular variance analysis (AMOVA) revealed significant genetic differentiation (p < 0.001) among populations within groups (farmed vs wild) but no variation explained by groups overall or within populations (Table 5.5). Specifically, a significant 42.16% of the variance was due to differences among populations within groups (FST = 0.3228, p < 0.001) and although 67.19% of the genetic variance was found within populations (FCT = -0.093, p = 0.904).

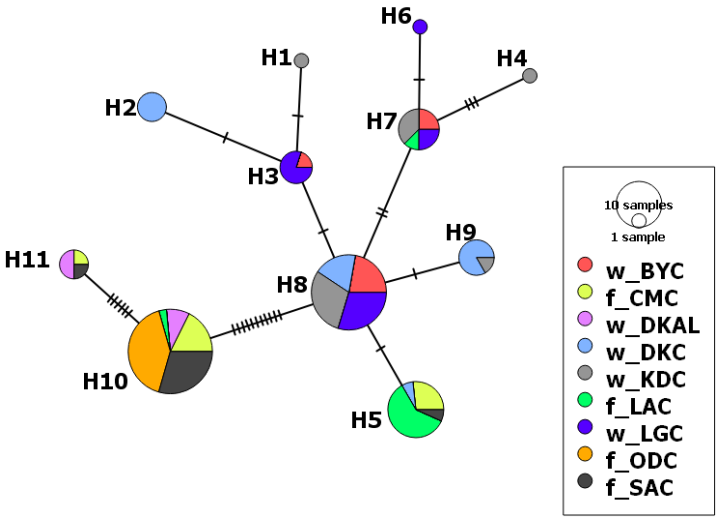

Figure 5.2 Haplotype network showing genetic relationships of farmed (f_) and wild (w_) *Clarias gariepinus* populations from Northeast (w_BYC, w_DKAL, w_DKC, w_KDC, w_LGC, and f_SAC) and Southwest (f_CMC, f_LAC, f_ODC) Nigeria. The Haplotype 8, shared among w_KDC, w_LGC, w_BYC, and w_DKC, appears to be the ancestral haplotype, with the most connections to other haplotypes. The albino C. gariepinus from Dadin Kowa dam (w_DKAL) is the only wild population which had the farmed-dominated haplotypes 10 and 11.

Table 5.3 Global distribution of haplotypes based on 100% match from BOLD Systems search.

Haplotype	Country and reference
5	Nigeria (n = 2): MG824580, MG824583 (Iyiola et al., 2018), BAFEN141-10 (Nwani et al., 2011b); Algeria (n = 4): ON643478, ON643477, ON643476, ON643475 (Behmene et al., 2022); Egypt (n = 1): MK335911 (unpublished)
7	Nigeria (n = 2): MG824581 (Iyiola et al., 2018), BAFEN140-10 (Nwani et al., 2011b)
8	Israel (n = 11): FWISR057-21, FWISR056-21, FWISR055-21, FWISR054-21, FWISR053-21, FWISR052-21, FWISR046-21, LKCOX059-19, LKCOX058-19, LKCOX057-19, LKCOX056-19 (Tadmor-Levi et al., 2023); Thailand (n = 3): JF292311, JF292314, MT571809 (Wong et al., 2011); Bangladesh (n = 1): MG988400 (unpublished); Egypt (n = 2): MK335909, MK335910, (Unpublished); Nigeria (n = 12): BAFEN129-10, BAFEN130-10, BAFEN131-10, BAFEN134-10, BAFEN137-10, BAFEN144-10, BAFEN145-10, BAFEN146-10, BAFEN147-10, BAFEN148-10, BAFEN150-10, BAFEN151-10 (Nwani et al., 2011b); Syria (n = 1): FFMBH2002-14 (Geiger et al., 2014); India (n = 1): FNWG200-16 (Patil et al., 2018)
9	Israel (n = 7): FWISR057-21, FWISR056-21, FWISR055-21, FWISR054-21, FWISR053-21, FWISR052-21, FWISR046-21 (Tadmor-Levi et al., 2023); Thailand (n = 1): JF292314 (Wong et al., 2011); Bangladesh (n = 1): MG988400 (unpublished); Egypt (n = 1): MK335909, MK335910 (Unpublished)
10	DR Congo (n = 5): BCOVR501-17 (Sonet et al., 2019), DCF305-15, DCF751-15, DCF752-1, DCF602-15 (unpublished); Brazil (n = 6): FUPR532-09, LBPV-31863, LBPV-31864, LBPV-31865, LBPV-31866, FUPR536-09 (Pereira et al., 2013);
11	DR Congo (n = 1) BIN ID: AAB2256 (Sonet et al., 2019)

Table 5.4 Origin of farmed and wild African catfish (*Clarias gariepinus*) collected from various states in Nigeria, indicating the sampling site (Population) and code, region, the type of population (Source), number of haplotypes (Na), number of segregating sites (S), haplotype diversity (Hd) ± standard deviation (s.d), and pairwise nucleotide diversity (pi ± s.d).

Population	Code	Region	Source	*Na*	*S*	*Hd ± s.d*	*pi ± s.d*
CMC farm	f_CMC	Southwest	Farmed	3	18	0.618±0.104	0.012±0.007
LAC farm	f_LAC	Southwest	Farmed	3	14	0.346±0.172	0.004±0.003
Dutch *Clarias*	f_ODC	Southwest	Farmed	1	-	-	-
SAC farm	f_SAC	Northeast	Farmed	3	18	0.318±0.164	0.005±0.003
Kiri dam	w_KDC	Northeast	Wild	5	7	0.659±0.123	0.003±0.002
Lake Geriyo	w_LGC	Northeast	Wild	4	4	0.667±0.099	0.002±0.002
River Benue Yola	w_BYC	Northeast	Wild	3	3	0.556±0.163	0.002±0.001
Dadin Kowa dam	w_DKC	Northeast	Wild	4	4	0.752±0.056	0.002±0.002
Dadin Kowa dam (Albino)	w_DKAL	Northeast	Wild	2	6	0.600±0.175	0.006±0.004

Table 5.5 Analysis of Molecular Variance (AMOVA) of mtDNA COI sequences in nine farmed and wild *C. gariepinus* populations from northeastern and southwestern Nigeria.

Source of Variation	d.f.	SS	Var	% Var	F-statistics	p-value
Among groups	2	9.995	-0.039 Va	-9.35	FSC = 0.386	0.000*
Among populations within groups	7	17.278	0.174 Vb	42.16	FST = 0.328	0.000*
Within populations	192	53.302	0.278 Vc	67.19	FCT = -0.093	0.904
Total	201	80.575	0.413	100		

Notes: SS, sum of squares; Var, variance component; Va, variance components among populations; Vb, variance components among populations within groups; Vc, variance components within populations; FSC, proportion of total genetic variance among groups; FST, proportion of genetic variance among populations within groups; FCT: proportion of genetic variance with populations; *significant value ($P < 0.05$).

5.3.4 3RAD reads summary

The demultiplexed library resulted in 1,583,447,229 reads, ranging from 1,198,636 to 35,931,402 per individual. All samples passed the FASTQ check with a sequence per base quality score ≥ 28. After mapping the reads to the *C. gariepinus* reference GCF_024256425.1, the mean read coverage was 109.5x but with varying distribution of reads across populations (Figure 5.3). A total of 1,663,643 loci were assembled, ranging from 37,000 to 100,000 loci per sample (Figure 5.4A). The mean rate of missing variant sites (i.e. the average percentage of genomic locations that do not have any sequence information) within populations was less than 0.05% for most samples, except for one sample in the albino w_DKC population with about 25% missing variant sites (Figure 5.4B). This sample was dropped from subsequent analysis.

