

POPULATION DENSITY, DISTRIBUTION AND HABITAT ASSOCIATIONS OF
***Callulina shengena*: AN ENDEMIC FROG SPECIES IN THE EASTERN ARC**
MOUNTAINS

FLORA LAWRENCE TESHA

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN
WILDLIFE MANAGEMENT AND CONSERVATION OF SOKOINE
UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

2020

ABSTRACT

Population density and habitat associations are fundamental aspects for conservation and management of wildlife species such as *Callulina shengena*. Being a Critically Endangered warty frog in the Eastern Arc Mountains, such vital information was missing and therefore demonstrated substandard conservation measures. Documented here are the information on population density, distribution, habitat traits, associations and threats with the aim of enhancing proper management of the *C. shengena*. Active searching was done in Chome Nature Forest Reserve in 12 plots during wet and dry seasons. Plots were searched thoroughly and when the species was encountered, the information about that site were recorded which included GPS location, altitude, percentage canopy cover, distance from water source, disturbance level, temperature and the amount rainfall. There was significant difference in population density of *C. shengena* between wet and dry seasons. Places that exhibited higher density of *C. shengena* had largely shady areas close to slowly moving fresh waters. Also the population density was higher in wet season suggesting that rainfall and temperature had influence on *C. shengena* distribution. It was found that *C. shengena* was highly distributed in the mid altitudes (1951 - 2050 m.a.s.l), of the western part of the forest where there was slow moving waters nearby. About 55% of *C. shengena* population was close to the forest boundary and this exposes *C. shengena* to higher chances of depopulation due to increased human activities in the forest, either legally or illegally.

The Chome Nature Forest Reserve was observed to experience threats mainly from illegal logging, gold mining and human trails and the development of tourism activities.

The government should put more emphasis on the societal knowledge about the resources and wildlife found in the reserve to maximize the conservation efforts to the protected areas.

DECLARATION

I, Flora Lawrence Tesha, do declare to the Senate of Sokoine University of Agriculture that, this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution

Flora Lawrence Tesha
(MSc. Candidate)

Date

The above declaration is confirmed by:

Dr. Geoffrey Soka
(Supervisor)

Date

COPYRIGHT

No part of this dissertation may be reproduced, stored in any retrieval system, or transmitted in any form or by any means without prior written permission of the author or Sokoine University of Agriculture in that behalf

ACKNOWLEDGEMENTS

My profound heartfelt and sincere gratitude are to The Almighty God for his endless graces and blessings that have made me get here. I am also highly grateful to The Holy Virgin Mary, Mother of God whose prayers and guidance gave me strength throughout my studies. I humbly appreciate Sokoine University of Agriculture for enrolling me into this degree programme and providing me with the best academic experience. I am and forever will be deeply thankful to my parents, Mr. and Mrs. Lawrence N. Tesha for their non-ending support on my study. They have supported me financially, mentally and spiritually and yet they have been there for me always; to push and encourage me towards the goal, getting my MSc in Wildlife Management and Conservation. Much thanks to my loving grandmother, Mrs. Enna Semadio, she has always been pouring me care, love, prayers and words of wisdom and encouragement which have made me strong all along my studies. I would like to thank my supervisor, Dr. Geoffrey Soka (Department of Wildlife Management) for his remarkable support, always believing in me and leading and encouraging me in my study. Many thanks to the wonderful staff of the Department of Wildlife Management, led by Dr. Nsajigwa Mbije for their remarkable support in my study which had surely made me understand and love wildlife conservation more. I also acknowledge the support from Chome Nature Forest Reserve office, for their permission to conduct the research in the forest. I would also like to express my appreciation to my sisters Diana Tesha, Odeta Mduda, Mwajuma Ally, Angela Tesha, Sophia Buni and my brother Samuel Tesha for their field assistance. Many thanks to Mr. Amon, Mr. Mchome, Mzee Ng'ele and many other people from Chome who did help in one way or the other.

DEDICATION

To my loving parents, Mr. and Mrs. Lawrence N. Tesha, you have done everything and sacrificed a lot, just for me to get the best and quality education. This work is all for you, I hope it will make you proud as I am proud and blessed to have you.

TABLE OF CONTENTS

ABSTRACT.....	ii
DECLARATION.....	iii
COPYRIGHT.....	iv
ACKNOWLEDGEMENTS.....	v
DEDICATION.....	vi
TABLE OF CONTENTS.....	vii
LIST OF FIGURES.....	ix
LIST OF PLATES.....	xi
LIST OF TABLES.....	ix
LIST OF ABBREVIATIONS.....	xii
CHAPTER ONE.....	1
1.0 INTRODUCTION.....	1
1.1 Background information.....	1
1.2 Problem statement and justification.....	4
1.3 Objectives.....	5
1.3.1 Overall objective.....	5
1.3.2 Specific objectives.....	5

1.4	Hypotheses.....	5
CHAPTER TWO.....		6
2.0	LITERATURE REVIEW.....	6
2.1	Eastern Arc Mountains and endemism.....	6
2.2	Population parameters and their importance in conservation.....	7
2.3	Factors influencing distribution and habitats of Amphibians.....	7
CHAPTER THREE.....		9
3.0	MATERIALS AND METHODS.....	9
3.1	Study area description.....	9
3.2	Study design.....	10
3.3	Data collection.....	10
3.4	Data analysis.....	12
CHAPTER FOUR.....		13
4.0	RESULTS.....	13
4.1	Population density of <i>C. shengena</i>	13
4.2	Distribution pattern of <i>C. shengena</i>	14
4.3	Factors affecting population density and distribution of <i>C. shengena</i>	15
CHAPTER FIVE.....		17
5.0	DISCUSSION.....	17
5.1	Population density of <i>C. shengena</i>	17

5.2	Distribution of <i>C. shengena</i>	18
5.3	Suitable habitat characters for <i>C. shengena</i>	19
CHAPTER SIX.....		23
5.0	CONCLUSION AND RECCOMENDATIONS.....	23
6.1	Conclusion.....	23
6.2	Recommendations.....	23
REFERENCES.....		25

TABLE

Table 1: Habitat characteristics of sites occupied by *Callulina shengena*.....16

LIST OF FIGURES

Figure 1:	Map of Chome Nature Forest Reserve.....	9
Figure 2:	Average density of <i>C. shengena</i> within three altitudinal zones for two seasons. Values are means and error bars present standard errors of the means (SEM).....	13
Figure 3:	Map showing the distribution pattern of <i>C. shengena</i> in Chome Nature Forest Reserve.....	14

PLATE

Plate 1: (a) Fresh water swamp in CNFR (b) *C. shengena* obtained in the field
data collection (c) Image of *C. shengena* (d) Open land after human
activities in the reserve.....11

LIST OF ABBREVIATIONS

CNFR	Chome Nature Forest Reserve
GIS	Geographical Information System
h	Hours
IUCN	International Union for Conservation of Nature
Km	Kilometer
m	Meter
mm	Millimeter
MNRT	Ministry of Natural Resource and Tourism
SUA	Sokoine University of Agriculture
SVL	Snout to Vent Length
UV	Ultra Violet

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Amphibians were among the first vertebrates to inhabit the dry land from the waters in some stages of their lives over millions of years ago (Bishop *et al.*, 2019). Despite being the pioneers of life on land, they are still the most susceptible vertebrate taxon as revealed by their rapid global declines due to habitat loss, climate change, and many other factors (Stuart *et al.*, 2003; Alroy, 2015; IUCN, 2020). Most of the world's amphibian species are inhabiting the major biodiversity hotspots found in the tropics especially in Central and South America and the sub-Saharan Africa (Whittaker *et al.*, 2013).

