Sokoine University of Agriculture

MSc Dissertation

Assessment of Species Composition and the Genetic Population Structure of the Endemic Fishes in the Rufiji River Basin, Tanzania

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Assessment of Species Composition and the Genetic Population Structure of the Endemic Fishes in the Rufiji River Basin, Tanzania

A dissertation submitted to the Sokoine University of Agriculture in fulfillment of the requirements for the degree of Master of Science with education (biology)

By

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EXTENDED ABSTRACT

The Rufiji River Basin (RRB) is the largest river basin in East Africa and is home to many endemic fish species that are important for local livelihoods and food systems. However, unsustainable fishing practices driven by rapid population growth and high consumer demand have led to a concerning decline in these endemic fishes. In response two protected sites were established including, the Kilombero Valley Floodplain Ramsar site in 2002 and Nyerere national park to protect the endemic fish species from overexploitation. Yet, fish stocks are declining, and it is still unknown whether the implemented measures are consistent with the genetic stock structure of endemic fishes in the basin. Therefore, the aim of this study was to assess the composition of endemic fishes and the genetic stock structure of endemic fish *Bagrus orientalis* in the RRB. This study used fishers, and the key informant's interviews, Focus Group Discussion (FGDs), and fishery surveys to assess the composition of endemic fishes. DNA barcoding was also employed to confirm species identities. Tissue samples of 46 different endemic fish species and 182 of *B. orientalis* were collected from fishers at six landing sites in the RRB including Kivukoni, Mofu, Dinari, Ngalimila, Kidatu, and Zombe and preserved in 99.9% ethanol. Genomic DNA was extracted from each sample using the TIANamp Genomic DNA kit (TIANGEN Biotech, Beijing) and fragments (620 base pairs) of the cytochrome oxidase subunit I gene were amplified. The results showed 33 different fish species, out of which 54.55% are endemic and 45.45% are exotic. Additionally, it was revealed that *Heterobranchus longifilis* (Mjongwa), *Citharinus congicus* (Mbala), *Labeo congoro* (Ningu), *Mormyrus longirostris* (Sulusulu), and *Labeobarbus leleupanus* (Mkuyu) are very rare in the catch, despite being classified as Least Concern by IUCN. This suggests that rare species needs reassessment and

reclassification by IUCN because their current criteria does not reflect their actual status on the ground.

Furthermore, the results revealed significant genetic divergence between the populations of *B. orientalis* in the RRB (F_{ST} = 0.33, $p < 0.01$). Hierarchical AMOVA revealed that populations in the Ramsar site are genetically connected with those from other sites within the Kilombero Valley Flood Plain (KVFP), but genetically distinct from the populations in Ruaha and Rufiji. This implies that conservation efforts within the Ramsar site might not directly benefit the population in Ruaha and Rufiji. Therefore, it is recommended to establish the protected sites in Ruaha and Rufiji to rescue this population from further decline. The findings also showed that the low genetic diversity among population in Kidatu is due to restricted genetic connectivity, highlighting the need for enhancing habitat connectivity in the area. Moreover, the poor body condition and small sized fish were observed in Kidatu population. This does not conclude overfishing in the area but further studies on population dynamics and fishing pressure should be conducted to understand factors affecting size structure of the fish.

Keywords: Fish identification, genetic connectivity, genetic diversity, population genetic structure, DNA barcoding

IKISIRI KUU

Bonde la mto rufiji ni bonde kubwa katika Afrika Mashariki lenye spishi nyingi za samaki wa kienyeji ambao ni muhimu kwa maisha ya wenyeji na mfumo wa chakula. Walakini, vitendo vya uvuvi haramu vinavyosababishwa na ongezeko kubwa la idadi ya watu na mahitaji makubwa ya watumiaji, yamepelekea kupungua kwa kasi kwa samaki hao wa kienyeji.

Kutatua changamoto hiyo, maeneo mawili yaliyohifadhiwa yalifunguliwa ikiwa ni pamoja na, eneo la bonde la mafuriko la Kilombero mwaka 2002 na hifadhi ya taifa ya Nyerere ili kulinda spishi za samaki wa kienyeji kutokana na uvuvi haramu. Walakini, akiba ya samaki inapungua, na bado haijulikani kama hatua zilizochukuliwa zinaendana na muundo wa jenetiki wa samaki wa kienyeji katika bonde hilo.

Kwa hiyo, lengo la utafiti huu lilikuwa ni kutathmini muundo wa samaki wa kienyeji na muundo wa jenetiki wa samaki wa kienyeji aina ya Kitoga wajulikanao kisayansi kama “*Bagrus orientalis”* katika bonde la Rufiji.

Utafiti huu ulishirikisha makundi mbalimbali ukiwahusisha wavuvi, wataalamu wa uvuvi, Majadiliano ya Kikundi cha Kufanya Uchunguzi, ili kubaini usambazaji wa zamani na wa sasa wa samaki wa kienyeji. Pia, njia ya vinasaba zilitumika kuthibitisha aina za samaki.

Sampuli za tishu za spishi 46 tofauti za samaki wa kienyeji na 182 za Kitoga *B. orientalis* zilichukuliwa kutoka kwa wavuvi katika maeneo sita ya uvuvi katika bonde ikiwa ni pamoja na Kivukoni, Mofu, Dinari, Ngalimila, Kidatu, na Zombe na kuhifadhiwa katika ethanoli ya 99.9%. Vinasaba vilipatikana kutoka kwa kila sampuli kwa kutumia vifaa vya TIANamp Genomic DNA kit (TIANGEN Biotech, Beijing) na vipande (620 misingi) ya cytochrome oxidase subunit I vilikuzwa. Utafiti huu ulithibitisha spishi 33 tofauti za samaki,

kati ya hizo 54.55% ni za kienyeji na 45.45% ni za kigeni. Zaidi ya hayo, iligundulika kwamba Mjongwa *Heterobranchus longifilis*, Mbala *Citharinus congicus*, Ningu *Labeo congoro*, Sulusulu *Mormyrus longirostris*, na Mkuyu *Labeobarbus leleupanus* ni nadra sana katika mavuvi, licha ya kufanywa kama wasiwasi wa Chini na Chama cha Kimataifa cha Uhifadhi wa Asili (IUCN). Hii inaonyesha kuwa spishi nadra inahitaji tathmini upya na uainishaji upya na IUCN kwa sababu vigezo vyao vya sasa havionyeshi hali yao halisi ardhini.

Zaidi ya hayo, matokeo yalionyesha tofauti kubwa ya kijenetiki kati ya jamii za Kitoga *B. orientalis* katika bonde la mto Rufiji ($FST = 0.33$, $p < 0.01$).

AMOVA ya kihierarkia ilionyesha kuwa jamii ya Kitoga katika eneo lililohifadhiwa la Kilombero zinafanana kijenetiki na zile kutoka maeneo mengine ndani ya bonde la Kilombero, lakini zimejitenga kijenetiki na jamii kutoka Ruaha na Rufiji. Hii inaonyesha kuwa juhudi za uhifadhi ndani ya eneo la Kilombero huenda hazitawanufaisha moja kwa moja jamii ya Kitoga katika Ruaha na Rufiji.

Kwa hivyo, inapendekezwa kuweka maeneo yaliyohifadhiwa katika Ruaha na Rufiji ili kuokoa jamii hii isipotee zaidi.

Utafiti pia ulionyesha kuwa utofauti mdogo wa kijenetiki kati ya jamii katika Kidatu ni kwa sababu ya uunganisho mdogo wa kijenetiki, ikisisitiza umuhimu wa kuboresha uunganisho wa mazingira katika eneo hilo.

Zaidi ya hayo, hali dhoofu ya kimwili na samaki wenye saizi ndogo walionekana katika jamii ya Kidatu. Hii haimaanishi kuwa kuna uvuvi kupita kiasi katika eneo hilo lakini utafiti zaidi juu ya mienendo ya samaki na shinikizo la uvuvi unapaswa kufanywa ili kuelewa mambo yanayowaathiri muundo wa saizi ya samaki.

Ufunguo wa maneno: *utambuzi wa samaki, unganisho la jenetiki, utofauti wa jenetiki, vinasaba, muundo wa kijenetiki*

DECLARATION

I, **Jackson Lendoya Saiperaki**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work done within the period of registration, and that it has neither been submitted nor been concurrently submitted in any other institution.

_____ _____

Jackson Lendoya Saiperaki Date

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The above declaration is confirmed by

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Dr. Cyrus Rumisha Date

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Dr. Prisila Andrea Mkenda Date

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Dr. Elly Josephat Ligate Date

(Supervisor)

LIST OF PAPERS AND MANUSCRIPT

Saiperaki, J.L., Materu, S.F., Mkenda, P.A., Ligate, E.J. & Rumisha, C. (2024) Restricted genetic connectivity and conservation prospects of Bagrid catfish, *Bagrus orientalis*, populations in the Rufiji River basin, Tanzania. *Fisheries Management and Ecology*, 00, e12686. https://doi.org/10.1111/fme.12686

Saiperaki, J.L., Materu, S.F., Mkenda, P.A., Ligate, E.J. & Rumisha, C. Field and DNA-Barcode Based Surveys Reveal Evidence of Rare Endemic Fishes in the Rufiji River Basin. Under review in the *Tanzania journal of science*.

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Equally, I extend my thanks to my family, including my mother, brother, and friends, for their constant encouragement and support during the challenging moments of my academic pursuit. Their belief in me was a driving force.

Finally, I express my sincere appreciation to everyone who contributed to the realization of this dissertation. Your contributions have played a vital role in shaping this research endeavor.

DEDICATION

This dissertation is dedicated to my beloved mother Naseriani Lendoya and my beloved brother Emmanuel Lendoya

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

AMOVA	Analysis of Molecular Variance
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Data system
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CoEF	Conservation of Endemic Fishes
COI	Cytochrome Oxidase Subunit I
DNA	Deoxyribose nucleic acid
FGD	Focus group discussion
GIS	Geographic information system
GPS	Geographical positioning system
h	haplotype diversity
IUCN	International Union for Conservation of Nature
KVFP	Kilombero valley Floodplain
KVFPRS	Kilombero valley Flood Plain Ramsar site
LC	Least concern
NCBI	National Centre for Biotechnology Information
NT	Near Threatened
PCR	Polymerase chain reaction
RRB	Rufiji River Basin
SUA	Sokoine University of Agriculture
SUARIS	Sokoine University of Agriculture Research and Innovation Support
TZS	Tanzanian shillings
URT	United Republic of Tanzania
USD	United States Dollar
VU	Vulnerable
π	Nucleotide diversity

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Freshwater fish have long contributed to dietary animal protein, household income, and employment in riparian communities worldwide (Smith & Bennett, 2019). About 200 million Africans derive high-quality and low-cost proteins from fish (Obiero *et al*., 2019). In 2020, freshwater fisheries accounted for over 86% of the total fish production in Tanzania and the annual revenue from the industry was about two billion TZS (URT, 2021). However, as a result of unsustainable fishing practices fueled by rapid population growth and high demand for fish protein, many freshwater fish stocks in Tanzania, particularly those in the Rufiji River Basin (RRB), are declining rapidly (URT, 2019; Gebrekidan *et al*., 2020). In some areas within the RRB some endemic fish species have entirely disappeared in the catch (Msangameno & Mangora, 2016).

The decline in fish stocks is largely due to overfishing, destructive fishing practices and poor water quality resulting from unsustainable agriculture and overgrazing (Gebrekidan *et al*., 2020). For many years, the RRB has been used by the native tribes such as Ndamba and Pogoro, who traditionally engage in farming and fishing mainly for food support and cash income. A study by Turpie (2008) showed that indigenous people and local communities have a special connection with the dietary habits and taste of their food sources such as endemic fish species, in such a way that the disappearance of some fish species may lead to limited source of animal protein to some households.

