Small-scale movements and foraging areas of *Rousettus aegyptiacus* in Limpopo Province, South Africa

By

Matthew Roger Wood

Supervisor: Professor Wanda Markotter

Co-supervisor: Doctor Jonathan Epstein and Doctor Low de Vries

Submitted in partial fulfilment of the requirements for the degree

M.Sc. Medical Virology

in the Faculty of Health Sciences

University of Pretoria

January 2023

Declaration

I, Matthew Roger Wood, declare that the dissertation hereby submitted to the University of Pretoria for the M.Sc. degree in Medical Virology and the content therein is my own original work and has not previously been submitted for a degree at any tertiary institution.

SIGNATURE:

DATE: 31 January 2023

Acknowledgements

Research funding

This research was funded by the DSI-NRF South African Research Chair (SARHCHi) in Infectious Diseases of Animals (Prof. W. Markotter) together with a grant-holder linked bursary (Grant no. 98339). The opinions expressed and conclusions made are those of the author and are not necessarily to be attributed to the NRF. This research was also supported by the USA Department of Defence, Defence Threat Reduction Agency (HDTRA12010025). The content of the information does not necessarily reflect the position or the policy of the federal government and no official endorsement should be inferred.

Fieldwork

I hereby acknowledge the staff and students from the Biosurveillance and Ecology of Emerging Zoonoses (BEEZ) research group part of the Centre for Viral Zoonoses (CVZ) from the University of Pretoria for their assistance with fieldwork activities. Specifically, Dr L. de Vries, Tedson Nkoana and Myles Bushell for assisting directly with data collection.

Special thanks to the following:

Professor Wanda Markotter for the research opportunity, academic input and supervision.

Doctor Low de Vries for his support and patience during the research period as well as guidance and input for all writing aspects of the research project.

The staff members and students of the CVZ for their support during difficult times.

I would also like to thank my family and friends for helping me stay motivated towards the end and maintain a sense of humour despite setbacks.

Research outputs

Publication in preparation

Wood MR., de Vries JL, Monadjem A., Markotter W. *Submitted to Journal of Mammalogy*. Critical review and meta-analysis of correlates of home range size in bats.

Wood MR., de Vries JL., Epstein JH., Markotter W. *Manuscript in preparation*. Variations in small-scale movements of a viral host, *Rousettus aegyptiacus*, across a seasonal gradient.

Research presentations

Wood MR., Markotter W., de Vries JL. Small scale movements of *Rousettus aegyptiacus* in Limpopo Province, South Africa and implications for disease risk. 19th International bat research conference, Austin, Texas, USA

Executive summary

Movement is an integral component within the animal kingdom as species respond to internal and external stimuli to ensure their survival. As animals move, they interact with the environment in different ways that help to maintain ecological connectivity. These species interactions are often mutually beneficial such as pollination and seed dispersal. However, there are cases where the interactions between different species have negative implications. The emergence of medically important zoonotic and potentially zoonotic pathogens worldwide has increased over the past 50 years and emphasis has recently been placed on investigating the link between the movement patterns of a host species and potential disease spillover risk. Bats have been recognized as important hosts and especially viral hosts. However, the ecological context of bats as viral hosts is relatively poorly understood and consequently, an important component is often lacking from assessments of viral transmission risk. Bats exploit a wide array of different habitat types but the likelihood for contact between bats and people is increasing as humans encroach on their natural habitats. As such, studying the movements of bats may help understanding their potential role in spillover events by identifying the risk of contact with people or other potentially susceptible host species. This research study aimed to integrate virological and ecological data to provide a better understanding of transmission risk. Specifically, the ecological aspects of bat movement patterns were assessed alongside virological data to improve our understanding of the potential role bats may play in viral transmission events.

Bats exhibit wide forms and functions and consequently, their movement patterns may vary according to their specific requirements. There are, however, metrics such as home range size estimations that can be used as a benchmark to assess how the different ecological and biological characteristics of bats are linked to movement patterns. Understanding which traits influence bat movement patterns and how they are linked may enable predictions about a species' range requirements and provide insight into the potential scope of their species interactions. Similar previous assessments have been performed but with limited sample sizes and a restricted geographical range. Expanding the scope to include bat species around the globe will provide greater species representation with a wider diversity and encompass a broader array of ecological and biological traits. The findings can then be applied as a theoretical framework for species range requirements and movement patterns. This is important for data deficient and threatened species where not much is known about their range requirements. Moreover, it is especially relevant for assessing a species' interactions with the environment such as seed dispersal or, in the case of zoonotic hosts, potential pathogen spread and transmission. Through a global meta-analysis, specific characteristics of bat species were identified that have a clear relationship with bat home range size.

At a local scale, the movement patterns of a known zoonotic viral host, *Rousettus aegyptiacus* (Egyptian rousette bat), were assessed in the rural village of Fertilis, Limpopo Province, South Africa across a seasonal gradient to determine risk of contact with a human population. The *R. aegyptiacus* colony inhabits Matlapitsi cave which is within 500m of human dwellings and was specifically selected due to previously being shown to host several zoonotic viruses or viruses with zoonotic potential. The bats were tracked with radiotelemetry for between seven and nine nights every month over a 12-month period to determine their movements which were then compared to human presence. Their movements were further assessed relative to fruit availability that was estimated as the percentage of fruit cover on fruiting trees distributed around the study site. These comparisons were performed throughout the year to assess whether fruit availability influences movement patterns across a seasonal gradient. There were distinct seasonal differences in movement patterns with bats preferentially foraging within residential areas during the dry winter months when subsistence fruit trees provide constant sources of food. These findings are important as the peak in bat activity within residential areas coincides with previously identified periods of high risk of viral shedding. The findings, therefore, suggest that the risk of spillover during this period is high and highlight the importance of incorporating ecological data with virological data for more detailed risk assessments.

Key words: Chiroptera, contact risk, ecological interactions, Egyptian rousette bat, home range, movement ecology, radiotelemetry, spatial overlap, zoonoses, zoonotic spillover

Table of Contents

List of tables

List of figures

List of abbreviations

AEC	Animal Ethics Committee
AICc	Corrected Akaike Information Criterion
AICcWT	Corrected Akaike Information Criterion Weight
ARGOS	Advanced Research and Global Observation Satellite
a.s.l.	above sea level
BEEZ	Biosurveillance and Ecology of Emergin Zoonoses
BNC	Bayonet Neill-Concelman
CAPR	Controlled Air-Purifying Respirator
C.I.	Confidence Interval
CVZ	Centre for Viral Zoonoses
DBH	Diameter at Breast Height
DALRRD	Department of Agriculture, Land Reform and Rural Development
DEA	Department of Environmental Affairs
d.f.	degrees of freedom
DSI	Department of Science and Innovation
DUVV	Duvenhage virus
EGIS	Environmental Geographic Information System
F	Flying
GLMM	Generalised Linear Mixed Model
GPS	Global Positioning System
GVIF	Generalised Variance Inflation Factor
ha	hectare
HPIV-2	Human Parainfluenza virus 2
HR	Home range
JSTOR	Journal Storage
K	Parameters
KDE	Kernel Density Estimation
Km^2	Square kilometres
LMM	Linear Mixed Model
LoCoH	Local Convex Hull
LSCV	Least Squares Cross-Validation
MARV	Marburg virus
MCP	Minimum Convex Polygon
MDD	Mammal Diversity Database
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
M.Sc.	Masters of Science
MuV	Mumps virus
MVT	Marginal Value Theorem
NF	Non-flying
NRF	National Research Foundation
NY	New York
OHHLEP	One Health High-Level Expert Panel
PAPR	Powered Air-Purifying Respirator
PGLS	Phylogenetic Least Squares Regression
PIT	Passive Integrated Transponder
PPE	Personal Protective Equipment
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analysis
PTT	Platform Transmitter Terminal
QGIS	Quantum Geographic Information System
REC	Research Ethics Committee
RNA	Ribonucleic acid
SANLC	South African National Landcover
SARCHI	South Africa Research Chair Initiative
SARS-CoV-1	Severe Acute Respiratory Syndrome Coronavirus 1
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SE	Standard error
SOSV	Sosuga virus
UAV	Unmanned Aerial Vehicle
UHF	Ultra High Frequency
USA	United States of America
VHF	Very High Frequency
w.i.	selection ratio

Chapter 1: Literature review

Introduction

Ecosystems are complex ecological systems comprising multiple biotic and abiotic factors that are intricately interlinked (Perino et al. 2019). The actions of one individual or species, therefore, can influence several other individuals within the ecosystem. The movements of animals from one location to another are one such example of an activity that can have wide-ranging impacts (Nathan et al. 2008; Shaw 2020). These impacts can range from mutually beneficial activities such as seed dispersal or pollination to agonistic interactions such as predator-prey dynamics (Garcia et al. 2013; Allen and Singh 2016). However, movements can also carry negative implications, often as unintended side effects such as the introduction of parasites or pathogens to new areas (Allen and Singh 2016; Shaw 2020). Zoonotic pathogens are biological agents of animal origin that have the potential to infect humans and are responsible for more than 70% of emerging infectious diseases (Allen et al. 2017). Bats have been identified as important hosts for several zoonotic pathogens, especially viruses, with the potential for large-scale outbreaks and serious consequences for public health (Luis et al. 2013; Olival et al. 2017; Mollentze and Streicker 2020). Viral biosurveillance of bat species has received increasing focus over the last 30 years since the initial discovery of Hendra virus in 1994 (Murray et al. 1995; Chua et al. 2000; Peiris et al. 2003; Zaki et al. 2012). The advancement of nucleic acid sequencing techniques paired with an increased focus on bat biosurveillance has greatly contributed towards the discovery of novel bat-borne viruses with zoonotic potential, although the spillover and outbreak capacity of these viruses is unknown (Tan et al. 2021). However, the ecological link between bats and pathogen transmission is poorly understood as ecological data is often lacking in viral transmission risk assessments (K. Zhao et al. 2022). For instance, the movement patterns of bats may have direct consequences for pathogen transmission as the bats' foraging activities can result in interfaces with contact between multiple species that may facilitate spillover (Hassell et al. 2017). Ecological data such as movement patterns and foraging areas, therefore, can provide crucial insight for risk assessments by delineating the behaviours and dynamics of host populations that may facilitate spillover (Randhawa et al. 2020). Ecological data may also reveal potential patterns for viral maintenance or reintroduction in susceptible colonies, especially if the species forms a metapopulation with movements and interactions between multiple, spatially separate populations (Driscoll 2007; Epstein et al. 2020). This literature review explores the role bats play as viral hosts, potential determinants of bat movements and how the movements of bats may impact the risk of pathogen spillover. Specific reference is given to *Rousettus aegyptiacus* as it is a species in South Africa that has been associated with several viruses with zoonotic potential (Jansen can Vuren et al. 2018; Paweska et al. 2018; Mortlock et al. 2019; Paweska et al. 2020; Mortlock et al. 2021).

1.1 Movements and home ranges

The four components that influence the movements of organisms are: 1) an organism's internal physiology and psychology, 2) its motion capacity, 3) navigation capabilities and 4) all biotic and abiotic factors that can influence movements (Nathan et al. 2008; Shaw et al. 2020). Movements are a response to requirements for survival (Holyoak et al. 2008) with different movement types linked to varied responses to different selective pressures and drivers. The three most common types of movements are defined as foraging movements, dispersal and migration (Jeltsch et al. 2013). Foraging movements are performed within an animal's home range and often multiple times per day while dispersal is more circumstance-dependent resulting in an animal transitioning from one area to another, typically to avoid competition with conspecifics or kin (Clobert et al. 2012; Jeltsch et al. 2013). Finally, migration refers to specific movements performed on typically a seasonal basis to reach areas that are suitable for breeding and reproduction or to avoid adverse climatic conditions (Jeltsch et al. 2013; Fleming 2019). Migratory movements can also be linked with changing resource availability, although they are distinct from range shifts to track seasonally, ephemeral resources that are simply an extension of a previously established home range (Hale et al. 2016; Fleming 2019).

Aside from seasonal changes, predation pressure or the need to find a mate (Doherty et al. 2019), the availability of limiting resources such as food, water and suitable foraging or resting areas is generally referred to as the key factor impacting movement patterns (Hodgkison et al. 2004; Leblond et al. 2010). Therefore, some animals limit their movements within a specific region that provides essential resources for survival and is commonly referred to as a home range (Burt 1943). The establishment of a home range can also impact movement patterns as animals weigh up the costs and benefits of maintaining these areas or moving to alternative areas based on resource availability (Stamps 1995). Movement patterns typically mirror the availability of food as it varies on a seasonal basis (Richter and Cumming 2006). However, land-use changes, such as the conversion of natural habitats to agricultural fields or urbanisation may also influence movement patterns. Agricultural fields and trees planted within urban areas may inadvertently increase resource availability and encourage movements into these areas (Plowright et al. 2011; Russo and Ancillotto 2015). For instance, fruit bats in Australia have been observed expanding their ranges outside of their regular foraging areas due to an increased density of fruiting trees associated with trees planted along streets that increase food availability throughout the year (Williams et al. 2006). In Bangladesh, the Indian flying fox, *Pteropus medius*, preferentially roosted in small forest fragments near residential areas rather than undisturbed forested areas as the domestically grown fruit trees likely provided more diverse food sources than natural forests (Hahn et al. 2014). Similarly, the development of artificial wetlands for agricultural irrigation has resulted in equal or higher bat species diversity and abundance due to increased insect prey availability (Sirami et al. 2013). However, land-use change most often negatively impacts a species through habitat fragmentation that can decrease the size of suitable foraging sites (Quesada et al. 2004). This may translate to increased travel distances and range expansions as species in fragmented habitats are forced to locate multiple foraging sites to obtain sufficient resources when compared to intact areas (Doherty et al. 2019).

1.2 Methods for studying and analysing movements

There is a wide variety of options available to track the movements of animals including radio telemetry, satellite telemetry, acoustic telemetry and geolocation (Kranstauber et al. 2011). However, the two most prominent methods for tracking the movements of animals are satellite or radio telemetry as they provide the most reliable means to study movement patterns (Kays et al. 2011). Early satellite tracking efforts made use of Platform Transmitter Terminals (PTTs) linked to the Advanced Research and Global Observation Satellite (ARGOS) system (Fancy et al. 1988). These PTTs possess a set frequency that allows the location of the tag to be estimated based on the Doppler shift for frequencies received by the linked satellites as they orbit the earth (Fancy et al. 1988). Location data are subsequently available for download from anywhere in the world which greatly reduces fieldwork requirements. A major drawback of PTTs, however, is the relative inaccuracy of location estimates being two orders of magnitude less accurate than conventional Global Position System (GPS) transmitters (Soutullo et al. 2007). Satellite telemetry with GPS transmitters provides higher-resolution movement data than PTTs and can be configured to report movements at specific periods and intervals (Kays et al. 2015). However, these tags tend to be expensive and the size and the battery requirements for these devices have largely limited their applicability to larger species that can support the weight of the transmitter (Richter and Cumming 2008; Řeřucha et al. 2017). Some GPS trackers are equipped with solar batteries that reduced the overall mass of the tag, but these are not suitable for many nocturnal animals and specifically, species that inhabit caves or dense foliage during the day as the batteries unable to recharge (Epstein and Newman 2011; Toledo et al. 2020). GPS trackers are best used in inaccessible areas or areas with difficult terrain and for animals that travel large distances as the tag provides positional fixes from distance and does not require personnel in the field to actively track the tagged animals (Epstein et al. 2009; Řeřucha et al. 2017). Radio telemetry makes use of tags that emit radio signals at different frequencies: Very High Frequency (VHF) and Ultra High Frequency (UHF) are best suited to animals with small- to medium-sized home ranges and that occupy easily accessible areas (Millspaugh et al. 2012). The advantage of VHF tags over GPS tags is that the VHF tags are more affordable, there is a wide range of available sizes and weights and can be used on large and small species (Kays et al. 2011; Millspaugh et al. 2012). The disadvantages of VHF tracking are that it is labour-intensive, requiring people in the field to directly track the tagged animals, it can be difficult to track individuals across rough terrain and signals can be lost or misleading if there are elevational gradients and mountainous areas (Kays et al. 2011; Gerber et al. 2018; Kirol et al. 2020).

After movement data has been collected, several questions can influence the analysis process. For instance, researchers may be interested in specific areas of use or patterns of resource selection (Manly et al. 2002). Alternatively, researchers may be interested in the home range size of the study species for which there are several different analytical methods (Gregory 2017; Noonan et al. 2019). Home range size estimations offer insight into the spatial requirements of a study species and can identify variations in movement patterns brought about by differences in seasonal conditions or specific biological characteristics of the study species (Powell and Mitchell 2012). Historically, four methods have been used to calculate home range size: Minimum Convex Polygons (MCP) (Mohr 1947), Kernel Density Estimation (KDE) (Worton 1989), Local Convex Hulls (LoCoH) (Getz and Wilmers 2004) and Anderson Fourier analysis (Anderson 1982) with MCP and KDE being the most frequently utilized methods (Laver and Kelly 2008). The MCP method is the simplest to perform as it constructs a polygon around the outer limits of a pre-determined percentage of locations (Mohr 1947). This method enables researchers to compare estimates across multiple studies, however, it is a rudimentary process with high sensitivity to outlying locations and small sample sizes that can result in overestimated home ranges (Gregory 2017; Sillero et al. 2021). The comparability across multiple studies is commonly the reason given for the use of MCP methods despite the relative inaccuracy (Laver and Kelly 2008), however, ecological niche modelling can increase the accuracy of MCP estimates and provide robust predictions of space use (Sillero et al. 2021). MCP analysis may also be used for specific investigations into the occasional movements of animals outside of their predicted range (Laver and Kelly 2008). As an alternative, KDE is a complex, but more precise estimation method as it is less susceptible to outliers, relying on the density of locations rather than a polygon that simply encompasses the locations (Worton 1989; Fleming et al. 2015). The complexity of this method stems from the various ways in which it can be implemented as the calculations require an additional variable, termed the smoothing factor, that can influence the estimations and limit comparability across multiple studies (Laver and Kelly 2008; Noonan et al. 2019). Anderson Fourier analysis is a non-parametric estimation method that calculates home range size from the utilization distributions of spatial points through a Fourier transformation (Anderson 1982). This method, however, does not allow researchers to compare estimates across multiple animals and is heavily biased by sample size (Anderson 1982). LoCoH was developed as a more accurate alternative to traditional MCP or kernel estimations with the advantage that LoCoH accounts for physiographic features that may influence spatial patterns (Getz and Wilmers 2004). However, LoCoH requires large samples of high-resolution data, such as that obtained through GPS tracking, to generate accurate home range estimations and therefore, is not suitable for studies with small sample sizes (Getz et al. 2007).

1.3 Chiroptera

Bats belong to the order Chiroptera, which is the second largest mammalian order after Rodentia (Mammal Diversity Database 2022), with over 1400 recorded species from 21 families (Simmons and Cirranello 2022). The order Chiroptera was previously divided into two suborders, namely Megachiroptera and Microchiroptera (Dobson 1875), however, these suborders have since been replaced by Yinpterochiroptera and Yangochiroptera that group bats together based on recent molecular findings (Hutcheon and Kirsch 2006; Tsagkogeorga et al. 2013). They occur on six of the seven continents, inhabiting all biome types excluding the polar regions. Bats occupy a variety of different roosts during the day such as within foliage, abandoned buildings or structures or in specialised roost sites like caves (Monadjem et al. 2020). Bats are unique among mammals as they are the only group capable of self-powered flight (Gunnell and Simmons 2005) and comprise a wide variety of dietary niches including frugivory, insectivory, carnivory and sanguivory (Kunz 2013a). As a nocturnal group, most bat species are capable of echolocating which enables foraging in low-light conditions (Schnitzler and Kalko 2001). It was originally hypothesised that no fruit bat species were capable of echolocating as it was an unnecessary evolutionary adaptation for their foraging strategies (Jones and Teeling 2006). However, the genus *Rousettus*, are also capable of echolocating with tongue-clicks and, although this method is not commonly considered as precise as the laryngeal echolocation of insectivorous species (Jones and Teeling 2006), it enables effective navigation between obstacles with comparable accuracy (Yovel et al. 2011).

1.4 Movements of bats related to food availability and ecological factors

Resource availability is a key variable influencing movements as animals must balance their energy budgets with resources from the surrounding areas. The varying degrees of resource abundance and, or richness can also dictate movements according to marginal value theory (MVT) as animals may be willing to travel further to reach resource-rich areas (Owen-Smith et al. 2010). A model generated by Ford (1983) predicts that central-placed foragers located in areas with a low abundance of sparsely distributed resources would travel further and occupy larger areas compared to individuals in resource-rich areas. Support for this prediction was later shown by Boutin (1990) in a review of food supplementation experiments with terrestrial vertebrates. The review included birds, mammals, amphibians and reptiles and there was a consistent trend for animals inhabiting areas with more resources, through experimental supplementation, to occupy smaller areas than animals without food supplementation.

Bats display a similar reaction to resource availability, and it has been suggested they can track food availability and adjust their small-scale movements accordingly (Hodgkison et al. 2004; Richter and Cumming 2006; Páez et al. 2018). Probably the most famous occurrence of bats adjusting their movement patterns to target ephemeral food sources is during the mass migration of straw-coloured fruit bats, *Eidolon helvum*, across Africa to Kasanka National Park (Richter and Cumming 2006; Hurme et al. 2022). Hurme et al. (2022) demonstrate that at several points along their migratory route, peaks in colony size for *E. helvum* coincide with regional peaks in resource availability, suggesting that bats were not only targeting these regions but also timing their arrival to specifically coincide with peak fruit availability. Similarly, at Kasanka National Park the arrival and presence of *E. helvum* mirrors fruit availability as the first bats start arriving just before the resource peak, aggregating in a colony of several million individuals during the resource peak, before dispersing once fruit availability starts to decrease again (Richter and Cumming 2006). As long-lived species relative to their body size, *E. helvum* may retain some spatial memory of key foraging sites along their migratory routes which could facilitate their movements (Fagan et al. 2013), although this hypothesis is untested. In Australia, *Pteropus* spp. are known to shift roosting sites over hundreds of kilometres to track ephemeral flowering events (Roberts et al. 2012; Eby et al. 2022). McConkey and Drake (2007) also suggest that bats could track fruit availability among islands separated by several kilometres as fruit bat abundance is correlated with fruit availability on the different islands, although further investigations would be required to confirm this hypothesis. The mechanisms by which bats track fruiting events, however, remain unclear (Hurme et al. 2022).

Resource availability is closely linked to vegetation type and structure with open areas having more patchily distributed resources than cluttered areas with dense vegetation (Ofstad et al. 2016). The activities of humans such as urbanisation and agriculture can impact resource availability as habitat fragmentation during land conversion procedures can reduce resource density (Quesada et al. 2004). Agricultural activities such as livestock pastures and croplands have been the most prominent contributor to habitat fragmentation over the past 300 years (Hooke et al. 2012) and approximately 10^9 ha of the remaining natural ecosystems are expected to be converted to agricultural lands by 2050 (Tilman et al. 2001; Tilman et al. 2011; Oakleaf et al. 2015). Despite the rate of land being converted into agriculture slowing down (Hooke et al. 2012), these areas are now being more intensely managed to increase overall yield with the consequence of decreased biodiversity in these areas (Beckmann et al. 2019). The specific type of agriculture may influence movement patterns for bats (Wickramasinghe et al. 2003; Fuentes-Montemayor et al. 2013). For example, fruit bats may be attracted to fruit orchards due to the high food availability allowing the bats to be successful in these areas (Lučan et al. 2016), yet insectivores in similar landscapes may not be able to exploit these areas as successfully. Aerial hawking bats require open spaces to forage, and the open areas linked with agriculture may benefit these bats (Noer et al. 2012) but exclude bats that preferentially forage in cluttered areas (Fuentes-Montemayor et al. 2013; Weier et al 2021). By contrast, livestock and crop farming may benefit insectivorous bats by increasing prey items that are attracted to dung, the livestock themselves or the cultivated crops (Downs and Sanderson 2010; Boyles et al. 2011; Ancillotto et al. 2017) but exclude fruit bats due to reduced food density (Estrada et al. 1993). The conversion of natural habitats to anthropogenically altered areas can alter the movement patterns of bats (Kessler et al. 2018; McKee et al. 2021) which may increase the likelihood of pathogen transmission due to altered ecological dynamics causing novel interspecific interactions (Pulliam et al. 2012; Plowright et al. 2021). Fragmented habitats are a prominent feature in many ecosystems and, with human population growth, are likely to become increasingly dominant. Therefore, understanding how the movements of bats are affected in fragmented areas becomes important for future conservation planning and mitigating risks associated with interspecific contact between bats and livestock or people.

Bats may also adjust their foraging strategies according to varying degrees of urbanisation as this can influence the distribution, abundance and diversity of available resources (Egert-Berg et al. 2021). Artificial light associated with urban areas can impact insectivorous bats' foraging patterns as it may be an attractant for insect prey items (Schoeman 2016; Fleming and Bateman 2018). Furthermore, domestic gardens and city planning can inadvertently increase the number of fruiting trees present within cities and may result in a higher diversity of food items for fruit bats (Williams et al. 2006; Egert-Berg et al. 2021). These fruit sources may also fruit year-round and provide alternative options when natural sources are depleted, foregoing the need for seasonal migrations due to resource shortages (Plowright et al. 2011; Hahn et al. 2014; Giles et al. 2016; Eby et al. 2022).

As stated above, climatic variability influences movement patterns, however, this variability is more pronounced at higher latitudes and warrants physiological or behavioural adaptations to survive (Gutiérrez-Pesquera et al. 2016). Migration is one method to overcome unfavourable conditions (Nathan et al. 2008) and may be short distances (<500km) between summer and winter roosts or long-distance trips (>1000km) between seasonal roosts (Fleming and Eby 2003; Fleming 2019). Migration is more common for temperate bats that experience dramatic seasonal differences than tropical species where variable resource availability is the critical factor determining whether a species migrates (Fleming 2019). Wing morphology is typically linked to migratory capabilities and with most migrant species having a high wing-loading and aspect ratio that enable energetically efficient flight (Norberg and Rayner 1987; Fleming and Eby 2003; Fleming 2019). Species with a high aspect ratio and wing loading typically forage in constant flight within open areas away from vegetation or clutter (Schnitzler and Kalko 2001). Species that forage in cluttered areas or near vegetation, instead have lower wing-loading and aspect ratio that enable highly manoeuvrable flight at the cost of aerodynamic efficiency (Norberg and Rayner 1987). Foliage-gleaning and clutter foragers rarely migrate as long-distance flights are energetically expensive (Monadjem et al. 2009), although there are records of clutter foragers migrating long distances such as the bushveld horseshoe bat, *Rhinolophus simulator* (Laycock 1973). However, the wing adaptations observed in migratory species are likely a primary result of evolution for different foraging strategies and dietary niches and the suitability for migration was a side-effect of these evolutionary adaptations (Fleming and Eby 2003). Therefore, given the increased seasonal variability at higher latitudes (Hurme et al. 2022), temperate species likely exhibit more variable seasonal movement patterns compared to tropical species which may translate to overall larger range sizes for the temperate species.

Group size can also influence movement patterns as individuals may disperse further to reduce intraspecific competition (Meyer et al. 2005). This trend has been observed in bats with species occupying large colonies occupying larger ranges and travelling further for foraging compared to solitary species or species in small colonies (Bonaccorso et al. 2002). Although, Calderón-Capote et al. (2020) show that movements for *E. helvum* were largely independent of colony size, as these bats shifted roost sites according to resource availability. While group size can affect movement patterns, the converse is also true as group size may fluctuate on a seasonal basis as bats aggregate for the breeding season (Jacobsen and du Plessis 1976; Cheng and Lee 2004; van Haarten et al. 2022).

1.5 Monitoring of fruit availability and fruit tree density

To assess how movements may relate to food availability, an estimate of suitable food sources in the area must be made. There are two methods whereby plant abundance, distribution and density can be assessed within a region: direct observations in the fields or remote sensing either through drone footage or satellite imagery (Cruzan et al. 2016; Tay et al. 2018). Traditional vegetation survey methods involved distance sampling with quadrats or transects (McIntyre 1953) where a portion of the study area is sampled, direct counts are made of the target species and the results are extrapolated to the larger study area (Thomas et al. 2010). These approaches are simple to perform and well-suited to small study sites but are labour-intensive and may suffer from observer bias (Thomas et al. 2010; Cruzan et al. 2016). Remote sensing employs unmanned aerial vehicles (UAVs) such as drones or satellites to capture imagery of the study site and enables a survey of a much larger area compared to traditional methods (Tay et al. 2018; Morgan et al. 2022). While the use of drones is relatively inexpensive and can provide high-resolution imagery, their application may be limited by technical constraints or legal restrictions linked to privacy (Martinez et al. 2021; Smigaj and Gaulton 2021). Satellite imagery does not have the same technical or legal limitations but can result in low-resolution images which prevent accurate assessments of individual species or discernment between landscape features and can also be impeded by cloud cover (Cruzan et al. 2016; Morgan et al. 2022).

In addition to estimating the overall abundance of fruiting trees, estimating the availability of fruit for each tree is also important for comparisons between frugivore movements and food availability (Chapman et al. 1992). The distribution of frugivores typically mirrors fruit availability and fruit availability can influence frugivores movements on a small-scale, such as within a home range (van der Heide et al. 2012; García et al. 2013). The techniques to estimate fruit availability are associated with traditional vegetation survey methods as they require direct observations or measurements. There are a variety of different methods that can be used to estimate total fruit availability such as Diameter at Breast Height (DBH) measurements, direct counts (which can be linked to the percentage of fruit availability or scored on a relative scale), crown volume and fruit traps (Chapman et al. 1992). Each method has its advantages and disadvantages, for instance, DBH measurements are often not appropriate for trees with buttressed roots as the diameter can be artificially inflated. Direct counts are best suited to trees with large, conspicuous fruits and can be difficult to perform accurately if the canopy is high above the ground. After fruit availability estimates have been calculated, an important subsequent measure to link frugivore behaviour and movements is the approximate density of different fruiting trees (Chapman et al. 1994). For example, a tree species with 100% fruit cover but a density of 1 individual per hectare will provide an artificially inflated estimate of fruit availability compared to a tree species with 50% fruit cover and a density of 10 individuals per hectare.

1.6 Resource and habitat selection modelling

A habitat is defined as ‘the resources and conditions present in an area that produce occupancy' (Hall et al. 1997), while habitat type is the vegetation association in an area (Daubenmire 1968, pp 27-32). The specific patterns of use for different habitat types by a species can provide further information regarding that species’ movement patterns and resource requirements. Different habitat types are not all equivalent in terms of their structure, resource availability or use by different species (Manly et al. 2002). Therefore, habitat selection or avoidance is an important metric for populations of interest as it can illustrate patterns for long-term resource requirements within an area (Manly et al. 2002).

One method to assess this is through habitat suitability modelling in which a combination of environmental variables is used to predict the potential distribution of a species as well as its presence within or absence from specific habitat features (Kearney 2006; Hirzel and Le Lay 2008). This can be especially useful for species of specific interest such as threatened species or hosts of disease (Hahn et al. 2014), however, habitat suitability modelling is largely predictive and more focused on areas in which a species could theoretically occur rather than identifying patterns of specific use within an area. Patterns of active habitat selection can be assessed with resource selection functions (Boyce and McDonald 1999) by comparing the relative usage of a specific habitat type against its proportional availability within the study area (Manly et al. 2002). These location data are commonly obtained directly through tracking (Kurek et al. 2020) or via passive methods such as acoustic monitoring or camera traps (Gili et al. 2020; Salom-Pérez et al. 2021).

1.7 Viruses and diseases associated with bats

Chiropterans were believed to carry more viruses per species than any other mammalian order (Luis et al. 2013). However, Olival et al. (2017) argued that research effort for the different species and phylogenetic relatedness to humans should first be considered before such a distinction is made. Mollentze and Streicker (2020) further expanded on this topic by assessing the number of human-infecting viruses present across mammalian and avian orders. Mollentze and Streicker (2020) showed a proportional relationship between the number of zoonoses and species present within the order, which is in contrast to the previous findings that bats carry a disproportionately high number of zoonoses per species (Luis et al. 2013). Regardless, bats have been associated with numerous viruses with zoonotic potential or identified as reservoir hosts for emerging zoonotic diseases (Hayman et al. 2013; Moratelli and Calisher 2015; Markotter et al. 2020). Several studies have identified ecological traits of bats that may increase their suitability as reservoirs for such a wide diversity of viruses (Hayman et al. 2013; Luis et al. 2013; Guy et al. 2020). These traits include highly gregarious aggregations that increase the probability of contact between species or with conspecifics, broad geographic ranges that may facilitate contact and potential viral sharing with species across a wide range and long lifespans (Hayman et al. 2013; Luis et al. 2013; Guy et al. 2020). Beyond the factors that may aid viral presence and persistence in bats, flight allows bats to travel large distances and cross geographical barriers that would normally inhibit movement for non-volant mammals of similar sizes (Breed et al. 2010). Therefore, bats are capable of widespread contact with a diverse array of species which may constitute the risk of widespread transmission of viruses (Hayman et al. 2013).

Prominent viral families for which bats have been identified as reservoirs include *Coronaviridae*, *Paramyxoviridae*, *Filoviridae* and lyssaviruses from *Rhabdoviridae* (Wang and Anderson 2019; Markotter et al. 2020). From these families, specific viruses have spilled over into human populations causing outbreaks with varying degrees of severity and fatalities, the most notable of which are Hendra virus (*Paramyxoviridae*) in Australia (Murray et al. 1995; Young et al. 1996; Mahalingam et al. 2012), Nipah virus (*Paramyxoviridae*) in Malaysia, Bangladesh, the Philippines and India (Chua et al. 2000; Hsu et al. 2004; Luby et al. 2009; Ching et al. 2015; Alam 2022) and Marburg virus (*Filoviridae*) in Africa (Towner et al. 2009; F. Zhao et al. 2022). Despite being hypothesised as a reservoir for Ebola for many years and viral RNA and Ebola virus-specific antibodies being detected in fruit bats (Leroy et al. 2005; Pourrut et al. 2007), isolation of live virus from wild specimens has thus far not occurred and therefore, it is not possible to determine yet whether fruit bats are true reservoirs for Ebola virus or whether they have played a role in infecting humans (Leendertz et al. 2016). Bats are regarded as the predominant reservoirs for lyssaviruses but the precise reservoirs are unconfirmed as several lyssaviruses are associated with multiple species (Markotter et al. 2020). For example, Duvenhage virus (DUVV) has been linked to isolated cases of fatal human infection and is associated with Egyptian slit-faced bats, *Nycteris thebaica* (Coertse et al. 2020). However, the limited detections prevent definitive conclusions regarding the role of *N. thebaica* as the reservoir host for DUVV (Markotter et al. 2020). Similarly, for SARS-CoV-1 and SARS-CoV-2, bats have been implicated as hosts for closely related SARS-like coronaviruses (Li et al. 2005) and SARS-CoV-2 related viruses (Zhou et al. 2021). The case is slightly different for MERS-CoV as dromedary camels (*Camelus dromedarius*) are specifically linked to human infections (Mohd et al. 2016), yet closely related viruses detected in bats suggest bats may be the ancestral origin for MERS-CoV (Corman et al. 2014). However, the specific viruses have not yet been detected in bats and therefore, the exact reservoir for these viruses remains unknown (Hu et al. 2015; Zhou et al. 2021), although the genus *Rhinolophus* is strongly associated with SARS-CoV and SARS-CoV-2 being identified as hosts for closely related viruses (Balboni et al. 2012; Alkhovsky et al. 2022; Ruiz-Aravena et al. 2022). One consistent trend with these outbreaks is that all of these are RNA viruses which possess high rates of mutation and may be able to adapt to multiple hosts through rapid evolution

(Woolhouse et al. 2013; Williams et al. 2021) meaning they are of particular concern for risk of future outbreaks (White and Razgour et al. 2020).