It is estimated that at least one third of some 6000 currently known amphibian species are threatened with extinction, making amphibians one of the most threatened groups of animals on earth (Collins, 2010; IUCN, 2020). Higher numbers of species in this group are now inhabiting many biodiversity hotspot areas, but yet the decline has still been taking place in a higher rate (Ceballos *et al.*, 2015; Kitzes *et al.*, 2017).

Biodiversity hotspots are considered to be ecologically richest places globally since they have higher endemism levels and yet facing enormous threats (Hirschfeld *et al.*, 2016). About 70% of natural vegetation is said to be lost in these hotspots and the fauna inhabiting them are facing vast challenges to ride with the changing habitats (Operations and Town, 2005). Anthropogenic and environmental aspects can aggravate the decline and extinction of amphibian populations due to versatility of this group to emergent issues like diseases (Fouquet *et al.*, 2010; Blaustein *et al.*, 2018).

Among the most common wildlife conservation approaches used to manage threatened species is the introduction of protected areas (PAs), aiming to provide maximum and sustainable conservation of biodiversity with the lowest possible cost (Campos *et al.*, 2016). One of these areas is the Eastern Arc Mountains (EAMs) of Tanzania and Kenya which is one of the hotspots and harbors a higher number of amphibian species with high level of endemism (Malonza and Kinyatta, 2008).

Ancestral-area reconstructions show the existence of Brevicipitids since the Oligocene, with the central EAMs as the initial epicenter of divergence of the forest species (Gereau *et al.*, 2016). Brevicipitids are small to moderate-sized rain frogs (most 30–50 mm SVL) found in the Sub-Saharan east and southern Africa. They are nearly spherical in shape, with the head barely distinguishable from the body, short limbs and robust (Figure 2(b,c)). Their globular glandular appearance is further enhanced by a tendency to inflate the body when disturbed (Vitt and Caldwell, 2014). The EAM forest habitats had persevered stable conditions which ensured the accumulation of Brevicipitid frog species over a long span of geological time (Gereau *et al.*, 2016). Within this chain, there are multiple patches of mountains including South Pare Mountains where Chome Nature Forest Reserve (CNFR) is located.

The CNFR is a home to endemic frog species, *Callulina shengena* (IUCN, 2020). *C. shengena* is a warty frog species of family Brevicipitidae firstly reported in 2010 during a survey in the rainforests on northern part of Eastern Arc Mountains (Menegon *et al.*, 2011). *C. shengena* is highly characterized by a glandular bulbous body of 5cm snout-vent length maximum and a very small head lacking a tympanum and truncated toes. *C. shengena* is different from its close relative species *Callulina laphami* which is

considered to be its sister species lacking a tympanum, smooth, less granular skin, truncate finger and toe tips, and the presence of a bright color in the ocular region (Loader *et al.*, 2010; Menegon *et al.*, 2011; Bwong, 2017).

Upon the urge to comprehend the core factors for amphibian ecology, herpetologists realized that the spatial distribution and population density are important matters to work on since species distribution is never random (Malonza and Veith, 2012). In particular, we should strive to better understand the population density and dynamics of amphibian populations and the factors that govern the distribution of species and species diversity at particular sites (Schmidt, 2004). Population size and density estimates are crucial for the determination of the status of species and guides how to utilize resources for the management and conservation of that species (Kanagavel *et al.*, 2018).

Habitat loss, land degradation and climate change are among the frontline causes of biodiversity declines (Raxworthy *et al.*, 2008; Swartz *et al.*, 2020). Whenever the habitat changes, there can surely be visible or invisible impacts to the ecosystem functioning and individual species performance and survival (Rustad *et al.*, 2012). Habitat loss and fragmentation are among the major threats to amphibian populations and anthropogenic activities in the protected areas, along with climate change may exacerbate amphibians' extinction (Ashrafzadeh *et al.*, 2019).

Appropriate management of wildlife populations begins with acquiring the essential baseline information about target species and the habitats characteristics they associate with (Ehwan *et al.*, 2018). Predicting changes in population sustainability is a fundamental challenge, because knowledge of population status is required and research is needed to enumerate these parameters over appropriate time-scales (Mccaffery *et al.*,

2012). Proactive approaches before declines are essential to avoid cost full management strategies to sustain threatened populations because it is obvious that responsive management tactics don't bring about proper outcomes for amphibian conservation (Sterrett *et al.*, 2019).

Amphibians in tropical areas, are distributed in small patches which restrict them to thin altitudinal belts or in the isolated geographical ranges (Whittaker *et al.*, 2013). They are sensitive to changes in thermal and hydric environments due to their unshelled eggs, highly permeable skin and unique biphasic life-cycles (Mccaffery *et al.*, 2012; Whittaker *et al.*, 2013; Gould *et al.*, 2017). This explains why many species have gone extinct because climate have higher influence on the temperature and moisture of their corresponding habitats (Museo de Zoología (Barcelona), 2001; Lawler *et al.*, 2010; Duarte *et al.*, 2012).

1.2 Problem statement and justification

One of the focal constraints on understanding amphibians' impulsive decline is that there is no enough and long-term information about their populations which could be useful on creating better ways to respond to their decline. The IUCN Red list (2019) has listed *C. shengena* as Critically Endangered because its extent of occurrence is estimated to be less than 100 km². It is known to exist at only a single location but there is no information about its population size and trend, therefore IUCN urges and recommends more research to be done on that scope. Knowledge on the habitat characteristics of the area where the target species is found is very crucial for wildlife conservationists. Characteristics like altitudinal ranges, distance from water, availability of shade and other factors including disturbance are observed in many studies to influence the distribution and diversity of amphibian species (Qian *et al.*, 2007; Behangana *et al.*, 2009; Siqueira *et al.*, 2014;

Khatiwada *et al.*, 2019). In many African countries, declines in amphibian populations are highly attributed to the habitat alterations (Hirschfeld *et al.*, 2016).

To manage and conserve any species effectively, a proper knowledge of population size or density and its habitat associations is critical (Gomez-Salazar *et al.*, 2012; Kanagavel *et al.*, 2018). Populations with lower densities are under high extinction risk than larger populations and therefore understanding and forecasting deviations in population of species is a fundamental matter (Funk *et al.*, 2003).

Information on population density, distribution and the factors attributing to their distributions are highly required and research is needed to communicate those factors over appropriate time-scales to monitor their trends (Yannic, 2007). The data from this study will therefore enhance the proficiencies of conservationists to manage *C. shengena* and avert any odds of any possible threats and extinction risk. This study will enlighten and capacitate the understanding and clearer insight of the population status as well as the habitat characteristics pertaining to *C. shengena*'s survival.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to determine the population status and distribution of *C. shengena* and its habitat associations in Chome Nature Forest Reserve.

1.3.2 Specific objectives

- i. To estimate the population density of *C. shengena* in Chome Nature Forest Reserve;
- ii. To establish the *C. shengena* spatial distribution pattern in Chome Nature Forest Reserve;

- iii. To examine the influence of habitat characteristics and human disturbance on the *C. shengena*'s distribution in Chome Nature Forest Reserve.