5.3.5 Genetic diversity within farmed and wild *C. gariepinus* populations

Genetic diversity parameters were compared between farmed and wild *C. gariepinus*. The results based on 14,410 variant sites from 20,126 loci including albino samples considered as wild population revealed that observed heterozygosity (*Ho*), expected heterozygosity (*He*), and nucleotide diversity (*pi*) for both farmed and wild sampling sites exhibit similar ranges. When albino sample was considered as wild sample, the wild sampling sites had a slightly broader range and higher maximum values for *Ho* (0.109 – 0.165), *He* (0.111 – 0.216), and *pi* (0.125 – 0.225) compared to the farmed sampling sites (*Ho* = 0.118 – 0.147, *He* = 0.112-0.144, *pi* = 0.117 – 0.151). *Ho* comparison between farmed

(mean = 0.135) and wild (mean = 0.138) was not significant (t = -0.258, df = 6.753, p = 0.804). Likewise, He mean between farmed (mean = 0.127) and wild (mean = 0.172) was not significant (t = -2.277, df = 5.012, p = 0.072). Mean *pi* comparison between farmed (0.133) and wild (0.183) was also not significant (t = -2.511, df = 5.148, p = 0.052). However, inbreeding coefficient comparison between farmed (mean = 0.001) and wild (mean = 0.127) was significant (t = -3.472, df = 6.707, $p < 0.05$).

In a similar analysis excluding albino samples revealed lower but not significant (t = -1.045, df = 5.932, p = 0.337) *Ho* in farmed (mean = 0.135) compared to wild (mean = 0.188). *He* comparison between farmed (mean = 0.127) and wild (mean = 0.145) was also not significant (t = -3.897, df = 4.347, p = 0.015). However, *pi* in farmed (mean = 0.133) in comparison to wild (mean = 0.198) was significant (t = -3.883, df = 4.329, $p < 0.05$). Likewise, *Fis* comparison between farmed (mean = 0.001) and wild (mean = 0.149) was significant (t = -4.496, df = 5.742, $p < 0.05$).

When albino sample was included among the farmed samples, the average *Ho* in the farmed (*Ho* = 0.130) was less than the wild (*Ho* = 0.145) but their mean difference was not significant (t = -1.502, df = 6.863, p = 0.178). *He* in wild (average = 0.188) was significantly higher (t = -4.175, df = 4.152, $p < 0.05$) compared to the farmed (average = 0.124). Average *pi* comparison between farmed (*pi* = 0.132) and wild (*pi* = 0.198) was statistically significant (t = -4.118, df = 3.911, $p < 0.05$). *Fis* in the farmed (average = 0.008) in comparison to the wild (average = 0.149) was significantly lower (t = -4. 519, df = 5.577, $p < 0.05$).

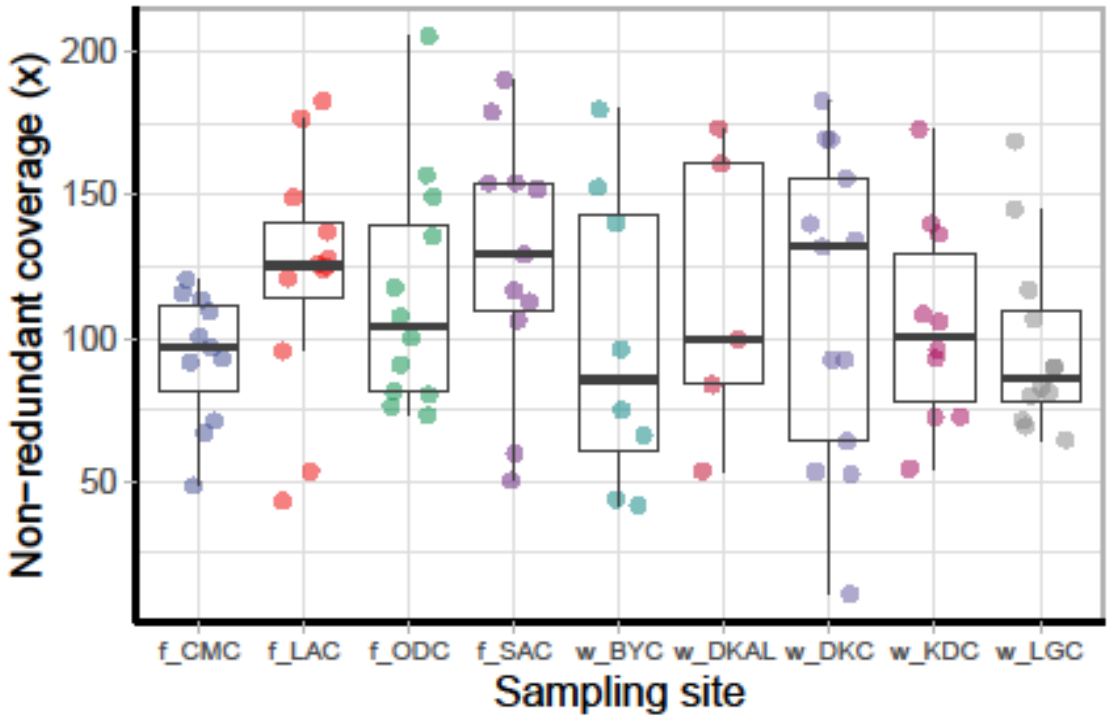

Figure 5.3 Boxplot illustrating the distribution of non-redundant read coverage, indicating the number of reads mapped to specific genomic regions or features for each population. The boxplot provides a visual summary of the read distribution, with the box indicating the interquartile range (IQR) and the median read coverage, and the whiskers extending to the minimum and maximum values or to a specified range. The prefixes f and w on the x-axis indicate farmed and wild samples, respectively.