The RRB contains the Kilombero Valley Floodplain (KVFP) which is the largest seasonal freshwater lowland floodplain

in East Africa, covering approximately 6,300 km^2 (Dinesen, 2016). The floodplain provides home to a large number of endemic fish species, as the results it has been designated as a Ramsar site since 2002. However, the floodplain continue to shrink and endemic fishes are declining dramatically due to pressures from agriculture and unsustainable fishing practices ranging from the use of poisons, dynamite fishing, beach seine nets and other destructive fishing techniques (BTC, 2015). Furthermore, despite the establishment of two protected sites within the RRB, namely the KVFP Ramsar site and the Nyerere National Park (Kijazi, 2020; Ramsar, 2002), it remains uncertain whether the fish stocks in the RRB consist of a singular stock connected to these protected sites or if there are distinct stocks necessitating separate management measures. Hence, there is a need to evaluate the genetic stock structure of endemic fishes in the RRB in order to ascertain whether the established protected sites are in line with the stock structure of endemic fishes. In addition, the overreliance of fishers and resource managers on local fish identification methods which primarily involve assessing the morphological traits of the fish such as body shape, scale counts, fin rays and behavioral patterns (Fischer 2013) has led to the mismanagement of the fish species. Therefore, accurate fish identification is essential for effective planning of sustainable management and conservation strategies (Ghouri et al. 2020).

Recently, genetic methods such as mitochondrial DNA analysis have been widely employed for fish species identification and stock validation (Rumisha et al., 2023; Trivedi, 2016). Likewise, these methods have proven valuable in identifying distinct fish populations, assessing genetic diversity, understanding migration patterns and estimating effective population size (Filho et al., 2018; Simwanza et al., 2023). The use of genetic methods has

become essential in sound fish stock management enabling informed decisions regarding sustainable fisheries management and conservation genetic diversity (Appleyard *et al*., 2021; Waples & Naish, 2009).

1.2. Statement of the problem

The RRB is a natural wetland ecosystem comprising a myriad of rivers, which make up the largest river basin in East Africa, covering approximately 177,429 km^2 (Manongi, 2023). The basin is threatened by livestock grazing, crop farming and unsustainable fishing practices (Gebrekidan *et al*., 2020), which imperil the efforts of the government to ensure the conservation of biodiversity hotspots as stated in the National Biodiversity Strategy and Action Plan (URT, 2020). The RRB is among the biodiversity hotspots with significant number of endemic fish species (Ramsar, 2002). *B. orientalis* is among of the endemic fish in the RRB that is highly fished and preferred by indigenous people due to its flavour and larger size reaching up to 44.7 cm at maturity. Apart from being rich in biodiversity, the basin is endowed with a good climate, fertile soil, minerals and other resources including hydroelectric potentials (Kiwia, 2013). These potentials have led to a rapid increase in human population and a rising demand for fish proteins (Mwalyosi, 1990). The fast population growth (URT, 2022) and escalating fish demand have led to habitats degradation due to intensified human activities and increased use of destructive fishing methods (Munishi & Jewitt, 2019). These resulted into a rapid decline of fish catch and disappearance of some fish species (Msangameno & Mangora, 2016). This affects negatively the household income, food security and nutrition of the riparian communities across the fish value chains. To offset the effects, the Ramsar site was designated within the KVFP in 2002 (Ramsar, 2002), and Nyerere National Park was established (Kijazi, 2020). Yet it is not known whether the

basin contains panmictic fish stocks that are connected to these protected sites. Therefore, this study used partial fragments of COI to reveal the composition of endemic fishes and the patterns of genetic connectivity of *B. orientalis* populations in the RRB.

1.3. Research Objectives

1.3.1. General objective

To assess the composition and the genetic stock structure of endemic fish species in the RRB to guide conservation and management decisions.

1.3.2. Specific objectives

a. To determine the composition of endemic fishes in the RRB through social surveys and DNA barcoding to pinpoint biodiversity hotspots.

b. To determine the genetic population structure of the endemic fish *Bagrus orientalis* in the RRB.

1.4. Research questions.

a. What is the composition of endemic fishes in the RRB?

b. Does the endemic fish *Bagrus orientalis* in the KVFP constitute a homogenous stock?

CHAPTER TWO

Field and DNA-Barcode Based Surveys Reveal Evidence of Rare Endemic Fishes in the Rufiji River Basin.

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The material contained in this chapter is has been submitted and is under review in the *Tanzania journal of science*.

ABSTRACT

Endemic fish species have long supported the livelihoods of local communities in the Rufiji River Basin (RRB). However, destructive fishing practices have led to a concerning decline in endemic fish stocks. To assess these changes, this study employed key informant interviews, focus group discussions (FGDs), and fishery surveys to assess the historical and contemporary distribution of endemic fishes within the RRB. DNA barcoding was also used to verify species identities. Out of 37 reported fish species, 33 species (54.55% endemic and 45.45% exotic to RRB) were confirmed through DNA barcoding and morphological characteristics. About 5 species including, *Heterobranchus longifilis, Citharinus congicus, Labeo congoro, Mormyrus longirostris,* and *Labeobarbus leleupanus* were rarely found in the field, despite being classified as Least Concern by IUCN. Additionally, five species that were reported to be present in the RRB by experienced fishers were not captured during sampling. This highlights the need for validation of the existence of such species through eDNA metabarcoding. Moreover, due to the rarity of some species in the area, their IUCN assessment should be revisited.

Keywords: DNA barcoding, fish conservation, fish identification, phylogenetic analysis, Tanzania.

1. Introduction

Freshwater fish have traditionally been a significant source of animal protein, income and employment to riparian communities globally (Sayer et al. 2018). In 2020 freshwater fisheries in Tanzania contributed for over 86% of total fish production and generated around two billion TZS (URT 2021). However, unsustainable fishing practices driven by rapid population growth and high demand for fish protein (URT 2019, Gebrekidan et al. 2020) have resulted in a rapid decline of freshwater fish stocks, particularly in the Rufiji River Basin (RRB).

The decline in fish stocks in the RRB can be attributed to destructive fishing practices, such as poison fishing, dynamite fishing and the use of beach seine nets, as well as poor water quality from unsustainable agriculture and overgrazing (BTC 2015, Gebrekidan et al. 2020). Additionally, the basin continues to shrink and the number of endemic fish species are declining due to land use change (Msofe et al. 2020). The native tribes of the RRB such as Ndamba and Pogoro have been engaged in fishing since time immemorial, but in the last 1-2 decades there is a great shift to crop farming as alternative source of food and livelihood support. Ndamba and Pogoro have a strong connection with endemic fish species, and thus their disappearance could have severe implications for household animal protein sources (Turpie 2008).

Despite the designation of the Kilombero Valley Floodplain (KVFP) as a Ramsar site in 2002 and the establishment of the Nyerere National Park within the RRB (Ramsar 2002, Kijazi 2020), the conservation of fish stocks in the RRB remains a critical issue. Unprotected areas within the RRB face significant fishing pressure, raising concerns about the potential disappearance of certain species from local catches

(Nindi et al. 2014). Currently, the available information on the species composition in the region dates back over 20 years, originating from a study that identified 23 fish species in the RRB (Utzinger and Charlwood 1996). However, this study relied solely on morphological identification methods alone, which raise concerns about the potential existence of cryptic species and the possibility of misidentification (Dudgeon et al. 2012). Such inaccuracies can skew population assessments and conservation priorities, potentially leading to inadequate protection measures for vulnerable species. This lack of accurate and up-to-date information on species composition hinders conservation efforts, making it challenging to implement targeted interventions to protect vulnerable species and maintain ecosystem balance. Additionally, prevalent illegal fishing activities in the RRB (Nindi et al. 2014) exacerbate these challenges, posing a direct threat to fish populations, especially rare and vulnerable species. Without accurate data on species composition and population dynamics, addressing and mitigating the impacts of illegal fishing activities become even more difficult. Hence, there is an urgent need to implement comprehensive monitoring programs that integrate advanced molecular techniques like DNA barcoding alongside traditional methods. This study integrated DNA barcoding and morphological identification techniques to reveal the composition of endemic fish species in the RRB. These approaches have been previously used in the country to uncover non-targeted tilapias among farmed fish and unveil protected elasmobranchs in Tanzanian fish markets (Mbilinyi et al. 2023; Rumisha et al. 2023). These approaches will provide more accurate assessments of species composition in the RRB, enabling better-informed conservation strategies and ensuring the long-term sustainability of its fish stocks.

2. Material and methods

2.1 Study Site

The present study was conducted in the RRB which consists of the Kilombero River, the Great Ruaha, the Rufiji River and other small rivers (Mcclain and Williams 2016). Six landing sites in the RRB including Kidatu, Kivukoni, Mofu, Dinari, Ngalimila and Zombe were selected based on the availability and accessibility of landing sites (Figure 1). The RRB lies between 5.7° to 10.5°S and 33.5° to 39°E, covering an area of about 177,429 km2, which accounts for 20% of the total land area of Tanzania (Mwalyosi 1990). The RRB includes the KVFP, the largest seasonal freshwater lowland floodplain in East Africa which is rich in biodiversity (Ramsar 2002). It contains the Kilombero Valley Ramsar site (KVRS), an internationally recognized site of local and international importance. The Ramsar site covers an area of 796,735ha with the wetland catchment area of 40,000 km^2 (Ramsar 2002). The RRB also contains Nyerere National Park, which is the largest National Park in Africa covering an area of over 30,000 km^2 (Kijazi 2020, Mkwizu 2022).The main economic activities in the RRB are fishing, crop production and livestock keeping (Wilson et al. 2017). The climatic condition of the RRB varies from tropical humid in the east to temperate in the southern highlands. In the east, the mean daily annual temperature is around 39 °C while it is around 23 °C in southern highlands (Mwalyosi 1990). The rainfall ranges from 250 mm in some areas to over 1800 mm on the east of the Udzungwa Mountain (Mwalyosi 1990).

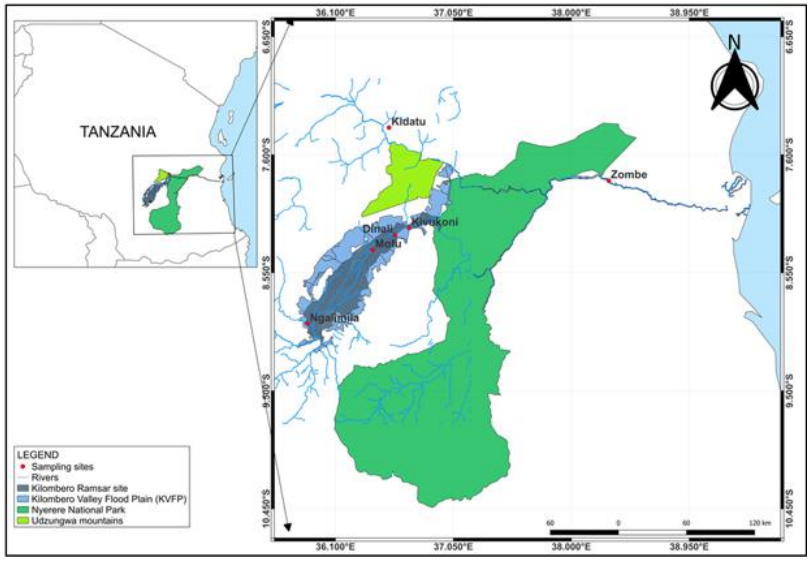

Figure 1: Map of Map showing the sampling sites in the Rufiji River Basin (RRB). Source: Created with the quantum GIS software ver. 3.32 and shapefiles from the Database of Global Administrative Areas (https://gadm.org/maps/TZA.html. Accessed 10 June 2022

2.2 Data collection

Fish sampling was conducted during two sampling seasons, between July 2022 (the onset of the dry season) and January 2023 (the onset of the wet season). A total of 46 different species were collected at six landing sites of the RRB. Fish were initially identified using the available fish identification keys (Eccles 1992; Genner et al. 2018). Fish species that showed potential differences from those already sampled were specifically collected from each landing site. For every landing site, where samples were taken (Table 2.1), coordinate points were recorded using a Geographical Positioning System (GPS) device. Fin clip tissues of about 0.05 grams were cut from each fish, stored in 1.5 ml microcentrifuges, and preserved using 99.9% ethanol until

further analysis. Additionally, three focus group discussions were conducted to gather information about species composition, local fish identification techniques, fishing trends and fish management strategies. In-depth interviews were conducted with 4 groups of key informants including village elders, environmental management officers, fisheries officers, and village chairpersons to gather information about the composition of fish in the RRB, and local fish identification techniques.