1.4 Hypotheses

- i. Habitat characteristics have no influence on *C.shengena*'s density and distribution.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Eastern Arc Mountains and endemism

The Eastern Arc Mountains is a chain of 13 ancient, crystalline blocks, arranged as an arc, and located in Eastern Africa (Tanzania and Kenya) overlooking the Indian Ocean (Malonza and Kinyatta, 2008). This chain and adjoining coastal forests of Tanzania and Kenya have been listed as world biodiversity hotspots with high endemism (Poynton *et al.*, 2007). Historically, the Eastern Arc Mountains were probably covered by a mosaic of rainforests. These rainforests were concentrated mostly on the eastern slopes and on the upland plateau and were interspersed with open grassland areas and with dry, semi-deciduous forests on the western slopes (Finch *et al.*, 2017).

The majority of endemic species occur in the wet forest that covers parts of the eastern slopes of the mountains (Menegon, 2015). Although the forest environment has been well-studied, the grasslands and ecotones could represent a further frontier of batrachological exploration. Although many species of amphibians have been described from Africa in recent years, amphibians as a group remain poorly studied relative to other vertebrate taxa (Menegon, 2015).

At least 96 vertebrate species are endemic, split as follows: 10 mammal, 19 bird, 29 reptile and 38 amphibian species (Moyer *et al.*, 2006), including this Warty frog, *C. shengena* (Loader *et al.*, 2010). Many of the endemic species are confined to high-altitude sites and their distribution patterns are shrinking with time. *C. shengena* displays a 'single site' distribution, since they are confined to a single valley or parts of it, at certain altitudes, and yet are surrounded by apparently suitable habitat (Menegon, 2015).

2.2 Population parameters and their importance in conservation

Population density is often regarded as an important factor in explaining the loss of species (Almeida-Santos *et al.*, 2011). Most amphibian declines go undetected until populations have dropped precipitously or gone extinct which is too late to infer causes of declines, prevent future declines, or restore populations (Unk *et al.*, 2003). Before launching the strategies to tackle population declines, wildlife managers should know which species are declining, where and the rate that they are declining (Schmidt, 2004).

Furthermore, it is critical that this information is gathered quickly and the only reliable way to gather this information is through well-designed amphibian population monitoring programs (Unk *et al.*, 2003). This involves estimating population parameters of interest over time to test for significant declines or increases in the parameters (Thompson *et al.*, 1998). The parameters of interest in population monitoring programs are usually abundance (the absolute number of animals) or density. There is an increasing interest among conservation biologists to learn about the effects of density dependence on life history traits and the dynamics of natural populations (Benedikt, 2006). This is because the response of a natural population to negative impacts, such as habitat loss, illegal harvesting depends crucially upon density-dependent population responses (Sutherland and Norris, 2002).

2.3 Factors influencing distribution and habitats of Amphibians

The distribution of amphibian populations is highly influenced by precipitation, temperature, relative humidity, light intensity along with canopy cover and ground moisture (Jameson and Jameson, 2013). Habitat conditions may cause variation in apparent abundance (the number of individuals that are active and can be detected) that may be more important than variation in true abundance (Tanadini and Schmidt, 2011). Timely monitoring the change in species composition and inferring effects of species interactions should thus be of high priority for conservation policy and population management (Dornelas *et al.*, 2014; Sentis *et al.*, 2014).

Changes in temperature drive changes in precipitation and the availability of water are perhaps more paramount to the survival of amphibians than it is for any other tetrapod group. Whitfield *et al.* (2007) documented ~75% decline in both amphibian and reptile communities at La Selva Costa Rica for over 35 years and suggested human induced and climate-driven loss of leaf litter as a cause. McMenamin *et al.* (2008) correlated a 50% decline in amphibian populations in Yellowstone National Park (the world's oldest nature reserve) with an increase in temperature and decrease in precipitation over 60 years.

Human disturbance has been one of the main causes of amphibian loss in forests (Vallan, 2002). Depending on their physiological and adoption capabilities, amphibians may respond differently whenever there is an anthropogenic disturbance or environmental fluctuation from the normality (Malonza and Kinyatta, 2008). Rovito *et al.* (2009) documented widespread and severe declines of upland salamanders in Mexico and

Guatemala and presented a convincing case for causation by changes in precipitation and humidity associated with massive human activities mainly deforestation in the areas. Donnelly and Crump (1998) predicted that tropical amphibians will suffer reduced reproductive success, reduced food supply, and a disruption in breeding behavior and periodicity by the ongoing human invasion and disturbance to their ranges. They predict that the effects will be greatest on endemic species, those species restricted to a specific location and which usually have specialized ecological requirements.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area description

The study was conducted in Chome Nature Forest Reserve (CNFR) located in the South Pare Mountains (Fig. 1). CNFR is located between $4^{\circ}10'$ – $4^{\circ}25'$ south and $37^{\circ} 53'$ – 38° East and includes the highest ridge of the range and the highest peak of Shengena (2463 m.a.s.l), but it slopes down to an altitude of 1250 m.a.s.l on its Eastern edge. The CNFR is among of twelve (12) Nature reserves found in Tanzania (Eastern Arc Mountains). Rainfall ranges between 700 – 1400 mm / year with a mist effect at higher altitudes. The dry season extends between June and October. Temperatures vary from 25 °C max (March) to 16 °C min (July) (Doggart *et al.*, 2008).

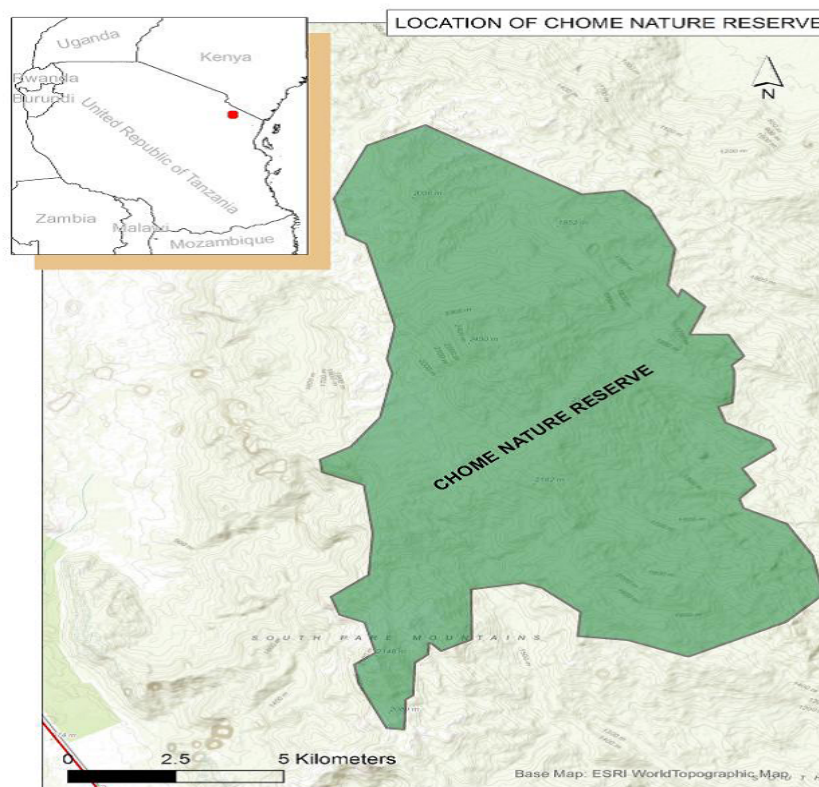


Figure 1: Map of Chome Nature Forest Reserve

3.2 Study design

A total of 12 plots were established in the reserve, in terms of altitudinal ranges, three zones were established to cover all the vegetation types of the mountain (URT, 2010). Low altitudes less than 1950 m.a.s.l, mid altitudes between 1951 and 2050 m.a.s.l, and higher altitudes more than 2051 m.a.s.l. In each altitudinal zone, four rectangular plots were established with size of 50 m × 70 m (0.35 ha) while the distance between plots was not less than 300 m. Nested plot sampling method was used where dominant trees species were recorded in 50 m x 20 m quadrats within the permanent plots, and shrubs in 10 m x 10 m quadrats where grasses were covered in 1m x 1m quadrats.