Bats play a role in the persistence and transmission of viruses, some with zoonotic potential and serious health implications (Cheetham and Markotter 2021) which emphasises the importance of continued surveillance of viral presence and diversity in bats. Investigations into bat ecology and the factors that may contribute towards spillover are also crucial to understanding and reducing the risk of future outbreaks (Letko et al. 2020). Linked to this is an assessment of excretion dynamics for these viruses that may help researchers make recommendations for how to prevent spillover events. For instance, Edson et al. (2015) identified bat urine as the major route of viral excretion for Hendra virus and suggested that the likely route of transmission occurs through bats contaminating water and grazing pastures with urine (Edson et al. 2015). Therefore, possible mitigation strategies may include removing fruiting trees or potential roost sites from horse pastures to reduce the likelihood of spillover. In Malaysia, Nipah virus was found to be shed in saliva and urine and it is hypothesised that the original outbreaks occurred when pigs fed on partially eaten fruit that had been dropped by the fruit bats and likely contaminated with saliva (Field et al. 2001; Chua et al. 2002). Similarly, Nipah virus outbreaks in Bangladesh have been attributed to people drinking raw date palm sap that has been contaminated by fruit bat excreta or saliva whilst they are feeding on the sap (Gurley et al. 2017) with shedding observed in saliva, urine and faeces (Epstein et al. 2020). These discoveries highlighted potential mitigation strategies to reduce future outbreaks including removing fruiting trees from pig farms (Chua et al. 2002) as well as constructing barriers around date palm sap collection receptacles, preventing the bats from drinking and potentially contaminating the sap (Gurley et al. 2017).

1.8 Relationship between movements and disease transmission risk

The necessity of studying the movements of any species stems from the species' interactions within the ecosystem and the impacts they may have on the surrounding areas (Harris et al. 1990; Voigt et al. 2017; Randhawa et al. 2020). Bats have a high species diversity, wide distribution and provide various important ecosystem services (Fleming and Muchhala 2008; Boyles et al. 2011; Kunz et al. 2011). Therefore, studying the movements of bats provides insight into the range over which their multitude of impacts may be felt (Voigt et al. 2017). Linked to the movements of bats, is the risk of disease transmission as bats have been identified as hosts for several viruses with the potential to infect humans or that have already caused outbreaks in humans with varying case fatality rates (Luby et al. 2009; Hayman et al. 2013; Moratelli and Calisher 2015; Markotter et al. 2020). Zoonotic diseases are responsible for most pandemics over the last 100 years (Morse et al. 2012; Bernstein et al. 2022) but predicting future outbreaks is difficult due to the numerous suitable pathogens that have the potential to jump the species barrier and infect humans (Holmes et al. 2018). Yet through extensive research, a trend has become clear: outbreaks are more likely to occur in areas with high species diversity and interactions between humans, livestock and wildlife (Allen et al. 2017; Olivero et al. 2017; Roberts et al. 2021; Stephens et al. 2021). A relatively novel attitude in the field of zoonoses and zoonotic transmission has been to include and integrate expertise from multiple fields such as virology, ecology, anthropology and sociology in line with the One Health approach (OHHLEP et al. 2022) for a better perspective for risk assessments (Morse et al. 2012; Dougherty et al. 2018). Most studies looking at emerging zoonoses in bats, however, only investigate virological aspects like viral prevalence or diversity within a population and lack potentially important ecological data obtained from movement or home-range studies (Letko et al. 2020; Randhawa et al. 2020; K. Zhao et al. 2022). Movement data can help identify key areas in the landscape that are favoured by a study species and may provide evidence of spatial overlap and risk of contact between species (Dougherty et al. 2018; McClure et al. 2020). Movements studies can be performed for species that are known hosts of potential zoonotic pathogens and, if paired with pathogen prevalence and excretion dynamics, can be extrapolated for risk of spillover events or disease transmission (de Jong et al. 2013; Plowright et al. 2017; Dougherty et al. 2018; Epstein et al. 2020). For example, a high proportion of Hendra virus spillover events occurred within the foraging ranges of *Pteropus* colonies, emphasising the importance of studying their small-scale movements in connection with the risk of disease transmission (Plowright et al. 2011). Furthermore, Field et al. (2015) show that there were strong seasonal fluctuations in viral excretion that correlated with seasonal alterations in bat distributions, showing that the risk of spillover may vary on a spatial and temporal scale. Spillover events are rare and certain processes must occur before spillover can take place (Holmes et al. 2018), but land use changes that disrupt natural processes are a crucial step in the process of spillover events (Olivero et al. 2020; Roberts et al. 2021). Being able to assess the risk factors associated with disease transmission can help aid management decisions to prevent or combat future outbreaks and, potentially, significantly reduce costs associated with an outbreak (Dobson et al. 2020).

Risk modelling approaches and the use of animal movement data can help to identify areas where the risk of transmission is high, however, the risk of spillover is not only dependent on the presence of viral hosts but also the levels of interspecific contact between bats and other species such as humans (Pigott et al. 2015). Surveys can be used in areas with the known presence of reservoirs or with a predicted risk of zoonotic transmission to identify the regularity with which people come into contact with reservoir species and enable a quantification of the risk of transmission (Pigott et al. 2015; Letko et al. 2020). The degree of contact between a host species and humans can further be influenced by land-use change which may alter the ecological dynamics in the system resulting in increased contact events between species (Gottdenker et al. 2014; McKee et al. 2021; Eby et al. 2022). For effective risk assessments, a proper understanding of a host's small-scale movement patterns and proximity to susceptible populations is required (Dougherty et al. 2018; McClure et al. 2020). Knowledge of the pathogen dynamics within the host species, such as excretion dynamics and viral sharing are also important for risk assessments (Pigott et al. 2015; Letko et al. 2020).

1.9 Human effect

The interface between wildlife, humans and or livestock is one of the key variables in zoonotic transmission (Magouras et al. 2020). As the human population increases in size, the degree of contact between humans and wildlife is inevitably going to increase as more and more natural habitats are being converted into production landscapes or residential areas (White and Ward 2011; White and Razgour 2020). Consequently, risks of spillover are increasing due to the expansion of human activities that are encroaching on natural habitats and increasing the likelihood of contact between species (Allen et al. 2017; Dobson et al. 2020).

Land-use change can potentially impact the movement patterns of animals through altered resource availability (Plowright et al. 2011; Russo and Ancillotto 2015) and may result in human-livestock-wildlife interfaces that could contribute to pathogen spillover and transmission (Allen et al. 2017; White and Razgour 2020). There are several instances where land-use change has been attributed as the factor leading to a spillover event (Plowright et al. 2011; Pulliam et al. 2012; Olivero et al. 2017; Olivero et al. 2020). In these examples, the ultimate reason for the spillover was human disturbance and alteration of natural landscapes that introduced novel species interactions and altered disease dynamics (Morse et al. 2012; Gottdenker et al. 2014). The outbreaks of Hendra virus in Australia were due to the overlap between farming areas, horse pastures and foraging areas for *Pteropus* spp. (Plowright et al.

2011) but a sequence of key events led to this overlap. Pteropid bats are historically nomadic, travelling long distances to track ephemeral resources (Fleming and Eby 2003; Eby et al. 2022). These bats typically only form permanent aggregations during food shortages when they form small groups near to reliable food sources (Fleming and Eby 2003). However, the loss of large portions of natural forest for agricultural developments along the Australian east coast has decreased the availability of natural food sources for *Pteropus* spp. (Plowright et al. 2011; Bradshaw 2012). Consequently, bats are being forced to locate alternative foraging areas and are experiencing periods of food shortages more frequently (Eby et al. 2022). Trees planted along the streets in Melbourne offer reliable food sources year-round and therefore, bats have responded by shifting their historical range and preferentially roosting within or near urban and agricultural areas in multiple small, permanent groups (Williams et al. 2006; Plowright et al. 2011; Eby et al. 2022). The larger urban bat populations mean that contact and the risk of disease transmission are both more likely to occur. These areas with a high risk of contact were not historically present but land-use change caused the destruction of natural food sources that ultimately drove the permanent occupancy of bats within urban areas and led to the contact between reservoirs and potential hosts (Morse et al. 2012; Gottdenker et al. 2014). With the Nipah virus outbreaks in southeast Asia, the intensification of farming activities, specifically pig farming, led to an overlap between agricultural lands with the bat reservoir’s natural foraging areas, ultimately resulting in an interface where pigs were being exposed to viral contaminants shed by the bats (Pulliam et al. 2012). However, this overlap between bats and pigs, and eventually humans, was not a chance occurrence. There was a long series of events that preceded the outbreak that had an impact on the overall outcome. The major events that enabled the spillover conditions included the three-fold increase in the extent of both mango and pig farming in the area (Pulliam et al. 2012). Moreover, the farming system and number of pigs on the farms facilitated long-term viral persistence due to the constant introduction of susceptible individuals, while a large population ensured a large outbreak could be sustained (Pulliam et al. 2012). Rapid population growth is leading to more extensive agricultural practices which have consequences for free-ranging livestock as there is no management of water or food sources and, therefore a high risk of contamination if the rangelands overlap with bat foraging areas (Mbu'u et al. 2019). These two events enabled the conditions for the contact between bats and suitable spillover hosts, pigs in this instance, that likely would not have occurred or would have been far less extensive if not for the intensification of agricultural practises in the area (Pulliam et al. 2012).

However, it is not necessarily only the presence of bats in an area that translates to the risk of spillover (Pigott et al. 2015; Gurley et al. 2017; McKee et al. 2021). For example, the majority of human Marburg virus outbreaks occur directly after people enter the mine in which the infected bats were roosting (Pigott et al. 2015) and in Bangladesh, Nipah virus outbreaks were linked with a higher proportion of households in which people consumed raw date palm sap that was likely contaminated with virus shed from the bats (Gurley et al. 2017; McKee et al. 2021). Activities such as these that bring people into direct potential contact with hosts should be noted as important avenues for potential viral spillover although the possibility of direct and/or indirect contact at foraging sites should also not be ruled out (K. Zhao et al. 2022).

1.10 *Rousettus aegyptiacus*

The Egyptian rousette bat, *R. aegyptiacus*, is one of seven species within the genus *Rousettus* (Yinpterochiroptera, Pteropodidae) (Mammal Diversity Database 2022; Simmons and Cirranello, 2022). Within the species *R. aegyptiacus*, there are six subspecies distributed throughout Africa and the Middle East (Juste and Ibáñez 1993; Albayrak et al. 2008; Benda et al. 2012). These subspecies include *R. aegyptiacus aegyptiacus, R. a. arabicus, R. a. leachii, R. a. princeps, R. a. tomensis* and *R. a. unicolor* (Simmons and Cirranello, 2022). *Rousettus aegyptiacus* is a medium-sized frugivorous bat with a mean body mass of 126.8 (± 25.29) g and forearm length of 92 (± 5.46) mm for males and 113.8 (± 21.9) g and 92.6 (± 4.54) mm for females (Monadjem et al. 2020). This bat has a wide geographic distribution ranging throughout most of Africa, north into parts of the Middle East and east into Pakistan (Benda et al. 2011; Benda et al. 2012). Within South Africa, there are a few known colonies in Limpopo (Jacobsen and du Plessis 1976; Jacobsen et al. 1986), Western Cape (Barclay and Jacobs 2011), Eastern Cape (Herzig-Straschil and Robinson 1978) and KwaZulu-Natal (Taylor et al. 1999) indicating the widespread distribution of this species. They are predominantly frugivorous typically targeting figs from a variety of native *Ficus* spp. although they also feed on cultivated fruit such as litchis (*Litchi chinensis*) and bananas (*Musa* spp.) (Jacobsen and du Plessis 1976; Monadjem et al. 2020). However, *R. aegyptiacus* is also a generalist and is known to include pollen, flowers and insects in its diet (Korine et al. 1999; Barclay et al. 2006). They are obligate cave or cavity dwellers roosting in natural cave systems or abandoned man-made structures in colonies that can range from a few hundred to several thousand individuals (Kwiecinski and Griffiths 1999; Barclay and Jacobs 2011). Bats in the genus *Rousettus* are capable of echolocating which is unique among fruit bats, and it is believed that this adaptation enables them to inhabit cave systems as they can effectively navigate the dark passages (Waters and Vollrath 2003). These bats have been associated with several potentially zoonotic viruses (Markotter et al. 2020). Most notably, *R. aegyptiacus* has been identified as a reservoir host for Marburg virus, which causes haemorrhagic fever with high case fatality rates similar to Ebola, both of which are from the *Filoviridae* family (Paweska et al. 2018; Paweska et al. 2020). *Rousettus aegyptiacus* tend to discard a large proportion of partially eaten fruit while foraging as well as spitting out fruit parts which include the skin and seeds (Jacobsen and du Plessis 1976; Kwiecinski and Griffiths 1999). These spit-outs have the potential to be contaminated by the bat's saliva and could serve as a potential route of transmission (Amman et al. 2015b; Amman et al. 2021). These risks may be especially prevalent if their foraging areas overlap with rangelands or pastures where livestock feed.

1.11 Viruses identified in *R. aegyptiacus*

Marburg virus (MARV), *Marburg marburgvirus*, belongs to the *Filoviridae* family which is characterised by thread-like or filamented structures (Barrette et al. 2011; Shi et al. 2018; Amarasinghe et al. 2019). It causes a haemorrhagic fever, similar to Ebola virus infection with case fatality rates of up to 90% (Amman et al. 2020a). There have been several sporadic outbreaks of MARV affecting multiple African countries (F. Zhao et al. 2022), the largest of which was in Angola (2004-2005) with 252 infections and 227 deaths (Amman et al. 2020a). Most primary infections in natural outbreaks have been associated with people entering caves inhabited by bats (Amman et al. 2020a) suggesting bats may play a role in MARV transmission and subsequent studies have identified *R. aegyptiacus* as the natural reservoir hosts for MARV (Towner et al. 2009; Amman et al. 2015b; Schuh et al. 2017). Viral shedding has been observed through saliva and urine and has also been detected in rectal swabs in *R. aegyptiacus* suggesting possible routes of transmission through faecal or urinary contamination or discarded fruit (Amman et al. 2015b; Schuh et al. 2017; Paweska et al. 2020). A subsequent study revealed that viable MARV could still be detected on artificially inoculated fruit up to six hours post-inoculation (Amman et al. 2021) and alludes to the potential for indirect contact and exposure outside of the foraging periods for bats. Within South Africa, MARV was identified in a population of *R. aegyptiacus* inhabiting Matlapitsi cave in the Limpopo Province (Paweska et al. 2018; Paweska et al. 2020). The studies both identified distinct seasonal patterns of seropositivity within juveniles indicating potential periods for increased shedding risk (Paweska et al. 2018; Paweska et al. 2020).

Paramyxoviridae is another viral family associated with bats with previous spillover events and human fatalities (Mahalingam et al. 2012; Alam, 2022). Thus far, however, the only paramyxovirus identified in *R. aegyptiacus* with recorded spillover and human infection is Sosuga virus (SOSV) (Amman et al. 2015a) with a single, non-fatal case being recorded to date (Albariño et al. 2014). SOSV is believed to originate and circulate naturally within *R. aegyptiacus* populations, although evidence supporting the reservoir status for these bats is still required (Amman et al. 2015a; Amman et al. 2020b). Experimental inoculation of *R. aegyptiacus* with SOSV revealed viral shedding through faecal and urinary excretion as well as being detected in oral swabs suggesting possible routes of transmission (Amman et al. 2020b).

Numerous other paramyxoviruses were identified from the Matlapitsi cave *R. aegyptiacus* colony including *Rubula*- and related viruses (Mortlock et al. 2019) and henipa-related viruses (Mortlock et al. 2021), although there have been no recorded cases of human infection. Among these viruses, two were highlighted due to being closely related to human parainfluenza virus (HPIV-2) and mumps virus (MuV) (Mortlock et al. 2019), both of which were detected in urine samples suggesting urinary excretion as a potential route for viral transmission. A novel adenovirus was also isolated from liver and spleen samples from an *R. aegyptiacus* from Matlapitsi cave (Jansen van Vuuren et al. 2018). The virus isolate was closely related to two viruses isolated from *Miniopterus schreibersii* faeces in China (Tan et al. 2017) which suggests contact with faecal material as a potential route of exposure. Rotaviruses were detected in *R. aegyptiacus* on two separate occasions in Kenya (Waruhiu et al. 2017) and Zambia (Sasaki et al. 2018). The strain detected in *R. aegyptiacus* from Kenya was obtained from faecal matter and was closely related to a human group A rotavirus, emphasising its zoonotic potential and faecal excreta as a plausible route for exposure (Waruhiu et al. 2017). *Rousettus aegyptiacus* have also been associated with Lagos bat virus (Coertse et al. 2021) and two Nairoviruses, one of which was linked to the infection of four laboratory workers that had handled bat samples (Kalunda et al. 1986).

Several other viral families and specific viruses have also been associated with *R. aegyptiacus* although they are not known to specifically infect humans with no spillover events thus far being recorded (Table 1).

Table 1: Summary of specific viruses and additional viral families associated with *R. aegyptiacus* from several African countries and Israel

Viral family	Virus	Details	Source
Astroviridae	Several *Astrovirus* spp.	Several viral strains were detected for the first time within *R. aegyptiacus* in Kenya and were distantly related to porcine strains suggesting possible interspecies transmission, although the public health impact is unknown.	Waruhiu et al. 2017
Caliciviridae	*Sapovirus* sp.	A novel strain of sapovirus was identified in *R. aegyptiacus* in Kenya, the first instance of calicivirus detection in this species. Further study of the strain is required before its zoonotic potential can be assessed.	Waruhiu et al. 2017
Coronaviridae	Several coronavirus spp.	A high diversity of coronaviruses has been detected in *R. aegyptiacus* in Kenya, none of which are known to infect humans.	Tong et al. 2009
Herpesviridae	Unclassified β-herpesvirus	An unclassified β-herpesvirus was isolated from *R. aegyptiacus* in South Africa although its pathogenicity and transmissibility to humans were not investigated.	Dietrich et al. 2018
Flaviviridae	Several viruses	Flaviviruses have been detected in *R. aegyptiacus* across a wide geographic range although the role they play in viral replication and transmission is unknown.	Kading et al. 2018
Nairoviridae	Crimean-Congo haemorrhagic fever-like virus	Typically, a tick-borne virus but it has been detected in *R. aegyptiacus* as well as other cave-dwelling species in Congo and Gabon, suggesting its potential role in the virus life cycle. The potential for human infection is unknown.	Müller et al. 2016
Orthomyxoviridae	Influenza A virus	A novel Influenza A virus was detected in *R. aegyptiacus* from Egypt with the potential for cross-species transmission including to	Kandeil et al. 2019

		humans, although no such cases have been reported.	
Phenuiviridae	Rift Valley Fever phlebovirus	Neutralizing antibodies to Rift Valley Fever phlebovirus were detected in Uganda in a region with recent human cases. The role bats play during the infection cycle or as reservoirs is unknown.	Fagre and Kading 2019
Polyomaviridae	Several virus spp.	*Rousettus aegyptiacus* from Kenya and Zambia have been associated with a high diversity of polyomaviruses, none of which have been recorded infecting humans, although they may play a role in viral transmission for other species through coinfection dynamics.	Tao et al. 2013 Carr et al. 2017
Poxviridae	Israeli poxvirus	A novel poxvirus was isolated from a visibly infected bat in Israel and is the first instance of a poxvirus bat infection in Israel. Poxviruses can infect humans, but the zoonotic potential of the Israeli poxvirus is unknown	David et al. 2020

1.12 Routes of viral shedding and excretion dynamics

Two important aspects to consider when assessing the risk of disease transmission are possible routes of viral shedding and the excretion dynamics of the virus (Mortlock et al. 2019; Letko et al. 2020). The possible routes of viral shedding suggest the most likely activities during which viral excretion may occur while knowing the excretion dynamics can highlight key periods through the year where viral shedding and risk of transmission are likely to be higher (Mortlock et al. 2019; Letko et al. 2020). Longitudinal sampling of a host population is likely the best way to assess excretion dynamics as it entails the sampling of a single population over multiple time points (Becker et al. 2019). Longitudinal sampling, however, has limitations as it can be expensive to conduct longitudinal surveys and require significant effort for sample collections (Plowright et al. 2016; Becker et al. 2019). As such, there can be a trade-off between sampling across a wide spatial area which would facilitate geographical mapping of risk populations/areas compared to sampling over a longer temporal scale that would provide information regarding viral dynamics for a given period (Becker et al. 2019). Longitudinal studies can also provide information regarding the specific infection dynamics for a given host species. For example, the findings by Plowright et al. (2008) suggested that immunity against Hendra virus infection does not last long in little red flying fox (*Pteropus scapulatus*) populations compared to other flying fox populations. This has important implications for the emergence or re-emergence of the pathogen and the little red flying fox's role as a host. The waning of maternal antibodies and innate immunity are suggested to be prominent factors influencing viral dynamics within host populations and may play a role in viral maintenance over long periods (Plowright et al. 2008; Breed et al. 2011; Plowright et al. 2016).

For the published studies that identified zoonoses or viruses with zoonotic potential in South Africa (Jansen van Vuren et al. 2018; Paweska et al. 2018; Mortlock et al. 2019; Paweska et al. 2020; Mortlock et al. 2021), longitudinal sampling helped identify periods throughout the year with higher potential risk for viral transmission and spillover for Marburg virus and various paramyxoviruses (Paweska et al. 2018; Mortlock et al. 2019; Mortlock et al. 2021). The studies that detected Marburg virus in the *R. aegyptiacus* colony revealed a noticeable period when juvenile seropositivity was significantly lower than adults that may suggest a period of higher risk of infection and shedding (Paweska et al. 2018; Paweska et al. 2020). Differences in Marburg virus prevalence between new pups and juveniles up to seven months old suggest that maternal antibodies prevent infection early which could explain the decreased seropositivity observed by Paweska et al (2020) as passive immunity begins to wane (Amman et al. 2012). Viral shedding has been observed in saliva as well as being detected in rectal swabs (Amman et al. 2015b; Paweska et al. 2020). The presence of viral shedding through saliva suggests fruit discarded by foraging bats is a significant route for potential transmission especially given that viable Marburg virus can still be detected on fruit six hours after inoculation (Amman et al. 2021). The studies that detected a high diversity of paramyxoviruses within the *R. aegyptiacus* colony report distinct peaks in viral shedding during winter and spring coinciding with waning maternal antibodies, the beginning of the birthing season and lactation (Mortlock et al. 2019; Mortlock et al. 2021). These peaks correspond to periods when bats may experience increased energetic constraints and nutritional stress either through reduced fruit availability (Jacobsen and du Plessis 1976) or reproduction which increases the energetic burden on females and may translate to immunosuppression due to increased stress (Markotter et al. 2020). An important finding regarding the identification of viruses similar to HPIV-2 and MuV was the detection of these viruses in individual and pooled urine samples, suggesting urinary excretion may act as a possible route of transmission (Mortlock et al. 2019). By identifying periods of higher risk of viral infection or shedding as well as the potential routes of viral shedding, we can better understand the dynamics of transmission risk, especially if combined with movement data delineating areas that are frequented by the bats.

1.14 Study rationale and brief background

Bats belong to the order Chiroptera and makeup approximately 20% of all mammalian species (Burgin et al. 2018). They are the only group of mammals capable of self-powered flight and exploit a wide diversity of niches, occurring on six continents within all habitat types excluding polar regions and extreme deserts (Monadjem et al. 2020). They are known to host a high diversity of viruses including Marburg, Hendra, and Nipah viruses that are responsible for several outbreaks in humans with varying case fatality rates (Murray et al. 1995; Chua et al. 2000; Towner et al. 2009). Intensification of viral biosurveillance in bats paired with advancements in nucleic acid sequencing techniques has resulted in an increasing diversity of novel bat-derived viruses being detected (Tan et al. 2021). However, the zoonotic potential of these viruses is largely unknown and viral risk assessments have historically lacked expertise and perspectives from other fields that may critically influence the assessment of spillover risk (Bird and Mazet 2018).

From an ecological perspective, one important factor that can influence the risk of pathogen transmission and spillover is the movement patterns of a viral host (Dougherty et al. 2018; McClure et al. 2020; Randhawa et al. 2020). Several components of a species' movement patterns can also be investigated such as daily or nightly foraging movements, seasonal movements or home range sizes (Breed et al. 2010). Foraging movements and home ranges specifically can be used to identify critical areas utilized by the study species that could feasibly translate to interfaces for the contact between species and potential spillover (Randhawa et al. 2020). As such, the integration of ecological movement data with virological data can provide a geographical and potentially temporal scale for an increased likelihood of contact and spillover risk (Randhawa et al. 2020; K. Zhao et al. 2022).

Rouesttus aegyptiacus is one species that has been associated with several viral species (Jansen van Vuren et al. 2018; Pawęska et al. 2018; Mortlock et al. 2019; Markotter et al 2020; Mortlock et al. 2021; Ramanantsalama et al. 2022). In South Africa, and specifically a colony from Matlapitsi cave, Limpopo, these bats have been associated with Marburg virus (Pawęska et al. 2018), rubula- and henipa-like viruses (Mortlock et al. 2019; Mortlock et al. 2021) and adenoviruses (Jansen van Vuren et al. 2018). In terms of the movement patterns for this bat in South Africa, only two studies have been performed (Jacobsen et al. 1986; Barclay and Jacobs 2011) but the movements for the colony from Matlapitsi cave have never been assessed and the most recent movement study for *R. aegyptiacus* in Limpopo occurred more than 40 years ago (Jacobsen et al. 1986).

1.14.1 Problem statement

Bats have an almost ubiquitous global distribution and due to anthropogenic expansion are experiencing encroachment into their natural habitats that is causing the distributions of bats and people to overlap (Jung and Threlfall 2016). They are a highly diverse order exhibiting multiple unique characteristics that influence their flight capabilities, movement patterns and range requirements (Voigt et al. 2017). There is evidence that the ecological traits of some insectivorous bats are linked to their nightly foraging distances and spatial requirements (Laforge et al. 2021), but whether these correlates are applicable to the diversity of species around the globe is unknown. Identifying if certain ecological or biological traits are linked to contrasting spatial requirements may aid with predictions of a species' potential range of impacts. The prominence of bats as viral hosts (Olival et al. 2017), the proximity many bat species share with humans and their movement patterns are cause for concern and warrant further investigations into the potential for contact and spillover risk. In Limpopo Province, South Africa a colony of *R. aegyptiacus* is an example where a known viral host (Pawęska et al. 2018) occurs near human populations. The cave roost is situated approximately 500 m from human dwellings (Figure 1) and human residents commonly cultivate fruiting trees on their properties which could serve as supplementary foraging sites for the bats. These bats are known hosts of Marburg virus (Paweksa et al. 2018; Pawęska et al. 2020) which has been shown capable of remaining on discarded fruit up to six hours after contact (Amman et al. 2021). Therefore, the presence of *R. aegyptiacus* foraging in human-occupied areas could result in direct or indirect contact with people and domestic animals and may constitute a risk of spillover. However, the risk of contact between bats and people is unknown as the small-scale movement patterns for this colony have never been assessed and therefore, it is unknown whether these bats exploit areas occupied by humans, potentially facilitating spillover and transmission.

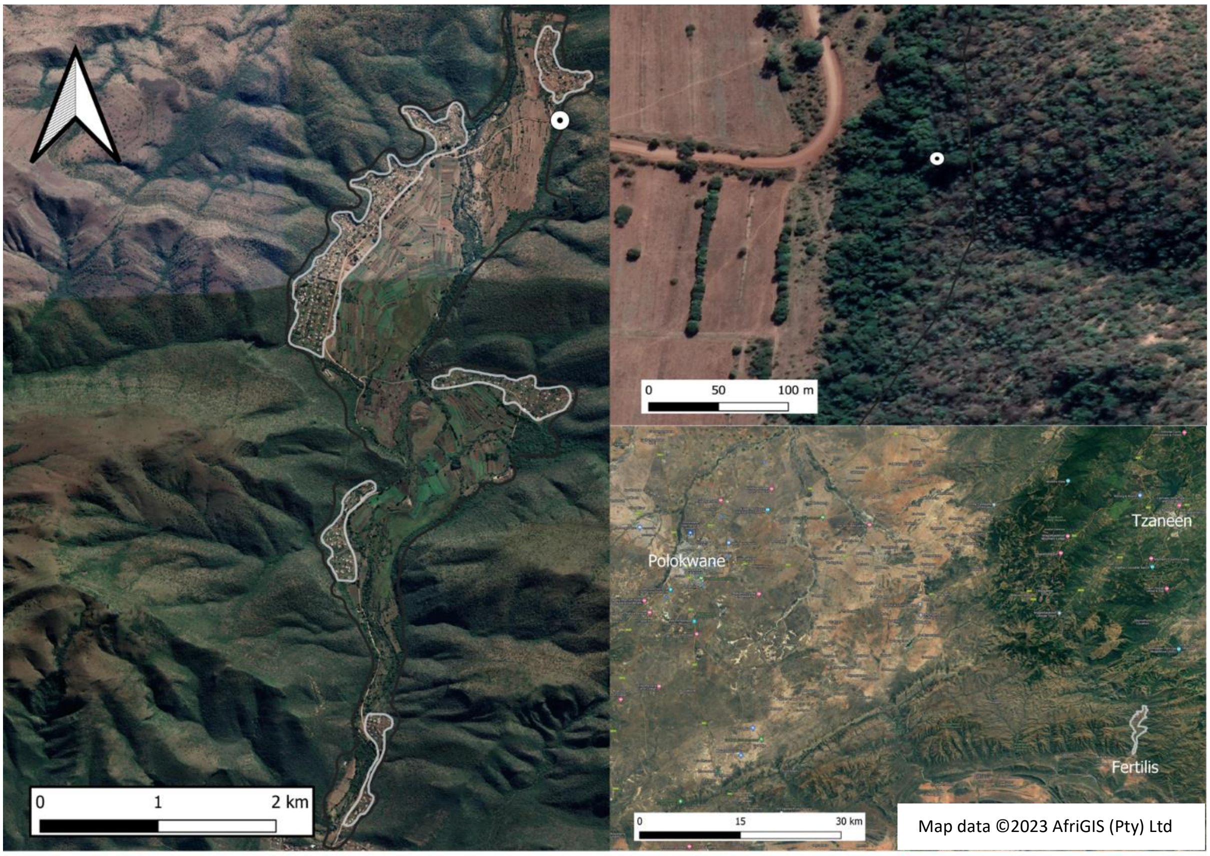


Figure 1: Satellite view of the Fertilis valley with the five major residential areas highlighted by the white encircled areas (left). Matlapitsi cave, as denoted by the white point, is situated in dense natural vegetation but is within 500 metres of the nearest residential area (top right). The study site is located approximately 30 kilometres south of Tzaneen and approximately 100 kilometres southeast of Polokwane (bottom right)

1.14.2 Research questions

- Can bat home range size be linked back to species-specific ecological and biological traits?
- Can the nightly foraging movement patterns of bats influence the risk of disease transmission through contact with people?
- Do the foraging movement patterns of *R. aegyptiacus* within the Fertilis valley vary on a seasonal basis in line with the fruiting periods for different tree species?
- Is there a risk of contact between *R. aegyptiacus* and people living in the rural settlements of the Fertilis valley in Limpopo Province, South Africa?

1.14.3 Research objectives

This study investigated the movement patterns of bats, different variables that may influence their movements and how their movements may translate to an increased risk of contact with people and by extension transmission risk of pathogens. The objectives of this research are as follows:

1. Identify ecological and biological variables that may influence movement patterns and bat home range size (Chapter 2)

- Systematic review of literature pertaining to bat home ranges to identify variables hypothesized to influence home range size.
- Compile published home range data from species around the globe and analyse through a meta-analysis to determine which variables are associated with bat home range size.

2. Assess the movement patterns of an *R. aegyptiacus* colony relative to nearby human population to determine whether there is the risk of contact (Chapter 3)

- Track the movement patterns of *R. aegyptiacus* around a rural settlement using radiotelemetry.
- Determine whether the movements vary across a seasonal gradient and follow fruiting patterns for fruit trees.
- Assess the risk of contact between bats and humans by plotting the recorded movements of *R. aegyptiacus* against residential areas to look for areas of spatial overlap.

Chapter 2: Critical review and meta-analysis of correlates of home range size in bats

(Formatted for publication in Journal of Mammalogy)

Critical review and meta-analysis of correlates of home range size in bats

Matthew R. Wood[1], J. Low de Vries[1], Ara Monadjem[2, 3], Wanda Markotter[1, *]

[1] *Centre for Viral Zoonoses, Department of Medical Virology, University of Pretoria, Pretoria 0001, South Africa*

[2] *Department of Biological Sciences, University of Eswatini, Kwaluseni M201, Eswatini*

[3] *Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa*

* Corresponding author

Email: wanda.markotter@up.ac.za

2.1 Abstract

Home range is an ecological concept that affects many aspects of the life of vertebrates and hence understanding how it varies between species is crucial. Mammalian home range size has been linked to body size and diet, but these studies were based on terrestrial species only and specifically excluded bats. As the only group of flying mammals, bats experience distinctly different constraints on movement, and hence home range. However, despite their diversity, relatively little is known about the home ranges of bats, and this is the first global review assessing correlates of their home range size. Our hypothesis is that home range will be impacted by different ecological conditions experienced by the bats and by the biological traits of the bats themselves. We performed a meta-analysis based on published data for 81 bat species to identify variables that contribute most to home range size. Sex, wing loading, functional group, colony size, dietary class, distance from the equator (latitudinal region), habitat type, and the interaction between habitat type and latitudinal region were all important variables explaining differences in the home range of bats. Wing loading was positively associated with their home range size, while females, open-air foragers, large colony sizes, and bats in temperate regions consistently had large home ranges. Understanding the correlates of home range has important implications for example for bat conservation and for assessing the risk of pathogen spillover of zoonotic pathogens from bats to humans and livestock.

Keywords: Chiroptera, colony size, ecological interactions, functional group, morphology, movement patterns

2.2 Introduction

Movements of animals within and between different areas occur daily while searching for *inter alia* food, mates, water sources or resting sites. Various factors may influence the decision to remain in an area, but whether animals show fidelity to one area or move frequently varies between species. When an animal shows a degree of site fidelity, remaining in a restricted area for prolonged periods, it is defined as a home range (Powell 2000; Powell and Mitchell 2012). The concept of a home range was initially introduced by Burt (1943) defining it as "that area traversed by the individual in its normal activities of food gathering, mating and caring for young". The basis of establishing a home range is that the area will provide benefits to the individual, such as reliable resources. However, home ranges are not equivalent among all species or individuals of the same species (Gompper and Gittleman 1991), and it has been hypothesised that a myriad of factors, ranging from seasonal food availability to body mass, are responsible for variability in home range sizes (McLoughlin and Ferguson 2000).

Being able to quantify the home range of an animal is important for understanding its potential ecological role within the ecosystem (Abedi-Lartey et al. 2016) as well as the negative implications of its presence in an area, for example as a host to a zoonotic disease (Epstein et al. 2020). The presence of a species that is a known host of zoonotic pathogens may pose a risk of transmission to intermediate hosts and people if there is spatial and temporal overlap with the host's distribution (Breed et al. 2010; Rulli et al. 2021). Home range analysis will help delineate the extent of a species' range requirements and the overall range of potential impact but analysing the patterns of a species' range use within that home range may assist in identifying regions where spatial and temporal overlap may occur (Randhawa et al. 2020; K. Zhao et al. 2022).

One overarching trend among mammalian species is that home range size scales allometrically with body mass according to the equation $HR = aM^b$ (McNab 1963) where HR represents home range, M represents body mass, a represents a species-specific, normalisation constant and b represents the scaling exponent giving the relationship between body mass and home range size (Swihart et al. 1988; Noonan et al. 2020). Larger animals require more resources to satisfy their energetic requirements and occupy larger areas (Kelt and Van Vuren 2001). In addition, resource distribution and availability are two features, unrelated to body mass, that can also impact home range size (Ford 1983; Boutin 1990). Resource acquisition is an integral component of survival for animals, and they should thus adapt the size of their ranges as resource availability changes. Boutin (1990) reviewed studies looking at the effect of food supplementation on home range size and showed that mammals inhabiting areas with higher resource availability had smaller average home ranges than mammals without food supplementation. This trend supported a model by Ford (1983), that predicted the home ranges of central-placed foragers would fluctuate in size according to temporal changes in resource availability and density, with home ranges being smaller in resource-rich compared to resource-poor areas. Habitat can also contribute to the variation in home range size as open areas with patchily distributed resources require animals to maintain larger home ranges than those living in more homogenous situations (Ofstad et al. 2016). Finally, sex and species-specific mating strategies also affect home range (Henry et al. 2005), with polygynous males tending to have larger home ranges than females as they travel widely in search of females, with the opposite expected for polyandrous species (Priotto et al. 2002; Henry et al. 2005).

Bats belong to the order Chiroptera, and despite being the second-largest mammalian order (Altringham 2011), have received little attention regarding their home ranges. They are unique among mammals as the only group capable of self-powered flight (Gunnell and Simmons 2005), however, flight is energetically costly (Voigt et al. 2017) and bats experience unique energetic constraints associated with movement. In addition, bats exhibit a wide variety of diets including frugivory, insectivory, carnivory and sanguivory (Kunz 2013a), and exploit a diverse array of habitats and ecological niches. Given their unique ecology and diversity, the home range of bats may be influenced differently by the aforementioned factors compared with other mammals in addition to being constrained by order-specific characteristics.