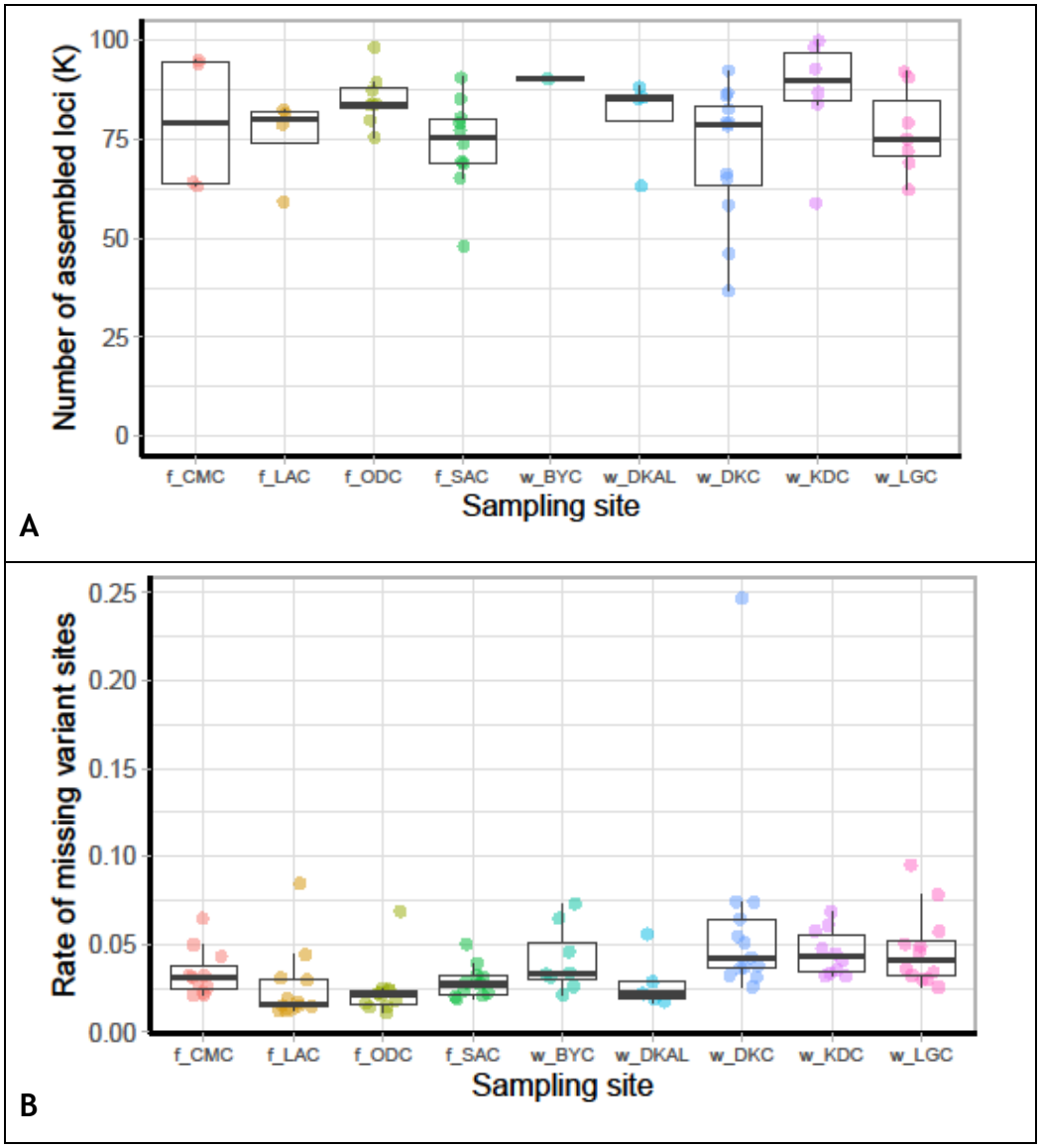

Figure 5.4 Boxplot illustrating the distribution of assembled loci across different sampling locations (A) and the rate of missing variant sites (B) for the 3RAD sequencing data. The y-axis indicates the number of loci in thousands and the x-axis represents the sampling locations. Each point on the plot represents a sample within a location. The prefixes f and w on the x-axis indicate farmed and wild samples, respectively.

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Table 5.6 Summary of observed (Ho) and expected heterozygosity (He), nucleotide diversity (pi), and inbreeding coefficient (Fis) estimated from 20,126 3RAD loci and 17,836 SNPs showing the genetic diversity in farmed (f_) and wild (w_) Clarias gariepinus in Nigeria.

# Pop ID	Code	N	*Ho*	*He*	*pi*	*Fis*
CMC farm	f_CMC	10	0.147	0.144	0.151	0.017
Lagos farm	f_LAC	12	0.142	0.121	0.127	-0.031
Ogun Dutch	f_ODC	12	0.132	0.112	0.117	-0.034
SAC	f_SAC	11	0.118	0.130	0.137	0.052
River Benue	w_BYC	9	0.144	0.205	0.220	0.196
Dadin Kowa Albino	w_DKAL	5	0.109	0.111	0.125	0.038
Dadin Kowa	w_DKC	11	0.165	0.216	0.225	0.163
Lake Geriyo	w_LGC	12	0.137	0.155	0.162	0.076
Kiri Dam	w_KDC	8	0.133	0.174	0.184	0.160

5.3.6 Genetic differentiation and population structure between farmed and wild populations

The pairwise *Fst* comparisons between sampling sites from the 3RAD genotypic data showed lower genetic differentiation among the wild sampling sites (0 – 0.26) compared to the farmed sites (0.16 - 0.28) (Figure 5.5). Pairwise *Fst* among wild sampling sites was significant except between w_BYC and w_KDC (*Fst* [95% bootstrapped CI] = -0.00 [-0.003–0.002]). Among the farmed sampling sites, *Fst* was also significant. Comparison between the albino samples (w_DKAL) and the farmed sampling sites showed consistently low but significant *Fst* values ranging from (*Fst* [95% bootstrapped CI] = 0.16 [0.150–0.168]) between f_CMC to (*Fst* [95% bootstrapped CI] = 0.28 [0.264–0.286]) between f_LAC. In comparison to the other wild sampling, *Fst* values between albino sample was higher, ranging from (*Fst* [95% bootstrapped CI] = 0.33 [0.383–0.400]) between w_DKC to (*Fst* [95% bootstrapped CI] = 0.42 [0.407–0.430]) between w_LGC. The lowest *Fst* (-0.00) among the wild sampling sites was observed between Kiri Dam (w_KDC) and River Benue Yola (w_BYC). At the top and left sides of the *Fst* heatmap are two dendrograms, each representing the hierarchical clustering of the farmed and wild sites (Figure 5.5). The rows and columns are grouped into two main clusters, with one large cluster of farmed sampling sites f_CMC, f_SAC, f_LAC, f_ODC, and the wild w_DKAL. The second cluster shows the wild w_DKC, w_BYC, w_KDC, and w_LGC separated from the farmed group. The blue and yellow

colours depict populations that are more similar to each other while the orange and red represents sampling sites that exhibited higher genetic differentiation between them. There was an observed low genetic differentiation among wild sampling sites in Adamawa state (w_BYC, w_KDC, and w_LGC) ranging from *Fst* = -0.00 (between w_BYC and w_KDC) to *Fst* = 0.03 (between w_BYC and w_LGC). When the northeast samples are considered to excluding the albino, the *Fst* values increases slightly from 0.10 between w_BYC and w_DKC to 0.26 between w_DKC and w_LGC.