Table 1: Central coordinates and numbers of fins clips sampled within the Rufiji River Basin (RRB) between July 2022 and January 2023.

Site	**Coordinates**		**Number of fin clips sampled**
	Latitudes (º S)	Longitudes (º E)	
Rufiji River			
Zombe	7.80	38.30	2
Ruaha River			
Kidatu	7.38	37.92	2
Kilombero River			
Kivukoni	8.19	36.69	34
Mofu	8.36	36.40	1
Dinari	8.24	36.58	2
Ngalimila	8.96	35.87	5
Total			**46**

2.3 DNA extraction, COI amplification, and sequencing

Genomic DNA was extracted from each sample using the TIANamp Genomic DNA kit (TIANGEN Biotech, Beijing) according to the manufacturer's protocol. Then the quality of each DNA extract was checked on 1% agarose gel before further analysis. Thereafter, fragments (620 base pairs) of the Cytochrome Oxidase Subunit I gene (COI) were amplified from the DNA extracts of each sample in a T100™ Thermal cycler machine (Bio-Lab Inc, GA, USA) using the Forward primer FishFI: 5'-TCAACCAACCACAAAGACATTGGCAC-3'and the reverse primer FishR1: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'. Amplification reactions were done in a total volume of 35 µL consisting of 2 µL template DNA, 1 x OneTaq 2X Master Mix with Standard Buffer (New England BioLabs Inc., MA, USA), 5 mg bovine serum albumin and 0.3 µM of each primer. Each reaction was initially denatured at 94 °C for 5 min, followed by 35 cycles of 94 °C for 40 s, 54 °C for 45 s and 72 °C for 60 s. The final extension of 72 °C for 15 min was added to ensure complete elongation. The quality of each PCR product was checked on a 1% agarose gels. The successful PCR amplicons were Sanger sequenced by Macrogen Europe Laboratory in the ABI 3730XL automated sequencer using the BigDye Terminator v3.1 technology (Applied Bio systems, Foster City, USA).

2.4 Data analysis

A total of 46 samples were successfully analysed. The obtained sequences were edited to trim the ends and aligned using ClustalW algorithm as implemented in the program MEGA ver. 11 (Tamura et al. 2021) to obtain sequences with equal length of 600 base pairs. Each sequence was then compared with COI sequences in the GenBank Nucleotide Database using the BLAST (Basic Local Alignment Search

Tool) and BOLD (Barcode of Life Data System). The sequences were then submitted to GenBank and accession numbers (OQ908874- OQ918545) were provided. At least 90.91% of the unknown fish were identified to species level and the samples were classified to family, genus and species following the Linnaean taxonomy.

The evolutionary history was inferred by using the maximum likelihood method and Tamura-Nei model. The bootstrap consensus tree inferred from 500 replicates was used to analyse the evolutionary history of the identified species. Branches corresponding to partitions reproduced in less than 75% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test 500 replicates was shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The COI sequence of Leopard whip ray *Himantura leoparda* with the accession number JX263418 were retrieved from GenBank and included in the dataset as an outgroup.

3. Results

3.1 Fish diversity

Fishers and the key informants mentioned a total of 37 different fishes found within the RRB. About 5 fish species were not verified during fishery survey suggesting that they are either no longer abundant in the wild or they are present in a very low number (Table 2.2). About 5 species including Mjongwa *H. longifilis,* Mbala *C. congicus,* Ningu *L. congoro,* Sulusulu *M. longirostris, and* Mkuyu *L. leleupanus* were rarely found in the field. Moreover, Fishers in the RRB used the local identification techniques such as fish morphology

including the size of the fish and structure of fins to identify fish. This knowledge was obtained from village elders and the experienced fishermen. The provided local names, however, do not reflect the Linnaean taxonomy and the DNA barcoding results. For example, species of Ngogo *Synodontis multipuctatus* was named as Ngogo ng'andu and Ngogo mwanajeshi while *Labeo congoro* was named as Mtuku and Ningu depending on morphological characteristics and stage of development. Additionally, one local name was given to more than one species, particularly those with similar morphologies. For example, two different species; *Glossogobius giuris* and *Eleotris klunzingerii* were reported as Bubu mchanga while *Hippopotamyrus* spp*.* and *Petrocephalus affinis* were reported as Ndipi (Table 2.2). However, although fishers could distinguish matured Bula *Schilbe moebiusii* and Luepe *Eutropiellus longifilis*, they could not distinguish juveniles of these species due to their similar morphologies**.**

3.2 Confirmation of morphologically identified species through DNA barcoding.

A total of 46 COI barcode sequences representing 33 different species belonging to 24 different genera, 11 different families and 8 different orders were obtained from the sampled specimens. About 18 (54.55%) out of 33 species were endemic while 15 (45.45%) species were exotic to RRB (Fig. 2.3). The endemic species revealed from this study were Njuju *Brycinus affinis*, Mtuku or Ningu *Labeo congoro*, Bula *S. moebiusii*, Ndipi mdomo mrefu *Marcusenius macrolepidatus*, Ndungu *Distochodus petersii*, Njege *Hydrocynus tanzaniae*, Kitoga *Bagrus orientalis*, Ngogo ng'andu *Synodontis rufigiensis*, Ngogo mweusi *Synodontis rukwaensis*, Mbala *C. congicus*, tilapia *Oreochromis urolepis*, Benasongo *Enteromius apleurogramma*, Mbewe *Brycinus* spp, Luepe *E. longifilis*,

Mgundu *Alestes stuhlmanni*, Ndipi *Petrocephalus affinis*, Ndipi *Hippopotamyrus* spp and Ndipi mdomo mfupi *Marcusenius livingstonii*. About 13 different fish species were identified using GenBank and BOLD databases. However, higher identities (98.87%-99.84%) failed to confirm Ndipi *P. affinis* and Sulusulu *Mormyrus longirostris* while low identities confirmed Mbala *C. congicus* (93.96%) and Ndipi kongwe *Pollimyrus nigrican* (96.73%) in GenBank database (Table 2.3). The taxonomic identity of 20 different fish species were not confirmed using DNA barcode alone due to lack of reference barcodes in the GenBank and BOLD databases. Therefore, the integration of DNA barcode results and morphological identification was used to confirm the identity of the 20 fish species. Yet, the identification sheets were poor for Ndipi *Hippopotamyrus* spp, Mbewe *Brycinus* spp and Gugutuu *Ctenopoma* spp.

3.3 Phylogenetic analysis of experimental fish species

The maximum likelihood phylogenetic tree was constructed from 46 nucleotide sequences with the total of 639 positions present in the final dataset. Phylogram (Fig. 2.2) was divided into 7 main clades. Clade I, II, III, IV, V and VII consisted of species from different orders and families. Within each clade, species belonging to the same family were clustered together. However, certain species within these clades such as Luepe *E. longifilis*, Bubu mchanga *G. giuris*, Njuju mkapa *Brycinus sadleri* and Ndungu *D. Petersii*, Kitoga *B. orientalis* and Bula *S. moebiusii* originated separately from their respective clades. Six different species (Mjongwa *H. Longifilis*, Ngurufi *Labeo coubie*, Perege *O. urolepis*, Ndipi mdomo mfupi *M. livingstonii*, Njege *H. tanzaniae* and Gugutuu *Ctenopoma* spp) do not originate from any of the clades. Clade VI included 10 species, showing difference in the position of Kitoga *B. orientalis* (family Bagridae) that

originated separately from the clade. Sheta *Clarias werneri* and Kambale *C. gariepinus* belonging to the order Siluriformes and family Clariidae originated from the same cluster. The same is true for Ngogo dongo or Ngogo mwanajeshi *S. multipuctatus* and Ngogo ng'andu *S. rufigiensis* belonging to the same order and family Mochokidae. This indicates that the amplified barcodes correctly identified the species. However, Mkuyu *Labeobarbus leleupanus* (order Cypriniformes) and Mbewe *Brycinus* spp (order Characiformes) were clustered together.

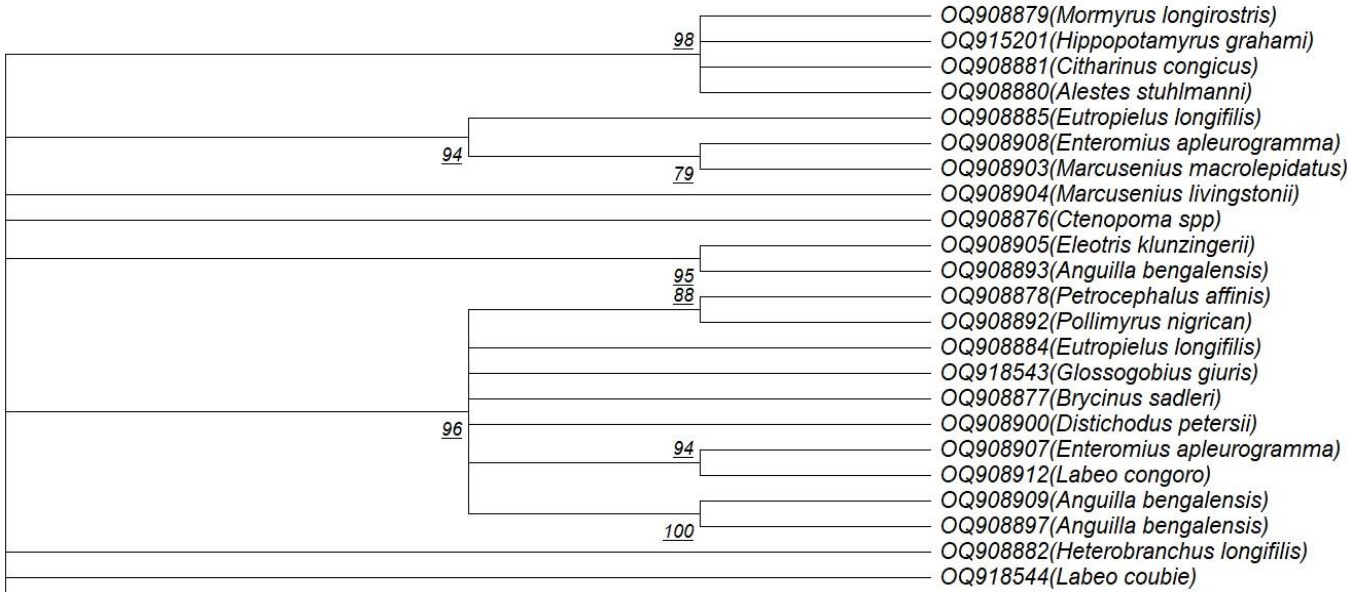

Figure 2a: Phylogenetic analysis of endemic and exotic fish species sampled in the Rufiji River Basin (RRB) between July 2022 and January 2023 based on the COI gene.

