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DEPARTMENT OF BIOLOGY

RAPID ASSESSMENT SURVEY OF THE ENDANGERED SILVER BLADDER REED FROG (*Hyperolius cystocandicans*) IN SOUTHERN SLOPES OF MOUNT KENYA FOREST

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DECLARATION

Declaration by Candidate

This is my original work and has not been presented for a degree or any other award in any university.

Signature: ………………………………… Date: 29/11/2023……………………………

Anthony Mwangi Karani

Declaration by Supervisors

This thesis is submitted for examination with our approval as university supervisors

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DEDICATION

∞

To the late Augustine Karani (1943 – 2021)

He is my dear father, a friend and a role model

∞

To the late Joel K. Makumi, (1939 – 2016)

He is a renowned writer, a friend and a mentor

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ABBREVIATIONS AND ACRONYMS

AES – Audial/Acoustic Encounter Survey
AICc – Akaike’s Information Criteria
ANOVA – Analysis of Variance
CFAs – Community Forest Associations
CJS – Cormack Jolly Seber
CMR – Capture Mark Recapture
DCA – Detrended Covariate Analysis
EABH – Eastern Afro-montane Biodiversity Hotspot
AOO – Area of Occurrence
GoK – Government of Kenya
IBA – Important Bird Area
IUCN – International Union for the Conservation of Nature and Natural Resources
KFS – Kenya Forest Service
KWS – Kenya Wildlife Service
MANOVA – Multivariate Analysis of Variance
NMK – National Museums of Kenya
POPAN – Population Analysis
SVL – Snout to Vent Length
UNESCO – United Nations Educational, Scientific and Cultural Organization
VES – Visual Encounter Survey

ABSTRACT

Reed frogs are a good case for investigating diversity of reproductive traits associated with co-occurrence, resource partitioning and habitat heterogeneity. Objectives of this study were to: (1) establish patterns of co-occurrence and spatial distribution among frogs in relation with characteristics of habitat patches; (2) identify the distance from water, spacing between conspecifics and perching height of silver bladder reed frog (*Hyperolius cystocandicans*) and other reed frogs, and; (3) estimate the population sizes and density of reed frogs in the forest on Southern Slopes of Mt. Kenya. Frogs were searched opportunistically through visual encounter surveys and acoustic encounter surveys, complemented by use of drift fences and pitfall traps with funnel traps. Capture mark recapture method was used to generate individual capture history file that was used to estimate population sizes, capture probability, probability of entry and probability of apparent survival using POPAN Jolly Seber model in MARK. Co-occurrence was estimated using pairwise probabilistic model of species co-occurrence using presence-absence data. The results of this survey showed that *H. cystocandicans* co-occurs positively with *Amietia nutti*, *Hyperolius glandicolor*, *Hyperolius montanus* and *Phrynobatrachus kinangopensis* and randomly with *Ptychadena mahnerti*, *Kassina senegalensis*, *Sclerophrys kerinyagae* and *Xenopus borealis*. There was interspecific variation in height and spacing of egg masses of *H. cystocandicans* and *H. montanus* but both species deposited their eggs away from water (terrestrial eggs). Interspecific variation was established in the height of perching adult reed frogs (*H. glandicolor, H. cystocandicans* and *H. montanus*) but not distance of perching site from water and spacing between conspecifics. The population sizes of reed frogs per site were: *H. glandicolor* (123±20.05, 154±99.86, 162±14.85), *H. montanus* (308±52.12, 50±23.30, 133±10.80; *H. cystocandicans* (194±18.24, 101±6.43, 298±38.07). Population estimates were skewed towards males in *H. cystocandicans* and *H. montanus* but not *H. glandicolor*. With the exception of *H. montanus,* the estimated population densities of *H. cystocandicans* and *H. glandicolor* did not vary across survey sites. Population densities did not vary significantly across species. All species have aquatic larvae but *H. glandicolor* has aquatic oviposition site which differs with terrestrial oviposition sites of *H. montanus* and *H. cystocandicans.* It was concluded that this variation drives spatial reproductive partitioning in distribution of reed frogs in breeding sites which was explained by interspecific difference in spacing between egg masses, distance of egg masses from water, height of perching adults and height of egg masses. This study provides baseline population data that will inform conservation of reed frogs. There is need for further estimates to determine whether the populations are declining. Future research should explore genetic diversity in order to estimate the effective population size and operational sex ratio in male-biased populations.

Keywords: Population size, Capture Mark Recapture, habitat characteristics, *Hyperolius cystocandicans,* Reed frogs, Co-occurrence, Mt. Kenya, Rapid Assessment Survey.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

It has been established over the past three decades that amphibians are declining at a global scale (Green *et al.,* 2020). Close to 40% of all extant amphibian species are threatened with extinction where nearly 500 of frogs and toads are rapidly declining across the world (Baillie *et al*., 2004). There are 7468 Anuran species (frogs and toads) globally (Frost, 2022). Their natural habitats are under increasing pressures due to factors associated with anthropogenic activities (Kiesecker *et al.*, 2001; Blaustein *et al.,* 2011). There is no single cause culpable for the decline in population sizes and numbers of amphibian populations but it is supposed that a multitude of factors act together (Whittaker *et al.,* 2013; Green *et al.,* 2020). Chief among these factors is habitat loss due to changes in land use (Baillie *et al.*, 2004; Stuart *et al.*, 2008).

In Sub-Saharan Africa, the extensive and diverse clade *Afrobatrachia* Frost, 2006 has been a subject in many studies of interest, such as sexual dichromatism (distinct color morphology between females and males) and evolution of reproductive diversity (Veith *et al.,* 2009; Portik *et al,* 2019). While many anuran families are found in Africa, the Afrobatrachian clade has only four families but it comprises of more than half of Africa's amphibian diversity (Frost, 2022). One of these families is *Hyperoliidae* Laurent, 1943 which is known for numerous colorful frogs that occupy diverse biogeographic regions across Sub-Saharan Africa (Schiøtz, 1999; Frost, 2022). The family has diverse life history traits, behavior and ecological morphology (Wieczorek *et al*, 2001; Starnberger *et al.*, 2013). Majority of the species in the family are sexually dichromatic (more than 60 species; Portik *et al*, 2019), mostly pronounced in females, where some species exhibit ontogenetic color change to a distinct adult female color while some males or all males retain juvenile coloration (Hayes and Menendez, 1999; Schiøtz, 1999).

Adults of the African mainland reed frogs in genus *Hyperolius* Rapp, 1842 are arboreal with free-swimming exo-trophic tadpoles. Although the genus (*Hyperolius*) has been faced with multiple disagreements in its taxonomy, over 145 species have been described making it relatively species-rich (Frost, 2022). Reproductive modes in the genus vary as result of choice of breeding location which includes terrestrial, aquatic, and arboreal oviposition sites (Drewes and Altig, 1996; Lawson *et al.*, 2018). All known species have tadpoles that develop inside lentic waters (Viertel *et al.*, 2007; Lehr *et al.*, 2015). However, in some species, males call from emergent vegetation near water bodies and eggs are deposited on those calling sites while others call from locations far away from water and others yet, lay their eggs inside water (Schiøtz, 1999). As such, reed frogs exhibit robust microhabitat preferences that have been traced to ancestral lineages (Portik *et al.*, 2016).

In East Africa, studies have shown that populations of many frog species are threatened by anthropogenic activities (Channing and Howell, 2006; Poynton *et al.*, 2007). Several populations of the African mainland reed frogs such as Silver Bladder Reed Frog (*Hyperolius cystocandicans* Richards and Schiøtz, 1977) are reported to be declining for reasons that are not fully understood (Stuart *et al.*, 2008; IUCN, 2021). It was conceptualized that habitat characteristics influence population structures, behavior (such as occurrence, co-occurrence and distribution within breeding sites) and shape intra- and interspecific interactions (Gichuki, *Pers. Comm.*). Being a species-rich taxon, reed frogs occupy a wide range of ecosystems; occur in sympatry with other reed frogs (Lötters *et al.*, 2004; Portik, *et al.*, 2015) and co-occur with a diverse frog species (Lötters *et al.*, 2006; Behangana *et al.*, 2019). Co-occurrence of frogs can provide insights into their niche differentiation, partitioning of resources, microhabitat preferences and competition dynamics. This knowledge would give insights towards informing species-specific and site-specific conservation actions (Hodgson *et al.*, 2011; Joly, 2019).

1.2 Statement of the problem

There is limited knowledge on resource partitioning efforts and behavioral patterns of reed frogs such as aggregation of frogs, breeding site and calling site selection of reed frogs within the study area. These include the height of calling males, distance between reproductive adults and distribution of these adults relative to sites of egg deposition and location of larval development. No studies have been conducted to establish population characteristics such as presence in habitat patches, frequency of co-occurrence with other species, population sizes, density and distribution of reed frogs in Mt. Kenya Forest. Current knowledge on species traits is based on general observations from closely related species. Consequently, there is inadequate reference data to inform research and conservation of threatened species.

1.3 Objectives

1.3.1 Main Objective

The main objective of the study was to contribute to knowledge of *Hyperolius cystocandicans,* other reed frogs and the frog community in Southern Slopes of Mount Kenya by estimating population sizes, co-occurrence and local distributional patterns in relation with habitat characteristics.

1.3.2 Specific Objectives

The specific objectives were:

i. To establish patterns of co-occurrence and spatial distribution among frogs in relation to the characteristics of habitat patches
ii. To identify the distance from water, spacing between conspecifics and perching height of reed frogs (*H. cystocandicans, H. glandicolor* and *H. montanus*)
iii. To estimate the population size and density of reed frogs (*H. cystocandicans, H. glandicolor* and *H. montanus*)

1.4 Hypotheses

H_{01}. The distance from water, spacing between conspecifics and perching height of reed frogs (*H. cystocandicans, H. glandicolor* and *H. montanus*) do not vary with habitat characteristics

H_{02}. The population density of reed frogs (*H. cystocandicans, H. glandicolor* and *H. montanus*) do not vary with habitat characteristics and across species

1.5 Research questions

The study had the following questions:

i. What is the spatial distribution of frog species (*Anura*) that occur together with the silver bladder reed frog (*H. cystocandicans*)?
ii. What are the characteristics of the habitat patches where the species co-occur?

iii. How does distance from water, spacing between conspecifics and perching height of reed frogs (*H. cystocandicans, H. glandicolor* and *H. montanus*) change with habitat characteristics?
iv. What are the population sizes and densities of reed frogs (*H. cystocandicans, H. glandicolor* and *H. montanus*)?

1.6 Justification of the study

Investigating demographic parameters of rare and threatened species such as *H. cystocandicans* is important for conservation (IUCN, 2021). Understanding the environments occupied by reed frogs and their distributions across breeding sites is vital for informing decision making, management and restoration of ecosystems (Sinsch *et al.,* 2011). The conservation status of *H. cystocandicans* in IUCN Red List has moved from Vulnerable (VU) to Near Threatened (NT) and to Endangered (EN) in the recent past (Baillie *et al.,* 2004; IUCN, 2021). Its conservation needs however, should be better informed and understood, through for instance by using capture mark recapture to estimate population sizes, capture probability, probability of apparent survival, and probability of entry. Conservation of co-occurring species should also be targeted because the niche of reed frogs is narrowly partitioned (Lötters *et al.,* 2004; Portik *et al.,* 2018). In addition to IUCN, there are other biodiversity databases (such as Global Biodiversity information Facility) that utilize observational data of live and preserved specimen of threatened species (Petersen *et al.*, 2021). Such data can be used to model the extinction risks for *H. cystocandicans*; develop species distribution models and predict conservation scenarios under future environmental changes (Ngwava *et al.,* 2021)

The establishment of Wildlife Conservation and Management Act (Government of Kenya, 2013) lead to legal protection of species such as *H. cystocandicans* which is listed in the sixth schedule alongside other threatened reed frogs like *H. rubrovermiculatus* Schiøtz, 1975. Without background data on the population dynamics of listed species, the realization of conservation management strategies targeted to these species will be limited. Studying *H. cystocandicans* as a species that co-occurs with other frog species gives a nuanced understanding of species-specific threats resulting from co-occurrence in a montane frog community.

CHAPTER TWO: LITERATURE REVIEW

2.1 Amphibian populations and conservation

Globally, amphibians are declining at unprecedented rates that exceed those of other vertebrates. These rates are said to double the rate of decline of mammals and birds (Blaustein *et al*., 2011). There are 788 species of amphibians in Africa, of which 437 belong to the Afrobatrachian clade (Frost, 2022). Slightly more than a fifth (235 species) of amphibians in Sub-Saharan Africa is threatened with extinction while 201 species are Data Deficient (Stuart *et al.,* 2008). For three decades, the real nature of amphibian declines has remained a mystery (Green *et al.,* 2020). These declines are of great concern due to the important ecological roles played by amphibians and their sensitivity to environmental changes (Hocking and Babbit, 2014).

Several factors that contribute to amphibian population declines include: habitat loss and degradation, chytrid fungus (*Batrachochytrium dendrobatidis* Longcore, Pessier and Nichols, 1999), changes in temperature and precipitation patterns, chemical pollutants including pesticides and herbicides, overexploitation for the pet trade and food (Alford and Richards, 1999; Kiesecker *et al.,* 2001; Armstrong, 2005; Blaustein *et al.,* 2011; Whittaker *et al.,* 2013). Other threats include invasive species, increased UV radiation; isolation of populations due to habitat fragmentation that reduce genetic diversity and make amphibians more vulnerable to extinction (Fahrig, 2003; Baillie *et al.,* 2004; Bwong *et al.,* 2017). These stressors induce evolutionary and phenotypic changes in amphibian populations globally in response to environmental degradation (Pabijan *et al.,* 2019). Efforts to mitigate these declines include habitat conservation, establishment of protected areas, captive breeding and reintroduction programs, research into disease management practices, and raising public awareness about the importance of amphibian conservation (Falk *et al.,* 2006; Griffiths and Pavajeau, 2008; Hodgson *et al.,* 2011).

The structure of populations is not the same for all species and habitats because the response to the drivers of population declines is often species-specific and context-dependent (Green, 2003; Ngwava *et al.,* 2021). Conservation of amphibian habitats is complicated by their complex life cycles being dependent on both land and water (Petranka, 2007; Sinsch *et al.,* 2011). Amphibian populations depend upon life history and habitat of the individual species because abundance varies a lot between stages, habitats and time (Alford and Richards, 1999; Honeycutt *et al.,* 2019). This demands that changes in population sizes are estimated for adults, tadpoles and egg masses across time and space; a relatively difficult effort (Corn *et al.,* 2005; Rombough, 2012). It is also argued that a trend in amphibian populations is ambiguous because population fluctuates widely by season and span in similar wetlands (Tanadini and Schmidt, 2011; Greenberg *et al.,* 2018). For instance, population size may decline for a number of years but increase incredibly in just one year to surpass the population size prior to the decline (Green, 2003). Population sizes and statuses of many frogs have not been estimated, but these gaps have been addressed vaguely with the use of population indices such as measure of diversity and relative abundance (Engeman, 2003; Newell *et al.,* 2013; Luikart *et al.,* 2021).

Frogs are indicators of ecosystem health (Hocking and Babbit, 2014). The presence and well-being of frogs in ecosystems are important for maintaining ecological balance and for the well-being of many other species, including humans (Pabijan *et al.,* 2019). Efforts to conserve frog populations can have positive ripple effects on the health and stability of ecosystems (Falk *et al.,* 2006; Griffiths and Pavajeau, 2008). Frogs have contributed significantly to scientific research, including genetics and other medical advances (Hayes and Menendez, 1999). Frogs are predators and also, their abundance provides sustenance for various animal species including birds, reptiles and mammals and thereby contributing to nutrient flow in aquatic and terrestrial ecosystems (Luiselli *et al.,* 2004; Hughes *et al.,* 2018). Most importantly is that they are insectivores and play a key role to control populations of insects, like mosquitoes and other agricultural pests (Hocking and Babbit, 2014).

2.2 *Hyperolius cystocandicans* and its co-occurrence of with other species

The silver-bladder reed frog (*Hyperolius cystocandicans* Richards and Schiøtz, 1977) is endemic to Central Kenya Highlands, inhabiting montane headwaters (Köhler *et al.,* 2005). These headwaters are continually threatened by habitat loss as the Afro-montane forests are being encroached uphill (Lambrechts *et al*., 2003). The species was split from the Kenya montane reed frog (*Hyperolius montanus* Angel, 1924) based on differences in color and skin patterns (on dorsal, lateral and ventral surfaces) and by having distinct advertisement calls. Evidence showed that these two species are reproductively isolated (Richards and Schiøtz, 1977). Majority of the populations of *H. cystocandicans* have individuals that are yellowish or brownish in color except the population of a regional color morph from the Southern Slopes of Mount Kenya which also includes greenish and golden dorsal patterns (Köhler *et al*., 2005). However, the species has closely related morphology, habitats and most often occurring syntopically with *H. montanus*. 'Syntopic' means that closely related species of the same genus co-occur and coexist without necessarily competing over resources and space. Such co-occurrences are common among reed frogs (Liedtke *et al.,* 2004; Sinsch *et al.,* 2011; Portik *et al.,* 2018).

The population trend is not known, but the population of *H. cystocandicans* is thought to be declining in areas of suitable habitats due to habitat loss (Stuart *et al.,* 2008; IUCN, 2021). It is known that declines in frog populations are complex and differ from species to species, as well as across populations and life stages. An important prerequisite to establish that population is declining is the availability of baseline population data, which is not currently available for *H. glandicolor*, *H. cystocandicans* and *H. montanus* (IUCN, 2021). It is also recorded that *H. cystocandicans* occurs in less than ten threat-defined locations (associated with habitat loss) with an area of occurrence (AOO) of approximately 52 km^2 (IUCN, 2021). A study by Bielby (2008) insinuated that there is low abundance of *H. cystocandicans* in Central Kenya Highlands. The reported decline of the populations of *H. cystocandicans* has no empirical scientific evidence as much as the reasons for this decline are not fully understood (Baillie *et al.*, 2004; Stuart *et al.*, 2008). It is however assumed to result from the ongoing diminishing quality and extent of the habitat due to livestock grazing, illegal logging and reclamation of wetlands (Bielby, 2008).

Conservation status of *H. cystocandicans* has most recently, moved from Vulnerable (VU) to Near Threatened (NT) and Endangered (EN). Little has been done to understand nature of the threats affecting this species (Bwong, *Pers. Comm.*). Studies have been targeted to the species to demystify the identity of Mount Kenya regional morph using bioacoustics (Köhler *et al.,* 2005); and to assess the species vulnerability to fungal infection (Bielby, 2008). These studies are species-specific but do not emphasize breeding habitats of *H. cystocandicans* and it co-occurrence with other species. It is reported that the population of *H. cystocandicans* has been declining while its other co-occurring reed frogs are perceived as stable in shared habitats (IUCN, 2021). These reed frogs include *H. glandicolor* Peters, 1878 and *H. montanus* but it is notable that more reed frogs such as *Hyperolius howelli* Du Preez and Channing, 2013 have been identified or re-described.

The distribution, biogeographic range and diversity of reed frogs have widely been documented by multiple studies (Frost, 2022). The presence of reed frogs has been confirmed in multiple locations throughout the highlands of Central Kenya. In Lolldaiga hills and Mpala Research Centre, three reed frogs co-occur. These are *H. cystocandicans, H. glandicolor* and *H. viridiflavus* Duméril and Bibron, 1841 complex. Other regions of co-occurrence with these species include Limuru, Kimandi, Nyambene, Tigoni, Thompson Falls, Nyeri, and Mt. Kenya (Lötters *et al.,* 2006). However, possible co-occurrences may happen in regions where they have not been detected and may vary with season because presence-absence data does not distinguish between complete species absence and difficulties in detectability.

Understanding co-occurrence patterns is essential in ecology for identifying potential ecological interactions and conserving biodiversity. Structure of ecological communities, species coexistence and biodiversity are all founded on the ways in which species co-occur with one another (Veech, 2013). Co-occurrence and sympatry are not restricted to the species of the same genus or family. While it is not known how these species interact, a comprehensive list by Lötters *et al.,* (2006) indicated a number of species collected from same locations as *H. cystocandicans*. In these same locations, Malonza (2015) delimited *H. cystocandicans* and *H. montanus* along altitudinal gradients. Understanding co-occurrence patterns of frogs can help researchers better understand the mechanisms that allow these similar species to coexist in the same ecosystem and for the conservation of imperiled species.

2.3 Reproductive ecology of reed frogs

2.3.1 Reproductive physiology and life history strategies

The afro-batrachian family *Hyperoliidae* Laurent, 1943 comprises of: African wart frogs, reed frogs of Madagascar; stripped frogs (two genera) and running frogs; wax frogs; spiny or banana frogs; reed frogs of mainland Africa, and other nine monotypic genera (Schiøtz, 1999; Portik and Blackburn 2016; Frost, 2022; Jaynes *et al.,* 2022).

These frogs have a constricted phylogenetic relatedness and ecological similarity (Wieczorek *et al.,* 2001). Studies have been conducted on their physiology and reproductive endocrinology (Hayes and Menendez, 1999); embryogenesis (Lehr *et al.,* 2015); and larval development (Viertel *et al.,* 2007). A common feature of many reed frogs is their patterning and bright coloration, where colors are highly variable within species and across species, driven by sexual dichromatism and the presence of color polymorphism (Veith *et al.,* 2009; Portik *et al.,* 2019).

Males are usually variable and more conspicuous sex in reed frogs where females change color at adult stage while some males or all males retain the color of juvenile stage (Hayes and Menendez, 1999; Schiøtz, 1999). Color polymorphism may have arisen as a reproductive strategy but it is possible that some color morphs help the frog camouflage from predators or adapt to the environment. For instance, Kobelt and Linsenmair (1986) studied the adaptations of *H. viridiflavus nitidulus* Peters, 1825 to arid environments and found that the species change colors of the skin with temperature during the dry season. Such seasonality has invited many questions regarding amphibian breeding phenology (Grant *et al.,* 2009). Environmental stochasticity influences physiology and behavior of *Hyperolius* species (Schmuck *et al.*, 1988; Grafe *et al.*, 1992). There are multiple studies on the evolution of life histories and diversification of ecological morphologies (Portik and Blackburn, 2016); but the evolutionary significance of this variability is still largely unknown.

