UNIVERSIDADE TÉCNICA DO ATLÂNTICO
INSTITUTO DE ENGENHARIA E CIÊNCIAS DO MAR

WEST AFRICAN SCIENCE SERVICE CENTRE ON CLIMATE CHANGE AND ADAPTED LAND USE

Master Thesis

MORPHOLOGICAL AND GENETIC CHARACTERISATION OF MANGO TILAPIA, *Sarotherodon galilaeus* (LINNAEUS, 1758) FROM BAFING AND BAKOYE RIVERS IN MALI

DJIBRIL KONATE

Master Research Program on Climate Change and Marine Sciences

São Vicente
2022

UNIVERSIDADE TÉCNICA DO ATLÂNTICO
INSTITUTO DE ENGENHARIA E CIÊNCIAS DO MAR

WEST AFRICAN SCIENCE SERVICE CENTRE ON CLIMATE CHANGE AND ADAPTED LAND USE

Master Thesis

MORPHOLOGICAL AND GENETIC CHARACTERISATION OF MANGO TILAPIA, *Sarotherodon galilaeus* (LINNAEUS, 1758) FROM BAFING AND BAKOYE RIVERS IN MALI

DJIBRIL KONATE

Master Research Program on Climate Change and Marine Sciences

Supervisor | Dr. Heino Fock
Co-supervisor | Dr. Reinhold Hanel & Dr. Evandro P. Lopes

São Vicente
2022

UNIVERSIDADE TÉCNICA DO ATLÂNTICO
INSTITUTO DE ENGENHARIA E CIÊNCIAS DO MAR

WEST AFRICAN SCIENCE SERVICE CENTRE ON CLIMATE CHANGE AND ADAPTED LAND USE

Morphological and Genetic characterisation of Mango tilapia, *Sarotherodon galilaeus* (Linnaeus, 1758) from Bafing and Bakoye rivers in Mali

Djibril Konate

Master's thesis presented to obtain the master's degree in Climate Change and Marine Sciences, by the Institute of Engineering and Marine Sciences, Atlantic Technical University in the framework of the West African Science Service Centre on Climate Change and Adapted Land Use

Supervisor

Dr. Heino Fock
Thünen Institute

Co-supervisors

Prof. Dr. Reinhold Hanel
Thünen Institute

Dr. Evandro P. Lopes
ISECMAR-UTA

São Vicente
2022

UNIVERSIDADE TÉCNICA DO ATLÂNTICO
INSTITUTO DE ENGENHARIA E CIÊNCIAS DO MAR

WEST AFRICAN SCIENCE SERVICE CENTRE ON CLIMATE CHANGE AND ADAPTED LAND USE

Morphological and Genetic characterisation of Tilapia Mango, *Sarotherodon galilaeus* (Linnaeus, 1758) from Bafing and Bakoye rivers in Mali

Djibril Konate

Panel defense

President

_____

Examiner 1

_____

Examiner 2

_____

São Vicente
2022

Financial support

The German Federal Ministry of Education and Research (BMBF) in the framework of the West African Science Service Centre on Climate Change and Adapted Land Use (WASCAL) through the WASCAL Graduate Studies Program in Climate Change and Marine Sciences at the Institute for Engineering and Marine Sciences, Atlantic Technical University, Cabo Verde.

Dedication

Life is just a flash, and a successful day is beautiful.

I dedicate this thesis:

To my dear father Madala and my dear mother Sadio Dia

for the education and great love, which they surrounded me with since my birth.

To my dear brothers, sisters, and friends.

Acknowledgements

This Master thesis has been the biggest challenge of my life so far, I thank above all the good God and his prophet Muhamad peace be upon him, who gave me the strength, the patience, and the courage to accomplish this work, and huge of gratitude to many people who have provided guidance, support, and motivation.

I want to express my gratitude to my principal supervisor Dr. Heino Fock senior researcher at the Thünen - Institute of Sea Fisheries, for accepting me as a Master's Student under his scholastic supervision. Many thanks for your support, requirement, and availability and for communicating his passion for this research to me. Words cannot convey my gratitude and most profound appreciation to you.

I feel short of word to express my profound gratitude to Prof. Dr. Reinhold Hanel, head of the Thünen-Institute of Fisheries Ecology and Dr. Evandro P. Lopes, Lecturer at the Institute of Engineering and Marine Sciences PhD in Biodiversity, as my co-supervisors. My sincere, most incredible gratitude to my supervisors for their precious help, answers and suggestions for this thesis, especially the genetic part and time they gave to this work through their availability, patience and communicative enthusiasm.

I would like to thank Dr. Corrine Almeida, Dr. António Pinto, Dr. Estanislau Lima, Ms. Lysine Silva and all the staff at Wascal Cabo Verde for giving me this opportunity and supporting me so diligently to completion. Thanks for your kind encouragement, help and guidance throughout this programme and the writing of the thesis.

I acknowledge the Ministry of Education and Research (BMBF) for founding this great programme. And huge thanks to Universidade Técnica do Atlântico (UTA) – Cabo Verde for hosting this programme.

My special thanks are grateful to my English teachers at Myrtle School Mindelo for supporting and educating me during this programme, which helped me a lot in writing my thesis.

My sincere thanks go to Nouhoum BERTHE Director of Department National Fish Mali Bekaye TANGARA, head of the Fisheries and Aquaculture Division of Department National Fish, for their help in getting permission and responses to carry out this research on the Bafing and Bakoye Rivers in Mali and answering on the field. And Sidiki DRAME, the head of the Manantali fisheries sector, and Karim DEMBELE, the director of the Bafoulabé fisheries

antenna, many thank for the hospitality, accommodation, company and invaluable help in finding fish among the fishermen.

Mohamed MARIKO, graduate in Fisheries and Aquaculture at IPR/IFRA of Katibougou, I would like to thank you for your support, help and effort in collecting data for this thesis.

Without distinction, thank all the lecturers of this program for the quality of the education received during these years.

I would like to express my sincere gratitude to Dr. Henrike Andersen, research associate at the Thünen-Institute of Marine Ecology, and his family for their hospitality and support during my workshop in Bremerhaven, my supervisor.

Finally, thanks to my colleagues for their help, moral support and late-night feedback sessions, who influenced and inspired me.

Resumo

O estudo visava comparar a caracterização Morfológica e Genética da tilápia manga, Sarotherodon galilaeus (Linnaeus, 1758) dos rios Bafing e Bakoye no Mali. Cento e vinte e nove exemplares foram recolhidos dos rios Bafing e Bakoye.

De cada espécime de 18 medidas morfométricas, foram registadas contagens de 5 medidaserísticas. Para a genética molecular, amostras de tecido de brânquias e músculos foram colhidas de cada espécime e conservadas em álcool absoluto para estudos moleculares. Os índices de proporção, morfométrica, merística, comprimento na primeira fase de maturação, relação comprimento/peso e índices D-loop mtDNA foram utilizados para estudar e comparar semelhanças e diferenças entre Sarotherodon galilaeus colhidos do Bafing e Bakoye. Além disso, o teste Wilcoxon rank sum e o teste T foram utilizados para determinar se existiam diferenças morfológicas e merísticas significativas. As diferentes fases das gónadas foram determinadas pela inspecção visual da aparência adaptada por Marcano et al. (2007) para resolver o Lm50. O resultado da relação comprimento-peso indicava uma ligeira variação nos pesos a um determinado tamanho, referidos como resíduos. Além disso, os valores R-quadrados foram de 0,90 para Bafing e 0,88 para Bakoye. O modelo é significativo para ambos os rios com valores alométricos < 3. O Lm50 era de 16,99 cm para as fêmeas e 15,09 cm para os machos para Bafing e 11,37 cm para as fêmeas e 16,33 cm para os machos para Bakoye. As observações sobre a distribuição da frequência do número total de S. galilaeus mostraram frequências mais elevadas de peixes entre 11 e 17 cm TL para ambos os rios. As medidas morfométricas de três partes do corpo (BD, CPD, CPL) moldam as três partes da cabeça (HL, CHD, LAD) mostraram uma diferença significativa, e as contagens merísticas mostram uma diferença considerável entre DF e LONG. A análise genética de 311 amostras da localização de Bafing, Bakoye, quatro de Israel, e três do Gana revelou variações no D-loop do mtDNA. As populações ganesas e israelitas apresentaram o menor número de haplótipos e os menores valores de diferenças de nucleótipos e diversidade de haplótipos, e a maior diversidade de haplótipos e nucleótipos foram encontrados no Mali. Este estudo mostra que existem diferenças significativas entre os rios a nível genético e morfológico, indicando uma diferenciação populacional.

Palavras-chave: *Sarotherodon galilaeus*, morfométricas, merísticas, Rios Bafing e Bakoye.

Abstract

The study aimed to compare the Morphological and Genetic characterisation of Mango tilapia, *Sarotherodon galilaeus* (Linnaeus, 1758) from Bafing and Bakoye rivers in Mali. One hundred twenty nine specimen were collected form Bafing and Bakoye rivers.

From each specimen 18-morphometric measurements, 5-meristic counts were recorded. For molecular genetics, tissue samples from gills and muscles were from each specimens and preserved in absolute alcohol for molecular studies. The ratio indices, morphometric, meristic, length at the first maturity stage, length weight relationship and D-loop mtDNA indices were used to study and compare similarities and differences between *Sarotherodon galilaeus* collected from the Bafing and Bakoye. Further, the Wilcoxon rank sum test and the T-test were used to perform whether there were significant morphological and meristic differences. The different stages of the gonads were determined by visual inspection of the appearance adapted by Marcano et al. (2007) to resolute the Lm50. The result of the length-weight relationship indicated a slight variation in the weights at a particular size, referred to as residuals. Further, the R-squared values were 0.90 for Bafing and 0.88 for Bakoye. The model is significant for both rivers with allometric values < 3. The Lm50 was 16.99 cm for females and 15.09 cm for males for Bafing and 11.37 cm for females and 16.33 cm for males for Bakoye. The observations on the frequency distribution of the total number of *S. galilaeus* showed higher frequencies of fish between 11 and 17 cm TL for both rivers. The morphometric measures of three parts of the body (BD, CPD, CPL) shape the three parts of the head (HL, CHD, LAD) showed a significant difference, and the meristic counts show a considerable difference between DF and LONG. Genetic analysis of 311 samples from the location of Bafing, Bakoye, four from Israel, and three from Ghana revealed variations in the D-loop of the mtDNA. The Ghanaian and Israeli populations showed the lowest number of haplotypes and the lowest values of nucleotide differences and haplotype diversity, and the highest haplotype diversity and nucleotide were found in Mali. This study shows that significant differences between the rivers exist on a genetic and a morphological level, indicating population differentiation.

Keywords: *Sarotherodon galilaeus*, morphometric measures, meristic counts, genetic structure, Bafing and Bakoye Rivers.

Abbreviations and acronyms

BMBF	German Federal Ministry of Education and Research
DNA	Deoxyribonucleic Acid
DnaSP5	Sequence polymorphism Deoxyribonucleic acid
GB	GenBank
Hd	Haplotype Diversity
ISECMAR	Institute of Marine Science and Engineering
LWR	Length/Weight Relationship
mtDNA	Mitochondrial Deoxyribonucleic Acid
OMVS	Senegal River Basin Development Authority
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic
UTA	Universidade Técnica do Atlântico
WASCAL	West African Science Service Centre on Climate Change and Adapted Land Use

General Index

Figure Index

Table Index

1. Introduction

1.1 Background and Context

Cichlid (family *Cichlidae*) fish have become an important model for studying evolutionary biology, particularly speciation and adaptive radiation (Burress 2014). Cichlids constitute one of the biggest freshwater fish lineages (McMahan et al. 2013), having 1500 species from 220 genera (Turner 2007). Nonetheless, although many more cichlids have yet to be properly described, this continues to be regarded an underestimation. There are several hypotheses regarding the causes of rapid evolution in cichlids, including speciation due to exploitation of novel habitats (Hulsey et al. 2010), hybridisation between existing lake specialists (Seehausen 2004; Genner & Turner 2012), multiple colonial expansion of riverine species (Loh et al. 2013; Tyers, 2013), and responsive radiation observed in African haplochromine cichlids.

In line with the considerable economic value of tilapia species for aquaculture and fisheries, these fish play a crucial role in ecosystems in tropical rivers and water bodies where they occurred naturally or were introduced (R. H. Lowe-McConnell, 2000). Thus, introducing tilapia species in the same fragile ecosystem without proper planning and cultivation is likely to affect the environment negatively (Canonico et al., 2005). Therefore, the introduction of tilapia species into the same fragile ecosystem without proper planning and culture is likely to affect the environment negatively (El-Sayed, 2020)(El-Sayed, 2020; Cucherousset, 2012 and Alves, 2007).

Fish and fish products play a hugely important role in the nutritional picture because they are a rich source of nutrients, provide a good balance of protein, vitamins, and minerals, and have relatively low caloric content (Abdel-Hamid et al., 2014). In addition, they are also excellent sources of polyunsaturated fatty acids such as omega-3 fatty acids, which appear to have significant effects in reducing the risk of cardiovascular diseases. And are linked with positive benefits in many other pathological conditions, mainly certain types of cancer and arthritis (Pal et al., 2018). The fish has a good taste with a moderate flavor, is widely recognized as a food fish, is used in many cuisines, and its intake is not banned by religious observers. It is also a highly plentiful and commercially significant fish in Nigeria's natural and artificial lakes (Omotayo et al., 2016).

The taxonomic classification of tilapia species or tilapia stocks has not been easy, often relying on differences in reproductive behaviour and discrimination between species at the generic or subgeneric level. However, Fish taxonomy is concerned with describing fish species and placing each species within a taxon system that shows its relationships to other species. For more than a century, systematics relied on external and internal morphology to define and organise these species into genera. Recently, these groupings have been confirmed or altered when examined with molecular approaches (Omer et al., 2020). Therefore, tilapia is the common name for over 70 tropical African cichlid fish species. They inhabit various freshwater habitats, including shallow streams, ponds, rivers, and lakes (El-Sayed, 2020).

The morphometric characteristic and meristic traits analyses are parts of rigorous critical tools used to differentiate closely related species of an organism having massive similarity indices of various parameters (Oladimeji et al., 2020). They are also used to identify fish stocks and to determine the evolutionary linkages between fish fauna (Deesri et al., 2009). In addition, a Length-weight relationship is an essential tool in fish biology to distinguish units of a population, given that feeding opportunities are different in different habitats (Lalèyè, 2006). These studies are used for fish ecology and conservation in several parts of the world, providing information on the condition, growth pattern, ontogenic changes, and fish population dynamics (Alam et al., 2013).

1.2 Problem Statement

The main obstacle to the scientific management of fish stocks in West Africa is the lack of reliable data on target stocks that is especially true for inland fisheries. Due to the low availability of stock and population data and the limited number of regional fisheries experts, government institutions and researchers primarily rely on simple catch statistics and empirical models to estimate fish production and potential yields (FAO, 2015)

The purpose is to figure out the difference between local populations of *Sarotherodon galilaeus* from the Bakoye and Baking rivers, two major tributaries to the Senegal river in Mali, by using morphometric, meristic and genetic data. Genetic markers offer significant advantages by allowing the direct assessment of genetic diversity. In addition, adequate characterisation of autochthonous wild strains is a prerequisite for better managing aquatic genetic resources and developing targeted conservation programs.

The information collected from Fishermen along both rivers showed that the cichlid *Sarotherodon galilaeus* (Linnaeus, 1758) was the most abundant species in the fish catch in terms of numbers and weight. This species is usually harvested by local people using gillnets, pots, and seines and is a valuable food source. Considering the economic importance of *Sarotherodon galilaeus*, numerous studies have been undertaken on the biology and demography of this species in Africa. The present research will quantify a series of population traits for *S. galilaeus*, including size at first maturity, external morphology, and genetics. This study will provide an excellent comparative framework for the *Sarotherodon galilaeus* from Bafing and Bakoye environments.

1.3 Research Questions

To contribute to morphometric, meristic, and genetic knowledge of the Mango tilapia specie *Sarotherodon galilaeus* from the two rivers?

Following are the main questions that describe the driving motivations of this thesis.

- Is there a difference in size at maturity between the two rivers?
- Is there a difference in length and weight relationships between the two rivers?
- What are the distinctions in morphological and meristic characteristics, genetic markers, and condition indices that might suggest the presence of a local subspecies?

1.4 Relevance and Importance of the Research

Relying on the result of the research, we look forward to contributing to the scientific analysis of morphometric, meristic and genetics of the species *Sarotherodon galilaeus*, which is economically significant in the two regions.

As well as seven subspecies of *Oreochromis niloticus*: *Oreochromis niloticus* from West Africa and the Nile, *Oreochromis niloticus eduardianus* from Lakes George, Edward, and Tanganyika, *Oreochromis niloticus cancellations* from the Awash. (Seyoum & Kornfield, 1992) described a new subspecies, *Oreochromis niloticus tana* from Lake Tana in Ethiopia, using genetic mitochondrial deoxyribonucleic acid (mtDNA) characteristics. In addition, the genetic markers provide significant benefits by directly evaluating gene variety and population variability in line with morphological and meristic assessments. They are effective instruments for recognising the genetic uniqueness of individual species or subspecies.

1.5 Objectives of the work

The main objective is to investigate a potential population structure of Mango tilapia *Sarotherdon galilaeus* sampled in different locations of the two rivers, Bafing and Bakoye in Mali, using morphometric characteristics and mitochondrial genetic markers. Specifically, this study aims to:

- Compare Mango tilapia *Sarotherdon galilaeus* from Bafing river and Bakoye river employing a set of morphometric characters as well as sex ratio and size at maturity;
- Compare the length/weight relationship (LWR) of Mango tilapia *Sarotherdon galilaeus* in Bafing and Bakoye rivers with their maturity stages;
- Genetic markers assess population differences of Mango tilapia *Sarotherdon galilaeus* from the Bafing and Bakoye rivers.

1.6 Structure of the work

This thesis is grouped into six sections: section 1 presents the introduction. Section 2 highlights relevant literature to situate future research within the extant theoretical paradigms and emphasizes areas where further study in *Sarotherdon galilaeus* from Bafing and Bakoye rivers is needed. Section 3 explains and justifies the research methodology, describing the data collection methods with a particular focus on the scope and the parameters used. Section 4 presents the results of this study's statistical analysis and genetic analysis. In section 5, discuss the research findings, followed by the limitations of this study and avenues for future research, and followed by a conclusion and recommendation in section 6.

2. Literature Review

Cichlid fish (Cichlidae) has become a popular model for researching evolutionary biology, particularly speciation and adaptive radiation (Burress, 2015). The Family of Cichlidae includes at least 1500 scientifically described species, and the taxonomic status of at least 600 species remains undecided, making Cichlids the third largest fish family (Ebraheem, 2012). The classification of species has been primarily based on variation in dentition, bone, structure, pigmentation, squamation characteristics and general body morphology (Trewavas, 1983). Galman (1988) used morphological description and analysis of morphometric measurements and meristic counts to differentiate tilapia species.