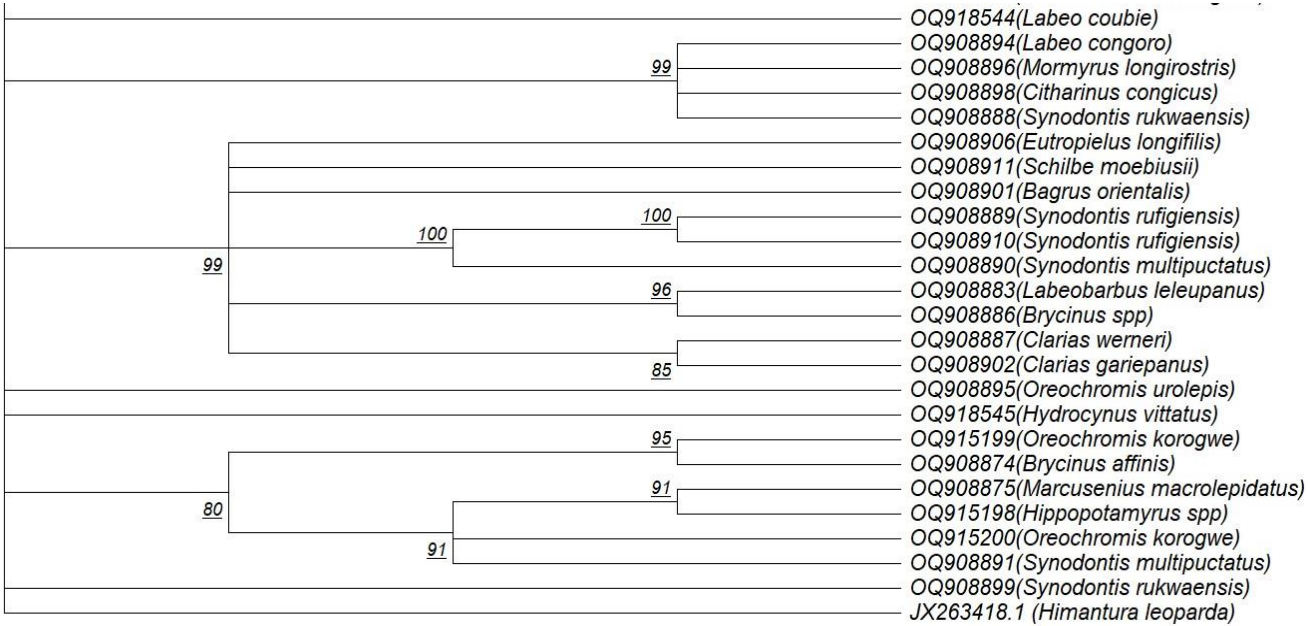

Figure 2b: Phylogenetic analysis of endemic and exotic fish species sampled in the Rufiji River Basin (RRB) between July 2022 and January 2023 based on the COI gene

3.4 Conservation status

About 90.91% (30 different fish species) of the identified species are categorised by IUCN as least concern (LC), 3.03% as near threatened (NT), and 6.06% as vulnerable (VU; Table 2.3). Hence, none of the sampled fish species is either endangered or critically endangered. Similarly, none of the sampled fish species is either CITES-protected or protected by Tanzanian laws.

Table 2: The local names of endemic fishes sampled in the Rufiji River Basin (RRB) between July 2022 and January 2023 and their corresponding Linnaean classification according to DNA barcode results.

		Linnaean classification		
No	**Local name**	**Family**	**Genus**	**Species**
1	Benasongo	Cyprinidae	Enteromius	*Enteromius apleurogramma*
2	Bubu mchanga	Gobiidae	Glossogobius	*Glossogobius giuris*
3	Bubu mchanga	Eleotridae	Eleotris	*Eleotris klunzingerii*
4	Bula	Schilbeidae	Schilbe	*Schilbe moebiusii*
5	Gugutuu	Anabantidae	Ctenopoma	*Ctenopoma* spp
6	Jwalajwala	**Not verified**		
7	Kambale	Clariidae	Clarias	*Clarias gariepinus*
8	Kibenamdenge	**Not verified**		
9	Kitoga	Bagridae	Bagrus	*Bagrus orientalis*
10	Luepe	Schilbeidae	Eutropielus	*Eutropiellus longifilis*
11	Mbala	Citharinidae	Citharinus	*Citharinus congicus*
12	Mbewe	Alestidae	Brycinus	*Brycinus* spp
13	Mgundu	Alestidae	Alestes	*Alestes stuhlmanni*
14	Mjongwa	Clariidae	Heterobranchus	*Heterobranchus longifilis*

No	Local name	Linnaean classification		
		Family	**Genus**	**Species**
15	Mkunga	Anguillidae	Anguilla	*Anguilla bangelensis*
16	Mkuyu	Cyprinidae	Labeobarbus	*Labeobarbus leleupanus*
17	Mtuku	Cyprinidae	Labeo	*Labeo congoro*
18	Ndipi	Mormyridae	Petrocephalus	*Petrocephalus affinis*
19	Ndipi	Mormyridae	Hippopotamyrus	*Hippopotamyrus* spp
20	Ndipi kongwe	Mormyridae	Pollimyrus	*Pollimyrus nigrican*
21	Ndipi mdomo mfupi	Mormyridae	Marcusenius	*Marcusenius livingstonii*
22	Ndipi mdomo mrefu	Mormyridae	Marcusenius	*Marcusenius macrolepidatus*
23	Ndipi miera	**Not verified**		
24	Ndipi namani	Mormyridae	Hippopotamyrus	*Hippopotamyrus grahami*
25	Ndungu	Distichodontidae	Distichodus	*Distichodus petersii*
26	Ngogo dongo	Mochokidae	Synodontis	*Synodontis multipuctatus*
27	Ngogo mwanajeshi	Mochokidae	Synodontis	*Synodontis multipuctatus*
28	Ngogo mweusi	Mochokidae	Synodontis	*Synodontis rukwaensis*
29	Ngogo ng’andu	Mochokidae	Synodontis	*Synodontis rufigiensis*

No	Local name	Linnaean classification		
		Family	**Genus**	**Species**
30	Ngolya	**Not verified**		
31	Ngurufi	Cyprinidae	Labeo	*Labeo coubie*
32	Ningu	Cyprinidae	Labeo	*Labeo congoro*
33	Njege	Alestidae	Hydrocynus	*Hydrocynus tanzaniae*
34	Njuju	Alestidae	Brycinus	*Brycinus affinis*
35	Njuju mkapa	Alestidae	Brycinus	*Brycinus sadleri*
36	Perege 1	Cichlidae	Oreochromis	*Oreochromis korogwe*
37	Perege 2	Cichlidae	Oreochromis	*Oreochromis urolepis*
38	Sheta	Clariidae	Clarias	*Clarias werneri*
39	Sulusulu	Mormyridae	Mormyrus	*Mormyrus longirostris*
40	Sulusulu vihondi	**Not verified**		

Table 3: Fish species identification from GenBank and BOLD databases, conservation status and the number of samples obtained from fish species sampled between July2022 and January 2023 in the Rufiji River Basin (RRB). LC= least concern, VU= vulnerable and NT= near threatened

Scientific name	Accession no.	GenBank Species name	Identity (%)	BOLD Species name	Identity (%)	Number of samples	IUCN Red list category
Brycinus affinis	OQ908874	***Alestes spp***	**92.57**	**No match**	**0**	1	LC
Labeo congoro	OQ908912 OQ908894	***Labeo lineatus***	**96.89**	***Labeo lineatus***	**97.40**	2	LC
Schilbe moebiusii	OQ908911	***Schilbe intemedius***	**93.98**	**No match**	**0**	1	LC
Marcusenius macrolepidatus	OQ908903 OQ908875	***Campylomormyrus numenius***	**93.27**	***Marcusenius livingstonii***	**98.34**	2	LC
Oreochromis korogwe	OQ915200 OQ915199	*Oreochromis korogwe*	99.66	*Oreochromis korogwe*	99-100	2	LC
Distichodus petersii	OQ908900	*Distichodus petersii*	98.04	*Distichodus petersii*	98.16	1	VU
Hydrocynus tanzaniae	OQ918545	***Hydrocynus vittatus***	94.92	**No match**	**0**	1	LC

Scientific name	Accession no.	GenBank Species name	Identity (%)	BOLD Species name	Identity (%)	Number of samples	IUCN Red list category
Clarias gariepinus	OQ908902	*Clarias gariepinus*	99.84	*Clarias gariepinus*	99.84	1	LC
Bagrus orientalis	OQ908901	***Bagrus caeruleus***	**93.13**	**No match**	**0**	1	LC
Synodontis rufigiensis	OQ908910 OQ908889	***Synodontis spp***	**94.96**	**No match**	**0**	2	LC
Synodontis rukwaensis	OQ908899 OQ908888	*Synodontis rukwaensis*	99.38	*Synodontis rukwaensis*	99.67	2	LC
Citharinus congicus	OQ908898 OQ908881	*Citharinus congicus*	93.96	**No match**	0	2	LC
Anguilla bengalensis	OQ908897 OQ908893 OQ908909	*Anguilla bengalensis*	100	*Anguilla bengalensis*	100	3	NT
Labeo coubie	OQ918544	***Labeo forskalii***	**95.57**	**No match**	**0**	1	LC
Hippopotamyrus grahami	OQ915201	***Pollimyrus isidori***	**90.15**	**No match**	**0**	1	LC

Scientific name	Accession no.	GenBank Species name	Identity (%)	BOLD Species name	Identity (%)	Number of samples	IUCN Red list category
Hippopotamyrus spp	OQ915198	***Ciphomyrus discorhynchus***	**96.20**	**No match**	**0**	1	LC
Mormyrus longirostris	OQ908896 OQ908879	***Mormyrus rume***	**98.87**	***Mormyrus tapirus***	**98.71**	2	LC
Oreochromis urolepis	OQ908895	***Oreochromis korogwe***	**97.93**	*Oreochromis urolepis*	98.36	1	LC
Pollimyrus nigrican	OQ908892	*Pollimyrus nigrican*	96.73	**No match**	**0**	1	LC
Enteromius apleurogramma	OQ908908 OQ908907	*Enteromius apleurogramma*	100	*Enteromius apleurogramma*	100	2	LC
Brycinus sadleri	OQ908877	***Brycinus sp.epulu***	97.03	***Brycinus lateralis***	**98.69**	1	LC
Synodontis multipuctatus	OQ908891 OQ908890	***Synodontis victoriae***	96.08	***Synodontis victoriae***	**97.07**	2	LC
Clarias werneri	OQ908887	***Clarias alluaudi***	**95.94**	**No match**	**0**	1	LC

Scientific name	Accession no.	GenBank Species name	Identity (%)	BOLD Species name	Identity (%)	Number of samples	IUCN Red list category
Brycinus spp	OQ908886	***Brycinus nurse***	**91.31**	**No match**	**0**	1	LC
Eutropielus longifilis	OQ908906 OQ908885 OQ908884	***Schilbe intemedius***	**91.06**	**No match**	**0**	3	LC
Labeobarbus leleupanus	OQ908883	***Labeobarbus robertsi***	**96.27**	**No match**	**0**	1	VU
Heterobranchus longifilis	OQ908882	*Heterobranchus longifilis*	98.72	***Heterobranchus spp***	**98.86**	1	LC
Alestes stuhlmanni	OQ908880	***Alestes baremoze***	**91.58**	**No match**	**0**	1	LC
Petrocephalus affinis	OQ908878	***Petrocephalus catostoma***	**99.84**	***Petrocephalus catostoma***	**97.18**	1	LC
Ctenopoma spp	OQ908876	***Ctenopoma muriei***	**93.81**	**No match**	**0**	1	LC
Glossogobius giuris	OQ918543	*Glossogobius giuris*	98.35	*Glossogobius giuris*	98.8	1	LC