Reed frogs especially in the Afro-montane radiation are characterized with low fecundity and shorter lifespan (Sinsch and Dehling, 2017); suggesting that their populations are regulated by juvenile recruitment but not adult longevity. Some species actually exhibit this trait especially short-lived species inhabiting highly seasonal habitats which builds to the key premise in the model that recruitment rather than adult survival regulates amphibian populations (Alford and Richards, 1999). Populations are sustained through complex life history strategies in amphibian behavioral ecology (Müller *et al.,* 2013; Andrade *et al.*, 2016). Both *H. kivuensis* Ahl, 1931 and *H. viridiflavus* are short-lived, and their longevity is two to three years in the wild habitat (Sinsch *et al.,* 2021). Within one rainy season, two consecutive generations of reproductive males of *H. nasutus* Gϋnther, 1865 are present at the same pond (Rödel *et al.*, 2006). Although, studies by Grafe and Linsenmair (1989) were based on a *H. viridiflavus* frog from the savannah, they associate this group of species with high mortality and high juvenile recruitment. It was hypothesized that no adults survive beyond post-reproductive phase (Grafe *et al.,* 1992).

Common reed frogs (*H. viridiflavus* complex) are widespread in East Africa (Wieczorek *et al.,* 2001). The complex has been split into several species including *H. glandicolor* and *H. mariae* (Barbour and Loveridge, 1928). A study was on *H. viridiflavus taetanus* froglets by Schmuck *et al.,* (1988) pointed out that skin adaptation increases survival of the species through environmental stochasticity and water scarcity. Energy accumulated in lipids was used utilized for a fortnight by calling males of South African reed frog *H. marmoratus* (Grafe *et al.,* 1992). A study on the Montane Common Reed Frog (*H. glandicolor,* formerly known as *H. viridiflavus ommatostictus*) showed that females change into functional males depending on sex ratio, judging from advertisement calls in the surroundings, and this suggests that population densities induce physiological plasticity (Grafe and Linsenmair, 1989).

2.3.2 Eggs, oviposition and distribution in breeding sites

Studies involving eggs of African reed frogs have received little attention with the exception of *H. marmoratus* whose reproductive behavior is well studied (Davies, 2014). This is partly motivated by the general assumption that reed frogs exhibit similar reproductive strategies where eggs are attached to vegetation at varying heights above the water surface or deposited in water (Schiøtz, 1999). Forest species attach their eggs on substrates above water while deposition of eggs in water is common among savannah dwelling species such as *H. viridiflavus* and is presumably a strategy to avoid desiccation (Rödel *et al.*, 2006).

Reed frogs typically lay their eggs in or near water, as their eggs and tadpoles require an aquatic environment to develop (Mϋller *et al.,* 2018). These sites can include aquatic vegetation, shallow water bodies, or even water-filled tree holes, depending on the species life history traits and local habitat (Lötters *et al.,* 2004; Portik *et al.,* 2018). The eggs are usually laid in clusters and can range in size and quantity depending on the species (Lawson *et al.,* 2018). In some species, the gel-like egg mass adheres to aquatic vegetation to keep the eggs in place and protect them from predators during embryogenesis (Drewes and Altig, 1996; Lehr *et al.,* 2015). Once the tadpoles hatch, they go through a period of development in water before metamorphosing into juvenile frogs (Chapman *et al.,* 1999). Oviposition and subsequent development of eggs and tadpoles in an aquatic environment are critical phases in the life cycle of reed frogs. The eggs that are deposited away from water hatch in the egg gelatin and following embryogenesis, tadpoles are washed to the nearby water body. Larvae of reed frogs have been associated with developmental plasticity. Environmentally cued hatching has been documented for *H. spinigularis* Stevens, 1971 and *H. cinnamomeoventris* Bocage, 1866 in response to presence of egg-eating frogs and larvae (Warkentin, 2011).

The choice of oviposition sites, as well as the timing of egg deposition, are essential for the survival of their offspring and can be influenced by environmental cues and species life history traits (Lawson *et al.,* 2018). Several forest reed frogs are either explosive breeders or intermediate between explosive and prolonged breeders (Lötters *et al.,* 2004). Explosive breeders are those that spawn within a few days after a rainfall event while prolonged breeders have breeding period extending into weeks or even months. An example of a prolonged breeder is *H. nasutus* Günther, 1865 that start reproducing 2-5 months after the rainy season begins (Rödel *et al.*, 2006).

2.3.3 Calls of reed frogs and distribution in breeding sites

Studies in the Mount Kenya Region and the Central Highlands by extension include Spawls (1996) and Lötters *et al.,* (2006). Advertisement calls of aggregating males of *H. cystocandicans* were recorded at Irangi forest in Mount Kenya by Köhler *et al.,* (2005) while studying acoustics of several montane species. The calls of *H. cystocandicans* consist of a single short note repeated at irregular intervals (Richard and Schiøtz, 1977; Köhler *et al.*, 2005). The frogs were found in swampy areas within the secondary forest, calling at a height of about two meters.

Understanding the height of calling males, distance to potential oviposition sites and distance between calling males can be used to account for call energetics (Rodriguez *et al.,* 2020); and construct niche partitioning (Lötters *et al.,* 2004; Portik *et al.*, 2018). Reed frogs were found calling on the ground during the day (*H. montanus*) and close to water (*H. tuberculatus* Moquard, 1897). There is a general assumption that reed frogs are nocturnal species but males of various species are often found in the breeding sites during the day (Köhler *et al.,* 2005). Majority of reed frogs are nocturnal and there are no known strictly diurnal species (Schiøtz, 1999). The fact that *H. montanus* was found calling on the ground during the day invokes questions about its circadian pattern. This diel activity pattern (cathemerality) is defined as non-adherence to a strict nocturnal activity (Pincheira-Donoso *et al.,* 2020).

The calls of the frogs from *H. viridiflavus* complex and *H. marmoratus* are the most studied, indicating similarities in choice of calling sites (Passmore *et al.,* 1992; Davies, 2014). The montane common reed frog *H. glandicolor*, calls from equal heights but has longer calls with slightly higher dominant frequency than the common reed frog *H. viridiflavus* (Köhler *et al.,* 2005). The calls of various species available in literature such as *H. mariae, H. montanus* and *H. sheldricki* Duff-Mackay and Schiøtz, 1971 were recorded from frogs calling from low vegetation (Schiøtz, 1999; Wieczorek *et al.,* 2001). Similarly, *H. acuticeps* Ahl, 1931*, H. glandicolor* and *H. kuligae* Mertens, 1940, call from short vegetation in primary forests, secondary forests, swampy areas and ponds.

2.4 Habitats and populations of reed frogs

2.4.1 Relationship between habitats and distribution of reed frogs

Reed frogs have been recorded from diverse habitats and altitudinal ranges, but exhibit high species richness and co-occurrence in rainforests and montane forests (Köhler *et al.,* 2004; Müller *et al.,* 2013). More than a dozen reed frog species were recorded in near sympatry and yet exhibiting similar reproductive traits (Portik *et al.,* 2018). It has been hypothesized that taxa with high number of co-occurring species may have interspecific competition, resource partitioning and inherent divergence of reproductive traits such as distribution in acoustic spaces, selection of calling sites, selection of oviposition sites and diversity of larval forms (Nowakowski *et al.,* 2017). Sympatric reed frogs are also partitioned through production of chemicals associated with sexual selection and habitat choice (Müller *et al.,* 2013). For example, the mixtures of chemicals from the vocal sac glands were found to be unique for every species in *Hyperolius* genus (Starnberger *et al.,* 2013).

Some species are associated with disturbed habitats while others are restricted to pristine areas such as primary forests (Bell *et al.,* 2014; Behangana *et al.,* 2019). For instance, *H. cinnamomeoventris* inhabits forest clearings and disturbed forest (Schiøtz, 1999). Similarly, *H. dintelmanni* Lötters and Schmitz, 2004 occurs in the outer margins of disturbed primary forest where it calls from lower grass vegetation with ferns. *Hyperolius viridiflavus* and *H. kivuensis* are tolerant to severe human disturbances on habitats (Sinsch *et al.,* 2021). They prefer habitats such as naturally flooded and artificial habitats in agricultural areas and roadside ditches. On the contrary, there are strictly forests species, endemic species, transition species and montane forest species that have low adaptability to altered and disturbed habitats (Stuart *et al.,* 2008). Habitat selection and utilization differs with habitat quality and species adaptation (Bwong *et al.,* 2020). Syntopic species sharing common habitats are shielded from competition through diverse adaptations in their habitat utilization (Lötters *et al.,* 2004). At the scale of habitat patches, species may still compete and often out-compete each other in preferred patches. The stability of a healthy coexistence depends on fitness differences between species based on variability, niche differentiation and competition (Liedtke *et al.*, 2004).

Dispersal to suitable habitats has been found to be fundamental to the biogeography of *Hyperolius* species, even across oceanic islands (Bell *et al.,* 2014). At local scales, individual reed frogs are in constant struggle to identify habitat types that have minimum risks and increase potential for reproductive success (Portik *et al.,* 2018; Mϋller *et al.,* 2018). Some habitats are suitable for feeding and escaping predators but do not have the capacity of supporting the population through reproduction while other habitat patches function as corridors through which species disperse (Whittaker *et al.,* 2013). Suitability of calling sites free from disturbance, predation and competition explains reasons for dispersal. The quality requirements may occur in the natal habitat patch or beyond in which case these habitat patches must be connected to facilitate dispersal (Smith and Green, 2005; Armstrong, 2005).

2.4.2 Significance of montane forests to reed frogs

Afromontane forests are hotspots for biodiversity which include many species of reed frogs. More than 10% (25 species) of reed frogs in Africa were evaluated as threatened (Stuart *et al.,* 2008). They are mostly forests species (including transition and montane forests) and the species have low adaptability to altered and disturbed habitats. Out of these species, nine are associated with montane forests and montane grasslands including: *H. tannerorum* Schiøtz, 1977; *H. riggenbachi* Nieden, 1910; *H. minutissimus* Schiøtz, 1975; *H. leleupi* Laurent, 1950; *H. kihangensis* Schiøtz and Westergaard, 1999; *H. frontalis* Laurent, 1950; *H. cystocandicans*; *H. discodactylus* Ahl, 1931 and *H. castaneus* Ahl, 1931 (Baillie *et al.,* 2004; Stuart *et al.,* 2008). Additionally, a third of the reed frogs studied by Müller *et al.* (2013) inhabits either montane forests or montane grasslands and their reproductive modes are either terrestrial eggs with aquatic larvae or aquatic eggs with aquatic larvae. Indeed, it was posited that terrestrially breeding species have strong affinities with features found in montane forests (Müller *et al.,* 2013).

Montane forests have numerous fast flowing streams which have been associated with evolution of reed frogs (Portik and Blackburn, 2016; Müller *et al.,* 2018). For instance the Endangered Torrent Reed Frog *H. torrentis* Schiøtz, 1967 has been observed calling from emergent vegetation near waterfalls or fast flowing streams in forested areas (Stuart *et al.,* 2008). The male *H. chelaensis* Conradie, Branch, Measey, and Tolley, 2012 calls half-submerged against a rock in slow flowing water in cascade stream habitats patches of Afro-montane Forest in Serra da Chela Mountains.

Widespread montane reed frogs are important because they serve as umbrella species for conservation (Sinsch *et al.,* 2011). However, the majority of Afro-montane species of reed frogs are endemic in regions where they occur, exhibiting relatively small distributions and restricted to a few localities (Müller *et al.*, 2013). These include *H. davenporti* Loader, Lawson, Portik, and Menegon, 2015 that occupy shallow ponds on the fragmented forest patches*; H. burgessi* Loader, Lawson, Portik, and Menegon, 2015 that inhabits high altitudes in, and at the edges of sub-montane forests, and *H. minutissimus* Schiøtz, 1975 which is found in both forest and grassland habitats (Loader *et al.*, 2015).

The silver bladder reed frog relies on high altitude environments for breeding purposes (the type locality is at 2200 m asl.) which have a restricted range (Bielby, 2008). The species co-occurs with *H. montanus*. Afro-montane forests in Mount Kenya provide diverse microclimates with many habitat patches suitable for breeding of reed frogs (Müller *et al.,* 2013). These amphibian breeding habitats in montane forests and surrounding areas are highly disturbed and fragmented. The root causes of habitat destruction demand commitment from communities in restoration (Griffiths and Pavajeau, 2008; Sinsch *et al.,* 2011; Pabijan *et al.,* 2019).

2.4.3 Habitat fragmentation in the context of metapopulations

Most amphibian populations occur in metapopulations, living in isolated habitat patches that are connected through seasonal dispersal (Whittaker *et al.,* 2013). Metapopulations are subpopulations occupying a network of suitable and unsuitable habitat patches that are sustained through series of extinction and colonization events (Akçakaya *et al.,* 2007; Hanski *et al.,* 2017). Species occupancy alternates around these patches where large habitat patches support diversity of species and smaller ones play the role of sources or stepping-stones for dispersal (Armstrong, 2005). Connectivity of these patches is significant to the viability and long-term survival of the subpopulations (Hodgson *et al.,* 2011; Whittaker *et al.,* 2013). This long-term survival includes the possibility of all habitat patches becoming extinct simultaneously and therefore the extinction of a metapopulation (Fahrig, 2003).

Long-term survival of amphibian populations depend on complex dispersal patterns, occasional gene-flow and re-colonization of habitat patches rendered vacant by disturbances including habitat loss and fragmentation (Armstrong, 2005; Corn *et al.,* 2005). These patterns result to increased survival and reproductive success and thereby influencing the size and number of their populations (Smith and Green, 2005; Petranka, 2007; Honeycutt *et al.,* 2019). Highly fragmented habitats can be understood by studying species distribution and demography (Akçakaya *et al.,* 2007). Patterns of habitat patch occupancy (presence/absence) can be established within a short study period and used to infer the relationship between distribution and population (Alford and Richards, 1999; Falk *et al.,* 2006).

Population declines in metapopulations result from isolation of habitat patches, threatened survival due to inaccessibility of resources such as food, cover and mates trapped in another patch as well as reduced genetic and reproductive vigor due to inbreeding (Müller *et al.,* 2013). This is because some habitat patches are more productive than others and dispersal between these habitats form sources and sinks

(Corn *et al.,* 2005). Connectivity of habitats and dispersal are at the core in the conservation of threatened species occupying fragmented patches (Hodgson *et al.,* 2011). Akçakaya *et al.* (2007) posits that management of such populations depends on the structure and survival of habitat patches.

The fit for Hanski's metapopulation paradigm for *H. cystocandicans* was intuitively appealing in literature (IUCN, 2021). First, small local breeding populations were identified in the Central highlands of Kenya (Richard and Schiøtz, 1977). Second, the population is declining due to habitat loss and fragmentation (Bielby, 2009). The patches are isolated but subsequent studies have found persisting populations hinting to metapopulation synchrony (Köhler *et al.,* 2005; IUCN, 2021). Notably however, not all threatened amphibian species have the spatial structure of a metapopulation (Smith and Green, 2005).

2.5 Population sizes of reed frogs

In spite of being a species-rich genus with numerous endangered species, population sizes of reed frogs are largely unknown. Known estimates include those of Pickersgill's reed frog (*H. pickersgilli* Raw, 1982) and *H. nasutus* which are respectively estimated at 159 calling males per hectare and 28 – 54 males per 100 m transect (Tarrant and Armstrong, 2014; Rödel *et al.*, 2006). It is known that estimation of amphibians' populations can be difficult and expensive compared to raw counts or other indices such as measure of relative abundance (Engeman, 2003). The demographic data available for most species were acquired for other purposes other than detecting trends in population size (Green, 2003).

Estimating frog population sizes can be challenging because they inhabit various environments, ranging from coastal wetlands to montane forests, and their populations may vary greatly across these habitats and often have seasonal breeding and migration patterns, making it difficult to assess their populations throughout the year (Petranka, 2007; Sinsch *et al.,* 2011). Additionally, frogs go through different life stages (eggs, tadpoles, adults) that can be challenging to track and count (Carlon and Langkilde, 2013; Honeycutt *et al.,* 2019). Many frog species are nocturnal and have cryptic behaviors, making them hard to observe, especially in the wild and while some frog species live in remote or hard-to-reach areas, it is logistically challenging to conduct surveys (Green, 2003; Engeman, 2003). Conservation efforts aimed to protect frog populations and their habitats, and ongoing research is essential to better understand and monitor these species, especially given their ecological importance and sensitivity to environmental changes (Falk *et al.*, 2006; Hocking and Babbit, 2014).

2.6 A review of methods and techniques

2.6.1 Population estimation methods

The methods of estimating amphibian populations include distance sampling and mark-recapture sampling (Hutchens and DePerno, 2009). In distance sampling, amphibians are counted along line transects while estimating the distances from the line (Nomani *et al.*, 2012). This way, probability density function is calculated which allows for estimation of the abundance of observed and non-observed population. In capture mark recapture studies, a large sample of amphibians is caught from the population and marked individually (sometimes with a batch specific mark) and released to mix freely with unmarked animals (Lettink and Armstrong, 2003; Lettink, 2012). After allowing some time, another sample is taken from the same population, and also marked and released (Sen and Akçakaya, 2022).

The proportion of marked individuals and unmarked individuals is used to infer population sizes based on capture probability (White and Burnham, 1999). A continuum of models is available to estimate ecological and demographic parameters of marked populations (Lettink, 2012). The accuracy of estimates depends on proportion of captured animals and the frequency of capture (Lettink and Armstrong, 2003). Several studies that utilize capture mark recapture have applied such population estimates to offer insights into the life history, habitats and ecological settings of species (Kissel *et al.*, 2013; Newell *et al.*, 2013; Joly, 2019).

2.6.2 Rapid assessment survey

With the exception of a few species in the *H. viridiflavus* complex, the current knowledge on reed frogs has been gathered from numerous short-term studies. Rapid assessment survey though rarely used is emerging as a promising method to study threatened species (Vonesh *et al.,* 2010). Rapid assessment surveys have been recommended by Howell (2002) for studies on forest biodiversity in Africa. Rödel and Ernst (2004) further recommends opportunistic trapping, visual and acoustic encounter techniques for rapid assessment surveys (Penner and Rödel, 2007; Vonesh *et al.,* 2010). These methods were found to be especially effective for their ability to provide quantitative and qualitative data useful for rapid assessment surveys, ease in handling, low environmental impacts as well as time and cost efficiency; allowing more efficient sampling and analysis of effects and magnitude of environmental factors that may be related to population regulation (Falk *et al.,* 2006). Rapid assessment surveys are easy and reliable for presence-absence data necessary to estimate populations than technically sophisticated surveys (Howell, 2002; Sinsch *et al.,* 2011, Rombough, 2012).

2.7 Summary of the research gap

The large family of reed frogs is still growing as new species are being described (Frost, 2022). It has been guesstimated that the genus is comprised of numerous cryptic taxa and reed frogs that were initially thought to belong in *Hyperolius* are being placed in new genera such as *Congolius* Nečas *et al.* (2021). As a result, generalizing the reproductive ecology and population trends of reed frogs based on studies from a few species can be misleading. Estimates of population densities of reed frogs documented in science are based on ephemeral studies; usually aimed at direct conservation or for testing contextual hypotheses. Making generalizations to apply to all reed frogs based on these estimates is unreliable. The diversity of habitats occupied by reed frogs is noteworthy and is coming to considerable pressures lately (Bell *et al.,* 2014; Sinsch and Dehling, 2017). As a result, our understanding of reed frogs is continually improving which requires that studies be done on the basis of individual species, especially when dealing with co-occurring species.

CHAPTER THREE: STUDY AREA, MATERIALS AND METHODS

3.1 Study area

3.1.1 Mount Kenya Ecosystem

Mount Kenya is located in the highlands of Central Kenya, covering parts of Nyeri, Kirinyaga, Embu, Tharaka Nithi and Meru Counties (KFS, 2010). The mountain is an extinct volcano with five snow-capped peaks and 12 remnant glaciers, has a base of 120 km and touches the equator to the North (Nature Kenya, 2019). The peak of Mt. Kenya (Batian) stands at 5199 m above sea level making it the second highest point in Africa after Mt. Kilimanjaro. Mt. Kenya is an Important Bird Area (IBA) and in 1997, it was designated a World Heritage Site by United Nations Educational, Scientific and Cultural Organization (UNESCO) (Bennun and Njoroge, 1999). Mt. Kenya is a water tower, maintaining biological diversity and life-support of an extensive watershed (KFS, 2010).

The mountain area is characterized with unique Afro-alpine biodiversity, accounts for nearly 15% of Kenya’s native forest and forms part of the Eastern Afro-montane biodiversity hotspot (EABH) (Zhou *et al.,* 2018). Mt. Kenya hosts 75% of Kenya’s mountain endemic birds and 53 of the 67 African highland biome bird species in Kenya (Bennun and Njoroge, 1999; Nature Kenya, 2019). The ecosystem is a protected area with both a National Park and a National Reserve. Mt. Kenya National Park (69,406 ha) is managed by Kenya Wildlife Service (KWS) and is located above the altitude of 3500 m while Kenya Forest Service (KFS) is responsible for Mt. Kenya Forest Reserve (213,083 ha). The forest reserve is divided into 18 forest stations and is associated with several satellite stations (KFS, 2010). Each forest station is a discrete management unit area (Nyongesa and Vacik, 2019). Several Community Forest Associations (CFAs) are associated with the forest reserve (KFS, 2010).

3.1.2 Location of the study sites

The study area covered small forested sections of Nyeri and Kirinyaga Counties in the southern slopes of Mt. Kenya (Figure 1). Cumulatively, the area covered was approximately 200 km^2 (20000Ha). The work was distributed unevenly with most of the work taking place in three Forest Stations (Kangaita, Chehe and Ragati), but also extending to parts of Hombe Forest to the west and eastwards to Castle Forest. A complete list of all breeding sites is shown in Appendix 1.1.