Body mass is expected to scale with home range size for bats, similar to non-volant mammalian species (Swihart et al. 1988; Kelt and Van Vuren 2001), and Fenton (1997) predicted that larger bats would travel further compared to smaller bats. This was based on the observation of a limited number of species, most of which weighed more than 15 g and therefore excluded many smaller bat species. A recent review reported that the home ranges of European and North American bats were positively associated with body mass (Laforge et al. 2021).

Flight patterns and behaviour in bats are dictated by wing aerodynamics (Norberg and Rayner 1987), and two important metrics influencing flight patterns are aspect ratio and wing loading. Aspect ratio is the ratio of the wingspan to the wing surface area, while wing loading is the ratio between body mass and wing surface area (Aldridge and Rautenbach 1987; Norberg and Rayner 1987). Differences in aspect ratio and wing-loading of bats result in a trade-off between the cost of transport, flight speed, and manoeuvrability and thus determine the habitats available to different species (Norberg and Rayner 1987). For bats with high aspect ratio and wing loading, high-speed flight is a requirement to generate sufficient lift (Voigt and Holdried 2012) and, consequently, these bats are restricted to foraging in open areas with few obstacles as manoeuvring at lower speeds incurs a high energetic cost (Norberg and Rayner 1987). For example, the Brazilian free-tailed bat, *Tadarida brasiliensis*, specifically forages at high altitudes in open areas where it utilises its high flight speed hunting aerial insects (Wilkins 1989). Alternatively, bats with low wing loading and aspect ratio have slow flight speeds but manoeuvre efficiently in cluttered space (Norberg and Rayner 1987) such as the woolly false vampire bat, *Chrotopterus auritus*, which hunts in wooded situations (Vleut et al. 2019). It has been suggested that wing morphology also impacts home range size (Aldridge and Rautenbach 1987) with Laforge et al. (2021) recently providing support for this idea in their review. Species adapted to open spaces are typically capable of long-distance travel at reduced energetic costs given that “a long, narrow wing works better than a short, wide one with the same area and profile, because it develops more lift, and less induced drag at the same angle of attack” (Pennycuick 2008, p. 68). Hence, these species are more likely to occupy larger home ranges (O’Donnell 2001; Noer et al. 2012), while the opposite appears to be the case for clutter specialists (Monadjem et al. 2009).

Kelt and Van Vuren (2001) demonstrated that non-volant carnivorous mammals have larger home ranges than non-volant omnivorous or herbivorous species of comparable body mass (Carbone and Gittleman 2002; Tucker et al. 2014), but this trend remains untested for bats. Given that frugivorous and nectarivorous bats target stationary food items that are located on randomly distributed fruiting or flowering trees, their home ranges typically vary as resources are depleted or renewed (Fahr et al. 2015). But may also depend on landscape structure, as fragmented habitats can affect the presence and distribution of fruiting or flowering trees (Quesada et al. 2004). Furthermore, their home ranges may differ between seasons as resource abundance fluctuates (Lučan et al. 2016). Insectivorous and carnivorous bat species target prey items that, similarly to fruit availability, fluctuate in abundance on a seasonal basis, but are also mobile and, therefore, variable in space and time (Conenna et al. 2019). Therefore, their movement patterns are reliant on the temporal availability of prey items but also different hunting strategies, which may result in variable use of space, distances flown, and home range size (Meyer et al. 2005). Bats are typically assigned to three functional groups based on their foraging habitats: clutter, edge and open-air foragers with each group possessing specialised flight characteristics and echolocation systems to best exploit cluttered, clutter-edge or open areas (Schnitzler and Kalko 2001). Species that hunt in cluttered space are adapted for highly manoeuvrable but slow flight that is energetically inefficient for long-distance flights, whereas species foraging in open spaces or along edge habitats are adapted for high-speed flight at the cost of manoeuvrability (Norberg and Rayner 1987; Abbott et al. 2012) and therefore are more likely to occupy larger areas since long-distance flights are less energetically expensive for them.

Bat species roost in a spectrum of social groups ranging from large colonies, which can number in the tens to hundreds of thousands, to roosting solitarily, with most species falling somewhere in between (Kunz 2013b). A colony has been defined as a group of bats forming a social unit within the confines of a specific roost site or several adjacent roost sites (Kunz et al. 2009). For species roosting in large colonies, higher levels of intraspecific competition are predicted to result in increased dispersal distances during foraging bouts, leading to large home ranges (Meyer et al. 2005). In a study focusing on several colonies of different sizes, flight distances and home ranges were greater for individuals from the larger colonies (Acharya et al. 2015). In contrast, Calderón-Capote et al. (2020) showed that distances travelled by straw-coloured fruit bats, *Eidolon helvum*, while foraging were not strongly influenced by colony size. Individuals often switched roost sites to occupy highly productive areas rather than return to a single roost site and increase commuting distance, although foraging flights still ranged from 10 – 100 km regardless of colony size, representing extensive commuting distances. By comparison, solitary species such as the Hawaiian hoary bat (*Lasiurus cinereus semotus*) may defend small areas with key foraging patches (Bonaccorso et al. 2015) resulting in smaller home ranges.

Mammalian home range size is predicted to differ between sexes due to different daily activity patterns and energy requirements with females more strongly influenced by the energetic constraints of reproduction (O'Donnell 2001; Henry et al. 2002) while males are influenced by mate availability and could limit movement distances to defend females or roost sites (Meyer et al. 2005). However, no clear trend is thus far evident for bats as Safi et al. (2007) recorded male parti-coloured bats, *Vespertilio murinus*, having significantly larger home ranges than females, while Meyer et al. (2005) suggest that female long-legged bat, *Macrophyllum macrophyllum* home ranges are likely significantly larger than males. Dechmann et al. (2014) also showed no significant differences in home range size or habitat use between males and females, but Mackie and Racey (2007) identified varied use of different habitat types by females of different reproductive conditions.

The specific landscape in which a bat occurs can influence its movement patterns as the landscape structure dictates resource abundance and distribution and may impose limitations on movements by creating ecological or geographical barriers (Wickramasinghe et al. 2003; Fuentes-Montemayor et al. 2013). Areas that have been fragmented by anthropogenic activities can increase landscape heterogeneity and the corresponding availability of food or roosting sites (Williams et al. 2006; Hahn et al. 2014; Egert-Berg et al. 2021). However, disturbances more commonly result in isolated resource patches and an overall decrease in the availability of resources, forcing bats to travel further each night to obtain sufficient resources (Quesada et al. 2004). Latitude may also play a role in movement patterns as higher latitudes (i.e. distances further away from the equator) experience greater seasonal variation in conditions compared to tropical areas which may influence local resource conditions (Fleming and Eby 2003). The dramatic changes between seasons and the corresponding resource availability may influence movements as bats must adjust to compensate for increased or decreased resource availability (Richter and Cumming 2006; Páez et al. 2018). Given the more pronounced seasonal variation in temperate regions, movement patterns may be more variable among temperate species compared to tropical species (Fleming and Eby 2003).

Home range size may be influenced by specific methodological variables such as the mass of the transmitter relative to the tagged individual (Aldridge and Brigham 1988) as bats burdened with excessively heavy tags may experience difficulties maintaining normal flight patterns (O'Mara et al. 2014). Meanwhile, the number of locations obtained may influence the statistical power of the estimate, whereas the different estimation methods used may give variable results (Laver and Kelly 2008; Noonan et al. 2019).

Home range studies provide insight into the movement patterns of bats and their potential interactions with other species. As bats provide several ecosystem services and are hosts for potential zoonotic pathogens, their movements carry importance for ecosystem well-being but also affect the risk of pathogen spread and disease transmission to people (Kunz et al. 2011; Letko et al. 2020). Therefore, understanding how their movements are affected by ecological and biological variables is vital to delineating their importance across different fields (Randhawa et al. 2020). Laforge et al. (2021) reviewed variations in bat home range size, but only for North American and European species and the review only assessed the effect of landscape composition, body mass and wing morphology and did not include important variables such as colony size, functional group, or diet. Therefore, this review aims to assess how ecological and biological variables are associated with home ranges for bats on a global scale and determine whether there are any consistent patterns that could prove useful for future studies looking at general movements for bats as well as their associated impacts on the environment.

Our hypothesis is that home range will be influenced by different ecological conditions experienced by the bats and by the biological traits of the bats themselves. In particular, we predict that: 1) aspect ratio and wing loading will be positively associated with home range size; 2) larger bats will have larger home ranges; 3) species roosting in larger colonies will have larger home ranges; 4) open-air foragers will have larger home ranges than edge and clutter foragers; 5) females will have larger home ranges than males; 6) disturbed landscapes will contribute towards larger home ranges than natural landscapes; 7) bats from temperate regions will have larger home ranges than those from tropical regions.

2.3 Materials and Methods

Data collection. —We searched for peer-reviewed publications including journal articles, review papers, and book chapters on home range sizes of bats published around the globe up until the end of November 2022 following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al. 2009). Searches were performed using the Institute for Scientific Information’s Web of Science database, ScienceDirect, Scopus, JSTOR, and Google Scholar with the following keywords: “Chiroptera” OR “bats” AND “home range” OR “foraging range” OR “movement ecology” OR “movements” OR “telemetry” OR “radio-tracking” OR “GPS tracking”. From these searches, we identified species for which home range had been reported. We reviewed the publications by reading the abstracts, or full articles where necessary, and excluded articles that did not report home range sizes. Of the publications that did report home range size, the decision to further retain or exclude an article was made based on the home range data reported as only articles in which home range data were reported for the species at an individual level were retained for the analysis. Therefore, publications where home range size had been reported as a mean for multiple individuals or as a colony estimate were excluded. The decision to only include home range statistics reported at the individual level was to allow for meaningful comparisons between sexes or where studies included multiple study sites with different landscape types and vegetation mosaics. Where needed, further searches to obtain supplementary data such as colony size, functional group, and wing morphology metrics for the individual species obtained from the literature search were performed using the keywords “foraging ecology”, “mammalian species”, “wing morphology” or “population ecology” paired with the species name. Taxonomic classifications for the species included in the dataset were confirmed using the Mammal Diversity Database (MDD 2022) and the ‘Bats of the World’ database (Simmons and Cirranello 2022). There were no cases of ambiguous species classifications in our dataset as any cryptic species had had their taxonomic classifications resolved by the time of this study. All searches were performed using the current species name and all previous names if there had been a taxonomic revision. All publications not written in English were translated to assess their relevance for the analysis.

Statistical analysis. —We extracted information regarding the habitat and landscape of the study area, the latitudinal region in which the study occurred, body mass, sex, diet, functional group, colony size, wing morphology, and home range size of the bats directly from the selected articles. Home range measurements were reported using either hectares (ha) or square kilometres (km^2) but were all converted to hectares before analysis to ensure comparability across the same unit. Habitat type and landscape classifications for the study area were further supplemented with data from worldwide habitat type ranges (Jung et al. 2020) and by satellite imagery from Google Earth respectively. The habitat types were defined using the definitions given in Jung et al. (2020) and were identified by comparing the global vegetation maps against the locations of the sites for the home range studies. Landscapes were assessed using the historical imagery feature to determine landscape structure and cover during the respective study periods. Categorical variables were divided into separate classes (Table 2). We had initially wanted to include data on the reproductive status and age of the bats, and the season in which each study was performed, however, due to inconsistent reporting among publications, these variables could not be included in our analyses.

Table 2: Summary of the categorical variables and respective classes used in Linear Mixed Models for the home range analysis

Variable	Classes [a]
Diet	Frugivore; Nectarivore; Insectivore; Carnivore
Functional group	Clutter forager; Edge forager; Open-air forager
Habitat	Arid; Forest; Savanna
Landscape	Disturbed; Natural
Region	Temperate; Tropical
Colony size	Solitary; Small colony; Large colony
Sex	Male; Female
Family [b]	Furipteridae; Hipposideridae; Megadermatidae; Miniopteridae; Molossidae; Mystacinidae; Myzopodidae; Noctilionidae; Nycteridae; Phyllostomidae; Pteropodidae; Rhinolophidae; Vespertilionidae
Home range [b] estimator	Minimum Convex Polygon; Kernel Density Estimation; Local Convex Hulls; Anderson Fourier

[a] For more detail on how the variables were defined refer to the paragraph below

[b] Family and home range estimators were included as random effects in the analysis

The sex of the bats was obtained from the corresponding articles. Habitats were broadly defined as ‘arid’, ‘forest’ or ‘savanna’ as bats from these ecoregions tend to be ecologically quite different. A more specific definition of habitat was not possible due to the inconsistent way in which this was described in the different papers, however, since this review is focused on a global scale, the use of such broad definitions is necessary as a finer-scale classification would result in too many variables for reliable statistical comparisons. The landscape for the study areas was defined as either ‘natural’ or ‘disturbed’ based on the descriptions given and historical satellite imagery, with ‘disturbed’ areas being considered as areas in which agricultural practices were present, intensive vegetation management is ongoing, or urbanisation had occurred. The classification of a ‘disturbed’ site was based on the proportional habitat availability described in the home range studies. Areas where the majority (>50%) of the landscape has been transformed to either agricultural fields, grazing lands or urban areas were considered as disturbed. The scope for each study site was defined by the home range sizes of the bats in the study and were therefore, unique for each study site. Region refers to the latitudinal distance from the equator and was defined as ‘tropical’ for studies performed between the latitudes of 23.5° N and 23.5° S, or ‘temperate’ for studies performed further north or south of 23.5° N and 23.5° S respectively. The diet and functional group for each species were identified from supplementary literature pertaining to that species where that information was not included in the home range publication. Dietary classes were broadly defined as insectivore, carnivore, frugivore and nectarivore, although there are instances where species exhibit generalist dietary requirements such as some phyllostomids that include fruit, nectar, and insects in their diet in varying proportions (Stevens and Amarilla-Stevens 2021). For example, the great fruit-eating bat, *Artibeus lituratus*, is primarily frugivorous but molecular analysis has revealed the inclusion of insects in the diet as well (Ingala et al. 2021). Therefore, dietary classifications were based on the primary food items targeted by the different species. Bat species were assigned to functional groups according to the definitions given by Schnitzler and Kalko (2001) of clutter, edge and open-air foragers. Colony sizes were defined as solitary, small colony (less than 100 individuals) or large colony (more than 100 individuals) similar to the classifications used by (Tournant et al. 2013), with the word “colony” used when the relevant publication used this term.

A phylogenetic generalised least-squares (PGLS) regression was run to search for a phylogenetic signal pertaining to home range size in the dataset using the mammalian phylogeny generated by Upham et al. (2019). As we were interested in the potential effect of phylogeny on home range size, given the variables in the dataset, we constructed the PGLS using body mass, aspect ratio, wing loading, and all categorical variables (Table 2) as predictors with home range size as the response (Mundry 2014). We ran two separate PGLS regressions using the ‘caper’ and ‘ape’ packages in R (Paradis and Schliep 2019; Orme et al. 2018) to estimate the maximum likelihood of the parameter lambda (λ). Lambda is a metric that scales from 0 to 1 (0 representing no phylogenic signal, 1 representing a strong phylogenic signal and one can assume evolution by a Brownian motion model where trait data are fully explained by phylogeny) and is used to determine the extent to which phylogeny explains the variation in a dataset (Symonds and Blomberg 2014). Both PGLS regressions demonstrated that there was no phylogenic signal in the estimates of home range; $\lambda= 0$, meaning the effect of the different covariates on home range size was not biased by phylogeny (Revell 2010). Therefore, we performed linear mixed models (LMMs) to determine which variables had an influence on home range size. Home range estimates were log-transformed to account for outliers and achieve an approximately normal distribution. Although data were not normally distributed post-transformation (Shapiro-Wilk: $w = 0.997$; $p = 0.008$), regression models with a Gaussian distribution have been shown to be robust to violations of normality (Knief and Forstmeier 2021). Body mass was also log-transformed to account for high variability within the dataset.

We assessed for potential collinearity between variables in the full model using generalised variance inflation factors (GVIF) from the ‘car’ package in R (Fox and Weisberg 2019). Upon the recommendations by Fox and Monette (1992), we used the squared ratio generalised variance inflation factor, $GVIF^{1/(2 * df)}$, which enables effective comparisons for variables with multiple coefficients. We included wing loading, aspect ratio, log of body mass, diet, functional group, colony size, habitat type, sex, region and landscape as fixed effects. Interaction terms were considered for inclusion in the models only if they carried biological relevance that may influence home range size variation. Two interaction terms were considered: firstly, between body mass and sex to address the potential effect of sexual dimorphism on home range size and secondly, between habitat type and latitudinal region due to potential regional variability in vegetation structure and, or resource availability between similar habitat types. We investigated whether the number of locations, proportional tag mass or statistical methods influenced home range estimates. Unfortunately, the number of locations obtained for each bat was inconsistently reported among publications and was not available for a large proportion of the dataset, therefore, it could not be assessed. The proportional mass of the transmitters deployed on the bats was, however, available and we included it as a covariate in the analysis. The best measure to assess its potential influence was to analyse it for species where multiple studies had been performed. Therefore, a separate analysis was performed using a subset of the dataset including only species with multiple home range studies. Due to the nature of the morphological predictor variables we collected (i.e. body mass, aspect ratio, wing loading), their variation is driven by phylogenetic relatedness. For instance, the average body mass for Pteropodidae was higher than that for Vespertilionidae. However, as they were predictor variables, we included family as a random effect in the LMMs to account for trends across the dataset due to phylogenetic relatedness. Four different home range estimation methods had been employed to calculate home range size in the different studies, which can confound comparative assessments given the lack of comparability between different statistical methods (Laver and Kelly 2008). However, as we had multiple species and families represented for each of the different estimation methods, we included home range estimator as a second random effect to control for potential variability between the statistical methods. Both family and home range estimator were included as random intercepts. Model selection was performed using the ‘AICcmodavg’ and ‘lme4’ packages in R (Bates et al. 2015; Mazerolle 2020) by generating all relevant candidate models and assessing the AICc (corrected Akaike Information Criterion) and ΔAICc values. The model with the lowest AICc value was selected as the best model, and any models that were within $\Delta AICc < 2$ were considered as competing (Burnham and Anderson

2004). To assess the reliability of the output from the top-ranked model, we performed a Bonferroni test assessing for the presence of outliers (Cook and Prescott 1981), although none were detected and overall model fit was assessed with the marginal and conditional R^2 values using the 'MuMIn' package in R (Barton 2020). Post-hoc Tukey tests were also performed for variables with >2 classes using the 'emmeans' package (Lenth 2022) to determine interclass differences. All analyses were performed in R v4.0.2 (R Core Team 2021) using the Rstudio v1.3.1073 (Rstudio Team 2021) interface and statistical significance was always assessed using an alpha of 0.05.

2.4 Results

The dataset. —Our literature search returned 4239 articles relevant to our search terms, which, after the removal of duplicate articles, conference results, non-peer-reviewed datasets, off-topic publications, or articles reporting home range at population or colony level, was reduced to 91 publications. Subsequent searchers for species-specific supplementary data included an additional 75 publications resulting in a total of 166 publications encompassing 1260 individuals from 81 species and 13 families for our final dataset with sample size per species ranging from 1 – 83 individuals (Supplementary Data SD1). Studies that were retained comprised peer-reviewed journal articles as well as a single unpublished report.

Summary of species collected in home range studies. —The species for which we obtained home range data were distributed across the six continents where bats are found: Africa (14), Asia (7), Europe (19), North America (18), Oceania (8), South America (15) and included insectivores, frugivores, nectarivores and carnivores. From the six continents, species were distributed across tropical (44) and temperate regions (37). In terms of representation across the different families, our species collectively belonged to 13 families: Furipteridae (1), Hipposideridae (1), Megadermatidae (2), Miniopteridae (1), Molossidae (2), Mystacinidae (1), Myzopodidae (1), Noctilionidae (1), Nycteridae (1), Phyllostomidae (22), Pteropodidae (17), Rhinolophidae (3), and Vespertilionidae (28). Home range sizes varied from 0.2 ha (Atacama myotis, *Myotis atacamensis*; (Rodriguez-Pinto et al. 2022)) to 124,413.8 ha (Straw-coloured fruit bat, *Eidolon helvum*; (Randhawa et al. 2020)).

Home range estimates and model selection. —There was no evidence of collinearity within the dataset with all GVIF^($^{1}/_{2 * \mathrm{df}}$) values <3 (Supplementary Data SD2), well below previously suggested values of 10 when assessing severe multicollinearity (Hair et al. 1995; O'Brien

2007). Model selection revealed the best performing model was one that included sex, wing loading, colony size, the interaction between habitat type and region, functional group, and diet with family and home range estimator as random intercepts ($R^2m = 0.29$; $R^2c = 0.81$) (Table 3). Residuals were not normally distributed according to statistical tests (Shapiro-Wilk: $w = 0.99$, $p < 0.001$) but Knief and Forstmeier (2021) suggest that these violations of normality do not impact the accuracy of estimates nor the significance of variables if sample sizes are sufficiently large ($n \geq 1000$). The separate analysis assessing the influence of proportional tag mass on home range size for the data subset revealed a negative relationship with home range size ($\beta = -0.20$; $p < 0.001$). When included in the top-ranked model, the proportional mass of transmitters on the tagged bats still had a negative influence on home range size ($\beta = -0.07$; $p = 0.001$), supporting the idea that tag mass may influence movement patterns (Aldridge and Brigham, 1988) and may bias home range estimates. However, its inclusion did not change the significance or effects of the remaining variables and therefore, did not influence the overall results (Supplementary Data S3). There were four competing models that included the interaction between body mass and sex, body mass as a separate covariate, and landscape in different combinations (Table 3).

Table 3: Summary of the top 10 candidate models generated during model selection with the full model for comparison with AICc scores, model weight (AICcWt) and parameters (K) examining the effect of biological and ecological variables on bat home range sizes

	Candidate models	K	AICc	ΔAICc	AICcWt
1	S + WL + C + H*R + FG + D	18	4075.60	0.00	0.24
2	log(m)*S + WL + C + H*R + FG + D	20	4076.30	0.70	0.17
3	S + WL + C + H*R + FG + D + L	19	4076.55	0.95	0.15
4	log(m)*S + WL + C + H*R + FG + D + L	21	4077.13	1.53	0.11
5	log(m) + S + WL + C + H*R + FG + D	19	4077.55	1.95	0.09
6	log(m)*S + AR + WL + C + H*R + FG + D	21	4077.98	2.38	0.07
7	log(m) + S + WL + C + H*R + FG + D + L	20	4078.38	2.78	0.06
8	log(m)*S + AR + WL + C + H*R + FG + D + L	22	4078.94	3.34	0.04
9	log(m) + S + AR + WL + C + H*R + FG + D	20	4079.21	3.61	0.04
10	log(m) + S + AR + WL + C + H*R + FG + D + L	21	4080.17	4.57	0.02
Full	log(m) + S + AR + WL + C + H + R + FG + D + L	19	4206.50	130.90	0.00

Variable definitions: m = mass; S = sex; AR = aspect ratio; WL = wing loading; C = colony size; H = habitat; D = diet; FG = functional group; R = region; L = landscape; species and home range estimator were included as random effects

* Denotes an interaction between variables

Wing loading, sex, functional group, habitat type, latitudinal region, the interaction between habitat type and region, colony size, and diet all influenced home range size (Table 4; Supplementary Data S4). None of the variables included in the competing models were shown to influence on home range size. Their inclusion also did not alter the signs or significance of the other covariate estimates except for models with the interaction between body mass and sex. For these models, sex no longer had a direct influence on home range size, and the estimate changed from a negative to a positive value, although its inclusion in the interaction likely caused this difference. Wing loading had a positive influence on home range size (Supplementary Data S5). Females had significantly larger home ranges than males. Temperate home ranges were significantly larger than in tropical regions, home ranges within savanna habitats were significantly smaller than in arid areas, and pairwise comparison revealed they were also significantly smaller than in forested areas (Table 5). However, since habitat type was involved in a significant interaction with latitudinal region, the interpretation of the pairwise comparisons for the main effects in isolation may not be reliable. Considering the interaction, home ranges were significantly larger within temperate forests and arid areas compared to tropical regions for these two habitat types, however, tropical savannas had significantly larger home ranges than temperate savannas. Species roosting in large colonies had significantly larger home ranges than both small colonies or species roosting solitarily, with no difference between small colonies and solitary species. Home ranges for open-air foragers were significantly larger than both edge and clutter foragers, while edge foragers also had larger home ranges than clutter foragers. Finally, carnivores were shown to have significantly larger home ranges compared to insectivores and frugivores but not nectarivores. Although, it should be noted that the small sample size for carnivores in our dataset (n = 34) likely influenced these results. There were no other differences in home range size between the other dietary classes (Table 5).

Table 4: Coefficient estimates, standard error, t-values and 95% confidence intervals for the best performing LMM assessing the effects of ecological and biological variables on bat home range sizes. Statistical significance was assessed with an alpha threshold of 0.05

Variable	Coefficient estimate	Standard error	t – values	95% CI
Intercept	**5.17**	**0.94**	**5.51**	**3.32 – 7.01**
Wing loading	**0.08**	**0.02**	**4.88**	**0.04 – 0.12**
Sex				
Female	[a] Ref lev			
Male	**-0.28**	**0.10**	**-2.72**	**-0.48 – -0.08**
Colony size				
Large	Ref lev			
Small	**-1.34**	**0.15**	**-8.69**	**-1.63 – -1.05**
Solitary	**-1.54**	**0.18**	**-8.67**	**-1.89 – -1.19**
Habitat				
Arid	Ref lev			
Forest	0.00	0.47	0.00	-0.92 – 0.92
Savanna	**-4.31**	**0.52**	**-8.26**	**-5.33 – -3.29**
Region				
Temperate	Ref lev			
Tropical	**-5.60**	**0.90**	**-6.25**	**-7.36 – -3.84**
Functional group				
Clutter	Ref lev			
Edge	**0.41**	**0.12**	**3.37**	**0.17 – 0.65**
Open-air	**1.60**	**0.18**	**9.10**	**1.25 – 1.95**
Diet				
Insectivore	Ref lev			
Frugivore	-0.01	0.28	-0.04	-0.56 – 0.54
Nectarivore	0.68	0.38	1.79	-0.06 – 1.42
Carnivore	**1.72**	**0.32**	**5.36**	**1.09 – 2.35**
Habitat_Forest * Region_Tropical	**4.10**	**0.98**	**4.19**	**2.18 – 6.02**
Habitat_Savanna * Region_Tropical	**10.09**	**1.02**	**10.66**	**8.09 – 12.09**

Significant variables given in bold.

[a] Reference level variable

Table 5: Coefficient estimates, standard error and 95% confidence intervals for post-hoc pairwise comparisons of functional group, colony size, and dietary class. Statistical significance was assessed with an alpha threshold of 0.05

Comparison	Coefficient estimate	Standard error	d.f.	t-ratio	95% CI
Clutter – Edge	**-0.41**	**0.12**	**1148**	**-3.34**	**-0.65 – -0.17**
Clutter – Open	**-1.60**	**0.17**	**1150**	**-9.02**	**-1.93 – -1.27**
Edge – Open	**-1.19**	**0.17**	**1150**	**-6.91**	**-1.52 – -0.86**
Large – Solitary	**1.54**	**0.18**	**1145**	**8.57**	**1.19 – 1.89**
Large – Small	**1.34**	**0.16**	**1153**	**8.59**	**1.03 – 1.65**
Small – Solitary	-0.21	0.15	1151	-1.34	-0.50 – 0.08
Insectivore – Frugivore	0.01	0.28	1148	0.04	-0.54 – 0.56
Insectivore – Nectarivore	-0.68	0.39	1122	-1.77	-1.44 – 0.08
Insectivore – Carnivore	**-1.71**	**0.32**	**1146**	**-5.32**	**-2.34 – -1.08**
Frugivore – Nectarivore	-0.69	0.30	1148	-2.29	-1.28 – -0.10
Frugivore – Carnivore	**-1.73**	**0.33**	**1150**	**-5.27**	**-2.38 – -1.08**
Nectarivore – Carnivore	-1.03	0.45	1142	-2.32	-1.91 – -0.15

Significant comparisons given in bold.

The above results (Table 4 and 5) show how some individual variables contribute towards home range size variation, however, investigating the different variables in conjunction provides a better picture of home range size variation and can highlight where different species fall along the continuum of home range sizes. As an example, large colony size, open-air foraging strategies and temperate regions all contributed towards larger home ranges (Table 4). By comparison, solitary species, clutter foragers and tropical regions had smaller home ranges. A comparison of home range sizes for species from our dataset, with these combinations of variables, was included to assess whether the variables that individually, positively influence home range size effected the same result when assessed simultaneously (Figure 2). The figure also shows the positive relationship between home range size and wing loading.

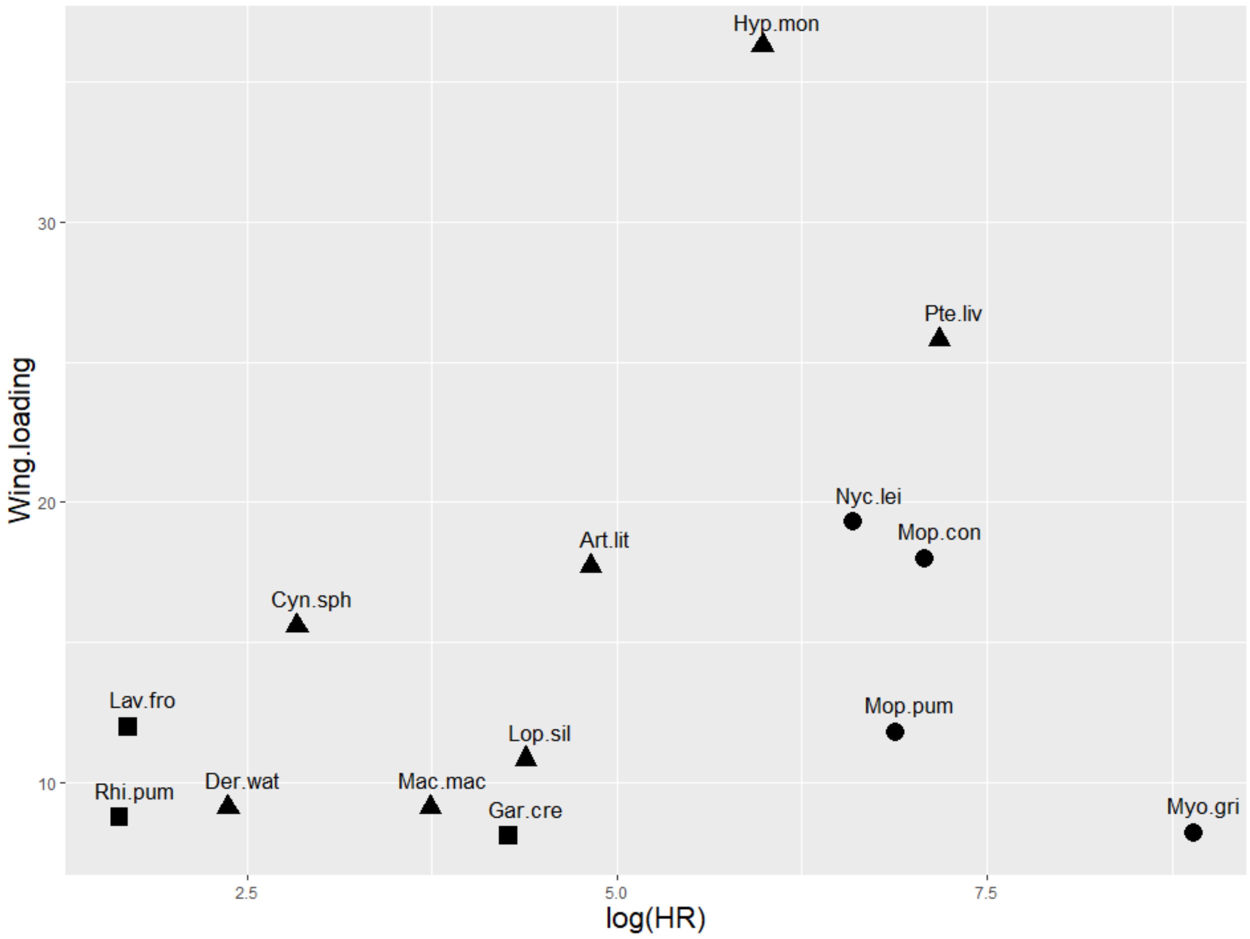


Figure 2: Graphical representation of the combined effect of colony size, functional group, and latitudinal region on home range size compared against wing loading. Circles represent open-air foragers, in large colonies from temperate regions: *Myotis grisescens* (Myo.gri), *Mops condylurus* (Mop.con), *Mops pumilus* (Mop.pum), *Nyctalus leisleri* (Nyc.lei). Triangles represent edge foragers, in small colonies from tropical regions: *Hypsignathus monstrosus* (Hyp.mon), *Pteropus livingstonii* (Pte.liv), *Artibeus lituratus* (Art.lit), *Lophostoma silvicolum* (Lop.sil), *Macrophyllum macrophyllum* (Mac.mac), *Cynopterus sphinx* (Cyn.sph), *Dermanura watsoni* (Der.man). Squares represent solitary, clutter foragers from tropical regions: *Gardnerycteris crenulatum* (Gar.cre), *Lavia frons* (Lav.fro), *Rhinophylla pumilio* (Rhi.pum)

2.5 Discussion

Referring back to our original predictions: 1) wing loading, but not aspect ratio was positively associated with home range size; 2) body mass did not affect home range size; 3) colony size was linked with home range size with larger colonies having larger home ranges; 4) open-air foragers had larger home ranges than both edge and clutter foragers; 5) females had larger home ranges than males; 6) level of landscape disturbance did not affect home range size; 7) bats from temperate regions had larger home ranges than bats from the tropics, but there were also habitat-specific trends present across latitudinal regions.

We found a weak, but positive association between the wing loading of bats and home range size. Wing loading is primarily linked to flight speed in bats, with higher wing loading correlating to higher flight speeds to generate sufficient lift (Norberg and Rayner 1987; Voigt and Holderied 2012). Higher flight speeds would enable bats to cover a greater distance in a shorter space of time and could translate to larger range sizes (Laforge et al. 2021). Contrary to our predictions, we did not find an association with aspect ratio, despite a higher aspect ratio corresponding with higher aerodynamic efficiency which may facilitate long-distance flight (O'Donnell 2001). Body mass was also predicted to have a positive effect on home range size as bats experience unique energetic constraints associated with flight (Thomas and Suthers 1972), which increase with higher body masses (Norberg and Rayner 1987; Fenton 1997). Therefore, it seems reasonable that larger bats would occupy larger home ranges to obtain sufficient resources to balance their energy budgets. Yet, body mass did not contribute to the top model and when it was included in competing models, it did not influence home range size. This contrasts with the findings of Laforge et al. (2021) and may be due to the higher diversity of species and families present within our dataset. Regardless, we similarly showed that wing loading was positively associated with home range size (Laforge et al. 2021).

Like most mammals, bats are hypothesised to experience sex-specific energetic constraints linked to reproduction that may influence movement patterns and range sizes (Henry et al. 2002). Several previous studies have reported contrasting results regarding sex-specific range requirements (Meyer et al. 2005; Safi et al. 2007; Dechmann et al. 2014), yet females consistently had larger home ranges than males in our dataset. Female bats experience stringent energetic constraints during pregnancy and lactation that males do not encounter and therefore may need to alter their foraging behaviours with one option being to expand their ranges over a large area to satisfy their increased energetic requirements (O'Donnell 2001; Henry et al. 2002). Similarly, the nightly activities may differ between sexes which could also influence movement distances. For instance, males were observed foraging closer to the day roost than females during the breeding season to defend females or potentially sneak copulation (Meyer et al. 2005).