Scientific name	Accession no.	GenBank Species name	Identity (%)	BOLD Species name	Identity (%)	Number of samples	IUCN Red list category
Eleotris klunzingerii	OQ908905	*Eleotris klunzingerii*	100	*Eleotris klunzingerii*	100	1	LC
Marcuseniu s livingstonii	OQ908904	***Ciphomyrus discorhynchus***	**93.23**	**No match**	**0**	1	LC

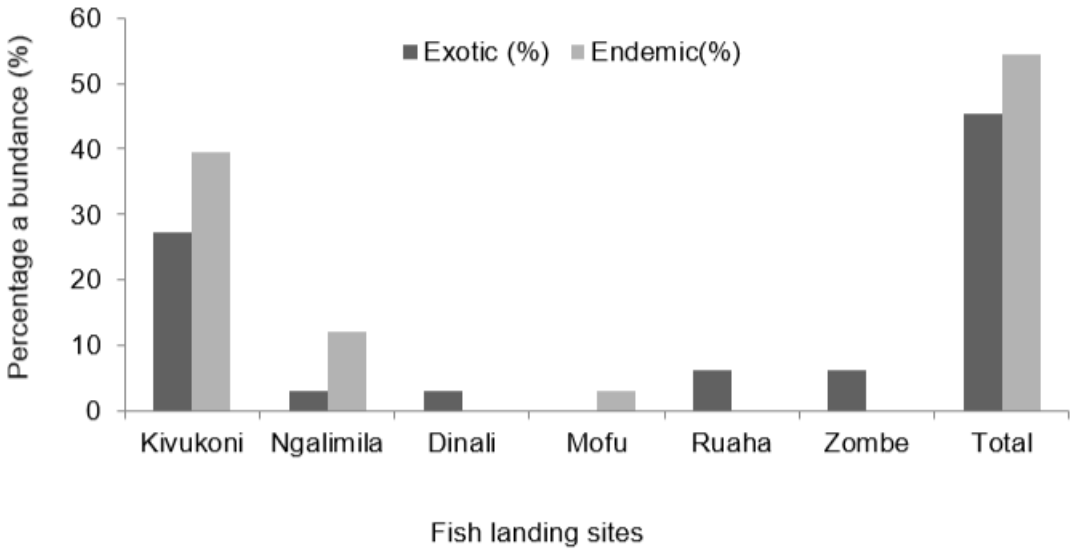

Figure 3: Percentage of endemic and exotic fish sampled between July 2022 and January 2023 in six different landing sites within the Rufiji River Basin (RRB).

4. Discussion

The present study revealed 33 different fish species in the RRB. This number is higher than the number reported in a previous study (Utzinger and Charlwood 1996) which showed that there were only 23 different fish species. The variation in results can be attributed to differences in limited sampling sites and shorter duration of sampling. Therefore, a total of 10 fish species identified in this study were not reported in the earlier studies. These newly identified species include, Bubu mchanga *G. giuris* and *E. klunzingerii,* Gugutuu *Ctenopoma* spp, Luepe *E. longifilis*, Mkuyu *L. leleupanus*, Ndipi *P. affinis*, Ndipi kongwe *P. nigrican*, Ngogo mwanajeshi *S. multipuctatus*, Ngogo ng'andu *S. rufigiensis* and Ndipi mdomo mrefu *M. macrolepidatus*. Eighteen out of 33 species were endemic to RRB while 15 were exotic. The presence of a high number of exotic fish species poses a serious threat to the endemic fish populations. Some of these exotic species can act as competitors, predators, or even hybridize with the endemic species, further exacerbating the risk of extinction (Marine et al. 2018). The present study also confirmed the presence of *H. longifilis, C. congicus* and *L. coubie* contrary to study conducted by (Mombo et al. 2011) which revealed that the species have disappeared in the RRB. However, the fact that these species were rare in the catch suggests that the current IUCN assessment of them as Least Concern should be revisited. This is particularly critical for *H. longifilis* because it was found at only one site and was reported by experienced fishers to be among the fishes that were highly abundant in the past but are currently vary rare.

The local fish identification techniques used was found to be inaccurate, leading to numerous contradictions, especially when distinguishing closely related species. Despite using the Field guide for freshwater fishes of Tanzania, there were limitations in the identification sheets, particularly for certain fish species. This is similar to the study conducted in the

study area (Utzinger and Charlwood, 1996) which showed the limitation of the identification sheets in identifying Mbewe *Brycinus* spp and Sheta *C. werneri*.

DNA barcoding alone confirmed identities of 13 species. However, low identities were used to confirm some species in the GenBank database while higher identities failed to confirm the identity of Ndipi *P. affinis* and Sulusulu *M. longirostris*, suggesting a high probability of tentative, incorrect or low-quality sequences being submitted to the database (Wong et al. 2011). BOLD database confirmed less species than GenBank. However, most of the confirmed species were identified with 99-100% identities. This reveals that BOLD database has greater resolution than GenBank database (Panprommin et al. 2019). The COI sequences of 21 fish species have not been recorded in the GenBank database, and the COI sequences of 17 fish species do not match any sequence in the BOLD database. Thus, this study was able to increase the number of COI gene sequences of these fish species in both databases. Furthermore, fish species identified from this study would help to solve the problem of unidentified species from the previous studies (Utzinger and Charlwood 1996, Mombo et al. 2011). Some fish species were however, not verified through DNA barcoding alone due to absence of corresponding COI sequences in the GenBank and BOLD Database. The integration of DNA sequencing information with the morphological traits of the fish showed great efficiency.

The constructed phylogenetic tree provided a similar classification concerning the taxonomy and morphological traits of the fishes. All closely related species were clustered under the same nodes revealing that the amplified barcodes correctly identified the species. However, there were instances where species from different orders and families clustered together suggesting potential similarities or evolutionary relationships between these species' despite of

their taxonomic differences. This is consistent with the study by Nakatani et al. (2011), which revealed that one of the two suborders in the family Characiformes is more closely related to Siluriformes than to its suborder. The study also revealed some divergence or unique evolutionary history as certain species originated separately from their respective clades. Six species did not fall into any of the identified clade indicating either distinct genetic characteristics or their evolutionary relationship were not captured within the analyzed data set. This is in consistent with the study conducted by Reza et al. (2016) which revealed distinct genetic lineages among the species of the genus Capoeta in the family Cyprinidae. The presence of closely related species within different clades and the occurrence of distinct genetic characteristics in some species highlighted the complex nature of fish diversification and evolutionary history (Reza et al. 2016, Betancur-r et al. 2017).

The results of the present study indicate that none of the sampled fish in the RRB are classified as endangered or critically endangered according to the IUCN. However, due to the rarity of some species in the catch, their IUCN assessment should be revisited. This is critical for species such as Mjongwa *H. longifilis*, Mbala *C. congicus*, Ningu *L. congoro*, Sulusulu *M. longirostris,* and Mkuyu *L. leleupanus* because they were particularly rare. These rare species require reassessment and reclassification as their current IUCN criteria does not accurately reflect their actual status on the ground. Also, because none of the rare species are listed in either CITES Appendices or the Third Schedule of the Tanzania Fisheries (Amendment) Regulations of 2009. This implies that there are currently no specific legal measures in place to regulate or protect these fish species from overexploitation or illegal trade. This highlights the need to update CITES Appendices and the Third Schedule of the Tanzania Fisheries (Amendment) Regulations of 2009 to

include the above-mentioned rare species if they are to be protected from extinction. Furthermore, the absence of some reported species during sampling does not conclusively indicate their complete disappearance in the RRB; instead, it calls for further studies employing environmental DNA (eDNA) to confirm the presence of these species.

5. Conclusion

The present study confirmed 33 different species in the RRB, including species that were reported to have disappeared. However, some species were rarely found in the field despite being classified as Least Concern by the IUCN, suggesting the need for their IUCN Red List status to be reevaluated. Additionally, the presence of rare species suggests the need to protect them in the RRB to prevent further decline in fish populations. This can be achieved through promoting sustainable fishing practices by raising awareness among local fishers about techniques that minimize harm to fish populations and their habitats. Furthermore, the expansion of protected areas within the RRB could provide safe havens for rare species, potentially reversing the observed declining trends. Moreover, the findings of this study should be validated using environmental DNA (eDNA) to confirm the existence of species reported to have disappeared.

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Data Availability Statement

The data that support the finding of this study are available in NCBI databases under the accession number (OQ908874- OQ918545).

Declaration of interest

The authors affirm that they have no conflict of interest to declare.

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CHAPTER THREE

Restricted genetic connectivity and conservation prospects of Bagrid catfish, *Bagrus orientalis*, populations in the Rufiji River basin, Tanzania

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ABSTRACT

The Bagrid catfish, *Bagrus orientalis*, historically sustained communities in the Rufiji River basin (RRB), Tanzania, but has rapidly declined due to high consumer demand and unsustainable fishing. Consequently, a Ramsar site was designated within the RRB, although its potential to revitalize overexploited populations beyond its boundaries is uncertain because of limited information on genetic connectivity. To address this uncertainty, 158 partial cytochrome oxidase subunit I sequences of *B. orientalis* were analyzed to quantify genetic connectivity in the RRB. We observed significant genetic differentiation, indicating limited connectivity among populations. Populations in the Ramsar site were genetically connected to those in the Kilombero Valley Floodplain (KVFP), but were distinct from those in Ruaha and Rufiji, which clustered separately. Our findings suggested the Ramsar site could revitalize overexploited KVFP populations and emphasized the need for sustained efforts against its encroachment. However, limited genetic connectivity with Ruaha and Rufiji implied that conservation measures in the site might have restricted effect in these areas. Conservation efforts should extend beyond the Ramsar site, by promoting sustainable fishing and enhancing habitat connectivity in Ruaha and Rufiji.

Keywords: Population genetic structure, gene flow, genetic diversity, haplotype diversity, mitochondrial COX1, Bagrid catfish *Bagrus orientalis.*

1. Introduction

Freshwater ecosystems globally support crucial fisheries vital for global food security and actively shape local climate while nurturing intricate ecological networks (Sayer et al., 2018). These ecosystems, particularly those in the tropics, are biodiversity hotspots that harbor a rich diversity of endemic fish that play fundamental roles in supporting and sustaining local communities (Hamerlynck et al., 2011; Hamerlynck & Doody, 2003; Nindi et al., 2014). In particular, Bagrid catfishes, widely distributed in Africa and Asia, are of exceptional importance as a critical food resource. Renowned for their high omega-3 polyunsaturated fatty acid content, low cholesterol levels, and high-quality protein, demand for and consumption of these fish have recently increased (Widjaja et al., 2009). However, as the human population expands, especially in Africa, these fish have come under heavy fishing pressure that results in population declines (Gebrekidan et al., 2020; Msangameno & Mangora, 2016; URT, 2019). Increasing demand for these fish from local and distant communities, driven by a need for cash income by fishers to sustain families, has also triggered a rise in unsustainable fishing practices, such as use of poisons, dynamite, and beach seines that further exacerbate fishing pressure.