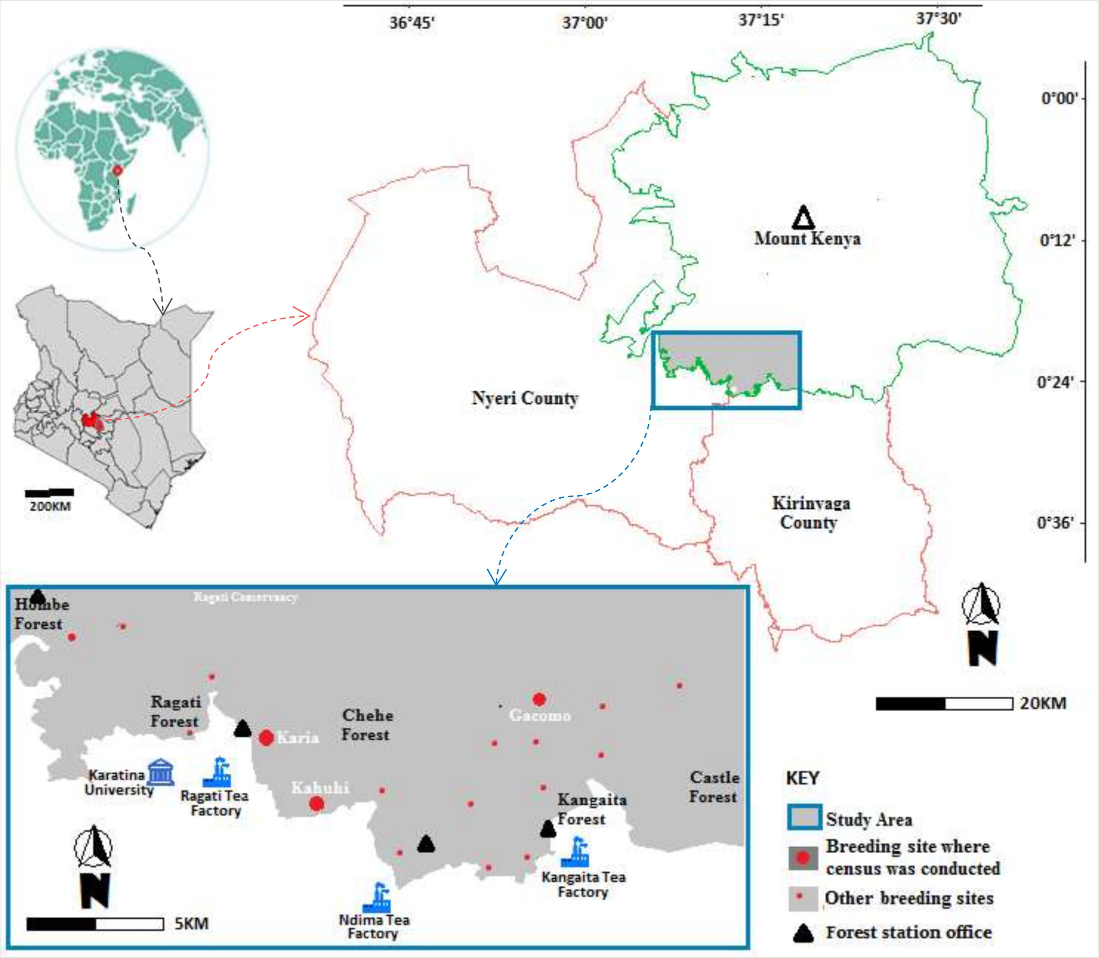

Figure 1: Map of Kenya showing Mount Kenya Ecosystem and location of the study area and breeding sites in Nyeri and Kirinyaga counties

Data source: Counties: Global Administrative boundaries (www.gadm.com) and Protected areas shape file: (World Resources Institute).

3.1.3 Topography, drainage, soils and climate

The study area falls between 2000-3500 meters above sea level (KFS, 2010). The area is hilly and with many rugged ravines, V-shaped valleys as well as gentle river valleys with steep ridges. The slopes of the study area direct southwards and lead gently to undulating plains. There are also flat swampy grasslands, montane streams with extensive floodplains and numerous temporary puddles. The area cuts across several watersheds exhibiting a dendritic drainage pattern. The main rivers (streams) are Mukengeria, Rundu, Ruiru, Rwamuthambi, Chehe, Kururu, Muhuhi, Nyanyaga, Ragati and Muthira. The rivers flow southwards to join Tana River, which eventually drains into Indian Ocean (Nature Kenya, 2019). The soils of the southern slopes of Mount Kenya are *Nitisols* and *Andosols* which have colour hues of brown to red brown to dark reddish brown to deep reddish brown (Mutuma *et al.,* 2017). There are also dark tenacious *Histosols* in poorly drained wetlands (Kapkiyai *et al.,* 1999). They are volcanic soils with geology of basaltic origin, dating in the late Pleistocene (Muchena and Gachene, 1988; Mutuma *et al.,* 2017). These soils are deep, highly leached, well drained, fertile; and suitable for agriculture. As a result, amphibian breeding habitats are highly disturbed and fragmented.

The phenology of amphibians is dependent on climate, as breeding events occur in the rainy season (Grant *et al.,* 2009). Precipitation of the humid Eastern and Southern Slopes of Mount Kenya ranges between 1800 – 2500 mm annually (KFS, 2010). The region has two rainy seasons; long rains (March – June) and short rains (October – November). The driest months are January and February. The average maximum temperature in the area (Source: Kangaita Tea Factory) is 25^0 C, with an average minimum temperature of 9.2^0 C. The months of June to August experience the lowest temperatures (KFS, 2010). Habitat heterogeneity is evident due to irregular topography and many local microclimates within this region. These microclimates also host multiple habitat patches suitable for amphibian breeding (Müller *et al.,* 2013).

3.1.4 Floral diversity and conservation status

Mount Kenya has unique biodiversity distributed across six zones: the lower montane humid and dry forests, bamboo zone, upper montane forest, heath zone and the afro-alpine zones (Young and Evans, 1993; Zhou *et al.,* 2018). The forests hosts about 880 floral species in 146 families, which include 150 near endemic plants(Nature Kenya, 2019). Strictly endemic flora includes *Lobelia keniensis* (Asch.) Hemsl, *Lobelia telekii* Schweinf, *Dendrosenesio keniodendron* B.Nord, *Dendrosenesio keniensis* (Baker.f) Mabb and *Sedum keniense* Zhou, Hu and Wang.

The lower montane humid forest (study area) has high diversity of woody plant species (Zhou *et al.,* 2018). Some threatened flora include: *Vitex keniensis* Turrill (EN), *Premna maxima* T.C.E. Fr (EN), *Ixora scheffleri* ssp *keniensis* Bridson (CR), *Polyscias kikuyuensis* Summerh (NT), *Croton alienus* Pax (EN) and *Prunus (Pygeum) africana* (Hook.f.) Kalkman (VU). Once feared to be extinct, *Ixora s. keniensis* was rediscovered at Ragati Forest (Ngari and Newton, 2010). There are reforestation efforts of indigenous plants and exotic plantations. The study area is largely forested with tree species like *Kuloa (Ocotea) usambarensis* (Engl.) Trofimov and Rohwer, *Olea capensis* L., *Syzygium guineense* Wall, and *Croton macrostachys* Hochst ex Delile among others including many native trees and liana (Nature Kenya, 2019).

3.1.5 Faunal diversity and conservation status

The study area is characterised by dense forests, montane grasslands, wetlands and montane headwater streams that support an assemblage of endemic amphibian species. The forest is also a home to many migratory birds as well as endemic species such as the forest dependent Hartlaub's turaco (*Turaco hartlaubi* Fisher and Reichenow, 1884) and the rare Abbott's starling (*Alizeropsar femoralis* Richmond, 1897) (Bennun and Njoroge, 1999). Threatened insect species encountered in the study area include Kenya Jewel (*Platycypha amboniensis* Martin, 1915) – (CR), Giant Sprite *(Pseudagrion bicoelurans,* Martin, 1907) – (VU) and Elegant bushcricket (*Meruteranna elegans,* Sjöstedt, 1912) – (CR), among others (Clausnitzer *et al.*, 2011; Hemp and Hemp, 2018).

There are numerous species of mammals known to occur in the mountain area (Young and Evans, 1993). Threatened mammalian species include the African elephant (*Loxodonta africana* Brumenbach, 1797) – (EN); Leopard (*Panthera pardus* Linnaeus) – (VU); Alpine mole rat (*Tachyoryctes rex* Heller, 1920) – (EN); Mountain bongo (*Tragelaphus eurycerus isaaci* Thomas, 1902) – (CR); and Mount Kenya shrew (*Surdisorex polulus* Hollister, 1916) – (VU).

There are threatened herpetofauna species in the study area which include Silver bladder reed frog (*H. cystocandicans*) – (EN), Kinangop puddle frog (*Phrynobatrachus kinangopensis* Angel, 1924) – (VU), Giant puddle frog *(Phrynobatrachus irangi* Drewes and Perret, 2000) – (CR), Lonnberg's toad (*Mertensophryne lonnbergi* Anderson, 1911) – (VU), Mount Kenya side-striped chameleon (*Trioceros schubotzi* Sternfeld, 1912) – (NT), Mount Kenya sentinel chameleon (*Kinyongia excubitor* Barbour, 1911) – (VU), Mount Kenya worm snake

(*Leptotyphlops keniensis* Broadley and Wallach, 2007) – (DD); and Mount Kenya bush viper (*Atheris desaixi* Ashe, 1968) – (EN) (Lambrechts *et al.,* 2003; Köhler *et al.,* 2005; Malonza, 2015).

3.1.6 Economic activities

The main economic activities around Mount Kenya are livestock keeping and agriculture especially coffee (*Coffea Arabica* L.), tea (*Camellia sinensis* L.), and horticultural crops (KFS, 2010). The greater Upper Tana River catchment area provides waters that are used for domestic and municipal consumption, hydropower generation and irrigation agriculture (Nature Kenya, 2019). The study area is a tourist destination that host diverse activities such as trout fishing, camping and conferencing. Kenya Forest Service issues grazing permits to CFA members who also participate in reforestation projects, ecotourism and fire prevention. The area has a history of human-wildlife conflicts, illegal logging and forest fires which threatens local biodiversity (Nyongesa and Vacik, 2019). Human activities that threaten frogs in the study area include use of agro-chemicals, reclamation of wetlands and grazing of livestock in the forest reserve, especially in wetlands.

3.2 Materials and methods

3.2.1 Study design

This study design was based on a rapid assessment survey method following the guidelines of Vonesh *et al.,* (2010). The methods utilised are similar to those employed for amphibian diversity in tropical forests by Howell (2002), Rödel and Ernst (2004), Penner and Rödel (2007) and Sinsch *et al.,* 2011.

3.2.1.1 Reconnaissance

Before commencement of the actual data collection, there was a reconnaissance, conducted between October and December 2019 aimed at testing efficiency of sampling techniques and applicability of marking methods. A list of sites considered suitable for breeding of frogs (breeding sites) was generated during this period.

3.2.1.2 Sampling procedure

The study area include two strata: 1) Kangaita (Kirinyaga); 2) Chehe and Ragati (Nyeri). Breeding sites were selected randomly from the list developed during reconnaissance (detailed in section 3.2.1.1 above) by using the function [=index(range, randbetween(range)] in MS Excel™ which was run separately for sites in Nyeri and Kirinyaga. A breeding site contained several well connected habitat patches within close proximity to each other. Breeding sites spread throughout the study area were identified using a stratified random sampling technique as defined by Williams and Brown (2019) resulting to a total of 15 breeding sites (eight in Nyeri and seven in Kirinyaga). In this study, an area characterised a discrete habitat type (section 3.2.2) was considered as an independent habitat patch. There were a total of 44 such habitat patches (22 in Nyeri and 20 in Kirinyaga).

Capture Mark recapture (detailed in section 3.2.4) was conducted on three breeding sites that were identified as easily accessible, secure and representative of different habitat characteristics considered in this study. The first breeding site was a swamp at *Karia ka Ndumanu* (Karia) located East of Ragati Tea Factory in Ragati Forest Station. The second site was a flood plain along *Kahuhi* Stream near *Kangiri village* in Chehe Forest Station. The third site was a temporary puddle at *Karia ga Gacomo* (Gacomo) located 5 km North West of Kangaita Forest Station.

3.2.1.3 Timing of sampling period

The first survey was conducted in all sampled habitat patches during the long rainy season occurring between March and May 2020. This was replicated during the short rains occurring between October and December 2020. Additionally, reed frogs were captured, marked, released and recaptured at three sites during the second survey undertaken between October and December 2020. The reason for this timing was to establish a population size at the peak of the breeding season within habitat patches suitable for breeding. Reed frogs are sporadic breeders that remain cryptic during the dry period but resume breeding activity starting at the onset of the rainy season.

3.2.2 Identification and classification of habitat patches

Habitat patches were visually assessed by slowly traversing the patch and were classified using descriptive features used by Sakané *et al.* (2011) and Rödel and Ernst (2004). These include wetland morphology, source of water and the height of dominant vegetation. The habitat patches were classified as marshy bogs, montane grasslands, reed swamps, temporary puddles, stream flood plains and ephemeral pools. At each habitat patch, source of water of the wetland was assessed visually and categorized as either surface run-off, spring or river overflow. Morphology of the wetland was categorized as either flat downstream or concave. The flow of surface water was observed and the wetlands were categorized as waterlogged, temporarily flooded, permanently flooded or not flooded. The height of dominant vegetation in lower strata (0.5 – 1.5m) and understory (<0.5) was measured using a tape measure following the guidelines of Rödel and Ernst (2004). This was important in order to distinguish between a reed swamp and a marshy bog.

A habitat patch that was swampy, water logged and dominated by common reeds (0.5 – 1.5m tall) and reed-like plants, shrubs and trees was categorised as a reed swamp. The category of marshy bog was assigned to a waterlogged wetland near a stream or spring with slow moving water, dominated by sedge (<0.5m tall). Montane grassland category included patches at high elevations, with a large open area covered with grasses, sedge, rushes and scattered shrubs. Temporary pools of stagnant or slow moving water, flooding seasonally after rains, runoff or springs was categorised as temporary puddles. A stream floodplain was identified as a generally flat area of land next to a river or stream stretching between the river bank and river valley. An ephemeral pool of water included short-lived pools and ditches caused by streams, flash floods and overflow of reservoirs.

Once identified and classified, the altitude and GPS coordinates of each habitat patch were taken and recorded. The distance between breeding sites was measured by drawing a straight line using GPS points marked in the field from the edge of one breeding site to another on Google Earth (Google Inc., 2020). The purpose of measuring this distance was to approximate the linear distance covered by reed frogs dispersing from one breeding site to the nearest breeding site.

3.2.3 Determining of species spatial distribution within breeding sites

3.2.3.1 Opportunistic encounter surveys

In order to identify co-occurrence, frogs were searched in all habitat patches opportunistically at night (between approx. 1900–2200 h) by slowly traversing the patches with the aid of low-powered head torches. Presence-absence value was recorded for all species identified in all habitat patches following the guidelines of

Veech (2013); Veech (2014) and Griffiths *et al*. (2016). Counts of adults, juveniles, tadpoles and egg masses were recorded. Identification of individual egg masses of reed frogs follows the description by Richards and Schiøtz (1977) for *H. cystocandicans* and *H. montanus,* and Lawson *et al.* (2018) for *H. glandicolor*. For all other species, only egg masses of known parentage were considered (and therefore egg masses were not included in data analysis for species co-occurrence).

Tadpoles were searched at selected suitable locations within each habitat patch (characterised with presence of stagnant or flowing water) using a dip net following the guidelines of Howell (2002) and Rombough (2012). In addition to Visual Encounter Surveys (VES), the locations of adult frogs were identified through Acoustic Encounter Surveys (AES). Vocalising individuals were followed to their calling location and identified visually to confirm species identity. Direct manual recordings of advertisement calls were made using Voice Memos app and in-built microphone in Apple iPad 7 A1671 (software version 13), placed within 50 cm from the vocalising individual as recommended by Köhler *et al.* (2017). Each individual adult's snout vent length (SVL) was also measured using vernier callipers (accuracy ± 0.01 mm). SVL is a reliable delimiting factor used in species identification.

In order to estimate the distribution of reed frogs in breeding sites, whenever a reed frog was encountered, the height of perching for adults (both females and males) was measured using a tape measure (accuracy ± 1.00 cm). The distance from water body and distance between conspecifics were also measured. Similarly, whenever an egg mass of a reed frog was identified, the height of the spawning site, the distance from water body and distance between conspecifics were measured. These data were necessary for testing the relationship between the distance from water, perching height and distance between adults and egg masses of each species of reed frogs.

3.2.3.2 Drift fences and pitfall traps

Opportunistic sampling (*sensu* Williams and Brown, 2019) was complemented by the use of drift fences with pitfall traps. The method used here was a modification of Rödel and Ernst (2004) in order to fit to a rapid assessment survey as detailed by Penner and Rödel (2007) and Vonesh *et al.* (2010). The method was necessary for more comprehensive coverage of cryptic species and to obtain morphological characteristics that may not have been sufficiently covered using the opportunistic survey. Each drift fence was made of three lines of 5m from the centre forming a Y-shape as used by Hutchens and DePerno (2009). Each line was erected with plastic sheeting (0.5m high) and supported with stakes.

Along the drift fence, pitfall traps made from small plastic buckets (5 litres) were submerged and covered with plastic funnels. The purpose of funnel traps was to prevent frogs from escaping from the pitfall trap. A total of 86 drift fences were erected (32 in the first survey and 54 in the second survey). Each pitfall trap was checked at least once every day for four days (20 days in first survey and 60 days in second survey). Catching success using this method was very low (accounting for only 5.1% of all specimen encounters). Whenever a specimen was collected and identified, it was released to the habitat nearest to the collection pit. This data (presence-absence of species) was used to test for species co-occurrence and species composition in various types of habitat patches.

3.2.4 Preservation of collected specimen

The method of preserving amphibian specimen is detailed in Rombough (2012). First, photographs of live encounters for all species including eggs, tadpoles, juveniles and adults were taken in the field using a camera. Additionally, voucher specimens of adult frogs were collected, euthanized, fixed in 10% formaldehyde and later preserved in 70% ethanol at National Museums of Kenya (NMK). In the field, specimen were preserved in 4% formaldehyde (literally 2 - 4 drops in a 50 ml specimen jar full of water). Egg masses were not collected but tadpoles (and juveniles with tails) were fixed in 10% formaldehyde and later preseved in 4 - 7% formaldehyde.

3.2.5 Estimating the population of reed frogs

A single large quadrat located 20 m from the habitat edge was marked in three breeding sites. The locations of breeding sites have been detailed in section 3.2.2 and 3.2.3 above. The quadrat measured 40 x 40 meters. The method of capture-mark-recapture (CMR) as used in this study follows the recommendations of Lettink and Armstrong (2003) and Lettink (2012) in estimating amphibian population sizes using marking method as initiated by White and Burnham (1999). Reed frogs were searched for opportunistically at night (between approx. 1900–2200 h) with aid of low-powered

LED head torches by slowly traversing the quadrat. Whenever encountered, reed frogs were caught by hand and the following details were recorded: date of the capture, site of capture, capture number, the individual who captured it, the specific name and sex of the frog. The natural colours of the frog, natural markings and other peculiarities were noted in the field notebook. Snout vent length (SVL) for each captured individual was measured using vernier callipers (accuracy ± 0.01 mm).

The reed frog was marked on dorsal surface with a mark for individual identification in the field. The colours used for marking were red, green, blue and black. The frog was then released at the point of capture. Capturing, marking and releasing continued until all the reed frogs encountered and captured within the quadrat were marked. Recapturing (second to fifth capture occasions) was done for four nights at a regular interval of every two days (a total of five capture occasions). On the occasion of subsequent capture (recapture), the date of its first capture was recorded. In subsequent capture occasions, reed frogs with no capture history were marked in the same way as in the initial capture occasion. After marking, frogs were released at approximately the same capture location and therefore allowing it to mix freely with unmarked frogs. There were 15 survey nights where each of the three species of reed frogs were counted (45 censuses in total). The purpose of these data was to generate a capture history file used to estimate species population size.

3.2.6 Data analysis

Mark recapture data were analysed and tested for significance in the program MARK while all other tests were conducted in R Software versions x64: 4.0.3 (R Core Team, 2020). Prior to the actual data analysis, preliminary tests were conducted with the aim of testing suitability of the data for model. There were no major setbacks identified in these tests.

3.2.6.1 Species co-occurrence and composition

The absence-presence values of each species in each habitat patch (detailed in section 3.2.3 above) was subjected to the R package '*cooccur*' which uses probabilistic model of estimating species co-occurrence initiated by Veech (2013), Veech (2014) and Griffiths *et al*. (2016) to plot species co-occurrence matrix. The model analyses pairwise patterns of species co-occurrence based the frequency at which species occupy a set of spatial locations and gives assessment of whether the observed frequency is random or exceeds the expectations of a random distribution (positive co-occurrence) or is less than expected in a random distribution (negative co-occurrence).

Species composition was calculated in R package '*vegan*' using the method of Bray-Curtis dissimilarity following the guidelines of Oksanen *et al.* (2020). The pairwise distances between species in habitat patches were presented in a Detrended covariate analysis (DCA) bi-plot. This method is robust with low diversity and is scored to measure of species turnover. The summary statistics of DCA are presented as eigenvalues and gradient lengths (standard deviation units).

3.2.6.2 Analysis of advertisement calls

Advertisement calls (recorded in section 3.2.3.1) were analyzed using Raven Pro version 1.5 (Center for Conservation Bioacoustics, 2019) from where individual sound notes were identified and used to generate oscillograms and spectrograms of sampled calls. The call terminologies used in this study are those defined in Köhler *et al.* (2017).

3.2.6.3 Analysis of variance

The distance of reed frogs from water, perching height and distance between reed frogs of the same species were subjected to analysis of variance (ANOVA) in order to test whether they vary within species across different habitat types and; to test whether they vary across species. ANOVA was used to measure the variation in the distance of egg masses from water across different types of habitat patches. This was repeated separately for the height and distance between egg masses and separately for adults. ANOVA was used to measure the variation in distance of egg masses from water across species. This was repeated separately for the height and distance between egg masses and separately for adults.

3.2.6.4 Multivariate analysis of variance

The significant difference in the height of reed frogs across species and across different types of habitat patches were measured using multivariate analysis of variance (MANOVA). This was repeated separately for distance from water and distance between reed frogs for both adults and also separately for egg masses.

3.2.6.5 Linear regression

A linear regression model was developed to test relationship between species traits. These traits included distance from water, perching height and distance between conspecifics. A linear regression was also used to test relationship of the aforementioned traits against the distance to the nearest breeding. This was done separately for each species for both adults and egg masses.

3.2.6.6 Selecting the best fitting model

Individual capture history file (generated in subsection 3.2.4) of each reed frog captured, marked and released during the survey was organized to conform to a series of models that are available in the program MARK as initiated by White and Burnham (1999). Capture mark recapture estimates the population using detection probabilities when animals are marked across capture occasions and released into the population. Validity of capture recapture data in POPAN Jolly Seber model depends on probability of entry (β or pent) to a patch at particular time (t); capture probability (p of π) and probability of apparent survival between capture sessions (Φ of phi). Probability of apparent survival accounts for losses due to mortality and permanent emigration. Probability of entry refers to the probability that a frog was not available for capture at a particular capture occasion (due to recruitment, dispersal or arrival from another patch).

Capture history file was imported in program MARK using the guidelines of White and Burnham (1999). Data were specific to CJS live recaptures model with nine groups (three species, three breeding sites) and five capture occasions without covariates and specified to a sampling interval of two days. A total of 16 pre-defined models were run based on combinations of constant and time dependent probabilities of apparent survival, entry and capture across all groups. Model selection was done using Akaike's information criteria (AICc) with no sample correction. The best-fitting (parsimonious) model was determined as the one with the lowest Akaike's Information Criterion (AIC).

The program RELEASE (this program is embedded in MARK) was used to test goodness of fit to the model. Dispersion (departure of the data from model assumptions) was determined using the variance inflation factor (ĉ, c-hat), the chi-square (χ^2) statistic and its associated p-values. Chi-square (χ^2) test was used to measure capture heterogeneity (measure of trap dependence, test 2) and heterogeneity in apparent survival (measure of transience between capture occasions, test 3).