The three functional groups, open-air, edge and clutter foragers, each have unique flight characteristics and patterns imposed by their wing shape and aerodynamic constraints (Norberg and Rayner 1987; Schnitzler and Kalko 2001). Examples for each group include Angolan free-tailed bats, *Mops condylurus*, which forage in open spaces over vegetation (Noer et al. 2012), soprano pipistrelles, *Pipistrellus pygmaeus*, which forage along the edge of tree lines

(Kirkpatrick et al. 2018), and Egyptian slit-faced bats, *Nycteris thebaica*, which forage in heavily cluttered areas (Monadjem et al. 2009). Open-air foragers, with their typically high aspect ratio and wing loading, experience greater drag at lower speeds, so faster flight is needed to achieve the optimum balance between energetic output and flight speed, but at the cost of manoeuvrability (Pennycuick 2008; Voigt and Holderied 2012). In contrast, clutter foragers typically possess shorter, rounder wings with a lower wing loading and aspect ratio, increasing manoeuvrability and making slower flight more economical, but limiting flight speed and distances travelled as the broader wings are less aerodynamically efficient (Norberg and Rayner 1987). These traits preclude open-air foragers from exploiting cluttered areas as manoeuvring through cluttered areas is not energetically viable while clutter foragers will typically avoid crossing large open areas as such commutes are energetically costly (Norberg and Rayner 1987; Voigt and Holderied 2012). There was a trend for home range sizes to increase in line with increasing requirements for open space. Open-air foragers had the largest home ranges followed by edge and clutter foragers. As open-air foragers typically possess above-average wing loading and aspect ratio (Norberg and Rayner 1987), they are constrained in their ability to utilise cluttered areas and must target open areas which can lead to increased travel distances to locate suitable foraging sites (Abbott et al. 2012). Clutter foragers are better adapted for slow, highly manoeuvrable flight in cramped spaces compared to open-air foragers while edge foragers represent an intermediate between the two extremes, capable of exploiting areas around background clutter (Abbott et al. 2012; Denzinger and Schnitzler 2013). The wing structures that enable manoeuvrability in clutter could inhibit long-distance commutes as these are not energetically viable, thereby encouraging the occupancy of a smaller overall range (Abbott et al. 2012).

There are fundamental differences among habitat types in terms of vegetation structure, density and heterogeneity that can influence resource distribution and availability (Egert-Berg et al. 2021). The specific vegetation structure within different habitats may limit the types of foragers present in these areas which may result in different bat assemblages with traits specifically evolved to exploit a particular habitat type (Fahr and Kalko 2011). As a result, there may be contrasting movement patterns for bats from these different habitats. There were differences in patterns of range size between different habitat types, but this was also influenced by latitudinal region. As latitude increases further north and south of the equator, seasonal variation in climatic conditions becomes more pronounced, which may influence resource availability and, thus movement patterns (Fleming and Eby 2003). Therefore, we might expect movement patterns to be more variable within temperate compared to tropical habitats which was the case for arid and forested areas in our dataset. The small sample size for temperate (n = 19) and tropical (n = 43) arid habitats may have confounded this effect, however, tropical forests are typically densely vegetated with high levels of structural complexity and species diversity (Bonaccorso et al. 2005). The structural complexity of tropical forests limits accessibility to species that are adapted for slower flight and higher manoeuvrability (Norberg and Rayner 1987; Schnitzler and Kalko 2001) while the dense concentration and diversity of suitable resources may enable species to satisfy their energetic requirements within a smaller area compared to temperate forests (Rothenwoehrer et al. 2011). For savanna habitats, there was a trend for home range to be larger in tropical regions than in temperate. This trend, however, may have been an artefact of sampling bias as all but one of the species from tropical savannas were from South America that has experienced extensive habitat fragmentation and alteration (Bernard and Fenton 2007) which may have influenced their movement patterns.

Colony size was an important predictor for variation in home range size. Large colony size was linked to larger home range areas, supporting previous hypotheses that intraspecific competition in colonial species drives wider dispersal to avoid potential resource competition (Meyer et al. 2005; Corman et al. 2016). Acharya et al. (2015) alluded to a similar trend where range size and flight distances were greatest for bats from larger colonies. There are, however, reports of bat movement distances that appear independent of colony size. For example, Calderón-Capote et al. (2020) show that *E. helvum* dispersal distances did not increase proportionately with increases in colony sizes. However, mean nightly dispersal distances still ranged from 10 – 100 km which are extensive movement distances for any bat species and may have confounded potential correlations with colony size (Calderón-Capote et al. 2020). Dietary class was also linked to home range size variation. Carnivorous bats had larger home ranges than insectivores and frugivores, although the limited number of carnivores may have masked the true differences between these groups as the small sample size is more sensitive to extreme values. The theory of whether diet influences home range size for bats has thus far remained untested and, although our results suggest carnivorous bats may require larger ranges, further investigations are required with greater representation of carnivorous species before such a conclusion can be drawn. Landscape status did not affect home range size, despite predictions that sparsely distributed resources in disturbed areas would force bats to travel further to reach isolated resource patches or to avoid undesirable areas such as urban centres or large, open agricultural fields (Quesada et al. 2004). However, responses to habitat fragmentation may be species-specific as habitat specialists are typically more susceptible to disturbance than generalists (Webala et al. 2019). Furthermore, the disturbance or alteration of natural habitats may benefit bats through increased heterogeneity that provides greater foraging opportunities year-round or potential roosting sites that could forego the need for larger ranges and travel distances (Hahn et al. 2014; Egert-Berg et al. 2021; Laforge et al. 2021). Similarly, bats foraging at high altitudes may be less susceptible to fragmentation as their flight paths allow them to circumnavigate areas of disturbance without being directly affected (Jung and Threlfall 2016).

As known viral hosts, the movement patterns of bats are potentially important for their role in pathogen spillover events (Breed et al. 2010; Dougherty et al. 2018). Most studies investigating emerging zoonoses in bats, however, do not consider movement data that could highlight potential areas of contact between species (Letko et al. 2020; Randhawa et al. 2020). Home range analysis can help provide a broad idea of the potential range over which contact may occur, however, a more useful measure may be to explore patterns of range use within a home range (Randhawa et al. 2020; McKee et al. 2021). Range use analysis, such as kernel density estimates that identify areas with higher intensity usage, could provide crucial information regarding specific regions of overlap and potential risk of contact for pathogen spillover (K. Zhao et al. 2022). Additionally, by assessing the patterns of range use within bat home ranges, researchers can gain more specific insight into which habitat features are vital to their survival to make informed conservation decisions (Kunz et al. 2003; Zeale et al. 2012).

Data were available for a relatively limited number of species given their overall diversity with >1400 known species (Simmons and Cirranello 2022), and there was relatively poor representation for species from Asia and Oceania. The different home range estimators used in the different studies brought in further complexity through the lack of comparability between methods (Laver and Kelly 2008). We addressed this by controlling for the estimator method across the different studies. Although this may not have accounted for all potential bias inherent between the different statistical methods, it helped to control for some of the variation in the available data without sacrificing statistical power with smaller sample sizes or fewer species in the dataset. Regardless, we feel that the findings of this meta-analysis provide important insight into how the home ranges of bats across the globe are affected by different biological and ecological variables. Future studies should look to build on these findings by focusing on additional species once home range data become available as the high diversity of bats may potentially provide contrasting trends not observed within our dataset. Although future efforts will still be constrained by the availability of appropriately sized tags that may prevent investigations for smaller species. Smaller tags are being developed which will increase accessibility to smaller species, however, the costs associated with more compact units may limit the number of bats that can be tracked. The inclusion of reproductive status in general home range studies can provide important information regarding energetic constraints and movement patterns during these periods, especially for males due to the scarcity of published data regarding home range sizes for males of different reproductive statuses. The inconsistent or lack of reporting for the season(s) in which studies were performed prevented the comparison of home ranges and movement patterns across seasonal gradients. However, perhaps a more useful metric may be to report periods of food availability around the study area as this would likely be more informative than season or rainfall periods as habitat dynamics can vary between different ecoregions and allow for comparisons of home ranges given an understanding of fluctuating resource availability.

2.6 Conclusions

This study highlighted key ecological variables and biological traits that are linked with bat home ranges and can help improve our understanding of variations in their movement patterns. These findings may serve as a preliminary step towards predicting potential range requirements for different species if their ecological and biological traits are known. As a highly diverse order that is responsible for several key ecosystem services but is also facing widespread population declines and threats (Frick et al. 2020), understanding the movements and range requirements of bats can aid in their protection by identifying key areas of importance. Furthermore, as viral hosts, their movements carry implications for disease transmission and understanding how different variables influence range requirements could aid with risk assessments if supplemented with specific investigations into patterns of range use within a home range.

2.7 Supplementary Data

Supplementary Data S1. —Results of literature search and list of publications used in the analysis including species sample size and study locations.

Supplementary Data S2. —Results from collinearity test with Generalised Variance Inflation Factors for variables in the full model.

Supplementary Data S3. —Comparison of the top-ranked model when proportional mass of the transmitter is included as a covariate.

Supplementary Data S4. —Graphical visualization of statistical outputs from top model detailing the scale of effects for the different covariates on home range size.

Supplementary Data S5. —Graphical plots detailing the effects on home range size for (A) sex, (B) wing loading, (C) colony size, (D) functional group, (E) diet, and (F) habitat type, latitudinal region interaction.

Supplementary Data S6. —Full dataset. https://doi.org/10.17605/osf.io/jbc4s.

2.8 Acknowledgements

This study was funded by the DSI-NRF South African Research Chair (SARCHi) in Infectious Diseases of Animals (Prof. W Markotter) together with a grant-holder linked bursary (Grant no. 98339). The opinions expressed and conclusions arrived at are those of the authors and are not necessarily to be attributed to the NRF. The study was also funded by the USA Department of Defence, Defence Threat Reduction Agency (HDTRA12010025). The content of the information reported here does not necessarily reflect the position or the policy of the federal government, and no official endorsement should be inferred.

Chapter 3: Variations in small-scale movements of a viral host, *Rousettus aegyptiacus*, across a seasonal gradient

(Formatted for Frontiers in Zoology)

Variations in small-scale movements of a viral host, *Rousettus aegyptiacus*, across a seasonal gradient

Matthew R. Wood[1], J. Low de Vries[1], J.H. Epstein[2], Wanda Markotter[1*]

[1] Centre for Viral Zoonoses, Department of Medical Virology, University of Pretoria, Pretoria, South Africa

[2] EcoHealth Alliance, New York, NY, USA

* Corresponding author

Email: wanda.markotter@up.ac.za

3.1 Abstract

Background: Bats are recognized as hosts for several viruses, some of which are zoonotic and carry the potential for spillover within human and livestock populations. Biosurveillance studies focused on assessing the risk of pathogen transmission, however, have largely focused on the virological component and have not always considered the ecological implications of different species as viral hosts. The movements of known viral hosts are an important component for disease risk assessments as they can potentially identify regions of higher risk of contact and spillover. As such, this study aimed to synthesize data from both virological and ecological fields to provide a more holistic assessment of the risk of pathogen transmission from bats to people.

Results: Using radiotelemetry, we tracked the small-scale movements of *Rousettus aegyptiacus*, a species of bat known to host Marburg virus and other viruses with zoonotic potential, in a rural settlement in Limpopo Province, South Africa. The tracked bats exhibited seasonal variations in their movement patterns including variable usage of residential areas which could translate to contact between bats and humans and may facilitate spillover. We identified a trend for increased usage of residential areas during the winter months with July specifically experiencing the highest levels of bat activity within residential areas. July has previously been identified as a key period for increased spillover risk for viruses associated with *R. aegyptiacus* from this colony and paired with the increased activity levels, illustrates the risk for spillover to human populations.

Conclusion: This study emphasizes the importance of incorporating ecological data such as movement patterns with virological data to provide a better understanding of the risk of pathogen spillover and transmission.

Keywords: Chiroptera, contact risk, Egyptian rousette bat, spatial overlap, zoonoses

3.2 Background

Movements of animals are linked to changes in their internal state or environmental conditions that induce a critical response to satisfy essential requirements for survival (Holyoak et al. 2008). Aside from the myriad of factors that may affect movement patterns such as seasonal changes, predation pressure or the need to find a mate (Doherty et al. 2019), the availability of limiting resources such as food, water and suitable habitats is generally considered the most prominent determinant of movement patterns (Hodgkison et al. 2004; Leblond et al. 2010). Bats, belonging to the order Chiroptera, are very diverse with 1 456 reported species (Simmons and Cirranello 2022) and are unique as the only mammals capable of self-powered flight (Gunnell and Simmons 2005). As such, they face unique constraints for their movement patterns that are linked to flight mechanics, energetics and wing morphology (Norberg and Rayner 1987; Voigt et al. 2017). For instance, while flight enables individuals to traverse larger areas compared to similar-sized terrestrial species and cross geographic barriers that would typically inhibit movements (Epstein et al. 2009; Breed et al. 2010) it has far higher energetic costs compared to non-flying movements (Voigt et al. 2017). Linked to flight, the morphological characteristics of their wings influence their movement patterns with some wing types limited to high-speed flight through open areas whereas others trade flight speed for manoeuvrability enabling flight through highly cluttered areas (Norberg and Rayner 1987). Furthermore, bats can be categorised into different functional groups according to their foraging strategies (Schnitzler and Kalko 2001) which are in turn influenced by wing morphology and flight characteristics and therefore, also influence small-scale movement patterns.

Chiroptera is the second-most speciose order after Rodentia and was theorised to carry a higher proportion of zoonotic viruses per species than any other mammalian order (Luis et al. 2013). Although Olivero et al. (2017) and Mollentze and Streicker (2020) suggested that the number of zoonotic viruses was proportional to what would be expected given their species diversity. It has been hypothesised that the cause for their prominence as viral hosts is linked to key ecological traits that increase their suitability as viral reservoirs (Hayman et al. 2013; Luis et al. 2013; Guy et al. 2020). These traits include highly gregarious aggregations in some species that increase the chance for contact between species or with conspecifics, and long lifespans (Hayman et al. 2013; Luis et al. 2013; Guy et al. 2020). Furthermore, flight is an important factor as it enables widespread movements, and the broad geographic ranges for some species may facilitate contact and potential viral sharing with species across a wide range (Luis et al. 2013) and, in some cases, across national borders (Breed et al. 2010; Randhawa et al. 2020).

Bats are associated with several viral families, including *Filoviridae*, *Paramyxoviridae* and *Coronaviridae* as well as lyssaviruses from the *Rhabdoviridae* family, that have already demonstrated spillover and the potential for high pathogenicity (Wang and Anderson 2019;

Ruiz-Aravena et al. 2021). Within these families, there are notable examples where bats have been identified as the virus reservoir hosts namely Marburg virus (Towner et al. 2009), Nipah virus (Halpin et al. 2011) and Hendra virus (Murray et al. 1995; Young et al. 1996). Amongst these bats is *R. aegyptiacus,* the Egyptian rousette bat (Ramanantsalama et al. 2022). It is a medium-sized fruit bat (mean body mass – 120 g; mean forearm length: 92 mm) (Monadjem et al. 2020) with a wide geographic distribution across Africa and into the Middle East (Benda et al. 2012). They roost in large colonies that can range from a few hundred to several thousand individuals (Monadjem et al. 2020) and are typically frugivorous, although there is evidence of them feeding on pollen, flowers, leaves and insects (Barclay et al. 2006; Ramanantsalama et al. 2022). *Rousettus aegyptiacus* has been identified as the reservoir host for a viral haemorrhagic fever, Marburg virus (Towner et al. 2009; Paweska et al. 2018) in addition to being associated with paramyxoviruses, lyssaviruses and coronaviruses, several with zoonotic potential (Mortlock et al. 2019; Markotter et al. 2020). This bat is highly mobile, capable of travelling 24 km in a single night (Jacobsen et al. 1986) with one individual previously being recorded travelling over 500 km between cave roost sites (Jacobsen and du Plessis 1976), illustrating this species' capacity for long-distance movements between suitable habitats. The role of *R. aegyptiacus* as a reservoir host for zoonoses or viruses with zoonotic potential, paired with their regular proximity to human populations (Barclay and Jacobs 2011; Bachorec et al. 2020), a widespread distribution and the ability for long-distance movements highlights *R. aegyptiacus* as a prominent species that warrants further studies into its ecology, viral diversity and viral dynamics.

A relatively novel attitude in the field of zoonoses and virus transmission has been to integrate expertise from multiple fields including virology, ecology, anthropology and sociology to provide a better understanding of risk assessments (Morse et al. 2012; Dougherty et al. 2018). Virological studies, while highlighting important components such as viral prevalence, diversity and excretion dynamics, lack the ecological perspective that could provide context and potential scale for disease transmission risk (Dougherty et al. 2018; Randhawa et al. 2020). Similarly, while focused ecological studies can provide insight into a species' interactions with other species and potential spillover hosts, without the virological background, those data are effectively meaningless in the context of disease risk assessments (Dougherty et al. 2018). The integration of virological and ecological data, however, can provide a more holistic understanding of risk assessments. For instance, movement data can help identify key areas in the landscape that are utilised by a study species and may provide evidence of spatial overlap with humans, wildlife or livestock species, and by extension, the likelihood of contact between humans, wildlife and livestock species (Dougherty et al. 2018; Randhawa et al. 2020; K. Zhao et al. 2022). Furthermore, movement studies may identify patterns of interconnectedness between separate populations which can influence pathogen dynamics, maintenance and evolution within a system (Epstein et al. 2020). If the movement data are obtained for species that are known hosts of potential zoonotic pathogens and paired with data regarding pathogen prevalence, excretion and shedding dynamics, researchers can extrapolate the data to assess the risks of spillover events or disease transmission (Plowright et al. 2017; Dougherty et al. 2018). Spillover events are rare and certain processes must occur and conditions met before spillover can take place (Holmes et al. 2018), but the alteration of land use that disrupts natural processes has been shown to be a crucial step (Olivero et al. 2020; Roberts et al. 2021). These disruptions can alter pathogen transmission dynamics and potentially open up new routes for transmission (Gottdenker et al. 2014; McKee et al. 2021; Eby et al. 2022). Spillover events are more likely to occur in areas with high species diversity and interactions at human, livestock and/or wildlife interfaces (Allen et al. 2017; Magouras et al. 2020; Tajudeen et al. 2022). Such interfaces are occurring with increasing regularity as anthropogenic expansion encroaches on natural areas, and this may influence the degree of contact between a reservoir species and potential susceptible hosts, further increasing the risk of spillover (Plowright et al. 2021). For effective risk assessments, a proper understanding of a host's movements and proximity to susceptible populations is required (Dougherty et al. 2018; McClure et al. 2020) as well as knowledge of pathogen dynamics within the host species, such as excretion dynamics and viral sharing (Pigott et al. 2015; Letko et al. 2020). Such risk assessments may aid management decisions to reduce the risk of future outbreaks and associated costs (Dobson et al. 2020).

Therefore, this study aimed to assess the movement patterns of a single population of *R. aegyptiacus*, a known viral host, relative to a human population in South Africa. Specifically, we focused on: 1) determining whether their nightly foraging movements varied on a seasonal basis linked to different fruiting periods for natural or cultivated fruit trees and, 2) whether their movement patterns overlapped with human presence, translating to the risk of contact and potential spillover.

3.3 Methods

3.3.1 Study area

Fieldwork was performed around Fertilis, a rural settlement (24º07’30” S 30º06’17” E), north of the town of Ga Mafefe in Limpopo Province, South Africa. The area is located in a valley with elevation ranging from 740 m above sea level (a.s.l) at the bottom of the valley to 990 m a.s.l on the mountain slopes. The climate can be described as typical humid subtropical with a warm, wet season from October until March and a relatively cooler, dry season from April until September (Figure 3) (Additional file 1) (The World Bank Group 2021). The landscape contains large areas of agricultural lands, which make up approximately 35% of the study area with patches of natural vegetation occurring along the Mohlapitsi river and on the mountain slopes, accounting for approximately 45% of the study area. Rural residential areas constitute the remaining 20% of landcover, with inhabitants residing in the five major residential areas (Figure 4). Free-roaming livestock, including donkeys (*Equus asinus*), cattle (*Bos taurus*), goats (*Capra hircus*) and chickens (*Gallus domesticus*), are also prominent throughout the valley. A large proportion of households have fruiting trees on their properties including mangos (*Mangifera indica*), papayas (*Carica papaya*), litchis (*Litchi chinensis*), avocados (*Persea americana*), lemons (*Citrus limon*), oranges (*C. sinensis*) and bananas (*Musa* spp.) which are used for subsistence. Native fruiting trees in the area include a variety of *Ficus* spp., including *Ficus sycomorus*, *F. sansibarica*, *F. ingens*, *F. abutilifolia* and *F. sur* as well as other trees such as Cape Ash (*Ekebergia capensis*) and Marula (*Sclerocarya birrea*). Matlapitsi cave (24º06’52” S 30º07’16” E) is located in the northern section of the valley and serves as a maternity roost for an *R. aegyptiacus* colony that is the focus of this study (Mortlock et al. 2019). This cave has previously been used for religious and cultural practices and, although these have since been ceased, there is still evidence of people entering and exiting the cave (*personal observation*). The bat community residing within Matlapitsi cave have been the focal point for long-term virological studies focusing on zoonotic disease in bats that started in 2012 (Mortlock et al. 2019). The bat community includes *R. aegyptiacus*, *Rhinolophus simulator*, *Rh. blasii*, *Rh. clivosus*, *Myotis tricolor*, *Miniopterus natalensis* with *Hipposideros caffer* and *Cloeotis percivali* recorded infrequently. *Rousettus aegyptiacus* from this colony have been identified as hosts for Marburg virus and paramyxoviruses with zoonotic potential (Paweska et al. 2018; Mortlock et al. 2019; Paweska et al. 2020; Mortlock et al. 2021).

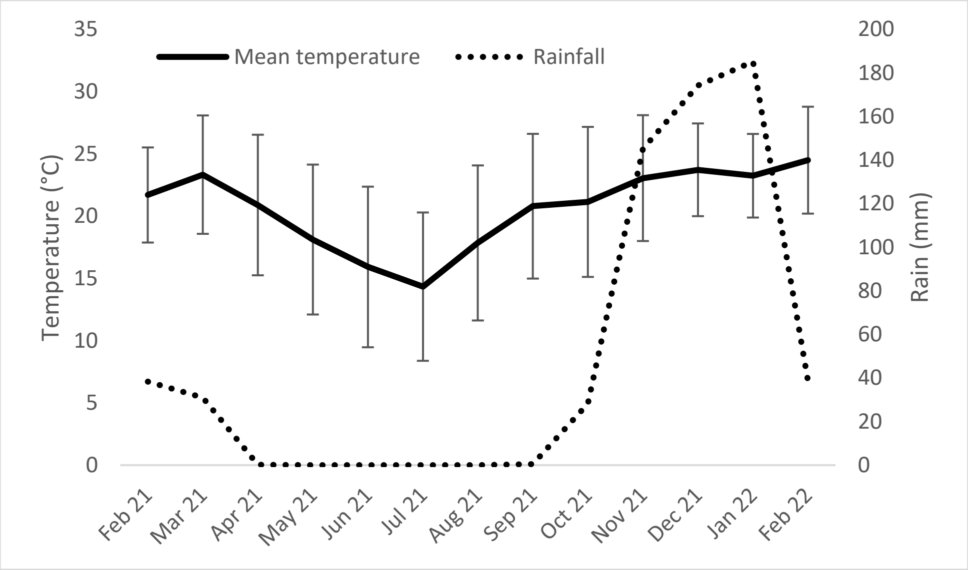


Figure 3: Weather station data for the Fertilis valley during the study period depicting the mean monthly temperatures (± standard deviation) and the total monthly rainfall (Additional file 1). The warm, wet season spans October to March, while the cooler, dry season is between April and September

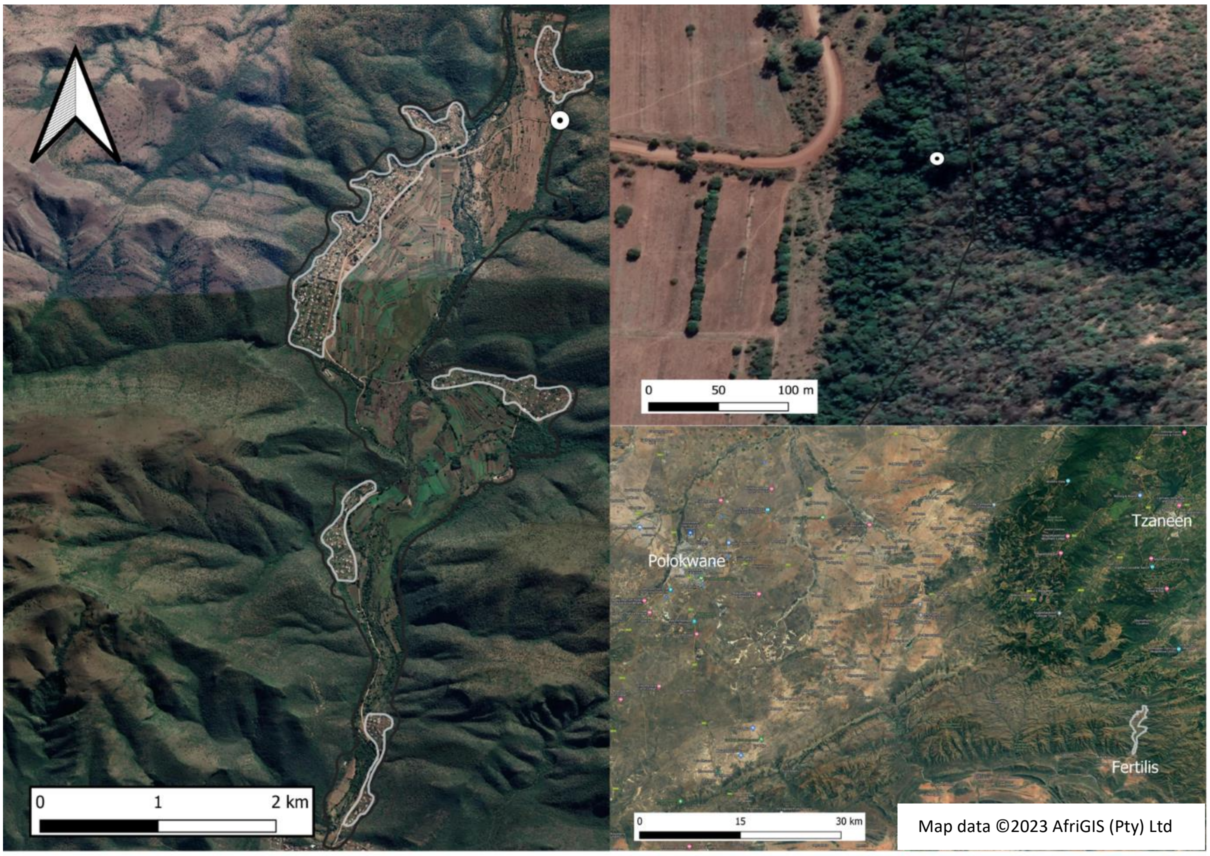


Figure 4: Satellite view of the Fertilis valley with the five major residential areas highlighted by the white encircled areas (left). Matlapitsi cave, as denoted by the white point, is situated in dense natural vegetation but is within 500 metres of the nearest residential area (top right). The study sight is located approximately 30 kilometres south of Tzaneen and approximately 100 kilometres southeast of Polokwane

3.3.2 Small-scale movements

Capture and tagging

Rousettus aegyptiacus were captured as they emerged from the cave using a 4.2 m^2, 2-bank harp trap and a 1.2 m^2 3-bank harp trap (Faunatech Austbat, Bairnsdale, Victoria Australia). The traps were set up outside the cave entrance 60 minutes before sunset with the remainder of the cave entrance closed off by a tarpaulin to prevent bats from flying around the traps. All individual bats were weighed, forearm length measured, and sex, age and reproductive status were determined. Age was determined by assessing the fusion of the epiphyseal gap of the phalangeal bones in the wing and based on forearm length measurements (Brunet-Rossinni and Wilkinson 2009). Bats with a forearm length greater than 89 mm were considered adults, whereas bats with a forearm of 89 mm and less were labelled as subadults (Monadjem et al. 2020). For reproductive status, females were separated into three categories: pregnant, non-pregnant and lactating, while males were separated into scrotal (having testes visibly descended into the scrotum) or non-scrotal.

A total of 26 bats were tagged, 15 male and 11 females. Very High Frequency (VHF) radio transmitters (PD-2C, Holohil, Ontario, Canada) weighing 4.1 g were attached to the dorsal side of the bats with a collar and were also secured with a cyanoacrylate adhesive (Alteco Chemical Pte Ltd). The tag was positioned so that the transmitter sat between the scapulae and the antenna extended over the back and did not interfere with wing movements. We specifically did not tag pregnant females to avoid over-burdening them and risking injury. Collars were individually measured and fitted to each bat to ensure they would not fall off prematurely, or risk constriction around the neck. The collars were encased in Tygon® tubing to prevent injury to the bat through chafing and were connected by a small metal ferrule that was designed as a weak point enabling the eventual discarding of the tags after a few months, thus ensuring the tags would not remain permanently attached similar to the methods proposed by O’Mara et al. (2014). In cases where tags had fallen off and were recovered again, they were first disinfected before being redeployed on newly captured bats using the same methods as described above. As the tags were designed to eventually fall off, the number of active tags varied each month (Table 6). The tags had an additional position-sensitive feature that enabled us to determine whether the bats were flying or not as the frequency of signal pulses doubled when the tag was parallel to the ground, indicative of flight. Bats were also tagged with Passive Integrated

Transponders (PIT tags) to aid in identification should they be recaptured in the future. The total handling time for the bats, including measurements, PIT-tagging and collaring was less than 10 minutes.

Biosafety

All personnel operating the traps and handling the bats donned suitable Personal Protective Equipment (PPE) including disposable Tyvek® coveralls (Dupont™), a double pair of nitrile gloves (Lasec®) (the bottom layer of which is duct taped to the Tyvek coverall to create a sealed suit), handling gloves which are worn over the nitrile gloves, respiratory protection with Powered Air-Purifying Respirators (PAPR, Versaflow, Maxair, CAPR system) and gumboots (Bata Industrials, KZN, South Africa). Once all capture and handling activities were finished and the tagged bats were released, all personnel disinfected with sodium hypochlorite (1:10 dilution of domestic bleach). Once disinfected, disposable PPE was discarded into double-bagged biohazard bags which were transported to the BSL4 NHLS facility for autoclaving and incineration.

Table 6: A summary of the number of active VHF tags on *R. aegyptiacus* for each month of the study period

Year	Month	No. active tags
2021	February	6
	March	6
	April	12
	May	10
	June	8
	July	6
	September	14
	October	12
	November	12
	December	12
2022	January	8
	February	5

Tracking

Tags were calibrated with the receiver before deployment to allow for distance estimates by determining relative signal strengths at increasing distances away from the tags. The detection distance for tags, while still maintaining a reliable signal, was approximately 300m. Once released, and after all personnel had been disinfected and removed their PPE, bats were tracked using a four-element Yagi antenna (VHF BNC 4-element Yagi, Africa Wildlife Tracking, Rietondale, Pretoria, South Africa) paired with a receiver (TR-4 Telonics, Africa Wildlife Tracking, Rietondale, Pretoria, South Africa). Tracking was performed by driving the available roads and walking transects in areas inaccessible to vehicles. Bats were tracked for between seven and nine nights each month, except for December 2021 where only five nights of tracking were performed due to Covid-19 cases affecting the research team. Furthermore, August 2021 was not sampled due to logistic constraints with tags not being available to deploy. Tracking started at sunset each night and continued until an hour before sunrise except during periods of heavy rainfall or thunder during which tracking was temporarily halted. Once a bat was detected with the receiver, the distance to the bat was estimated based on signal strength, the bearing taken, and the GPS coordinates of the location recorded. The bearing and distance estimates enabled the approximate location of the bat to be determined using the following coordinate conversion calculations, where la1 = latitude of first point; la2 = latitude of second point; lo1 = longitude of first point; lo2 = longitude of second point; Ad = distance/Earth radius; ϴ = bearing in radians with bearing described as the clockwise angle from true north:

$$(la2) = \arcsin(\sin(la1)*\cos(Ad) + \cos(la1)*\sin(Ad)*\cos(\Theta)$$

$$(lo2) = lo1 + \text{arctan2}(\sin(\Theta)*\sin(Ad)*\cos(la1), \cos(Ad) - \sin(la1)*\sin(la2))$$

If there were repeat detections of the same bat in the same location, the location was only recorded if the detections were greater than 10 minutes apart to ensure independence with the collected data. Additionally, the landcover category in which the bats were detected, and the activity of the bats were recorded. Bats were labelled as either ‘flying’ or ‘non-flying’ depending on signal impulse frequency and landcover categories was labelled as natural, agricultural or residential areas according to classifications from the South African National Land-Cover (SANLC) 2020 dataset. In addition to recording the locations of the tagged bats, we searched for fruit bats and other wildlife with a spotlight (Zartek®) by looking for eye shine while driving and these locations were also recorded.

3.3.3 Fruit availability estimates

We walked line transects (McIntyre 1953) to identify fruiting trees within the valley. A line transect is a simple method to survey biological populations whereby an observer walks an imaginary line counting individuals or objects of interest within a certain distance from the line (Burnham et al. 1980). The locations for the transects were randomly selected within the valley, but we chose areas that represented all landcover types within the valley. We walked four transects at each of the seven locations (terrain dependent) along the four cardinal directions (North, East, South and West). Each transect was 500m long and all trees within 50m on either side of the transect line were counted. Tree identification was performed with the aid of an identification guide (van Wyk and van Wyk 2013). For fruit availability estimates, we selected one fruiting tree of each species per transect, representing a variety of native and cultivated fruit trees, to serve as proxies for assessing seasonal variations in fruiting patterns for different species, in different areas throughout the study period (Additional file 2). These trees were then monitored each month for the duration of the study. Estimations were calculated as a percentage of total fruit cover, similar to the methods employed by Chapman et al. (1994).

3.3.4 Risk of contact and habitat selection

To assess the risk of contact between bats and people, all tracked locations for the bats were plotted onto a satellite map of the study area in QGIS (Quantum Geographic Information System) 3.12 (QGIS Development Team 2022) using the SANLC 2020 dataset to delineate the different landcover types. The dataset was downloaded from the South African Department of Environmental Affairs (DEA) Environmental Geographic Information Systems (EGIS) website (https://egis.environment.gov.za/data_egis/data_download/current). The proportion of locations obtained for bats within residential areas was calculated each month similar to the method used by Randhawa et al. (2020) where they included locations within 100m of urban areas in their risk assessment. We assessed the proportion of all locations within residential areas but also specifically assessed non-flying locations as these represent locations where bats are likely to spend longer durations which could increase the risk of direct and indirect contact. The proportional cover of different landcover types within the study area was estimated using the landcover map which enabled us to quantify the proportional usage of each landcover type by the bats each month.

3.3.5 Statistical analyses

Movements

We performed Generalised Linear Mixed Models (GLMMs) with Poisson distribution and log link function to analyse the number of locations obtained each month for different landcover types and activities and whether there were significant differences across the study period. Model selection was performed using the ‘lme4’ and package in R (Bates et al. 2015) through backward, stepwise logistic regression where candidate models were generated by sequentially removing variables from the full model until the best-performing model was obtained. We included landcover (residential/agricultural/natural) and the activity of the bat (flying/non-flying) as fixed effects. Age and sex of the bats was not included in the analysis as we were interested in the broader question of whether there was risk of contact rather than if specific sexes or age cohorts exhibited increased risk for contact with people. To control for potential monthly variation across a seasonal gradient, month was included as a random slope over season (dry/wet). The number of GPS locations obtained each month was used as the response variable. Model performance was assessed with Akaike’s Information Criterion (AIC) values, with the lowest AIC value corresponding to the best-performing model. Models that were within $\Delta AIC < 2$ were considered as competing (Burnham and Anderson 2004). Results from the models were interpreted using the ‘lmertest’ (Kuznetsova et al. 2017) and ‘emmeans’ (Lenth 2022) packages with marginal and conditional R^2 values reported from the MuMIn package (Barton 2020).

We were unable to include variables linked to sampling effort in the main GLMMs as the number of nights, hours tracked and the number of active tags were specific to each month and as such there were singularities in the dataset which would confound the analysis. Therefore, to assess whether sampling effort influenced the number of locations obtained each month, we ran additional linear models. For these models, the number of nights, hours tracked and active tags each month were used as predictor variables with the number of GPS locations obtained each month as the response.

Resource selection

Landcover use and resource selection was assessed using the landcover maps with the tracked locations plotted onto the maps in QGIS. Landcover types with similar classifications were concatenated into single landcover classes. For example, formal residential, informal residential and scattered village areas were all grouped as residential areas as all residential areas within the study area were similar with the presence of cultivated fruit trees. After simplifying the landcover classifications, three distinct landcover classes remained: residential, natural and agricultural areas (Figure 5). The area of each of these habitats was measured as well as the area of the whole study area using polygons in QGIS to obtain an estimate of proportional availability for each landcover type in the study area. After this, the number of locations within each habitat was counted and compared against the proportion of habitat availability in the study. We performed a resource selection ratio analysis using the 'adehabitatHS' package v 3.15 in R (Calenge 2006) using the 'widesI' analysis as the data were pooled for all bats with the same proportion of habitat available in all cases (Thomas and Taylor 1990). Manly selectivity plots were generated for resource selection ratio analyses as a way to visually represent the patterns of landcover use. Selection ratios were performed for the different activities (flying/non-flying) to determine if there were any preferences apparent between the categories. The terms 'positive selection' and 'negative selection' were used when referring to habitat usage rather than 'preference/avoidance' or 'presence/absence' as these latter terms can be ambiguous and difficult to accurately quantify (Hirzel et al. 2002). For instance, determining the avoidance of an area can, similarly to absence records, be incorrectly recorded if the animal is not detected despite its presence in the area, a feature we may likely have encountered, given the difficulty of tracking small, flying animals such as bats (Moussy et al. 2013). The tests generated a selection score (wi) which related to whether landcover types were selected for or not. A selection score greater than 1 represented positive selection while a score less than represented negative selection.