Tilapia is the commonly used term for three genera and species of fish in the Cichlidae family: *Oreochromis, Sarotherodon, and Tilapia*. These species are native to Africa and the Middle East but have spread worldwide (Watanabe et al., 2002). The term "tilapia" comes from the African Bushman word for "fish" (Trewavas, 1982). Smith first described the genus Tilapia in 1840. Based on breeding behaviour and feeding habits, it was split into two subgenera: Tilapia substrate spawners and *Sarotherodon* brush-toothed or mouth-brooders. Mouth brooders incubate fertilised eggs and newly born fry in the mouths of the male, female, or both parents. Later, the subgenus *Sarotherodon* was elevated to a genus and further divided into two genera: *Oreochromis* mountain cichlids and *Sarotherodon,* depending on whether the mouthbrooding is conducted by the female *Oreochromis,* the male *Sarotherodon,* or both parental sexes of *Sarotherodon* (Smith, 1840).

The initial classification consists of five genera: *Tilapia*, *Sarotherodon*, *Oreochromis, Tristromella*, and *Danakilia*. Seven species belong to these five genera: *Hemichromis fasciatus, Hemichromis bimaculatus, Haplochromis strigigena, Haplochromisdes fontainesi, Oreochromis niloticus, Sarotherdon galilaeus,* and *Tilapia Zilli*. Later classifications have just one genus, with seven subgenera: *Heterotilapia*, *Pelmatilapia*, *Sarotherodon*, *Oreochromis*, *Nyasalapia*, *Alcolapia*, and *Neotilapia* have been described, but there is significant discussion as to whether these species are different. The taxonomic classification of Tilapia is still ambiguous and susceptible to frequent revisions. It is mainly due to the overlap and similarity of their morphological characteristics, as well as the fact that several species of Tilapia freely hybridise in nature (Froese & Pauly, 2018).

Tilapias are the generic name of a group of cichlids endemic to Africa. The group consists of three important genera in aquaculture, *Oreochromis, Sarotherodon,* and *Tilapia*. Many

characteristics distinguish these three genera, but possibly the most critical relates to reproductive behaviour. Both female and male *Sarotherodon* and only female *Oreochromis* are mouth brooders (Sturmbauer, 1998; Trewavas, 1983). In *Oreochromis* spp., the male excavates a nest at the pond bottom (generally in water shallower than 1m) and males with many females. After a short mating ritual, the female spawns in the nest; the male fertilises the eggs, and the female broods them. Fry remain in the female's mouth through yolk sac absorption and often seek refuge in her mouth for many days after they begin to feed (Popma & Masser, 1999). They are known to reach sexual maturity at a small size, about 8-10 cm body length, and at a young age (sometimes 2-3 months). Adult fish are known to live six to eight years, but some up to 11 or 12 years of age have been reported (F.J. et al., 2019). They can survive in dissolved oxygen less than 0.3 mg/L but grow better at 0.7 to 0.8 mg/L, with a pH range of 6 to 9 (Dampin et al., 2012).

Sarotherodon galilaeus is native to Africa and the Middle East, although it is now widely distributed in Europe and some regions of the United States (GBIF Secretariat 2018). It has been brought to various Asian countries for aquaculture reasons, including Japan and China Luo, C., Yang, P., & Wang, S. (2021). The body is usually pale in colour, with fins that are uniform or inconspicuously marked except for the pink caudal border (Fishbase.se). *Sarotherodon galilaeus* has 14-17 dorsal spines, 13 dorsal soft rays, three anal spines, and 9-12 anal soft rays. As a deep-bodied species' depth is usually 43-56% standard length (SL), rarely as low as 38% (Base, 2004). It has been brought to various Asian countries for aquaculture reasons, including Japan and China Luo, C., Yang, P., & Wang, S. (2021).

Temperature is a significant factor in determining the properties of the water necessary for growing Tilapia throughout its various development phases. The average temperature (water) range for tilapias is 20 to 30°C. But, they can also withstand lower temperatures (Wohlfarth & Hulata, 1983). Since *Sarotherdon galilaeus* can survive the winter temperatures of Egypt and the Mediterranean rivers of Israel, it must be more cold-resistant than *O. mossambicus*, but its confinement to shallow water during the winter likely slowed its growth under the described conditions (Jensen, 1958). When the snow waters from Mount Hermon flowed into the lake, it was reported that there was a high mortality rate (Bertram, 1942; Bodenheimer, 1935). Ricardo-Bertram reported that the water temperature was 9°C. Although most Tilapia are freshwater species, their exceptional adaptability to varying salinities is known (Stickney, 1986). In addition, before the spawning season in Lake Tiberias, *S. galilaeus* is present in large schools whole the lake. The schools separate in March when couples come to

the shores to look for the spawning places where the sand or the gravels and plants are abundant (Ben-Tuvia, 1958). Wohlfarth and Hulata, (1983) report that, in the Lakes of Egypt, the species *S. galilaeus*, *S. niloticus* and *T. zillii* were found at salinities between 13.5‰ and 22.4‰ and the maximum reproductive salinity for *Sarotherodon aureus* is 19‰, but it may be adapted to grow in salinities between 36‰ and 45‰.

Sarotherodon galilaeus exhibited a variety of reproductive activities. Including mouth brooding, nest guarding, and parental care. It reach sexual maturity (Lm50) at a total Length (TL) of 131 mm and 106 mm for males and females, respectively, in Lake Amazon. (Olele, N.F, 2010) the maturity Ogive revealed that the male specimens matured at a total length of 98.00 mm while the females matured at a total length of 89.00 mm. Reproduction is rapid in this species. The breeding season in Lake Tiberias extends from the end of March or the beginning of April to August. During this time, a female produces two or more broods. The participation of sexes males and females in parental care was first reported by Liebmann (1933).

Sarotherodon galilaeus is an omnivore and verified this by the varieties of food items found in its gut. However, the importance of plant food (algae, diatoms, and macrophyte fragments) in its diet was earlier demonstrated. However, according to a previous study, while *S. galilaeus* had a diverse diet, its stomach mostly contained phytoplankton, chironomids, and insect larvae (Amisah & Agbo, 2008). In addition, (Fagade, 1971) confirmed that *Sarotherdon galilaeus* is a phytoplanktophagous species that feeds various algae, desmids, and diatoms.

Sarotherodon galilaeus is an abundant and economic species in the Bafing and Bakoye rivers during flood periods. Because of the lack of data on the morphological characterisation and genetics of *Sarotherodon galilaeus* wild populations. There is a need to investigate whether this species has morphotypes. However, this study was conducted in locations where a large and indiscriminate harvest of this fish occurs daily. This input will help our understanding of this fish species taxonomy, facilitating its effective management, sustainable production, and conservation.

In addition, *Sarotherodon galilaeus* consists of several subspecies also; thus, proper identification is required. For instance, the samples of subspecies belonging to the family of Cichlidae were obtained from Niger river (upper and middle), Senegal river, lake Chad, and the Gambia River identified the *Sarotherodon galilaeus galilaeus, Sarotherodon galilaeus multifasciatus, Sarotherodon galilaeus borkuanus, Sarotherodon galilaeus sanagaensis,*

Sarotherodon galilaeus boulengeri the map and the description show the different variations (Trewavas, 1983)(Trewavas, 1983)(Trewavas, 1983).

In considering other tilapia species, *Oreochromis niloticus* is very similar to *Sarotherodon galilaeu*s has a mouth smaller than that of *Oreochromis niloticus*, not extending beyond the nostrils, gill rakers on the smaller part of the first-gill arch number 21-26 and very small, numerous densely crowded teeth on the lower pharyngeal bone. Dorsal fins consist of 16-17 spines and 12-14 rays, and anal fins with three spines and 10-12 rays. The pectoral fin is as in *O. niloticus* but more extended, and the caudal fin is truncated or emarginated. The caudal peduncle is short, and deep *Sarotherodon galilaeus* has 2-3 rows of scales below the eyes (Abu Gideiri, 1984; Hassan, H., & Mahmoud, Z. 2021). The colour is yellowish to brownish or olive-green, uniform or with small dark spots, or with ill-defined darker streaks along the series of scale, more or less distinct dark opercula spot and, pectoral fins greenish or brown, without markings in adult. Young fish with 5-9 dark bars on the body and oblique dark streaks on the dorsal and anal fins. Both parents stay close to each other. Eggs and fry brooded in the oral cavity up until they were ready for release. Fry are between 7-9 mm at first feeding with well-developed fins for swimming (Ahmed & Ebraheem, 2012).

Length frequency distribution of the data and population dynamics are tools to identify and predict species at greater risk of being endangered and to avoid small catches. (Onimisi & Oniye, 2010) The length-weight relationship is known primarily to measure its growth pattern, an essential component of biological production. The range of sizes of *S. galilaeus* from the Ogun coastal estuary measured 11 to 41 cm. (Ebenezer, 2010) reported a size range of 7.0 to 33.3 cm for *S. galilaeus* in Weija reservoir, Ghana. (Abdul, 2015) also reported a size range of 22-34 cm for *S. galilaeus* from the same water body when investigating the impact of Iken Brush Park fishing practice on the species.

Morphometric, meristic and molecular data are collected from Bafing and Bakoye. This thesis will investigate characteristics such as the length-weight relationship, the size-at-maturity (Lm50), morphometric measures and meristic comparison, and the molecular study using the D-loop control region of the mitochondrial DNA (mtDNA). A length-weight relationship is an essential tool in fish biology, physiology, ecology, fisheries assessment, and fish conservation. It is also used for fish conservation in several parts of the world, providing information on the condition, growth pattern, ontogenic changes and fish population dynamics (Alam et al., 2013).

It is also helpful for converting growth-in-length equations to growth-in-weight for stock assessment models and estimating stock biomass from limited sample sizes (Kullander, 2003).

The length at first maturity (Lm50), defined the size of fish at which half the number of the species attain sexual maturity, represents an essential parameter in fish stock management and exploitation. Size and age at maturity stages influence population model estimates of sustainable harvest rates (Kendall et al., 2014). They are also used in predicting the risk of overexploitation of stocks (Reynolds et al., 2005).

In fishery and aquaculture, a higher Lm50 is desirable so that a suitable size for the market still is obtained from breeding fish. If the size at first maturity is small, then spawners will not grow to reach a good size for the market since energy is allocated to reproduction at the expense of somatic growth (Quince et al., 2008). Furthermore, since sexual maturation influences physiological and behavioural changes in a fish, size at first maturity is essential in inferring information on fish growth, maximum size and longevity (Lederoun et al., 2016). Various methods are used to estimate the Lm50: linear interpolation, fitting of a logistic curve, or estimation from a plot of the percentage of mature fish samples over Length (Fontoura et al., 2009).

Morphometric measurements and meristic counts have been successfully applied in defining species (Abu Gideiri, 1984; Dampin et al., 2012; Fagbuaro et al., 2015; Moushomi & Saha, 2015; Oladimeji et al., 2020; Hassan, H., & Mahmoud, Z. 2021); Akindele & Fagbuaro, 2022). It is rare to find studies that present a hypothesis of relationship or taxonomic status that ignores the similarity and dissimilarity of these characters (Stepien & Kocher, 1997). For descriptive purposes, all measurements except standard length are expressed as ratios of a reference length. For instance, body measurements and the head length are expressed as a percentage of standard length as ratio indices (Sturmbauer, 1998).

Molecular techniques have characterised and identified Tilapiines species (Seyoum & Kornfield, 1992). However, most or all of these characters overlap and may fail to identify species or subspecies unambiguously due to inter-population variation and slight differences among species (Seyoum & Kornfield, 1992)(Seyoum & Kornfield, 1992)(Seyoum & Kornfield, 1992). Apart from its straight forward structure, devoid of complex introns that otherwise interrupt genes but do not code for amino acids, mtDNA is abundant, usually 500-1,000 copies per cell as one linkage group compared to only two copies of nuclear DNA (Giddelo et al.,

2002). A variation in DNA markers has been reported for tilapia species identification, including the mtDNA control region (D-loop) (Yang et al., 2020). The D-loop sequences are non-coding regions of mtDNA, with a high rate of evolution and no recombination, which have become one of the most commonly used mtDNA sequences for addressing the evolutionary relationship of close relatives and subspecies (Murgia et al., 2002). The D-loop sequences have been widely used in genetic analyses for culture species, primarily related to the genetic structure (Jiang et al., 2019). It has a high rate of evolutionary change. Therefore D-loop control region of the mtDNA has become a helpful marker in studying rapid evolutionary trends in species or populations, taxonomy (Agnèse et al., 1997), and genetic structure and biogeography (Giddelo et al., 2002).

3. Materials and Methods

3.1 Study Area

This section aims to characterise the habitat where *S. galilaeus* was collected (Bafing and Bakoye rivers Mali). They join to form the Senegal river in the town of Bafoulabé in western Mali (Figure 1). The Senegal river is the second largest waterway in West Africa after the Niger river, with a length of 1800 km and a catchment area of 300,000 km^2 in Mali. In Bafoulabé, downstream from Manantali, the Bafing river joins the Bakoye river, which originates in Mont's Ménien in Guinea at an altitude of 760 m. After entering the Bafing, the Bakoye receives the Baoulé on the north side. The Senegal river receives the Kolimbiné, then the Karokoro on the right and the Falémé on the left, 50 km upstream from Bakel. The Falémé's source is in the northern part of the Fouta Djallon in Guinea, at 800 m in altitude. At Bakel, the mean annual volume of the Senegal river's flow is 22 billion m^3. The basin has a roughly ovoid shape with a significant axis-oriented SW-NE. It extends from latitude 10°20' N to latitude 17°00' N and lies between meridians 7° W and 12°20' W (OMVS Rapport, 2018).

Bafing river has its source in the "Fouta-Djallon" massif, at about 800 m of altitude, a source that is located about fifteen km northwest of Mamou, in Guinean territory. Its upper course is tormented and flows between granitic and dolerite massifs, which force him to take very different directions. From the 600 m altitude mark at river 97 km, it brings to its confluence with the Bale, the first right bank tributary, and SW-NE direction. From the 600m line onwards to the Dakka-Saidou station at 377 km, it crosses, through a series of rapids, the dolerite zone located to the west of Dinguiraye. It then enters the sandstone plateau and crosses this area of low relief, describing the numerous meanders. In this part of its course and up to Dibia at 657 km, several small rapids are present due to sills formed by banks of infra-Cambrian sandstone. From 600 km onwards, it takes an E-W direction, and at Dibia, it takes an S-N direction until Bafoulabé, where it receives the Bakoye after 750 km. In addition, the hydroelectric dam at Manantali, constructed in 1987, is located 90 km upstream of Bafoulabé on the Bafing River. It generates Lake Manantali, Mali's largest artificial lake (OMVS Rapport, 2018).

Bakoye river has a catchment area of 85,600 km^2 and its source is at an altitude of 760 m in the Ménien Mountains 11°50 N - 9°40' W, northwest of Siguiri. It quickly reaches the 400 m mark after 14 km of crossing this granite region through a series of falls and rapids. From this point onwards, it passes through a flat, featureless region. It is an indictment of the Birimian shale and infra-Cambrian sandstone. The Bakoye then describes the river has many meanders:

the general direction of its course is S-N. On the right bank, it receives the Baoulé after 445 km before it flows into the Bafing 561 km, a little upstream of Bafoulabé. The river is seasonal, with a maximum flow in September and almost no between January and June (OMVS, 2018).

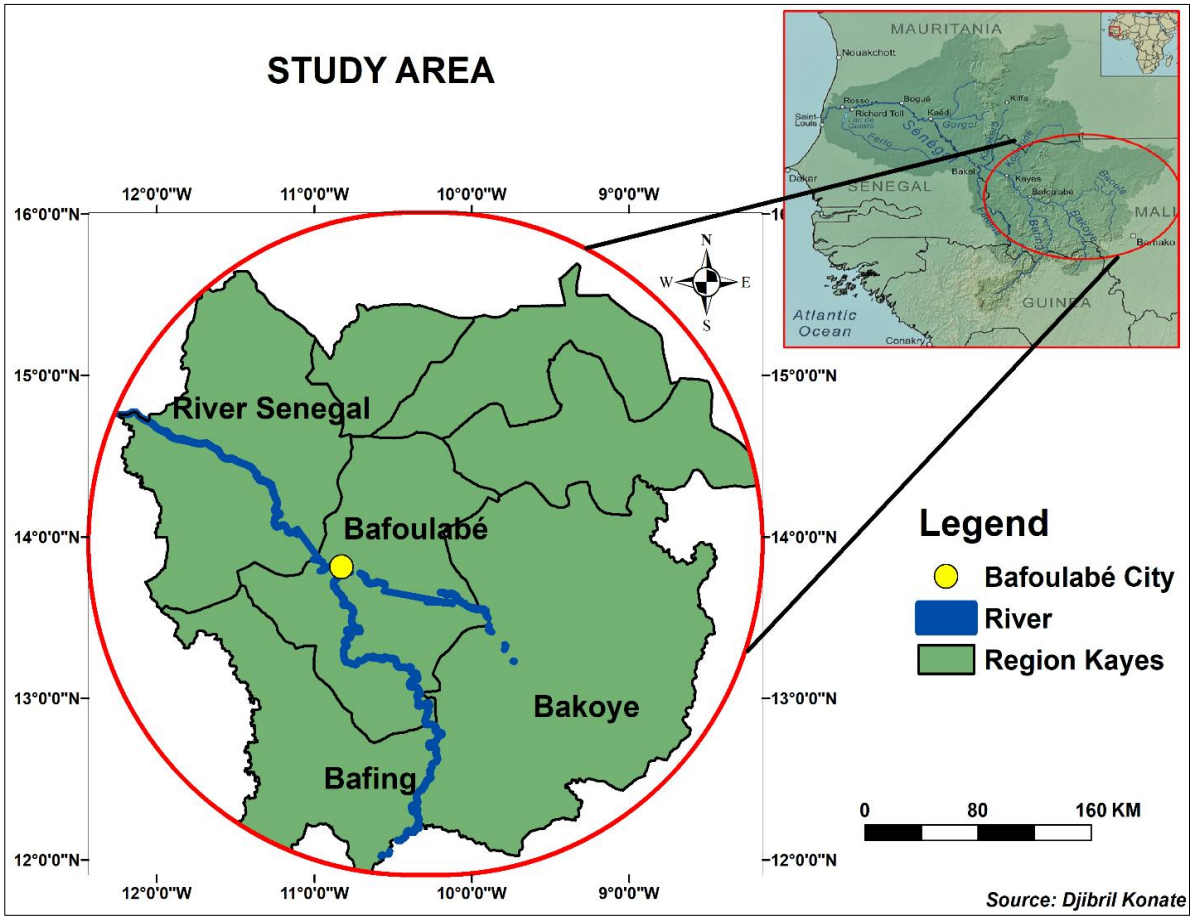

Figure 1: The Senegal River drainage basin, the study area of Bafing and Bakoye River

3.2 Data collection

The *Sarotherodon galilaeus* were randomly collected from each river, Bafing and Bakoye, totalling 139 samples. The fish samples were collected from landing sites by Fishermen at each study site and then brought to the Fish Department of this city. The fish samples were identified using a standard identification key prepared based on the methodology shown in FAO (1995). Twenty morphometric measurements and seven meristic parameters cited by Snoeks (1994) were recorded in 139 individuals using a digital Vernier calliper (INCGO). In addition, a piece of tissue was cut from each specimen and then preserved in tubes containing 96% alcohol. These tissues were used to carry out the genetic study at the ISECMAR-UTA laboratory. The characteristics of the different water bodies and sites are presented in Table 1.