3.3 Data collection

Active search was executed during dry season (October, 2019) and wet season (March, 2020) and this was conducted five (5) times per season. In each block, seven (7) people conducted a search by walking in parallel lines of 2 m each until the whole plot was sufficiently searched. In a method adopted and modified from Kanagavel *et al.* (2018) the area search took place from 0800 h to 1600 h and this was done with same search efforts throughout. The same plots were searched in wet season and dry seasons to determine the effects of seasonal differences on the population density and distribution of *C. shengena*.

In each plot numbers of individuals per plot were recorded, coordinates of each plot and habitat variables. These variables were: altitude measured using an altimeter, air temperature using thermometer, percentage canopy cover by ocular estimation at each plot surveyed (Behangana *et al.*, 2009). Proximity to water source by estimating between

the distance between the plot and the nearest water point, human disturbance level by estimating the number of logged trees, bare land size left after mining and human trails (Plate 1).



(d)

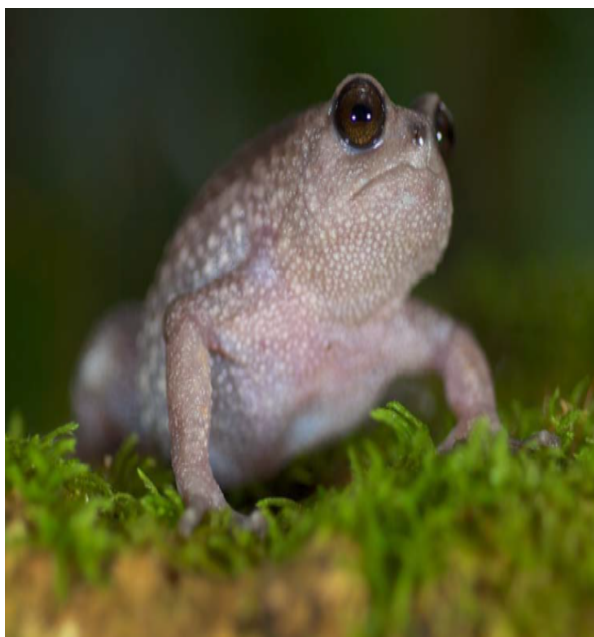


Plate 1: (a) Fresh water swamp in CNFR (b) *C. shengena* obtained in the field data collection (c) Image of *C. shengena* (d) Open land after human activities in the reserve

3.4 Data analysis

The population density of *C. shengena* was estimated by calculating the average number of individuals obtained in a unit area (ha). The distribution was mapped using Kernel Density in Geographic Information System GIS. The locations that were recorded in the field and the associated altitudes were used to prepare distribution maps. This is to show how *C. shengena* is distributed in their ranges.

To assess the main effects and interactions of the recorded habitat factors with the population density a General Linear Model (GLM) in SPSS 16.0 was run. Disturbance levels was used as a covariate while altitude, canopy cover, water distance, annual rainfall and mean monthly temperature were used as random factors. This was to determine how each factor had influenced the distribution or density of individuals.

CHAPTER FOUR

4.0 RESULTS

4.1 Population density of *C. shengena*

A total of 180 individuals were spotted and recorded during an active search in the study site. Average density of *C. shengena* was between 15 individuals/ha to 22 individuals/ha during dry and wet season respectively. The total number of individuals varied significantly between sampling seasons ($t_{11} = 5.9$, $p < 0.001$) with a minimum of 63 in dry season and a maximum of 117 in wet season. There was a significant variation in density with altitudes where the recorded highest density was in the mid altitudes followed by the lower altitudes (ANOVA; $F_{1,5} = 3.55$, $p < 0.001$). About 60% of the total density was confined in the mid altitude areas compared to the higher or lower altitudes (Figure 2). The higher altitude areas had the lowest density which was only about 7.2% of the average density.

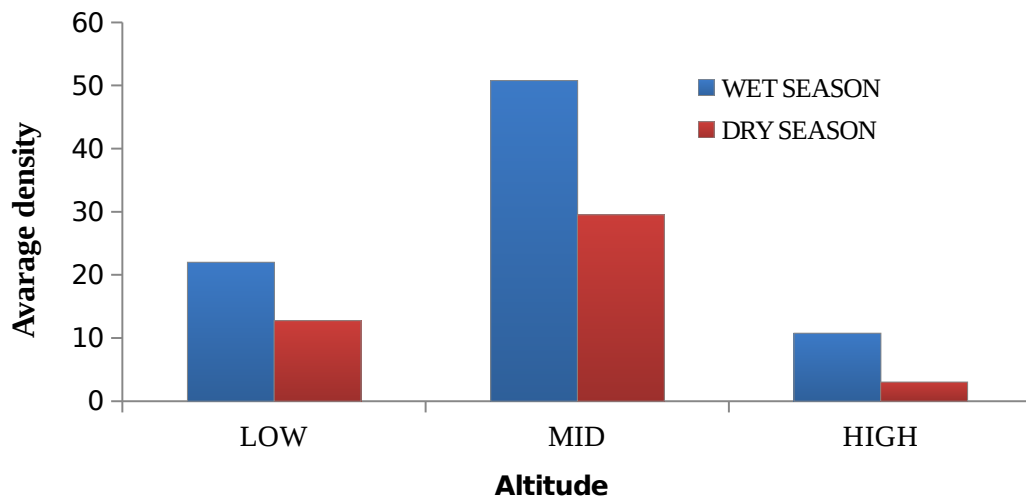


Figure 2: Average density of *C. shengena* within three altitudinal zones for two seasons. Values are means and error bars present standard errors of the means (SEM)

4.2 Distribution pattern of *C. shengena*

Distribution of *C. shengena* varied substantially across the reserve. *C. shengena* was recorded to be mostly abundant in the western part of the mountain (less than 2100 m.a.s.l) in more undisturbed sites with slow moving waters. Some were recorded in the higher altitudes of the reserve. Figure 3 displays this pattern with the high density on western areas than the eastern sides.