3.2.6.7 Estimating the population size

The population sizes (Ň), standard errors and confidence limits were estimated using the POPAN formulation of the Jolly Seber Method based on the best fitting model. Population density was calculated by dividing population sizes (Ň) with area (1600 m^2). Analysis of variance was used to determine the difference in population density across sampled sites and again separately across species. A chi square (χ^2) test was used to test deviation of sex ratio (proportion of males: proportion of females) from hypothetical 1:1 sex ratio.

CHAPTER FOUR: RESULTS

4.1 Species spatial distribution in the breeding sites

4.1.1 Distributional differences in species

The survey encountered 421 individuals of nine species that belong in six anuran families (Table 1). Advertisement calls and brief account of the egg masses of these frogs (with exception of *Xenopus borealis*) have been presented in Appendix 2.1. Compositional differences in breeding sites based on Bray-Curtis dissimilarity as plotted in a DCA bi-plot show that the highest dissimilarity was recorded in reed swamps and montane grasslands (Figure 2). Flood plains, temporary puddles, ephemeral pools and marshy bogs recorded high abundance of species. Six species clustered close to the axes centroid showing low gradient lengths which indicates limited within-species variance in breeding sites.

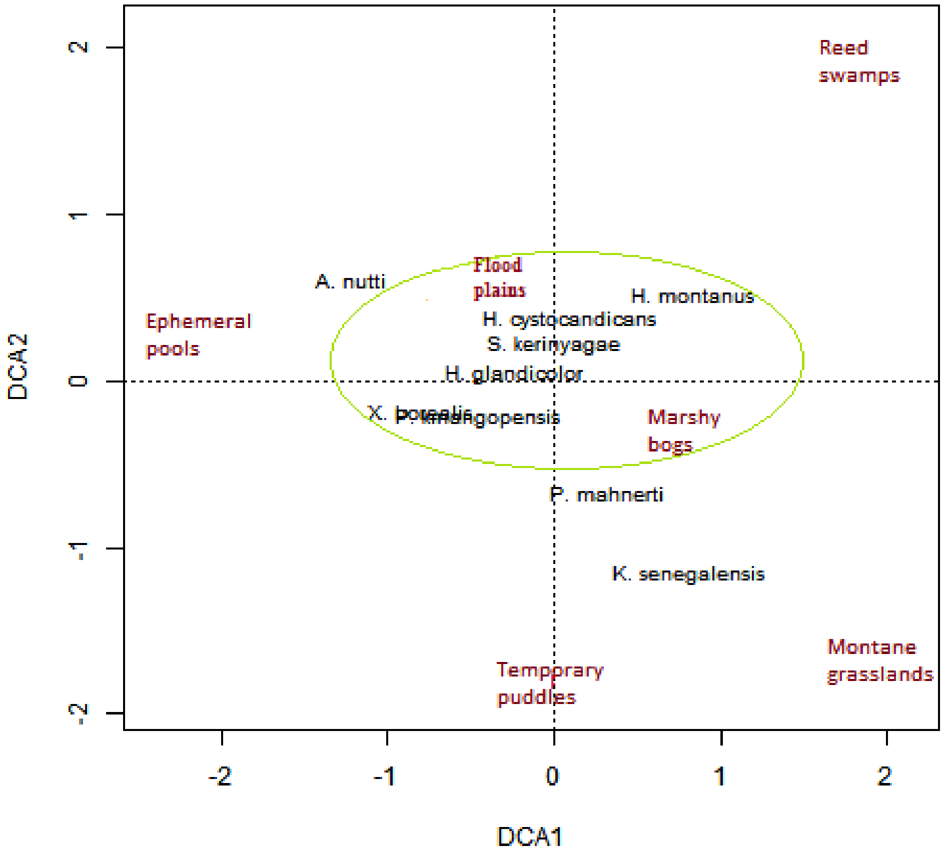

Figure 2: DCA bi-plot based on Bray-Curtis dissimilarity scores: Species (black), habitat types (red) and the green circle indicate a cluster with most species.

Table 1: The species and specimen encountered in opportunistic surveys and pitfall traps with funnel traps along drift fences in the study area grouped at different life stages; adults, juveniles, tadpoles and egg masses.

Family	**Species**	**Adults**	**Juveniles**	**Tadpoles**	**Egg masses**
Pyxicephalidae	*Amietia nutti* Boulenger, 1896	7	5	17	-
Ptychadenidae	*Ptychadena mahnerti* Perret, 1996	7	20	21	3
Hyperoliidae	*Hyperolius cystocandicans* Richards and Schiøtz, 1977	34	20	40	5
	Hyperolius glandicolor Peters, 1878	32	14	24	1
	Hyperolius montanus Angel, 1924	23	13	23	5
	Kassina senegalensis Dumeril and Bibron, 1841	2	2	10	3
Phrynobatrachidae	*Phrynobatrachus kinangopensis* Angel, 1924	34	10	3	7
Bufonidae	*Sclerophrys kerinyagae* Keith, 1968	5	2	19	4
Pipidae	*Xenopus borealis* Parker, 1936	8	7	19	-
	Total	**152**	**93**	**176**	**28**

Note: The images of tadpoles and adults of these frogs have been detailed in Appendix 2.1. Also included are the advertisement calls recorded in this survey and a comprehensive list of specimen collected and curated at National Museums of Kenya. The egg masses in this table were not included in analysis (only their parents were). Additionally, further sampling effort was targeted towards reed frogs in objective two (a total of 422 reed frogs and 84 egg masses) and objective three (a total of 841 reed frogs).

4.1.2 Species co-occurring with *H. cystocandicans*

The frequency (presence/absence) of occurrence of species subjected to pairwise probabilistic model (*sensu* Veech, 2014) showed that, out of 36 possible pairs involving nine encountered species, 19 pairs were randomly distributed (Figure 3). Four pairs co-occurred fewer times than expected in a random distribution (negative co-occurrence). These included *A. nutti* and *K. senegalensis; A. nutti* and *H. montanus; X. borealis* and *H. montanus* and; *K. senegalensis* and *X. borealis.* However, 13 pairs were co-occurring more times than expected in a random distribution (positive co-occurrence). The results show that *H. cystocandicans* co-occurs with eight species, either randomly (four species) or positively (four species). Positive co-occurrence implies non-random association between species and suggests that they have ecological interactions or similar habitat preferences.

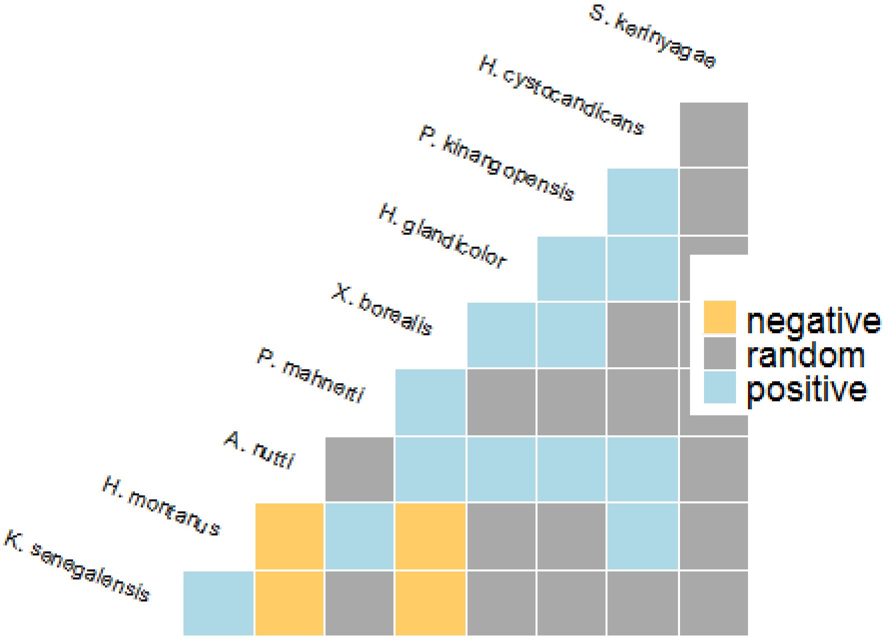

Figure 3: Species co-occurence matrix output using co-occur package.

This figure shows comparisons of expected and observed frequencies of species co-occurrence based of presence absence data. The blue boxes indicate a high likelihood of species co-occurrence (positive co-occurrence), while the yellow boxes indicate a low likelihood of co-occurrence (negative co-occurrence) and the gray boxes indicate no discernible pattern (random).

4.2 Distribution of egg masses in the breeding sites

There was intraspecific variation in the distance of egg masses from water in *H. cystocandicans* (F $_{(5, 46)}$ = 2.865, p=.025). The egg masses of *H. cystocandicans* were found away from water at an average distance of 7.39±0.44 m (mean±sd). However, there was no intraspecific variation across habitat patches in the spacing between individual egg masses and the height of egg masses. Average distance between egg masses was 2.62±0.13 m and the height of egg masses was 58.09±5.53 cm in *H. cystocandicans* as shown in Figure 4.

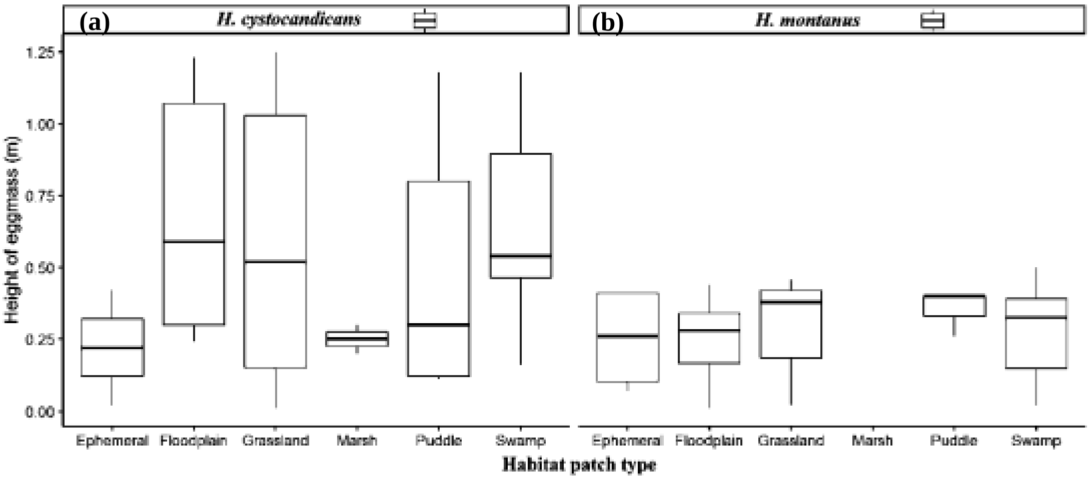

Figure 4: Height of egg masses in different habitat patches for *Hyperolius cystocandicans* (a) and *H. montanus* (b), showing: median (horizontal lines); 25th and 75th quantile ranges (box), and; upper and lower ranges (whiskers).

There was intraspecific variation in the space between egg masses (F $_{(4, 38)}$ = 3.867, p=.01) and distance of egg masses from water (F $_{(4, 38)}$ = 2.576, p=.05) in *H. montanus*. The average distance between the egg masses of *H. montanus* was 3.47±0.3 m while the average distance of egg masses from water and average height of egg masses was 7.27±0.48 m and 27.88±2.26 cm respectively (Figure 5).

Egg masses of *H. montanus* were found away from water showing no interspecific variation between *H. cystocandicans* and *H. montanus* (p=.804). However, there was interspecific variation between *H. cystocandicans* and *H. montanus* in the height of egg masses across habitat patches (F $_{(1, 92)}$ = 11.596, p<0.001) and distance between egg masses (F $_{(1, 92)}$ = 5.164, p=.008).

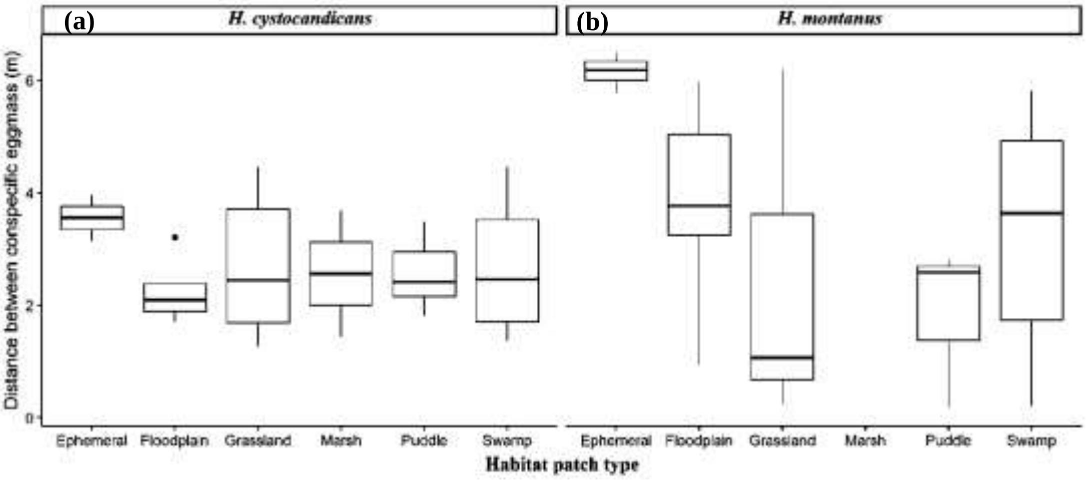

Figure 5: Distance between egg masses in different habitat patches for *H. cystocandicans* (a) and *H. montanus* (b), showing: median (horizontal lines); 25th and 75th quantile ranges (box), upper and lower ranges (whiskers), and; outliers (dots).

There was a relationship between distance of the egg masses from water (Figure 6) and distance of the egg mass from those of its conspecific for *H. cystocandicans* ($F_{(1, 46)} = 5.735$, p=.02) but not for *H. montanus* ($F_{(1, 46)} = 5.735$, p=.17).

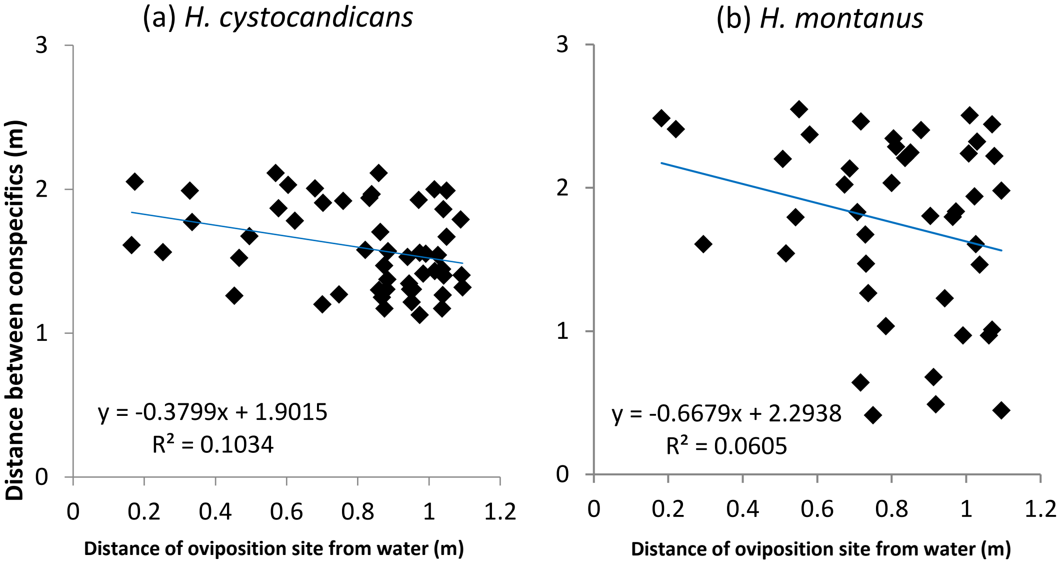

Figure 6: Linear regression of the distance from conspecifics againts the distance from water for eggmasses of (a) *H. cystocandicans* and (b) *H. montanus*.

4.1 Distribution of adults in the breeding sites

There was no interspecific variation and no intraspecific variation in spacing of conspecifics. The distance between males was (mean±sd) 2.86±0.98 m and 2.96±0.94 m for females of *H. cystocandicans* (Figure 7). The distance between males of *H. glandicolor* was 6.20±2.62 m and 5.90±2.30 m for females, 3.00±1.04 m for males in *H. montanus* and 2.92±0.91 m for females.

There was neither intraspecific nor interspecific variation in distance of the perching site from water among all species. The distance of perching site from water was (mean±sd) 6.73±3.29 m for males and 7.85±2.93 m for females in *H. montanus*, 7.06±3.22 m for males of *H. cystocandicans* and 7.17±3.08 m for females, 0.39±0.24 m for males of *H. glandicolor* and 0.42±0.23 m for females.

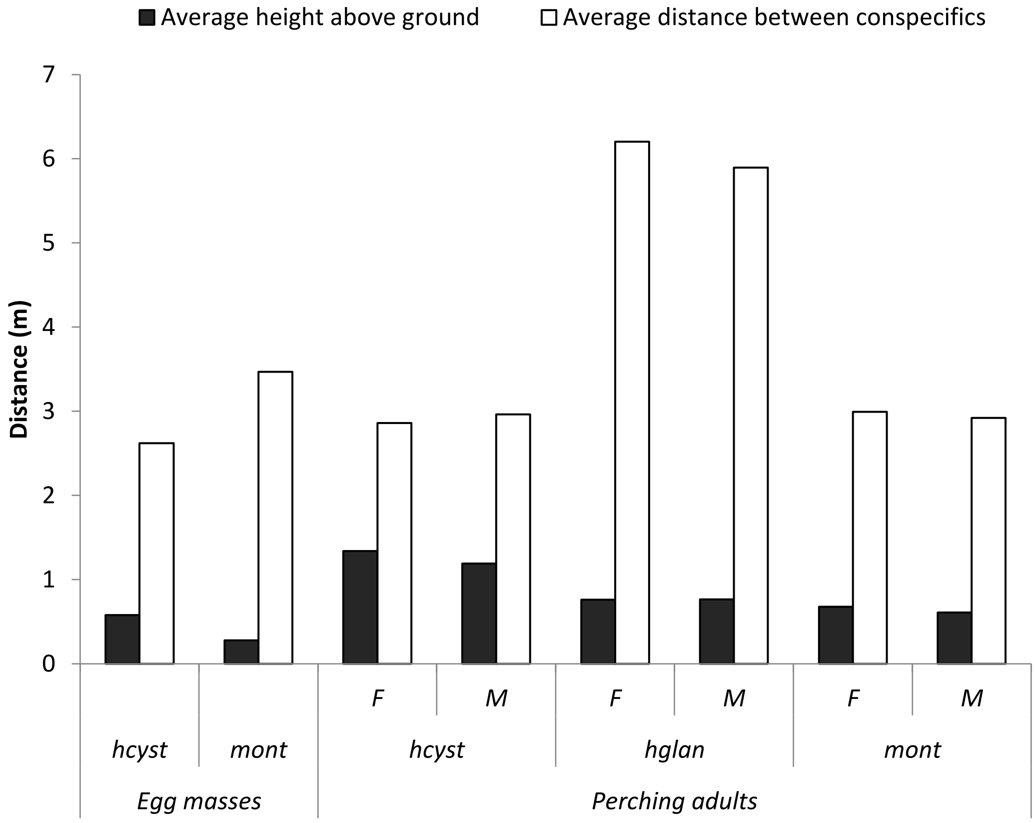

Figure 7: Spacing between conspecifics (light bar) and height above the ground (dark bar) for egg masses and adults for hcyst – *Hyperolius cystocandicans*; mont – *H. montanus*; hglan – *H. glandicolor*

There was interspecific variation in height of perching adult among *H. glandicolor*, *H. cystocandicans* and *H. montanus* (F $_{(2,\ 422)}$ = 3.93, p=.048). However, intraspecific variation in height of perching adults was not observed in all species. The height of perching adult was 0.61±0.36 (mean±sd) for males and 0.68±0.4 m for females in *H. montanus,* 1.19±0.75 m for males in *H. cystocandicans* and 1.34±0.77 m for females, 0.77±0.43 for males in *H. glandicolor* and 0.76±0.42 for females. While the distance between conspecifics was not predicted by the distance from water for *H. montanus* (Figure 8), the distance between conspecifics reduced with increase in the height of perching adult *H. montanus* (F $_{(1,\ 120)}$ = 11.04, p=.002), indicating intraspecific competition and preference for oviposition sites closer to the ground.

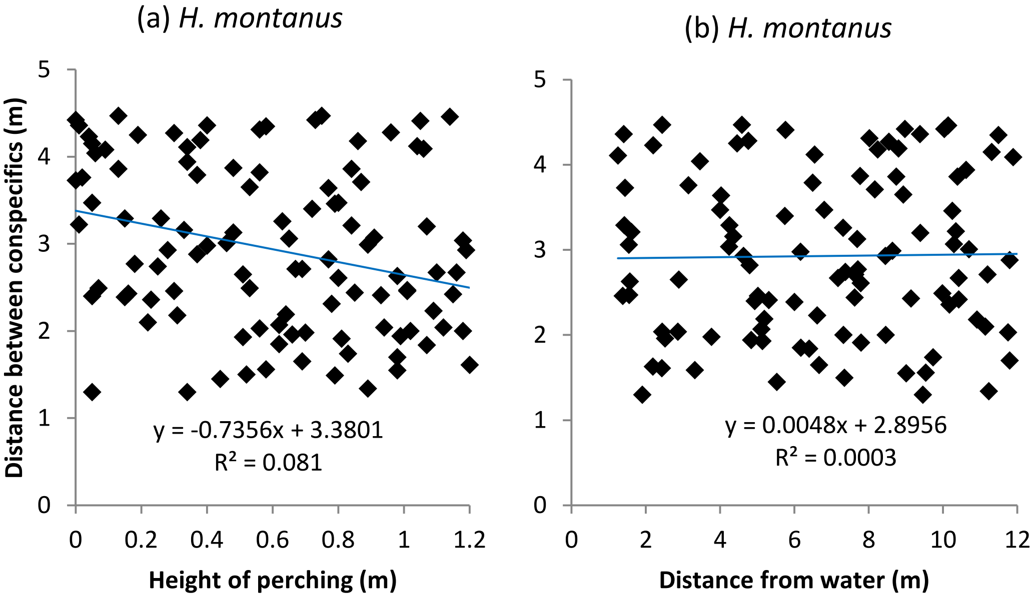

Figure 8: Linear regression of the distance between conspecifics against the distance of the perching site from water and the height of perching adult for *H. montanus*

There was a significant relationship (p=.004) between the distance between conspecifics for *H. cystocandicans* and the distance to the nearest breeding site (km) (Figure 9). This indicates aggregation of conspecifics in breeding sites that are close together. A number of observations on interactions of reed frogs have been highlighted in Appendix 2.2, including: sharing of habitats; aggression and intraspecific competition over breeding space; road mortality; desiccation of egg masses and tadpoles, and; predation, infections and parasites. There was no relationship between the distance between conspecifics and the distance to the nearest breeding site (km) for *H. glandicolor* (p=.15) and *H. montanus* (p=.47). Similarly, there was no relationship between the distance from water and the distance to the nearest breeding site (km) for *H. montanus* (p=.35), *H. glandicolor* (p=.32) and *H. cystocandicans* (p=.54). There no relationship between perching height and the distance to the nearest breeding site (km) for *H. glandicolor* (p=.16), *H. montanus* (p=.79) and *H. cystocandicans* (p=.59).