Table 1: The characteristics of the sample areas. Legends: Water Body (WB); LS- Landing Sites (LS); Number of Specimens collected from each site

WB	LS	N	GPS Coordinates	
			Latitude	Longitude
Bafing river	Kéniékéniéko	08	13°14'10.34"N	10°24'31.21"W
	Manantali	19	13°12'14.71"N	10°25'46.06"W
	Mahina	17	13°13'27.61"N	10°28'16.46"W
	Bakouroufata	14	13°25'57.16"N	10°42'19.05"W
Bakoye river	Diabougou	16	13°47'06.71"N	10°46'16.03"W
	Diguila	18	13°43'35.47"N	10°39'31.84"W
	Niakalénessiraya	19	13°43'08.24"N	10°35'11.82"W
	Bodiarinko	28	13°26'37.23"N	10°26'36.23"W

3.3 Morphometric measurements

The morphometric data were taken from samples collected along the Rivers Bafing and Bakoye Following the protocol. The weight was taken with the balance, and a calliper was used to measure the level of each fish of the samples. The different morphometric parameters measured and their definitions are demonstrated below, and a schematic figure (Figure 5).

- **Total Length (TL):** the distance between the upper jaw's rostral point and the caudal fin's dorsal lobe (not showing in figure 5).
- **Standard Length (SL):** from the rostral tip of the upper jaw to the middle of the commencement of the caudal fin.
- **Body Depth (BD):** the greatest depth of the body in front of the pelvic fin, measured vertically from the base of the dorsal fin.
- **Head Length (HL):** the distance between the rostral tip of the upper jaw and the posterior point on the gill cover border.
- **Head Width (HW):** with the operculum in a ducted, typical position. Just above the dorsal extremities of the preopercular bones, these bony structures correspond to two well-defined spots on the skull.
- **Inter Orbital Width (IOW):** minimal width of the bony orbits' dorsal edge.

- **Snout Length (SNL):** the distance from the rostral tip of the upper jaw to the nasal point of the bony orbital border.
- **Lower Jaw Length (LJL):** distance between the rostral and ventrocaudal end of the lower jaw.
- **Premaxillary Pedicle Length (PPL):** from the point of the upper jaw's nostril to the tip of the premaxilla ascending process.
- **Cheek Depth (CHD):** the distance between the dorsal corner of the lower jaw and the ventral point of the bony edge of the orbit.
- **Eye diameter (ED):** maximal eye length from the orbit's most anterior to most posterior points. From the rostral corner of the bony orbit to the rostral corner of the lachrymal.
- **Lachrymal depth (LAD):** from the rostral corner of the bony orbit to the rostral corner of the lachrymal.
- **Length of the anal fin base (AFB):** the distance between the most caudal point and the most rostral point of the anal fin.
- **Pre-dorsal distance (PRD):** the distance between the rostral tip of the upper jaw and the rostral most point of the base of the dorsal fin.
- **Pre anal distance (PRA):** from the most rostral point of the anal fine base to the most rostral point of the upper jaw.
- **Pre-pectoral distance (PRP):** the distance between the rostral tip of the upper mouth and the most rostral point of the pectoral fin.
- **Pre pelvic distance (PRV):** from the most rostral point of the pelvic fin base to the most rostral tip of the upper mouth.
- **Length of the caudal peduncle (CPL**): the vertical distance between the caudal point of the anal fin insertion and the caudal border of the hypural.
- **Caudal peduncle Depth (CPD):** minimum depth of caudal peduncle

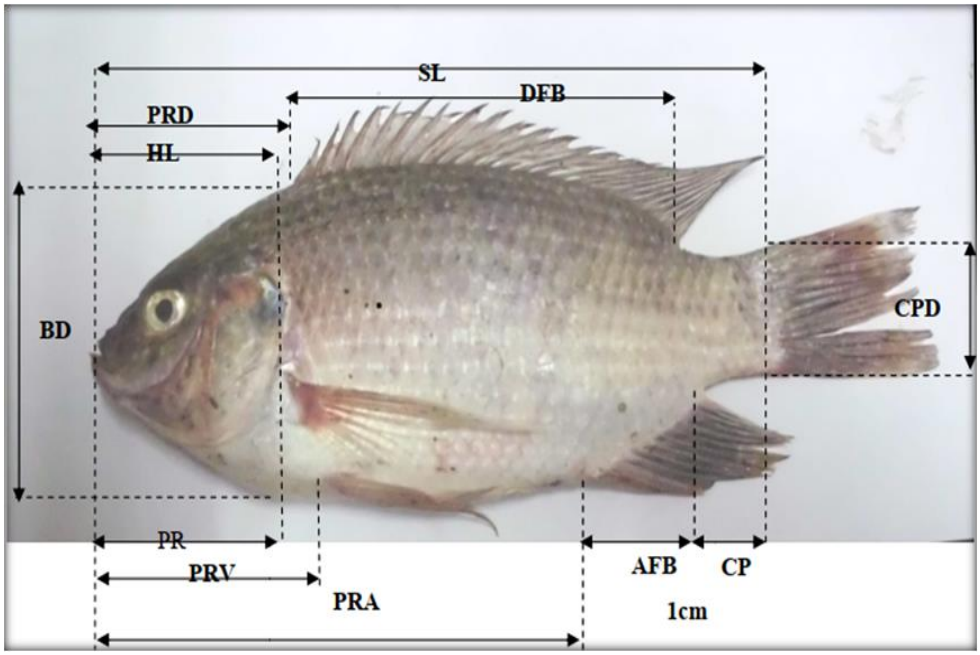

Figure 2: Morphometric measurements of the body part of S. galilaeus (Ebraheem, 2012).

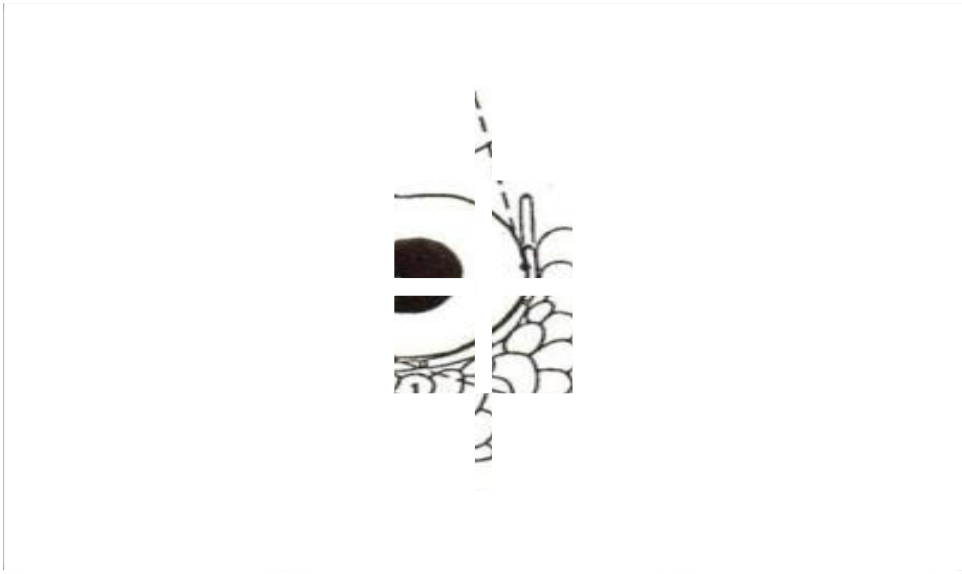

Figure 3: Morphometric measurements of the head part of S. galilaeus (Snoeks, 2004).

3.4 Meristic counts

The meristic counts following Snoeks (1994) methods and the different parameters were taken, and their definitions are shown below, and a schematic figure (Figure 6).

- **Dorsal fin formula (DF):** As a number of dorsal fin rays according to custom spine (DSPINES), delicate fine rays are soft (DOSFT). Examine the posterior portion of the fin for the existence of thin, tiny fin rays.

- **Anal fin formula (AF):** number of rays in the anal fin Spines (SPINES) and soft rays of the fin (ASOFT).
- **Pectoral fin formula (PC):** number of rays on the pectoral fin.
- **Pelvic fin formula (PV):** number of pelvic fin rays. Spine and soft rays of the fin.
- **Longitudinal line scales (LONG):** number of scales on the upper lateral line plus the number of scales on the lower lateral line that are caudal to the final scale per lateral line scale.

3.5 Determination of sex and dissection

The sex of the *S. galilaeus* specimens was identified, as illustrated in Figure 7, following the FAO report 1995 approach (Thabet, 2017). The sexual dimorphism of the genitalia can be observed, and the distinction between males and females is possible. Indeed, in males, the genital papilla is protuberant in the shape of a cone and carries a urogenital pore at the end. On the other hand, in females, it is small, rounded and has a transverse slit in the middle (oviduct) located between the anus and the urethral orifice (urogenital pore) located at the end of the figure 4 (Thabet, 2017). A ventral incision to expose gonads will determine the sex and gonadal development stage. The sex of each specimen will be recorded. The dissection will be done on the field to ascertain their sex, sex ratio, and the level of maturation of their gonad. The gonads samples were tallied and weighted.

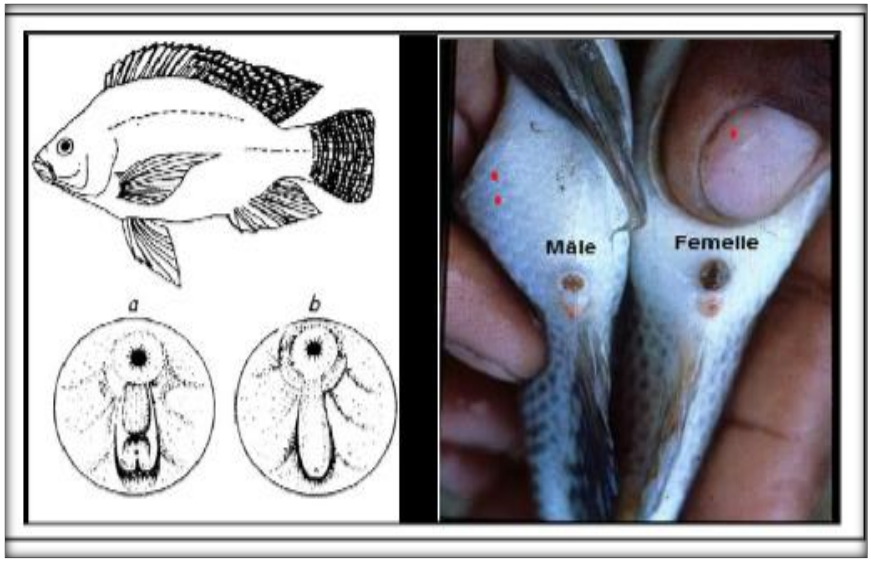

Figure 4: Genital papillae of female (a) and male (b) Tilapia (FAO, 1995)**.**

3.6 Gonad measurements

The different stages of the gonads were determined by visual inspection of the appearance adapted by Marcano et al. (2007) (Table 2).

Table 2: Macroscopic criteria for determining the maturity stages of the gonads

Stage	Classification	Macroscopic appearance	
		Testes	**Ovaries**
I	Virgin	Small and flat, translucent. Non identified sex	Small, transparent. Non identified sex
II	Immature	Whitish, poorly developed, with reduced fringes	Translucent and not very voluminous
III	Maturing	Whitish, with voluminous fringes	Large orange-pale, oocytes may be visible through the ovary tunic
IV	Mature	Very large, firm, white colour	Very large occupying part of the abdominal cavity. Yellow oocyte turgescence
V	Spermatid /Ovulated	Full developed, turgid fringes, milky-whitish in colour. Milt run out of the fish	Occupying the entire abdominal cavity. Ovulated oocytes can be fully expelled from the oviduct with gentle pressure
VI	Spent/Spawned	Bloody and flaccid fringes	Flaccid, red-brown or bloody in colour

3.7 Statistical analysis

3.7.1 Total length and standard length relationship

Size class was determined using the histogram to classify the 139 *S. galilaeus* collected specimens in Bafing and Bakoye Rivers in February 2022. The relationship between total Length (TL) and standard Length (SL) was determined using the linear regression method (Craig, 2007) in RStudio software, and the relationship fits the equation below:

$$\boldsymbol{TL = a + b * SL} \qquad \textbf{(1)}$$

Where:

- TL/SL = total length/standard length,
- a and b are linear regression constants.

3.7.2 Weight and Length Relationship

The weight of each specimen was measured using a top loading balance to the nearest 0.1g. Total lengths (TL) were measured using a calliper. After the linear relationship analysis

between TL and SL, the total lengths were used. The data generated were statistically analysed by fitting the length-weight relationship described by Le Cren (1951). The Length-weight relationship can be expressed as:

$$\boldsymbol{W = aTL^{b}} \qquad \textbf{(2)}$$

The logarithmic transformation gives the linear equation:

$$\boldsymbol{Log\ W = \log a\ + b * log\ TL} \qquad \textbf{(3)}$$

Where:

- **W** = weight in gram
- **L** = length in cm
- **a** = is a constant being the initial growth index and **b** = growth coefficient.

The constant 'a' represents the point at which the regression line intercepts the y-axis and 'b' the slope of the regression line. The relationship between Length and Weight was determined for males and females separately by transforming the values of both variables to logarithmic values and fitting a straight line by the method of least squares, and R-square ($R^{2)}$ were estimated. Analysis of covariance (ANCOVA) was used to investigate differences in the mean values of dependent variables related to the effect of the controlled independent variables while accounting for the influence of the uncontrolled independent variables (Paternoster, R et al., 1998).

3.7.3 Determination of Length at first maturity (Lm50)

The data collected on the Total Length (TL) and gonadal stages of the fish *S. galilaeus* were used to determine the length at first maturity (Lm50). These values were used to plot graphs for males and females using the software RStudio 4.1.0 to estimate the length at which 50% of the population is sexually mature. The percentages of males and females considered mature were used to determine the percentage maturity for the selected class size. Moreover, the gonads at stages 2 to 5 were considered mature, and stage 1 was considered immature.

Using the binary coding means transforming “YES” and “NO” which is needed for logistic regression so that their probabilities can be analysed. YES has the P, and then NO has the probability 1-P. Logistic regression uses the *logit* – transformation,

Example 80 % (YES)| $\boldsymbol{logit(YES) = log\frac{P}{1-P} = log\frac{0.8}{0.2}}$ **= 1.38**

Example 20 % (YES)| $\boldsymbol{logit(YES) = log\frac{P}{1-P} = log\frac{0.2}{0.8}}$ **= -1.38**

A linear equation in slope-intercept form:

$$\mathbf{y = a + bx} \qquad \mathbf{(4)}$$

$$\boldsymbol{logit(YES - being\ mature) = log\frac{P_{mat}}{1 - P_{mat}} = a + b\ (SL)}$$

At Lm 50 P_{mat} by definition, is 0.5, which can be substituted in the logistic equation to calculate Lm50. The formula obtains probability P = 50% in the equation at Lm50:

$$\boldsymbol{log\frac{P_{mat}}{1 - P_{mat}} => \ log\frac{0.5}{0.5} = log1 = 0 = a + b\ (TL)}$$

$$\boldsymbol{0 = a + b\ (Lm50)}$$

$$\boldsymbol{\frac{-a}{b} = \ Lm50}$$

3.7.4 Comparisons of morphometric measures

Basic descriptive statistics were computed for the morphometric measurements, including minimum value, maximum value, mean, and range. However, each morphometric parameter is divided by the total Length (TL) to obtain a ratio of size to find differences between the *S. galilaeus* from two rivers: BD/TL, HL/TL, HW/TL, DFB/TL, PRD/TL, PRA/TL, PRP/TL, PRV/TL, SL/TL, IOW/TL, SNL/TL, PP/TL, CHD/TL, ED/TL, LAD/TL, AFB/TL, CPL/TL, and CPD/TL. The standardisation of data is essential for allowing intra- and interspecific comparisons. Lastly, in the Wilcoxon rank sum test for this case, the non-normal distributed data was used to performed whether there were significant morphological differences ($p < 0.05$) between the *S. galilaeus* from the two rivers (Bafing and Bakoye).

3.7.5 Comparisons of meristic counts

The descriptive statistics, such as minimum and maximum values, mean and standard deviation, were performed for the meristic counts. The Student T-test was used to determine whether species varied substantially from one another in the five meristic features (DF, AF, PC, PV, and LONG) (Omotayo, 2015).

3.8 Phylogenetic analysis

Fish samples were bought from Fishermen's landings sites from natural populations at the two tributaries, Bafing river, and Bakoye river. At each landing site, samples and approximately 25 mg pieces of muscle tissues were obtained and stored in a sterile cryovial tube of 1.5 ml. Each tube was clearly labelled with the specimen number for Bafing River (A) and Bakoye (B), e.g. A1, A25, B1, B33, randomly selected at each landing site. During fieldwork, the tissues of 61 samples of Mango tilapia *S. galilaeus* were taken, 35 from Bafing and 26 from Bakoye, and preserved in ethanol 96% provided by the ISECMAR/UTA molecular biology laboratory. The samples were later sent to the ISECMAR/UTA molecular biology lab for DNA extraction and PCR amplification.

3.8.1 Extraction procedure and the purification of tissue

The DNA extraction procedure and purification followed the protocol of E.Z.N.A Tissue DNA Kit with some specific features: 30 mg tissue was transferred to a 1.5 mL microcentrifuge tube and mixed with 200 µL of TL Buffer and 25 µL of Proteinase K Solution. The tubes were incubated at 55°C in a shaking water bath. The cleaning process starts with centrifugation at maximum speed (≥10,000g) for 5 minutes, collecting 200 µL of the supernatant to a sterile 1.5 mL microcentrifuge tube to join to 220 µL BL Buffer and incubation at 70°C for 10 minutes. The precipitation of DNA starts with the addition of 220 µL 100% ethanol, 500 µL HBC Buffer diluted with 100% isopropanol, filtration in HiBind® DNA Mini Column and washed with 700 µL of DNA Wash Buffer (diluted with 100% ethanol) intercalated with several centrifugation steps. The last step includes the elution of the DNA in 100-200 µL Elution Buffer heated to 70°C. DNA quality was checked with 0.8% agarose gel electrophoresis ad stained with Syber save. The extracted DNA was stored at -20ºC for the next steps.

3.8.2 Polymerase chain reaction (PCR) primers

DNA amplification was achieved through polymerase chain reaction (PCR) for two mitochondrial fragments of the Control region (D-loop) gene and cytochrome oxidase subunit (COI). Universal primers for D-loop by Lee et al. (1995) (CR-A, 5′ TTCCACCTCTAACTCCCAAAGCTAG-3′ and CR-E,5′-CCTGAAGTAGGAACCAGATG 3′) were used for 400pb amplification fragments and Ward et al. (2005) for 600 bp of COI (FishF2: 5′-TCGACTAATCATAAAGATATCGGCAC-3′; FishR2: 5′ ACTTCAGGGTGACCGAAGAA TCAGAA-3′). The PCR amplifications were conducted in a 25 µl total volume containing 10–20 ng/µl of template genomic DNA, 0.5 µl of forward and

reverse primers, and 12.5 µl of Phusion High-Fidelity PCR Master Mix with HF buffer, following the manufacturer's specifications. PCR cycling was performed in an Eppendorf thermocycler (Eppendorf AG, Hamburg, Germany) using an initial denaturation step at 95°C for 15 min. Then, followed by 35 cycles of denaturation at 94°C for 45 s, optimum annealing (Tm D-loop = 52°C and Tm COI = 50°C) for 45 s, and extension at 72°C for 1 min and the last extension step at 72°C for 10 min. The amplification products were examined by electrophoresis (1% agarose gel stained with Syber save). Sequencing was performed on an automated Sanger sequencer, and the results were compared with the sequences available in the GenBank using Geneious (Drummond et al., 2006). The blast output in GenBank was used to create a taxonomic classification.