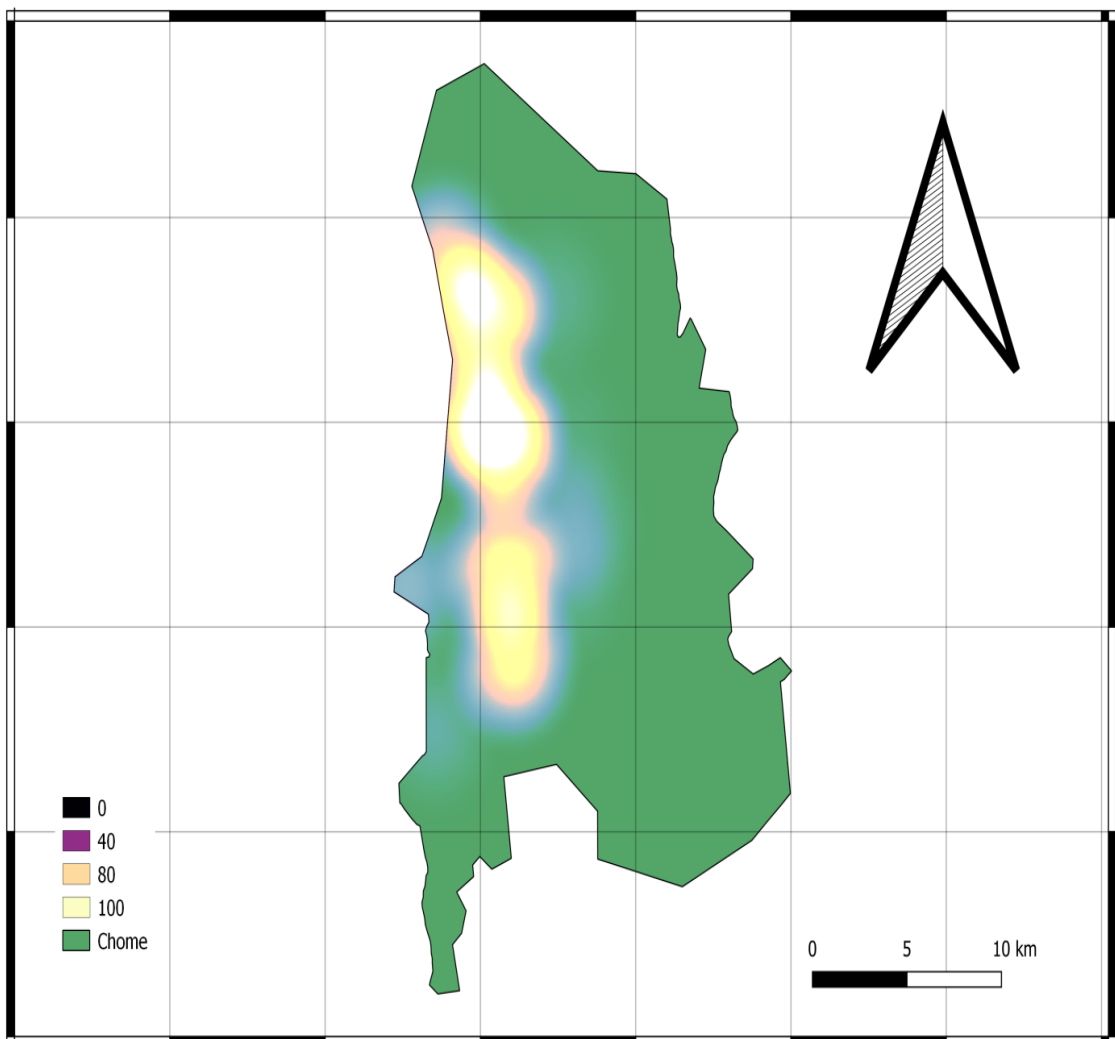


Figure 3: Map showing the distribution pattern of *C. shengena* in Chome Nature Forest Reserve

4.3 Factors affecting population density and distribution of *C. shengena*

Habitat attributes that were investigated in this study included altitude, canopy cover, distance from the water sources and the levels of disturbance. General Linear Model was run to test the relationship between these attributes to the population of the study species. There was a main effect of the altitudes on populations density of *C. shengena* ($F_{1,11} = 22.78$, $p = 0.001$). Mid altitudes exhibited highest population density of *C. shengena* compared to the lower or higher altitudes.

Canopy cover had a significant influence of *C. shengena* distribution ($F_{1,11} = 18.1$, $p = 0.002$). 66% of the plots were having canopy of 75% and more. Large trees that had broad branches and leaves were more abundant in the plots which had higher counts.

Furthermore, the plots were set within various distances from the water sources. Plots which were closer to water source (less than 50m) had a higher density of *C. shengena* (57%) while those far from water had lower density. There were significant variations of population density as there were changes in distances from water sources ($F_{1,11} = 69.88$, $p = 0.004$). This suggests that *C. shengena* density declines from water sources.

The mid altitudes were dominated by *Ocotea spp*, *Macaranga spp* and *Podocarpus spp*, Fern (*Drynaria volkensii*), *Cladonia usambarensis* as the understoreys. The higher altitude zone was dominated by *Ocotea spp*, *Syzygium spp*, *Podocarpus spp* and *Rapanea sp*.

The lower zone is dominated by *Acacia spp*, (exotic) *Albizia spp* and grass *Sporobolus spp*. Lower zone was previously heavily disturbed by illegal gold

mining activities and logging which left many bare spaces and holes with natural vegetation removed.

Human disturbance on the site was among the factors assessed for distribution and density of *C. shengena*. It was observed that disturbances induced by anthropogenic activities varied within the reserve. This had a statistically significant but yet negative influence on distribution of the species ($F_{1,11} = 31.1, p < 0.0001$).

The microclimatic data recorded were surface temperature and rainfall in both wet and dry seasons. Average monthly temperatures were $12.2 \pm 1.14^\circ\text{C}$ and $25.3 \pm 1.06^\circ\text{C}$ for wet and dry seasons respectively. The average monthly rainfall was higher for wet season (268.4 ± 34 mm) than for dry season (160 ± 18 mm). Throughout the study duration, monthly temperature showed significant influence on the population density of *C. shengena* ($F_{1,1} = 25.94, p = 0.001$). Furthermore, rainfall had a significant effect on *C. shengena* density ($F_{1,11} = 18.13, p = 0.001$). This suggests that *C. shengena* density increase with increasing rainfall.

Table 1: Habitat characteristics of sites occupied by *Callulina shengena*

Variable	Range	Mean \pm Se
Altitude (m.a.s.l)	1900-2400	2066.4 \pm 132.6
Air Temperature ($^\circ\text{C}$)	12-26 $^\circ\text{C}$	12.2 \pm 1.14 $^\circ\text{C}$
		25.3 \pm 1.06 $^\circ\text{C}$
Rainfall (mm)	98-378mm	268.4 \pm 34 mm
		160 \pm 18 mm.
Proximity to Water Source (m)	5-125m	
Percentage Canopy Cover (%)	50-100%	
Disturbance Levels	1High, 2Moderate, 3-Low	

CHAPTER FIVE

5.0 DISCUSSION

5.1 Population density of *C. shengena*

This study recorded the maximum average density of *C. shengena* to be 22 individuals/ha. This shows that the population of *C. shengena* is very small and threatened (IUCN Species, 2012). This average density varies along the forest, where the mid altitudes harbored a higher percentage of the total density which provides more favorable and conducive conditions which sustain *C. shengena*. The same pattern was also observed by Malonza (2012) in Taita Hills in Kenya where there was a higher density of anuran species in the mid altitudes than the lower or higher altitudes.