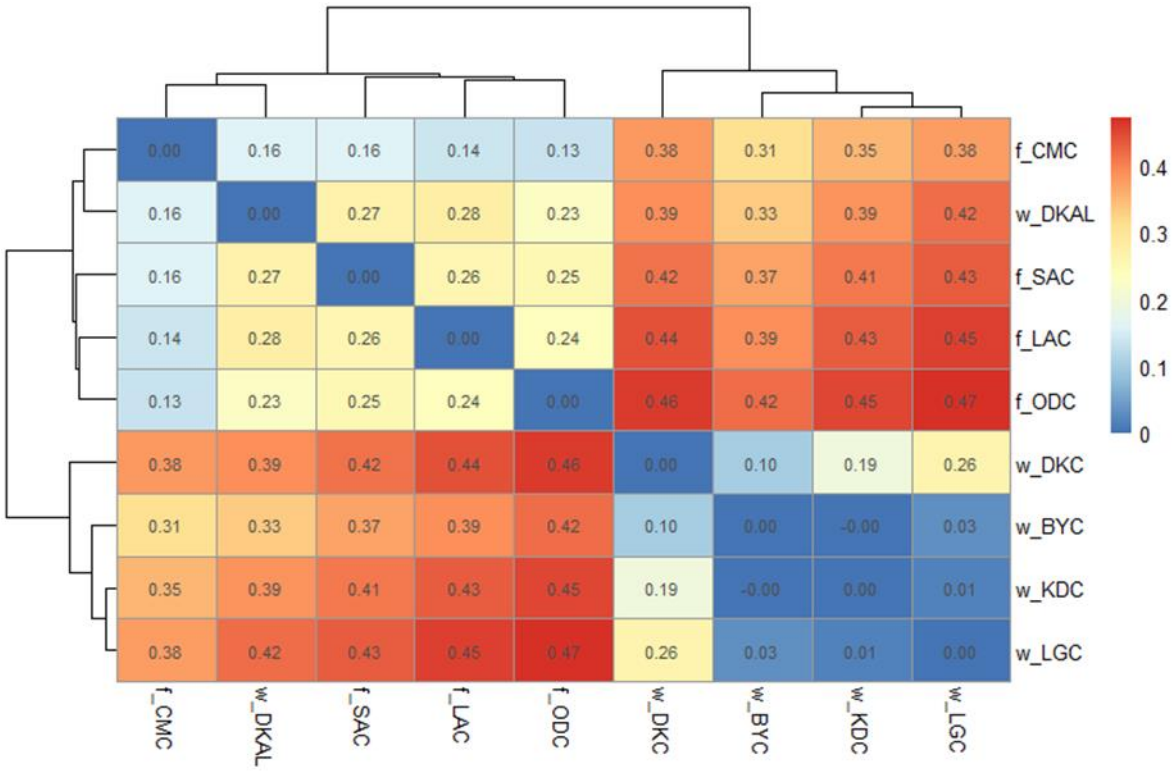

Figure 5.5 Pairwise genetic differentiation (Fst) among nine *Clarias gariepinus* from farmed and wild populations based on SNP data. f_CMC: CMC farm, f_ODC: Dutch Clarias, f_LAC: Lagos farm, f_SAC: SAC farm, w_BYC: River Benue Yola, w_LGC: Lake Geriyo, w_DKAL: Albino Dadin Kowa, w_DKC: Dandin Kowa dam, w_KDC: Kiri dam.

The PCA plot separated the wild samples based on their states of origin as well as between farmed and wild except for the “wild” albino samples that clustered with the farmed individuals (Figure 5.6). Principal components 1 (PC 1) and 2 (PC 2) explained 20.72% and 13.26% of the variance, respectively. The distribution of individuals and populations on the PCA plot showed a major cluster of all the farmed samples along the PC1 axis, but with the albino samples (green triangle) interestingly clustering with them. The remaining wild sampling sites were distributed across the PC 1 and PC2 axes with most of the wild

samples from Adamawa (w_BYC: River Benue Yola, w_LGC: Lake Geriyo, and w_KDC: Kiri dam) clustering at the bottom (i.e. lower values of PC2) while the Gombe samples (w_DKC: Dadin Kowa dam) were distributed at the top of the PC 2 axis. Samples from w_BYC were distributed across both wild clusters.

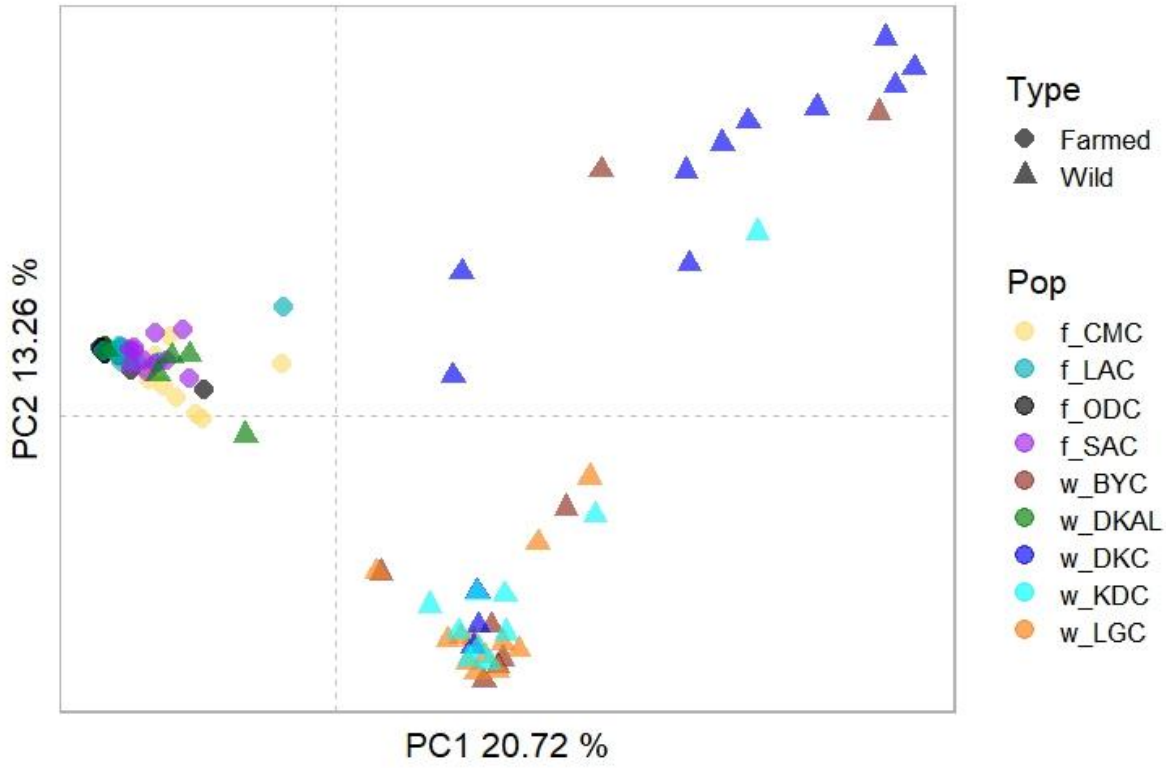

Figure 5.6 Figure 5. A Principal Component Analysis (PCA) plot of farmed and wild *C. gariepinus* generated from SNP data. The x-axis and y-axis represent the first and second principal components, respectively, which are linear combinations of SNP alleles that capture the maximum amount of variation in the data. PC1 (explaining 20.72% of the variation) separates the wild samples (triangles) from the farmed samples (circles), except the wild albino population (w_DKAL) sampled from Dadin Kowa dam. PC2 (explaining 13.26% of the variance) separates some of the wild populations from one another; note that w_BYC is found in both main wile clusters.

Based on the lowest cross-validation error value for the admixture analysis (Figure 5.7), the best K value to describe the population structure was 5. At K = 2, there was obvious differentiation between the farmed and wild sampling sites but again, the albino wild population (w_DKAL) shared the same cluster with the farmed groups. More population structure was revealed among the farmed sampling sites at K = 3 and among the wild sites at K = 4. The f_SAC was differentiated from the other farmed populations at K = 3 but clustered with the f_ODC and w_DKAL populations at K = 4. Like the PCA result, the Adamawa sampling sites (w_BYC: River Benue Yola, w_LGC: Lake Geriyo, and w_KDC: Kiri dam) appeared to be differentiated from the Gombe samples (w_DKC: Dadin

Kowa dam) at K=4; there was evidence of admixture within and between w_BYC and w_DKC, with some individuals from w_KDC also showing admixture with the main cluster in w_BYC. At the best cluster (K = 5), f_SAC from the northeast was differentiated from their farmed counterparts from the southwest. Within the southwest populations, the CMC farm (f_CMC) was genetically distinct from the Dutch *Clarias* (f_ODC) but all individuals from f_LAC showed evidence of admixture including all of the farmed clusters. Some of the individuals from f_SAC also showed admixture with the other farmed clusters. At K=5, clustering of the albino wild population (w_DKAL) with Dutch *Clarias* (f_ODC) was even more striking than at the lower values of K.