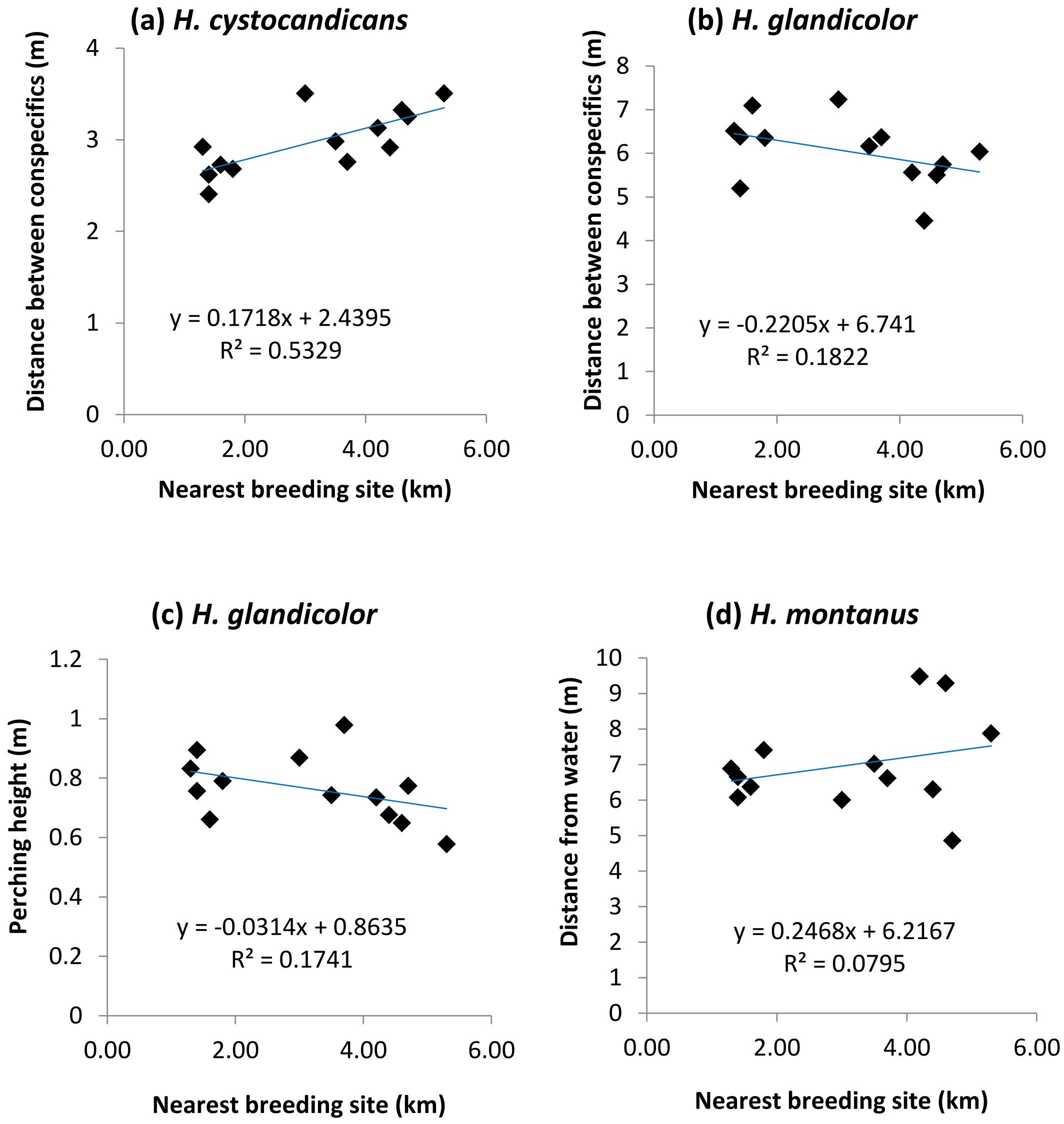

Figure 9: Linear regression of the distance to the nearest breeding site (km) and distance from water of *H. cystocandicans* (a) and *H. glandicolor* (b); perching height of *H. glandicolor* (c) and; distance between conspecifics of *H. montanus* (d).

4.2 Estimates of the population size

4.2.1 The number of reed frogs marked and recaptured

A total of 841 uniquely identified and marked reed frogs were encountered 1213 times. Out of these encounters, 372 were recaptures where 156 individuals were recaptured more than once. These include *H. cystocandicans* (342 frogs, 118 recaptured), *H. montanus* (216 frogs, 69 recaptured) and *H. glandicolor* (283 frogs, 185 recaptured). These encounters generated 31 unique capture histories for five capture occasions at three breeding sites (Appendix 1.2).

4.2.2 Probabilities of capture and apparent survival

The data fitted to the CJS live recaptures model (χ^2 = 57.54, df = 54, p = .345). There was a slight over-dispersion (median-ĉ = 1.099). The study revealed neither trap dependence (χ^2 = 10.79, df = 14, p = .703) nor transience across capture occasions (χ^2 = 47.058, df = 40, p = .206). Results for Goodness of Fit tests are detailed in (Appendix 1.6). The most parsimonious model [*phi*(g) *p*(g*t)] had a constant probability of apparent survival that varies across groups with a time dependent capture probability that varied across groups (Appendix 1.3).

The estimates of entry (pent, β_i), apparent survival (phi, Φ_i) and capture probabilities (p, π_i) were comparable across capture occasions for all species in breeding sites (Appendix 1.4). On average (mean ± se), the estimates for capture probability oscillated about 0.423 ± 0.059 for *H. cystocandicans* and were lower than the mean capture probabilities for *H. montanus* (0.544 ± 0.064) and *H. glandicolor* (0.514 ± 0.054). The significance of capture probability is that it quantifies the proportion of frogs that were available for capture and thereby accounting for partial observation in the estimated population sizes (section 4.2.3).

The mean estimate of probability of entry was higher for *H. glandicolor* (mean ± se) (0.034 ± 0.016) than *H. montanus* (0.027 ± 0.015) and *H. cystocandicans* (0.022 ± 0.016). This probability (despite being small in this study) incorporates into the estimated population sizes, a proportion of frogs that were not available for capture but arrived later during the course of census based on model predictions. Similarly, the mean apparent survival was lower for *H. glandicolor* (0.649 ± 0.106) as compared to *H. cystocandicans* (0.794 ± 0.063) and *H. montanus* (0.706 ± 0.107). This probability (ranging from 0.348 to 0.834 in this study) standardises the population size to account for frogs that remained in the breeding site between capture occasions (even when they were not actually recaptured).

4.2.3 Population sizes and density

A total population of 1863 (LCI=1510, UCI=2316) was estimated for three reed frog species (*Hyperolius glandicolor, H. montanus, H. cystocandicans*) in 3 breeding sites covering an area of 4800 m^2. The estimated average population was 197 (LCI=160, UCI=243) for *H. cystocandicans*, 263 (LCI =217, UCI=315) for *H. glandicolor* and 164 (LCI=124, UCI=242) for *H. montanus*. Population size estimates at each breeding site are shown in Table 2. The sample sizes and estimates of population size of every census are summarized in Appendix 1.5.

Table 2: Estimates of population sizes (Ň) showing standard errors, lower and upper confidence intervals (LCI, UCI) at 95% limits of SE.

Site	Species	Ň	SE	LCI	UCI
Kangaita	*H. glandicolor*	123	20.05	89	169
	H. montanus	308	52.12	237	451
	H. cystocandicans	194	18.24	161	233
Chehe	*H. glandicolor*	154	99.86	64	581
	H. montanus	50	23.30	21	119
	H. cystocandicans	101	6.43	89	114
Ragati	*H. glandicolor*	162	14.85	135	193
	H. montanus	133	10.80	113	156
	H. cystocandicans	298	38.07	232	382

Population densities were (frogs per 100 m^2): *H. glandicolor* (7.7, 9.6, and 10.1), *H. montanus* (19.3, 3.1, and 8.3); *H. cystocandicans* (12.1, 6.3, and 18.6). There was no interspecific variation in population density ($F_{(2, 42)} = 3.825$, $p = .059$). The population density was not different across survey sites for *H. glandicolor* ($F_{(2, 12)} = 0.27$, $p=0.748$) and *H. cystocandicans* ($F_{(2, 12)} = 2.764$, $p=0.103$). However, the population density of *H. montanus* varied across survey sites ($F_{(2, 12)} = 6.987$, $p<0.05$). Estimates of population sizes by sex are shown in Figure 10. The population was skewed towards males in *H. cystocandicans* ($\chi^2 = 43.188$, df = 2, $p<0.05$) and *H. montanus* ($\chi^2 = 337.49$, df = 2, $p<0.05$). However the population of *H. glandicolor* did not deviate from the hypothetical 1:1 sex ratio ($\chi^2 = 5.746$, df = 2, $p=0.057$).

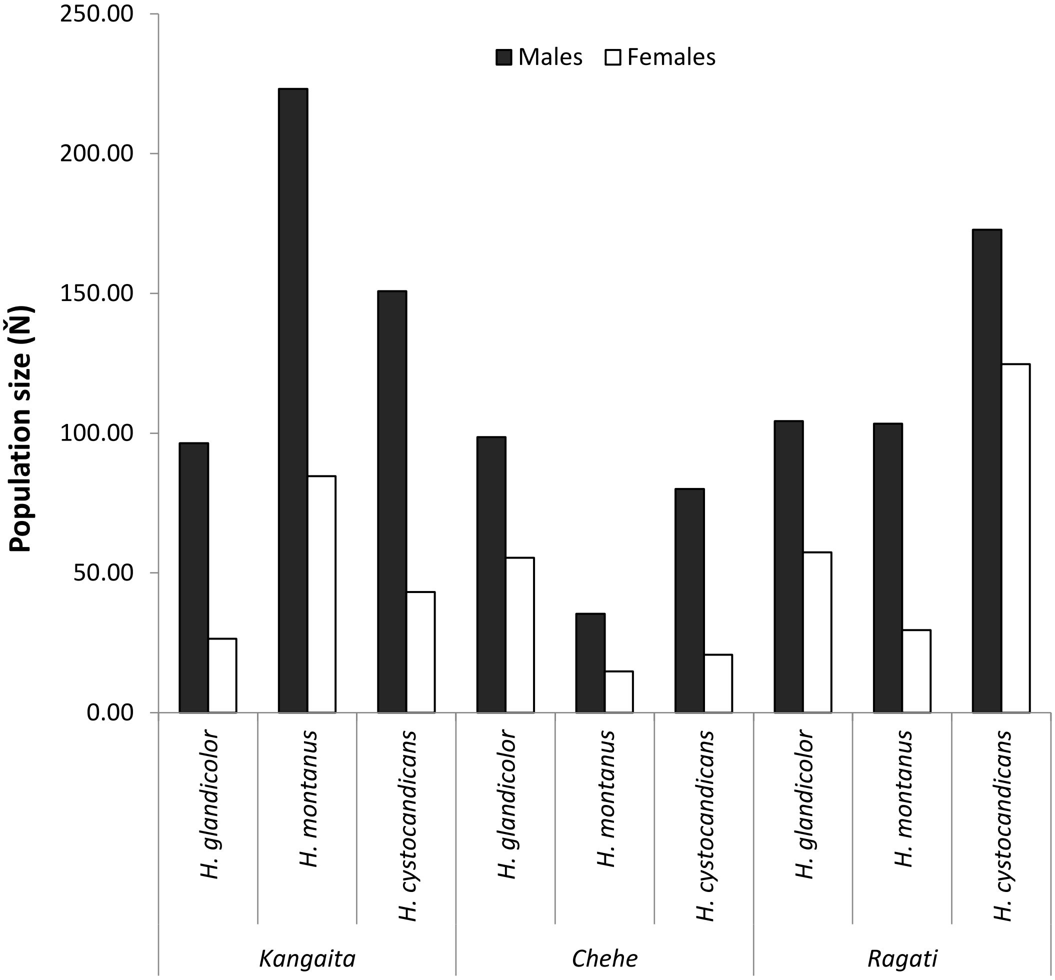

Figure 10: A histogram of the population size showing the number of males (dark bar) and females (light bar).

CHAPTER FIVE: DISCUSSIONS

5.1 Species co-occurrence and distributional differences

The forest on the southern slopes of Mt. Kenya is a region of unique herpetofaunal biodiversity. In a rapid assessment survey within the study area, nine frog species were encountered. Two of these species (*H. cystocandicans* and *P. kinangopensis*) are threatened species while *H. montanus* is Kenyan endemic and several near endemic species. These results are comparable with a cross-sectional study by Malonza (2015) that encountered six amphibian species along altitudinal gradients from the South Eastern slopes. That study however was not timed entirely in the amphibian breeding season (Malonza, 2015). In addition, this study compares to a study by Lötters *et al.* (2006) that documented 17 anuran species occurring in Mount Kenya with additions by Wasonga (*Pers. Comm.*). This high number of species is justifiable because the studies by Lötters *et al*. (2006) were based on specimen collected over an extended period and covered the entire ecosystem.

The occurrence of *H. cystocandicans* in a habitat shared with other reed frogs was not unexpected. Indeed, quite a number of studies such as Portik *et al.* (2018) and Lötters *et al.* (2006) have documented sympatry among reed frogs. In addition to reed frogs, *H. cystocandicans* also co-occurred positively with other frog species. Positive co-occurrence can have various ecological explanations such as sharing similar habitat requirements or having similar resource preferences. While co-occurrence does not necessarily mean coexistence, it would mean that species have fewer antagonistic interactions (Blanchet *et al.,* 2020). Related species that co-occur also display interspecific competition for reproductive resources and inherent divergence of reproductive traits (Bell *et al.,* 2014; Strauβ *et al.,* 2018; Portik *et al.,* 2018). There were however, cases of negative co-occurrence of related taxa such as, *H. montanus* and *K. senegalensis* (family *Hyperoliidae*).

This study shows that the study area is not dominated by a few frog species but species were not uniformly distributed in all habitat types. Although Bray-Curtis dissimilarity emphasizes on rare species than common species, the distances among habitats and among species represent ecological distances (Ricotta and Podani, 2017). Given that this study was undertaken during the breeding season, these results could mean that species were aggregated in habitats suitable for breeding. It is hypothesized that, rather than encouraging competition, frogs attract densities into breeding sites by interspecific information transfer using acoustic signals (Goodale *et al.,* 2010; Tóth *et al.,* 2020). However, besides indicating dissimilarity in species distributional composition and species co-occurrence, this study is limited in that it does not quantify how much individual species (such as *H. cystocandicans*) contributes to the overall diversity.

The use of combination of visual encounter surveys and acoustic encounter survey as used in this study is suitable because it detects both adults and larvae. Identification of tadpoles is crucial in rapid assessment surveys because in the absence of adults, they serve as indicators of presence or absence of a species, especially during periods when adults are inactive or otherwise less frequently encountered (Channing *et al.,* 2019; Dehling and Sinsch, 2019). For the first time, this study documents and collects specimen of larval stages from all species encountered. Such specimen will aid the description of the tadpoles of *Phrynobatrachus kinangopensis, Ptychadena mahnerti* and *Sclerophrys kerinyagae.* While it was possible to identify pairs in amplexus and incorporate egg masses in this study, there is still limited work in the identification of eggs to species level without reference to adults, particularly for species-rich taxa such as *Hyperoliidae* (Lawson et al., 2018).

5.2 Distribution of egg masses of reed frogs

The diversity of reproductive modes in reed frogs is largely as a result of different locations of egg deposition, in respect to the height of oviposition site and source of water for larval development (Refsnider and Janzen, 2010; Müller *et al.,* 2013). Despite preferring different habitat patches, *H. cystocandicans* and *H. montanus* have non-aquatic oviposition sites and were similar in terms of the distance of oviposition site from water and spacing between egg masses. Such terrestrial eggs of reed frogs are known to hatch outside water and developing embryos persist for an extended period nourished by their large yolks (Chipman *et al.,* 1999). The tadpoles hatching from these terrestrial eggs are washed into water during flash floods caused frequently by rainfall in the area (Lehr *et al.,* 2005). However, if these tadpoles do not make their way into a water body, there is a high chance of desiccation. As such, some reed frogs that deposit eggs from water such as *H. mossaicus* Perret, 1959 utilize tree holes (Lötters *et al.,* 2004).

The height of oviposition varied where *H. cystocandicans* attached its eggs higher on twigs and herbs while *H. montanus* attached its eggs on lower herbs, fallen wood and on grass. In hyperoliids, terrestrial eggs have been attributed with predator avoidance, including cannibalism (Drewes and Altig, 1996). Other species that deposit eggs on leaves include *inter alia*: *H. drewesi* Bell, 2016; *H. kivuensis, H. lateralis* (Lötters *et al.,* 2004). The eggs of these species are fewer and larger, which means a large yolk to facilitate terrestrial hatching (Chipman *et al.,* 1999; Lawson *et al.,* 2018). These species differed from *H. glandicolor* whose eggs are deposited in water. The clutch size recorded for *H. glandicolor* by Lawson *et al.* (2018) doubles the known clutch size of *H. cystocandicans* established by Richards and Schiotz (1977). Nowakoski *et al.* (2017*)* proposed that such high fecundity is a trade-off for decreased survival in altered habitats and thereby increasing population persistence.

5.3 Distribution of adult reed frogs in the breeding site

Reed frogs were partitioned in the breeding sites exhibiting interspecific differences in breeding site selection. While call parameters are important in understanding species boundaries in reed frogs (Köhler *et al.,* 2005; Rödder and Böhme, 2009; Köhler *et al.,* 2017; Bamba-Kaya *et al.,* 2019), distribution patterns of individuals in breeding sites offers insights on interactions of co-occurring species. These patterns include the height of calling males when calls are propagated, distance between calling males and distance to locations where larvae develop.

Intraspecific variations were not registered but the spatial structure of adult reed frogs presented a unique pattern of vocal dispersion among species based primarily on the structure of habitat patches. The calls of *H. glandicolor* are produced from calling positions closer to water as compared to other reed frogs. This bares similarity with a sect of reed frogs such as *H. nasutus* and *H. viridiflavus* (Viertel *et al.,* 2007; Rödder *et al.,* 2006; Lawson *et al.,* 2018; Sinsch *et al.,* 2021). The perching height was lower for *H. montanus* as compared to *H. glandicolor* and *H. cystocandicans.* However, both *H. montanus* and *H. cystocandicans* called from perching sites away from water. These reed frogs are similar with species that have terrestrial eggs such as *H.*

dintelmanni Lötters and Schmitz, 2004 (Portik *et al.,* 2019); and the results align with earlier observations by Köhler *et al.,* (2005) and Richards and Schiøtz (1977).

Occurrence of multiple reed frogs in a single habitat is associated with the existence of diverse habitat types (Portik *et al.,* 2018). Several studies point out to the significance of such habitat heterogeneity in determining niche overlap among *Hyperolius* frogs. A study in Kakamega forest on species traits and niche divergence of sympatric *Hyperolius* species found that *inter alia*, these species vary significantly in the choice of their breeding sites (Lötters *et al*., 2004). Syntopic reed frogs choose different calling sites, calling intervals, duration of daily calling periods and height of calling sites (Rödel *et al.,* 2006). Calling height is related with call energetics (Grafe, *et al.,* 1992). For example, calls that are produced from higher perches have lower amplitude that matches similar louder calls that are produced from the ground while males producing higher amplitude sounds had a narrower space between them (Rodriguez *et al.,* 2020).

Aggregation of males during the breeding season was as a result of social interactions within species, between species and the distribution of suitable breeding sites. The study by Rodriguez *et al.* (2020) further showed that distance between calling males increases with an increase in calling perch height. This spatial distribution is likely to reduce confrontations among calling males. Male-male competition at breeding sites was observed for *H. cystocandicans* in this study (see Apendix 2.2). Males have digital glands and are able to grip on substrates with their hands and strike rival males with their hind limb (Richards and Schiøtz, 1977). Intraspecific male-male competitions were also observed for *H. glandicolor* and are thought to contribute to the overall fitness of the population by influencing spatial distribution and spacing (Dyson and Passmore, 1992). Competition within and between species is a fundamental ecological process that influences the structure and functioning of ecosystems, maintaining ecological balance and biodiversity.

Distribution of adults in a breeding site can expose different species to predation risk and parasites both in the terrestrial and aquatic realms. For instance, while calling at elevated heights augments auditory and visual conspicuousness to females and other males in territorial species, it tends to increase vulnerability to predators and parasites such as chigger mites (Rodriguez *et al.,* 2020). This also applies to species with aquatic eggs, because eggs remain sedentary until hatching. In this study, some tadpoles had been attacked by aquatic predators especially beetles (*Coleoptera*), dragonfly larvae (*Odonata*) and predatory leeches (*Huridinea*). These jawed leeches were also common on adults (see Appendix 2.1) and have been observed in other studies such as Hughes *et al.,* (2018). Additionally, this rapid assessment survey documents reed frogs with parasites (fungus and flukes) and an infection that is yet to be identified (see Appendix 2.2). Notably, species that occur closer to the forest edge are at risk of contaminations from agro-chemicals (Bwong, *pers. comm*). Growing body of evidence shows that co-occurrence of reed frogs has resulted to diversification and a common pool of parasites such as helminths and flukes (Sinsch *et al.,* 2020; Sinsch *et al.,* 2021). A study by Bielby (2008) also alluded vulnerability of *H. cystocandicans* in Central Highlands to chytrid fungus (*Batrachochytrium dendrobatidis,* Bd*)* on the basis that other unspecified reed frogs were infected.

In addition to egg masses and adults, large aggregations of juveniles of reed frogs were found at the end of the breeding season, aestivating at various heights in the breeding sites, even when no adult was detected. This agrees with other studies that there is a period when the abundance of juveniles surpasses that of adults (Grafe and Linsenmair, 1989; Sinsch and Dehling, 2017). Reed frogs are cryptic and call in the open at night but disperse to concealed locations in order to avoid predators and therefore require different environments in order to survive. The pristine habitats in higher altitudes suits *H. montanus* more than other reed frogs for calling during the day (Köhler *et al.,* 2005). All encountered species of reed frogs were heard calling during the day at the onset of the breeding period, which is associated with sporadic breeders, but breeding activities peaked at night. It is probable that calls during the day are meant for other purposes (such as territoriality) other than reproduction.