Results of this study have shown that the population of *C. shengena* is explained by a combination of factors both physical and microclimatic. Population density influences population trend in combination with habitat characteristics with climatic attributes (Benedikt, 2006). The combination of these factors are mainly used by many researchers and they can properly determine the ecology of different amphibian species as observed by Ra *et al.* (2010) in the study conducted on Gold-spotted pond frog.

Density is a crucial parameter in amphibian ecology as it may act as a potential cause of decline and with the inputs of extrinsic factors like global climatic change; the species population may decline dramatically if the density is low and the rate of restocking is slow. In such a small reported density, most of it is in the mid and lower altitudes due to the corresponding warmer temperatures and significant amounts of moisture essential for ectotherms survival.

5.2 Distribution of *C. shengena*

In this study, *C. shengena* had a humped distribution with highest density within the mid elevations. It is found on the western side of CNFR, mostly on the mid altitudes of the mountain in the reserve. Its distribution pattern as shown in Figure 3, the mid altitudes of the mountain provide more ideal environments for the sustainability of *C. shengena*. This distribution pattern is observed as many amphibian species tend to have their population peak in the mid altitudinal ranges (Chettri and Acharya, 2020).

C. shengena will still be categorized as Critically Endangered (B1ab + 2ab) as per the IUCN Red list because its distribution is restricted to an area estimated to be 35 km² (IUCN, 2019). This range is potentially at risk of shrinkage due to the on-going human activities and possible climatic changes that are happening all over the globe (Ecosystems, 1995; WWF-france *et al.*, 2015).

C. shengena is known from one locality, where maximum distribution range and quality of its habitat is expected to decline due to the ongoing development of large-scale tourism activities and human trails that are cutting through the forest. These activities that have been highly promoted by the management of the reserve pose a high risk of shrinkage of the presently small range of *C. shengena*.

One of the probable limiting factors that subject *C. shengena* to mid and lower altitudes is the higher chance and availability of water that is decreasing as the altitudes increase. This is supported by Spesies *et al.* (2018) in a study conducted on the species richness and distribution along the Malaysian forests. CNFR has many of its water sources in the

mid altitudes of the western part but are emptying these waters in the eastern side of the mountain.

The mid altitudes of the CNFR are occupied by many large trees with large canopy that obstructs most of the Ultra Violet (UV) radiation, fresh water streams and swamps with warm temperatures slightly higher than the higher altitudes. This had shown to be more preferable and conducive habitat to *C. shengena* and therefore its peak population in those altitudes.

5.3 Suitable habitat characters for *C. shengena*

Amphibian species depend on multiple habitat characteristics which are necessary for completion of life circle and sustainability of the whole population (Bishop *et al.*, 2019). These include water, shade or vegetation canopy cover, rainfall, temperature and the disturbance in the area could either support or suppress the given population. With the increase in altitude, temperature declines monotonically, whereas precipitation normally peaks at mid-altitudes (Chettri and Acharya, 2020).

It was observed that *C. shengena* is highly dependent on proximity of streams of fresh water. Distance from water source has an impact on the occurrences of amphibian species throughout the world (Bagne *et al.*, 2007). Most of the individuals were closer to water bodies and many would run to the water after being disturbed. The slowly moving waters or swamps had more individuals. This explains the way they use calm or slow moving waters for reproduction and it is highly beneficial for their oxygen absorption and survival tactics in case of disturbances.

Average density of *C. shengena* was positively influenced by canopy cover. Plots which

had a high percent of canopy cover were harboring a high number of *C. shengena*. The shade provided by the trees had shown to be better refuges for the species to avoid direct and too much sunlight. This is because the shades are more preferable to the amphibian physiology and camouflage from predators and disposal to UV-light (Corn, 2005).

Vegetation canopy also acts as a blanket to the ground dwelling organisms including *C. shengena* during harsh weather conditions. In CNFR, the mid altitudes had about 80% percentage of canopy cover and this allows optimal passage of sun rays and warm temperatures for the reproduction and other normal physiological activities.

In the Eastern Arc Mountains, rainfall increases with altitude while temperature decreases (Shemsanga *et al.*, 2010). *Ocotea spp*, *Macaranga spp* and *Podocarpus spp* were the most abundant trees in the mid altitudes but there is an increase of invasive and introduction of non-native tree species. These trees were more abundant in the areas that had higher densities of *C. shengena* and this suggests that they are more advantageous to the survival of this population due to their large canopy for shade and moisture retention.

As the ectothermic organisms, it is widely recognized that warmer and moderate temperature and precipitation in low and mid altitudes locations usually support more species and individuals (Khatiwada *et al.*, 2019). Temperature and precipitation are crucial to the physiological functioning and survival of the amphibian species everywhere (Qian *et al.*, 2007). Climatic variables mainly temperature and rainfall affect the survival and existence of amphibians in an area (Rustad *et al.*, 2012).

During high rainfall season, more individuals were spotted easily because the weather

provided wet conducive environments for the species survival, which when integrated with intrinsic population characteristic, can sustain the individual species.

In this study, rainfall had a higher influence on the number of occurrences recorded during both wet and dry season. The rainfall significantly increases the volume of swamps and ponds that are highly used by *C. shengena* and it is clearly observed in the dropping of counts during dry season.

Despite the high rainfall at high altitudes in CNFR, much of the water as observed elsewhere settles on mid-altitudes and the rest on the foot of the mountain creating breeding sites for open water breeders (Hofer *et al.*, 2000). This is one of the reasons to the high density of *C. shengena* observed in the mid elevations of the mountain in the reserve because it is the ideal condition to support amphibian populations.

The changes in rainfall seasons were reported in many parts of Tanzania in 2019 and early 2020. The months that were to experience low rainfall or drought received extremely high rainfalls in the past 40 years as reported on the Statement on the Status of Tanzania Climate (TMA, 2019). The longer dry seasons and abnormal rainfalls have been associated with the increase of deadly amphibian fungi that has been sweeping off global amphibian populations (Catenazzi *et al.*, 2010; Collins, 2010; Alroy, 2015; Bishop *et al.*, 2019; Blaustein *et al.*, 2018).

These changes in weather in many areas also affect the life circles of amphibians and alter their reproductive rates since their eggs are highly affected by temperature and humidity of the corresponding habitats. The unexpected scenarios like this are tampering the typical

ecosystems behavior and if the species are not adaptive to the harsh turnovers, it could lead to the disruption of the gene flow of the individual species and the whole ecosystem.

In this study, frog counts were negatively correlated to human induced disturbances in the forest. Humans' trails, illegal mining and logging that have been taking place in the forest are pushing the frogs into a smaller habitat range. This is supported by the study that was previously reported in Albertine Rift Valley done by Behangana *et al.* (2009) where the disturbance had negative impact on amphibian abundance and caused more damage to the species survival.

The previous illegal mining had left the low altitudes of the Eastern Part with multiple holes and despite of the introduction and plantation of the trees by the UNDP to reestablish the vegetation of the area, the natural trees have been removed. Human activities, either legal or illegal reduce habitat range of species and might subject them to more threats and fuel their population decline.

The increasing rate of tourism activities that are highly encouraged by the government through the Ministry of Natural Resources and Tourism poses a higher risk of reducing *C. shengena* range, provided that it is endemic to the corresponding forest (Doggart *et al.*, 2017). Human trails across the forest tend to be among the major causes of introduction and dispersion of exotic tree species in the forest. Species like *Acacia mearnsii* and *Eucalyptus spp* were not reported in the forest decades ago but now the dispersal of their seeds by human movements have moved it to the forest.