3.8.3 Phylogenetic analysis

The Geneious Basic (Drummond et al., 2006), a user-friendly biological sequence alignment editor, was used to visualise the chromatograms and make the global alignment. For phylogenetic analyses, for logistical reasons, COI sequences of specimens were not used (serving only for species identification). The haplotype network was generated using the parsimony method implemented in the TCS Software (Clement et al. 2000) with the final manipulation in tcsBU (Múrias Dos Santos et al., 2015). Pairwise distance values for all haplotypes were estimated above the 95% level to create the network, whit completely separation with differences higher than 5 %.

DNA Sequence Polymorphism DNASP v6 (Rozas et al., 2018) was used to infer the mtDNA haplotypes and calculate the differentiation among specimens based on D-loop haplotypes and genetic diversity: Number of sequences (Ns), number of haplotypes (h), haplotype gene diversity (Hd), nucleotide diversity, (π) the average number of, nucleotide differences (k), Tajima's D, Test Fu & Li's Test, and R^2. The pairwise differences and the neutrality test Fu & Li's., Tajima's D test, and R^2 were also applied to compare the two populations of *S. galilaeus* from Bafing and Bakoye.

4. Results

4.1 Length frequency distribution

This part aims to summarise the numbers of individuals for all analyses and to classify the total number of *S. galilaeus* into size classes as a function of the total length TL. Length-frequency distribution of total *S. galilaeus* in Bafing and Bakoye by sex is illustrated in (Figure 5). One hundred thirty-nine specimens were collected from Bafing and Bakoye Rivers, with 61 females and 78 males. The distribution was polymodal, with a maximum of 26.24 cm TL for males and 21.25 cm TL for females of the *S. galilaeus*. The histograms show that the females and males from the two rivers were more frequent in class sizes from 11 cm TL to 17 cm TL.

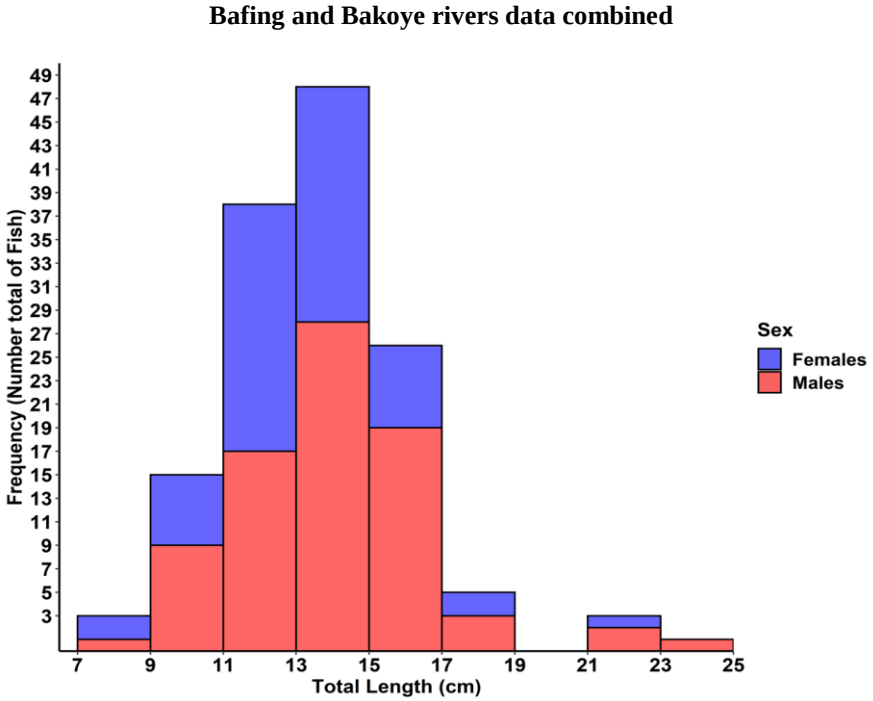

Figure 5: Length-Frequency Distribution of *S. galilaeus* collected from each river Bafing and Bakoye data combined.

A total, 72 specimens (42 males and 30 females) were collected in the Bafing river, and 67 specimens (36 males and 31 females) in the Bakoye river (Figures 6). The higher frequencies of fish were observed between 11 and 17 cm TL, with a larger size in males for both rivers, Bafing and Bakoye rivers.

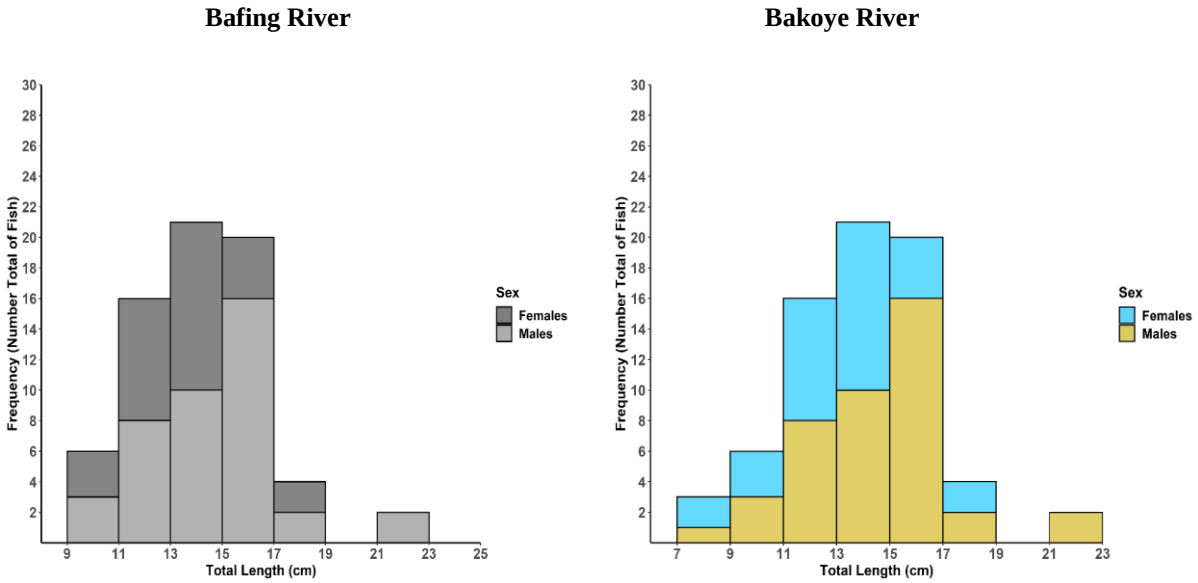

Figure 6: Length-Frequency Distribution of *Sarotherodon galilaeus* collected from Bafing and Bakoye Rivers data separate

4.2 Relationship between Total Length and Standard Length

The relationship is investigated to indicate the appropriate length scale for the analysis of maturity, length-weight relationships, and morphometric measures and to convert these measures into SL units for comparisons with literature data.

The relationship between total Length (TL) and standard Length (SL) a linear relationship is shown in (Figure 11). However, three points are outliers. In two cases, the standard length SL is more significant than the total length TL, which by definition cannot be the case. The third outlier case had a very low standard length SL as compared to the total length TL. The removal of the three outliers gives a new SL and TL relationship (Figure 7). There was an improved correlation between standard length SL and total length TL (see Table 3). Lastly, all analyses hereafter will be conducted with TL after removing outliers.

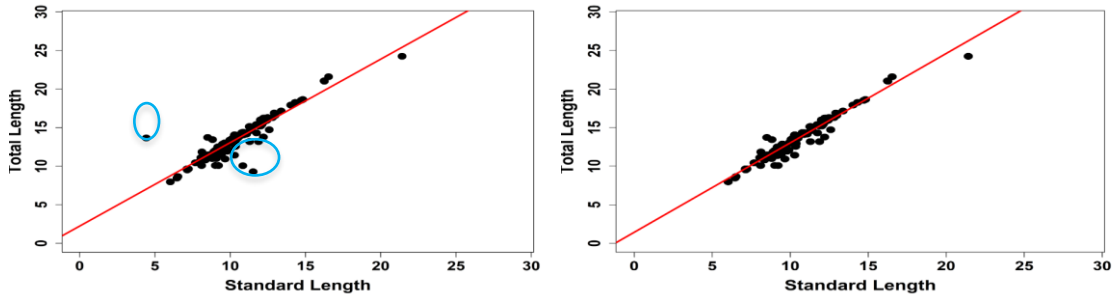

Figure 7: Relationship between total length TL and standard length SL for 139 *Sarotherodon galilaeus* collected in Bafing and Bakoye Rivers (left) and three outliers (blue circle) removed (right) .

Table 3: Summary of the output of the model for the SL-TL

Coefficients	Intercept	Standard Length
All Data	2.236	1.082
Without outliers	1.432	1.160

The length-length relationship used for calculating indices for further comparisons with literature data is The Length–length relationship was estimated by linear regression analysis:

$$\mathbf{TL = a + b*SL}$$

Where:

- **a** is the intercept
- **b** is the slope of the linear regression. Calculating SL

$$\mathbf{(TL-2.236) /1.082=SL}$$

$$\mathbf{(TL-1.432) /1.160=SL}$$

This equation can be used if TL data are available. For comparison with literature data, the following conversion can be used where the intercept is suppressed.

$$\mathbf{TL = b\ SL}$$

- b can be used as a conversion factor.

$$\mathbf{TL = 1.287SL}$$

4.3 Relationship between weight and total length (LWR)

This part conducts the weight-length relationships most often modelled with simple linear regression. Moreover, the statistical test of analysis of covariance is used to detect differences between Bafing and Bakoye rivers. Diagnostic plots were applied to check for normal distribution, and the homoscedasticity of residuals and assumptions was violated. The adequacy of these assumptions is frequently better checked by evaluating two graphs: the residual graph and the residual histogram.

The residual plot in (Figure 8) for both data combined with Bafing and Bakoye Rivers shows the residuals about the predicted values. The values are scattered around a horizontal line, i.e. which is centred on zero. The error variance does not appear to be constant because

there is an obvious funnelling of the residuals from left to right with the significant outliers highlighted with the line number of the outliers 88, 49, and 61. The histogram of the residuals in the same (Figure 8) is slightly asymmetric, with an extended positive tail of residuals. They indicated that the model fit was not perfect. The exponential value of the length-weight relationship 'b' is 2.63, as observed from the above logarithm equations indicating negative allometric growth (b<3). The regression results are summarised in (Table 4), and the relationship between weight and total length is significant with p-value = < 2e-16 *** with R-squared $R^{2 =}$ 0.89.

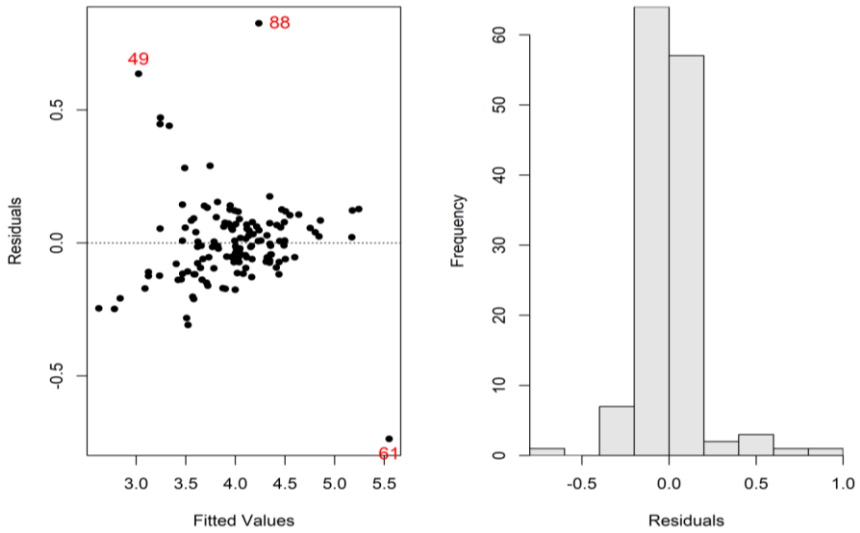

Figure 8: Modified residual plot (Left) and histogram of residuals (Right) from linear regression to the log-transformed weights and lengths of *S. galilaeus* captured in the combined Bakoye and Bafing rivers data.

Table 4: Summary of the residuals for the Bafing and Bakoye data separate

River	Coefficients	Estimate	Std. Error	t value	P (>|z|)
	Intercept	-2.8344	0.20255	-13.99	<2e-16 ***
Bafing and Bakoye	log(TL)	2.62890	0.07798	33.71	<2e-16 ***

The residual plots in Figure 14 and Figure 15 for the two separate data of the Bafing and Bakoye rivers show the residuals about the predicted values. The values are further around a horizontal line for the Bakoye (Figure 15) than for the Bafing (Figure 14). The error variance does not appear to be constant as there is an apparent funnel of residuals from left to right, with the significant outliers highlighted by line numbers 49 and 61 for Bafing and 24 for Bakoye.

The histogram of the residuals of Bafing in (Figure 9) is slightly asymmetric with an extended positive tail of the residuals than the Bakoye in (Figure10). The model shows that the exponential value of the length-weight relationship 'b' is 2.48 for Bafing and 2.70 for Bakoye, as observed from the above logarithm equations indicating negative allometric growth (b<3). Therefore, there is a relationship between weight and total length for both Bafing and Bakoye rivers with the exact significant p-value = <2e-16 *** and a slight difference of the R-square R^2 (0.88 & 0.90) for Bafing and Bakoye (Table 5).

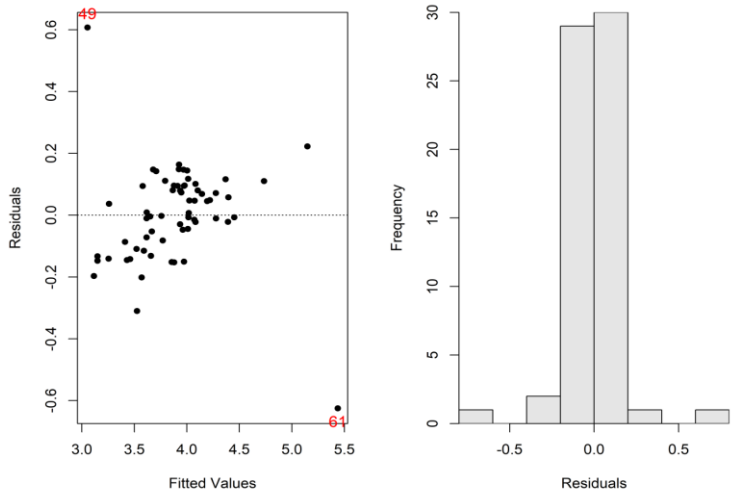

Figure 9: Modified residual plot (Left) and histogram of residuals (Right) from fitting a linear regression to the log-transformed weights and lengths of *S. galilaeus* captured in the Bafing River.

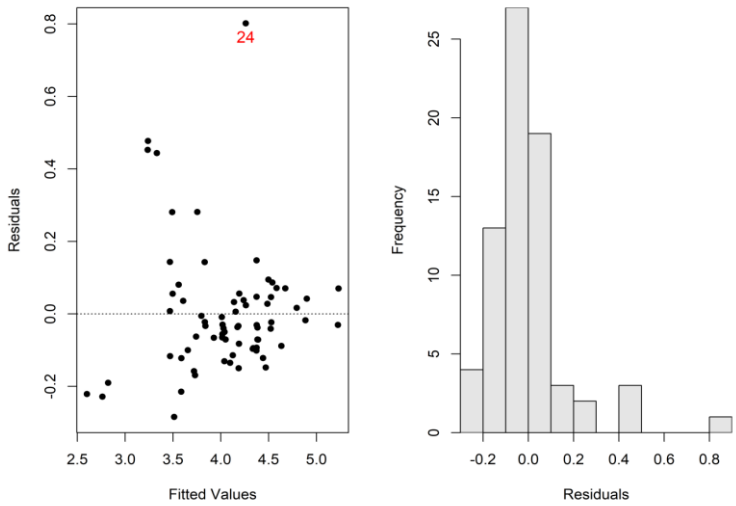

Figure 10: Modified residual plot (Left) and histogram of residuals (Right) from fitting a linear regression to the log-transformed weights and lengths of *S. galilaeus* captured in the Bakoye River.

Table 5: Summary of the residuals for the Bafing and Bakoye data separate

River	Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Bafing	Intercept	-2.4784	0.2946	-8.411	7.67e-12 ***
	log(TL)	2.4821	0.1147	21.648	< 2e-16 ***
Bakoye	Intercept	-3.0245	0.2805	-10.780	<2e-16 ***
	log(TL)	2.7085	0.1070	25.320	<2e-16 ***

Table 6 shows the summary of the analysis of covariance test (ANCOVA), which was used to investigate differences in the mean values of dependent variables related to the effect of the controlled independent variables while accounting for the influence of the uncontrolled independent variables.

4.4 Length at first maturity (Lm50)

This part aims to estimate the size at first maturity for males and females of *S. galilaeus*. Data from the two rivers, Bafing and Bakoye, were combined for the analysis. In total, 139 specimens were analysed. The fitted logistic curves for length at first maturity of males (red) and females (blue) show the data combined of *S. galilaeus* from the Bafing and Bakoye rivers. All fish over 0.5% were mature, with the remaining under 0.5% being immature (Figure 11).

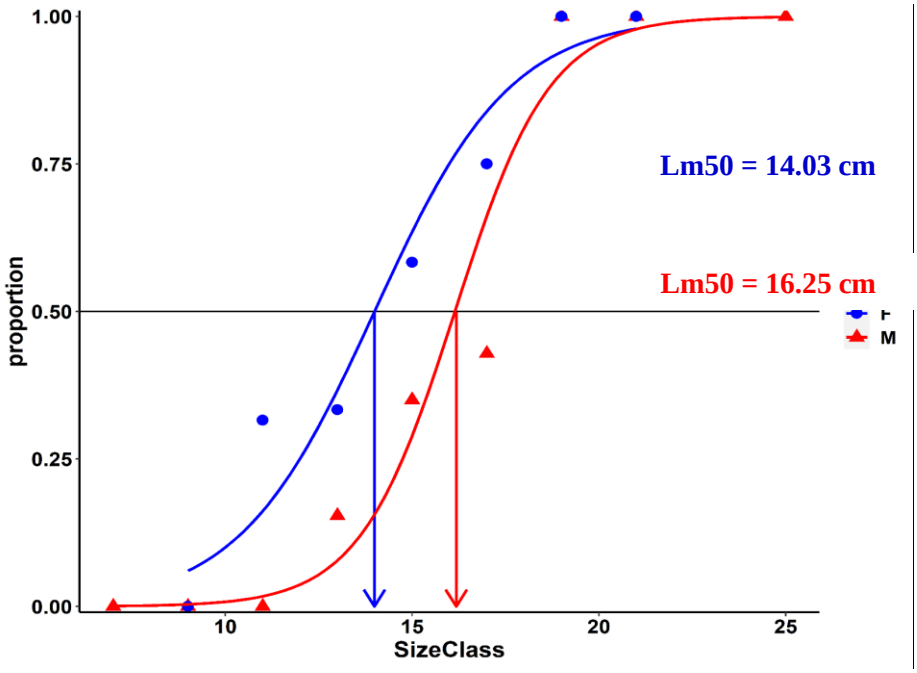

Figure 11: A logistic curve fits the percentage of the data combined of Bafing and Bakoye rivers classified as an adult by size. The size of Lm50 maturity is then estimated by evaluating the logistic curve at 50%.