Bagrus orientalis is among the Bagrid catfishes that have been heavily exploited throughout its native range in Tanzania and Malawi. Once a dominant species in the Rufiji River basin (RRB), it was highly preferred by locals due to its flavour and large size that can reach 44.7 cm in length. Consequently, the species commanded high prices in local markets that range $2-5 per kg, depending on location (Siima et al., 2012; Turpie, 2008). However, the number and size of fish in the RRB declined dramatically due to unsustainable fishing practices and habitat degradation. These issues stem from increased livestock keeping (free

grazing) and mechanized agriculture, both of which are associated with use of agrochemicals that degrade water quality (Materu et al., 2021; Materu & Heise, 2019). In response, a Ramsar site covering an extensive area of 796,735 hectares was designated in the Kilombero Valley Floodplain (KVFP) in 2002. This designation recognized its ecological significance, biodiversity, and critical role in supporting local communities (Ramsar, 2002). However, limited information on genetic connectivity among *B. orientalis* populations in the RRB makes it uncertain if the Ramsar site can rejuvenate fish stocks beyond its boundaries. Existing genetic data in the RRB primarily stems from DNA barcoding studies of farmed fish (Mbilinyi et al., 2023; Mndeme et al., 2020).

Bagrus orientalis like other bagriids species breeds seasonally, with bimodal spawning peaks coinciding with rainy seasons (Mbabazi et al., 2023: Aruho et al 2013). The first spawning peak is from March to May, while the second peak is from September to November. During the breeding season, mature individuals migrate from deep to shallow waters to find suitable spawning grounds (Anja et al., 2009). Like many other freshwater fish, *B. orientalis* are fertilized externally when females release eggs into water to be fertilized by sperm from males. However, hydropower dams in the RRB, like the Kidatu dam on the Ruaha River (Dye & Hartmann, 2017; Tesha et al., 2003), are barriers to fish migration that hinder dispersal of larvae and adults between the Ramsar site and areas outside its boundaries (Arantes et al., 2019). When combined with habitat degradation and unsustainable fishing practices, these factors can reduce gene flow and increase genetic differentiation among populations (Martinez et al., 2018; Rumisha et al., 2023). Therefore, we quantified the extent of genetic connectivity between populations of *B. orientalis* in the Kilombero Valley Floodplain Ramsar site (KVFPRS) and those in other areas within the RRB.

2. 0 Material and methods

2.1 Study area

The study area in the RRB encompassed the Kilombero, Great Ruaha, and Rufiji rivers. Sampling was at six landing sites at Kidatu, Kivukoni, Mofu, Dinari, Ngalimila, and Zombe (Fig 3.1). The RRB is the largest river basin in East Africa and is rich in fish biodiversity (Hamerlynck & Doody, 2003). The basin also has a good climate, fertile soil, minerals, and human resources, including hydroelectric potential (Kiwia, 2013). The basin covers 177,429 km^2 that is 20% of the total area of Tanzania (5.7° to 10.5°S and 33.5° to 39°E; Mwalyosi, 1990). The RRB includes the KVFP, the largest seasonal freshwater lowland floodplain in East Africa that is also rich in biodiversity. The KVFPRS is part of the larger KVFP and was designated in 2002 owing to its critical ecological significance, biodiversity richness, and profound support to local communities. Covering an area of 796,735 hectares within a wetland catchment area of 40,000 km^2, this internationally recognized site provides sanctuary and plays a pivotal role in conserving overexploited fish populations by offering protected habitats (Ramsar, 2002). However, despite its designation, limited information is available about genetic connectivity between fish populations within the Ramsar site and those in other areas within the RRB.

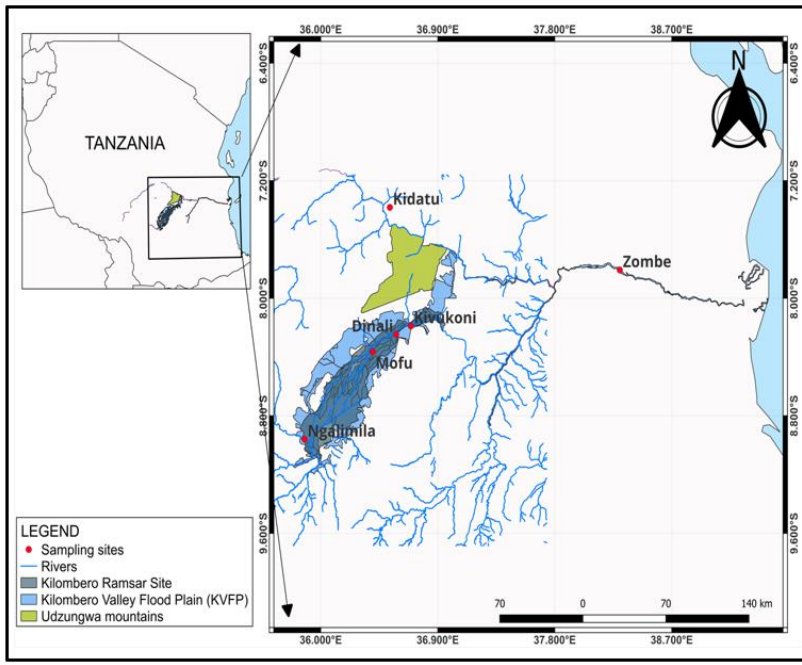

Figure 1: Locations within the Rufiji River Basin where *Bagrus orientalis* were sampled between July 2022 and July 2023

2.2 Sampling

Sampling of *B. orientalis* was conducted between July 2022 and July 2023. Of 182 fish collected by fishers at six landing sites, 60 were from the KVFPRS and the remaining 122 were from areas outside the Ramsar site. Sampling locations were marked using Geographical Positioning Systems (GPS) (Table 3.1). *B. orientalis* were initially identified on-site using a published fish identification key (Eccles, 1992). For each sampled fish, weight was measured in grams using a sensitive beam balance and length was measured in centimeters on a measuring board. Fin-clip tissues of 0.5 grams were cut from the caudal fin of each fish, stored in 1.5 mL microcentrifuges, preserved in 99.9% ethanol, and

transported to the Molecular Biology laboratory at the Sokoine University of Agriculture for analysis.

Table 1: Central latitude and longitude coordinates and numbers of fin-clip tissues sampled from Bagrus orientalis in the Rufiji River Basin between July 2022 and July 2023.

Site	Coordinates		Number of fin clips sampled
	Latitudes (º S)	Longitudes (º E)	
KVFP			
Kivukoni	8.2	36.7	30
Ngalimila	8.58	35.55	29
KVFPRS			
Dinari	8.19	36.2	30
Mofu	8.19	36.6	30
Ruaha River			
Kidatu	7.38	37.9	41
Rufiji River			
Zombe	7.59	38.44	22
Total			182

2.3 DNA extraction, COI amplification, and sequencing

Genomic DNA was extracted from each fin-clip tissue sample using the TIANamp Genomic DNA kit (TIANGEN Biotech, Beijing) according to manufacturer protocol. Quality of each DNA extract was checked on 1% agarose gel before analysis. Thereafter, fragments (620 base pairs) of the cytochrome oxidase subunit I gene (COI) were amplified from DNA extracts of each sample in a T100™ Thermal cycler machine (Bio-Lab Inc, GA, USA) using the Forward primer FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and the reverse primer FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'). Amplification reactions were in a total volume of 35µL consisting of 2µL template DNA, 1 x OneTaq 2X Master Mix with Standard

Buffer (New England BioLabs Inc., MA, USA), 5 mg bovine serum albumin, and 0.3 µM of each primer. Temperature profiles were 94°C for 5 min, followed by 35 cycles of 94°C for 40 s, 54°C for 45 s and 72°C for 60 s. Final extension of 72°C for 15 min was added to ensure complete elongation of PCR amplicons. Quality of each PCR reaction was checked on 1% agarose gels. Successful PCR amplicons were sequenced by Macrogen Europe Laboratory using Sanger's Dideoxy sequencing technology and the primer FishF1.

2.4 Data analysis

Out of 182 samples that were initially analyzed, 24 samples were removed because they produced unclear peaks that could not be used for analysis. The remaining sequences were edited to trim ends and aligned using the ClustalW algorithm in the program MEGA ver. 11 (Tamura et al., 2021). Edited COI sequences were then translated into amino acid sequences using the vertebrate genetic code in MEGA ver. 11 to check for the presence of sequencing artefacts and nuclear pseudogenes (Mgeleka et al., 2023; Rumisha et al., 2023). After cleaning, 127 COI sequences remained and were submitted to the National Centre for Biotechnology Information (NCBI) under accession numbers OR138542–OR138668 and OR759455–OR759484. Sequences were then collapsed into haplotypes using FaBox DNA collapser ver. 1.61 (Villesen, 2007). The number of haplotypes and nucleotide diversities ($\boldsymbol{\pi}$) were calculated using ARLEQUIN version 3.5.1.2 (Excoffier and Lischer, 2010). Analysis of molecular variance (AMOVA) and hierarchical AMOVA used the same software to quantify genetic variation among and within populations. Pairwise F_{ST} values and significance levels were calculated with the same software to quantify genetic connectivity among subpopulations. A minimum spanning haplotype network was constructed with PopART ver. 1.7 (Leigh and Bryant 2015) to assess relationships among haplotypes. Bayesian phylogenetic analysis using BEAST ver. 2.5 (Bouckaert et

al., 2019) was used to assess evolutionary relationships among haplotypes. The analysis used a relaxed uncorrelated log-normal molecular clock and a general-time-reversible (GTR) evolutionary model that ran for 10-million generations while sampling parameters every 1000 generations. The phylogenetic tree was annotated using the TreeAnnotator ver 1.10 and visualised using the FigTree ver. 1.4 software. One COI sequence of the African sharptooth catfish (*Clarias gariepinus*) was retrieved from GenBank (accession number OQ908902) and included as outgroup. Resulting outputs were then analyzed using Tracer ver. 1.7, to generate a Bayesian skyline plot highlighting potential changes in population size over time (Rambaut et al., 2018).

The length-weight relationship was modeled using $W = aL^b$ (Nehemia et al., 2012), where W = weight of each fish (g), L = total length of each fish (cm), and a and b are parameters that describe the change in weight with length. The equation was log-transformed to estimate a and b parameters. Isometric growth is indicated when b = 3 and allometric growth is indicated when b <> 3. Fulton's condition factor was estimated using the equation, $K= 100W/L^3$ (Nash et al., 2006), where K = Fulton's condition factor and W and L are as defined for the weight-length model. The length-frequency distribution was depicted for 10-cm total length intervals.

3. Results

3.1 Genetic connectivity among populations

Genetic differentiation was significant between sampling sites (AMOVA, $F_{ST} = 0.33$, $p < 0.01$, 1023 permutations). Variation among populations accounted for 33% of total variation. Populations in KVFPRS were genetically similar to other KVFP populations but distinct from those in Kidatu and the Lower Rufiji River (Table 2). The three groups, KVFP, Kidatu, and Lower Rufiji, exhibited significant genetic differentiation (Hierarchical AMOVA $F_{CT} = 0.44$, $p < 0.0001$), with differences between groups explaining 43% of total genetic variation. Restricted genetic connectivity was also

indicated by the presence of private haplotypes among populations. Haplotypes from Lower Rufiji and two haplotypes from Kidatu clustered into separate clusters (Fig. 2 and 3).

Table 2: Pairwise F_{ST} values among populations of *Bagrus orientalis* sampled from the Rufiji River Basin between July 2022 and July 2023. Bolded values are significant after Holm- Bonferroni sequential correction.