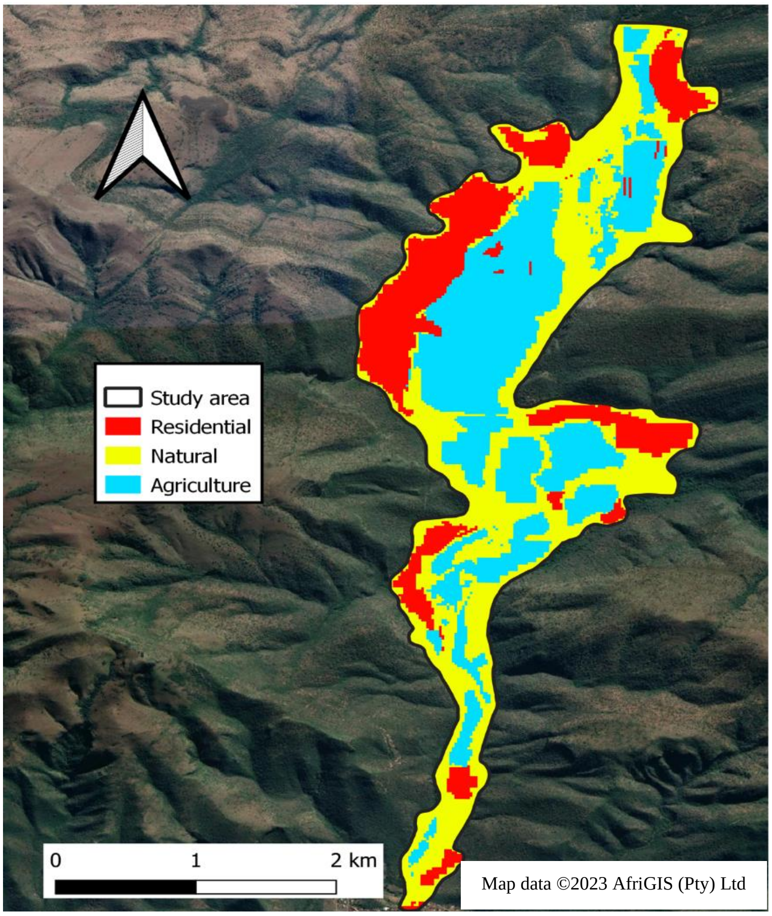


Figure 5: Satellite image of the Fertilis valley detailing the three different landcover types present within the study site that were obtained from the SANLC 2020 dataset

Utilization distributions

As a further metric of habitat usage, we calculated utilization distributions using kernel density estimates (Worton 1987) for bat movements each month. Although we had sufficient points to calculate home range estimates for some individual bats (i.e. more than 30 locations for an individual) (Seaman et al. 1999), the layout of the study area prevented us from tracking the bats if they left the valley and therefore, any home range estimations would be heavily limited towards our study area. Therefore, the utilization distributions were calculated to identify specific areas of usage rather than to estimate home range sizes. Location data were first projected into the Universal Transverse Mercator coordinate system (EPSG:32736 - WGS/UTM zone 36S) for ease of analysis and import into QGIS. Kernel density estimates were calculated using the ‘adehabitatHR’ package v 4.19 in R (Calenge 2006) using the least squares cross-validation (LSCV) method for selecting bandwidth (Gitzen et al. 2006). Kernel estimates generate contours around a pre-specified proportion of points and for our analysis, we calculated density estimates for 50% and 95% locations. Kernels were estimated for non-flying activities during July (H_{ref} = 159.72) and January (H_{ref} = 125.27) as these represent the months with the highest and lowest levels of activity within residential areas and we wanted to compare the patterns of area use for these periods. The kernels obtained from the analysis were overlayed with the satellite map of our study site and the proportional usage of different landcover types was calculated as the area for each habitat encompassed by the kernels. Areas were calculated using polygons in QGIS. We assessed use across all three landcover types and compared the proportional use between wet and dry seasons for non-flying and all locations to determine whether use differed significantly between seasons, for specific activities or different landcover types. We assessed differences in proportional use between seasons or for non-flying activities using paired t-tests. The location data were not normally distributed ($w = 0.91$; $p < 0.05$) and therefore, non-parametric tests were used for the analyses. All analyses were performed in R v4.0.2 (R Core Team 2020) using the RStudio v1.3.1073 (RStudio Team 2020) interface and statistical significance was assessed at an alpha threshold of 0.05 unless otherwise stated.

3.4 Results

Tracking was performed for 95 nights totalling 627 tracking hours across the 12-month study period. We obtained 930 locations for the 26 tagged bats (Table 7) and additionally recorded 295 unique locations for bat sightings throughout the study period (Additional file 3).

Table 7: Summary of tagged *R. aegyptiacus* that were tracked during the study including sex, age, reproductive status, body mass, forearm length, the number of tracked locations and months tracked

Bat ID	Sex	Age	Reproductive status	Body mass (g)	Forearm length (mm)	Number of locations	Months tracked
UP21-02	F	SA	NP	75	84.3	55	6
UP21-03	M	A	S	127	92.2	39	4
UP21-04	M	A	S	130	93.8	21	3
UP21-05	M	SA	NS	82	83.5	34	5
UP21-06*	F	SA	NP	82	85.3	25	3
UP21-07	F	A	NP	104	89.7	38	5
UP21-08	F	A	NP	112	94.2	30	3
UP21-09	F	SA	NP	88	84.6	30	3
UP21-10	F	A	NP	133	90.0	34	4
UP21-11	M	A	S	119	93.2	30	3
UP21-12	M	A	S	113	92.8	28	4
UP21-13*	M	SA	NS	88	84.7	22	2
UP21-14	M	A	NS	127	90.4	13	2
UP21-15	F	A	NP	74	90.2	13	2
UP21-200	M	A	S	128	94.6	52	6
UP21-201	F	A	NP	122	94.0	55	6
UP21-202	M	A	NS	113	93.8	55	6
UP21-203	M	A	NS	107	89.9	39	4
UP21-204	F	A	NP	96	90.3	57	6
UP21-205	M	SA	NS	95	88.8	46	5
UP21-206	F	A	NP	109	90.3	20	4
UP21-207	M	A	NS	100	89.2	31	4
UP21-208	M	A	S	138	95.8	37	5
UP21-209	F	A	NP	97	92.4	65	6
UP21-210	M	A	S	137	89.5	35	5
UP21-211	M	A	S	126	93.1	27	4

*Discarded tags recovered and redeployed on UP21-14 and UP21-15

F = Female, M = Male; A = Adult, SA = Subadult; NP = Non-pregnant, S = Scrotal, NS = Non-scrotal

Model selection showed that the best-performing model included landcover, activity and the interaction between landcover and activity as fixed effects with month over season as the random slope (Table 8).

Table 8: All candidate models generated during model selection with full and null models for comparison with AIC scores and model weight examining the effect of landcover type and bat activity on the number of GPS locations recorded

Model	Candidate models	AIC	ΔAIC	Model weight
1	Locations ~ Landcover*Activity + (Season|Month)	553.6	0.00	0.96
2	Locations ~ Landcover + (Season|Month)	560.9	7.33	0.02
Full	Locations ~ Landcover + Activity + (Season|Month)	561.6	8.06	0.02
Null	Locations ~ 1 + (Season|Month)	694.7	141.03	0.00
5	Locations ~ Activity + (Season|Month)	695.7	142.18	0.00

Landcover type and activity independently had a significant influence on the number of GPS locations obtained each month as well as the interaction between habitat type and activity (Table 9).

Table 9: Summary of best-performing model output for GPS locations of *R. aegypiacus* including estimates, standard error, 95% confidence intervals, z-scores and p-values examining the effect of landcover type and bat activity on the number of GPS locations recorded

Coefficient	Estimate	Std.error	C.I.	z-score	p-value
Intercept	3.07	0.11	2.85 – 3.29	28.34	< 0.001
Landcover type					
Agricultural	Ref lev*				
Natural	0.28	0.10	0.08 – 0.48	2.71	< 0.05
Residential	-0.71	0.13	-0.96 – -0.46	-5.29	< 0.001
Activity					
Flying (F)	Ref lev				
Non-flying (NF)	-0.42	0.12	-0.66 – -0.18	-3.43	< 0.001
Habitat.Natural*Activity.NF	0.50	0.15	0.21 – 0.79	3.27	< 0.05
Habitat.Residential*Activity.NF	0.53	0.19	0.16 – 0.90	2.74	< 0.05

* Reference level variable

Throughout the study period, the number of locations obtained from natural areas was significantly higher than from agricultural and residential areas, whereas the number of locations obtained from agricultural areas was significantly higher than from residential areas. There was a significantly higher number of locations detected where bats were flying compared to non-flying. The pairwise comparisons for the interaction term showed that, statistically, the highest number of locations was obtained for non-flying bats in natural areas followed by flying bats in natural and agricultural areas. The lowest number of locations were recorded for non-flying bats in agricultural areas although this was not significantly different compared to locations recorded in residential areas for either activity (Table 10). The separate linear model assessing tracking effort showed that the number of active tags per month ($\beta = 5.15$, $p < 0.05$), had a significant, positive influence on the number of locations recorded. The number of nights ($\beta = 10.34$, $p = 0.14$) and number of hours tracked each month ($\beta = -0.05$, $p = 0.95$) had no statistical influence. Therefore, it is reasonable to assume that our results were not biased by tracking effort.

Table 10: Post-hoc pairwise comparisons of the interaction between landcover type and activity for the number of monthly locations, including the estimates, standard errors and p-values

Comparison	Estimate	SE	p-value
Agriculture.F – Natural.F	-0.28	0.10	0.07
Agriculture.F > Residential.F	0.71	0.13	**<0.05**
Agriculture.F > Agriculture.NF	0.42	0.12	**<0.05**
Agriculture.F < Natural.NF	-0.36	0.10	**<0.05**
Agriculture.F > Residential.NF	0.59	0.13	**<0.05**
Natural.F > Residential.F	0.98	0.13	**<0.05**
Natural.F > Agriculture.NF	0.69	0.12	**<0.05**
Natural.F – Natural.NF	-0.08	0.09	0.95
Natural.F > Residential.NF	0.87	0.12	**<0.05**
Residential.F – Agriculture.NF	-0.29	0.14	0.35
Residential.F < Natural.NF	-1.06	0.13	**<0.05**
Residential.F – Residential.NF	-0.11	0.15	0.98
Agriculture.NF < Natural.NF	-0.78	0.11	**<0.05**
Agriculture.NF – Residential.NF	0.17	0.14	0.81
Natural.NF > Residential.NF	0.95	0.12	**<0.05**

Significant comparisons given in bold

The above analyses, however, only utilized the mean number of locations and did not consider the effect of landcover availability within the study area. Landcover availability was defined as the proportional area size for the different landcover types identified from the SANLC database out of the total area for the study site. When the proportion of habitat availability was accounted for, there was clear positive and negative selection of different landcover types. Natural areas were significantly selected for more than their relative availability ($n = 470$; $wi = 1.11$; $p < 0.05$). By contrast, the number of locations obtained in agricultural areas was significantly lower than expected given its proportional availability within the study area (n = 282; wi = 0.88; p < 0.05) (Table 11). There was evidence of positive or negative selection in residential areas (n = 178; wi = 0.97; p = 0.60). The selectivity plot for habitat selection throughout the year illustrates the positive and negative selection for natural and agricultural areas respectively (Figure 6).

Table 11: Selection ratios for the full study period with proportional usage, proportional availability, selection ratios (w.i.) for landcover type and the corresponding p-values. Significant p-values were determined after Bonferroni adjustment (significant p-value < 0.0167)

	Used	Available	w.i.*	SE w.i.	p-value
Residential	0.191	0.198	0.966	0.065	0.597
Agricultural	0.303	0.345	0.879	0.044	<0.0167
Natural	0.505	0.457	1.107	0.036	<0.0167

* w.i. > 1 indicate positive selection; w.i. < 1 indicate negative selection

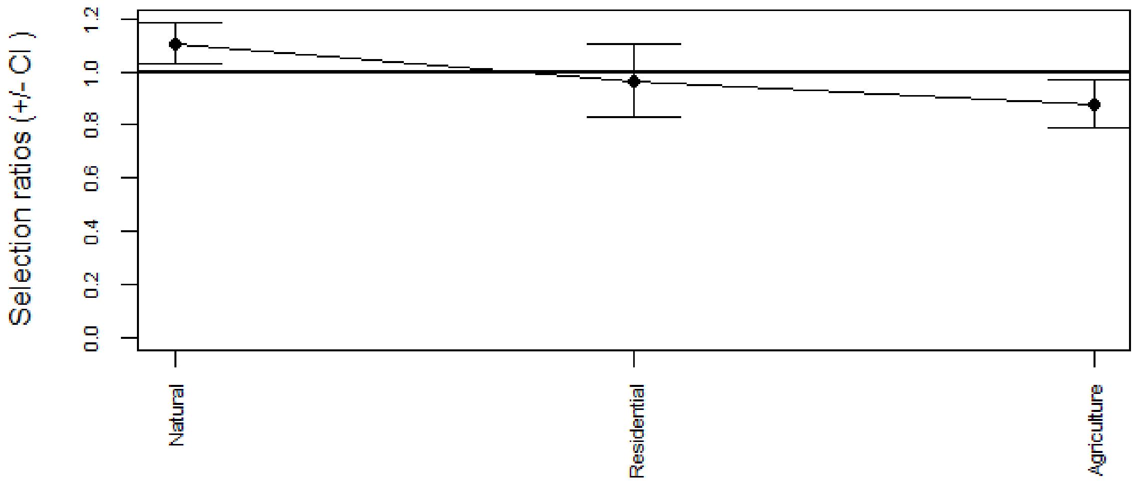


Figure 6: Manly selectivity measure with 95% confidence intervals for landcover selection by *R. aegyptiacus* throughout the study period. Where standard error bars overlap with the solid line, it indicates no selection. Selection ratios > 1 indicate positive selection; < 1 indicates negative selection

When habitat selection was assessed for different activities, there were no clear trends for significant selection of any habitats for flying activities. However, there was a clear positive selection for natural areas (wi = 1.19; p < 0.0167 after Bonferroni adjustment) and a negative selection of agricultural areas (wi = 0.72; p < 0.0167 after Bonferroni adjustment) for non-flying activities (Table 12). The selectivity plot follows a similar trend to the one for the full study period with all locations, although the negative selection for agricultural areas was even stronger for non-flying activities (Figure 7).

Table 12: Selection ratios for non-flying activities with the proportional usage, proportional availability, selection ratios (w.i.) for landcover type and the corresponding p-values. Significant p-values were determined after Bonferroni adjustment (significant p-value < 0.0167)

	Used	Available	w.i.	SE w.i.	p-value
Residential	0.208	0.198	1.049	0.096	0.61
Agricultural	0.248	0.345	0.718	0.059	<0.0167
Natural	0.544	0.457	1.192	0.051	<0.0167

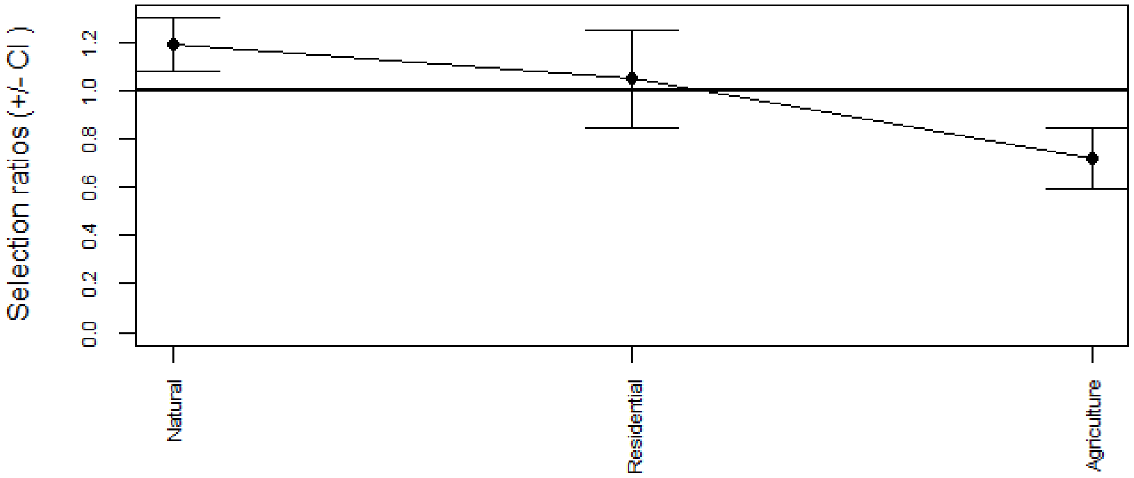


Figure 7: Manly selectivity measure with 95% confidence intervals for non-flying activities of *R. aegyptiacus* throughout the study period. Where standard error bars overlap with the solid line, it indicates no selection. Selection ratios > 1 indicate positive selection; < 1 indicates negative selection

The patterns of use of residential areas varied across the study period with the overall highest activity occurring in July 2021 and the lowest in January 2022 with the same pattern for non-flying and all locations (Figure 8). Important to note was the trend for a decrease in the use of residential areas after October, despite an increase in fruit availability estimates for the mango and banana trees we monitored in residential areas (Additional file 2), both of which are known food sources for *R. aegyptiacus* (Kwiecinski and Griffiths 1999). Bats likely did not need to rely on mangoes or bananas during this period given the patterns of increased fruit availability for our proxy trees within natural areas as well.

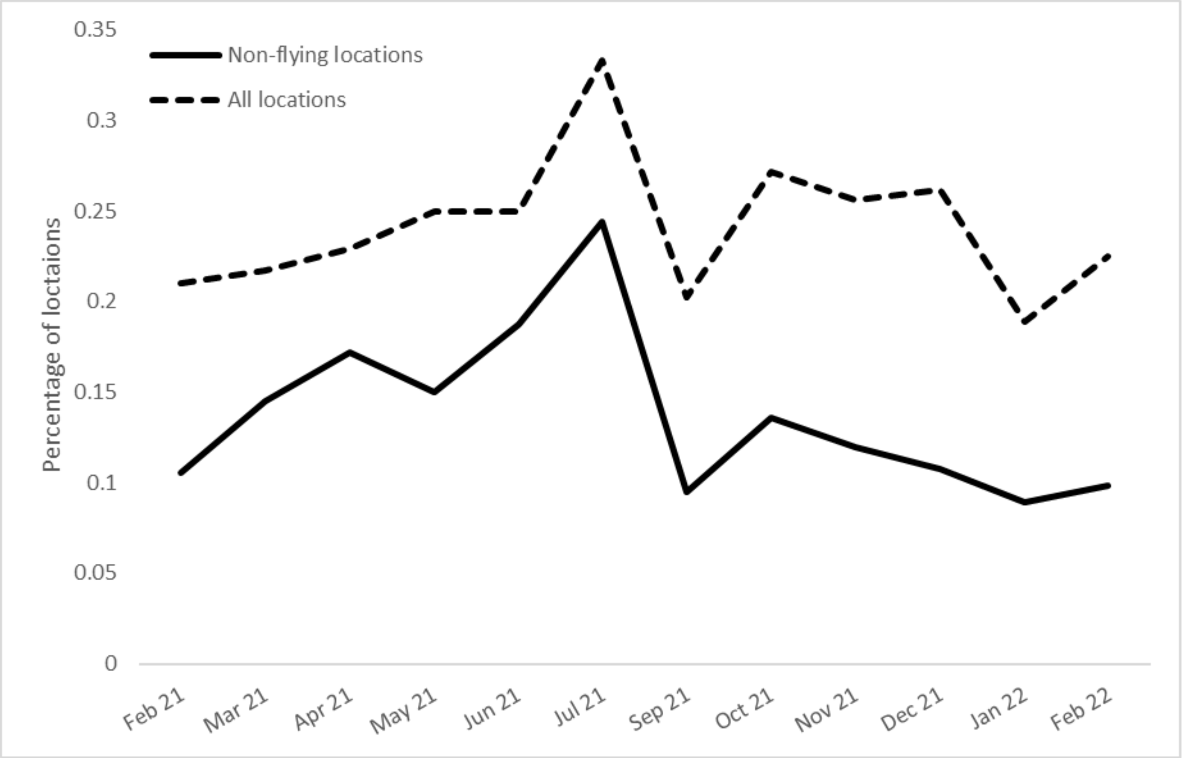


Figure 8: Percentage of non-flying and all locations for *R. aegyptiacus* that were detected each month in the residential areas of Fertilis

The utilization distributions between seasons further supported this trend as usage of residential areas was significantly greater for non-flying activities during the dry than the wet season ($t_{(5)} = -2.70$, $p < 0.05$). To expand on these trends, we further compared the utilization distributions for non-flying activities between the months with the highest (July 2021 – Figure 9a) and lowest activity levels within residential areas (January 2022 – Figure 9b) showing that the proportional usage of residential areas was greater in July than January.

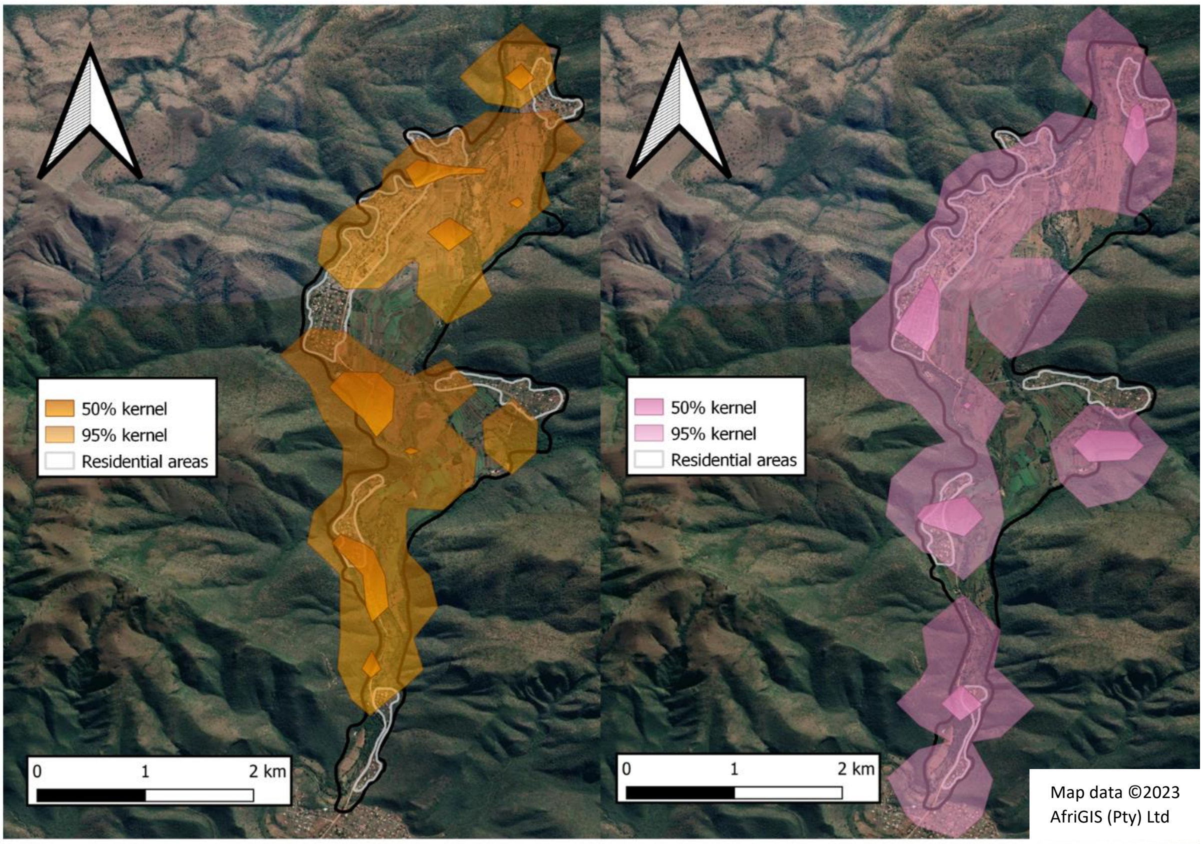


Figure 9: Kernel density estimates showing utilisation distributions for 50% and 95% of non-flying locations during July 2021 (a) and January 2022 (b) with specific reference to the residential areas. Darker areas depict regions with higher levels of activity

Not only that, but the proportional area size within residential areas for July was significantly larger than the other landcover types for both January and July (Table 13). Furthermore, the proportional usage of residential areas during January was significantly less than that observed for the other landcover types in January and July, although its usage was on par with natural areas in July (Table 14). This suggests that bats preferentially utilise residential areas for foraging during the dry season when natural fruit sources are scarce, but resort back to natural areas once the trees start fruiting again in the wet season.

Table 13: Paired t-tests assessing proportional area size utilised for non-flying activities within each landcover type during July (Jul) 2021 and January (Jan) 2022 with mean difference estimates, t-values and p-values

Comparison	Estimate	t-value	p-value
Jan.Agricultural < Jan.Natural	**-1.74**	**-3.35**	**0.020**
Jan.Agricultural > Jan.Residential	**7.76**	**5.90**	**0.002**
Jan.Agricultural > Jul.Agricultural	**5.62**	**3.94**	**0.011**
Jan.Agricultural > Jul.Natural	**8.29**	**4.74**	**0.005**
Jan.Agricultural < Jul.Residential	**-17.48**	**-6.41**	**0.001**
Jan.Natural > Jan.Residential	**9.49**	**5.35**	**0.003**
Jan.Natural > Jul.Agricultural	**7.35**	**3.89**	**0.011**
Jan.Natural > Jul.Natural	**10.02**	**4.96**	**0.004**
Jan.Natural < Jul.Residential	**-15.75**	**-5.90**	**0.002**
Jan.Residential < Jul.Agricultural	**-2.14**	**-3.18**	**0.024**
Jan.Residential – Jul.Natural	0.53	0.42	0.693
Jan.Residential < Jul.Residential	**-25.24**	**-7.35**	**<0.001**
Jul.Agricultural – Jul.Natural	2.67	1.42	0.214
Jul.Agricultural < Jul.Residential	**-23.10**	**-7.56**	**<0.001**
Jul.Natural < Jul.Residential	**-25.77**	**-5.93**	**0.002**

Table 14: Comparison of the proportional areas sizes (ha) within the different landcover types for non-flying activities in July 2021 and January 2022. Area sizes were calculated for 50% and 95% kernels

	July 2021			January 2022		
	Agriculture	Natural	Residential	Agricultural	Natural	Residential
50%	3.71	6.76	16.24	7.59	9.08	3.61
95%	67.62	60.98	85.86	69.13	69.48	63.41

3.5 Discussion

This study identified trends in seasonal movement patterns including periods of potential high contact risk between bats and people that could constitute a risk of pathogen spillover and transmission. This was only the third movement study for *R. aegyptiacus* in South Africa, (Jacobsen et al. 1986; Barclay and Jacobs, 2011), the first study since Jacobsen et al. (1986) to assess the movements of this species in Limpopo and the first ever study for this specific *R. aegyptiacus* colony. This species, and this colony specifically, have been identified as hosts for viruses with zoonotic potential (Mortlock et al. 2019; Mortlock et al. 2021) including Marburg virus (Pawęska et al. 2018; Pawęska et al. 2020) which is responsible for several outbreaks with high fatality rates (F. Zhao et al. 2022). In a landscape characterised by large expanses of agricultural areas and small residential settlements, we show evidence of habitat preferences for non-flying and flying activities. *Rousettus aegyptiacus*, however, do not feed while in flight (Kwiecinski and Griffiths 1999; Barclay and Jacobs 2011) and therefore, we can assume that non-flying activities equated to foraging or feeding. Furthermore, there was increased activity within residential areas during the dry, winter months, specifically July, which has been identified as a critical period for viral shedding for paramyxoviruses due to waning maternal antibodies in juveniles (Mortlock et al. 2019; Mortlock et al. 2021).

Bats utilised all landcover types available but showed a preference for natural areas and relative lower use of agricultural areas. Natural areas were used for commuting and foraging with equal likelihood; however, agricultural areas were used for commuting significantly more often than foraging. *Rousettus aegyptiacus* are capable of long-distance flights (Jacobsen et al. 1986) and possess internal navigation capacities that enable them to locate distant resources (Tsoar et al. 2011; Fenton 2020). They have been observed utilising open areas as flyways when traversing large distances (Tsoar et al. 2011), but at smaller scales have been reported avoiding large open fields for foraging (Lučan et al. 2016). Similarly, lesser short-nosed fruit bats, *Cynopterus brachyotis*, and frugivorous phyllostomids only used plantations as flyways when few to no suitable foraging sites were present (Heer et al. 2015; Lim et al. 2018). The agricultural fields in our study site are predominantly used for vegetable crop production or livestock grazing with few sparsely distributed fruiting trees and therefore, provide little incentive for fruit bats as foraging sites.

Residential areas were used as frequently as would be expected by random selection with no preference for foraging or commuting activities. However, bats used significantly larger proportions of residential areas than other landcover types for foraging during the dry season. The trees we monitored for fruit availability within natural areas had little to no fruit during the dry season, whereas the trees within residential areas, especially bananas, maintained moderate levels of fruit availability. Therefore, bats likely relied on residential areas for foraging during the dry season when food was scarcer in natural areas. Meanwhile, there were also contrasts between wet and dry season months with July 2021 experiencing higher levels of use in residential areas than January 2022. This suggests that residential areas may not be important foraging locations for bats during the wet season if resources are available elsewhere. This was further supported by our observations of specific fruit trees in natural areas which all saw an increase in fruit availability during the wet season. Barclay and Jacobs (2011) reported similar trends where *R. aegyptiacus* preferentially utilised native forests over residential areas during periods of fruit abundance. Several species of fruit bats have shown positive responses to urbanisation and residential expansion (Williams et al. 2006; Giles et al. 2016; Egert-Berg et al. 2021). Grey-headed flying foxes, *Pteropus poliocephalus*, have been recorded shifting their historical ranges to include urban areas (Williams et al. 2006). A variety of *Pteropus* spp., have also started forming permanent roost sites within urban regions due to increased resource availability and sustained availability throughout the year (Plowright et al. 2011). Meanwhile, straw-coloured fruit bats, *Eidolon helvum*, aggregate in large colonies across several African cities with the introduction of exotic and cultivated fruiting trees hypothesised as the cause for these trends (Fahr et al. 2015). *Rousettus aegyptiacus* have also been shown to opportunistically exploit human-modified areas as foraging sites (Lučan et al. 2016) and were observed altering movement patterns specifically to target orchards or cultivated crops when overall resource availability was low (Bachorec et al. 2020). The residential areas in Fertilis possess a high diversity and density of fruiting trees as residents rely on fruit trees planted on their property for subsistence. For example, a survey of one of the five residential areas, which is approximately 62 ha, revealed that there were more than 360 fruiting trees from nine species, seven of which are cultivated species (*personal observation*). The most common fruiting tree species were mangoes, bananas, and citrus, all known to be eaten by *R. aegyptiacus* (Kwiecinski and Griffiths 1999) and which fruit at different times of the year, meaning they offer potential food sources for the bats year-round or alternative food sources when natural resources are scarce or depleted. We observed *R. aegyptiacus* feeding in banana plants adjacent to houses during the study period and the Chief of the village has also reported observing *R. aegyptiacus* foraging in the banana plants next to his house. which further emphasises that bats are utilizing domestically grown fruit trees as food sources during periods of resource scarcity. The significantly large proportional area sizes utilized by the bats, as mirrored by the peak in activity, within residential areas during July coincided with an abundance of citrus in these areas while other food sources were scarce or depleted. This may have led to bats specifically targeting these areas during periods where foraging opportunities in natural areas were limited, similar to trends observed in northern hemisphere *R. aegyptiacus* populations (Lučan et al. 2016; Bachorec et al. 2020).

Contact between fruit bats and potential spillover hosts is likely more prevalent for species that are active at night since their periods of activity overlap. However, indirect contact with diurnal species such as livestock may also occur through faecal or urinary excretions or fruit discarded by bats while foraging (Kwiecinski and Griffiths 1999). *Rousettus aegyptiacus* typically chew fruits to extract the juices from the pulp and then discard the remainder of the fruit (Jacobsen and du Plessis 1976). Evidence of 'spit-outs' were present at several of the fruiting trees we monitored during the study period. These 'spit-outs' may be contaminated with saliva and recent research on captive *R. aegyptiacus* showed that viable Marburg virus was still detected on artificially inoculated fruits six hours post-inoculation (Amman et al. 2021). This suggests that early morning activities of people or livestock could feasibly overlap with periods where the virus is still viable. Previous outbreaks of bat-borne zoonoses were associated with the overlap in distributions of bats, people and livestock species that served as intermediate hosts (Edson et al. 2015; Gurley et al. 2017; McKee et al. 2021). Therefore, our evidence of bats utilising residential areas for commuting and foraging demonstrates the risk of contact, exposure and potential future spillover. Specifically, July represents the period of greatest risk, not only because activity levels in residential areas were highest, but also because July has been identified as a critical period for increased potential shedding of Marburg virus (Pawęska et al. 2018) and paramyxoviruses (Mortlock et al. 2021).

And yet, despite the historic proximity between the colony and human populations, evidence of spatial overlap between bats and humans and times during the year when the risk of viral shedding is increased, there have never been any recorded spillover events in the area. One possibility is that clinical cases simply were not documented as the nearest hospital is approximately an hour's drive away and transport is not easy to come by so patients may have opted for local care. The other possibility is that, to date, there have been no spillover events which leads to a follow-up question: why have no spillover events occurred? Plowright et al. (2017) outlined a series of key factors linked to viral and host ecology that may act as barriers to spillover and only if all of these barriers are overcome by the pathogen, can spillover occur.

A preliminary consideration is whether the distribution of the reservoir host overlaps with the potential spillover host. From our tracking data, we know that the distribution of fruit bats from Matlapitsi cave overlaps with humans within our study area, specifically during July when the risk of viral shedding is increased (Pawęska et al. 2018; Mortlock et al. 2021). However, we also observed an overall decrease in bat presence within the valley during winter with bats typically detected just after sunset and then only again just before sunrise. This suggests that the bats may be foraging outside of the valley and could feasibly be travelling anywhere within a 24 km radius of their roost site to forage (Jacobsen et al. 1986). Therefore, any residential areas, fruit orchards or natural regions within this range could be targeted for foraging and therefore, the risk of contact and overlap with humans is potentially not limited to Fertilis. Distant foraging sites are plausible as the Greater Tzaneen area, just north of our study site (Figure 4), is a prominent producer of a variety of fruit products in South Africa with many orchards as supported by the research of Tshilowa (2015) and there are several small towns nearby where smallholding fruit production also occurs.

Subsequent knowledge of the pathogen prevalence in the reservoir host can also help gauge the risk of contact between the reservoir host and susceptible recipient hosts within its distribution. These data are not available for our focal colony; however, previous longitudinal studies have identified periods throughout the year with increased levels of viral RNA (ribonucleic acid) detection (Mortlock et al. 2019; Mortlock et al. 2021) as well as decreased viral antibody seropositivity (Paweska et al. 2018; Paweska et al. 2020) both of which may correspond to increased likelihood for viral shedding. Routes of potential viral excretion and pathogen survival rates outside of the host can identify possible routes of contact and risk of exposure. Routes of viral shedding have been identified through urine, faecal excreta, faecal material from rectal swabs and saliva from oral swabs for Marburg virus and a variety of paramyxoviruses (Amman et al. 2015a; Mortlock et al. 2019; Paweska et al. 2020; Mortlock et al. 2021). Amman et al. (2021) showed that viable Marburg virus was still detectable on experimentally inoculated fruit six hours post-inoculation, thereby demonstrating a possible route for contact through fruits discarded by bats while foraging (Jacobsen and du Plessis 1976).

Finally, the internal characteristics of potential spillover hosts will influence the probability and severity of infection. Data for these aspects of the spillover process are unknown for the population in our study area, although previous, large-scale Marburg virus outbreaks have occurred in other parts of Africa (Brauburger et al. 2012; Amman et al. 2020a; F. Zhao et al. 2022) emphasising the potential for a spillover event. The other viruses associated with this *R. aegyptiacus* population are not known to cause infection in humans but are closely related to viruses with zoonotic potential (Mortlock et al. 2019; Mortlock et al. 2021) and therefore, are also important to consider when assessing the overall risk of potential viral spillover.