Calling males that are closer to oviposition sites may have advantages over other males. Similarly, in species where calling and spawning sites are the same, it is possible that females choose mating partners on the basis of quality of the breeding site. This is probably why males of *H. glandicolor* maintain space in breeding sites and aggressively defend their territories (Dyson and Passmore, 1992). Reed frogs preferentially select for calling space in similar habitats. The calling males of *H. nasutus* also aggregate and defend a small territory preferably on the sedge but the sympatric sharp-nosed reed frogs (*H. nitidulus*) prefer small, thin, stiff reed grasses that grow in water while *H. concolor* Hallowell, 1844 call mainly from plants growing on land (Rödel *et al.,* 2006). The sympatric species studied by Bell *et al.* (2014) differ in their breeding biology where *H. molleri* Bedriaga, 1892 breeds near still waters or slow-moving sections of streams water but *H. thomensis* Bocage, 1886 breeds exclusively in water-filled tree hole cavities.

Recent studies show that sympatric reed frogs have different calling positions within their shared habitats (Portik *et al.,* 2018). The advertisement calls of *H. kivuensis* are made on sedge, herbs, or papyrus reeds about 30–100 cm in height while *H. viridiflavus* calling at similar sites, prefer floating leaves of water lilies near the water surface (Sinsch *et al.,* 2021). The males of *H. guttulatus* Gϋnther, 1858 utilize well concealed calling sites covered by large leaves at heights up to 143 cm (Kouamé *et al.,* 2015). The males of *H. cinereus* Monard, 1937 are known to call on grass about 30–50 cm from the water surface while sympatric *H. raymondi* Conradie, Branch and Tolley, 2013 calls from tall grass and reeds at the edge of large pools (Conradie *et al.,* 2013). The calling height of *H. molleri* is 30 to 100 cm while *H. thomensis* calls from 500 cm above the ground and utilizes tree holes for oviposition (Strauβ *et al.,* 2018).

The availability (or lack thereof) of various resources that reed frogs utilize for reproduction such as calling substrates, coupled with intraspecific spacing of calling males can help control population abundances (Rödel *et al.*, 2006; Gilbert and Bell, 2018). In the context of multiple co-occurring species, these differential traits lead to niche differentiation, where species adapt to occupy different ecological niches, reducing direct confrontations and allowing multiple species to coexist in an ecosystem (Lötters *et al.,* 2004; Portik *et al.,* 2018). Interaction between species regulates the use of limited resources like food, water, and habitat, thereby promoting efficient resource allocation in ecosystems (Luiselli *et al.*, 2004). By preventing a single species from dominating, interspecific competition contributes to biodiversity by supporting a coexisting species, driving evolutionary adaptations, influencing the traits and behaviors of competing species over time and driving speciation (Liedtke *et al.,* 2004; Schick *et al.,* 2010; Bwong *et al.,* 2020).

5.4 Detection probabilities and population sizes of reed frogs

This study gives the baseline estimates of population sizes of *H. cystocandicans, H. glandicolor* and *H. montanus* in Mount Kenya Forest and is the first population estimates of a reed frog in East Africa. The demographic compositions of reed frogs across the three objectives of this survey included egg masses, tadpoles, juveniles and adults, which is an indication that the frogs in this population are reproducing. Reed frogs, like other frogs have complex life history traits and dependence on both terrestrial and aquatic habitats (Petranka, 2007; Sinsch *et al.,* 2011). However, only the population sizes of adult reed frogs were estimated, owing to the complications associated with marking and recapturing larvae, difficulties of estimating tadpole survival under field conditions, loosing marks and exposing tadpole to predation risk (Carlon and Langkilde, 2013).

The estimated population size in this study refers to the size at the breeding sites during the breeding season. Mean population size of *H. cystocandicans* (197) was comparable to that of other reed frogs in the range of co-occurrence; *H. glandicolor* (263) and *H. montanus* (164). This is also comparable to population sizes of reed frogs estimated elsewhere (Tarrant and Armstrong, 2014; Rödel *et al.*, 2006). Estimates of population sizes and statuses of *Hyperolius* species are however, sparse. In South Africa, it is estimated that the population density of the Endangered Pickersgill's reed frog (*H. pickersgilli*) was 159 calling males per hectare (Tarrant and Armstrong, 2014). A survey in 1996 counted an abundance of between 28 – 54 calling males per night along a transect 100 m long for *H. nasutus* in Ivory Coast, West Africa (Rödel *et al.,* 2006). These abundances were estimated through acoustic and visual encounters along transect lines (Rödel *et al.*, 2006).

Estimates from this study were higher than raw counts because the 'super population approach' of POPAN Jolly Seber estimates number of animals and include animals that were not captured but are accounted for by detection probabilities (Tanadini and Schmidt, 2011; Wagner *et al.,* 2011; Williams *et al.,* 2011). Population estimates by capture probabilities are therefore justifiable as compared to estimates of abundance by along transects (Tanadini and Schmidt, 2011; Pesarakloo *et al.,* 2020). In addition, the Jolly-Seber (JS) model relaxes the assumption of population closure in open-population, and allows multiple sampling occasions with possibilities of population increase (as a result of immigration or recruitment) or population decrease (as a result of emigration or mortality) (White and Burnham, 1999; Kissel *et al.,* 2020; Sen and Akçakaya, 2022).

A male-biased sex ratio in this study is attributed to the fact that during the breeding season, males congregate in large numbers every night in choruses in breeding sites, while females only return to the breeding site for oviposition purposes. Sex ratio among frogs are either skewed towards males (Loman and Madsen, 2010; Lambert *et al.,* 2021); or females (Alho *et al.,* 2008). However, sex ratio in the population of *H. glandicolor* in this study was not skewed. This species has weakly expressed sexual size dimorphism as compared to *H. montanus* and *H. cystocandicans* and has been found to regulate its sex ratio through sex change of females to reproductive males (Grafe and Linsenmair, 1989). Additionally, males mature early and die earlier than females (Lawson *et al.,* 2018). Other frogs that have recorded male-biased sex ratio at breeding sites include reed frogs of *H. nasutus* complex (Rödel *et al.*, 2006).

The sex ratio estimated in this study however, is not equivalent to operational sex ratio (in relation to the number of males who are in the habitat for breeding purposes) that is estimated using genetic studies (*sensu* Mangold *et al.*, 2015). Non-breeding males and females of reproductive age are often present in skewed populations (Alho *et al.,* 2008; Loman and Madsen, 2010). The results should therefore be interpreted with caution because marking did not distinguish periphery males included in the population survey (Honeycutt *et al.,* 2019). Studies based on genetic diversity have estimated effective population sizes, operational sex ratios and demonstrated that the number of active breeders in a population is less than the absolute population size (Mangold *et al.*, 2015; Luikart *et al.,* 2021; Jaynes *et al.,* 2022).

Using detection probabilities rather than abundance improves estimates of population sizes and accounts for imperfect detection (Tanadini and Schmidt, 2011). The probabilities of capture were moderate for all species because sampling was conducted in the rainy season when the frogs are aggregating at breeding sites, and not the dry season when frogs are less active and difficult to detect. Ideally, detection probabilities increase as abundance increases; thereby making estimates more reliable (Pesarakloo *et al.,* 2020). During the breeding season, sites are well connected by flooded plains, water-logged swamps and large temporary puddles. This triggers individuals in less populated sites to populous sites because species exploit auditory cues to attract density (Goodale *et al.,* 2010; Joly, 2019; Tóth *et al.,* 2020).

The highest probability of survival was recorded by *H. cystocandicans* and indicates that estimated population sizes accounts for a large proportion of individuals who remained within the breeding site across capture occasions. A major assumption of the model is that marks are not lost (White and Burnham, 1999; Lettink, 2012; Newell *et al.,* 2013). While it was expected from reconnaissance results that marked frogs retained the mark for 28-30 days, there were only nine days between the first capture occasion and the last day of recapturing at any given census site and therefore marks did not disappear over the study period. These reed frogs exhibit color polymorphism and natural markings (Kobelt and Linsenmair, 1986; Liedtke *et al.,* 2004; Lötters *et al.,* 2004; Schick *et al.,* 2010). Therefore, in addition to the use of administered marks, peculiarities and colors noted in field notebooks and photographs were utilized and thus, increasing the certainty of individual identification, especially in subsequent encounters of *H. cystocandicans* (Richards and Schiøtz, 1977).

The standard approach of capture-mark-recapture provides robust estimates of population sizes, while the underlying model assumptions are considered (Williams *et al.,* 2011; Kissel *et al.,* 2020). Notably, despite efforts to limit bias in this study, survival heterogeneity and heterogeneity in capture probabilities may exist to some degree. For example, non-breeding individuals and gravid females entering the breeding site are likely transient and therefore assuming equal survival probability when pooling encounter histories of individuals of different sexes and age classes might violate the assumption (Honeycutt *et al.,* 2019). POPAN Jolly Seber model is a powerful tool for estimating frog population sizes that allows for a constant sampling area and constant sampling effort, yet sampling time that is shorter than the sampling interval (White and Burnham, 1999; Lettink and Armstrong, 2003; Newell *et al.,* 2013). Since a variety of habitats was sampled and guidelines were adhered to, it is justifiable to assume that the result of this study are a reliable estimate of reed frogs' populations in the forests on the southern slopes of Mount Kenya.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

i. Silver bladder reed frog (*H. cystocandicans*) co-occurs with other frog species in the study area, which include reed frogs.

ii. All reed frogs encountered have aquatic larvae but differ in having aquatic eggs in *H. glandicolor* and terrestrial eggs in *H. montanus* and *H. cystocandicans.* This drives spatial reproductive partitioning in the distribution of reed frogs in breeding sites.

iii. The distance of egg masses of *H. montanus* and *H. cystocandicans* from water vary between species and within species across various habitats.

iv. Adult reed frogs also, differ in the height of perching; therefore the first hypothesis was rejected. However, there was no intraspecific and interspecific variation in spacing between conspecifics and distances from water.

v. The population density of *H. montanus* varied across survey sites; therefore the second hypothesis was rejected.

vi. The population size of *H. cystocandicans* is comparable to that of *H. glandicolor* and *H. montanus* in similar habitable patches at the peak of the breeding season. These estimates are a baseline population data that provide key information that will inform conservation of reed frog populations.

vii. The population estimates were skewed towards males in *H. cystocandicans* and *H. montanus* but not *H. glandicolor.*

6.2 Recommendations

i. Studies should be done to further understand the interaction between *H. cystocandicans* and other co-occurring species. Future research should focus on species’ functional traits that influence resource partitioning.

ii. There is need for further population estimates to determine whether the population is declining and for informed conservation of reed frogs.

iii. A demographic survey that incorporates genetic diversity is required in order to estimate effective population sizes and number of active breeders (operational sex ratio) in male-biased populations.

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APPENDICES

Appendix 1: Additional findings in support of objective 3

1.1: Breeding sites

Station	Name and types of patches identified*	South	East	Altitude
Ragati Forest	Hombe (P, B)	-0.352752	37.124026	1973
		-0.350976	37.139502	2017
	Muthira (R, B, S)	-0.397781	37.138877	1990
		-0.390781	37.168877	1962
		-0.386069	37.148956	2007
	Gatei (S, R, P, B, G)	-0.36895	37.158159	2027
		-0.378065	37.160091	2040
		-0.375967	37.158159	2042
	Karia (S, R, B)	-0.39583 (census)	37.169121	1987
		-0.384205	37.163081	1966
		-0.401059	37.164983	1928
Chehe Forest	Muhuhi (S, R)	-0.406609 (census)	37.174808	1920
		-0.41649	37.17897	1925
	Kinyororo (R, S)	-0.384904	37.216136	2146
		-0.379219	37.234791	2198
	Kururu (R, S)	-0.4055707	37.190002	1952
		-0.392007	37.216136	2091
		-0.398914	37.201621	2049
	Chehe (R, S, P)	-0.415669	37.208242	1966
		-0.410999	37.240229	2044
		-0.420999	37.200229	1933
Kangaita Forest	Ruiru (R, B)	-0.422643	37.227417	1913
		-0.402643	37.217417	1910
	Rundu (R, S, M)	-0.420476	37.240312	2019
		-0.429476	37.219312	1984
		-0.419476	37.249312	1966
		-0.41614	37.246094	2005
		-0.41209	37.24276	2035
	Mukengeria (R, S)	-0.393772	37.25725	2150
		-0.388143	37.25725	2076
		-0.389143	37.25025	2071
	Kiamikaragati (S, G)	-0.38273	37.256453	2120
		-0.38208	37.256453	2130
	Njabacabi (S, R, B)	-0.386855	37.23575	2207
		-0.386483	37.231718	2203
		-0.376483	37.238718	2111
	Gacomo (S, P, G)	-0.380493	37.240189	2230
		-0.380998 (census)	37.239724	2230
	Mairoinya (S, P, G)	-0.374918	37.285379	2072
		-0.375667	37.281249	2063
	Kirumiandu (S, P)	-0.387801	37.242491	2210
		-0.391964	37.239724	2198

Notes:

i. **Habitat types**: S – Reed Swamp; R – Flood plain; P – Temporary Puddle; M – Ephemeral pools of Water; B – Marshy Bogs; G – Montane Grasslands.

ii. **For census sites:** (shaded row) see information on Appendix 1.2 to 1.6.

1.2: Capture history file and notes on data analysis

history*↓	KANGAITA			CHEHE			RAGATI			
species*	**hglan**	**hmont**	**hcyst**	**hglan**	**hmont**	**hcyst**	**hglan**	**hmont**	**hcyst**	**Total**
00001	14	36	16	6	2	9	25	15	16	139
00010	13	16	25	9	4	7	17	11	15	117
00011	1	21	2	0	1	2	6	5	2	40
00100	10	19	16	6	3	7	3	14	14	92
00101	1	2	0	0	0	1	1	3	2	10
00110	2	3	2	2	1	6	3	3	5	27
00111	0	0	0	0	0	0	1	1	0	2
01000	7	14	13	2	1	3	5	5	25	75
01001	0	0	2	0	0	2	2	0	1	7
01010	0	1	0	0	0	4	2	1	0	8
01011	0	0	0	0	0	1	0	0	0	1
01100	0	7	3	0	0	0	10	2	2	24
01101	0	0	1	0	0	0	0	0	0	1
01110	2	0	1	0	1	0	2	2	1	9
01111	1	0	0	0	0	0	0	1	0	2
10000	8	27	11	9	6	10	13	11	37	132
10001	1	0	1	0	0	2	2	0	2	8
10010	1	3	5	0	0	1	3	3	8	24
10011	0	0	0	0	0	3	1	0	0	4
10100	1	2	3	0	0	3	3	3	5	20
10101	0	1	1	0	0	1	1	0	0	4
10110	0	2	0	0	0	0	1	1	1	5
10111	0	0	0	0	0	0	1	0	0	1
11000	2	6	9	1	2	8	6	6	3	43
11001	0	1	0	0	0	0	0	2	0	3
11010	1	1	0	0	0	1	0	1	1	5
11011	1	0	0	0	0	0	0	0	0	1
11100	0	2	5	1	1	9	2	4	1	25
11101	0	0	0	0	0	1	0	0	0	1
11110	1	2	1	0	0	2	2	0	1	9
11111	0	1	0	0	0	0	1	0	0	2

Notes

i. **Species:** hcyst – *H. cystocandicans;* hmont – *H. montanus;* hglan – *H. glandicolor*

ii. **History**: a capture history code was prepared by pooling together the times every individual was captured (1) or not captured (0) across all batch releases. For instance an individual with a history (10101) means that it was encountered on the first capture event and subsequently captured on the third and the fifth batch release.

iii. **Analysis:** (Detailed results, see Appendix 1.3 – 1.6) the POPAN Jolly-Seber model of estimating population sizes assumes that marked and unmarked frogs have the same capture probability. Population on the i^{th} sample ($\check{N}_i$) can be calculated using the number of marked animals at the time of i^{th} sample ($\check{M}_i$), the total number of animals caught in i^{th} sample (n_i), the number of animals that are released after i^{th} sample (R_i) and the number of animals in i^{th} sample that carry marks from previous captures (m_i).

The formula is: $\check{N}_i = M_{ij} \frac{n_i + 1}{m_i + 1}$

iv. **Input file:** For data analysis in MARK, an input file (without headings), separated with commas and each line ending with semicolons was created using table above and the file was saved from notepad with extension (.inp).

v. **PIMS:** The global model {Phi(g*t) p(g*t) pent(g*t) N(g*t)} was used. Parameter specific link functions used for p and phi was “Logit”, “MLogit” for pent and “Log” for N.

1.3: The model selected for population size estimation

Model	AICc	ΔAICc	Weight	Likelihood	Par	Deviance	-2log(L)
Phi(g) p(g*t)	1791.99	0	0.98965	1	37	185.713	1715.1
Phi(g*t) p(g)	1802.15	10.1571	0.00616	0.0062	39	191.5411	1720.8
Phi(t) p(g)	1803.22	11.2299	0.00361	0.0036	13	247.513	1776.8
Phi(g) p(g)	1807.25	15.2528	0.00048	0.0005	16	245.3504	1774.6
Phi(g*t) p(g*t)	1812.02	20.0226	0.00004	0	50	177.2688	1706.6
Phi(g) p(t)	1812.6	20.6062	0.00003	0	13	256.8893	1786.2
Phi(g*t) p(.)	1813.52	21.5298	0.00002	0	32	217.9866	1747.3
Phi(g*t) p(t)	1819.42	27.4264	0	0	35	217.4503	1746.7
Phi(.) p(g*t)	1823.52	31.5279	0	0	36	219.3987	1748.7
Phi(t) p(g*t)	1823.69	31.7012	0	0	38	215.252	1744.6
Phi(g) p(.)	1824.20	32.2079	0	0	10	274.6385	1803.9
Phi(t) p(.)	1835.83	43.8333	0	0	5	296.427	1825.8
Phi(.) p(t)	1838.44	46.448	0	0	5	299.0418	1828.4
Phi(t) p(t)	1839.49	47.4998	0	0	7	296.0406	1825.4
Phi(.) p(.)	1848.75	56.7559	0	0	2	315.3982	1844.7

Notes:

i. Akaike's Information Criteria (AICc) values of the candidate models for the estimation of apparent survival, capture probalility, probability of entry and abundance of the groups Phi – probability of apparent survival; p – capture probability; (g) – group dependent; (t) – time dependent; (.) – constant probability; AICc - Akaike's Information Criterion; ΔAICc – delta AICc; Par – number of parameters; -2log(L) – Negative $\log_2$ likelihood.

1.4: Summary of detection probabilities

Species	Station	Estimator	Estimate	SE	LCI	UCL
H. cystocandicans		**Average p (π)**	**0.4233**	**0.0588**	**0.3304**	**0.5512**
	Kangaita	p (π)	0.4110	0.0766	0.2993	0.5803
	Chehe	p (π)	0.5049	0.0607	0.4004	0.6285
	Ragati	p (π)	0.3540	0.0391	0.2916	0.4449
		Average pent (β)	**0.0219**	**0.0162**	**0.0054**	**0.1606**
	Kangaita	pent (β)	0.0455	0.0373	0.0095	0.4241
	Chehe	pent (β)	0.0124	0.0069	0.0041	0.0352
	Ragati	pent (β)	0.0080	0.0044	0.0025	0.0225
		Average phi (φ)	**0.7941**	**0.0632**	**0.3835**	**0.8717**
	Kangaita	phi (φ)	0.7780	0.0923	0.5757	0.8790
	Chehe	phi (φ)	0.8431	0.0566	0.1518	0.9075
	Ragati	phi (φ)	0.7610	0.0406	0.4231	0.8286
H. glandicolor		**Average p (π)**	**0.5144**	**0.0536**	**0.4324**	**0.6464**
	Kangaita	p (π)	0.3689	0.0599	0.2792	0.5117
	Chehe	p (π)	0.6331	0.0299	0.6051	0.7481
	Ragati	p (π)	0.5413	0.0711	0.4127	0.6795
		Average pent (β)	**0.0341**	**0.0155**	**0.0140**	**0.1652**
	Kangaita	pent (β)	0.0168	0.0092	0.0056	0.0462
	Chehe	pent (β)	0.0154	0.0114	0.0035	0.0620
	Ragati	pent (β)	0.0702	0.0260	0.0330	0.3874
		Average phi (φ)	**0.6493**	**0.1062**	**0.3017**	**0.7879**
	Kangaita	phi (φ)	0.7993	0.1086	0.3786	0.8759
	Chehe	phi (φ)	0.3483	0.1506	0.1176	0.6043
	Ragati	phi (φ)	0.8002	0.0595	0.4090	0.8836
H. montanus		**Average p (π)**	**0.5439**	**0.0639**	**0.3113**	**0.6806**
	Kangaita	p (π)	0.4892	0.0689	0.1668	0.6266
	Chehe	p (π)	0.7054	0.0659	0.4250	0.8609
	Ragati	p (π)	0.4371	0.0569	0.3421	0.5541
		Average pent (β)	**0.0272**	**0.0145**	**0.0095**	**0.1583**
	Kangaita	pent (β)	0.0430	0.0211	0.0161	0.3584
	Chehe	pent (β)	0.0222	0.0144	0.0061	0.0760
	Ragati	pent (β)	0.0166	0.0079	0.0063	0.0406
		Average phi (φ)	**0.7055**	**0.1065**	**0.2947**	**0.8591**
	Kangaita	phi (φ)	0.7593	0.0821	0.4716	0.8480
	Chehe	phi (φ)	0.5389	0.1862	0.2115	0.8338
	Ragati	phi (φ)	0.8183	0.0513	0.2011	0.8955

Notes:

i. The probability that a frog was captured in any occasion is (p, π) while the probability that it was not is (1-p); also, survival (Φ and 1-Φ) and entry probability (β, pent and 1-β).

ii. New entrants into the population at capture occasion i is calculated as ($\beta_i = Nb_i$).

iii. The superpopulation (Appendix 1.5) is estimated using the detection probability ($\check{N}i = \frac{ni}{pi} \pm 1.96SE$) {1} where$SE(\check{N}i) = \frac{ni(se[pi])}{p_i^2}$.

iv. Population estimates for successive occasions are calculated iteratively ($N_i = N_{i-1}\varphi_i\ 1\ \beta_i$).