Landing sites	Kivukoni (KVFP)	Ngalimila (KVFP)	Dinari (KVFPRS)	Mofu (KVFPRS)	Kidatu	Lower Rufiji
Kivukoni (KVFP)	*					
Ngalimila (KVFP)	0.06	*				
Dinari (KVFPRS)	-0.02	0.002	*			
Mofu (KVFPRS)	-0.03	0.06	-0.02	*		
Kidatu	0.52	0.44	0.47	0.52	*	
Lower Rufiji	0.42	0.33	0.38	0.43	0.50	*

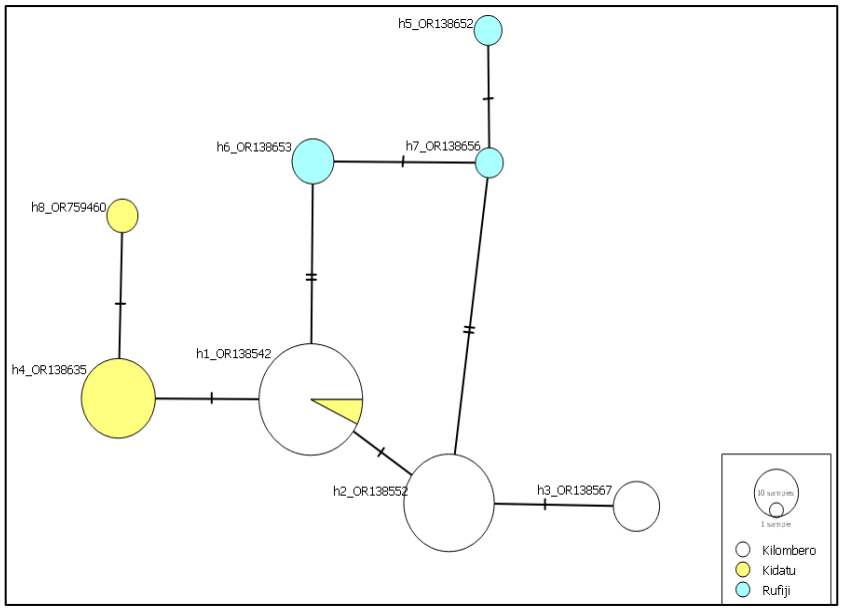

Figure 2: Minimum spanning haplotype network for relationships among cytochrome oxidase subunit I haplotypes of *Bagrus orientalis* sampled from the Rufiji River Basin between July 2022 and July 2023. Each circle represents a haplotype. The size of each circle is proportional to the number of individuals carrying each haplotype.

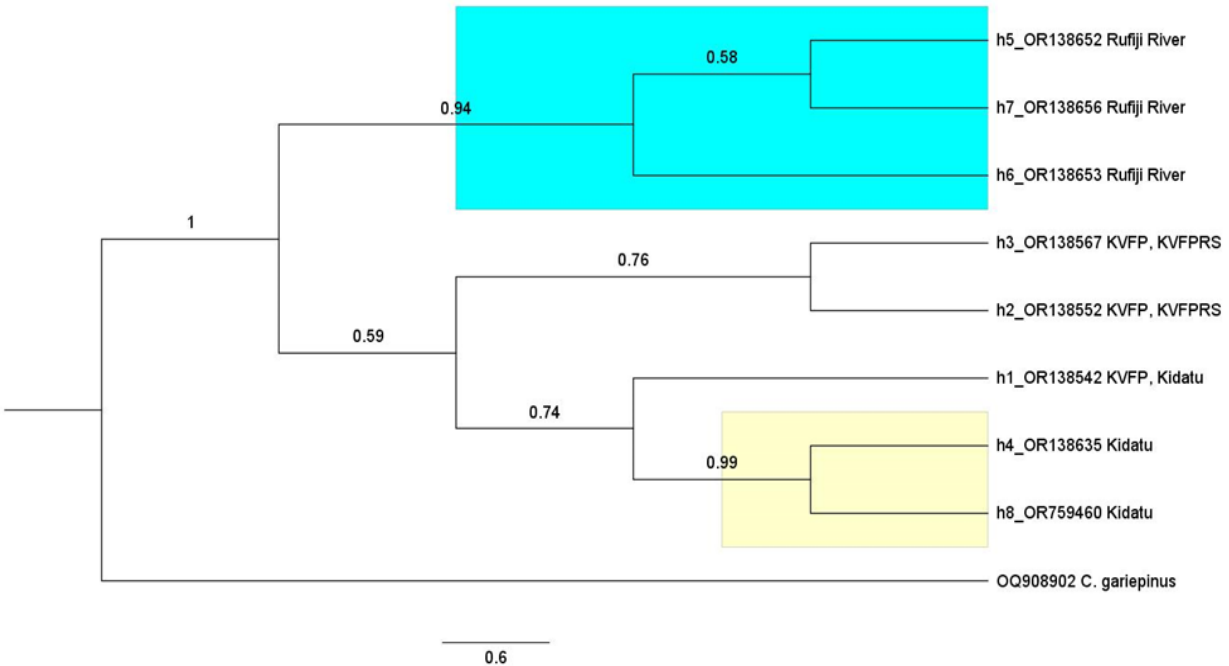

Figure 3: Phylogenetic relationships among cytochrome oxidase subunit I haplotypes of *Bagrus orientalis* sampled from the RRB between July 2022 and July 2023. KVFP, Kilombero Valley Floodplain; KVFPRS, Kilombero Valley Floodplain Ramsar Site; *C. gariepanus*

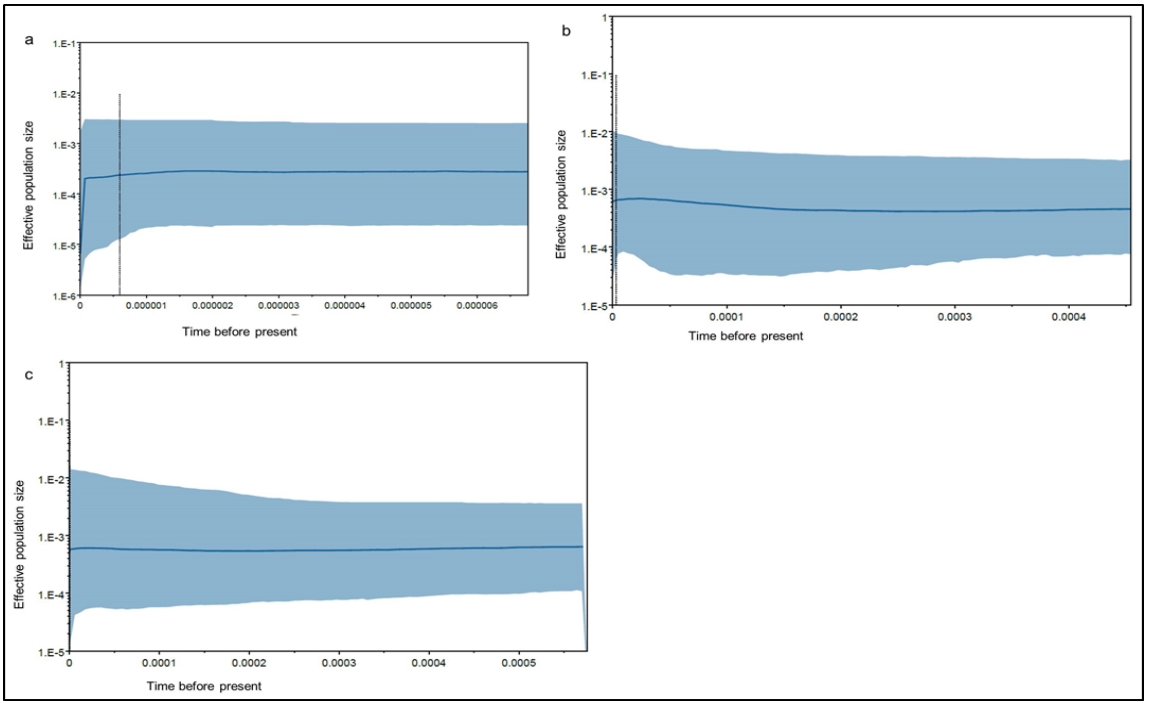

Figure 4: Bayesian skyline plots based on cytochrome oxidase subunit I sequences of *Bagrus orientalis* sampled from (a) Kilombero valley floodplain, (b) Kidatu, and (c) Lower Rufiji River between July 2022 and July 2023.

3.2 Genetic diversity among populations

For the 158 COI sequences analyzed, eight haplotypes differed from one another by one- or two-point mutations (Table 3). Haplotype diversity was higher for populations from Rufiji and KVFP, including KVFPRS, than the population from Kidatu (Table 3.3). Conversely, nucleotide diversity within populations was typically less than 0.2%. However, observed genetic variation was consistent with what would be expected under the neutral evolution hypothesis, because Tajima's D and Fu's Fs values did not differ significantly from zero (Table 3). Effective population size was relatively stable over time (Bayesian skyline plot, Fig. 4). However, most fish caught in Kidatu and KVFP were of small size (Fig 5) and average weight at length was lightest for fish from the population from Kidatu (Table 4).

Table 1: Haplotype and nucleotide diversities of B. orientalis sampled from the Rufiji River Basin between July 2022 and July 2023.

Site name	Number of samples	Number of haplotypes	Haplotype diversity(h)	Nucleotide diversity (%)	Number of mutations	Tajma's D Value (p> 0.05)	Fu's Fs value (p> 0.05)
KVFP & KVFPRS	104	3	0.59	0.12	2	1.23	1.61
Kivukoni	24	2	0.52	0.087	1	1.60	1.59
Ngalimila	28	3	0.68	0.15	2	1.64	1.34
Dinari	28	3	0.60	0.12	2	0.83	0.76
Mofu	24	2	0.50	0.085	1	1.51	1.53
Ruaha River							
Kidatu	37	3	0.40	0.07	2	1.35	0.85
Lower Rufiji							
Zombe	17	3	0.64	0.153	2	-0.16	-0.07

Table 3: Average length, average weight, Fulton's condition factor, and exponent of the weight-length relationship for *Bagrus orientalis* from three populations in the Rufiji River Basin sampled between July 2022 and January 2023.

Fish populations	Average total length (cm)	Average weight (g)	Fulton's condition factor (K)	Weight at unit length/ slope (b)
KVFP	34.24	514.69	0.8	3.30
Kidatu	27.50	162.15	0.7	2.91
Rufiji	59.66	1758.41	0.8	3.11

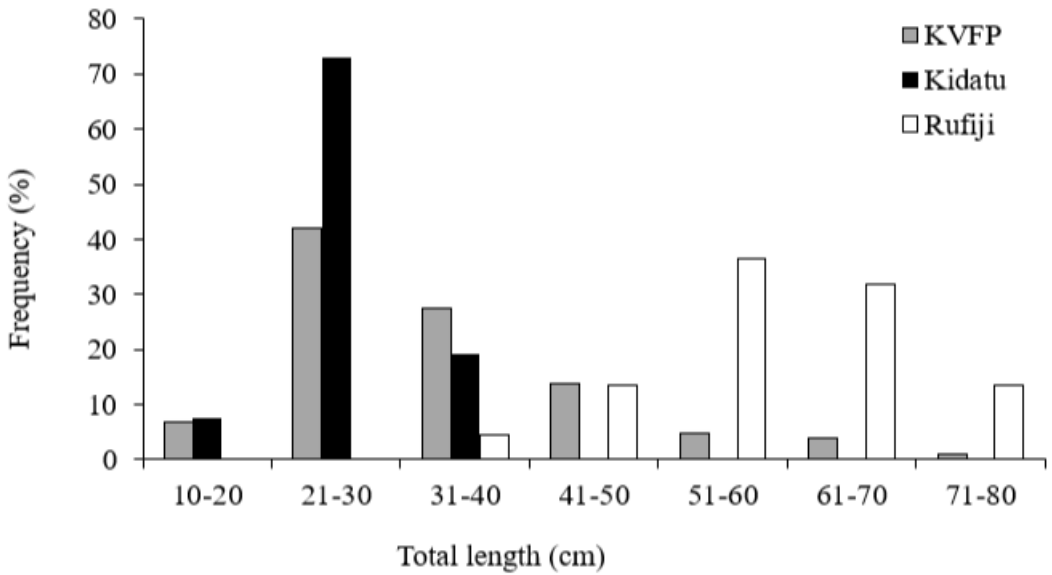

Figure 5: Length frequency distribution of *Bagrus orientalis* sampled from Kilombero Valley Floodplain (KVFP), Kidatu, and Lower Rufiji between July 2022 and July 2023.