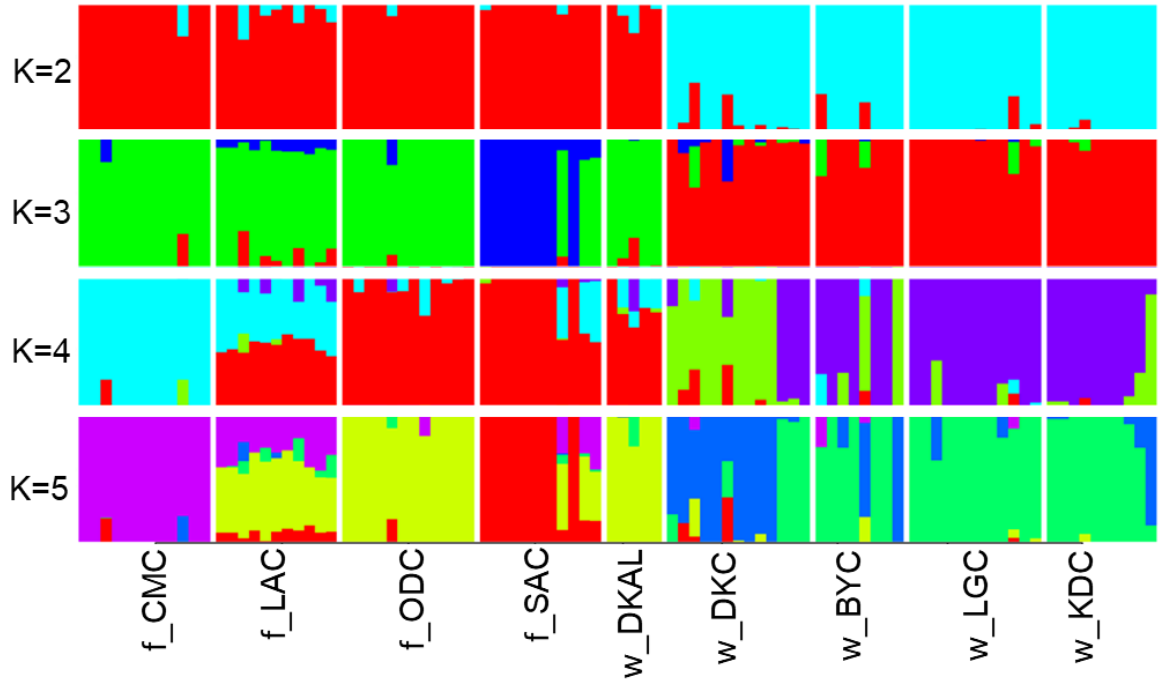

Figure 5.7 Admixture plot illustrating the genetic ancestry of individual fish samples from farmed and wild *C. gariepinus* populations based on SNP data. Each vertical bar in the plot represents an individual fish sample, partitioned into segments corresponding to inferred genetic clusters. The plot is grouped according to farmed (the first four) and wild (the last five) populations.

5.4 Discussion

In this study, the genetic diversity and differentiation of farmed and wild populations of *C. gariepinus* in Nigeria were investigated using the mtDNA COI gene and 3RAD sequencing approaches. The mtDNA analyses revealed higher haplotypes that were only found in wild populations, suggesting that genetic diversity is preserved in the wild populations for these specific haplotypes. Only two of the haplotypes unique to wild sampling sites had a 100% match in the

BOLD database but representative samples were distributed across the Middle East and Asia; for example, this haplotype had been found in a survey of freshwater fish diversity in Israel (Tadmor-Levi et al., 2023). This suggests a broader geographic distribution of these haplotypes in the Middle East and Asia. In contrast, all of the haplotypes found in farmed populations had also been described from other regions, which could reflect shared sources of farmed fish. Haplotype 5 found in predominantly farmed f_LAC and f_CMC as well as one wild samples (w_KDC) was reported in the wild in the Southeastern Algerian population (Behmene et al., 2022) and Southeast of Nigeria (Nwani et al., 2011a). The presence of Haplotype 5 in predominantly farmed sampling sites suggests a likely introduction from wild populations. This phenomenon is potentially attributed to common practices among catfish farmers in Nigeria, who often acquire gravid broodstock from fishers during the peak rainy season when wild *Clarias* spp. are about to begin breeding. Purchasing broodstock from the wild is preferred due to its cost-effectiveness compared to buying from farms, as breeders view wild-caught broodstock as more economically viable. However, this decision to buy broodstock from the wild is not driven by genetic considerations. Similarly, predominantly wild haplotype 7 which was also found in farmed f_LAC has in previous studies been reported to be a wild haplotype in both Northcentral (Iyiola et al., 2018) and Southeastern Nigeria (Nwani et al., 2011b). The presence of predominantly farmed haplotypes 10 and 11 in the albino samples w_DKAL, despite being caught in Dadin Kowa dam, raises questions about the true wild status of the w_DKAL samples. The absence of haplotypes 10 and 11 in previous DNA barcoding studies conducted in Nigeria, which primarily focused on wild samples, suggests that these haplotypes were not previously documented in sequences submitted to BOLD from Nigerian studies. However, both haplotypes 10 and 11 were identified in the Lower and Middle Congo Rivers, as well as in three major drainage basins of the Lower Guinean ichthyological province, namely Kouilou-Niari, Nyanga, and Ogowe (Sonet et al., 2019).

Pairwise nucleotide diversity for the mtDNA was higher in some of the farmed than the wild sampling sites, possibly because the farmed samples had come from a variety of sources and so included differentiated haplotypes (indicated by the higher number of segregating sites), which was also suggested by the high

admixture in the 3RAD analyses for some of the farmed sampling sites. Previous studies have confirmed that the farmed haplotypes are sourced from the wild as reported in Nigeria (Iyiola et al., 2018; Nwani et al., 2011b), Algeria (Behmene et al., 2022), and DR Congo (Sonet et al., 2019). The AMOVA analyses revealed that there was more variation among populations than between farmed and wild groupings, which is explained by the finding that all of the farmed mtDNA haplotypes were also found in wild populations. Intriguingly, an albino population sampled from the wild not only shared two of the farmed mtDNA haplotypes but also clustered with the farmed samples in both PCA and admixture analyses of the 3RAD data, even though other "normally pigmented" individuals from the same sampling site were highly distinctive. This could provide evidence that the albino fish are escapees from Dutch *Clarias*, which is the sampling site to which they showed highest similarity. The shared genetic similarities observed with the Dutch *Clarias*, along with the presence of haplotypes found in the albino samples in DR Congo (Sonet et al., 2019) which coincidentally happens to be one of the donor countries of *C. gariepinus* to Belgium and the Netherlands where Dutch *Clarias* was developed (Holčík, 1991; Huisman and Richter, 1987), could confirm their identity as escaped farmed Dutch *Clarias*. Except for the albino samples, wild sampling sites showed consistently higher genetic diversity based on the 3RAD data than farmed sampling sites but somewhat unexpectedly, farmed samples were less inbred than wild populations. Although this could again be due to breeding of farmed fish from multiple sources, since pairwise genetic differentiation (*Fst)* was lower among the wild than the farmed populations, it also could be a sign of bottlenecks in the wild populations. However, the high levels of admixture in some of the farmed sampling sites could also explain this lower *Fst*. Although the wild samples showed clustering by geography region based on the 3RAD data, there was also some evidence of admixture, suggesting that there could be transport of samples between regions to be used as stocks. In contrast, there was little evidence of admixture between wild sampling sites and genetic clusters from farmed sites, suggesting that interbreeding might not be common, despite the sharing of some of the mtDNA haplotypes between farmed and wild populations. Overall, my results emphasise the types of insights that can be gained by comparing patterns of genetic variation within and between farmed and wild populations.