If the above factors are considered, according to Plowright et al. (2017), the risk and potential for spillover are high yet there are some other possible explanations for the absence of a spillover event. It was suggested that the lack of local disease, despite the presence of zoonoses in the bat colony, could be attributed to the lack of activities such as hunting bats for bush meat or entering the cave for guano mining (Mortlock et al. 2021). All previous human infections of Marburg virus can be traced back to cave entries, sharing living areas with reservoir hosts or direct exposure to infected specimens (Brauburger et al. 2012; Amman et al. 2020a). While people do still enter the cave, it is not a common practice and therefore the most likely interface for contact with bats would be outside the cave where external factors or asynchronous windows of activity may prevent exposure and spillover. The possibility for viable Marburg virus to persist on discarded fruit (Amman et al. 2021) notwithstanding, the limited or complete lack of temporal overlap between bat and human activities is likely an important factor confounding the spillover process (K. Zhao et al. 2022). It may also be the case that the viruses with zoonotic potential have not yet evolved the specific mechanisms required for cell entry, replication and human infection (Letko et al. 2020). Alternatively, the spillover process may be incomplete and that transmission to an intermediate host may have occurred rather than direct transmission to humans. We observed close interactions between bats and other wildlife species while foraging, especially bushbabies (*Galago spp.*) and genets (*Genetta tigrina*), that could plausibly facilitate exposure and spillover. Genets were observed directly preying on *R. agyptiacus* while bushbabies were observed foraging within the same *Ficus* spp. as *R. aegyptiacus.* No viral surveillance of wildlife species has been performed in the area and should form part of future investigations to determine whether transmission to an intermediate host has occurred.

As this study was solely focused on assessing the spatial overlap and risk of contact between bats and people within Fertilis, we did not attempt to track the bats' movements outside of the study area. The VHF trackers we deployed are also not suitable for tracking the movements of *R. aegyptiacus* over a wide area outside the valley. However, information regarding the wider movement patterns of *R. aegyptiacus*, especially during the dry season when fewer bats remained in the valley, would help identify key potential foraging locations outside of the valley and potential secondary roost sites. Future studies should therefore look to expand on our findings by employing methods that can accurately track bats outside of the valley. GPS tags provide high-resolution data on movement patterns, without the need for personnel in the field and therefore could suitably identify alternative roosting or foraging sites for *R. aegyptiacus* when they are not in the valley. This may help identify additional interfaces with potential spatial overlap between *R. aegyptiacus* and people, expanding the range of risk of contact. This could also potentially identify migratory patterns which would explain the fluctuations in colony size during the year and provide an idea of where the bats are going during the dry season. The GPS trackers would, however, need to rely on onboard battery power rather than solar-powered batteries as they would not receive charge during the day since these bats roost in caves. Furthermore, an interesting follow-up would be to specifically assess movement differences between sexes or age cohorts as an indication of whether there are specific groups from the colony that may pose increased risk of contact. This should be performed in conjunction with dietary studies to investigate the food sources targeted in different seasons as this could identify specific periods where bats are more reliant on domestically grown fruiting trees than natural fruit sources.

3.6 Conclusion

This study provides insight into the seasonal movement patterns of a known viral host, an aspect which, up until recently, was often not an integral part of viral biosurveillance studies and risk assessments (Dougherty et al. 2018). Although no spillover events or outbreaks have yet been reported, we provide evidence for the potential of future spillover given the spatial overlap between bats and people in our study area. Even though there was no evidence for direct temporal overlap, the findings of Amman et al. (2021) that viable Marburg virus can be detected on discarded fruit for up to six hours illustrate the potential for indirect contact and exposure for humans. Specifically, we identify July as a high-risk period for spillover to humans as the potential for viral spillover is increased and bat activity in residential areas was highest during this period. Future studies could investigate the potential overlap between bats and livestock populations in the area given the historical evidence for livestock species to serve as intermediate hosts during spillover events (Letko et al. 2020).

3.7 Declarations

3.7.1 Ethical approval

All procedures were performed in accordance with ethical approval obtained from the University of Pretoria Faculty of Health Science Research Ethics Committee and the Faculty of Veterinary Science Animal Ethics Committee with the clearance numbers AEC – 254/2020 and AEC – 552/2020. Permission was also obtained for performing research in the area under Section 20 of the Animal Disease Act (Act No. 35 of 1984) from the Department of Agriculture, Land Reform and Rural Development of South Africa (12/11/1/1/8). Furthermore, a regional sampling permit was obtained for sampling in the cave from the Department of Economic

Development, Environment and Tourism of the Limpopo Provincial Government, South Africa (ZA/LP/100499).

3.7.2 Acknowledgements

We would like to thank the staff and students from the Biosurveillance and Ecology of Emerging Zoonoses (BEEZ) research group, part of the Centre for Viral Zoonoses (CVZ) from the University of Pretoria for their assistance with fieldwork and data collection activities. Furthermore, we would like to acknowledge Chief Cyril Mampa and the community members from Ga Mafefe for allowing us to perform our research within their village.

3.7.3 Funding

This research was funded by the DSI-NRF South African Research Chair (SARHCHi) in Infectious Diseases of Animals (Prof. W. Markotter) together with a grant-holder linked bursary (Grant no. 98339). The opinions expressed and conclusions drawn in this study are those of the authors and are not necessarily attributed to the NRF. Furthermore, funding was also obtained from the USA Department of Defence, Defence Threat Reduction Agency (HDTRA12010025). The content of the information reported here does not necessarily reflect the position or the policy of the federal government and no official endorsement should be inferred.

3.8 Additional files

Additional file 1.csv – Weather data. Weather station data from the Fertilis valley for the duration of the study period. 10.25403/UPresearchdata.22815410

Additional file 2.csv – Vegetation data. Fruit availability estimates for selected fruiting trees in the Fertilis valley. 10.25403/UPresearchdata.22815410

Additional file 3.xlsx – Tracking data. Location data for the 26 tracked bats and bat sightings in the Fertilis valley. 10.25403/UPresearchdata.22815410

Chapter 4: Final conclusion

This study explored the ecological context of bat movement patterns and how these can be related to risk assessments through two avenues of investigation. Firstly, a meta-analysis of home ranges for 81 bat species provided the first-ever global review assessing the influence of ecological and biological variables on bat home range size. Secondly, this study also included the first movement study for a colony of *R. aegyptiacus* in Limpopo for over 40 years (Jacobsen et al. 1986) and the first-ever study for the colony from Matlapitsi cave that are known to host viruses with zoonotic potential (Jansen van Vuren et al. 2018; Pawęska et al. 2018; Mortlock et al. 2019; Mortlock et al. 2021). The findings from the movement study identified evidence of distinct seasonal variations in the use of residential areas that may translate to periods of increased contact and spillover risk.

The meta-analysis supported previous findings from a study focusing on North American and European bats (Laforge et al. 2021) but also expanded on it by including a global scope and investigating additional variables not previously assessed. Sex, wing loading, functional group, colony size, dietary class, latitudinal region and habitat type were all shown to influence home range size and there were further interactions for habitats in different latitudinal regions that influenced bat home ranges. The findings for wing loading, functional group, colony size and latitudinal region supported previous hypotheses or suggestions that these variables may influence home range size (O'Donnell 2001; Meyer et al. 2005; Monadjem et al. 2009; Hurme et al. 2022). Although dietary class was shown to influence home range size, the small sample sizes for different classes likely masked the true differences between diets and further analyses with larger sample sizes would be required before accurate conclusions can be drawn. There were limitations in terms of the number of species obtained as they represented only 5% of the total bat species diversity, and several variables could not be included due to inconsistent reporting among the studies. However, these findings are still important for understanding the different characteristics that can influence bat movement patterns and which may aid conservation efforts but also contribute towards risk assessments.

The radio-tracking of *R. aegyptiacus* in Limpopo highlighted important trends in their movement patterns across a seasonal gradient and provided evidence for the risk of contact and potential viral spillover to humans. There were distinct patterns of landcover use between the dry and wet seasons as bats utilised residential areas more frequently and exploited a larger proportion of these areas during the dry season. These trends for increased usage of residential areas during the dry season coincided with periods of low availability of natural resources as many of the native fruiting trees did not bear fruit during the dry season. Fruiting trees grown for subsistence purposes within residential areas provided relatively stable sources of fruit year-round. Specifically, citrus was abundant during the dry season and the large number of banana plants meant that bananas were almost constantly available. The presence of these domestically grown fruit species likely resulted in bats exploiting these areas when alternative food sources were scarce and there were observations from the tracking team and Fertilis residents of bats foraging within banana plants during the study period to support this.

There were limitations for the tracking part of this study in terms of available manpower and technological constraints. Tracking efforts were limited to a single team as there were insufficient personnel available to assist which prevented the triangulation of locations or surveying different areas of the valley simultaneously, which may have affected the accuracy and number of locations obtained. Tracking was limited to the study area as bats could not be tracked if they flew out of the valley or into the mountains. Consequently, home range sizes could not be estimated for the tracked bats as the area would be heavily biased towards the study area. Additionally, the inability to track bats outside of the valley, meant that the larger-scale movement patterns of these bats remain unknown. However, this study was focused on the movements of *R. aegyptiacus* specifically for risk of contact within the valley.

Future studies should, therefore, look to expand on this research by performing follow-up tracking studies with GPS tags that would broaden the scope of the study area and provide data for bat movement patterns outside of the valley. Furthermore, more focus should be placed on the potential overlap between bats and livestock species given their free-ranging nature and their historical association as intermediate hosts during spillover events (Letko et al. 2020). An additional aspect to be included in future studies is the surveillance of humans within the valley. The basis of the study would include targeted surveys of villagers assessing their potential contact with bats, livestock or other wildlife species and then the testing of human blood and serum samples to investigate potential exposure to bat-borne pathogens. Ethical approval has already been obtained and the study is set to commence in 2023 and will provide a valuable perspective on the risks of contact with bats.

Chapter 5: References

Abbott I., Harrison S., Butler F. 2012. Clutter-adaptation of bat species predicts their use of under-motorway passageways of contrasting sizes–a natural experiment. Journal of Zoology 287: 124-132.

Abedi-Lartey M., Dechmann D.K., Wikelski M., Scharf A.K., Fahr J. 2016. Long-distance seed dispersal by straw-coloured fruit bats varies by season and landscape. Global Ecology and Conservation 7: 12-24.

Acharya P.R., Racey P.A., Sotthibandhu S., Bumrungsri S. 2015. Home-range and foraging areas of the dawn bat *Eonycteris spelaea* in agricultural areas of Thailand. Acta Chiropterologica 17: 307-319.

Alam A.M. 2022. Nipah virus, an emerging zoonotic disease causing fatal encephalitis. Clinical Medicine 22: 348-352.

Albariño C.G., Foltzer M., Towner J.S., Rowe L.A., Campbell S., Jaramillo C.M., Bird B.H., Reeder D.M., Vodzak M.E., Rota P., et al. 2014. Novel paramyxovirus associated with severe acute febrile disease, South Sudan and Uganda, 2012. Emerging Infectious Diseases 20: 211-216.

Albayrak İ., Aşan N., Yorulmaz T. 2008. The natural history of the Egyptian fruit bat, *Rousettus aegyptiacus*, in Turkey (Mammalia: Chiroptera). Turkish Journal of Zoology 32: 11-18.

Aldridge H., Brigham R. 1988. Load carrying and maneuverability in an insectivorous bat: a test of the 5%" rule" of radio-telemetry. Journal of Mammalogy 69: 379-382.

Aldridge H., Rautenbach I. 1987. Morphology, echolocation and resource partitioning in insectivorous bats. The Journal of Animal Ecology: 763-778.

Alkhovsky S., Lenshin S., Romashin A., Vishnevskaya T., Vyshemirsky O., Bulycheva Y., Lvov S., Gitelman A. 2022. SARS-like coronaviruses in horseshoe bats (*Rhinolophus* spp.) in Russia, 2020. Viruses 14: DOI: 10.3390/v14010113.

Allen A.M., Singh N.J. 2016. Linking movement ecology with wildlife management. Frontiers in Ecology and Evolution 3: DOI: 10.3389/fevo.2015.00155.

Allen T., Murray K.A., Zambrana-Torrelio C., Morse S.S., Rondinini C., Di Marco M., Breit N., Olival K.J., Daszak P. 2017. Global hotspots and correlates of emerging zoonotic diseases. Nature Communications 8: DOI: 10.1038/s41467-017-00923-8.

Altringham J.D. 2011. Bats: from evolution to conservation. Oxford University Press Inc., New York, USA.

Amarasinghe G.K., Ayllón M.A., Bào Y., Basler C.F., Bavari S., Blasdell K.R., Briese T., Brown P.A., Bukreyev A., Balkema-Buschmann A., et al. 2019. Taxonomy of the order Mononegavirales: update 2019. Archives of Virology 164: 1967-1980.

Amman B.R., Albariño C.G., Bird B.H., Nyakarahuka L., Sealy T.K., Balinandi S., Schuh A.J., Campbell S.M., Ströher U., Jones M.E., et al. 2015a. A recently discovered pathogenic paramyxovirus, Sosuga virus, is present in *Rousettus aegyptiacus* fruit bats at multiple locations in Uganda. Journal of Wildlife Diseases 51: 774-779.

Amman B.R., Bird B.H., Bakarr I.A., Bangura J., Schuh A.J., Johnny J., Sealy T.K., Conteh I., Koroma A.H., Foday I., et al. 2020a. Isolation of Angola-like Marburg virus from Egyptian rousette bats from West Africa. Nature Communications 11: DOI: 10.1038/s41467-020-14327-8.

Amman B.R., Carroll S.A., Reed Z.D., Sealy T.K., Balinandi S., Swanepoel R., Kemp A., Erickson B.R., Comer J.A., Campbell S., et al. 2012. Seasonal pulses of Marburg virus circulation in juvenile *Rousettus aegyptiacus* bats coincide with periods of increased risk of human infection. PLoS Pathogens 8: e1002877. doi:10.1371/journal.ppat.1002877.

Amman B.R., Jones M.E., Sealy T.K., Uebelhoer L.S., Schuh A.J., Bird B.H., Coleman-McCray J.D., Martin B.E., Nichol S.T., Towner J.S. 2015b. Oral shedding of Marburg virus in experimentally infected Egyptian fruit bats (*Rousettus aegyptiacus*). Journal of Wildlife Diseases 51: 113-124.

Amman B.R., Schuh A.J., Albariño C.G., Towner J.S. 2021. Marburg virus persistence on fruit as a plausible route of bat to primate filovirus transmission. Viruses 13: DOI: 10.3390/v13122394.

Amman B.R., Schuh A.J., Sealy T.K., Spengler J.R., Welch S.R., Kirejczyk S.G., Albariño C.G., Nichol S.T., Towner J.S. 2020b. Experimental infection of Egyptian rousette bats (*Rousettus aegyptiacus*) with Sosuga virus demonstrates potential transmission routes for a bat-borne human pathogenic paramyxovirus. PLoS Neglected Tropical Diseases 14: e0008092.

Ancillotto L., Ariano A., Nardone V., Budinski I., Rydell J., Russo D. 2017. Effects of free-ranging cattle and landscape complexity on bat foraging: implications for bat conservation and livestock management. Agriculture, Ecosystems & Environment 241: 54-61.

Anderson D.J. 1982. The home range: a new nonparametric estimation technique. Ecology 63: 103-112.

Bachorec E., Horáček I., Hulva P., Konečný A., Lučan R.K., Jedlička P., Shohdi W.M., Řeřucha Š., Abi-Said M., Bartonička T. 2020. Spatial networks differ when food supply changes: Foraging strategy of Egyptian fruit bats. PLoS ONE 15: e0229110. DOI: 10.1371/journal.pone.0229110

Balboni A., Battilani M., Prosperi S. 2012. The SARS-like coronaviruses: the role of bats and evolutionary relationships with SARS coronavirus. Microbiologica-Quarterly Journal of Microbiological Sciences 35: 1-16.

Barclay R.M., Barclay L.E., Jacobs D.S. 2006. Deliberate insectivory by the fruit bat *Rousettus aegyptiacus*. Acta Chiropterologica 8: 549-553.

Barclay R., Jacobs D. 2011. Differences in the foraging behaviour of male and female Egyptian fruit bats (*Rousettus aegyptiacus*). Canadian Journal of Zoology 89: 466-473.

Barrette R.W., Xu L., Rowland J.M., McIntosh M.T. 2011. Current perspectives on the phylogeny of Filoviridae. Infection, Genetics and Evolution 11: 1514-1519.

Barton K. 2020. MuMIn: multi-modal inference. R Package version 1.43.17.

Bates D., Mächler M., Bolker B., Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1-48.

Becker D.J., Crowley D.E., Washburne A.D., Plowright R.K. 2019. Temporal and spatial limitations in global surveillance for bat filoviruses and henipaviruses. Biology Letters 15: DOI: 10.1098/rsbl.2019.0423.

Beckmann M., Gerstner K., Akin-Fajiye M., Ceaușu S., Kambach S., Kinlock N.L., Phillips H.R., Verhagen W., Gurevitch J., Klotz S., et al. 2019. Conventional land-use intensification reduces species richness and increases production: A global meta-analysis. Global Change Biology 25: 1941-1956.

Benda P., Abi-Said M., Bartonička T., Bilgin R., Faizolahi K., Lučan R.K., Nicolaou H., Reiter A., Shohdi W.M., Uhrin M., Horáček I. 2011. *Rousettus aegyptiacus* (Pteropodidae) in the Palaearctic: list of records and revision of the distribution range. Vespertilio 15: 3-36.

Benda P., Vallo P., Hulva P., Horáček I. 2012. The Egyptian fruit bat *Rousettus aegyptiacus* (Chiroptera: Pteropodidae) in the Palaearctic: Geographical variation and taxonomic status. Biologia 67: 1230-1244.

Bernard E., Fenton B.M. 2007. Bats in a fragmented landscape: species composition, diversity and habitat interactions in savannas of Santarém, Central Amazonia, Brazil. Biological Conservation 134: 332-343.

Bernstein A.S., Ando A.W., Loch-Temzelides T., Vale M.M., Li B.V., Li H., Busch J., Chapman C.A., Kinnaird M., Nowak K., et al. 2022. The costs and benefits of primary prevention of zoonotic pandemics. Sciences Advances 8: DOI: 10.1126/sciadv.abl4183.

Bird B.H., Mazet J.A. 2018. Detection of emerging zoonotic pathogens: an integrated one health approach. Annual Review of Animal Biosciences 6: 121-139.

Bonaccorso F.J., Todd C.M., Miles A.C., Gorresen P.M. 2015. Foraging range movements of the endangered Hawaiian hoary bat, *Lasiurus cinereus semotus* (Chiroptera: Vespertilionidae). Journal of Mammalogy 96: 64-71.

Bonaccorso F.J., Winkelmann J.R., Byrnes D.G. 2005. Home range, territoriality, and flight time budgets in the black-bellied fruit bat, *Melonycteris melanops* (Pteropodidae). Journal of Mammalogy 86: 931-936.

Bonaccorso F.J., Winkelmann J.R., Dumont E.R., Thibault K. 2002. Home range of *Dobsonia minor* (Pteropodidae): a solitary, foliage–roosting fruit bat in Papua New Guinea. Biotropica 34: 127-135.

Boutin S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. Canadian Journal of Zoology 68: 203-220.

Boyce M.S., McDonald L.L. 1999. Relating populations to habitats using resource selection functions. Trends in ecology & evolution 14: 268-272.

Boyles J.G., Cryan P.M., McCracken G.F., Kunz T.H. 2011. Economic importance of bats in agriculture. Science 332: 41-42.

Bradshaw C.J.A. 2012. Little left to lose: deforestation and forest degradation in Australia since European colonization. Journal of Plant Ecology 5: 109-120.

Brauburger K., Hume A.J., Mühlberger E., Olejnik J. 2012. Forty-five years of Marburg virus research. Viruses 4: 1878-1927.

Breed A.C., Breed M.F., Meers J., Field H.E. 2011. Evidence of endemic Hendra virus infection in flying-foxes (*Pteropus conspicillatus*)—implications for disease risk management. PLoS ONE 6: e28816. DOI: 10.1371/journal.pone.0028816.

Breed A.C., Field H.E., Smith C.S., Edmonston J., Meers J. 2010. Bats without borders: long-distance movements and implications for disease risk management. EcoHealth 7: 204-212.

Brunet-Rossinni A., Wilkinson G. 2009. Methods for age estimation and the study of senescence in bats. In: Kunz T., Parsons S., editors. Ecological and behavioral methods for the study of bats. John Hopkins University Press, Baltimore; 315-325.

Burgin C.J., Colella J.P., Kahn P.L., Upham N.S. 2018. How many species of mammals are there? Journal of Mammalogy 99: 1-14.

Burnham K.P., Anderson D.R. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociological Methods & Research 33: 261-304.

Burnham K.P., Anderson D.R., Laake J.L. 1980. Estimation of density from line transect sampling of biological populations. Wildlife Monographs 72: 3-202.

Burt W.H. 1943. Territoriality and home range concepts as applied to mammals. Journal of Mammalogy 24: 346-352.

Calderón-Capote M.C., Dechmann D.K., Fahr J., Wikelski M., Kays R., O'Mara M.T. 2020. Foraging movements are density-independent among straw-coloured fruit bats. Royal Society Open Science 7: 200274. DOI: 10.1098/rsos.200274.

Calenge C. 2006. The package adehabitat for the R software: tool for the analysis of space and habitat use by animals. Ecological Modelling 197: 1035.

Carbone C., Gittleman J.L. 2002. A common rule for the scaling of carnivore density. Science 295: 2273-2276.

Carr M., Gonzalez G., Sasaki M., Ito K., Ishii A., Hang'ombe B.M., Mweene A.S., Orba Y., Sawa H. 2017. Discovery of African bat polyomaviruses and infrequent recombination in the large T antigen in the Polyomaviridae. Journal of General Virology 98: 726-738.

Chapman C.A., Chapman L.J., Wangham R., Hunt K., Gebo D., Gardner L. 1992. Estimators of fruit abundance of tropical trees. Biotropica 24: 527-531.

Chapman C.A., Wrangham R., Chapman L.J. 1994. Indices of habitat-wide fruit abundance in tropical forest. Biotropica 26: 160-171.

Cheetham S., Markotter W. 2021. Bats as reservoirs of viral zoonoses. In. Lim B.K., Fenton M.B., Brigham R.M., Mistry S., Kurta A., Gillam E.H., Russell A., Ortega J., editors. 50 years of bat research: Foundations and new frontiers. Springer Cham; 313-330.

Cheng H.-C., Lee L.-L. 2004. Temporal variations in the size and composition of Formosan leaf-nosed bat (*Hipposideros terasensis*) colonies in central Taiwan. Zoological Studies 43: 787-794.

Ching P.K.G., de los Reyes V.C., Sucaldito M.N., Tayag E., Columna-Vingno A.B., Malbas F.F. Jr., Bolo G.C. Jr., Sejvar J.J., Eagles D., Playford G., et al. 2015. Outbreak of henipavirus infection, Philippines, 2014. Emerging Infectious Diseases 21: 328-331.

Chua K., Bellini W., Rota P., Harcourt B., Tamin A., Lam S., Ksiazek T., Rollin P., Zaki S., Shieh W.-J., et al. 2000. Nipah virus: a recently emergent deadly paramyxovirus. Science 288: 1432-1435.

Chua K.B., Koh C.L., Hooi P.S., Wee K.F., Khong J.H., Chua B.H., Chan Y.P., Lim M.E., Lam S.K. 2002. Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes and Infection 4: 145-151.

Clobert J., Baguette M., Benton T.G., Bullock J.M. 2012. Dispersal ecology and evolution. Oxford University Press.

Coertse J., Geldenhuys M., le Roux K., Markotter W. 2021. Lagos bat virus, an under-reported rabies related lyssavirus. Viruses 13: DOI: 10.3390/v13040576.

Coertse J., Grobler C.S., Sabeta C.T., Seamark E.C., Kearney T., Pawęska J.T., Markotter W. 2020. Lyssaviruses in insectivorous bats, South Africa, 2003–2018. Emerging Infectious Diseases 26: 3056-3060.

Conenna I., López-Baucells A., Rocha R., Ripperger S., Cabeza M. 2019. Movement seasonality in a desert-dwelling bat revealed by miniature GPS loggers. Movement ecology 7: 1-10.

Cook R.D., Prescott P. 1981. On the accuracy of Bonferroni significance leves for detecting outliers in linear models. Technometrics 23: 59-63.

Corman A.M., Mendel B., Voigt C.C., Garthe S. 2016. Varying foraging patterns in response to competition? A multicolony approach in a generalist seabird. Ecology and Evolution 6: 974-986.

Corman V.M., Ithete N.L., Richards L.R., Schoeman M.C., Preiser W., Drosten C., Drexler J.F. 2014. Rooting the phylogenetic tree of Middle East Respiratory Syndrome Coronavirus by characterization of a conspecific virus from an African bat. Journal of Virology 88: 11297-11303.

Cruzan M.B., Weinstein B.G., Grasty M.R., Kohrn B.F., Hendrickson E.C., Arredondo T.M., Thompson P.G. 2016. Small unmanned aerial vehicles (micro-UAVs, drones) in plant ecology. Applications in Plant Sciences 4: DOI: 10.3732/apps.1600041.

Daubenmire R. 1968. Plant communities: a textbook of plant synecology. Harper & Row, New York, New York City, USA.

David D., Davidson I., Berkowitz A., Karniely S., Edery N., Bumbarov V., Laskar O., Elazari-Volcani R. 2020. A novel poxvirus isolated from an Egyptian fruit bat in Israel. Veterinary Medicine and Science 6: 587-590.

de Jong C., Field H., Tagtag A., Hughes T., Dechmann D., Jayme S., Epstein J., Smith C., Santos I., Catbagan D., et al. 2013. Foraging behaviour and landscape utilisation by the endangered golden-crowned flying fox (*Acerodon jubatus*), the Philippines. PLoS ONE 10: e0120945. DOI: 10.1371/journal.pone.0120945.

Dechmann D.K., Wikelski M., Varga K., Yohannes E., Fiedler W., Safi K., Burkhard W.-D., O'Mara M.T. 2014. Tracking post-hibernation behavior and early migration does not reveal the expected sex-differences in a "female-migrating" bat. PLoS ONE 9: DOI: 10.1371/journal.pone.0114810.

Denzinger A., Schnitzler H.-U. 2013. Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviours of microchiropteran bat. Frontiers in Physiology 4: DOI: 10.3389/fphys.2013.00164.

Dietrich M., Kearney T., Seamark E.C., Pawęska J.T., Markotter W. 2018. Synchronized shift of oral, faecal and urinary microbiotas in bats and natural infection dynamics during seasonal reproduction. Royal Society Open Science 5: DOI: 10.1098/rsos.180041.

Dobson A.P., Pimm S.L., Hannah L., Kaufman L., Ahumada J.A., Ando A.W., Bernstein A., Busch J., Daszak P., Engelmann J., et al. 2020. Ecology and economics for pandemic prevention. Science 369: 379-381.

Dobson G.E. 1875. Conspectus of the suborders, families and genera of Chiroptera arranged according to their natural affinities. Annals and Magazine of Natural History 16: 345-357.

Doherty T.S., Fist C.N., Driscoll D.A. 2019. Animal movement varies with resource availability, landscape configuration and body size: a conceptual model and empirical example. Landscape Ecology 34: 603-614.

Dougherty E.R., Seidel D.P., Carlson C.J., Spiegel O., Getz W.M. 2018. Going through the motions: incorporating movement analyses into disease research. Ecology Letters 21: 588-604.

Downs N.C., Sanderson L.J. 2010. Do bats forage over cattle dung or over cattle? Acta Chiropterologica 12: 349-358.

Driscoll D.A. 2007. How to find a metapopulation. Canadian Journal of Zoology 85: 1031-1048.

Eby P., Peel A.J., Hoegh A., Madden W., Giles J.R., Hudson P.J., Plowright R.K. 2022. Pathogen spillover driven by rapid changes in bat ecology. Nature DOI: 10.1038/ s41586-022-05506-2.

Edson D., Field H., McMichael L., Vidgen M., Goldspink L., Broos A., Melville D., Kristoffersen J., de Jong C., McLaughlin A., et al. 2015. Routes of Hendra virus excretion in naturally-infected flying-foxes: implications for viral transmission and spillover risk. PLoS ONE 10: e0140670. DOI: 10.1371/journal. pone.0140670.

Egert-Berg K., Handel M., Goldshtein A., Eitan O., Borissov I., Yovel Y. 2021. Fruit bats adjust their foraging strategies to urban environments to diversify their diet. BMC Biology 19: 1-11.

Epstein J.H., Newman S.H. 2011. The use of telemetry to understand bat movement and ecology. In. Newman S.H., Field H., Epstein J.H., de Jong C., editors. The role of bats in emerging zoonoses: Balancing ecology, conservation and public health interests. FAO Animal Production and Health Manual: 151-162.

Epstein J.H., Anthony S.J., Islam A., Kilpatrick A.M., Khan S.A., Balkey M.D., Ross N., Smith I., Zambrana-Torrelio C., Tao Y., et al. 2020. Nipah virus dynamics in bats and implications for spillover to humans. Proceedings of the National Academy of Sciences 117: 29190-29201.

Epstein J.H., Olival K.J., Pulliam J.R., Smith C., Westrum J., Hughes T., Dobson A.P., Zubaid A., Rahman S.A., Basir M.M., et al. 2009. *Pteropus vampyrus*, a hunted migratory species with a multinational home-range and a need for regional management. Journal of Applied Ecology 46: 991-1002.

Estrada A., Coates-Estrada R., Meritt Jr D. 1993. Bat species richness and abundance in tropical rain forest fragments and in agricultural habitats at Los Tuxtlas, Mexico. Ecography 16: 309-318.

Fagan W.F., Lewis M.A., Auger-Méthé M., Avgar T., Benhamou S., Breed G., LaDage L., Schlägel U.E., Tan W., Papastamatiou P., et al. 2013. Spatial memory and animal movement. Ecology Letters 16: 1316-1329.

Fagre A.C., Kading R.C. 2019. Can bats serve as reservoirs for arboviruses? Viruses 11: DOI: 10.3390/v11030215.

Fahr J., Abedi-Lartey M., Esch T., Machwitz M., Suu-Ire R., Wikelski M., Dechmann D.K. 2015. Pronounced seasonal changes in the movement ecology of a highly gregarious central-place forager, the African straw-coloured fruit bat (*Eidolon helvum*). PLoS ONE 10: e0138985. DOI: 10.1371/journal.pone.0138985.

Fahr J., Kalko E.K. 2011. Biome transitions as centres of diversity: habitat heterogeneity and diversity patterns of West African bat assemblages across spatial scales. Ecography 34: 177-195.

Fancy S.G., Pank L.F., Douglas D.C., Curby C.H., Garner G.W., Amstrup S.C., Regelin W.L. 1988. Satellite telemetry: a new tool for wildlife research and management. U.S. Fish and Wildlife Service Resource Publication 172, Washington D.C., USA.

Fenton M.B. 1997. Science and the conservation of bats. Journal of Mammalogy 78: 1-14.

Fenton B. 2020. Bats navigate with cognitive maps. Science 369: 142.

Field H., Jordan D., Edson D., Morris S., Melville D., Parry-Jones K., Broos A., Divljan A., McMichael L., Davis R., et al. 2015. Spatiotemporal aspects of Hendra virus infection in pteropid bats (flying-foxes) in eastern Australia. PLoS ONE 10: e0144055. DOI: 10.1371/journal.pone.0144055.

Field H., Young P., Yob J.M., Mills J., Hall L., Mackenzie J. 2001. The natural history of Hendra and Nipah viruses. Microbes and Infection 3: 307-314.

Fleming C.H., Fagan W.F., Mueller T., Olson K.A., Leimgruber P., Calabrese J.M. 2015. Rigorous home range estimation with movement data: a new autocorrelated kernel density estimator. Ecology 96: 1182-1188.

Fleming P.A., Bateman P.W. 2018. Novel predation opportunities in anthropogenic landscapes. Animal Behaviour 138: 145-155.

Fleming T.H. 2019. Bat migration. Encyclopedia of Animal Behavior 3: 605-610.

Fleming T.H., Eby P. 2003. Ecology of bat migration. In: Kunz T., Fenton B., editors. Bat ecology. University of Chicago Press, Chicago, USA; 156-208.

Fleming T.H., Muchhala N. 2008. Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. Journal of Biogeography 35: 764-780.

Ford R.G. 1983. Home range in a patchy environment: optimal foraging predictions. American Zoologist 23: 315-326.

Fox J., Monette G. 1992. Generalized collinearity diagnostics. Journal of the American Statistical Association 87: 178-183.

Fox J., Weisberg S. 2019. An R companion to applied regression. 3rd ed. Sage Publications Inc., Thousand Oaks California, USA.

Frick W.F., Kingston T., Flanders J. 2020. A review of the major threats and challenges to global bat conservation. Annals of the New York Academy of Sciences 1469: 5-25.

Fuentes-Montemayor E., Goulson D., Cavin L., Wallace J.M., Park K.J. 2013. Fragmented woodlands in agricultural landscapes: the influence of woodland character and landscape context on bats and their insect prey. Agriculture, Ecosystems & Environment 172: 6-15.

García D., Martínez D., Herrera J.M., Morales J.M. 2013. Functional heterogeneity in a plant-frugivore assemblage enhances seed dispersal resilience to habitat loss. Ecography 36: 197-208.

Gerber B.D., Hooten M.B., Peck C.P., Rice M.B., Gammonley J.H., Apa A.D., Davis A.J. 2018. Accounting for location uncertainty in azimuthal telemetry data improves ecological inference. Movement Ecology 6: 1-14.

Getz W.M., Wilmers C.C. 2004. A local nearest-neighbor convex-hull construction of home ranges and utilization distributions. Ecography 27: 489-505.

Getz W.M., Fortmann-Roe S., Cross P.C., Lyons A.J., Ryan S.J., Wilmers C.C. 2007. LoCoH: nonparametric kernel methods for constructing home ranges and utilization distributions. PLoS ONE 2: e207. DOI: 10.1371/journal.pone.0000207.

Giles J.R., Plowright R.K., Eby P., Peel A.J., McCallum H. 2016. Models of Eucalypt phenology predict bat population flux. Ecology and Evolution 6: 7230-7245.

Gili F., Newson S.E., Gillings S., Chamberlain D.E., Border J.A. 2020. Bats in urbanising landscapes: habitat selection and recommendations for a sustainable future. Biological Conservation 241: 108343. DOI: 10.1016/j.biocon.2019.108343.

Gitzen R.A., Millspaugh J.J., Kernohan B.J. 2006. Bandwidth selection for fixed-kernel analysis of animal utilization distributions. The Journal of Wildlife Management 70: 1334-1344.

Gompper M.E., Gittleman J.L. 1991. Home range scaling: intraspecific and comparative trends. Oecologia 87: 343-348.

Gottdenker N.L., Streicker D.G., Faust C.L., Carroll C. 2014. Anthropogenic land use change and infectious diseases: a review of the evidence. EcoHealth 11: 619-632.

Gregory T. 2017. Home range estimation. In: Fuentes A., editor. The international encyclopedia of primatology. John Wiley & Sons, Inc.; 575-578

Gunnell G.F., Simmons N.B. 2005. Fossil evidence and the origin of bats. Journal of Mammalian Evolution 12: 209-246.

Gurley E.S., Hegde S.T., Hossain K., Sazzad H.M., Hossain M.J., Rahman M., Sharker M.Y., Salje H., Islam M.S., Epstein J.H., et al. 2017. Convergence of humans, bats, trees, and culture in Nipah virus transmission, Bangladesh. Emerging Infectious Diseases 23: 1446-1453.

Gutiérrez-Pesquera L.M., Tejedo M., Olalla-Tárraga M., Duarte H., Nicieza A., Solé M. 2016. Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. Journal of Biogeography 43: 1166-1178.

Guy C., Ratcliffe J.M., Mideo N. 2020. The influence of bat ecology on viral diversity and reservoir status. Ecology and Evolution 10: 5748-5758.

Hahn M.B., Epstein J.H., Gurley E.S., Islam M.S., Luby S.P., Daszak P., Patz J.A. 2014. Roosting behaviour and habitat selection of *Pteropus giganteus* reveal potential links to Nipah virus epidemiology. Journal of Applied Ecology 51: 376-387.

Hair J.F.J., Anderson R.E., Tatham R.L., Black W.C. 1995. Multivariate data analysis. Macmillan, New York.