4. Discussion

We found evidence of restricted genetic connectivity among populations of *B. orientalis* in the RRB, which suggested that the designated Ramsar site in the RRB may not be benefiting all *B. orientalis* populations in the basin, unlike protected areas that are globally recognized as crucial tools in fisheries management, as sanctuaries for conserving overexploited fish populations by providing protected habitats (Roberts et al., 2017). The fact that populations in the KVFP were genetically connected with those in the KVFPRS suggests that unsustainable fishing in the KVFP could be mitigated by the Ramsar site, by aiding recovery of overexploited populations in the floodplain. This emphasizes the need for sustained protection of the Ramsar site from encroachment and unsustainable activities for long-term sustainability of floodplain fish populations. The high genetic connectivity we found indicated that populations in the Ramsar site interacted with those in the KVFP, despite spatial separation between these populations, perhaps because of seasonal changes in water flow (Roman et al., 2018). The KVFP is subjected to periodic flooding, which prompt fish to move across various sections of the floodplain, accessing new feeding grounds, breeding sites, and sheltered environments (Nindi et al., 2014). Migration during flood events fosters increased interactions among fish populations (Hurd et al., 2016) that potentially contribute to genetic connectivity among populations. Populations in the KVFP should be treated as a single genetic management unit in a comprehensive conservation strategy that accounts for potential interactions among populations. Therefore, integrating strategies that encompass the protection of the Ramsar site and efforts to understand and manage connectivity beyond its borders is essential for ensuring long-term sustainability of *B. orientalis* in the KVFP.

The lack of genetic connectivity we found between populations in Kidatu, Lower Rufiji, and those in the Ramsar site indicates limited interaction between populations in these areas, which suggests that efforts to safeguard habitats and fish populations within the Ramsar site might not enhance genetic diversity or resilience of *B. orientalis* populations in Kidatu and Lower Rufiji. The presence of a hydropower dam in Kidatu may act as a physical barrier that restricts genetic connectivity between Kidatu and other areas in the RRB, by limiting genetic exchange among populations (Dye & Hartmann 2017). Construction of the Julius Nyerere hydropower dam in the RRB (Paget, 2020) may increase the risk by further isolating *B. orientalis* populations in lower Rufiji, thereby elevating their vulnerability to extinction.

These findings underscore the necessity of establishing connectivity pathways between populations in Rufiji and Kidatu, through fish ladders, passages, and bypass channels around hydropower dams in the RRB, to allow fish to pass barriers, thereby restoring access to crucial spawning and feeding grounds, and potentially revitalizing or enhancing population sustainability (Zarri et al., 2022). Similarly, specific conservation measures should target populations in Kidatu and Lower Rufiji, such as habitat restoration, sustainable fishing practices, or community engagement programs, to ensure protection and enhancement of genetic diversity. Furthermore, within the Rufiji, the recent declaration of Nyerere National Park offers potential protected habitats for isolated *B. orientalis* populations in the area (Paget, 2020). Similar initiatives for demarcating protected areas should be considered for the isolated population in the Ruaha River.

The haplotype and nucleotide diversities we measured are similar to levels of other African catfishes (Mohammed-Geba, 2015; Nneji et al., 2020). However, the Kidatu population had low haplotype and nucleotide diversities,

possibly due to restricted genetic connectivity with other populations in the RRB or small effective population size. Nevertheless, the relatively stable effective population size in Kidatu over time suggested that low genetic diversity may have resulted from restricted genetic connectivity that reduced genetic exchange, increased genetic drift, and reduced genetic diversity like other isolated populations (Arantes et al., 2019; Baird & Hogan, 2023; Pavlova et al., 2017; Rumisha & Kochzius, 2023). Additionally, most fish caught in Kidatu were smaller in size and lower in body condition than other populations in our study, which did not conclusively indicate overfishing, but justifies further studies of population dynamics, fishing pressure, and recruitment to understand factors affecting size structure.

5. Conclusion

We found limited genetic connectivity among *B. orientalis* populations in Kidatu, Lower Rufiji and KVFPRS of the RRB, which suggested that protected areas like Ramsar sites, globally recognized as crucial in fisheries management, might not enhance genetic diversity or resilience of populations with limited genetic connectivity. However, the COI marker used in our study is maternally inherited, so our findings should be validated using hypervariable nuclear genetic markers like microsatellites. Nevertheless, high genetic connectivity we observed between populations in the KVFP and those in the Ramsar site underscores the potential importance of the Ramsar site for supporting overexploited populations within the floodplain. This hints at the importance of continuing efforts to prevent encroachment of the Ramsar site. However, the limited genetic connectivity we observed between populations in the Ramsar site and those in Kidatu and Lower Rufiji suggests that protective measures within the Ramsar site might have a limited impact on these populations. Hence, we recommend broader conservation

strategies beyond the Ramsar site to encompass and specifically target populations in Kidatu and Rufiji, by implementing localized conservation measures, promoting sustainable fishing practices, facilitating habitat connectivity, and considering specific needs of these distinct populations to enhance their genetic diversity and resilience.

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CHAPTER FOUR

GENERAL DISCUSSION

The finding from this study revealed that none of the sampled fish in the RRB are classified as endangered or critically endangered by IUCN. However, species such as Mjongwa *Heterobranchus longifilis*, Mbala *Citharinus congicus*, Ningu *Labeo congoro*, Sulusulu *Mormyrus longirostris*, and Mkuyu *Labeobarbus leleupanus* were classified as least concern by IUCN despite being rarely found in the RRB. This suggests that reassessment and reclassification are needed because their current IUCN criteria do not accurately reflect their actual status on the ground.

Also, the present study revealed the limitation of relying solely on local fish identification, especially when distinguishing closely related species. These limitations have been previously reported in other studies (Utzinger and Charlwood, 1996). In this study, unidentified fish species from previous studies (Utzinger and Charlwood 1996, Mombo et al. 2011) were successfully identified through DNA barcoding. However, the absence of corresponding CO1 sequences for some fish species in GenBank and BOLD databases presents limitations when relying solely on DNA barcoding. Therefore, it is recommended to integrate both morphological characteristics and DNA barcoding when identifying fish species. The present study also revealed that 60% of fishers use illegal fishing gears and methods, highlighting that there is a need to revisit the current enforcement measures in the basin.

Furthermore, this study revealed the presence of three distinct genetic stocks of *Bagrus orientalis* in the Rufiji River Basin (RRB): the KVFP, Ruaha, and Rufiji populations. This indicates limited genetic connectivity among B. orientalis within the RRB. However, the population within the Ramsar

site shares genetic similarities with the population from other sites within the KVFP but remains genetically distinct from the populations in Ruaha and Rufiji. Consequently, the Ramsar site may primarily benefit the KVFP populations, underscoring the necessity to establish protected sites in Ruaha and Rufiji to conserve and prevent further decline of these populations. The observed limited connectivity is probably caused by the presence of Kidatu hydropower dams. Hydropower dams limit fish migrations, dispersal of larva and adults , restricting genetic exchanges among populations (Dye & Hartmann, 2017). Limited genetic exchanges among these populations suggests that the population are at risks of extinctions if measures are not taken (Filho et al., 2018; Hague & Routman, 2016).

The study also revealed low genetic diversity among the population in Kidatu due to restricted genetic connectivity within this population. Therefore, the present study highlights the need to enhance habitat connectivity in the area to enable fish species to move across the dams, gaining access to nutrients and suitable breeding habitat, thus forming a population with higher genetic diversity. Moreover, the present study revealed a stable population size over time. Therefore, the poor body condition and small-sized fish observed in Kidatu, does not conclusively indicate overfishing, but further studies on population dynamics and fishing pressure should be conducted to understand the factors affecting the size structure of the fish.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

This study reported 33 different fish species including 5 species that are rarely found in the RRB. However, none of the reported species are classified as endangered or critically endangered by IUCN. Also, none of the reported fish species are listed in either CITES Appendices or the Third Schedule of the Tanzania Fisheries (Amendment) Regulations of 2009. This implies that there are currently no specific legal measures in place to regulate or protect these fish species from overexploitation or illegal trade. Therefore, it is advised that CITES Appendices and the Third Schedule of the Tanzania Fisheries (Amendment) Regulations of 2009 should be updated to include the rare species if they are to be protected from extinction. Moreover, there is a need for IUCN to conduct reassessments and reclassifications of the identified rare species. This is necessary because their current IUCN criteria do not accurately reflect their actual status on the ground.

In addition, it was revealed that the RRB contains three distinct stocks of *Bagrus orientalis*: the KVFP, Rufiji and Ruaha which need separate management approaches. This suggests that, although the Ramsar site was designated in the KVFP, but it does not help the declining stocks from Rufiji and Ruaha to recover. Therefore, it is advised to establish the protected sites in Ruaha and Rufiji to rescue the fish stocks from further decline. Furthermore, observed low genetic diversity in Kidatu highlights the need of enhancing habitat connectivity in the area by establishing bypass channels, passage, and fish ladders to enable fish to move across the dams, facilitating interaction among populations. Moreover, the findings of this study need to be validated using hypervariable nuclear markers such as microsatellites

because the marker used in this study is maternally inherited and is subjected to selection pressure making it difficult to estimate effective population size. Also, further studies need to be conducted in the area using environmental DNA (eDNA) to confirm the presence of rare species that were not confirmed but reported to be present in the RRB.

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Kuhusu Tasnifu Hii

Utafiti ulifanyika katika bonde la mto Rufiji, lenye samaki wengi wa kienyeji. Walakini, uvuvi haramu umepelekea kupungua kwa kasi kwa samaki hao. Kutatua hili, bonde la mafuriko la kilombero lililindwa, walakini, akiba ya samaki inapungua, na bado haijulikani kama hatua zilizotekelezwa zinaendana na muundo wa jenetiki za samaki. Kwa hivyo, utafiti huu ulilenga kutathmini muundo za spishi za samaki wa kienyeji na muundo wa jenetiki za samaki aina ya Kitoga. Kutekeleza hilo, utafiti ulitumia nija za vinasaba, wavuvi na wataalamu muhimu. Sampuli za tishu 46 kutoka kwa samaki tofauti na 182 za Kitoga zilichukuliwa.

Utafiti huu ulithibitisha spishi 33 tofauti za samaki na ulionyesha kuwa jamii ya Kitoga katika eneo lililohifadhiwa la kilombero zinafanana kijenetiki na zile kutoka maeneo mengine ndani ya bonde hilo, lakini zimejitenga na jamii kutoka Ruaha na Rufiji. Utafiti unapendekeza kuwekwa kwa maeneo yaliyohifadhiwa katika Ruaha na Rufiji ili kuokoa jamii hii isipotee.