1.5: Population estimates on every sampling occasion

Chehe Forest Station: Kahuhi stream near Kangiri (Forest edge)

Species	Survey	Captures (n_i)	Recaptured (m_i)	Marked (μ_i)	Released (R_i)	Population ($\check{N}_i$)
hcyst	First	41	0	41	41	90.03±5.91
hcyst	Second	31	21	10	51	90.03±5.91
hcyst	Third	33	19	14	65	90.03±5.91
hcyst	Fourth	30	21	9	74	73.1±23.32
hcyst	Fifth	22	13	9	83	20.93±4.01
hmont	First	9	0	9	9	48.76±29.09
hmont	Second	5	3	2	11	15.73±7.84
hmont	Third	6	2	4	15	6.29±2.42
hmont	Fourth	7	2	5	20	5.49±2.09
hmont	Fifth	3	1	2	22	2.14±1.26
hglan	First	11	0	11	11	203.9±154.85
hglan	Second	4	2	2	13	36.29±30.14
hglan	Third	9	1	8	21	9.07±2.96
hglan	Fourth	11	2	9	30	10.05±3.02
hglan	Fifth	6	0	6	36	5.36±2.2

Ragati Forest Station: Karia Swamp near Gatei Shopping Centre

hcyst	First	59	0	59	59	273.83±39.36
hcyst	Second	35	6	29	88	273.83±39.36
hcyst	Third	33	12	21	109	155.47±40.86
hcyst	Fourth	34	17	17	126	155.47±40.86
hcyst	Fifth	23	7	16	142	22.18±4.47
hmont	First	31	0	31	31	116.78±11.44
hmont	Second	24	13	11	42	116.78±11.44
hmont	Third	34	13	21	63	116.78±11.44
hmont	Fourth	40	24	16	79	71.42±23.41
hmont	Fifth	26	11	15	94	25.53±4.46
hglan	First	36	0	36	36	106.17±14.77
hglan	Second	32	11	21	57	80.68±12.69
hglan	Third	31	23	8	65	80.68±12.81
hglan	Fourth	39	16	23	88	75.79±16.58
hglan	Fifth	43	18	25	113	38.51±5.26

Kangaita Forest Station: Karia ga Gacomo Swamp

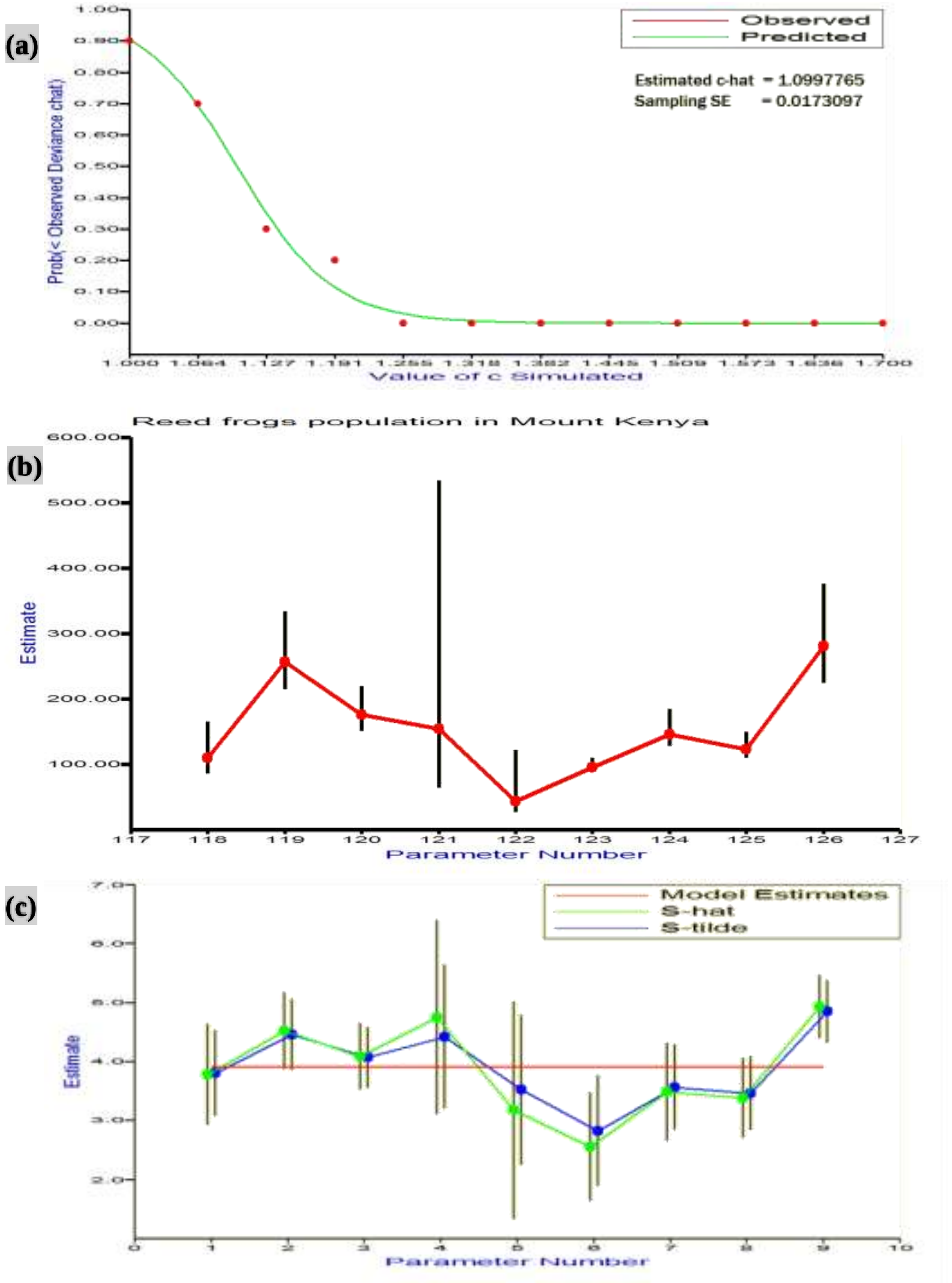
hcyst	First	36	0	36	36	145.46±30.14
hcyst	Second	35	15	20	56	145.46±30.14
hcyst	Third	33	15	18	74	145.46±30.14
hcyst	Fourth	36	9	27	101	112.42±64.5
hcyst	Fifth	23	7	16	117	21.72±4.31
hmont	First	50	0	50	50	190.18±32.34
hmont	Second	35	13	22	72	158.75±39.34
hmont	Third	43	19	24	96	118.25±36.64
hmont	Fourth	48	13	35	131	70.64±12.49
hmont	Fifth	43	7	36	167	41.1±5.62
hglan	First	16	0	16	16	107.32±27.05
hglan	Second	15	5	10	26	103.01±31.51
hglan	Third	18	5	13	39	76.49±22.63
hglan	Fourth	23	9	14	53	76.49±22.63
hglan	Fifth	19	5	14	67	17.42±3.79

Notes:

i. **Species:** hcyst – *Hyperolius cystocandicans;* hmont – *H. montanus;* hglan – *H. glandicolor*

ii. ($\check{N}_i$) – Population on the i^{th} sample given as N±SE; ($\acute{M}_i$) - number of marked frogs at the time of i^{th} sample; (n_i) - total number of frogs caught in i^{th} sample; (m_i) - number of frogs in i^{th} sample that carry marks from previous captures, and; (R_i) - cumulative number of frogs released after i^{th} sample and therefore contains marks after that occasion.

1.6: Model estimates in program MARK and goodness of fit



Notes:

i. Figure (a), the estimate of c-hat (CJS) showing predicted values (green line) and observed values (red dot). There was a slight over-dispersion (C-hat = 1.099±0.017se)

ii. Figure (b), population size (POPAN) for all occasions based on {Phi(g*t) p(g*t) pent(g*t) N(g*t)}. Parameter specific link function: p and phi is “Logit”; pent for “MLogit” and “Log” for N. (PS. Note the high estimate of standard error on the fourth occasion).

iii. Figure (c), estimates of population size parameters based on bootstrap simulation of the data at c-hat (1.5) for the global model with same parameter specific link functions.

iv. Goodness of fit results for Test 2 and Test 3 across the groups. There was no sufficient data to generate Chi-square values for the starred (**) group.

Site	Species	df	Chi-square	p-value
Kangaita	H. glandicolor	7	6.3603	0.4984
	H. montanus	7	10.5472	0.1596
	H. cystocandicans	7	6.1064	0.5274
Chehe	H. glandicolor **	2	0.0000	1.0000
	H. montanus**	3	0.0000	1.0000
	H. cystocandicans	7	18.6285	0.0094
Ragati	H. glandicolor	7	7.6231	0.3670
	H. montanus	7	2.6259	0.9173
	H. cystocandicans	7	5.6530	0.5808
Overall		**54**	**57.5446**	**0.3454**

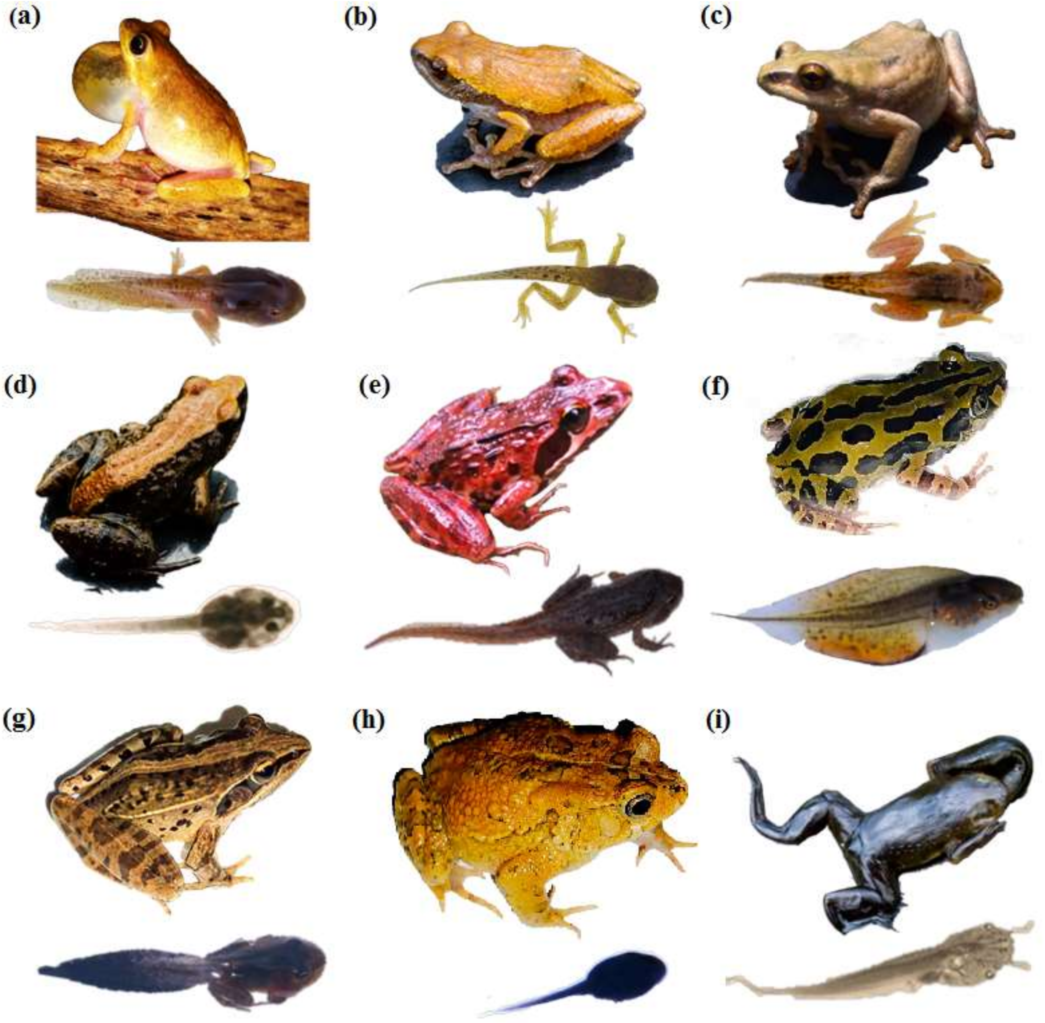
v. Goodness of fit results Test 1 across groups.

	Summary of TEST 1 (Between Groups Test) Results			
Component	**Chi-square**	**df**	**P-level**	**Sufficient Data**
1.R4	31.9856	7	0	Yes
1.T4	0.6195	6	0.9961	Yes
1.R3	5.6366	7	0.5828	Yes
1.T3	4.5949	6	0.5967	Yes
1.R2	22.1941	7	0.0024	Yes
1.T2	15.0935	7	0.0348	Yes
1.R1	29.5749	8	0.0003	Yes
TEST 1	**109.6991**	**48**	**-**	

Appendix 2: Additional findings in support of objective 1 and 2

2.1: Species list, specimen and analysed calls of encountered species

2.1.1: Species identity, dissimilarity and co-occurrence



Notes:

i. Figure in 2.1.1 (a – h) shows adults of frogs and nearly metamorphosed tadpoles (late stages) at which point they were easily distinguished in absence of detailed descriptions.

ii. Table (a) shows species relative detectability and patterns of breeding activity

Table (a)	**Relative detectability**			**Breeding activity**	
Species names	**Eggs**	**Tadpoles**	**Adult**	**Season**	**Diel**
a. Hyperolius glandicolor;	Poor	Poor	Good	Prolonged	Nocturnal
b. Hyperolius cystocandicans;	Good	Good	Good	Explosive	Nocturnal
c. Hyperolius montanus;	Good	Poor	Fair	Explosive	Nocturnal
d. Phrynobatrachus kinangopensis;	Fair	Poor	Good	Explosive	Diurnal
e. Amietia nutti;	Poor	Fair	Good	Prolonged	Nocturnal
f. Kassina senegalensis	Fair	Good	Poor	Explosive	Nocturnal
g. Ptychadena mahnerti;	Fair	Poor	Good	Explosive	Diurnal
h. Sclerophrys kerinyagae	Fair	Fair	Good	Prolonged	Diurnal
i. *Xenopus borealis*	Poor	Good	Fair	Prolonged	-

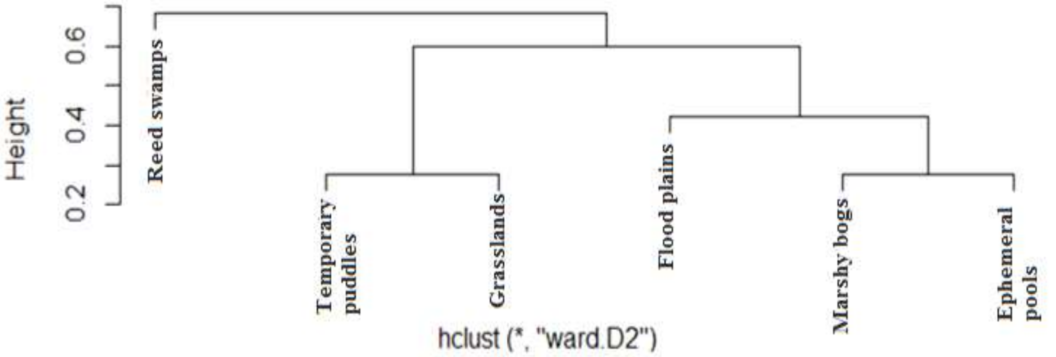
iii. Table (b) shows eigenvalues and ecological distances on DCA axes

Table (b)	**DCA1**	**DCA2**	**DCA3**	**DCA4**
Eigenvalues	0.259	0.197	0.189	0.105
Decorana values	0.269	0.131	0.016	0.004
Axis lengths	2.049	1.780	1.691	1.302

iv. Table (c) shows ecological distances of species composition (Bray Curtis)

Table (c)	***A. nutti***	***H. cystocandicans***	***H. glandicolor***	***H. montanus***	***K. senegalensis***	***P. kinangopensis***	***P. mahnerti***	***S. kerinyagae***
H. cystocandicans	0.484							
H. glandicolor	0.407	0.095						
H. montanus	0.630	0.238	0.368					
K. senegalensis	1.000	0.529	0.467	0.533				
P. kinangopensis	0.333	0.179	0.086	0.486	0.407			
P. mahnerti	0.500	0.486	0.419	0.484	0.391	0.357		
S. kerinyagae	0.412	0.436	0.357	0.357	0.600	0.280	0.524	
X. borealis	0.200	0.314	0.226	0.677	0.652	0.143	0.250	0.524

v. Cluster dendrogram for anuran species in different habitat types in the breeding sites based on Bray Curtis Dissimilarity index.



vi. Table (d) shows species turnover on DCA axes based on abundance (Bray Curtis) while Table (e) shows co-occurrence based on presence absence (same data) in ‘cooccur’.

Table (d)	**Dissimilarity (Bray Curtis)**				**Table (e)**	**Co-occurrence (cooccur)**	
	DCA1	**DCA2**	**DCA3**	**DCA4**	**Positive**	**Negative**	**Random**
A. nutti	-1.212	0.620	0.468	0.153	4 (50.0%)	2 (25%)	2 (25%)
H. cystocandicans	0.100	0.376	0.358	0.312	4(50.0%)	0(0%)	4(50%)
H. glandicolor	-0.235	0.045	-0.008	0.011	4 (50.0%)	0 (0%)	4 (50%)
H. montanus	0.837	0.521	0.638	0.138	3(37.5%)	2(25%)	3(37.5%)
K. senegalensis	0.809	-1.160	-1.053	-0.395	1(12.5%)	2(25%)	5(62.5%)
P. kinangopensis	-0.449	-0.218	-0.279	-0.231	4(50.0%)	0(0%)	4(50%)
P. mahnerti	0.327	-0.669	-0.590	0.153	2(25.0%)	0(0%)	6(75%)
S. kerinyagae	0.002	0.230	0.308	-0.984	0 (0%)	0 (0%)	8 (100%)
X. borealis	-0.807	-0.180	-0.300	0.318	4(50.0%)	2(25%)	2(25%)

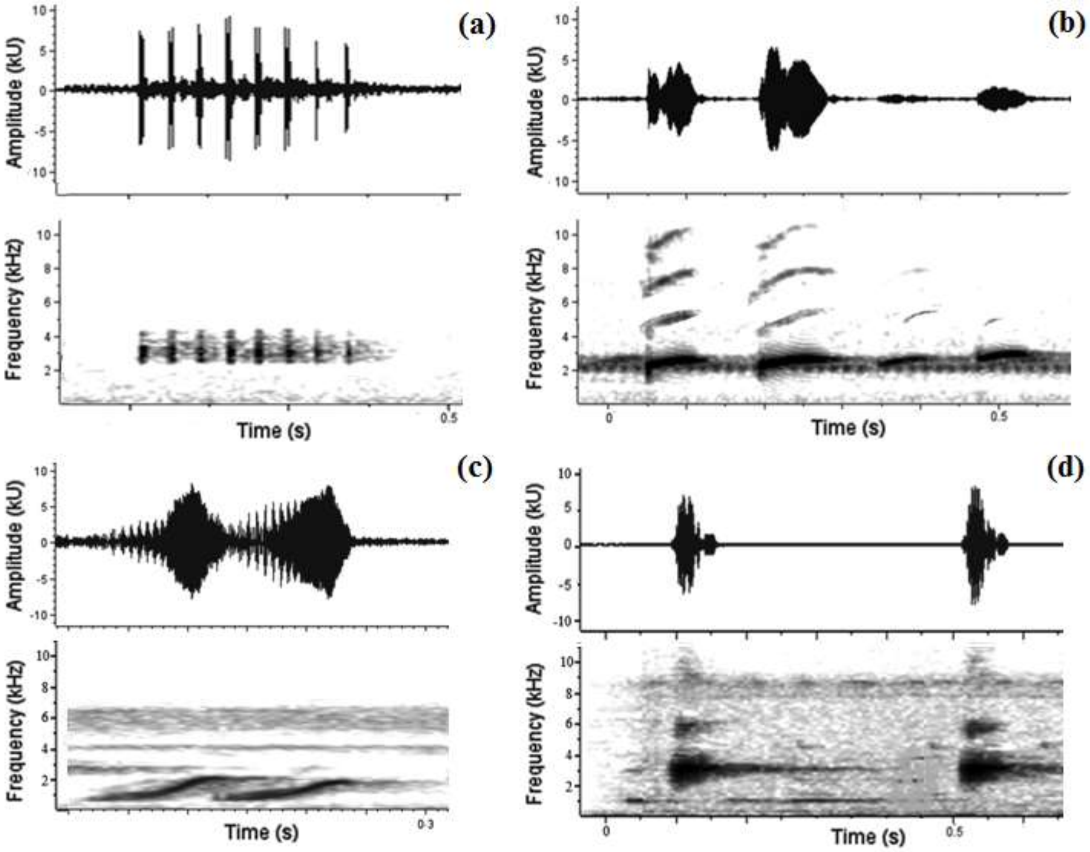
vii. **Specimen included in this study:** Kinangop Puddle Frog (*Phrynobatrachus kinangopensis* Angel 1924): (NMK623A/01, NMK623A/02, NMK612A/01 – NMK612A/12, NMK618A, and NMK637A/01 – NMK637A/20, NMK639A/01 – NMK639A/10). River Frogs (*Amietia nutti* Boulenger, 1896): (NMK640A/01 – NMK640/18). Mahnert's Grass Frog (*Ptychadena mahnerti* Perret, 1996): (NMK617A/01, NMK622A; NMK629A/01 – NMK629A/03; NMK625A). Kerinyaga Toad, *Sclerophrys kerinyagae* (Keith, 1968): (NMK611A/01 – NMK611/15; NMK626A/01 – NMK626A/12). Marsabit platanna (*Xenopus borealis,* Angel 1924): (NMK638A/01). Running Frog (*Kassina senegalensis* Bibron and Dumeril 1841): (NMK609A/01). Peter's Reed Frog (*Hyperolius glandicolor* Peters 1878): (NMK607A/01, NMK605A/01 – NMK605A/13, NMK628A/01 – NMK628A/28). Montane Reed Frog (*Hyperolius montanus* Angel 1924): (NMK614A/01 – NMK614A/03, NMK616A/01 – NMK616A/25; NMK619A/01 – NMK619A/28, NMK624A/01 – NMK624A/09; NMK632A/01). Silver bladder reed frog (*Hyperolius cystocandicans* Richards and Schiøtz, 1977): (NMK620A/01, NMK620A/02, NMK604A/01 – NMK604A/37, NMK613A, NMK606A/01 – NMK606A/16, NMK621A/01 – NMK621A/13; NMK632A/01 – NMK632A/35, NMK633A/01 – NMK633A/39; NMK635A/01 – NMK635A/37, NMK636A/01 – NMK636A/10).

viii. **Specimen not included in this study:** *Ptychadena nilotica* Seetzen, 1855 (NMK608A/01 – NMK608A/06, NMK634A01, NMK634A02); *Phrynobatrachus keniensis* Barbour and Loveridge, 1928 (NMK610A/01, NMK610A/02); *Phrynobatrachus scheffleri* Niedeni, 1911 (NMK615A/01 – NMK615A/06); *Sclerophrys gutturalis* Power, 1927 (NMK627A/01 – NMK627A/06); *Hemisus marmoratus* Peters, 1854 (NMK624A/01 – NMK624A/06) and *Hyperolius howelli* Du Preez and Channing, 2013 (NMK630A/01, NMK630A/02); *Mochlus sundevalli* Smith, 1849 (NMK595L); *Trachyleppis striata* Peters, 1844 (NMK596L/01, NMK596L/02); *Hemidactylus mabouia* Moreau de Jones, 1818 (NMK597L/01); *Trioceros hoehnelli* Steindachner, 1819 (NMK599L/01); *Kinyongia excubitor* Barbour, 1911 (NMK600L/01 – NMK600L/04); *Trioceros jacksoni* Boulenger, 1986 (NMK601L/01 – NMK601L/03); *Afrotyphlops lineolatus* Jan, 1864 (NMK602S/01); *Lycophidion ornatum* Parker, 1936 (NMK603S/01 – NMK603S/03).

ix. **Research and collection permit:** These specimens (notes vii and vii) were collected with authorization from Kenya Wildlife Service (Permit Ref No: KWS/BRP/5001) and are housed in herpetological collection of the National Museums of Kenya. Some specimens are yet to be catalogued but are available for public access.

x. **Images in public domain**: Some images were uploaded and made accessible via iNaturalist (www.inaturalist.org) and are therefore available in public domains. 128657603, *H. cystocandicans* (18.x.2020); 146323176, *H. cystocandicans* (24.v.2020); 149455636, *H. montanus* (15.iv.2020); 149455636, *H. montanus* (26.x.2019); 191836679, *P. kinangopensis* (09.iv.2020); 191836764, *A. nutti* (18.xi.2020); 191836810, *H. cystocandicans* (04.ix.2020); 191836857, *H. glandicolor* (04.xi.2020); 191836932, *A. nutti* (04.xi.2020); 191836959, *P. mahnerti* (20.xi.2020); 191837008, *P. kinangopensis* (18.xi.2020); 191837068, *H. cystocandicans* (04.xii.2020); 191837108, *H. cystocandicans* (08.xii.2020); 191837736, *H. montanus* (10.xii.2020); 191837606, *S. kerinyagae* (29.v.2020); 191837650, *S. kerinyagae* (06.xii.2020).