Table 7 below summarises the regression coefficients from the general linear model analysis of the first maturity size class. The model intercept for the proportion of maturity for females is -5.2477 and for males is -9.6034. That indicates for an increase in the maturity stage; the model relates the percentage to the logit of maturity size class using the logistic regression equation below. However, we expect an increase of 0.3742 for females and 0.5907 for males per cm in the maturity stage. The p values show that the males are slightly better than the females. The logistic regression equation is described in the methodology part. The example below determines the maturity of the virtual size class 50 cm for the females and the males for the combined Bafing and Bakoye rivers data:

$$\textbf{Females}: log\frac{p}{1-p} = -5.2477 + 0.3742\ (50) = 13.46$$

$$\textbf{Males:} log\frac{p}{1-p} = -9.6034 + 0.5907(50) = 19.90$$

- Where the probability is:

$$\textbf{Male; p} = \frac{e^{(13.46)}}{1+e^{(13.46)}} = 0.99 \textbf{ and Females; p} = \frac{e^{(19.90)}}{1+e^{(19.90)}} = 0.99$$

That means that the probability at size class 50 cm TL of females and males being mature is 99%.

Table 6: Regression coefficients from general linear model analysis of Lm50 females and males for the combined Bafing and Bakoye rivers data. Moreover ***, **, * represent the significance levels of $p < 0.001$, $p < 0.01$, $p < 0.05$. Size class measured as total length binned in 2 cm intervals.

Sex	Coefficients	Estimate	Std	z value	Pr(>|z|)
Females	Intercept	-5.2477	1.8626	-2.817	0.00484 **
	Size Class	0.3742	0.1399	2.675	0.00748 **
Males	Intercept	-9.6034	2.5456	-3.772	0.000162***
	Size Class	0.5907	0.1712	3.451	0.000558***

The fitted logistic curves for the length at first maturity of male and female *Sarotherodon galilaeus* in the Bafing River and in Bakoye rivers (Figure 12 and 13). The majority of the fish obtained were sexually immature, separating the data. In Bakoye River, females larger than 11.37 cm TL have a 50% chance of being sexually mature (Lm50).

Males with a size greater than 16.33 cm TL also have a more than 50% chance of being sexually mature. In addition, in Bafing river, the Lm 50 for females were 16.99 cm and for males 15.09 cm respectively.

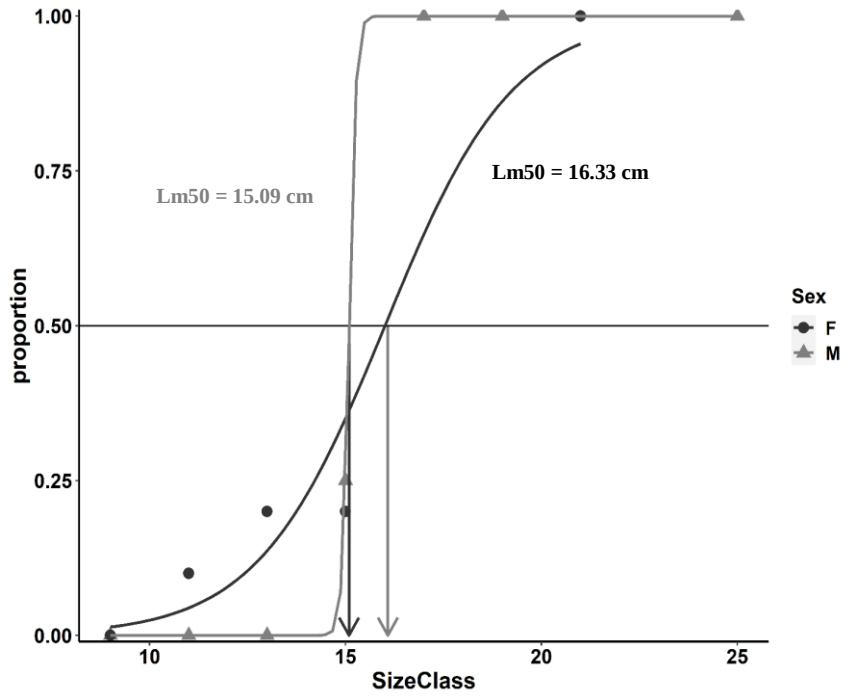

Figure 12: Fitted logistic regression for the proposition of females (blue) and males (red) from Bafing River *Sarotherodon galilaeus* mature by a total length of Lm50.

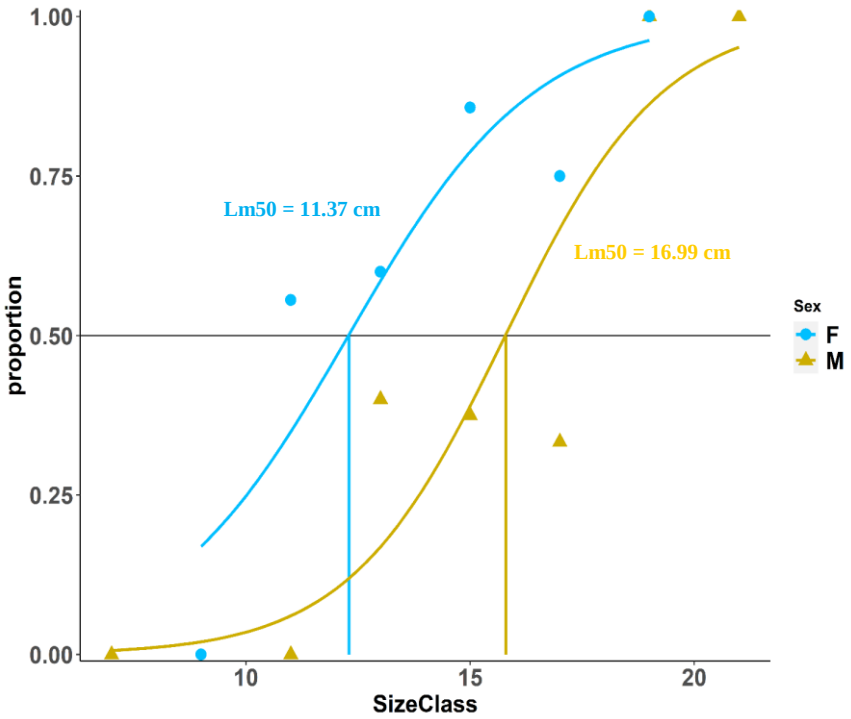

Figure 13: Fitted logistic regression for the proposition of females (blue) and males (red) from Bakoye River *Sarotherodon galilaeus* mature by a total length of Lm50

Table 8 summarises the regression coefficients for which data from the two rivers, Bafing and Bakoye, were used separately to find the Lm50 for males and females of *Sarotherodon galilaeus*. However, the data separated to illustrate the effect of reduced sample sizes not perfectly covering the required size range and, in particular, a low number of mature *S. galilaeus* in the Bafing river and the *p* values shown for the males Bakoye river are less significant.

Table 7: Regression coefficients from general linear model analysis of Lm50 females and males for each river Bafing and Bakoye data separate. And ***, **, * represent significance levels $p < 0.001$, $p < 0.01$, $p < 0.05$

River	Sex	Coefficients	Estimate	Std	z value	Pr(>|z|)
Bafing River	**Females**	Intercept	-6.808	3.401	-2.002	0.045*
		Size Class	0.402	0.250	1.611	0.107
	Males	Intercept	-186.960	379194.240	0	1.000
		Size Class	12.390	25279.620	0	1.000
Bakoye River	**Females**	Intercept	-4.092	2.416	-1.694	0.090
		Size Class	0.360	0.188	1.917	0.055
	Males	Intercept	-5.529	2.481	-2.229	0.026*****
		Size Class	0.339	0.164	2.061	0.039*****

4.5 Comparisons of Morphometric Measures

The observations were 67 for the Bafing river and 72 for Bakoye River. Each morphometric measure is expressed as the proportion of total Length (TL). Based on the TL - SL relationship, measurements can also be converted by the standard length (SL) scale.

Six out of eighteen morphometric measures show a significant difference (Table 9). Three measures are related to the body shape (BD/TL, CPD/TL, CPL/TL), and three are related to the head (HL/TL, CHD/TL, LAD/TL). Table 9 describes the morphometric ratios of *S. galilaeus*, with the minimum (Min), maximum (Max), mean (Mean), and standard deviation (SD) and test statistics of the Wilcoxon Rank Sum test.

Table 8: Morphometric ratios of *S.galilaeus* by the river, with minimal values (Min), maximal (Max), mean (M), and standard deviation (SD) expressed as a proportion of total length.

Morphometric ratios	River	Min	Max	Mean	SD	Wilcoxon *p*
BD/TL	Bafing	0.20	0.43	0.33	0.03	0.0025
	Bakoye	0.01	0.38	0.31	0.04	
HL/TL	Bafing	0.22	0.33	0.25	0.02	0.0120
	Bakoye	0.06	0.30	0.25	0.03	
HW/TL	Bafing	0.03	0.10	0.05	0.01	0.8500
	Bakoye	0.03	0.12	0.05	0.01	
DFB/TL	Bafing	0.09	0.62	0.47	0.06	0.6900
	Bakoye	0.39	0.64	0.48	0.04	
PRD/TL	Bafing	0.18	0.40	0.29	0.03	0.5600
	Bakoye	0.16	0.40	0.29	0.03	
PRA/TL	Bafing	0.31	1.00	0.58	0.08	0.1400
	Bakoye	0.52	0.78	0.60	0.05	
PRP/TL	Bafing	0.21	0.34	0.25	0.03	0.7000
	Bakoye	0.13	0.33	0.25	0.03	
PRV/TL	Bafing	0.21	0.34	0.25	0.03	0.7000
	Bakoye	0.13	0.33	0.25	0.03	
SL/TL	Bafing	0.32	1.24	0.77	0.10	0.3900
	Bakoye	0.72	0.91	0.77	0.03	
IOW/TL	Bafing	0.07	0.19	0.11	0.03	0.3200
	Bakoye	0.08	1.35	0.12	0.15	
SNL/TL	Bafing	0.04	0.21	0.08	0.03	0.1000
	Bakoye	0.08	1.35	0.08	0.02	
PP/TL	Bafing	0.07	0.19	0.11	0.03	0.3200
	Bakoye	0.08	1.35	0.12	0.15	
CHD/TL	Bafing	0.05	0.14	0.08	0.02	0.0141

	Bakoye	0.05	0.12	0.08	0.01	
ED/TL	Bafing	0.04	0.38	0.07	0.04	0.7400
	Bakoye	0.04	0.09	0.06	0.01	
LAD/TL	Bafing	0.04	0.14	0.06	0.02	0.0393
	Bakoye	0.02	0.11	0.06	0.10	
AFB/TL	Bafing	0.11	0.40	0.16	0.04	0.2100
	Bakoye	0.12	0.21	0.15	0.02	
CPL/TL	Bafing	0.06	0.15	0.08	0.02	0.0227
	Bakoye	0.06	0.14	0.09	0.01	
CPD/TL	Bafing	0.11	0.22	0.14	0.02	0.0232
	Bakoye	0.08	0.23	0.13	0.03	

The boxplots represent the variations of significant morphological measures (Table 9). The most significant variations are related to the body (BD, CPD, and CPL) and the head (HL, CHD, and LAD). The variability for BD/TL was more significant in Bakoye river with the range of 0.37 than in Bafing with the range of 0.23 (range defined by the difference between max and min). The difference in means was 0.02 and, therefore, small (Figure 14 (a)). The CPD/TL was smaller in Bafing River, with a range of 0.11 than in Bakoye, with 0.15. The mean difference was slight at 0.009 (Figure 14 (b)).

Moreover, the CPL/TL for Bakoye were greater than for Bafing with the range 0.09-0.08 (Figure 14c). The difference in means was 0.01 and, therefore, negligible. Altogether, the specimens from the Bakoye river appeared to be more slender and elongated. The variability for HL/TL was more significant in Bakoye River with a range of 0.24 and in Bafing with a range of 0.11 (Figure 15 (d)). The mean is the same at 0.08. The CHD/TL was larger in Baking River, with a range of 0.09, and in Bafing, with a range of 0.07. The same mean (Figure 15(e)). In addition, the LAD/TL was greater in Bakoye River with the range of 0.15 and in Bafing with the range of 0.11(Figure 15 (f)). The difference in means was 0.01 and, therefore, negligible. Altogether, the head shape of specimens from Bakoye River was larger and more elongated.

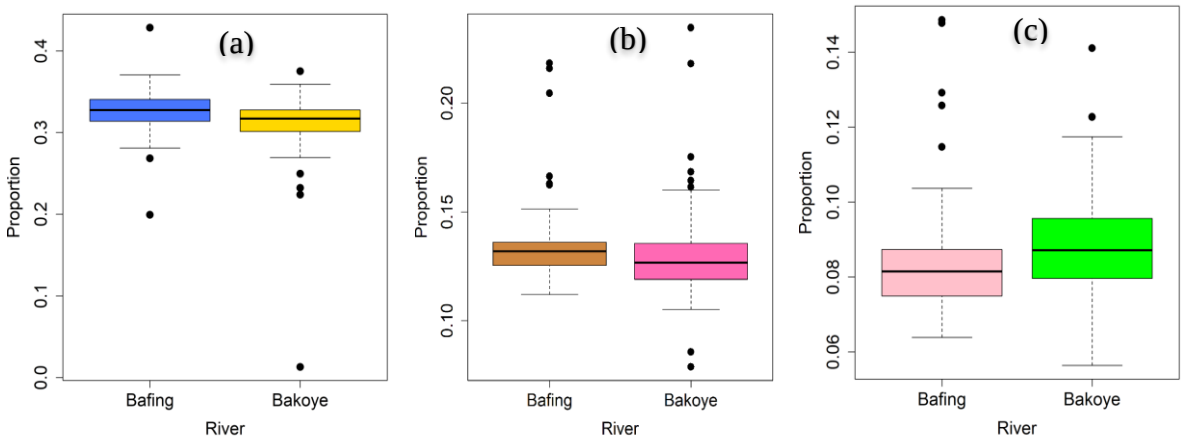

Figure 14: Boxplots representing the variations in body depth (a) (BD/TL), caudal peduncle (b) (CPD/TL), and the length of the caudal peduncle (c) (CPL/TL) of *S. galilaeus* from Bafing and Bakoye Rivers.

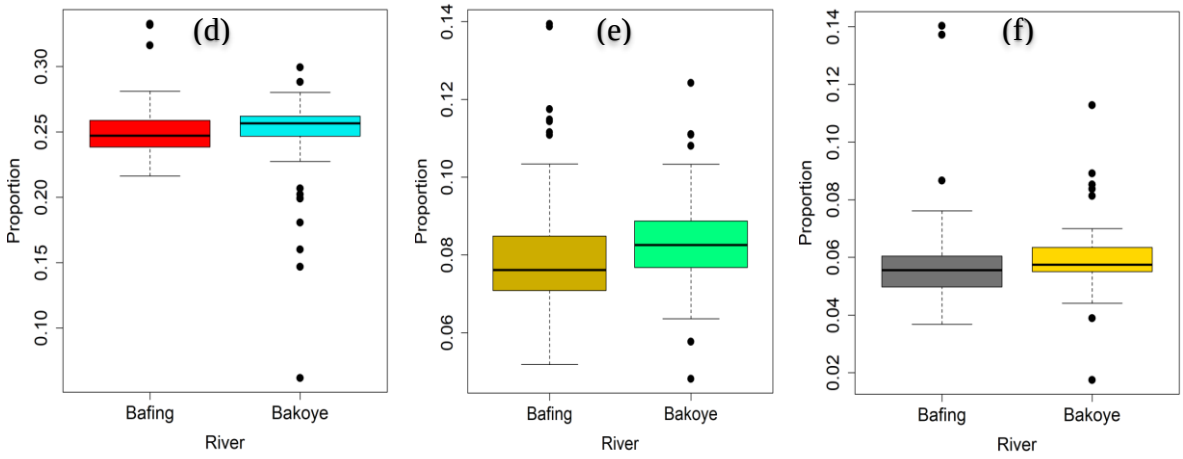

Figure 15: Boxplots representing the variations in head length (d) (HL/TL), cheek depth (e) (CHD/TL), and lachrymal depth (f) (LAD/TL) of *S. galilaeus* from Bafing and Bakoye Rivers.

4.6 Comparison of meristic counts

Five meristic counts (DF, AF, PC, PV, and LONG) of 139 *S. galilaeus* samples obtained from Bafing and Bakoye Rivers are shown in (Table 10). The AF, PC, and LONG ranges were similar in the two rivers. The slight variability in the mean was related to differences in the count (Table 10). In addition, two out of five meristic counts DF and LONG show a significant difference. The Dorsal fin (DF) T-test p = 0.0001 with SD (0.53- 35) and the mean (28.57 - 28.86). The Longitudinal line scale T - test p= 0.009 with SD (1.3- 1.3) and the mean (32.89 - 32.32).

Table 9: Meristic counts ratios of *S.galilaeus* by the river, with minimal values (Min), maximal (Max), mean (M), standard deviation (SD) and the T-test (*P*-value).

Meristic count	River	Min	Max	Range	Mean	SD	T-test *(p)*
DF	Bafing	27	29	2	28.57	0.53	0.0001
	Bakoye	28	29	1	28.86	0.35	
AF	Bafing	11	14	3	13.00	0.63	0.3100
	Bakoye	11	14	3	13.00	0.51	
PC	Bafing	10	13	3	11.82	0.80	0.3600
	Bakoye	10	13	3	11.94	0.80	
PV	Bafing	5	6	1	6.00	0.12	0.3000
	Bakoye	6	6	0	6.00	0.00	
LONG	Bafing	30	37	7	32.89	1.30	0.0090
	Bakoye	28	35	7	32.32	1.30	

4.7 Genetic differentiation and nucleo Diversity

Mitochondrial DNA (mtDNA) was extracted to reconstruct phylogenetic network haplotype and analyse population genetic diversity. Different from the analyses for morphometric and meristic data for which no external data were acquired, genetic data from Ghana and Israel are also included in the analysis of genetic similarity. The individuals sampled totalled 35 specimens ranging from a minimum of 26 for Bakoye river to a maximum of 35 for Bafing river. Sixty-one samples were sequenced, and an alignment was obtained with 455 bp of the D-loop gene. In addition, 30 haplotypes were obtained by joining the sequences from Bafing 27 haplotypes and Bakoye 23 haplotypes with the sequences obtained from the online databases (Ghana, 10 haplotypes, and Israel, 9 haplotypes).

Table 11 shows the differentiation among populations based on D-loop haplotypes of genetic diversity from the Bafing, Bakoye rivers, Ghana, and Israel. The alignment of 61 (35 Bafing + 26 Bakoye) D-loop sequences produced 30 haplotypes and GenBank accession codes, including polymorphic sites. Estimates of all parameters are summarised in Table 11. The data from Ghana and Israel populations showed the lowest number of haplotypes as well as the lowest values of haplotype diversity (h), the average number of pairwise differences (k) and nucleotide diversity (π) and the highest number of haplotypes was found in Bafing and Bakoye.

The π value is lower for the two Rivers, with respectively Bafing River at 0.048 and 0.016 for Bakoye River. These results reflect no big genetic structure difference between the two rivers.