Due to the previous logging activities that were heavily conducted in the reserve, native

species like *Ocotea usambarensis* and *Podocarpus latifolius* have declined and the exotic ones have taken over many parts. The native tree species were found to be more preferred by *C. shengena*. Despite of the conservation efforts and banning of commercial logging activities, the habitat preferences of *C. shengena*'s population could be threatened.

CHAPTER SIX

5.0 CONCLUSION AND RECCOMENDATIONS

6.1 Conclusion

Population density and habitat associations are key features in conservation and management of *C. shengena*. This study found an average density of about 22ind/ha and being in a very small area of occupancy of less than 100 km², it is categorized as critically Endangered as per the IUCN ranks. This population density is small but yet habitats strive to maintain its survival.

C. shengena was abundant in the mid altitudes of the reserve than the higher altitudes or lower altitudes. This is highly attributed to the availability of basic requirements for their survival including good shades, warm temperatures and fresh waters to sustain their population.

Mid altitudes, fresh waters, high canopy cover and rainfall were the crucial factors that prove to be more preferred by *C. shengena* while human disturbance and high temperature disturbed and negatively impacted it. There should be a focus on this species with its habitats to dodge any chances of population decline which may lead to the extinction in the future.

6.2 Recommendations

This study recommends that the management of water sources and natural vegetation in the forests should be prioritized for amphibian conservation. This is because the results from this study demonstrated the importance of water sources (rivers and swamps) inside closed canopied forests for maintaining high population densities.

Tourism activities should also be reviewed to minimize the impact of tourism on the habitat of *C. shengena* which is Critically Endangered and endemic to the CNFR. The presence of different and unofficial trails along the mountain should be avoided to minimize disturbances.

There should be immediate measures and more focus on the wildlife in the protected areas which are not considered as milestone species like the big five. Delaying species management of such small groups of wildlife in the forests lowers the chance of successful salvage, increases the risk of extinction, and can be expensive.

REFERENCES

- Almeida-Santos, M., Siqueira, C. C., Van Sluys, M. and Rocha, C. F. D. (2011). Ecology of the Brazilian Flea Frog *Brachycephalus didactylus* (Terrarana: Brachycephalidae). *Journal of Herpetology* 45(2): 251–255.
- Alroy, J. (2015). Current extinction rates of reptiles and amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 112(42): 13003–13008.
- Ashrafzadeh, M. R., Naghipour, A. A., Haidarian, M., Kusza, S. and Pilliod, D. S. (2019). Effects of climate change on habitat and connectivity for populations of a vulnerable, endemic salamander in Iran. *Global Ecology and Conservation* 19: 1 – 13.
- Bagne, K. E., Finch, D. M. and Friggens, M. M. (2007). *Vulnerability of Amphibians to Climate Change: Implications for Rangeland Management*. United States Department of Agriculture, USA. 1pp.
- Behangana, M., Kasoma, P. M. B. and Luiselli, L. (2009). Ecological correlates of species richness and population abundance patterns in the amphibian communities from the Albertine Rift, East Africa. *Biodiversity and Conservation* 18(11): 2855 – 2873.
- Benedikt, P. E. (2006). Density, climate and varying return points : an analysis of long-term population fluctuations in the threatened European tree frog. *Oecologia*

149: 65–71.

Bishop, P. J., Mainguy, G., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B. and Moreno, J. G. (2019). *The Amphibian Extinction Crisis - What Will it Take to Put the Action into the Amphibian Conservation Action Plan?*, Institute Veolia Environment, France. 17pp.

Bishop, P. J., Mainguy, G., Angulo, A., Lewis, J. P., Moore, R. D., Rabb, G. B. and Origin, T. (2019). The origin and evolution of vertebrates. **5: 697–735.**

Blaustein, A. R., Urbina, J., Snyder, P. W., Reynolds, E., Dang, T., Hoverman, J. T. and Hambalek, N. M. (2018). Effects of emerging infectious diseases on amphibians: A review of experimental studies. *Diversity* 10(3): 1 – 49.

Bwong, B. A. (2017). At the crossroads of two biodiversity hotspots: The biogeographic patterns of Shimba Hills, Kenya. Thesis for Award of PhD Degree at Universitat Basel, 230pp.

Campos, F. S., Llorente, G. A., Rincón, L., Lourenço-De-Moraes, R. and Solé, M. (2016). Protected areas network and conservation efforts concerning threatened amphibians in the Brazilian Atlantic Forest. *Web Ecology* 16(1): 9 – 12.

Catenazzi, A., Lehr, E., Rodriguez, L. O. and Vredenburg, V. T. (2010). *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the Upper Manu National Park, Southeastern Peru. *Conservation Biology* 25(2): 382 – 391.

- Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M. and Palmer, T. M. (2015). Accelerated modern human-induced species losses: Entering the sixth mass extinction. *Science Advances* 1(5): 9–13.
- Chettri, B. and Acharya, B. K. (2020). Distribution of amphibians along an elevation gradient in the Eastern Himalaya, India: Amphibians along the Himalayan elevation gradient. *Basic and Applied Ecology* 47: 57–70.
- Collins, J. P. (2010). Amphibian decline and extinction: What we know and what we need to learn. *Diseases of Aquatic Organisms* 92(3): 93 – 99.
- Corn, P. S. (2005). Climate change and amphibians. *Animal Biodiversity and Conservation* 28(1): 59 – 67.
- Doggart, N., Leonard, C., Perkin, A., Menegon, M. and Rovero, F. (2008). *The Vertebrate Biodiversity and Forest Condition of the Northern Pare Mountains*. Technical Paper No. 17. Tanzania Forest Conservation Group, Dar es Salaam, Tanzania. 81pp.
- Donnelly, M. A. and Sciences, B. (1998). Potential effects of climate change on two neotropical amphibian assemblages. *Climate Change* 39: 541 – 561.
- Duarte, H., Tejedó, M., Katzenberger, M., Marangoni, F., Baldo, D., Beltrán, J. F. and Gonzalez-Voyer, A. (2012). Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities.

Global Change Biology 18(2): 412 – 421.

Ecosystems, A. (1995). Consequences of climate change. *Environmental Health Perspectives* 103(12): 1085 –1086.

Fouquet, A., Ficetola, G. F., Haigh, A. and Gemmell, N. (2010). Using ecological niche modelling to infer past, present and future environmental suitability for *Leiopelma hochstetteri*, an endangered New Zealand native frog. *Biological Conservation* 143(6): 1375–1384.

Gereau, R. E., Cumberlidge, N., Hemp, C., Hochkirch, A., Jones, T., Kariuki, M. and Shirk, P. (2016). Globally Threatened Biodiversity of the Eastern Arc Mountains and Coastal Forests of Kenya and Tanzania. *Journal of East African Natural History* 105(1): 115–201.

Gould, P. R., Cecala, K. K., Drukker, S. S., McKenzie, B. A. and Van de Ven, C. (2017). Biogeographical factors affecting the distribution of stream salamanders on the Cumberland Plateau, USA. *Science of the Total Environment* 599(600): 1622–1629.

Hirschfeld, M., Blackburn, D. C., Doherty-Bone, T. M., Gonwouo, L. G. N., Ghose, S. and Rödel, M. O. (2016). Dramatic declines of montane frogs in a central African Biodiversity Hotspot. *PloS One* 11(5): 1 – 15.