Hale R. Swearer S.E. 2016. Ecological traps: current evidence and future directions. Proceedings of the Royal Society B 283: DOI: 10.1098/rspb.2015.2647.

Hall L.S., Krausman P.R., Morrison M.L. 1997. The habitat concept and a plea for standard terminology. Wildlife Society Bulletin 25: 173-182.

Halpin K., Hyatt A.D., Fogarty R., Middleton D., Bingham J., Epstein J.H., Rahman S.A., Hughes T., Smith C., Field H.E., et al. 2011. Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. The American Journal of Tropical Medicine and Hygiene 85: 946-951.

Harris S., Cresswell W., Forde P., Trewhella W., Woollard T., Wray S. 1990. Home-range analysis using radio-tracking data–a review of problems and techniques particularly as applied to the study of mammals. Mammal Review 20: 97-123.

Hassell J.M., Begon M., Ward M.J., Fèvre E.M. 2017. Urbanization and disease emergence: dynamics at the wildlife–livestock–human interface. Trends in Ecology & Evolution 32: 55-67.

Hayman D., Bowen R., Cryan P., McCracken G., O'shea T., Peel A., Gilbert A., Webb C., Wood J. 2013. Ecology of zoonotic infectious diseases in bats: current knowledge and future directions. Zoonoses and Public Health 60: 2-21.

Heer K., Helbig-Bonitz M., Fernandes R.G., Mello M.A., Kalko E.K. 2015. Effects of land use on bat diversity in a complex plantation–forest landscape in northeastern Brazil. Journal of Mammalogy 96: 720-731.

Henry C., Poulle M.-L., Roeder J.-J. 2005. Effect of sex and female reproductive status on seasonal home range size and stability in rural red foxes (*Vulpes vulpes*). Ecoscience 12: 202-209.

Henry M., Thomas D.W., Vaudry R., Carrier M. 2002. Foraging distances and home range of pregnant and lactating little brown bats (*Myotis lucifugus*). Journal of Mammalogy 83: 767-774.

Herzig-Straschil B., Robinson G. 1978. On the ecology of the fruit bat, *Rousettus aegyptiacus leachi* (A. Smith, 1829) in the Tsitsikama Coastal National Park. Koedoe 21: 101-110.

Hirzel A.H., Hausser J., Chessel D., Perrin N. 2002. Ecological-niche factor analysis: how to compute habitat-suitability maps without absence data? Ecology 83: 2027-2036.

Hirzel A.H., Le Lay G. 2008. Habitat suitability modelling and niche theory. Journal of Applied Ecology 45: 1372-1381.

Hodgkison R., Balding S.T., Zubaid A., Kunz T.H. 2004. Temporal variation in the relative abundance of fruit bats (Megachiroptera: Pteropodidae) in relation to the availability of food in a lowland Malaysian rain forest. Biotropica 36: 522-533.

Holmes E.C., Rambaut A., Andersen K.G. 2018. Pandemics: spend on surveillance, not prediction. Nature 558: 180-182.

Holyoak M., Casagrandi R., Nathan R., Revilla E., Spiegel O. 2008. Trends and missing parts in the study of movement ecology. Proceedings of the National Academy of Sciences 105: 19060-19065.

Hooke R.L., Martín Duque J.F., Pedraza Gilsanz J.d. 2012. Land transformation by humans: a review. GSA today 22: 4-10.

Hsu V.P., Hossain M.J., Parashar U.D., Ali M.M., Ksiazek T.G., Kuzmin I., Niezgoda M., Rupprecht C., Bresee J., Breiman R.F. 2004. Nipah virus encephalitis reemergence, Bangladesh. Emerging Infectious Diseases 10: 2082-2087.

Hu B., Ge X., Wang LF., Shi Z. 2015. Bat origin of human coronaviruses. Virology Journal 12: DOI: 10.1186/s12985-015-0422-1.

Hurme E., Fahr J., Eidolon Monitoring Network., Eric-Moise B.F., Hash C.T., O'Mara M.T., Richter H., Tanshi I., Webala P.W., Weber N., et al. 2022. Fruit bat migration matches green wave in seasonal landscapes. Functional Ecology 36: 2043-2055.

Hutcheon J.M., Kirsch J.A. 2006. A moveable face: deconstructing the Microchiroptera and a new classification of extant bats. Acta Chiropterologica 8: 1-10.

Ingala M.R., Simmons N.B., Wultsch C., Krampis K., Provost K.L., Perkins S.L. 2021. Molecular diet analysis of neotropical bats based on fecal DNA metabarcoding. Ecology and Evolution 11: 7474-7491.

Jacobsen N., du Plessis E. 1976. Observations on the ecology and biology of the Cape fruit bat *Rousettus aegyptiacus leachi* in the Eastern Transvaal. South African Journal of Science 72: 270-273.

Jacobsen N.G., Viljoen P., Ferguson W. 1986. Radio tracking of problem fruit bats (*Rousettus aegyptiacus*) in the Transvaal with notes on flight and energetics. Zeitschrift für Säugetierkunde 51: 205-208.

Jansen van Vuren P., Allam M., Wiley M.R., Ismail A., Storm N., Birkhead M., Markotter W., Palacios G., Paweska J.T. 2018. A novel adenovirus isolated from the Egyptian fruit bat in South Africa is closely related to recent isolates from China. Scientific reports 8: 1-8.

Jeltsch F., Bonte D., Pe'er G., Reineking B., Leimgruber P., Balkenhol N., Schröder B., Buchmann C.M., Mueller T., Blaum N., et al. 2013. Integrating movement ecology with biodiversity research-exploring new avenues to address spatiotemporal biodiversity dynamics. Movement ecology 1: 1-13.

Jones G., Teeling E.C. 2006. The evolution of echolocation in bats. Trends in Ecology & Evolution 21: 149-156.

Jung K., Threlfall C.G. 2016. Urbanisation and its effects on bats—a global meta-analysis. In. Voigt C.C., Kingston T., editors. Bats in the Anthropocene: conservation of bats in a changing world. Springer International Publishing; 13-33.

Jung M., Dahal P.R., Butchart S.H., Donald P.F., De Lamo X., Lesiv M., Kapos V., Rondinini C., Visconti P. 2020. A global map of terrestrial habitat types. Scientific Data 7: DOI: 10.1038/s41597-020-00599-8.

Juste J., Ibáñez C. 1993. Geographic variation and taxonomy of *Rousettus aegyptiacus* (Mammalia: Megachiroptera) in the islands of the Gulf of Guinea. Zoological Journal of the Linnean Society 107: 117-129.

Kading R.C., Kityo R.M., Mossel E.C., Borland E.M., Nakayiki T., Nalikka B., Nyakarahuka L., Ledermann J.P., Panella N.A., Gilbert A.T., et al. 2018. Neutralizing antibodies against flaviviruses, Babanki virus, and Rift Valley fever virus in Ugandan bats. Infection Ecology & Epidemiology 8: 1439215. DOI: 10.1080/20008686.2018.1439215.

Kalunda M., Mukwaya L., Mukuye A., Lule M., Sekyalo E., Wright J., Casals J. 1986. Kasokero virus: a new human pathogen from bats (*Rousettus aegyptiacus*) in Uganda. The American Journal of Tropical Medicine and Hygiene 35: 387-392.

Kandeil A., Gomaa M.R., Shehata M.M., El Taweel A.N., Mahmoud S.H., Bagato O., Moatasim Y., Kutkat O., Kayed A.S., Dawson P., et al. 2019. Isolation and characterization of a distinct influenza A virus from Egyptian bats. Journal of Virology 93: e01059-01018. DOI: 10.1128/JVI.01059-18.

Kays R., Crofoot M.C., Jetz W., Wikelski M. 2015. Terrestrial animal tracking as an eye on life and planet. Science 348. DOI: 10.1126/science.aaa2478.

Kays R., Tilak S., Crofoot M., Fountain T., Obando D., Ortega A., Kuemmeth F., Mandel J., Swenson G., Lambert T., et al. 2011. Tracking animal location and activity with and automated radio telemetry system in a tropical rainforest. The Computer Journal 54: 1931-1948.

Kearney M. 2006. Habitat, environment and niche: what are we modelling? Oikos 115: 186-191.

Kelt D.A., Van Vuren D.H. 2001. The ecology and macroecology of mammalian home range area. The American Naturalist 157: 637-645.

Kessler M.K., Becker D.J., Peel A.J., Justice N.V., Lunn T., Crowley D.E., Jones D.N., Eby P., Sánchez C.A., Plowright R.K. 2018. Changing resource landscapes and spillover of henipaviruses. Annals of the New York Academy of Sciences 1429: 78-99.

Kirkpatrick L., Graham J., McGregor S., Munro L., Scoarize M., Park K. 2018. Flexible foraging strategies in *Pipistrellus pygmaeus* in response to abundant but ephemeral prey. PLoS ONE 13: e0204511. DOI: 10.1371/journal.pone.0204511.

Kirol C.P., Kesler D.C., Walker B.L., Fedy B.C. 2020. Coupling tracking technologies to maximize efficiency in avian research. Wildlife Society Bulletin 44: 406-415.

Knief U., Forstmeier W. 2021. Violating the normality assumption may be the lesser of two evils. Behavior Research Methods 53: 2576-2590.

Korine C., Izhaki I., Arad Z. 1999. Is the Egyptian fruit-bat *Rousettus aegyptiacus* a pest in Israel? An analysis of the bat's diet and implications for its conservation. Biological conservation 88: 301-306.

Kranstauber B., Cameron A., Weinzerl R., Fountain T., Tilak S., Wikelski M., Kays R. 2011. The Movebank data model for animal tracking. Environmental Modelling & Software 26: 834-835.

Kunz T.H. 2013a. Ecology of bats. Springer Science & Business Media, Boston, Massachusetts, USA.

Kunz T.H. 2013b. Roosting ecology of bats. In: Kunz T.H., editors. Ecology of bats. Springer Science and Business Media, Boston, Massachusetts, USA; 1-56.

Kunz T.H., Betke M., Hristov N.I., Vonhof M.J. 2009. Methods for assessing colony size, population size, and relative abundance of bats. In: Kunz T.H., Parsons S., editors. Ecological and behavioral methods for the study of bats. Johns Hopkins University Press; 133-157.

Kunz T.H., De Torrez E.B., Bauer D., Lobova T., Fleming T.H. 2011. Ecosystem services provided by bats. Annals of the New York Academy of Sciences 1223: 1-38.

Kunz T.H., Lumsden L.F., Fenton M. 2003. Ecology of cavity and foliage roosting bats. In: Kunz T.H., Fenton B., editors. Bat ecology. The University of Chicago Press, Chicago, USA; 3-89.

Kurek K., Gewartowska O., Tołkacz K., Jędrzejewska B., Mysłajek R.W. 2020. Home range size, habitat selection and roost use by the whiskered bat (*Myotis mystacinus*) in human-dominated montane landscapes. PLoS ONE 15: e0237243. DOI: 10.1371/journal.pone.0237243.

Kuznetsova A., Brockhoff P.B., Christensen R.H.B. 2015. lmerTest package: tests in linear mixed effects models. Journal of Statistical Software 8:1-26.

Kwiecinski G.G., Griffiths T.A. 1999. *Rousettus egyptiacus*. Mammalian species 611: 1-9.

Laforge A., Archaux F., Coulon A., Sirami C., Froidevaux J., Gouix N., Ladet S., Martin H., Barré K., Roemer C., et al. 2021. Landscape composition and life-history traits influence bat movement and space use: Analysis of 30 years of published telemetry data. Global Ecology and Biogeography 30: 2442-2454.

Laver P.N., Kelly M.J. 2008. A critical review of home range studies. The Journal of Wildlife Management 72: 290-298.

Laycock P. 1973. Distribution and abundance of bats in the Natal midlands (Mammalia: Chiroptera). Annals of the Transvaal Museum 28: 207-229. Leblond M., Dussault C., Ouellet J.P. 2010. What drives fine-scale movements of large herbivores? A case study using moose. Ecography 33: 1102-1112.

Leendertz S.A.J., Gogarten J.F., Düx A., Calvignac-Spencer S., Leendertz F.H. 2016. Assessing the evidence supporting fruit bats as the primary reservoirs for Ebola viruses. EcoHealth 13: 18-25.

Lenth R.V. 2022. emmeans: estimated marginal means, aka least-squares means. R Package version 1.7.3.

Leroy E.M., Kumulungui B., Pourrut X., Rouquet P., Hassanin A., Yaba P., Délicat A., Paweska J.T., Gonzalez J.-P., Swanepoel R. 2005. Fruit bats as reservoirs of Ebola virus. Nature 438: 575-576.

Letko M., Seifert S.N., Olival K.J., Plowright R.K., Munster V.J. 2020. Bat-borne virus diversity, spillover and emergence. Nature Reviews Microbiology 18: 461-471.

Li W., Shi Z., Yu M., Ren W., Smith C., Epstein J.H., Wang H., Crameri G., Hu Z., Zhang H. 2005. Bats are natural reservoirs of SARS-like coronaviruses. Science 310: 676-679.

Lim V.-C., Clare E.L., Littlefair J.E., Ramli R., Bhassu S., Wilson J.-J. 2018. Impact of urbanisation and agriculture on the diet of fruit bats. Urban Ecosystems 21: 61-70.

Luby S.P., Hossain M.J., Gurley E.S., Ahmed B.-N., Banu S., Khan S.U., Homaira N., Rota P.A., Rollin P.E., Comer J.A. 2009. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerging infectious diseases 15: 1229.

Lučan R.K., Bartonička T., Jedlička P., Řeřucha Š., Šálek M., Čížek M., Nicolaou H., Horáček I. 2016. Spatial activity and feeding ecology of the endangered northern population of the Egyptian fruit bat (*Rousettus aegyptiacus*). Journal of Mammalogy 97: 815-822.

Luis A.D., Hayman D.T., O'Shea T.J., Cryan P.M., Gilbert A.T., Pulliam J.R., Mills J.N., Timonin M.E., Willis C.K., Cunningham A.A., et al. 2013. A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? Proceedings of the Royal Society B: Biological Sciences 280: 20122753. DOI: 10.1098/rspb.2012.2753.

Mackie I.J., Racey P.A. 2007. Habitat use varies with reproductive state in noctule bats (*Nyctalus noctula*): implications for conservation. Biological Conservation 140: 70-77.

Magouras I., Brookes V.J., Jori F., Martin A., Pfeiffer D.U., Dürr S. 2020. Emerging Zoonotic Diseases: Should We Rethink the Animal–Human Interface? Frontiers in Veterinary Science 7: DOI: 10.3389/fvets.2020.582743.

Mahalingam S., Herrero L.J., Playford E.G., Spann K., Herring B., Rolph M.S., Middleton D., McCall B., Field H., Wang L. 2012. Hendra virus: an emerging paramyxovirus in Australia. The Lancet Infectious Diseases 12: 799-807.

Manly B., McDonald L., Thomas D.L., McDonald T.L., Erickson W.P. 2002. Resource selection by animals: statistical design and analysis for field studies. Kluwer Academic Publishers, Dordrecht, Netherlands.

Markotter W., Coertse J., De Vries L., Geldenhuys M., Mortlock M. 2020. Bat-borne viruses in Africa: a critical review. Journal of Zoology 311: 77-98.

Martinez J.L., Lucas-Borja M.E., Plaza-Alvarez P.A., Denisi P., Moreno M.A., Hernández D., González-Romero J., Zema D.A. 2021. Comparison of satellite and drone-based images at two spatial scales to evaluate vegetation regeneration after post-fire treatments in a Mediterranean forest. Applied Sciences 11: 5423 DOI: 10.3390/app11125423.

Mazerolle M.J. 2020. AICcmodavg: model selection and multimodal inference based on (Q)AIC(c). R Package version 2.3-1.

Mbu'u C.M., Mbacham W.F., Gontao P., Sado Kamdem S.L., Nlôga A.M.N., Groschup M.H., Wade A., Fischer K., Balkema-Buschmann A. 2019. Henipaviruses at the interface between bats, livestock and human population in Africa. Vector-Borne and Zoonotic Diseases 19: 455-465.

McClure K.M., Gilbert A.T., Chipman R.B., Rees E.E., Pepin K.M. 2020. Variation in host home range size decreases rabies vaccination effectiveness by increasing the spatial spread of rabies virus. Journal of Animal Ecology 89: 1375-1386.

McConkey K.R., Drake D.R. 2007. Indirect evidence that flying foxes track food resources among islands in a Pacific archipelago. Biotropica 39: 436-440.

McIntyre G. 1953. Estimation of plant density using line transects. Journal of Ecology 41: 319-330.

McKee C.D., Islam A., Luby S.P., Salje H., Hudson P.J., Plowright R.K., Gurley E.S. 2021. The ecology of Nipah virus in Bangladesh: a nexus of land-use change and opportunistic feeding behavior in bats. Viruses 13: 169. DOI: 10.3390/v13020169

McLoughlin P.D., Ferguson S.H. 2000. A hierarchical pattern of limiting factors helps explain variation in home range size. Ecoscience 7: 123-130.

McNab B.K. 1963. Bioenergetics and the determination of home range size. The American Naturalist 97: 133-140.

Mammal Diversity Database. 2022. Mammal Diversity Database (Version 1.9.1). Zenodo. http://doi.org/10.5281/zenodo.4139818. Accessed 30 Nov 2022.

Meyer C.F., Weinbeer M., Kalko E.K. 2005. Home-range size and spacing patterns of *Macrophyllum macrophyllum* (Phyllostomidae) foraging over water. Journal of Mammalogy 86: 587-598.

Millspaugh J., Kesler D., Kays R., Gitzen R., Schulz J., Rota C., Bodinof C., Belant J., Keller B. 2012. Wildlife radiotelemetry and remote monitoring. In: Silvy N.J., editor. The wildlife techniques manual: (Volume 1: Research). Johns Hopkins University Press; 258-283.

Mohd H.A., Al-Tawfiq J.A., Memish Z.A. 2016. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) origin and animal reservoir. Virology Journal 13: DOI: 10.1186/s12985-016-0544-0.

Moher D., Liberati A., Tetzlaff J., Altman D.G., PRISMA Group 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Annals of internal medicine 151: 264-269.

Mohr C.O. 1947. Table of equivalent populations of North American small mammals. The American Midland Naturalist 37: 223-249.

Mollentze N., Streicker D.G. 2020. Viral zoonotic risk is homogenous among taxonomic orders of mammalian and avian reservoir hosts. Proceedings of the National Academy of Sciences 117: 9423-9430.

Monadjem A., Reside A., Cornut J., Perrin M.R. 2009. Roost selection and home range of an African insectivorous bat *Nycteris thebaica* (Chiroptera, Nycteridae). Mammalia 73: 353-359.

Monadjem A., Taylor P.J., Schoeman M.C. 2020. Bats of southern and central Africa: a biogeographic and taxonomic synthesis. Wits University Press.

Moratelli R., Calisher C.H. 2015. Bats and zoonotic viruses: can we confidently link bats with emerging deadly viruses? Memórias do Instituto Oswaldo Cruz 110: 1-22.

Morgan G.R., Hodgson M.E., Wang C., Schill S.R. 2022. Unmanned aerial remote sensing of coastal vegetation: a review. Annals of GIS 28: 385-399.

Morse S.S., Mazet J.A., Woolhouse M., Parrish C.R., Carroll D., Karesh W.B., Zambrana-Torrelio C., Lipkin W.I., Daszak P. 2012. Prediction and prevention of the next pandemic zoonosis. The Lancet 380: 1956-1965.

Mortlock M., Dietrich M., Weyer J., Paweşka J.T., Markotter W. 2019. Co-circulation and excretion dynamics of diverse rubula-and related viruses in Egyptian rousette bats from South Africa. Viruses 11: 37 DOI: 10.3390/v11010037.

Mortlock M., Geldenhuys M., Dietrich M., Epstein J.H., Weyer J., Paweşka J.T., Markotter W. 2021. Seasonal shedding patterns of diverse henipavirus-related paramyxoviruses in Egyptian rousette bats. Scientific reports 11: 1-12.

Moussy C., Hosken D., Mathews F., Smith G., Aegerter J., Bearhop S. 2013. Migration and dispersal patterns of bats and their influence on genetic structure. Mammal Review 43: 183-195.

Müller M.A., Devignot S., Lattwein E., Corman V.M., Maganga G.D., Gloza-Rausch F., Binger T., Vallo P., Emmerich P., Cottontail V.M., et al. 2016. Evidence for widespread infection of African bats with Crimean-Congo hemorrhagic fever-like viruses. Scientific reports 6: DOI: 10.1038/srep26637.

Mundry R. 2014. Statistical issues and assumptions of phylogenetic least squares. In: Garamszegi L.Z., editor. Modern phylogenetic comparative methods and their application in evolutionary biology. Springer, Berlin; 131-153.

Murray K., Selleck P., Hooper P., Hyatt A., Gould A., Gleeson L., Westbury H., Hiley L., Selvey L., Rodwell B. 1995. A morbillivirus that caused fatal disease in horses and humans. Science 268: 94-97.

Nathan R., Getz W.M., Revilla E., Holyoak M., Kadmon R., Saltz D., Smouse P.E. 2008. A movement ecology paradigm for unifying organismal movement research. Proceedings of the National Academy of Sciences 105: 19052-19059.

Noer C.L., Dabelsteen T., Bohmann K., Monadiem A. 2012. Molossid bats in an African agro-ecosystem select sugarcane fields as foraging habitat. African Zoology 47: 1-11.

Noonan M.J., Fleming C.H., Tucker M.A., Kays R., Harrison A.L., Crofoot M.C., Abrahms B., Alberts S.C., Ali A.H., Altmann J., et al. 2020. Effects of body size on estimation of mammalian area requirements. Conservation Biology 34: 1017-1028.

Noonan M.J., Tucker M.A., Fleming C.H., Akre T.S., Alberts S.C., Ali, A.H., Altmann J., Antunes P.C., Belant J.L., Beyer D., et al. 2019. A comprehensive analysis of autocorrelation and bias in home range estimation. Ecological Monographs 89: e01344. DOI: 10.1002/ecm.1344.

Norberg U.M., Rayner J.M. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London. B, Biological Sciences 316: 335-427.

Oakleaf J.R., Kennedy C.M., Baruch-Mordo S., West P.C., Gerber J.S., Jarvis L., Kiesecker J. 2015. A world at risk: aggregating development trends to forecast global habitat conversion. PLoS ONE 10: DOI: 10.1371/journal.pone.0138334.

O'Brien R.M. 2007. A caution regarding rules of thumb for variance inflation factors. Quality & Quantity 41: 673-690.

O'Donnell C.F. 2001. Home range and use of space by *Chalinolobus tuberculatus*, a temperate rainforest bat from New Zealand. Journal of Zoology 253: 253-264.

O'Mara M.T., Wikelski M., Dechmann D.K.N. 2014. 50 years of bat tracking: device attachment and future directions. Methods in Ecology and Evolution 5: 311-319.

Ofstad E.G., Herfindal I., Solberg E.J., Sæther B.-E. 2016. Home ranges, habitat and body mass: simple correlates of home range size in ungulates. Proceedings of the Royal Society B: Biological Sciences 283: DOI: 10.1098/rspb.2016.1234.

Olival K.J., Hosseini P.R., Zambrana-Torrelio C., Ross N., Bogich T.L., Daszak P. 2017. Host and viral traits predict zoonotic spillover from mammals. Nature 546: 646-650.

Olivero J., Fa J.E., Farfán M.Á., Márquez A.L., Real R., Juste F.J., Leendertz S.A., Nasi R. 2020. Human activities link fruit bat presence to Ebola virus disease outbreaks. Mammal Review 50: 1-10.

Olivero J., Fa J.E., Real R., Márquez A.L., Farfán M.A., Vargas J.M., Gaveau D., Salim M.A., Park D., Suter J. 2017. Recent loss of closed forests is associated with Ebola virus disease outbreaks. Scientific reports 7: DOI:10.1038/s41598-017-14727-9.

One Health High-Level Expert Panel (OHHLEP)., Adisasmito W.B., Almuhairi S., Behravesh C.B., Bilivogui P., Bukachi S.A., Casas N., Becerra N.C., Charron D.F., Chaudhary A., Ciacci Zanella J.R., et al. One Health: a new definition for a sustainable and healthy future. PLoS Pathogens 18: DOI: 10.1371/journal.ppat.1010537.

Orme D., Freckleton R., Thomas G., Petzoldt T., Fritz S., Isaac N., Pearse W. 2018. Caper: comparative analysis of phylogenetics and evolution in R. R Package version 1.0.1.

Owen-Smith N., Fryxell J., Merrill E. 2010. Foraging theory upscaled: the behavioural ecology of herbivore movement. Philosophical Transactions of the Royal Society B: Biological Sciences 365: 2267-2278.

Páez D.J., Restif O., Eby P., Plowright R.K. 2018. Optimal foraging in seasonal environments: implications for residency of Australian flying foxes in food-subsidized urban landscapes. Philosophical Transactions of the Royal Society B: Biological Sciences 373: DOI: 10.1098/rstb.2017.0097.

Paradis E., Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35: 526-528.

Pawęska J.T., Storm N., Markotter W., Di Paola N., Wiley M.R., Palacios G., van Vuren P.J. 2020. Shedding of marburg virus in naturally infected Egyptian rousette bats, South Africa, 2017. Emerging Infectious Diseases 26: 3051-3055.

Pawęska J.T., van Vuren P.J., Kemp A., Storm N., Grobbelaar A.A., Wiley M.R., Palacios G., Markotter W. 2018. Marburg virus infection in Egyptian rousette bats, South Africa, 2013–2014. Emerging infectious diseases 24: 1134-1137.

Pennycuick C.J. 2008. Modelling the flying bird. Elsevier.

Peiris J.S., Lai S.T., Poon L.L., Guan Y., Yam L.Y., Lim W., Nicholls J., Yee W.K., Yan W.W., Cheung M.T., et al. 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361: 1319-1325.

Perino A., Pereira H.M., Navarro L.M., Fernández N., Bullock J.M., Ceausu S., Cortés-Avizanda A., van Klink R., Kuemmerle T., Lomba A., et al. 2019. Rewilding complex ecosystems. Science 364: DOI: 10.1126/science.aav5570.

Pigott D.M., Golding N., Mylne A., Huang Z., Weiss D.J., Brady O.J., Kraemer M.U., Hay S.I. 2015. Mapping the zoonotic niche of Marburg virus disease in Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene 109: 366-378.

Plowright R.K., Field H.E., Smith C., Divljan A., Palmer C., Tabor G., Daszak P., Foley J.E. 2008. Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). Proceedings of the Royal Society B: Biological Sciences 275: 861-869.

Plowright R.K., Foley P., Field H.E., Dobson A.P., Foley J.E., Eby P., Daszak P. 2011. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (*Pteropus* spp.). Proceedings of the Royal Society B: Biological Sciences 278: 3703-3712.

Plowright R.K., Parrish C.R., McCallum H., Hudson P.J., Ko A.I., Graham A.L., Lloyd-Smith J.O. 2017. Pathways to zoonotic spillover. Nature Reviews Microbiology 15: 502-510.

Plowright R.K., Peel A.J., Streicker D.G., Gilbert A.T., McCallum H., Wood J., Baker M.L., Restif O. 2016. Transmission or within-host dynamics driving pulses of zoonotic viruses in reservoir–host populations. Plos Neglected Tropical Diseases 10: e0004796. DOI: 10.1371/journal.pntd.0004796.

Plowright R.K., Reaser J.K., Locke H., Woodley S.J., Patz J.A., Becker D.J., Oppler G., Hudson P.J., Tabor G.M. 2021. Land use-induced spillover: a call to action to safeguard environmental, animal, and human health. The Lancet Planetary Health 5: e237-e245.

Pourrut X., Delicat A., Rollin P., Ksiazek T., Gonzalez J.-P., Leroy E. 2007. Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species. The Journal of Infectious Diseases 196: DOI: 10.1086/520541.

Powell R.A. 2000. Animal home ranges and territories and home range estimators. In: Boitani L., Fuller T., editors. Research techniques in animal ecology: controversies and consequences. Columbia University Press, New York, USA; 65-110.

Powell R.A., Mitchell M.S. 2012. What is a home range? Journal of Mammalogy 93: 948-958.

Priotto J., Steinmann A., Polop J. 2002. Factors affecting home range size and overlap in *Calomys venustus* (Muridae: Sigmodontinae) in Argentine agroecosystems. Mammalian Biology 67: 97-104.

Pulliam J.R., Epstein J.H., Dushoff J., Rahman S.A., Bunning M., Jamaluddin A.A., Hyatt A.D., Field H.E., Dobson A.P., Daszak P., Henipavirus Ecology Research Group (HERG). 2012. Agricultural intensification, priming for persistence and the emergence of Nipah virus: a lethal bat-borne zoonosis. Journal of the Royal Society Interface 9: 89-101.

QGIS Development Team. 2022. QGIS Geographic Information System. Open Source Geospatial Foundation Project.

Quesada M., Stoner K.E., Lobo J.A., Herrerias-Diego Y., Palacios-Guevara C., Munguía-Rosas M.A., O.-Salazar K.A., Rosas-Guerrero V. 2004. Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat-pollinated Bombacaceous trees. Biotropica 36: 131-138.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria

Ramanantsalama R.V., Goodman S.M., Dietrich M., Lebarbenchon C. 2022. Interaction between Old World fruit bats and humans: from large scale ecosystem services to zoonotic diseases. Acta Tropica 231: DOI: 10.1016/j.actatropica.2022.106462.

Randhawa N., Bird B.H., VanWormer E., Sijali Z., Kilonzo C., Msigwa A., Ekiri A.B., Samson A., Epstein J.H., Wolking D.J., et al. 2020. Fruit bats in flight: a look into the movements of the ecologically important *Eidolon helvum* in Tanzania. One Health Outlook 2: DOI: 10.1186/s42522-020-00020-9.

Řeřucha Š., Jedlicka P., Hrabina J., Cizek M., Mikel B., Cip O., Bartonicka T., Helan R. 2017. Miniaturized GPS position logger for tracking of small mammals. 2017 6th Mediterranean Conference on Embedded Computing (MECO), IEEE. DOI: 10.1109/MECO.2017.7977242.

Revell L.J. 2010. Phylogenetic signal and linear regression on species data. Methods in Ecology and Evolution 1: 319-329.

Richter H., Cumming G. 2006. Food availability and annual migration of the straw-colored fruit bat (*Eidolon helvum*). Journal of Zoology 268: 35-44.

Richter H., Cumming G. 2008. First application of satellite telemetry to track African straw-coloured fruit bat migration. Journal of Zoology 275: 172-176.

Roberts B.J., Catterall C.P., Eby P., Kanowski J. 2012. Long-distance and frequent movements of the flying-fox *Pteropus poliocephalus*: implications for managemnet. PLoS ONE 7: e42532. DOI: 10.1371/journal.pone.0042532.

Roberts M., Dobson A., Restif O., Wells K. 2021. Challenges in modelling the dynamics of infectious disease at the wildlife-human interface. Epidemics 37: DOI: 10.1016/j.epidem.2021.100523.

Rodriguez-Pinto N.I., Diaz D.R., Medina C.E., Villalobos-Chaves D., Morales J.A., Bocardo E.F., Lopez E. 2022. Home range size of bats *Myotis atacamensis* (Vespertilionidae) and *Amorphochilus schnablii* (Furipteridae) in the coastal valleys of southern Peru. Revista Mexicana de Biodiversidad 93: DOI: 10.22201/ib.20078706e.2022.93.3855.

Rothenwoehrer C., Becker N.I., Tschapka M. 2011. Resource landscape and spatio-temporal activity patterns of a plant-visiting bat in a Costa Rican lowland rainforest. Journal of Zoology 283: 108-116.

RStudio Team. 2020. RStudio: Intergrated Development Environment for R. RStudio, PBC. Boston, MA

Ruiz-Aravena M., McKee C., Gamble A., Lunn T., Morris A., Snedden C.E., Yinda C.K., Port J.R., Buchholz D.W., Yeo Y.Y., et al. 2021. Ecology, evolution and spillover of coronaviruses from bats. Nature Reviews Microbiology 20: 299-314.

Rulli M.C., D'Odorico P., Galli N., Hayman D.T.S. 2021. Land-use change and the livestock revolution increase the risk of zoonotic coronavirus transmission from rhinolophid bats. Nature Food 2: 409-416.

Russo D., Ancillotto L. 2015. Sensitivity of bats to urbanization: a review. Mammalian Biology 80: 205-212.

Safi K., König B., Kerth G. 2007. Sex differences in population genetics, home range size and habitat use of the parti-colored bat (*Vespertilio murinus*, Linnaeus 1758) in Switzerland and their consequences for conservation. Biological conservation 137: 28-36.

Salom-Pérez R., Corrales-Gutiérrez D., Araya-Gamboa D., Espinoza-Muñoz D., Finegan B., Petracca L.S. 2021. Forest cover mediates large and medium-sized mammal occurrence in a critical link of the Mesoamerican Biological Corridor. PLoS ONE 16: e0249072. DOI: 10.1371/journal.pone.0249072.

Sasaki M., Kajihara M., Changula K., Mori-Kajihara A., Ogawa H., Hang'ombe B.M., Mweene A.S., Simuunza M., Yoshida R., Carr M. 2018. Identification of group A rotaviruses from Zambian fruit bats provides evidence for long-distance dispersal events in Africa. Infection, Genetics and Evolution 63: 104-109.

Schnitzler H.-U., Kalko E.K. 2001. Echolocation by insect-eating bats: we define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. Bioscience 51: 557-569.

Schoeman M.C. 2016. Light pollution at stadiums favors urban exploiter bats. Animal Conservation 19: 120-130.

Schuh A.J., Amman B.R., Jones M.E., Sealy T.K., Uebelhoer L.S., Spengler J.R., Martin B.E., Coleman-McCray J.A.D., Nichol S.T., Towner J.S. 2017. Modelling filovirus maintenance in nature by experimental transmission of Marburg virus between Egyptian rousette bats. Nature Communications 8: DOI: 10.1038/ncomms14446.

Seaman D.E., Millspaugh J.J., Kernohan B.J., Brundige G.C., Raedeke K.J., Gitzen R.A. 1999. Effects of sample size on kernel home range estimates. The Journal of Wildlife Management 63: 739-747.

Shaw A.K. 2020. Causes and consequences of individual variation in animal movement. Movement Ecology 8: DOI: 10.1186/s40462-020-0197-x.

Shi M., Lin X.-D., Chen X., Tian J.-H., Chen L.-J., Li K., Wang W., Eden J.-S., Shen J.-J., Liu L., et al. 2018. The evolutionary history of vertebrate RNA viruses. Nature 556: 197-202.

Sillero N., dos Santos R., Teodoro A.C., Carretero M.A. 2021. Ecological niche models improve home range estimations. Journal of Zoology 313: 145-157.

Simmons N., Cirranello A. 2022. Bat Species of the World: A taxonomic and geographic database. https://batnames.org/home.html. Accessed 30 Nov 2022.

Sirami C., Jacobs D.S., Cumming G.S. 2013. Artificial wetlands and surrounding habitats provide important foraging habitat for bats in agricultural landscapes in the Western Cape, South Africa. Biological conservation 164: 30-38.

Smigaj M., Gaulton R. 2021. Capturing hedgerow structure and flowering abundance with UAV remote sensing. Remote Sensing in Ecology and Conservation 7: 521-533.

Soutullo A., Cadahía L., Urios V., Ferrer M., Negro J.J. 2007. Accuracy of lightweight satellite telemetry: a case study in the Iberian Peninsula. Journal of Wildlife Management 71: 1010-1015.

Stamps J. 1995. Motor learning and the value of familiar space. The American Naturalist 146: 41-58.

Stephens P.R., Gottdenker N., Schatz A.M., Schmidt J.P., Drake J.M. 2021. Charcteristics of the 100 largest modern zoonotic outbreaks. Philosophical Transactions of the Royal Society B 376: DOI: 10.1098/rstb.2020.0535.

Stevens R.D., Amarilla-Stevens H.N. 2021. Dietary patterns of phyllostomid bats in interior Atlantic forest of eastern Paraguay. Journal of Mammalogy 102: 685-694.

Swihart R.K., Slade N.A., Bergstrom B.J. 1988. Relating body size to the rate of home range use in mammals. Ecology 69: 393-399.

Symonds M.R., Blomberg S.P. 2014. A primer on phylogenetic generalised least squares. In: Garamszegi L.Z., editor. Modern phylogenetic comparative methods and their application in evolutionary biology. Springer Berlin, Heidelberg; 105-130.

Tajudeen Y.A., Oladipo H.J., Oladunjoye I.O., Mustapha M.O., Mustapha S.T., Abdullahi A.A., Yusuf R.O., Abimbola S.O., Adebayo A.O., Ikebuaso J.G., et al. 2022. Preventing the next pandemic through a planetary health approach: a focus on key drivers of zoonosis. Challenges 13: DOI: 10.3390/ challe13020050.