Table 10: Differentiation among species based on D-loop haplotypes of genetic diversity: Number of sequences (Ns), number of haplotypes (h), haplotype gene diversity (Hd), nucleotide diversity (π), Average number of nucleotide differences (k), Tajima's D, Test Fu & Li's Test, and R2. The significance levels of *P<0.05 and ***P<0.001.

Sites	Ns	h	Hd	π	k	Tajima's Tests	Tajima's *P-value*	Fu & Li's Tests	Fu & Li's *P-value*	R2
Bafing	34	27	0.975	0.048	21.037	-0.24312	P > 0.10	0.94031	P > 0.10	0.1232
Bakoye	24	23	0.996	0.016	7.308	-0.83891	P > 0.10	-1.26590	P > 0.10	0.0980
Ghana	73	10	0.799	0.009	4.323	0.49750	P > 0.10	0.78051	P > 0.10	0.1189
Israel	91	9	0.336	0.001	0.365	-1.89084	P < 0.05	-1.91493	P > 0.05	0.0309

The results of the neutrality test of Fu's FS, based on the infinite site model and its p-values, showed negative values in Bakoye river (D loop = -1.26590) and Israel (D-loop = -1.91493). Overall, neutrality tests showed no signs of expansion for the marker in any site.

4.2.1 Haplotype network analysis

The parsimony haplotype network D-loop showed that all barn swallow populations were genetically admixed with noticeable divergence among haplotypes (Figure 16). Bafing and Bakoye show the higher diversity haplotype and lower diversity nucleotides. However, the Rivers Bafing and Bakoye result in higher mutations. In particular, five haplotypes from Bafing separated from all populations may be due to speciation or perhaps the new group population. Compared to the Ghana and Israel populations, whit lower genetic diversity with a higher number of samples, the Mali samples show a high number of haplotypes whit a high number of mutations between them. The mitochondrial haplotype networks D-loop of *S. galilaeus* for Bafing, Bakoye, Ghana and Israel geographic regions show high mutation between the different zones.

All three countries were shown to have unique haplotypes, with Mali and Ghana presenting two clades, while the samples from Israel presented only one clade. The mtDNA haplotype network D-loop showed the high ancestral central-most common haplotype connected too many haplotypes. Except for the five haplotypes from Bafing, speared from the

others are maybe the subspecies. This similarity of haplotypes in Bafing and Bakoye means there are no big significant differences in haplotypes between *S. galilaeus* in these two rivers.

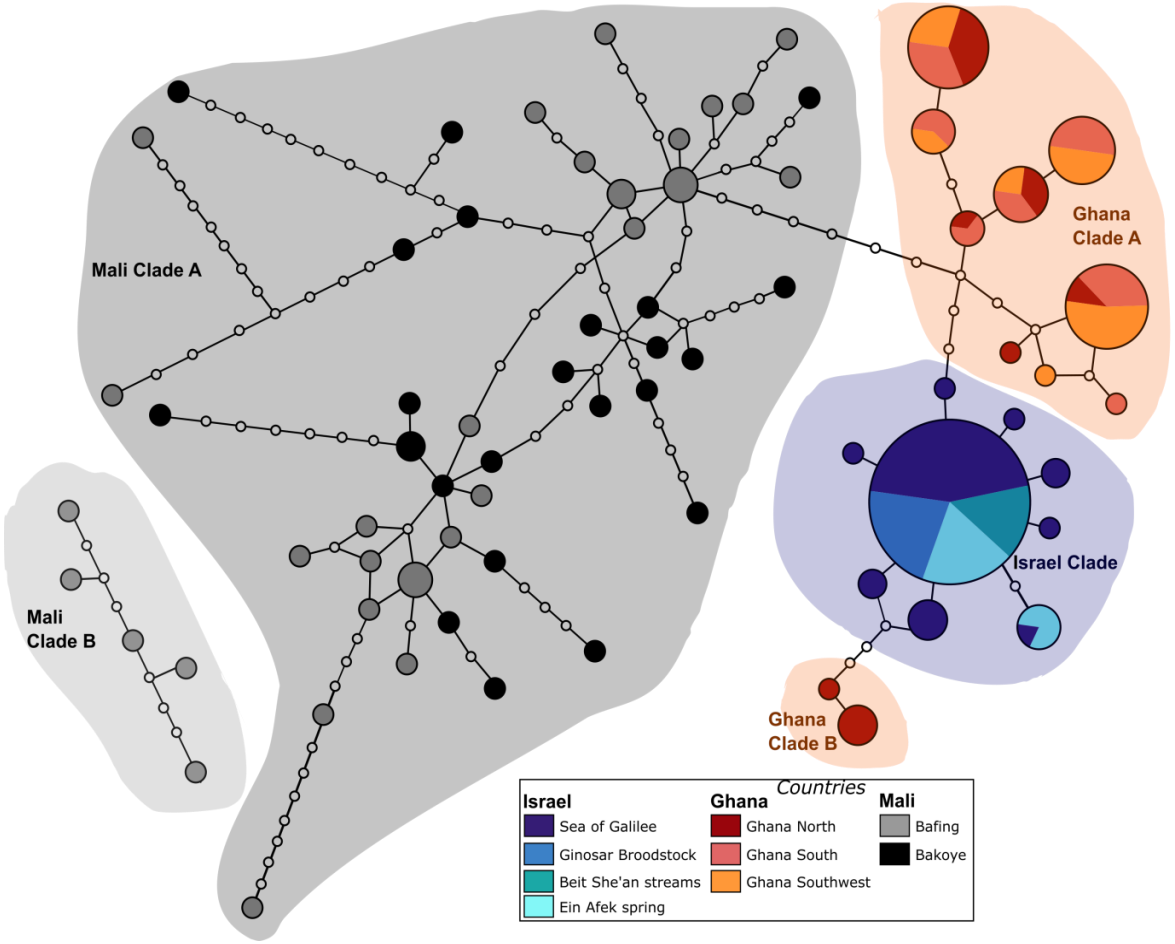

Figure 16: Network of haplotypes D-loop of *S. galilaeus* from Bafing, Bakoye from Mali, Ghana, and Israel places (95% connection limit); colors represent the sampling side, and the line represents the mutation steps. The white dots represent the missing haplotype. The areas of the circle are proportional to the number of individuals sharing the respective haplotypes.

4.8 Mitochondrial DNA (mtDNA) pairwise differences

The Mitochondrial DNA (mtDNA) pairwise differences between Bafing A and Bakoye B (Figure 17), also inferred by the statistics used, show no significance in all neutrality tests (D = -1.090, FS = 0.328 and R2 = 0.082, P > 0.10), and, it not possible to conclude an occurrence of a demographic expansion for the population. Indeed, the bell-shaped curve obtained for the Mismatch Distribution (MD) analysis of the pairwise differences indicated non-significant bimodal distribution in Bafing population samples. Further research is needed, especially regarding this population's movement pattern and separation rate.

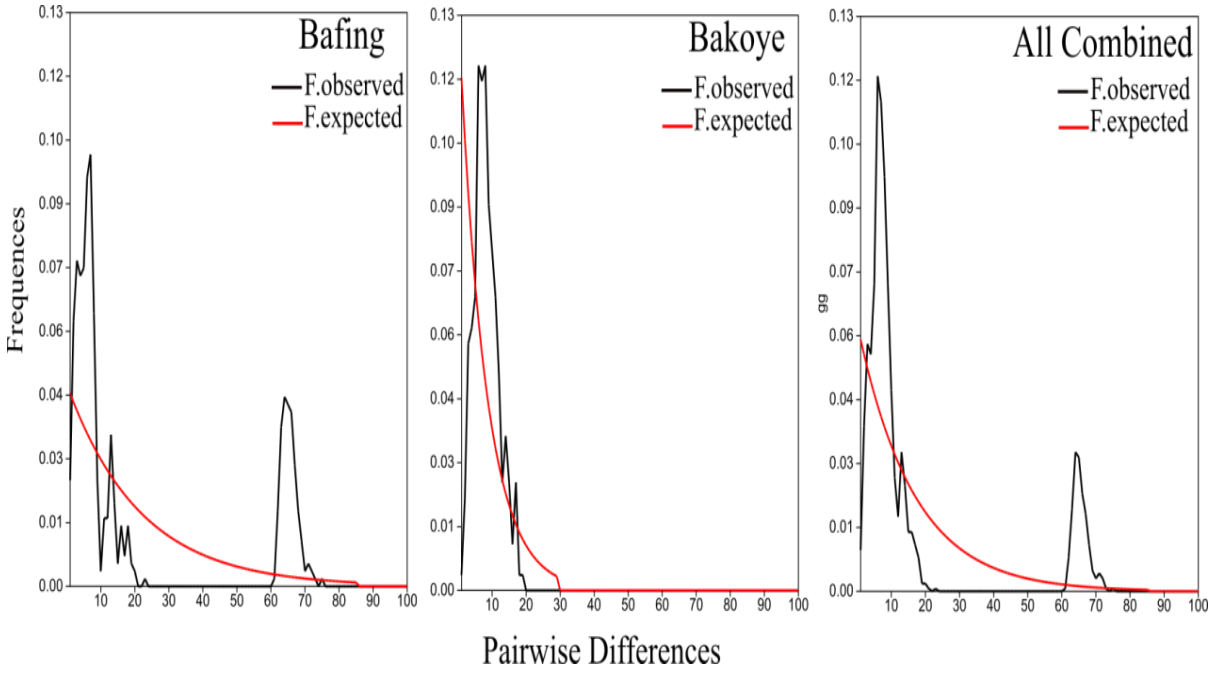

Figure 17: Mismatch distribution analysis showing bimodal (non-significant) analysis for the D-loop gene of *S, galilaeus* from Mali (Bafing, Bakoye). The expected and observed pairwise differences between the sequences with respective frequencies show a bimodal distribution in the Bafing river. The line in black shows the empirical pairwise-difference distribution, whereas the line in red is an equilibrium distribution with the same mean.

5. Discussions

5.1 Length-Frequency Distribution

The current study provided data sets on 20 morphometric indices, five meristic counts, weight, stage of gonads, and genetic data of *S. galilaeus* from Bafing and Bakoye rivers were used to characterise fish populations, as they are still reliable tools, especially in the field where more detailed diagnostic tools such as biochemical and molecular markers are not available. Furthermore, morphometric and meristic measurements are considered reliable tools as they are sensitive to environmental changes and susceptible to environmental changes (Fryer & Iles, 1972).

The length frequency distribution data have been used to identify and predict species at greater overfishing. The range of sizes of *S. galilaeus* from the Bafing and Bakoye rivers measured 7.98 cm to 21.41 cm. Abdul, W et al. (2015) reported a size range of 11 to 41 cm for the *S. galilaeus* in coastal Estuary Nigeria. Ebenezer (2010) reported a size range of 7.0 to 33.3 cm for *S. galilaeus* in Weija reservoir, Ghana. (Abdul et al. 2010) also reported a size range of 22-34 cm for *S. galilaeus* from the same water body when investigating the impact of the Iken brush park on the species.

The range of sizes of *S. galilaeus* from the Lake Guiers Senegal river measured 7.0 to 33.3 cm TL, and from the lake Ramitinga, Burkina Faso, measured 3.5 to 18.5 cm reported (Moreau et al.,1995). In this present study, the range length measured 7.98 to 24.41 TL cm from Bafing and Bakoye rivers in Mali. The more comprehensive size range in the present study and lake Guiers Senegal may be due to genetic factors and better environmental water conditions.

5.2 Total Length and standard length-weight total length relationship

The goals of this study were to determine the appropriate length unit for the analysis of the relationship between maturity, Length, and Weight, as well as morphometric analysis. Many researchers have studied the length-length relationship. Entsua-Mensah & Osei-Abunyewa (1995), Lalèyè (2006) and Kahn et al., (2004) observed a positive correlation between total Length (TL) and standard Length (SL), as this present study suggested. (Lalèyè,2006) reported that relationships between the total length and standard length of 50 fish species belonging to 18 families collected from the Ouémé River in Bénin (West Africa) were calculated using a total of 43.543 specimens. All relationships were highly significant (all $r2 > 0.90$, $P < 0.001$). The present study reports the highly significant (all $r2 > 0.90$, $P < 0.0001$ (Table 3).

The length-weight relationship of a fish is an essential factor in its productivity (Le Cren, 1951; Lalèyè, 2006; Tah et al., 2012). The total length (TL) of all the 139 specimens ranged between 7.98 and 24.41 cm and weighed between 10.80 g and 215.00 g. And the total Length (TL) of Bafing River, the 67 and 72 specimens sampled, ranged between 9.29 and 24.26 cm and weighed between 18.50 g and 215.00 g for Bafing River and then 7.98 and 21.07 cm and weighed between 10.80 g and 200.04 g for Bakoye River.

Du Feu, And Abiodun. J (1998) recorded positive allometric growth in Lake Kainji, Nigeria, and negative allometric growth was recorded for exploited *S. galilaeus* in the Buyo hydroelectrical dam on the Sassandra River. The negative allometric growth was recorded in Bafing and Bakoye Rivers. The present study also suggests that the slopes of the logarithmic weight-length relationships differed between the Bakoye River and the Bafing River. The log slope for log (Bakoye) is not significantly different from and less than the log slope for Bafing River of 2.482 (Table 6). The variation in the relationship between length-weight is not uncommon in *S. galilaeus* in African inland waters.

Sadio et al. (2021) reported the length-weight relationships of 19 fish species from two tropical artificial reservoirs (Manantali and Selingue) in Mali, with determination coefficient (R^2) values ranging from 0.90 to 0.99, thus the relationships between the total length and body weight are highly significant. In this present research, the R^2 coefficients were 0.88 from Bafing and 0.90 from Bakoye, and smaller than those shown by Sadio et al. (2021) and showed a significant relationship with negative allometry, i.e. the exponent b was < 3.

However, significant variability in length-weight relations may indicate changes in site environmental conditions, parasitism, harvest pressure, and other site-specific variables in the different reservoirs as in this present study by Bafing and Bakoye rivers.

Table 11: Length-weight relationships of some localities of *S. galilaeus* from the FishBase

Water Bodie	Country	N	R^2	TL Range cm	a	b	Reference
Bafing	Mali	67	0.883	7.98-21.07	0.083	2.482	Present study
Bakoye		72	0.901	9.29-24.26	0.048	2.708	
Lakes Doukon	Benin	1478	0.967	9.3 - 24.5	0.028	2.955	Lake Doukon, 2013
Lake Togbadji	Benin	1401	0.984	6.1 - 22.6	0.017	3.054	Lake Togbadji, 2013
Ouémé River	Benin	666	0.987	4.5 - 39.5	0.021	2.980	Ouémé River Basin, 1999-2001
Volta River	Burkina Faso	733	0.968	-	0.021	2.902	Béarez, P., 2003
Kainji Lake	Nigeria	59	0.982	12.4 - 35.7	0.014	3.136	Du Feu, T.A. & J. Abiodun, 1998

5.3 Length at first maturity (Lm50)

In fish stock management, size at first sexual maturity (Lm50) is essential. For example, in determining the optimal mesh size. The present study determined that the values for Lm50 in the Bakoye River were 16.99 - 15.09 cm TL for females and males respectively. Also, 11.37 - 16.99 cm TL for females and males, respectively, in the Bakoye River. Johnson (1974) reported 19.8 cm TL for *S. galilaeus* (males and females combined) from Lake Volta, Ghana. (Ita EO. 1982) also reported that the Lm50 were 15.5 - 16.8 cm TL for females and males, respectively, from Lake Kainji, Nigeria. Baijot and (Moreau 1997) measured the lengths in the small dams of Burkina-Faso were 12.6 and 13.8 cm TL for females and males, respectively.

Differences in maturity size between habitats could be related to food availability, quality, and fishing pressure (Pauly 1976; Panfili et al. 2006). However, due to the lack of mature data, it would be difficult to critically differentiate between the two rivers (Bafing and Bakoye) and the studies of other reservoirs and sampling efforts.

Table 12: shows the maturity length of some populations of *S. galilaeus* from the FishBase.

Water Bodie	Country	Lm50 females (cm)	Lm50 males (cm)	Reference
Bafing	Mali	16.99	15.09	Current study
Bakoye		11.37	16.99	
Lake Togbadji, 2013	Benin	11.07	12.04	Lederoun et al. (2016)
Small reservoirs, 1990	Burkina Faso	12.06	13.08	Baijot and Moreau, 1997
Volta Lake	Ghana	19.98		Johnson, 1974
Lake Tiberias or The Sea of Galilee	Israel	18		Ben-Tuvia, 1960

5.4 Morphometric measures and meristic counts

The morphological characters of 139 S. *galilaeus* from the Bafing and Bakoye rivers showed variations in the head (HL, CHD, and LAD) and the body (BD, CPD, and CPL) of 8 - 243 mm TL. Those reported by Trewavas (1983) focused on 19 fish of 62 - 223 mm SL from Huleh and Tiberias lakes (Kineret) and the Jordan River. As the proportions indicate in Table 13, these are deep-bodied fish with a small mouth, and the depth of the preorbital bone is from 22 - 5% length of the head at 70 mm SL. However, the results of Trewavas (1983) study revealed the differences in the body and head of *S. galilaeus*. Small heads were found in two specimens of 86 and 95 mm from the Jordan River (32-5 and 32-8%) and one of 201 mm from Khartoum (33-0%). Eleven specimens ranging from 62 to 270 mm SL from the Senegal and Gambia rivers are all deep-bodied, with a depth of 48-55% SL. The equivalent difference is revealed in the present study. Bakoye river specimens appeared slender and more elongated, and the head shape of the Bakoye River specimens was larger and more elongated.

The comparisons of meristic characters revealed a similarity to the morphometric characters and a slight variability between *S. galilaeus* specimens from Bafing and Bakoye. The AF, PC and LONG range were similar in the two rivers. The slight variability in the mean was related to the differences in counts (Table 14). And two of the five meristic counts AF and LONG also show a significant difference.

Other studies have reported similar analyses, in Hassan (2019) the mode of DFS, AFR, and PeFR were identical at the three sites studied. The slight variability in the mean was related to the differences in the number of features among the rivers. Abdul et al, (2019a) studied *S.*

galilaeus from the Ogun coastal estuary in Nigeria. They found that the mean values of most meristic traits were higher in the female populations than in the male populations, with a significant difference ($p < 0.05$) in pectoral fin radii. (Trewavas 1983) also reported the frequencies of the dorsal rays of the subspecies *S. galilaeus* in some water bodies like the Jordan Valley and West African rivers etc. His study showed that most frequencies are 28 and 29 dorsal fins. The present study suggests similar frequencies of 28 and 29 dorsal fins.

Table 13: Comparison of morphometric measurements of *S. galilaeus* from different water bodies. We convert the SL unit from Trewavas (1983) into a TL unit by applying the relationship SL = TL / 1.287.