5.4.1 mtDNA genetic diversity and differentiation

The mtDNA COI result suggests that wild sampling sites harbour a greater diversity of mtDNA haplotypes, which may reflect their larger effective population sizes as opposed to the farmed populations that are bred using fewer broodstock. The overall genetic diversity parameters (*Hd* and *pi*) obtained in this study are very low compared with results from previous studies. For examples, Kundu et al. (2023) using mtDNA COI to study *C. gariepinus* from the Nyong River in Cameroon reported 20 haplotypes with 81 segregating sites, haplotype diversity (*Hd*) = 0.854, and nucleotide diversity (*pi*) = 0.258. A similar study conducted in southwestern region of Nigeria using the mtDNA *cytb* gene revealed 53 haplotypes, with greater haplotype diversity (*Hd* = 0.999) and nucleotide diversity (*pi* = 0.073) than the current study (Popoola, 2022). Nyunja et al. (2017) studied five *C. gariepinus* hatchery populations in Kenya, and found 33 haplotypes, 60 segregating sites, high haplotype diversity (*Hd* = 0.988 ± 0.031), and low nucleotide diversity (pi = 0.024 ± 0.026). The relatively high genetic diversity (*Hd* = 0.618 ± 0.104, *S* = 18) found in the CMC farm can be explained by the periodic introduction of new broodstock into the hatchery to increase the genetic diversity and counteract the effects of genetic drift and inbreeding depression (Ibiwoye and Thorarensen, 2018). Alal et al. (2021) suggested that the genetic diversity in small *C. gariepinus* populations as observed in the Lake Kenyatta in Kenya, can be improved through stock augmentation by conservation scientists. For example, this approach could be used to boost declining *C. gariepinus* in lakes due to overfishing and periodic drying.

The genetic differentiation from AMOVA analysis showed high genetic differentiation among the nine populations (*Fst* = 0.382, $p < 0.0001$). The Analysis of Molecular Variance (AMOVA) indicated that the percentage of genetic variation was predominantly explained by differences among populations within farmed and wild categories, as well as within populations themselves. Also, after dropping the wild albino population that only shared haplotypes with the farmed populations from the AMOVA analysis, the *Fst* increased from 0.408 – 0.682. The absence of shared haplotypes between the albino population and the native Dadin Kowa dam population, from where it was captured, is noteworthy. Instead, the closer genetic relationship observed with the farmed population rather than the wild population suggests a recent escape event from the

aquaculture facility to the dam. Other studies using mtDNA markers have been conducted to investigate the genetic differentiation among *C. gariepinus* populations. For example, Alal et al. (2021) in their study of lacustrine and riverine *C. gariepinus* populations in Kenya using the mtDNA D-loop, observed distinct genetic patterns and population structures among lacustrine and riverine populations, with lower but significant genetic differentiation (*Fst* = 0.166, p < 0.001) among populations than what I found in this study. In a similar study using the cytb gene, Popoola (2022) found high genetic differentiation (Fst = 0.75 – 0.95) among three Southwest populations in Nigeria. Overall, the comparable results from my data compared with other studies have shown that the COI gene and other mtDNA markers including D-loop and cytb can be applied to successfully differentiate different populations of *C. gariepinus*. This cheap molecular tool compared to deep sequencing will be a vital tool for the conservation of *C. gariepinus* in Nigeria.

5.4.2 3RAD genetic diversity in farmed and wild populations

Overall, the genetic diversity of *C. gariepinus* reported in this study is lower than values reported for the species from studies in other locations using nuclear markers. The genome of *C. gariepinus* was only sequenced and assembled in 2022 by the Leibniz Institute for Farm Animal Biology (FBN) using an ecotype from the Netherlands. However, multiple studies have been conducted using microsatellite markers both within and outside Nigeria to investigate the genetic diversity of *C. gariepinus* studies, as provided in a recent review article (Kebtieneh et al., 2024). A similar study conducted in Nigeria on *C. gariepinus* from River Niger and Asejire dam using microsatellite markers found moderate to high observed heterozygosity (0.125 in River Niger and 0.409 in Asejire) and higher mean expected heterozygosity (0.55 in River Niger and 0.566 in Asejire dam). This difference was reflected in high positive inbreeding coefficients (*Fis* = 0.257, and 0.741, respectively) in the two locations. However, despite the lower genetic diversity observed in my study, the inbreeding coefficient was lower across all study sites (*Fis* = -0.034 – 0.196), but most especially among the farmed study sites. In a study exploring the genetic diversity of *C. gariepinus* hatchery populations across northeastern province and central Thailand utilising microsatellite markers, Wachirachaikarn and Na-Nakorn (2019) noted substantial heterozygosity, with values ranging from 0.52 to 0.72 for *Ho* and 0.67 to 0.77

for *He*. The low observed and expected heterozygosity in the wild sampling sites could be attributed to several factors, ranging from genetic bottlenecks due to a reduction in population size caused by overfishing and periodic drying of lakes and rivers during the extended dry season in Nigeria (Emmanuel et al., 2021). Within the farmed sampling sites, the lower genetic diversity observed compared to wild may have been influenced by the relatively limited number of breeders responsible for maintaining these strains. In a previous study, declining genetic diversity in farmed bream (*Abramis brama orientalis*) was primarily linked to the loss of rare alleles due to a small number of breeders maintaining the strains (Zeinab et al., 2014).

5.4.3 Genetic differentiation among farmed and wild populations

Overall, there was consistently high genetic differentiation between the farmed and wild *C. gariepinus* sampling sites (*Fst* = 0.31 – 0.47), suggesting that there is some level of genetic differentiation between the farmed and wild sampling sites. The low *Fst* among northeast sampling site from Adamawa (w_BYC, w_KDC, and w_LGC) compared to substantial genetic differentiation between another northeast sampling site from Gombe state (w_DKC) provide evidence for geographical differentiation within the northeast region. The lower *Fst* between the albino (w_DKAL) and the farmed sampling sites compared to the wild with higher degree of genetic differentiation suggest a closer genetic relationship between albino and the samples from farmed sites. This also indicate that the albino to be products escapes from a fish farm. This shared genetic similarities with farmed sampling sites is consistent with results in similar studies where farmed escapes show genetic similarities to their source populations. For example, Erkinaro et al. (2010) demonstrated that escaped Atlantic salmon caught in River Teno in northernmost Europe showed high genetic differentiation between wild from the same river compared to lower genetic differentiation with farmed salmon. In another study, a high *Fst* between escaped farmed seabass and their wild conspecifics in Adriatic sea, provided strong evidence of escape back to their farmed origin (Šegvić-Bubić et al., 2016). Additionally, more evidence on detecting farmed escapes in the wild were provided using 14-year dataset of farmed escapee from 54 rivers in western Norway (Mahlum et al., 2021). Using Bayesian inference, Mahlum et al. (2021) found abundance of farmed escapees in rivers to be correlated to aquaculture intensity.