2.1.3: Advertisement calls and eggs of Hyperoliids

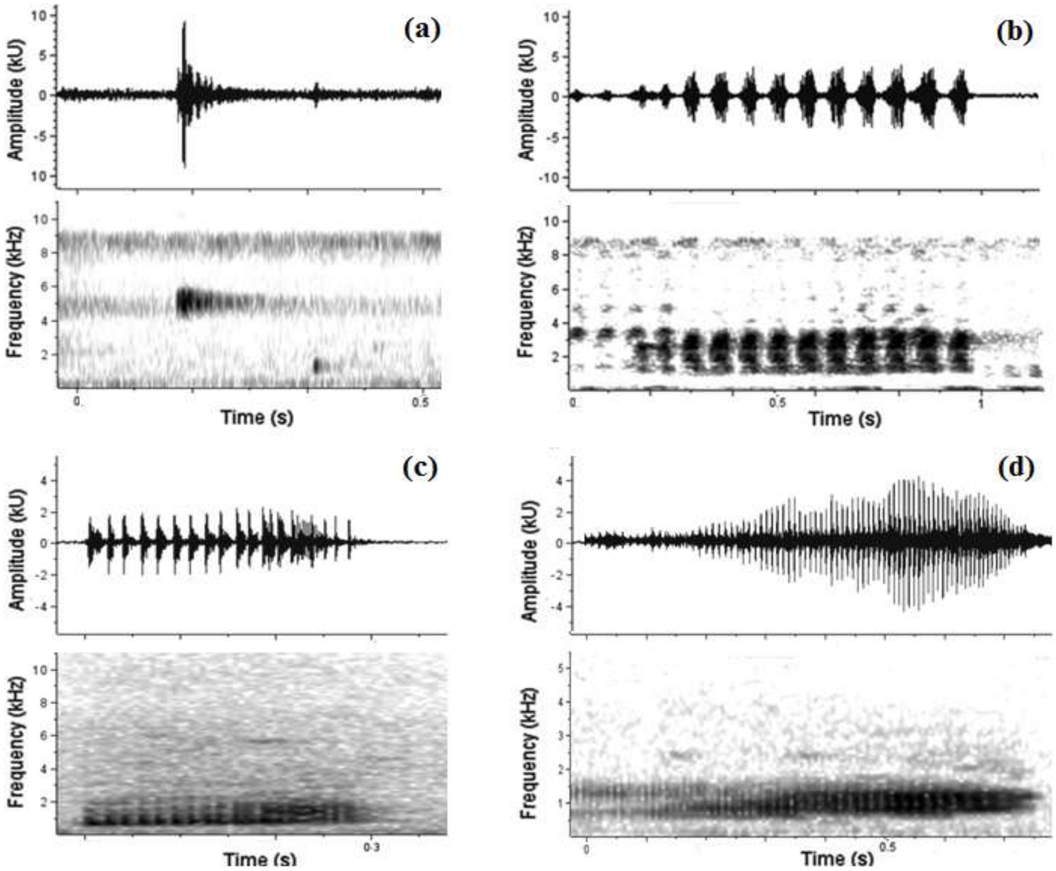


Notes

i. The figure shows advertisement calls of frogs in the family *Hyperoliidae*: (a) *Hyperolius montanus*, (release call shown here) a short scream; (b) *Hyperolius glandicolor*, a loud unpulsed whistle often repeated in quick succession; (c) *Kassina senegalensis*, a loud quoip sound with distinct pulses in the first half of the call, and; (d) *Hyperolius cystocandicans*, a short indistinctly pulsed note.

ii. The eggs of Hyperoliids are nearly similar: (a) *H. glandicolor* has large clutch, smaller eggs usually laid in water/lilies in water or on water surface; (b) *H. cystocandicans* has a gray vegetal pole, whitish animal pole and laid on reeds/shrubs; (c) *H. montanus* has a dark vegetal pole, yellowish animal pole and usually laid on grass/bare ground, and; (d) *Kassina senegalensis*, small eggs with a pale vegetal pole, neatly deposited on grass/vegetation at the edge of a pond or inside water.

2.1.4: Advertisement calls and eggs of other frogs



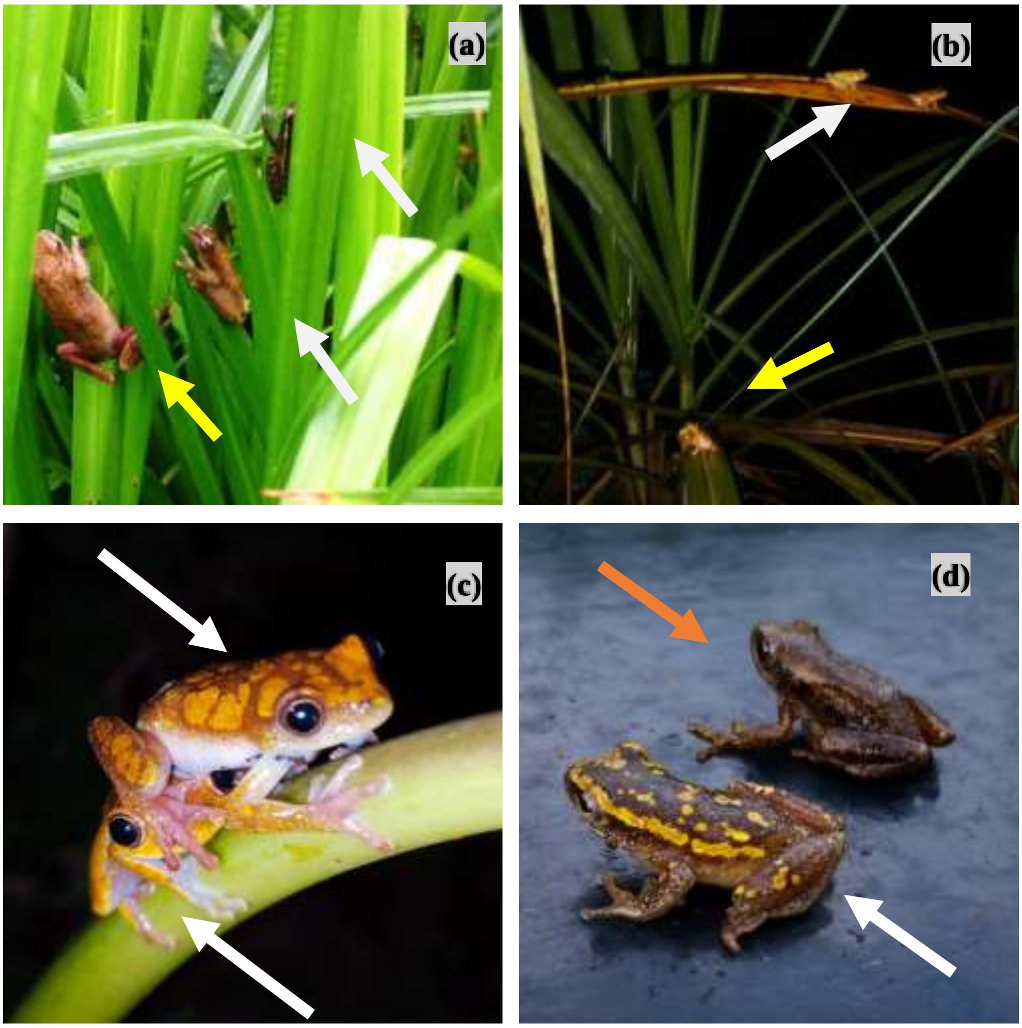
Notes

i. The figure shows the advertisement calls of frog species recorded from the study area: (a) *Phrynobatrachus kinangopensis*, a long buzz (narrowly spaced pulses) at 5kHz interspaced with sharp clicks; (b) *Ptychadena mahnerti*, a long trill made of several pulsed notes; (c) *Amietia nutti*, repetitive call made of single pulsed notes (13pulses per second) followed by a short escalating croak (shown here), and; (d) *Sclerophrys kerinyagae*, a long snore composed of many compacted pulses.

ii. The eggs of frogs: (a) *Sclerophrys kerinyagae*, a long string suspended in aquatic vegetation; (b) *Phrynobatrachus kinangopensis,* small brown eggs suspended in aquatic vegetation in puddles, and; (c) *Ptychadena mahnerti*, small dark eggs floating on water surface in a single thin layer, and; (d) *Amietia nutti*, large eggs covered by a thick membrane jelly and laid singly in muddy pools.

2.2: Distribution of adults in breeding sites

2.2.1: Interactions within species and among sympatric reed frogs



Notes:

i. Reed frogs sharing habitats: In figure (a), *H. glandicolor* (yellow arrow); *H. cystocandicans* (white arrow) were perching in a forest edge habitat during the day.

ii. In Figure (b), the same species in (a) are shown in the same habitat at night. Note that *H. glandicolor* male calls very close to the water surface while two males of *H. cystocandicans* call above the same site. Notably also, these males had a narrow spacing.

iii. In Figure (c) duelling males of *H. cystocandicans* are shown. These males were competing over breeding space with fights that involved physical touch. Both males were fighting actively. Males of *H. glandicolor* are well spaced and compete aggressively with other males (see Dyson and Passmore, 1992).

iv. In figure (d) *H. montanus* male (orange arrow) was found together on the same perch with *H. cystocandicans* (white arrow) both calling during the day in montane grassland.

2.2.1: Natural and unnatural means of population regulation

Notes

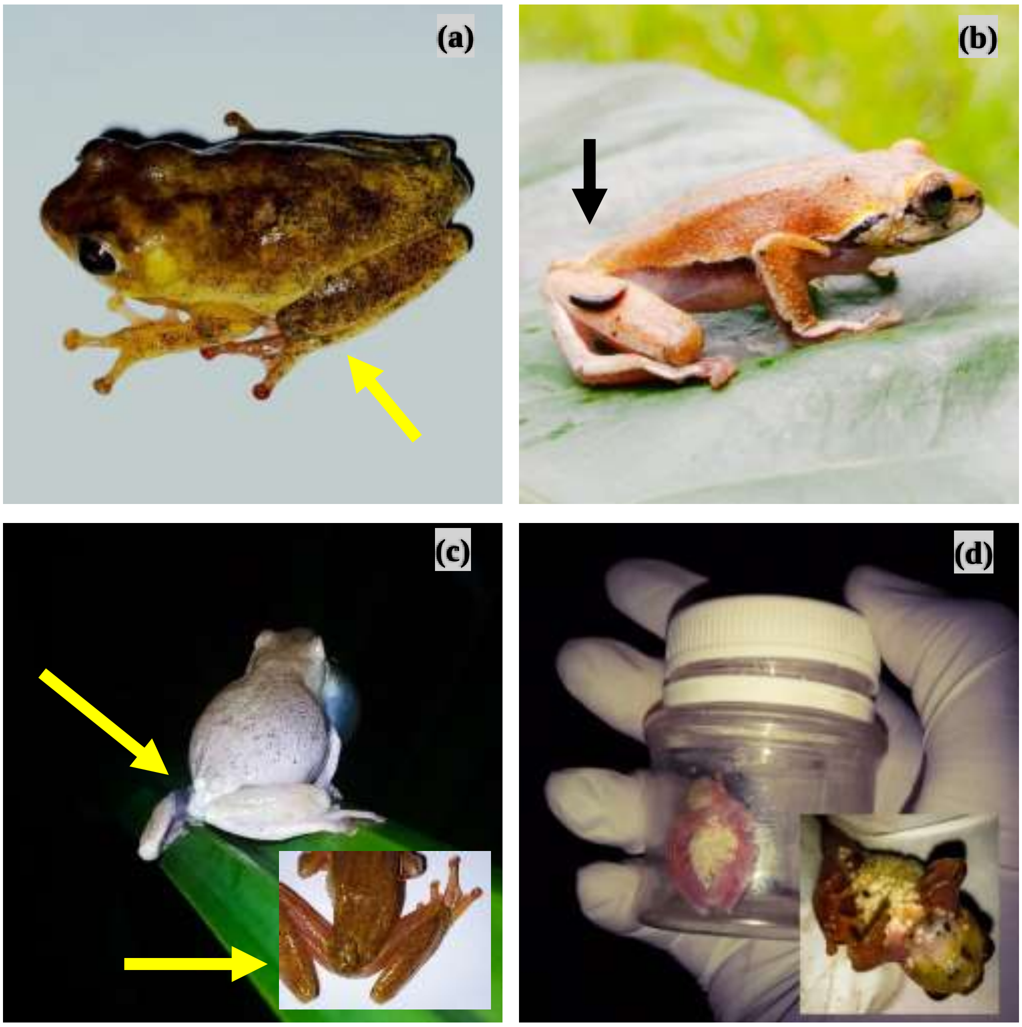
i. In Figure (a), female *H. cystocandicans* was dispersing to a breeding site across the road near Karatina University (following acoustic cues across the valley, ca 100 m from the forest) where it was ran over by a motorist. Such roadkills and many dead individuals (due to unnatural means) were encountered within and out of the protected area.

ii. In Figure (b), a calling male of *H. glandicolor* (white arrow) in a breeding site was traced acoustically by a white-lipped snake *Crotapopheltis hotamboeia* (red arrow). The frog was calling at a height of ca 50 cm at the edge of an ephemeral pool.

iii. In Figure (c), a habitat patch hosting an aggregation of breeding *H. montanus* (shown here) and multiple egg masses (including *Kassina senegalensis*) was raided (and probably exterminated) by a swarm of raiding driver ants, *Dorylus molestus* Wheeler, 1922. These species remain within the breeding sites and are often heard calling in grasslands or on bare ground during the day at the onset of the rainy season.

iv. In Figure (d), the eggs and tadpoles of several species dessicated after a temporary puddle dried out. Terrestrial eggs as well as aquatic eggs deposited in ephemeral pools of waters or tadpoles that hatch in the late season often desiccate when water dries. Water abstraction as well as draining of wetlands increases incidences of desiccation.

2.2.2: Parasites and infections associated with reed frogs



Notes

i. Figure (a), shows a male *H. glandicolor* with severe fungal infection. It was not confirmed whether this was *Batrachochytrium dendrobatidis*. This specimen showed signs of general weakness and change in call amplitude.

ii. Figure (b) arrow points at a jawed leech (*Huridinea*) attached to the thigh of a male *H. cystocandicans* (black arrow). These leeches, associated with aquatic species [see *X. laevis* in Appendix 2.1.1 (i)] are known parasites predating on frog species and leading to eventual death (similar results, see Hughes *et al*. 2018).

iii. In Figure (c), this calling male *H. glandicolor* hosts cysts of *Clinostomum chabaudi* Vercammen-Grandjean, 1960 (*Digenea*) Metacercariae near the urostyle (yellow arrow). Cysts were also found in the mouth (similar results, see Sinch *et al*. 2021).

iv. In Figure (d), the researcher displays a frog (*Hyperolius cystocandicans*) with a zoonotic disease that was not yet identified at the time of writing this thesis. This specimen is preserved at NMK. The frog had 'bubbles' on the ventral surface including on gloins and gular region (thereby preventing vocalisation). This infection was fatal and the specimen succumbed a few hours after collection.

2.2.3: Identification and marking of reed frogs

Notes

i. Figures (a - c) left to right: ventral surfaces of *Hyperolius glandicolor* female, *H. cystocandicans* male and *H. montanus* male.
   a. *Hyperolius glandicolor* has a minute hour glass pattern in males and juveniles, inner part of thighs is pink, ventrum is smooth but opaque, dorsum color variable but always continuous, no canthal or lateral stripe present.
   b. *Hyperolius cystocandicans* has white to pink inner thighs, ventrum is smooth and translucent, silver bladder visible in males and some females; dorsum color highly variable but continuous or mottled, often terminating sharply at the canthal stripe; males have a gular pouch.
   c. *Hyperolius montanus* has orange inner thighs, ventrum is rough and opaque, dorsum is brown to tan, lateral and canthal stripe often present.

ii. In figure (e - f) left to right; pairs of *Hyperolius glandicolor*, *H. cystocandicans* and *H. montanus* are shown in amplexus. Pairing often took place at the males calling site but the pair in amplexus then take position at a suitable spawning location or oviposition site. Initial identification of egg masses (Appendix 2.1.3 and 2.1.4) was based on eggs by pairs in amplexus.

iii. Based on the aforementioned, identification of individual frogs was based on individual peculiarities, natural markings (these reed frogs have diverse intraspecific colour variations) and on administered marks. For instance, the female in Appendix 2.2.3 (e) has a dark pigmentation and a yellow spot behind the eye, another mark just below the arm insertion; while the male has a canthal stripe edged with reddish and dark line. Reed frogs with marks administered on the dorsal surface were catalogued in a field data collection form of uniquely identified frogs (Appendix 3). History data (number of times or capture occasions when the uniquely identified frog was encountered) was registered in the catalogue.

Appendix 3: Additional documents

3.1. Field data collection form (capture mark recapture)

Created by: A. M. Karani												DATE						HABITAT								SPECIES					
History data for mark capture recapture																															
No	**S**	**Mark**	**1**	**2**	**3**	**4**	**5**	**No**	**S**	**Mark**	**1**	**2**	**3**	**4**	**5**	**No**	**S**	**Mark**	**1**	**2**	**3**	**4**	**5**	**No**	**S**	**Mark**	**1**	**2**	**3**	**4**	**5**
1		aaaa						53		adbb						105		bccc						157		cbdc					
2		aaab						54		adbc						106		bccd						158		cbdd					
3		aaac						55		adbd						107		bcda						159		ccaa					
4		aaad						56		adca						108		bcdb						160		ccab					
5		aaba						57		adcb						109		bcdc						161		ccac					
6		aabb						58		adcc						110		bcdd						162		ccad					
7		aabc						59		adcd						111		bdaa						163		ccba					
8		aabd						60		adda						112		bdab						164		ccbb					
9		aaca						61		addb						113		bdac						165		ccbc					
10		aacc						62		addc						114		bdad						166		ccbd					
11		aacd						63		addd						115		bdba						167		ccca					
12		aada						64		baaa						116		bdbb						168		cccb					
13		aadb						65		baab						117		bdbc						169		cccc					
14		aadc						66		baac						118		bdbd						170		cccd					
15		aadd						67		baad						119		bdca						171		ccda					
16		abaa						68		baba						120		bdcb						172		ccdb					
17		abab						69		babb						121		bdcc						173		ccdc					
18		abac						70		babd						122		bdcd						174		cdaa					
19		abad						71		baca						123		bdda						175		cdab					
20		abba						72		bacb						124		bddb						176		cdac					
21		abbb						73		bacc						125		bddc						177		cdad					
22		abbc						74		bacd						126		bddd						178		cdba					
23		abbd						75		bada						127		caaa						179		cdbb					
24		abca						76		badb						128		caab						180		cdbc					
25		abcb						77		badc						129		caac						181		cdbd					
26		abcc						78		badd						130		caad						182		cdca					
27		abcd						79		bbaa						131		caba						183		cdcb					
28		abda						80		bbab						132		cabb						184		cdcc					
29		abdb						81		bbac						133		cabc						185		cdcd					
30		abdc						82		bbad						134		cabd						186		cdda					
31		abdd						83		bbba						135		caca						187		cddb					
32		acaa						84		bbbb						136		cacb						188		cddc					
33		acab						85		bbbc						137		cacc						189		cddd					
34		acac						86		bbbd						138		cacd						190		daaa					
35		acad						87		bbca						139		cada						191		daab					
36		acba						88		bbcb						140		cadb						192		daac					
37		acbb						89		bbcc						141		cadc						193		daad					
38		acbc						90		bbcd						142		cadd						194		daba					
39		acbd						91		bbda						143		cbaa						195		dabb					
40		acca						92		bbdb						144		cbab						196		dabc					
41		accb						93		bbdc						145		cbac						197		dabd					
42		accc						94		bbdd						146		cbad						198		daca					
43		accd						95		bcaa						147		cbba						199		dacb					
44		acda						96		bcab						148		cbbb						200		dacc					
45		acdb						97		bcac						149		cbbc						Color ×		Red					
46		acdc						98		bcad						150		cbbd								Blue					
47		acdd						99		bcba						151		cbca								Black					
48		adaa						100		bcbb						152		cbcb								Green					
49		adab						101		bcbc						153		cbcc						Leg †	a	First *					
50		adac						102		bcbd						154		cbcd						Toe	b	Second					
51		adad						103		bcca						155		cbda						Back	c	Third					
52		adba						104		bccb						156		cbdb						Side	d	Fourth					

Notes |No: Uniquely identified animal | S - Sex | 1- 5: Capture ocassion | † Mark location | × Color | * Batch

3.2. Turnitin Originality Report

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