Water Bodies	Number	Measurements	TL range	Source
Huleh, Triberias Lakes,		Body Depth (BD)	0.33 – 0.43	
And Jordan Valley	19	L. head (HL)	0.24 – 0.30	Trewavas (1983)
Bafing		BD	0.2 – 0.43	
Bakoye	139		0.01 – 0.38	Present
Bafing		HL	0.22-0.33	study
Bakoye			0.06-0.3	

Kiithana Pillay G (2016) carried out the differentiation between *Oreochromis* populations by morphological and molecular analysis. The T-test comparison revealed significant differences in seven variables for *O. karongae* between Lake Itamba and Lake Malawi. *O. karongae* from Lake Itamba have larger heads and longer tails, as they were significantly more significant for the variables LJL, PPL, DFB, and CPL, while individuals from Lake Malawi have larger eyes and wider tails, as they were significantly more significant for the variables ED and CPD. In addition, comparing populations of *O. shiranus* and *O. karongae* from Lake Itamba revealed significant differences for all variables except for BD, LJL, PPL, and PRA. *Oreochromis karongae* has larger heads and shorter bodies with longer and wider caudal peduncles, while *O. shiranui* has smaller heads and longer bodies with shorter and wider tails. However, the present study shows the same significant differences in body depth (BD) and head Length (LH). The *S. galilaeus* from the Bakoye River appeared to be slimmer and more elongated, and the head shape of *S. galilaeus* from the Bakoye River was larger and more elongated even though there is a difference in the method test used.

Descriptive statistics and Students' T-test analysis to differentiate meristic traits between the Bafing and Bafing Rivers *S. galilaeus* samples were as follows. The AF, PC and LONG range were similar in the two Rivers. The slight variability in the mean was related to differences in the count (Table 10). And also, two out of five meristic counts AF and LONG show a significant difference. Other studies have reported similar analyses; Hassan & Mahmoud (2021) showed that the mode of DFS, AFR, and PeFR was identical in the three sites studied. The slight variability in the mean was related to differences in trait counts between Rivers. They suggested higher mean values in most meristic counts of female populations than male populations, with a significant difference ($p<0.05$) in pectoral fin radii.

5.5 Population genetic variation in *Sarotherodon galilaeus*

In total, 311 samples from the location of Bafing, Bakoye, four from Israel, and three from Ghana were analysed for variations in the D-loop region of mitochondrial DNA (mtDNA) (Table 11; Appendix 2). D-loop sequence alignment showed a high number of haplotypes. The data from Ghana and Israel populations had the fewest haplotypes and the lowest values for haplotype diversity (h), the average number of pairwise differences (k), and nucleotide diversity (π), whereas Bafing and Bakoye had the most haplotypes. The nucleotide diversity values for the two rivers are not greater than 0.048 for the Bafing and 0.016 for the Bakoye. These findings suggest that besides having many haplotypes, these haplotypes are very different from each other. This result was also evident in the haplotype network, where the Mali haplotypes showed many mutations between them as opposed to the haplotypes from Israel and Ghana. In the neutrality test, Tajima's D was insignificant except for Israel's Sea of Galilee population ($D = -1.89$, $P < 0.05$).

However, according to (Borovski et al. 2018), the 250 samples from Israel and Ghana were examined for changes in the D-loop region of mtDNA. The research demonstrates no differences between the samples from the different areas of the Sea of Galilee, indicating that the lake has a single population. A comparison of all samples' 1004-bp D-loop sequences revealed 39 polymorphic sites that produced 52 haplotypes, 19 in Israeli and 33 in Ghanaian fish (online resources, Appendix 2). Two Israeli haplotypes that differed by only one nucleotide were present in 82% of fish from all locales. The remaining 17 Israeli haplotypes were rare, with frequencies of 4% or less, and varied by no more than five nucleotides from the most prevalent haplotype.

In comparison, the frequency distributions of the 33 haplotypes in Ghanaian groups were more equal, with the most divergent ones differing by 19 places. In the end, genetic variation consistently for D-loop, fish from Israel showed significantly less genetic variation than from Ghana. Considered all together, and bearing in mind that we analysed only part of the D-loop (455 bp), the Mali fish (Bafing and Bakoye) show high genetic variation, possibly due to the presence of the dam in the Bafing river (Manantaly) and seasonality flow in the Bakoye river.

Soliman et al. (2017) reported similar data on the genetic structure of the population and the genetic diversity of the red-bellied cichlid (*Coptodon zillii*) in three different Egyptian waters: Brackish water (Lake Idku), marine water (Al Max Bay) and freshwater (Lake Nasser). Habitat differences, environmental factors and fishing pressure are the main characteristics of the sampling sites. Three mitochondrial DNA markers (COI: cytochrome oxidase subunit I; the D-loop; CYTB: cytochrome b) were used to assess differences in population structure among the three populations. The freshwater population of Lake Nasser showed higher haplotype diversity and nucleotide diversity based on the D- loop region (Hd = 0.7717; π = 0.0046) and combined sequences (Hd = 0.8116; π = 0.0021).

The fish from the three sampling sites were a representative sample of at least two distinct ancestral populations, as shown by the haplotypes of clades A and B. In our network, two genetic clades were found among the Ghanaian fish; however, the genetic differences did not correspond to geographical differences. The fish in clade B represented additional Ghanaian ancestry identified only in the northern sample site. The results show a substantial difference between the north and southern areas. The Mali samples demonstrate the same style, with clade B showing a high contrast between clade A and is separated from the other clades.

Borovski et al. (2018) also proposed that most differences between Israeli and Ghanaian fish were discovered in mtDNA and nuclear polymorphisms. Some cichlid species unique to Israel most likely came from Africa, initially colonising the western coastal rivers and then spreading farther east. It is also possible that the initial inhabitants came from the northern area of Africa rather than the Volta River system. However, the common ancestry of Israeli and Ghanaian fishes could be presumed because most of the individual D-loop mutations were common and hence likely traced back to ancestors. These ancestors also have the variations that separate the Ein Afek population from other eastern Israeli fish, corroborating the postulated migratory path. Given these shared ancestors, the differences between Israeli and Ghanaian fish are most likely the result of a mix of post-segregation events, such as genetic drift, adaptation to local environments, and the development of novel variations. The current

study finds the same results, with substantial mutation rates among fish endemic to both rivers (Bafing and Bakoye). Except for Mali clade B, all clades have a common ancestor.

5.6 *Sarotherodon galilaeus* populations in Bafing and Bakoye have a high genetic variation

The D-loop variation in dry *Sarotherodon galilaeus* scales was investigated and compared to variance in the contemporary Sea of Galilee population (Borovski et al. 2018). The collected samples permitted PCR amplification and sequencing of only short fragments (455 pb) from 35 samples, occasionally covering locations 15,737-16,050 of the D-loop region. Each archived sample's discontinuous D-loop sequence was increased by a current sequence, resulting in a 314 bp long alignment of archived and modern haplotypes. The short forward D-loop sequences were supplemented in the alignment by the forward D-loop sequences which strengthened the genetic data. The genetic diversity of historical samples ($\pi = 1.41$) was much greater than that of present lake fish ($\pi = 0.15$). And Tajima's D index (neutrality test) of archived samples was not statistically significant (0.6, P = 0.74). The same results were found when the Bakoye River was studied, indicating the greatest haplotype gene diversity (Hd = 0.996).

Samples were taken in January, during the dry season in Mali, and the river did not flow continuously, suggesting a weaker connection downstream. Golden et al. (2021) suggested that location and river dryness might influence genetic structure in a metapopulation of an important Arctic fish species. They discovered that the genetic structure of Arctic grayling on the North Slope was linked to river removal and environmental isolation (dry riverine areas). Both variables were related to limited gene flow across populations, which aided drift and contributed to genetic heterogeneity between sampling locations. Asymmetric downstream gene flow was also discovered in catchments with downstream dry zones. Additional genetic patterns, such as decreased allelic richness in upstream and heterozygosity, is not only a genetically varied ancestral population that is important but also the downstream flow of genetic variants that attain greater frequencies either through drift or selection.

However, Schliewen et al. (1994) found some of the finest evidence for sympatric speciation in any cichlid using mtDNA sequence data from a 340 bp fragment of the Cytochrome *b* gene and a 350 bp fragment of the mtDNA regulatory area sequence. The present study suggests that the five specimens of clade B from Mali may be undergoing the same process. This species has a high potential for speciation, with a demonstrated situation of high

variation that has allowed the creation of five subspecies described by Trewavas (1983). Eventually, significant work has to be done to fully understand the evolution and spread of tilapia species in a geologically and climatically unstable environment. And more lately, there has been a molecular study utilising genetic methods spurred on by interest in African tilapia species in evolution, biodiversity, and aquaculture. In general, the new methodologies have helped to corroborate previous findings while also opening up numerous new and exciting study paths.

6. Conclusions and recommendations

The results obtained in this study established slight morphological differences in the morphometric and meristic traits of *Sarotherodon galilaeus* inhabiting the two rivers of Bafing and Bakoye. The analysis was based on descriptive statistics, the Wilcoxon rank sum test, and the Student's T-test to analyse morphometric measures and meristic traits. The relationships between weight and length, most commonly modelled with simple linear regression, yielded the exact significant p-value = < 2e-16 *** and a slightly different R-squared R^2=0.88 - 0.90 for the two rivers, Bafing and Bakoye. In addition, for the Bafing and Bakoye rivers, the allometric coefficients b (2.48 and 2.70) were significantly less than three. Analysis of Covariance reveals that the log-slope for log (Bakoye) was not significantly different from and less than the Bafing River log-slope of 2.482. The logistic regression equation was used in this study to determine Lm50, but due to the lack of mature data, it would not be easy to differentiate critically between the two rivers (Bafing and Bakoye) and the other reservoirs and samplings in the study.

The molecular analysis then revealed that differentiation between populations based on D-loop haplotypes of genetic diversity from the Bafing and Bakoye rivers, Ghana and Israel was possible. The data from the Ghana and Israel populations showed the lowest number of haplotypes as well as the lowest values of haplotype diversity (h), an average number of pairwise differences (k) and nucleotide diversity (π), and the highest number of haplotypes was found in Bafing and Bakoye. The π for the two rivers was low than 0.048 for Bafing and 0.016 for Bakoye. The neutrality tests of Fu's FS showed negative values, a significant marker of no expansion at any point. The D-loop of the mtDNA haplotype network showed that the central, most common ancestral haplotype is linked to many haplotypes. Except for the five haplotypes from Bafing, which were overlapped by the others, they are possibly subspecies. This similarity of haplotypes in Bafing and Bakoye means that there are no major significant differences in the haplotypes of *S. galilaeus* in these two rivers.

The results of the study could be helpful for the future conservation and management of the native and endemic ichthyofauna in Mali. Furthermore, the introduction of alien species into these water bodies should be avoided as they threaten the existing native and endemic fauna. The species sampled in this study represent only a small part of the total *S. galilaeus* population inhabiting the waters of Bafing and Bakoye in Mali. Larger morphological and molecular (mtDNA) datasets would be required to fully understand hybridisation between

closely related *S. galilaeus* populations and the effects on speciation and evolution of the Bafing and Bakoye *S. galilaeus* fauna.

However, there is a need to determine the factors favouring the growth and evolution of *S. galilaeus* in further studies on the two rivers Bafing and Bakoye in Mali and to extend the study to the whole Senegal River, which Guinea shares, Mali, Mauritania and Senegal. Therefore, for future work we recommend:

- To carry out for one year in these two Rivers Bafing and Bakoye to compare variations and spawning periods and to collect many data on the mature fish stage in the wet and dry seasons.
- To conduct for different fishing gears to compare variations in length weight concerning the different gears and choose the ideal size mesh.
- Further study, especially on movement patterns and separation rates in this population.

7. References

Abdel-Hamid, Z. G., Abd El-Kader, H. A. M., Aboelhassan, D. M., & Mahrous, K. F. (2014). Genetic diversity in Egyptian tilapia species using PCR-RFLP of D-loop mitochondrial DNA gene. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *5*(6), 469–475.

Abdul, W. O., Omoniyi, I. T., Bashir, A. O., Adekoya, E. O., Owoade, T., Hamzat, I., & Ogunnusi, O. O. (2019). Multivariate analysis of morphological traits of sexually mature Sarotherodon galilaeus (Linnaeus, 1758) in Ogun State Coastal Estuary, Nigeria. *Tropical Freshwater Biology*, *28*(1), 65-76.

Abdul, WO. (2015). Life history change in Silver Catfish, *Chrysichthys nigrodigitatus*, family Bagridae, in Ogun State Coastal Estuary, Nigeria. IJS. III: 691-700

Abu Gideiri, Y. B. (1984). *Fishes of the Sudan*, Khartoum, University Press, Democratic Republic of Sudan, 122 p.

Adite, A., Winemiller, K. O. & Fiogbe, E. D. (2005). Ontogenetic, seasonal, and spatial variation in the diet of *Heterotis niloticus* (Osteoglossiformes : Osteoglossidae) *i*n the Sô River and Lake Hlan, Benin, West Africa. *Environmental Biology of fishes.* 73(4) 367–378.

Agnèse, J. F., Adépo-Gourène, B., Abban, E. K. & Fermon, Y. (1997). Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae). *Heredity*, *79*(1), 88–96.

Akindele, T. A. & Fagbuaro, O. (2022). The morphometric characteristics and meristic traits and condition factors of *Sarotherodon galilaeus* from three major reservoirs of Ekiti State, Nigeria. *Asian Journal of Advances in Research,* 12(1), 1–11.

Alam, M. M., Jahan, S. N., Hussain, M. A., De, M., Goutham-Bharathi, M. P., Barroso Magalhães, A. L., Ghaffar Mazlan, A. & Das Simon, K. (2013). Length-length relationship, length-weight relationship and condition factor of freshwater fish species of Bangladesh. *Aquaculture, Aquarium, Conservation & Legislation*, *6*(5), 498–509.

Alves, C. B. M., Vieira, F., Magalhães, A. L. B., & Brito, M. F. (2007). Impacts of non-native fish species in Minas Gerais, Brazil: present situation and prospects. *Ecological and genetic implications of aquaculture activities*, 291-314.

Amisah, S & Agbo, N. W. (2008). An investigation into the food and feeding ecology of a potential aquaculture candidate, *Sarotherodon galilaeus multifasciatus* in a meteoritic crater lake in *Ghana. Journal of Applied Sciences and Environmental Management*, 12(3), 15-18.

Bailey, R. G. (1994). Guide to the fishes of the River Nile in the Republic of the Sudan. *Journal of Natural History*, *28*(4), 937-970.

Ben-Tuvia, A. (1960). The Biology of the Cichlid Fishes of Lake Tiberias and Huleh. B*ulletin of the Sea Fisheries Research Station, Israël*, 27, 153-188.

Bertram, C. R. (1942). Death of St. Peter's Fish in Lake Huleh, Palestine. *Nature*, 150(3793), 58-58.

Bodenheimer, F. S. (1935). *Animal life in Palestine : An introduction to the problems of animal ecology and zoogeography*. L. Mayer, Jerusalem, pp 1–506

Borovski, T., Tadmor-Levi, R., Shapiro, J., Rubinstein, G., Agyakwah, S. K., Hulata, G., & David, L. (2018). Historical and recent reductions in genetic variation of the Sarotherodon galilaeus population in the Sea of Galilee. *Conservation Genetics*, *19*(6), 1323–1333.

Burress, E. D. (2015). Cichlid fishes as models of ecological diversification: patterns, mechanisms, and consequences. *Hydrobiologia*, *748*(1), 7–27.

Clement, M., Posada, D e Crandall, K. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 10, 1657-1659.

Craig, M. T. (2007). Preliminary observations on the life history of the white-streaked grouper, Epinephelus ongus, from Okinawa, Japan. *Ichthyological Research*, *54*(1), 81–84.

Cucherousset, J., Bouletreau, S., Martino, A., Roussel, J. M., & Santoul, F. (2012). Using stable isotope analyses to determine the ecological effects of non-native fishes. *Fisheries Management and Ecology*, *19*(2), 111-119.

Dampin, N., Tarnchalanukit, W., Chunkao, K., & Maleewong, M. (2012). Fish Growth Model for Nile Tilapia (*Oreochromis niloticus*) in Wastewater Oxidation Pond, Thailand. *Procedia Environmental Sciences*, *13*, 513–524.

Deesri, U., Cavin, L., Claude, J., Suteethorn, V. & Yuangdetkla, P. (2009). Morphometric and taphonomic study of a ray-finned fish assemblage (*Lepidotes buddhabutrensis*, Semionotidae) from the Late Jurassic-earliest Cretaceous of NE Thailand. *Geological Society, Lonon, Special Publication*, 315 (1), 115–124.

Drummond, A. J. & Ashton, B. M. C., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Thierer, T. & Wilson, A. (2009). Geneious v4.8; http://www.geneious.com/.

Du Feu, T. A. & Abiodun, J. (1998). Predictions of fish yields and the status of the Kainji lake fishery (Vol. 16). *New Bussa, Nigeria: Nigerian-German Kainji Lake Fisheries Promotion Project Tech*. Rep 17.

Ebenezer, D. E. (2010). Aspect of the Biology of the Nile Tilapia (*Oreochromis niloticus*) in Weija reservoir Ghana". M.Sc. Thesis. *University of Cape Coast: Ghana*.135p.

Ebraheem, H. A. H. (2012). *Morphometrics, Meristics and Molecular Characterization of from Kosti, Sinnar, Khashm El Girba and Al Sabloga.* MSc Thesis.

El-Sayed, A.-F. M. (2020). Taxonomy and basic biology. Chapter 2. In El-Sayed, A.-F. M. (Eds) *Tilapia Culture*, Second Edition. Academic Press, Egypt, pp*21–29*.

Entsua-Mensah M., A. Osei-Abunyewa, M. L. D. P. (1995). Length-weight Relationships of Fishes from Tributaries of the Volta River, Ghana: Part I Analysis of Pooled Data Sets. *Naga, the Iclarm Quarterly: Vol. 18* (1), 36–38.

F Idowu, A., A Adedoyin, I., M Titilayo, O., A Quddus, A., & O Micheal, A. (2019). Morphometric and Meristic features and Length-Weight Relationship as indicators of quality of Brycinus macrolepidotus in Lower River Ogun, Nigeria. *Egyptian Journal of Aquatic Biology and Fisheries*, *23*(2), 433-441.

Nihoreye, F.J, Nyongombe, U.N, Alunga, L.G, Akonkwa, B.D, Isumbisho, M.P, Okitayela, O.F. (2019). Reproductive performance of female Nile tilapia Oreochromis niloticus L. in South Kivu (DR Congo): comparison of two strains differing in origin and history of

domestication. *International Journal of Scientific Research and Management*, *7*(08), 224–236. https://doi.org/10.18535/ijsrm/v7i8.mp02

Fagade, S. (1971). The food and feeding habits of Tilapia species in the Lagos Lagoon. *Journal of Fish Biology*, 3(2), 151–156.

Fagbuaro, O., Oso, J., Olurotimi, M., & Akinyemi, O. (2015). Morphometric and Meristic Characteristics of Clarias gariepinus from Controlled and Uncontrolled Population from Southwestern Nigeria. *Journal of Agriculture and Ecology Research International*, *2*(1), 39–45.

FAO, 2015. Lakes and rivers key to livelihoods of millions. Retrieved from: http://www.fao.org/news/story/en/item/276122/icode/ (Accessed on 08/11/2017)

FAO. (1995). *Handbook on small-scale freshwater fish farming, FAO Training Series No. 24* (p. 205). Département des pêches et de l'aquaculture de la FAO.

Fontoura, N. F., Braun, A. S. & Milani, P. C. C. (2009). Estimating size at first maturity (L50) from Gonadossomatic Index (GSI) data. *Neotropical Ichthyology*, *7*(2), 217–222.

Froese, R.& Pauly, D. (Eds.) (2018). FishBase. World Wide Web Electronic Publication. In www.fishbase.org. Version*:* 06/2018.

Fryer, G. & Iles, T. D. (1972). *The Cichlid Fish of the Great Lakes of Africa*. Oliver and Boyd, Edinburgh, 641.