Overall, the *Fst* values observed in this study were higher than results obtained from other *C. gariepinus* studies using different markers including microsatellites. For example, in an assessment of genetic differentiation of *C. gariepinus* from three regions in India using microsatellites, Ezilrani and Christopher (2015) observed low to moderate *Fst* (0.159 – 0.200) values that were comparable to those observed between farmed sampling sites in this study (*Fst* = 0.16 – 0.25). The PCA and admixture analyses also supports the *Fst* results between the albino and the wild samples, providing more evidence that the albino is indeed is more related to the farmed than the wild group. With the combined evidence from the mtDNA analysis and the 3RAD results, it can be concluded that the albino samples is a case of an escape event from the farm to the wild. If the albino population is considered as farmed, it can be concluded based on the first admixture cluster of K = 2, and the PCA that *C. gariepinus* populations in Nigeria, despite the low genetic diversity can be differentiated according to farmed and wild using the genome-wide SNPs. The significant *Fst* values between farmed and wild sampling sites is an important indicator for the implementation of conservation strategies that will manage these different farmed and wild sites separately to preserve their unique diversity. Relevant scientific data such as this are relevant to the management of fish and aquaculture activities (Kemp et al., 2023).

5.5 Conclusions

These findings offer valuable insights for the formulation of conservation and management strategies aimed at preserving wild fish populations and regulating aquaculture activities to mitigate the risk of escape. Regulating breeding and effective management and conservation of natural populations are necessary and effective tools for protecting the worrying low genetic diversity in both farmed and wild populations of *C. gariepinus*. This 3RAD and COI data will be highly informative in proposing management strategies for conserving genetic diversity and maintaining population structure in both farmed and wild fish populations. Also, the implementation of genetic monitoring, habitat restoration, and sustainable aquaculture practices is needed to mitigate genetic risks and preserve the genetic integrity of native *C. gariepinus*. Future research directions should consider sampling across more geographical locations and

farms to further understand the dynamics of genetic diversity and population structure in farmed and wild fish.

Chapter 6 General discussion

This thesis set out to examine genetic variation in wild and farmed tilapia and catfish in Nigeria using mtDNA COI marker and two different RADseq approaches (ddRADseq for tilapia and 3RAD for catfish). It has shown that there was extensive hybridisation among farmed tilapia populations, leading to a lack of population structure. Conversely, wild populations, particularly from Badagry creek and Asejire dam, exhibited population structure with higher genetic diversity compared to farmed populations. Intergeneric hybridisation was observed in wild tilapia populations. In catfish, although genetic variation was low across both farmed and wild populations, there was minimal hybridisation among wild individuals, with clear differentiation between wild and farmed populations. The albino population was identified as originating from the farmed population based on mtDNA and 3RAD analyses. Despite the overall low genetic diversity, there was no evidence of negative impacts of aquaculture on wild populations, as genetic diversity remained preserved. In this final chapter, the findings will be synthesized to draw general conclusions in the context of fish conservation genetics.

6.1 Important mitochondrial markers in species identification

Fish species identification has traditionally relied on external morphological features. However, in numerous instances, identifying fishes by this method, particularly across various developmental stages, poses challenges due to the limitations of morphological characteristics (Teletchea, 2009), as clearly demonstrated by the complexity of variation within tilapia species in Nigeria that I described in Chapters 3 and 4. Species-level identification can become even more complex due to various factors such as the accumulation of variation over time in response to environmental changes (Moss and Cannon, 2011), and the existence of fish taxa such as the cichlids that frequently undergo evolutionary changes, leading to a wide array of morphologically distinct species (Svardal et al., 2021). Furthermore, farmed fish subjected to selective breeding may exhibit distinct colouration patterns (as evidenced in GIFT tilapia) compared to their wild counterparts, adding another layer of complexity to their identification when using only morphological traits. By harnessing the

advantages of both morphological characteristics and mitochondrial markers, researchers can delve deeper into species identification and uncover unique haplotypes in both wild and farmed species. This information can then be utilised to shape conservation and management strategies aimed at promoting sustainable aquaculture practices and biodiversity conservation initiatives. Employing the DNA barcoding approach using the mtDNA COI gene presented a valuable opportunity for identifying tilapia and catfish species in Nigeria in my study. This identification is crucial for biological research as it enables the assessment of species diversity, distribution, and ecological dynamics (Kürzel et al., 2022). Species identification is the first step for understanding native wild fish diversity as well as their aquaculture counterparts and is crucial for preserving biodiversity, fisheries management and forensic investigation (Cawthorn et al., 2012; Withler et al., 2004), as well as in the study of archaeological samples to provide evidence of evolutionary and ecological changes over time (Yang et al., 2004). Mitochondrial genes have emerged as powerful tools for helping to delineate phylogenetic and taxonomic relationships within both freshwater and marine fish taxa (Lakra et al., 2009; Nwani et al., 2011b), as well as for exploring intraspecific variation and defining species boundaries (Pérez-Rodríguez et al., 2009). The utility of mtDNA markers in stock discrimination has been exemplified in Atlantic herring (*Clupea harengus*), offering valuable insights into the genetic diversity and population structure of this marine pelagic teleost species (Hauser et al., 2001). The application of mitochondrial genes as molecular markers has significantly contributed to our understanding of fish phylogeny, taxonomy, intraspecific variation, and population structure. The utilisation of mitochondrial genes, particularly in under-studied species such as *C. gariepinus*, holds promise for providing valuable insights beyond current knowledge, particularly in terms of genetic diversity and stock discrimination. My finding that farmed fish escapes can be traced through genetic analyses is important because it provides a tool for monitoring at-risk areas.

6.2 Unplanned aquaculture development

Assessing the genetic diversity and population structure of commercially important fish species is a key strategy for enhancing the conservation of natural fish populations while also creating opportunities for increased aquaculture

production (Alal et al., 2021). In Chapter 2 (now published as Sanda et al. 2024) I reviewed “the potential impact of aquaculture on the genetic diversity and conservation of wild fish in sub-Saharan Africa” and advocated for the implementation of responsible guidelines in aquaculture practices to prevent genetic impacts on native populations of significant conservation value in the region.