ICUN (2020). Numbers of threatened species by major groups of organisms (1996 – 2020). [<https://www.iucnredlist.org/assessment/red-list-index>] site visited 14/8/2020.

- IUCN (2019). *Guidelines for Using International Union for Conservation of Nature Red List Categories and Criteria*. Version No 14. International Union for Conservation of Nature. Switzerland. 60pp.
- Jameson, D. L. and Jameson, D. L. (2013). The population dynamics of the Cliff Frog, Marnocki Syrrhophus. *The American Midland Naturalist* 54(2): 342 – 381.
- Kanagavel, A., Parvathy, S., Chundakatil, A. P. and Dahanukar, N. (2018). Distribution and habitat associations of the Critically Endangered frog Walkerana phrynodesma (Anura : Ranixalidae), with an assessment of potential threats , abundance , and morphology. *Phyllomedusa* 17: 21 – 37.
- Khawiwada, J. R., Zhao, T., Chen, Y., Wang, B., Xie, F. and Cannatella, D. C. (2019). Amphibian community structure along elevation gradients in eastern Nepal Himalaya. *BioMed Central Ecology* 19(19): 1–11.
- Kitzes, J., Berlow, E., Conlisk, E., Erb, K., Iha, K., Martinez, N. and Harte, J. (2017). Consumption-based conservation targeting: linking biodiversity loss to upstream demand through a global Wildlife Footprint. *Conservation Letters* 10(5): 531–538.
- Lawler, J. J., Shafer, S. L. and Blaustein, A. R. (2010). Projected climate impacts for the amphibians of the western hemisphere. *Conservation Biology* 24(1): 38–50.
- Loader, S. P., Gower, D. J., Ngalason, W. and Menegon, M. (2010). Three new species of Callulina (Amphibia: Anura: Brevicipitidae) highlight local endemism and

- conservation plight of Africa's Eastern Arc forests. *Zoological Journal of the Linnean Society* 160(3): 496 – 514.
- Malonza, Kinyatta, P. K. (2008). Amphibian biodiversity in Taita Hills, Kenya. Thesis for Award of Der Johannes Gutenberg, Univeritat Mains, 165pp.
- Malonza, P. K. (2012). Natural History Observations on a warty frog: *Callulina dawida* (Amphibia: Brevicipitidae) in the Taita Hills, Kenya. *International Scholarly Research Network Zoology* 2012: 1–9.
- Malonza, P. K. and Veith, M. (2012). Amphibian community along elevational and habitat disturbance gradients in the Taita Hills, Kenya. *Herpetotropicos* 7: 7–16.
- Mccaffery, R., Solonen, A. and Crone, E. (2012). Frog population viability under present and future climate conditions: A Bayesian state-space approach. *Journal of Animal Ecology* 81(5): 978 – 985.
- Menegon, M. (2015). *The Amphibian Fauna of the Eastern Arc Mountains of Kenya*. Lynx Edicions, London. 3pp.
- Menegon, M., Gower, D. J. and Loader, S. P. (2011). A remarkable new species of *Callulina* (Amphibia: Anura: Brevicipitidae) with massive, boldly coloured limb glands. *Zootaxa* 3095: 15 – 26.
- Moyer, D. C., Loader, S. P., Stanley, W. T., Cordeiro, N. J., Mbilinyi, B., Doggart, N. H. and Rovero, F. (2006). The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biological Conservation* 134(2): 209 – 231.

- Museo de Zoología, P. S. (2001). Climate change and amphibians. *Animal Biodiversity and Conservation* 28(1): 59 – 67.
- Operations, W. B. and Town, C. (2005). *Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya*. Critical Ecosystem Partnership Fund, Washington DC. 2pp.
- Poynton, J. C., Loader, S. P., Sherratt, E. and Clarke, B. T. (2007). Amphibian diversity in East African biodiversity hotspots: Altitudinal and latitudinal patterns. *Biodiversity and Conservation* 16(4): 1103–1118.
- Qian, H., Wang, X., Wang, S. and Li, Y. (2007). Environmental determinants of amphibian and reptile species richness in China. *Ecography* 30(4): 471 – 482.
- Ra, N. Y., Park, D., Cheong, S., Kim, N. S. and Sung, H. C. (2010). Habitat associations of the endangered gold-spotted pond frog (*Rana chosenica*). *Zoological Science* 27(5): 396 – 401.
- Rustad, L., Campbell, J., Dukes, J. S., Huntington, T., Lambert, K. F., Mohan, J. and Rodenhouse, N. (2012). Changing climate, changing forests: the impacts of climate change on forests of the Northeastern United States and Eastern Canada. *Forests* 7(12): 1 – 56.
- Schmidt, B. R. (2004). The role of density dependence in the dynamics of spatially structured populations View project *Rana dalmatina* in Central Romania View project declining amphibian populations. *Article in Herpetological Journal* 2004: 167–174.

- Shemsanga, C., Nyatichi, A. and Gu, Y. (2010). The cost of climate change in Tanzania : Impacts and Adaptations. *American Science* 6(3): 182–196.
- Spesies, K., Amfibia, T. and Kecerunan, S. (2018). Species richness and distributional pattern of amphibians along an elevational gradient at Gunung Raya, Pulau Langkawi, Kedah, Malaysia. *Sains Malaysian* 47(8): 1635 – 1644.
- Sterrett, S. C., Katz, R. A., Brand, A. B., Fields, W. R., Dietrich, A. E., Hocking, D. J. and Campbell Grant, E. H. (2019). Proactive management of amphibians: Challenges and opportunities. *Biological Conservation* 236: 404 – 410.
- Swartz, L. K., Lowe, W. H., Muths, E. L. and Hossack, B. R. (2020). Species-specific responses to wetland mitigation among amphibians in the Greater Yellowstone Ecosystem. *Restoration Ecology* 28(1): 206 – 214.
- Tanadini, L. G. and Schmidt, B. R. (2011). Population size influences amphibian detection probability : *Plos One* 6(12): 1 – 7.
- TMA (2019). *Statement on the Status of Tanzania Climate in 2019*. Tanzania Meteorological Authority, Dar es Salaam, Tanzania. 46pp.
- Unk, W. C. H. F., Einoso, D. I. A. L. and Ornos, F. E. N. O. (2003). Monitoring population trends of eleutherodactylus frogs. *Journal of Herpetology* 37(2): 245–256.
- URT (2010). *Nomination of Properties for Inclusion on the World Heritage List Serial Nomination : Eastern Arc Mountains Forests of Tanzania*. Ministry of Natural Resources and Tourism Tanzania, Dar es Salaam. 137pp.

- Vallan, D. (2002). Effects of anthropogenic environmental changes on amphibian diversity in the rain forests of eastern Madagascar. *Journal of Tropical Ecology* 18(5): 725 – 742.
- Whittaker, K., Koo, M. S., Wake, D. B. and Vredenburg, V. T. (2013). Global declines of amphibians. *Encyclopedia of Biodiversity* 3: 691 – 699.
- Wwf-france, C., Wwf-france, M. V., International, E. W. W. F. and Wwf-uk, S. C. (2015). *Impact of Climate Change on Species*. World Wildlife Fund, France. 21pp.
- Yannic, G. (2007). Estimating population size in the European tree frog (*Hyla*). *Population Trends* 28: 287 – 294.