Galman, O. R., Moreau, J., Hulata, G., & Avtalion, R. R. (1988). The use of electrophoresis as a technique for the identification and control of tilapia breeding stocks in Israel. *Pullin, T.; Bhukaswan, T*, 177-181.

Genner, M.J. & Turner, G.F. 2012. Ancient hybridization and phenotypic novelty within lake Malawi's cichlid fish radiation. *Molecular Biology and Evolution*, 29(1), pp.195–206.

Giddelo, C. S., Arndt, A. D. & Volckaert, F. A. M. (2002). Impact of rifting and hydrography on the genetic structure of Clarias gariepinus in eastern Africa. *Journal of Fish Biology*, *60*(5), 1252–1266.

Golden, H. E., Holsinger, K. E., Deegan, L. A., MacKenzie, C. J. A. & Urban, M. C. (2021). River drying influences genetic variation and population structure in an Arctic freshwater fish. *Conservation Genetics*, *22*(3), 369–382.

Hassan, A. H. A, Mahmoud, Z. N.; Elhg, A. A., El Sadig, A. Hagar & Hamid, M. M.(2020). *Meristic and Morphometric of Sarotherodon galilaeus from the Nile and its Tributaries, Sudan*, *IAR J Agri Res Life Sci*, 1(5)121–129.

Hassan, H., & Mahmoud, Z. (2021). Variability of Morphometric of Oreochromis niloticus, Sarotherdon galilaeus and Coptodon zilli from the Nile and its tributaries in Sudan. *Cross Current International Journal of Agriculture and Veterinary Sciences*, *3*(4–12), 12.

Hulsey, C.D., Hollingsworth, P.R. & Fordyce, J. A. 2010. Temporal diversification of Central American cichlids. *BMC evolutionary biology*, 10, p.279.

Jensen. (1958). Determination of age and growth of Tilapia nilotica L., T. galilaea Art., *T. zillii* Gerv. and *Lates niloticus* C. et V. by means of their scales. *Kongelige Norske Videnskabernes Selskabs Forhandlinger*, *30*, 150–157.

Jiang, B., Fu, J., Dong, Z., Fang, M., Zhu, W. & Wang, L. (2019). Maternal ancestry analyses of red tilapia strains based on D-loop sequences of seven tilapia populations. *PeerJ*, *7*, 1–14.

Kahn, R. G., Pearson, D. E. & Dick, E. J. (2004). Comparison of standard length, fork length, and total length for measuring west coast marine fishes. *Marine Fisheries Review*, *66*(1), 31–33.

Kendall, N. W., Dieckmann, U., Heino, M., Punt, A. E., & Quinn, T. P. (2014). Evolution of age and length at maturation of Alaskan salmon under size-selective harvest. *Evolutionary Applications*, *7*(2), 313–322.

Kpelly, E. D. (2010). *Aspects of the biology of the Nile tilapia (oreochromis niloticus L.) in the Weija reservoir (Ghana)* (Doctoral dissertation, University of Cape Coast).

Lalèyè, P. A. (2006). Length-weight and length-length relationships of fishes from the Oueme River in Benin (West Africa). *Journal of Applied Ichthyology*, *22*(4), 330–333.

Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *The Journal of Animal Ecology 20*(2) 201–219).

Lederoun, D., Vandewalle, P., Brahim, A. A., Moreau, J. & Lalèyè, P. A. (2016). Population parameters and exploitation rate of S*arotherodon galilaeus galilaeus* (Cichlidae) in Lakes Doukon and Togbadji, Benin. *African Journal of Aquatic Science*, *41*(2), 151–160.

Lee, W. J., Conroy, J., Howell, W. H. & Kocher, T. D. (1995). Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution*, *41*(1), 54–66.

Liebmann, E. (1933). Some observations on the breeding habits of Palestine Cichlidae. In *Proceedings of the Zoological Society of London* (Vol. 103, No. 4, pp. 885-888). Oxford, UK: Blackwell Publishing Ltd.

Loh, Y.H.E. et al., 2013. Origins of shared genetic variation in African cichlids. *Molecular Biology and Evolution*, 30(4), pp.906–917.

Lowe-McConnell, R. H. (2000). The roles of tilapias in ecosystems. *Tilapias: biology and exploitation*, 129-162.

Luo, C., Yang, P., & Wang, S. (2021). The complete mitochondrial genome of *Sarotherodon galilaeus* (Linnaeus, 1758) (Perciformes: Cichlidae) and its phylogenetic placement. *Mitochondrial DNA Part B*, *6*(3), 920-921.

Marcano, D., Cardillo, E., Rodriguez, C., Poleo, G., Gago, N. & Guerrero, H. Y. (2007). Seasonal reproductive biology of two species of freshwater catfish from the Venezuelan floodplains. *General and Comparative Endocrinology*, *153*(1–3), 371–377.

McMahan, C.D. et al. 2013. Temporal Patterns of Diversification across Global Cichlid 89 Biodiversity (Acanthomorpha: Cichlidae). *PLoS ONE*, 8(8), p.e71162.

Moreau, J., & Palomares, M. L. D. (1995). *Atlas d ,mographique des populations de poissons d'eau douce d'Afrique* (Vol. 1168). WorldFish.

Moreau, J., Palomares, M.L.D., Torres, F.S.B. & Pauly, D. (1995). Atlas demographique des populations de poissons d'eau douce d'Afrique. *ICLARM Tech. Rep*, 45–140.

Moushomi, R. & Saha, S. (2015). Landmark based shape variation of normal male, female and sex-reversed tilapia (Oreochromis niloticus) in Bangladesh. *Progressive Agriculture*, *26*(2), 168–172.

Murgia, R., Tola, G., Archer, S. N., Vallerga, S. & Hirano, J. (2002). Genetic identification of grey mullet species (Mugilidae) by analysis of mitochondrial dna sequence: Application to identify the origin of processed ovary products (Bottarga). *Marine Biotechnology*, *4*(2), 119–126.

Múrias dos Santos, A., Cabezas, M. P., Tavares, A. I., Xavier, R., & Branco, M. (2016). tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics*, *32*(4), 627-628.

Oladimeji, T. E., Awodiran, M. O., & Ola-Oladimeji, F. A. (2020). Morphological characterization of natural populations of *Sarotherodon galilaeus* (Linnaeus, 1758) from three selected reservoirs in south western, Nigeria. *Ife Journal of Science*, *22*(3), 85–96

Olumuyiwa, A. O., & Olatunde, F. O. (2015). Studies on aspects of morphometry and enteroparasitic infestation of S*arotherodon galilaeus* (Linnaeus, 1758), (Cichlidae) in Oba reservoir, Ogbomoso, Nigeria.

Omer, O. M., Abdalla, A. E. W. H., & Mahmoud, Z. N. (2020). Genetic Diversity of Two Tilapia Species () Using Random Amplified Polymorphic DNA. *The Open Biology Journal*, *8*(1).

Omotayo, F. (2015). Morphometric characteristics and meristic traits of *Tilapia zillii* from three major dams of a southwestern state, Nigeria. *Continental J. Biological Sciences*, *7*(1), 37–72.

Omotayo, F., Abayomi, O. J., Ola-Oladimeji, F. A., Tosin, O., & Oluwadare, A. (2016). Comparative biometric variations of two Cichlidae: Oreochromis niloticus and Tilapia zillii from a dam in Southwestern Nigeria. *American Journal of Research Communication*, *4*(5), 119-129.

OMVS (2018). Evaluation de la vulnérabilité du bassin du fleuve Sénégal face aux changements climatiques et élaboration d'un plan pour l'adaptation et le renforcement de la résilience du bassin, ainsi que l'actualisation des modèles de gestion des ressources

hydriques. Rapport de diagnostic d'evaluation de la vulnerabilite. Atelia Eau & Environnement-Branche ressources en eau et risques naturels, Echirolles, France, 450p

Onimisi, H.U. & Oniye, S. J. (2010). Length-weight relationship and condition factor of Auchenoglanis occidentalis in Zaria Reservoir, Nigeria. *Zoologist (The)*, 8, 20-24.

Pal, J., Shukla, B.N, Maurya, A. K., Verma, H. O., Pandey, G., & Amitha, A. (2018). A review on role of fish in human nutrition with special emphasis to essential fatty acid. *International Journal of Fisheries and Aquatic Studies*, *6*(2), 427–430.

Panfili, J., Thior, D., Ecoutin, J. M., Ndiaye, P., & Albaret, J. J. (2006). Influence of salinity on the size at maturity for fish species reproducing in contrasting West African estuaries. *Journal of Fish Biology*, *69*(1), 95-113.

Paternoster, R., Brame, R., Mazerolle, P., & Piquero, A. (1998). Using the correct statistical test for the equality of regression coefficients. *Criminology*, *36*(4), 859-866.

Pauly, D. 1976. The biology, fishery and potential for aquaculture of *Tilapia melanotheron* in a small West African lagoon. *Aquaculture* 7: 33-49.

Popma, T. & Masser, M. (1999). Tilapia Life History and Biology. *Southern Regional Aquaculture Center*, *USA*, *283*–286. in https://appliedecology.cals.ncsu.edu/wp-content/uploads/283.pdf.

Pullin, R. S. V., Management, I. C. L. A. R., & Pramong, T. K. (1988b). *The Second International Symposium on Tilapia in Aquaculture*. Department of Fisheries.

Quince, C., Abrams, P. A., Shuter, B. J., & Lester, N. P. (2008). Biphasic growth in fish I: Theoretical foundations. *Journal of Theoretical Biology*, *254*(2), 197–206.

Reis, R. E. (2003). *Check list of the freshwater fishes of South and Central America*. Edipucrs.

Reynolds, J. D., Dulvy, N. K., Goodwin, N. B., & Hutchings, J. A. (2005). Biology of extinction risk in marine fishes. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1579), 2337–2344.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S., & Sánchez-Gracia, A. (2018) DnaSP version 6 for 32-bit and 64-bit environments.

Sadio, O., Simier, M., Le Loch, F., & Tito De Morais, L. (2021). Length-weight relationships of 19 fish species from two tropical artificial reservoirs (Manantali and Selingue) in Mali, West Africa. *Cybium*, *45*(1), 59–62.

Schliewen, U. K., Tautz, D., & Pääbo, S. (1994). Sympatric speciation suggested by monophyly of Crater Lake cichlids. *Nature*, *368*(629–632).

Seehausen, O. 2004. Hybridisation and adaptive radiation. Trends in Ecology and Evolution, 19(4), pp.198-207.

Serajuddin, M., Prasad, L. & Pathak, B. C. (2013). Comparative Study of Length-Weight Relationship of Freshwater Murrel, *Channa punctatus* (Bloch , 1793) from Lotic and Lentic Environments. *World Journal of Fish and Marine Sciences*, *5*(2), 233–238.

Seyoum, S. & Kornfield, I. (1992). Identification of the subspecies of Oreochromis niloticus (Pisces: Cichlidae) using restriction endonuclease analysis of mitochondrial DNA. *Aquaculture*, *102*(1–2), 29–42.

Smith, A. (Smith, A. (1840)1840). Illustration of the Zoology of South Africa. *Smith, Elder and Co*, *4*, 5.

Snoeks, J. (1994). The Haplochromines (Teleostei, Cichlidae) of Lake Kivu (East Africa). In French. Musée Royal de l'Afrique Centrale, Tervuren, Belgique. In *Annales Sciences Zoologiques* (Vol. 270, pp. 1-221).

Snoeks, J. (2004). *The cichlid diversity of Lake Malawi/ Nyasa/ Niassa: identification, distribution and taxonomy.* Cichlid Press, El Paso, Texas.

Soliman, T., Aly, W., Fahim, R. M., Berumen, M. L., Jenke-Kodama, H., & Bernardi, G. (2017). Comparative population genetic structure of redbelly tilapia (*Coptodon zillii* (Gervais, 1848)) from three different aquatic habitats in Egypt. *Ecology and Evolution*, *7*(24), 11092–11099.

Stickney, R. R. (1986). Tilapia Tolerance of Saline Waters: A Review. *Progressive Fish Culturist*, 48 (3), 161–167

Streelman, J. T., Albertson, R. C. & Kocher, T. D. (2007). Variation in body size and trophic morphology within and among genetically differentiated populations of the cichlid fish, *Metriaclima zebra*, from Lake Malawi. *Freshwater Biology*, *52*(3), 525–538.

Sturmbauer, C. (1998). Explosive speciation in cichlid fishes of the African Great Lakes: A dynamic model of adaptive radiation. *Journal of Fish Biology*, *53*, 18–36.

Tah, L., Gouli, G. B., & Da Costa, K. S. (2012). Length-weight relationships for 36 freshwater fish species from two tropical reservoirs: Ayamé I and Buyo, Côte d'Ivoire. *Revista de Biologia Tropical*, *60*(4), 1847–1856

Thabet, R. (2017). Etude comparative de l ' élevage du Tilapia du Nil " Oreochromis niloticus " entre les eaux douces et géothermales en Tunisie. PhD Thesis, Institut Supérieur de Pêche et d'Aquaculture de Bizerte - Tunisie

Trewavas, E. (1982): Tilapias taxonomy and speciation. P. 3 – 13: in the biology and culture of Tilapias. R. S. V. Pullin and R. H., Lowe- Mc Connel (eds.) ICLARM conference proceedings 7, 432 p.

Trewavas, E. (1983). Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia, London, British Museum, 583 pp.

Turner, G.F. 2007. Adaptive radiation of cichlid fish. *Current Biology*, 17(19), pp.827–831.

Tyers, A. 2013. *Divergence and speciation of East African haplochromine cichlid fish.* Ph. D. thesis, Bangor University.

Ward, R. D. (2000). Genetics in fisheries management. *Hydrobiologia*, *420*(1), 191–201.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1847-1857.

Watanabe, W. O., Losordo, T. M., Fitzsimmons, K., & Hanley, F. (2002). Tilapia Production Systems in the Americas: Technological Advances, Trends, and Challenges. *Reviews in Fisheries Science, 10* (3–4) 465–498

Wohlfarth, G. W. & Hulata, G. I. (1983). *Applied genetics Tilapias*. ICLARM Studies and Reviews, 2nd edn. Vol. 6, 26 p.

Yang, L., Tang, H., Lin, X., Wu, Y., Zeng, S., Pan, Y., ... & Liu, X. (2020). OPA1-Exon4b binds to mtDNA D-loop for transcriptional and metabolic modulation, independent of mitochondrial fusion. *Frontiers in cell and developmental biology*, *8*, 180.

8. Appendix

8.1 Appendix 1: Fitting the Logistic Equation

The logistic equation has several applications in fishing. More than being used to indicate percent maturity as a function of size, it is also employed in toxicological investigations to define per cent survival as a function of dose level and to describe fishing mortality as a function of age (selection ogive). Until recently, the methods described in (Berkson 1944) were employed to fit the logistic equation to data. However, modern computer approaches for fitting nonlinear equations have rendered this method obsolete. Following is a quick explanation of how to fit the logistic equation and estimate the value of a and b equals fifty per cent. (Berkson's 1944) approach for fitting the logistic equation involves translating it into a linear form and then using weighted linear regression (Draper and Smith, 1966) to estimate the two parameters. The logistic equation may be written as:

$$\boldsymbol{log\frac{p}{1-p} = a + b\,SC}$$

$$\boldsymbol{\frac{p}{1-p} = e^{a+bSC}}$$

$$\boldsymbol{p = e^{(a+bSC)} * (1-p)}$$

$$\boldsymbol{p = e^{(a+bSC)} - pe^{(a+bSC)}}$$

$$\boldsymbol{1 = \frac{e^{(a+bSC)}}{p} - e^{(a+bSC)}}$$

$$\boldsymbol{1 + e^{(a+bSC)} = \frac{e^{(a+bSC)}}{p}}$$

$$\boldsymbol{p = \frac{e^{(a+bSC)}}{1 + e^{(a+bSC)}}}$$

Where p is the proportion of maturity of female or male, a and b are the coefficients of the equation and SC (size class)

$$\boldsymbol{L50\% = \frac{-a}{b}}$$

8.2 Appendix 2: Frequency distribution of haplotypes and Genbank (GB) accession numbers. The Israel and Ghana haplotypes illustrated here were taken from (Borovski et al. 2018).

Haplotype ID	GB accession number Numbers	**Israel**				**Ghana**			**Mali**	
		Seaof Galilee	Ginosar Broodstock	Beit She'an streams	Ein Afek spring	Ghana North	Ghana South	Ghana Southwest	Bakoye	Bafing
A1	KY940659	**10**	**10**	**5**	**9**					
A2	KY940660	**10**	**5**	**5**	**5**					
A3	KY940661	**6**								
A4	KY940662	**1**	**1**	**1**						
A5	KY940663	**3**								
A6	KY940664	**2**								
A7	KY940665	**2**								
A8	KY940666	**2**								
A9	KY940667	**1**								
A10	KY940668	**1**								
A11	KY940669	**1**								
A12	KY940670	**1**								
A13	KY940671				**4**					
A14	KY940672	**1**								
A15	KY940673	**1**								
A16	KY940674	**1**								
A17	KY940675				**1**					
A18	KY940676	**1**								
A19	KY940677	**1**								
B1	KY940678					**3**				
B2	KY940679					**1**				
B3	KY940680					**1**				
C1	KY940681					**1**	**4**	**3**		

C2	KY940682	**1**	**1**	**3**
C3	KY940683		**1**	**3**
C4	KY940684			**1**
C5	KY940685	**1**		
C6	KY940686		**1**	
C7	KY940687		**1**	
C8	KY940688			**1**
D1	KY940689		**3**	**3**
D2	KY940690	**2**	**3**	
D3	KY940691	**2**	**1**	**1**
D4	KY940692	**3**	**1**	
D5	KY940693		**1**	**2**
D6	KY940694		**2**	**1**
D7	KY940695			**2**
D8	KY940696		**1**	**1**
D9	KY940697		**1**	**1**
D10	KY940698		**1**	**1**
D11	KY940699		**2**	
D12	KY940700	**1**		
D13	KY940701			**1**
D14	KY940702	**1**		
D15	KY940703		**1**	
D16	KY940704		**1**	
D17	KY940705			**1**
D18	KY940706			**1**
D19	KY940707	**1**		
D20	KY940708	**1**		
D21	KY940709	**1**		
D22	KY940710		**1**	

H1	-	**1**
H2	-	**1**
H3	-	**1**
H4	-	**1**
H5	-	**1**
H6	-	**1**
H7	-	**1**
H8	-	**2**
H9	-	**1**
H10	-	**1**
H11	-	**1**
H12	-	**1**
H13	-	**1**
H14	-	**1**
H15	-	**1**
H16	-	**1**
H17	-	**1**
H18	-	**1**
H19	-	**2**
H20	-	**1**
H21	-	**1**
H22	-	**1**
H23	-	**1**
H24	-	**5**
H25	-	**1**
H26	-	**1**
H27	-	**1**
H28	-	**1**
H29	-	**1**

H30	-	**1**
H31	-	**1**
H32	-	**1**
H33	-	**1**
H34	-	**1**
H35	-	**1**
H36	-	**3**
H37	-	**1**
H38	-	**1**
H39	-	**1**
H40	-	**1**
H41	-	**1**
H42	-	**1**
H43	-	**1**
H44	-	**2**
H45	-	**1**
H46	-	**1**
H47	-	**1**
H48	-	**1**
H49	-	**1**