The establishment and expansion of aquaculture activities without adequate planning, regulation, or consideration of their environmental, social, and economic impacts can lead to a range of negative consequences, including habitat destruction, pollution, the spread of diseases, genetic introgression of farmed species into wild populations, displacement of local communities, and conflicts over resource use (Lind et al., 2015). Unplanned aquaculture development often occurs in response to market demand, government incentives, or technological advancements without proper assessment of carrying capacity, sustainability, and long-term consequences. Effective planning, regulation, and stakeholder engagement are essential to mitigate any negative impacts of aquaculture developments and promote sustainable practices that balance economic growth with environmental and social concerns (Sanda et al., 2024). The Nigerian government's dedication to enhancing the aquaculture sector to boost local production and lessen fish imports is evident through initiatives such as infrastructure development, introduction of superior fish strains, and farmer training. However, the focus on augmenting fish production has overlooked the potential negative genetic consequences such as fish escapes, hybridization between introduced and native species, and gene introgression, which can compromise the genetic diversity of wild fish. However, the extensive hybridisation observed in farmed tilapia and escapees detected in the wild highlights the importance of reevaluating the aquaculture policies. The confirmation of intergeneric hybridisation in wild populations highlights the need for improved conservation measures to mitigate the impacts of aquaculture. These measures should include regulations to address indiscriminate breeding practices, control the introduction of fish species, and establish designated aquaculture zones to minimize genetic interactions between farmed and wild populations.

Aquaculture production plays a pivotal role in ensuring global food security and the sustainable management of aquatic ecosystems. Balancing the management of the genetic diversity of wild populations with the pursuit of the United Nations Sustainable Development Goals (SDGs) on food security through aquaculture poses a significant challenge. It comes on top of the adverse effects of climate change on aquatic ecosystems, which include diminishing species diversity and decreased fish production. These effects are already evident in many parts of the world, including Nigeria (FAO, 2022). Additionally, unplanned aquaculture practices centred on non-native species, which are highly invasive, pose a further threat to native species in Nigeria and undermine conservation efforts. The unplanned introduction of domesticated tilapia strains for aquaculture purposes and the lack of regulations for the siting of farms can lead to escapes and introgressive hybridisation; evidence that this is already happening for tilapia in Nigeria is presented in Chapter 4.

6.3 Conservation approach for tilapia and *C. gariepinus*

The aquaculture of tilapia and the catfish *C. gariepinus* presents significant challenges, particularly concerning their taxonomy and management. Tilapia species are known for their propensity to interbreed with closely related species, posing difficulties in taxonomy resolution, as observed in both the mitochondrial and ddRADseq analyses presented in Chapters 3 and 4, respectively. Hybridization, a significant driver of speciation among cichlid fish (Rometsch et al., 2020), has further complicated the taxonomy of tilapia, rendering it challenging to resolve using a single maternally inherited DNA marker such as COI. Furthermore, hybridisation among cichlids has the potential to create new phenotypes that makes morphological identification complicated (Irisarri et al., 2018).

Indeed, hybridisation among cichlids, as highlighted by Irisarri et al. (2018), has the potential to generate novel phenotypes, further complicating morphological identification. This complexity is exacerbated by the lack of clear population structure and low genetic differentiation observed between introduced farmed and wild Oreochromis species in Nigeria, as shown in Chapter 4. Given their propensity for hybridization, managing these species presents a challenging genetic concern for Nigerian fisheries and the aquaculture industry. The recent

review of the Nigerian Fisheries Act of 2014 conducted in 2023, involving Government officials and researchers from tertiary institutions, missed an opportunity to integrate genetic tools into fisheries management. Instead, the focus was primarily on boosting aquaculture production by introducing improved strains. The review thus overlooked crucial genetic conservation measures such as aquaculture zoning, in which only areas suitable for aquaculture based on based on factors such as environmental suitability, infrastructure availability, socio-economic considerations, and ecological sustainability are considered for farming introduced species (Aguilar-Manjarrez et al., 2017).

Despite the observed low genetic diversity in *C. gariepinus* (as discussed in Chapter 5), there is hope for the species' conservation since there is no clear evidence of introgression between farmed and wild populations. Additionally, the use of mtDNA COI, which successfully identified wild albino populations as coming (and presumably escaped) from a farmed strain, presents a cost-effective genetic approach for identifying unique haplotypes that should be prioritised for conservation in both farmed and wild populations. The consistency of results between mtDNA COI and 3RAD further supports their potential utility in conservation efforts for this species. Maintaining genetic diversity in both farmed and wild populations is essential for long-term sustainability and resilience to environmental changes and diseases (Frankham, 1996; Rivera-Ortíz et al., 2015; Taylor et al., 2003; Whiteley et al., 2010).

6.4 Limiting factors affecting fish conservation genetic research in Nigeria

Genetic research on important commercial fish species in Nigeria has faced several limitations:

1. Lack of comprehensive sampling: Genetic studies require extensive sampling across different geographic regions and habitats to capture the full genetic diversity of the species. Limited sampling, especially in remote or inaccessible areas, may result in biased or incomplete representation of populations. The inability to sample some important fishing rivers in the Northern region of Nigeria due to the increasing political insecurity, threat of kidnapping and banditry that has led to the

loss of several lives including those of researchers is a major threat to fisheries research in the country.

2. Data quality and availability: Access to high-quality genetic data, including DNA sequences, is essential for robust analyses. However, limitations in local access to laboratory facilities, equipment, and expertise may hinder the generation of reliable genetic data in less well-developed countries.

3. Funding constraints: Genetic research often requires substantial financial resources to cover fieldwork, laboratory analyses, sequencing and computational resources for data analysis and storage. Limited funding opportunities and competing research priorities have restricted the scope and scale of genetic studies in Nigeria.

4. Infrastructure challenges: Research institutions in Nigeria are faced with inadequate infrastructure support, including limited access to reliable electricity supplies, internet connectivity, and research facilities. This lack of infrastructure has constrained opportunities for research, sample storage, data management, and communication with collaborators or research partners.

5. Limited collaborative networks: Collaborative partnerships between researchers, institutions, and stakeholders are essential for conducting comprehensive genetic research. However, limited collaboration and networking opportunities both within and outside Nigeria have hindered knowledge exchange, data sharing, and interdisciplinary research efforts.

6. Human resource constraints: Genetic research requires skilled personnel with expertise in molecular biology, bioinformatics, and population genetics. Shortages of trained researchers, technicians, and bioinformaticians within Nigeria have limited the capacity to conduct genetic studies.

7. Limited interest in fisheries research is also one of the major reasons why the level of funding has not been as high as that in other areas of applied biological research, such as plant science to ensure food security.

Addressing these limitations will require concerted efforts from researchers, policymakers, funding agencies, and other stakeholders to prioritize genetic

research, invest in infrastructure and capacity building, foster collaboration, and streamline regulatory processes. By overcoming these challenges, genetic research on tilapia species and catfish in Nigeria can contribute valuable insights that can aid fisheries management, conservation, and aquaculture development.

6.5 Directions for future research

Inevitably this project has revealed a number of areas where further research is needed. One such is a more detailed investigation of the extent of hybridisation between farmed and wild populations of tilapia as well as of the long-term genetic consequences of escaped farmed fish on wild populations. This should include assessing changes in genetic diversity and population structure over time. In my study I was only able to visit the sites in a single season so I have only a snapshot of genetic variation at a single timepoint. There is a need to examine the short- and long-term effectiveness of different management strategies, such as aquaculture zoning and genetic monitoring, in mitigating the genetic impacts of aquaculture on wild fish populations. Given the rate of development of genomic tools, such as whole-genome sequencing and genomic selection, there is still much scope for exploring their potential to improve the genetic management of farmed and wild fish populations, which would be beneficial for both the fisheries and aquaculture sectors in Nigeria. Finally, effective aquaculture policy development and implementation will require extensive collaborations between stakeholders, including government agencies, fish farmers, and conservation organisations, so as to develop integrated management plans that balance the needs of aquaculture production with the conservation of wild fish population.

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