Tan B., Yang X.-L., Ge X.-Y., Peng C., Liu H.-Z., Zhang Y.-Z., Zhang L.-B., Shi Z.-L. 2017. Novel bat adenoviruses with low G+C content shed light on the evolution of adenoviruses. Journal of General Virology 98: 739-748

Tan C.W., Yang X., Anderson D.E., Wang L.-F. 2021. Bat virome research: The past, the present and the future. Current Opinion in Virology 49: 68-80.

Tao Y., Shi M., Conrardy C., Kuzmin I.V., Recuenco S., Agwanda B., Alvarez D.A., Ellison J.A., Gilbert A.T., Moran D. 2013. Discovery of diverse polyomaviruses in bats and the evolutionary history of the *Polyomaviridae*. Journal of General Virology 94: 738-748.

Tay J.Y.L., Erfmeie A., Kalwij J.M. 2018. Reaching new heights: can drones replace current methods to study plant population dynamics? Plant Ecology 219: 1139-1150.

Taylor P., Cheney, C., Sapsford C. 1999. Roost habitat evaluation and distribution of bats (Chiroptera) in the Durban metropolitan region. Durban Museum Novitates 24: 62-71.

The World Bank Group. 2021. Climate Change Knowledge Portal. https://climateknowledgeportal.worldbank.org/country/south-africa/climate-data-historical.

Thomas D.L., Taylor E.J. 1990. Study designs and tests for comparing resource use and availability. The Journal of Wildlife Management 54: 322-330.

Thomas L., Buckland S.T., Rexstad E.A., Laake J.L., Strindberg S., Hedley S.L., Bishop J.R., Marques T.A., Burnham K.P. 2010. Distance software: design and analysis of distance sampling surveys for estimating population size. Journal of Applied Ecology 47: 5-14.

Thomas S.P., Suthers R.A. 1972. The physiology and energetics of bat flight. Journal of Experimental Biology 57: 317-335.

Tilman D., Balzer C., Hill J., Before B.L. 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences 108: 20260-20264.

Tilman D., Fargione J., Wolff B., D'antonio C., Dobson A., Howarth R., Schindler D., Schlesinger W.H., Simberloff D., Swackhamer D. 2001. Forecasting agriculturally driven global environmental change. Science 292: 281-284.

Toledo S., Shohami D., Schiffner I., Lourie E., Orchan Y., Bartan Y., Nathan R. 2020. Cognitive map–based navigation in wild bats revealed by a new high-throughput tracking system. Science 369: 188-193.

Tong S., Conrardy C., Ruone S., Kuzmin I.V., Guo X., Tao Y., Niezgoda M., Haynes L., Agwanda B., Breiman R.F. 2009. Detection of novel SARS-like and other coronaviruses in bats from Kenya. Emerging Infectious Diseases 15: 482-485.

Tournant P., Afonso E., Roué S., Giraudoux P., Foltête J.-C. 2013. Evaluating the effect of habitat connectivity on the distribution of lesser horseshoe bat maternity roosts using landscape graphs. Biological Conservation 164: 39-49.

Towner J.S., Amman B.R., Sealy T.K., Carroll S.A.R., Comer J.A., Kemp A., Swanepoel R., Paddock C.D., Balinandi S., Khristova M.L., et al. 2009. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathogens 5: e1000536. DOI: 10.1371/journal.ppat.1000536.

Tsagkogeorga G., Parker J., Stupka E., Cotton J.A., Rossiter S,J. 2013. Phylogenomic analyses elucidate the evolutionary relationship of bats. Current Biology 23: 2262-2267.

Tshilowa P.F. 2015. Land utilisation by small and emerging commercial farmers in the Greater Tzaneen Municipality in Mopani District of Limpopo Province. Master’s Thesis, University of South Africa, South Africa.

Tsoar A., Nathan R., Bartan Y., Vyssotski A., Dell'Omo G., Ulanovsky N. 2011. Large-scale navigational map in a mammal. Proceedings of the National Academy of Sciences 108: E718-E724.

Tucker M.A., Ord T.J., Rogers T.L. 2014. Evolutionary predictors of mammalian home range size: body mass, diet and the environment. Global Ecology and Biogeography 23: 1105-1114.

Upham N.S., Esselstyn J.A., Jetz W. 2019. Inferring the mammal tree: species-level sets of phylogenies for questions in ecology, evolution, and conservation. PLoS Biology 17: e3000494. DOI: 10.1371/journal.pbio.3000494.

van der Heide G., Fernandez-Duque E., Iriart D., Juárez C.P. 2012. Do forest composition and fruit availability predict demographic differences among groups of territorial owl monkeys (*Aotus azarai*). International Journal of Primatology 33: 184-207

van Harten E., Lawrence R., Lumsden L.F., Reardon T., Bennett A.F., Prowse T.A.A. 2022. Seasonal population dynamics and movement patterns of a critically endangered, cave-dwelling bat, *Miniopterus orianae bassanii*. Wildlife Research 49: 646-658.

van Wyk B., van Wyk P. 2013. Field guide to trees of southern Africa. 2nd ed. Penguin Random House South Africa, South Africa.

Vleut I., Carter G.G., Medellín R.A. 2019. Movement ecology of the carnivorous woolly false vampire bat (*Chrotopterus auritus*) in southern Mexico. PloS ONE 14: e0220504. DOI: 10.1371/journal.pone.0220504.

Voigt C.C., Frick W.F., Holderied M.W., Holland R., Kerth G., Mello M.A., Plowright R.K., Swartz S., Yovel Y. 2017. Principles and patterns of bat movements: from aerodynamics to ecology. The Quarterly Review of Biology 92: 267-287.

Voigt C.C., Holderied M.W. 2012. High manoeuvring costs force narrow-winged molossid bats to forage in open space. Journal of Comparative Physiology B 182: 415-424.

Wang L.-F., Anderson D.E. 2019. Viruses in bats and potential spillover to animals and humans. Current Opinion in Virology 34: 79-89.

Waruhiu C., Ommeh S., Obanda V., Agwanda B., Gakuya F., Ge X.-Y., Yang X.-L., Wu L.-J., Zohaib A., Hu B., Shi Z. 2017. Molecular detection of viruses in Kenyan bats and discovery of novel astroviruses, caliciviruses and rotaviruses. Virologica Sinica 32: 101-114.

Waters D.A., Vollrath C. 2003. Echolocation performance and call structure in the megachiropteran fruit-bat *Rousettus aegyptiacus*. Acta Chiropterologica 5: 209-219.

Webala P.W., Mwaura J., Mware J.M., Ndiritu G.G., Patterson B.D. 2019. Effects of habitat fragmentation on the bats of Kakamega Forest, western Kenya. Journal of Tropical Ecology 35: 260-269.

Weier S.M., Linden V.M.G., Hammer A., Grass I., Tscharntke T., Taylor P.J. 2021. Bat guilds respond differently to habitat loss and fragmentation at different scales in macadamia orchards in South Africa. Agriculture, Ecosystems & Environment 320: DOI: 10.1016/j.agee.2021.107588.

White P.C., Ward A.I. 2011. Interdisciplinary approaches for the management of existing and emerging human–wildlife conflicts. Wildlife Research 37: 623-629.

White R.J., Razgour O. 2020. Emerging zoonotic diseases originating in mammals: a systematic review of effects of anthropogenic land-use change. Mammal Review 50: 336-352.

Wickramasinghe L.P., Harris S., Jones G., Vaughan N. 2003. Bat activity and species richness on organic and conventional farms: impact of agricultural intensification. Journal of Applied Ecology 40: 984-993.

Wilkins K.T. 1989. *Tadarida brasiliensis*. Mammalian species 331: 1-10.

Williams E.P., Spruill-Harrell B.M., Taylor M.K., Lee J., Nywening A.V., Yang Z., Nichols J.H., Camp J.V., Owen R.D., Jonsson C.B. 2021. Common themes in zoonotic spillover and disease emergence: lessons learned from bat- and rodent-borne RNA viruses. Viruses 13: DOI: 10.3390/v13081509.

Williams N.S., Mcdonnell M.J., Phelan G.K., Keim L.D., Van Der Ree R. 2006. Range expansion due to urbanization: increased food resources attract Grey-headed Flying-foxes (*Pteropus poliocephalus*) to Melbourne. Austral Ecology 31: 190-198.

Woolhouse M.E.J., Adair K., Brierley L. 2013. RNA viruses: a case study of the biology of emerging diseases. Microbiology Spectrum 1: DOI: 10.1128/microbiolspec.OH0001-2012.

Worton B. 1987. A review of models of home range for animal movement. Ecological Modelling 38: 277-298.

Worton B.J. 1989. Kernel methods for estimating the utilization distribution in home-range studies. Ecology 70: 164-168.

Young P.L., Halpin K., Selleck P.W., Field H., Gravel J.L., Kelly M.A., MacKenzie J.S. 1996. Serologic evidence for the presence in Pteropus bats of a paramyxovirus related to equine morbillivirus. Emerging Infectious Diseases 2: 239-240.

Yovel Y., Geva-Sagiv M., Ulanovsky N. 2011. Click-based echolocation in bats: not so primitive after all. Journal of Comparative Physiology A 197: 515-530.

Zaki A.M., van Boheemen S., Bestebroer T.M., Osterhaus A.D., Fouchier R.A. 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. The New England Journal of Medicine 367: 1814-1820.

Zeale M.R., Davidson-Watts I., Jones G. 2012. Home range use and habitat selection by barbastelle bats (*Barbastella barbastellus*): implications for conservation. Journal of Mammalogy 93: 1110-1118.

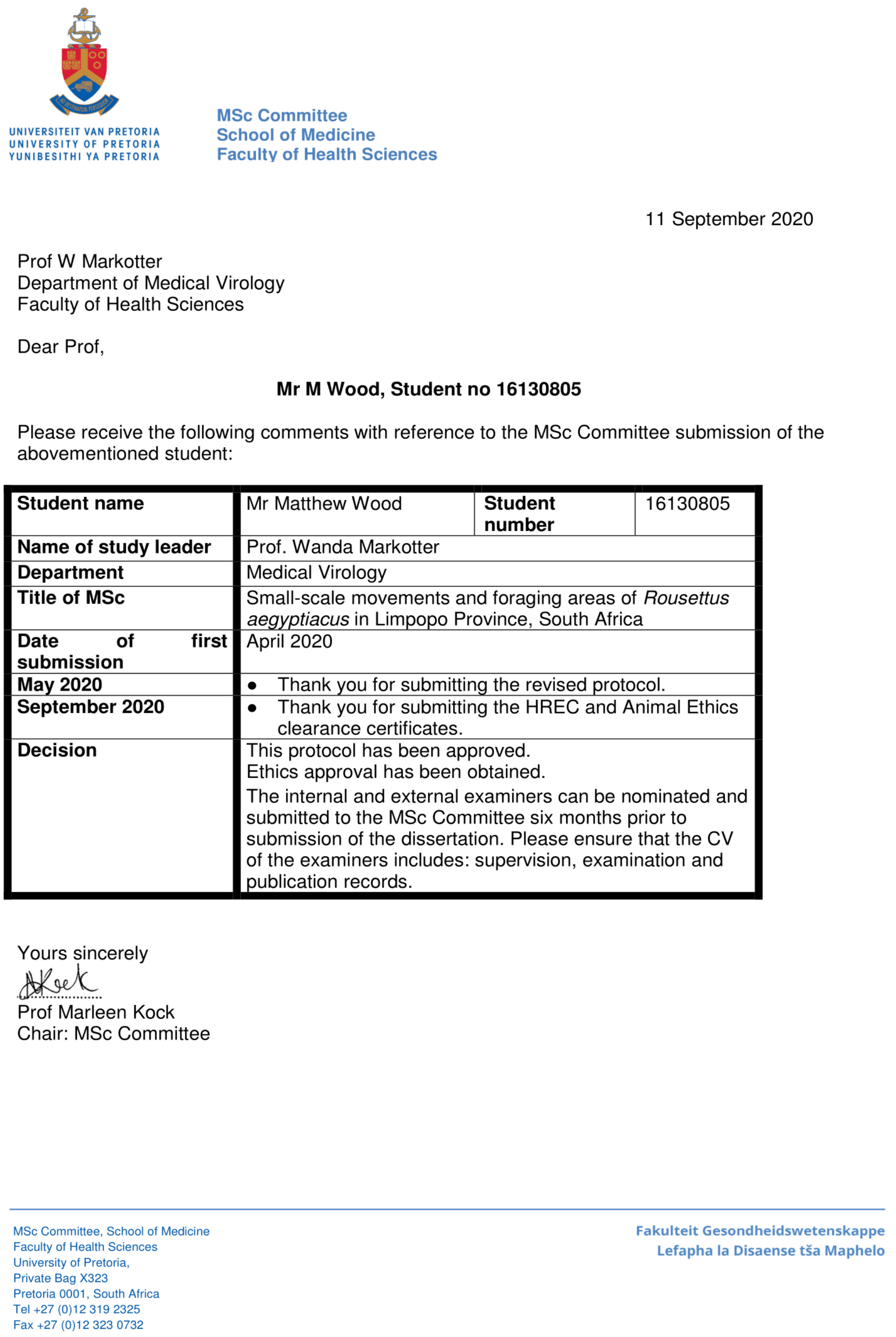
Zhao F., He Y., Lu H. 2022. Marburg virus disease: A deadly rare virus is coming. BioScience Trends 16: 312-316.

Zhao K., Zhang W., Li B., Xie S.-Z., Yi F., Jiang R.-D., Luo Y., He X.-Y., Zhang Y.-Z., Shi Z.-L., et al. 2022. Ecological study of cave nectar bats reveals low risk of direct transmission of bat viruses to humans. Zoological Research 43: 514-522.

Zhou H., Ji J., Chen X., Bi Y., Li J., Wang Q., Hu T., Song H., Zhao R., Chen Y., et al. 2021. Identification of novel bat coronaviruses sheds light on the evolutionary origins of SARS-CoV-2 and related viruses. Cell. 184: 4380-4391

Appendices

Appendix A: Final project approval from the M.Sc. committee in the School of Medicine in the Faculty of Health Sciences



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

MSc Committee
School of Medicine
Faculty of Health Sciences

11 September 2020

Prof W Markotter
Department of Medical Virology
Faculty of Health Sciences

Dear Prof,

Mr M Wood, Student no 16130805

Please receive the following comments with reference to the MSc Committee submission of the abovementioned student:

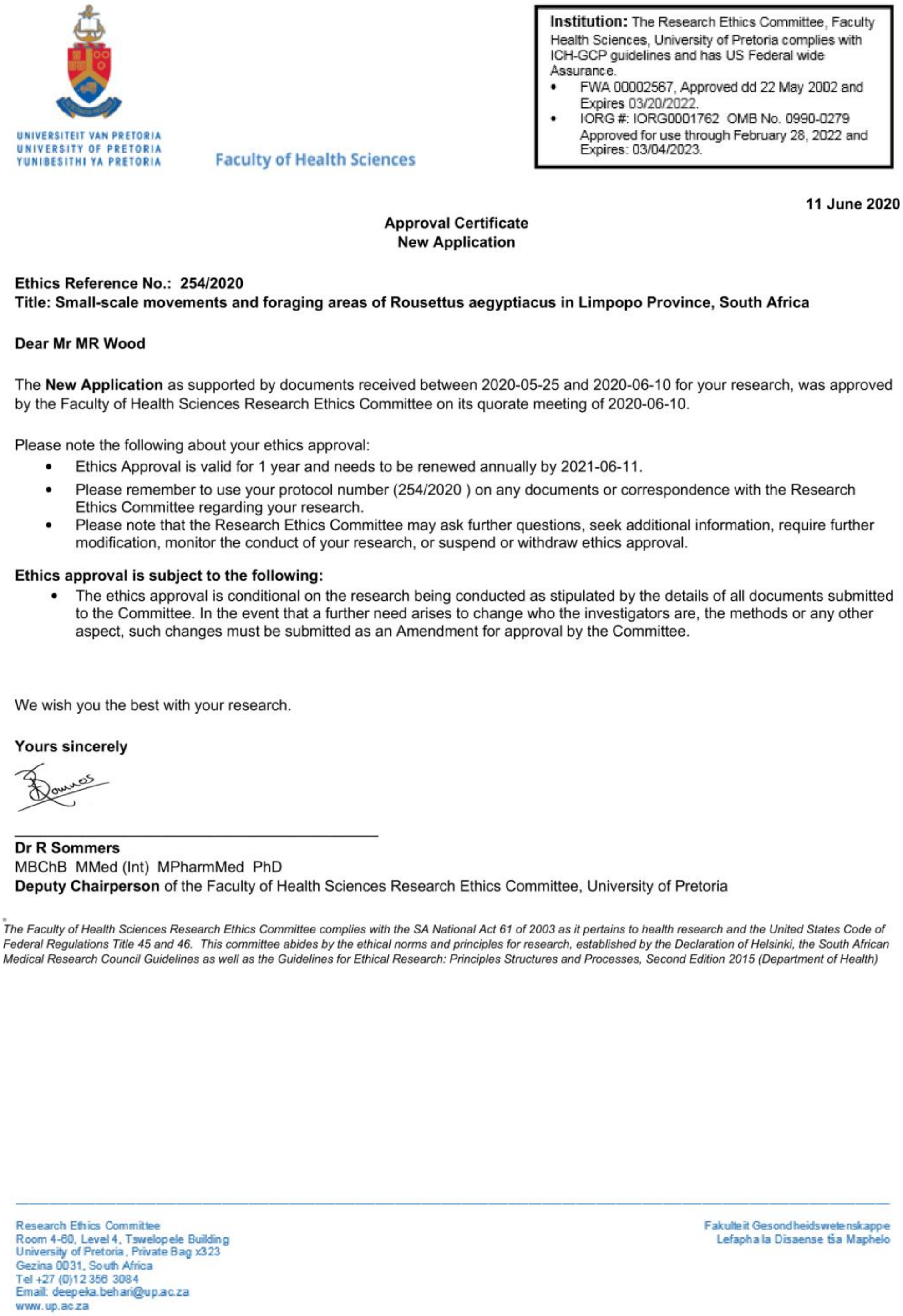
Student name	Mr Matthew Wood	**Student number**	16130805
Name of study leader	Prof. Wanda Markotter		
Department	Medical Virology		
Title of MSc	Small-scale movements and foraging areas of *Rousettus aegyptiacus* in Limpopo Province, South Africa		
Date of first submission	April 2020		
May 2020	● Thank you for submitting the revised protocol.		
September 2020	● Thank you for submitting the HREC and Animal Ethics clearance certificates.		
Decision	This protocol has been approved. Ethics approval has been obtained. The internal and external examiners can be nominated and submitted to the MSc Committee six months prior to submission of the dissertation. Please ensure that the CV of the examiners includes: supervision, examination and publication records.		

Yours sincerely
AKock
Prof Marleen Kock
Chair: MSc Committee

MSc Committee, School of Medicine
Faculty of Health Sciences
University of Pretoria,
Private Bag X323
Pretoria 0001, South Africa
Tel +27 (0)12 319 2325
Fax +27 (0)12 323 0732

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Appendix B1: Research ethics certificate 254/2020 from REC for 2020



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

11 June 2020

Approval Certificate
New Application

Ethics Reference No.: 254/2020
Title: Small-scale movements and foraging areas of Rousettus aegyptiacus in Limpopo Province, South Africa

Dear Mr MR Wood

The **New Application** as supported by documents received between 2020-05-25 and 2020-06-10 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 2020-06-10.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-06-11.
- Please remember to use your protocol number (254/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

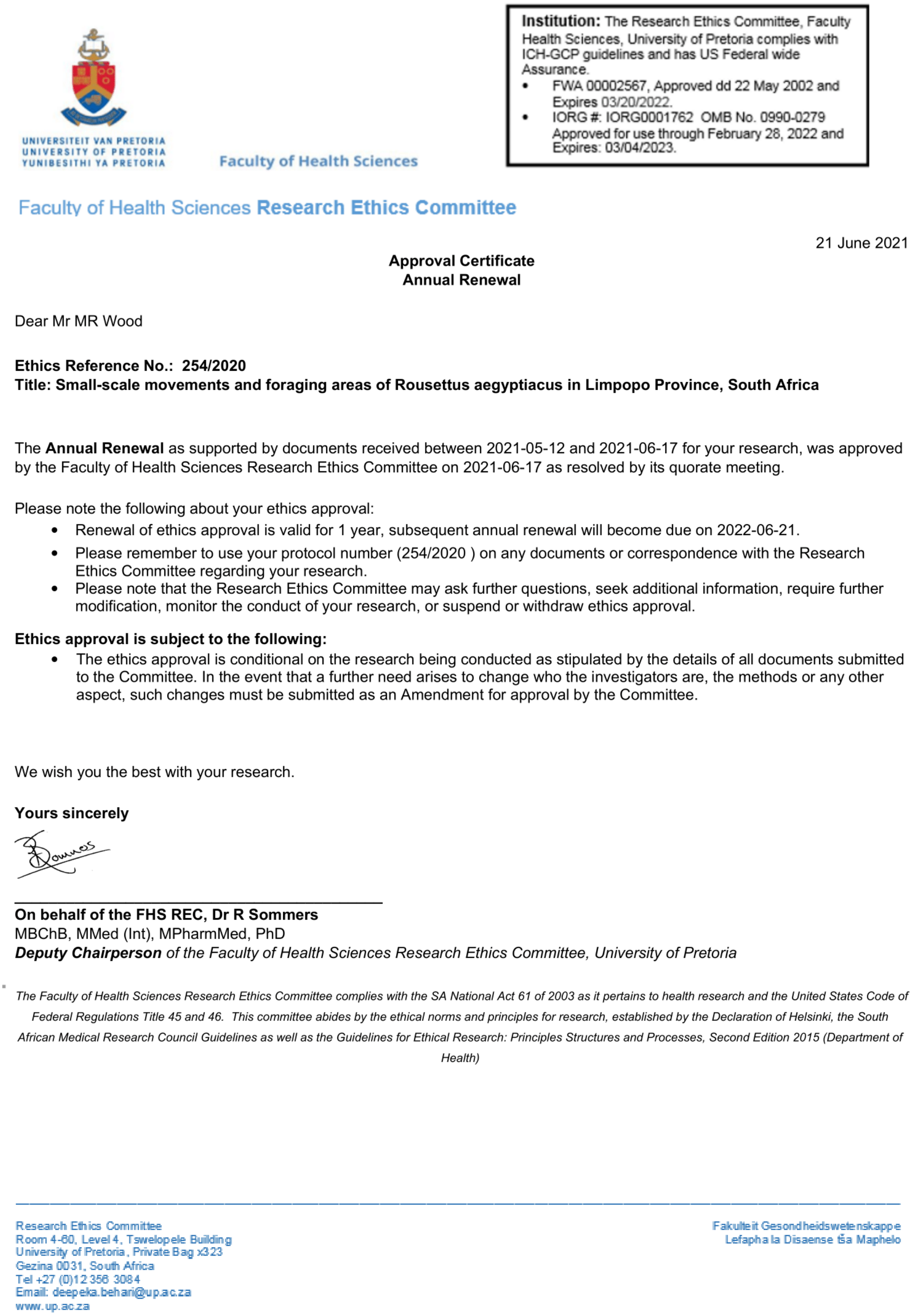
Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Appendix B2: Research ethics certificate for 254/2020 extension from the REC for 2021



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

Faculty of Health Sciences **Research Ethics Committee**

21 June 2021

Approval Certificate
Annual Renewal

Dear Mr MR Wood

Ethics Reference No.: 254/2020
Title: Small-scale movements and foraging areas of Rousettus aegyptiacus in Limpopo Province, South Africa

The **Annual Renewal** as supported by documents received between 2021-05-12 and 2021-06-17 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2021-06-17 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2022-06-21.
- Please remember to use your protocol number (254/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

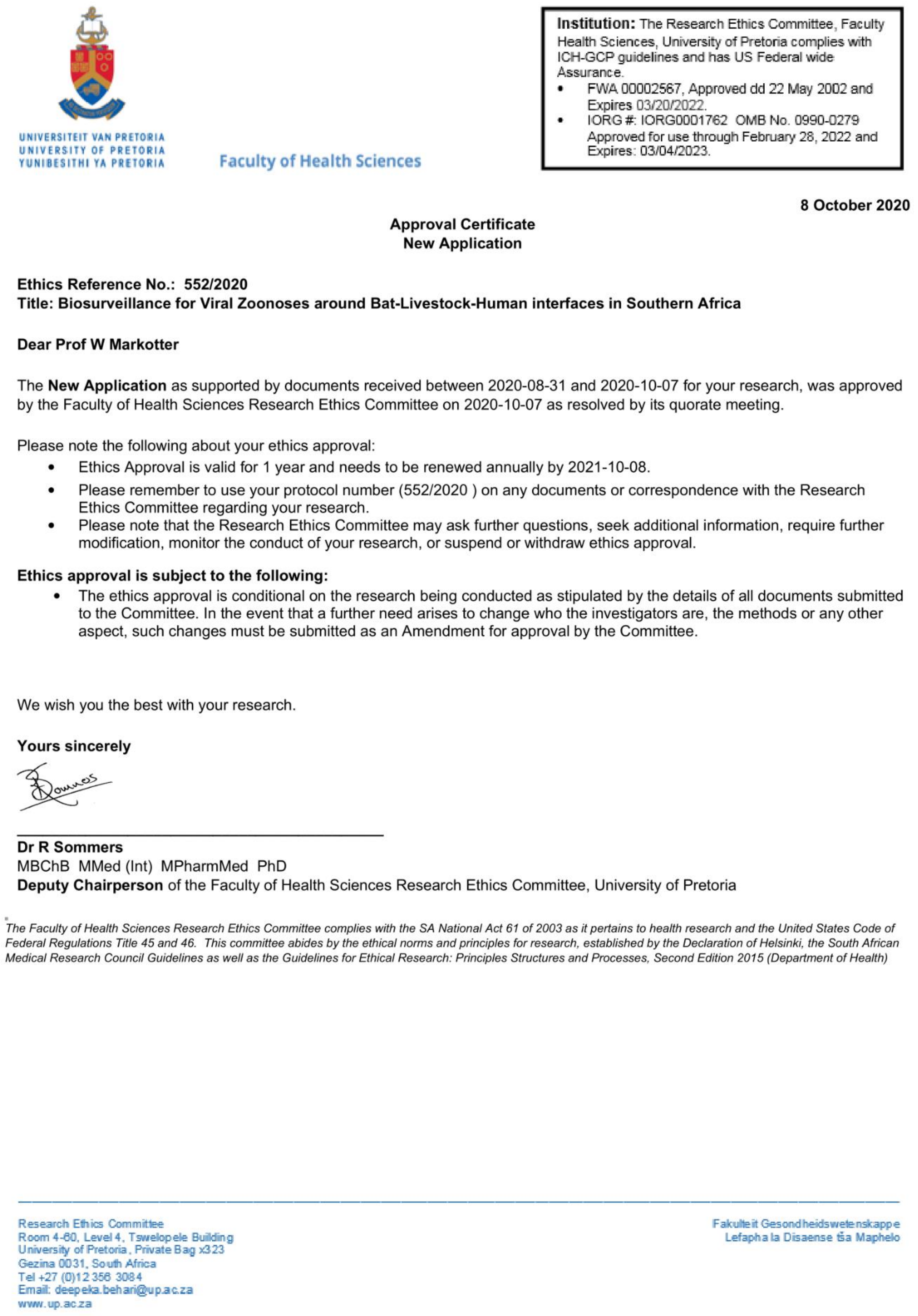
On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Appendix B3: Research ethics certificate for 552/2020 from the REC for 2020



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

8 October 2020

Approval Certificate
New Application

Ethics Reference No.: 552/2020
Title: Biosurveillance for Viral Zoonoses around Bat-Livestock-Human interfaces in Southern Africa

Dear Prof W Markotter

The **New Application** as supported by documents received between 2020-08-31 and 2020-10-07 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2020-10-07 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-10-08.
- Please remember to use your protocol number (552/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

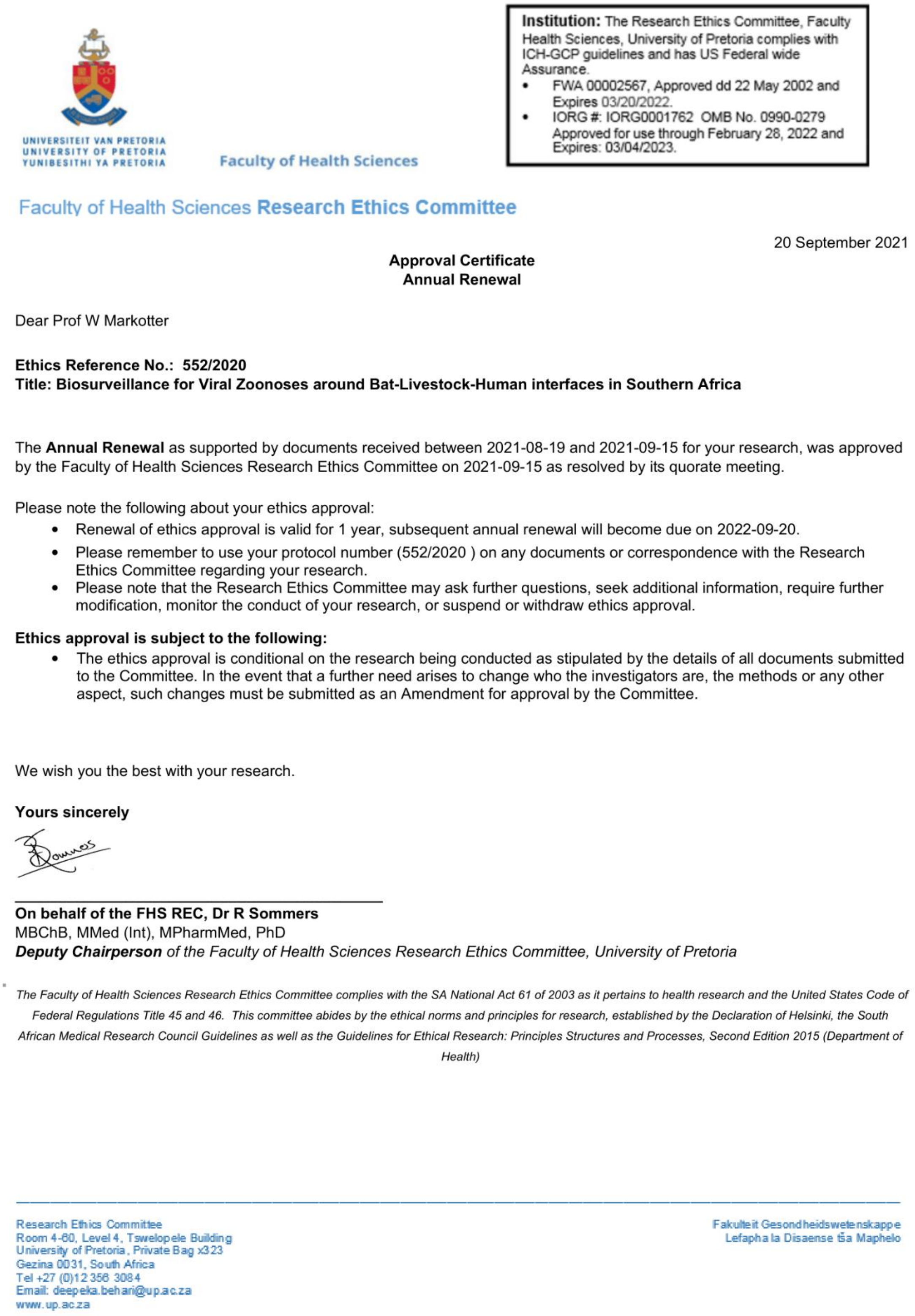
Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Appendix B4: Research ethics certificate for 552/2020 extension from the REC for 2021



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

Faculty of Health Sciences **Research Ethics Committee**

20 September 2021

Approval Certificate
Annual Renewal

Dear Prof W Markotter

Ethics Reference No.: 552/2020
Title: Biosurveillance for Viral Zoonoses around Bat-Livestock-Human interfaces in Southern Africa

The **Annual Renewal** as supported by documents received between 2021-08-19 and 2021-09-15 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2021-09-15 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2022-09-20.
- Please remember to use your protocol number (552/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

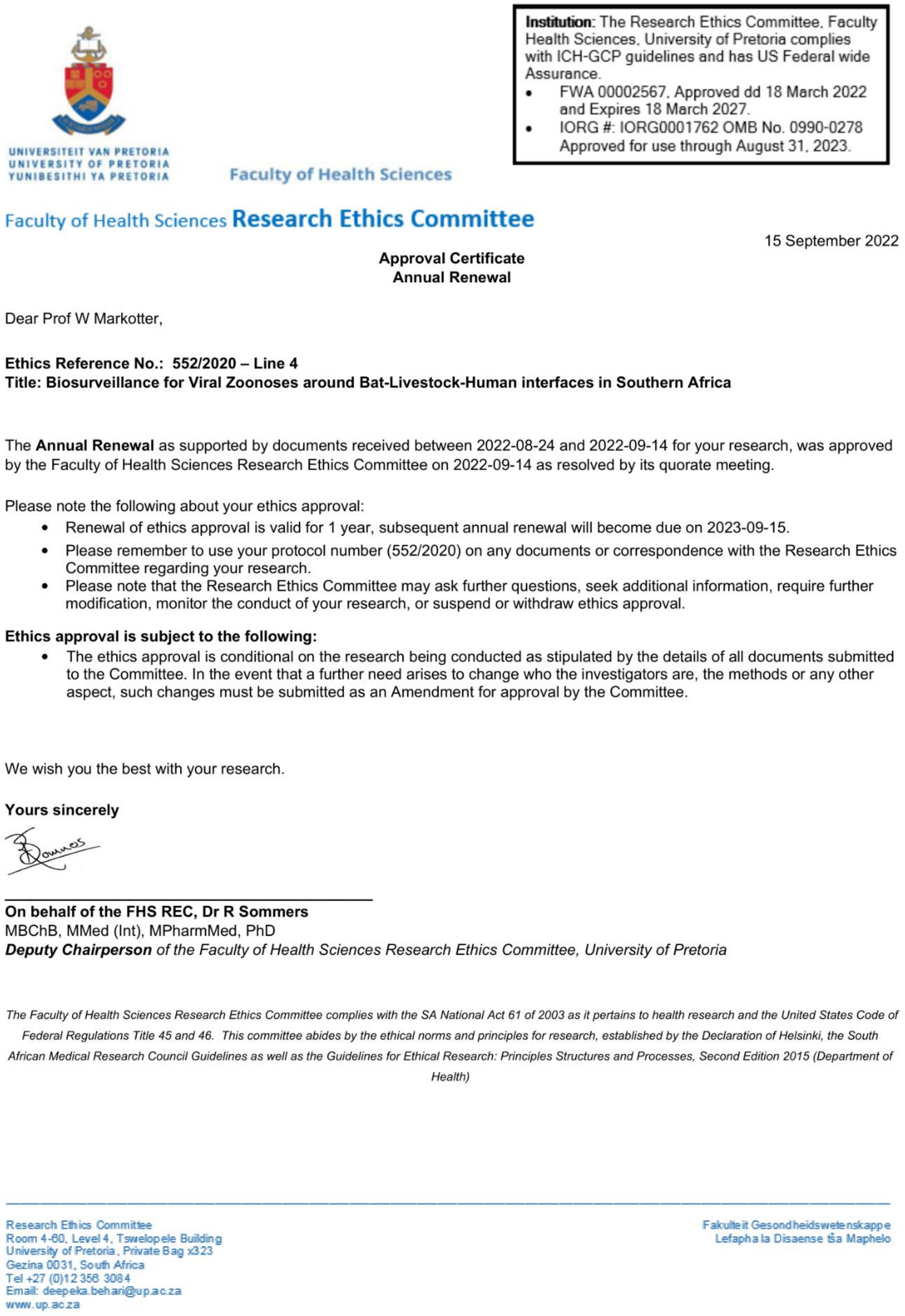
On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Appendix B5: Research ethics certificate for 552/2020 extension from the REC for 2022



Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Faculty of Health Sciences **Research Ethics Committee**

15 September 2022

Approval Certificate
Annual Renewal

Dear Prof W Markotter,

Ethics Reference No.: 552/2020 – Line 4
Title: Biosurveillance for Viral Zoonoses around Bat-Livestock-Human interfaces in Southern Africa

The **Annual Renewal** as supported by documents received between 2022-08-24 and 2022-09-14 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2022-09-14 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2023-09-15.
- Please remember to use your protocol number (552/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

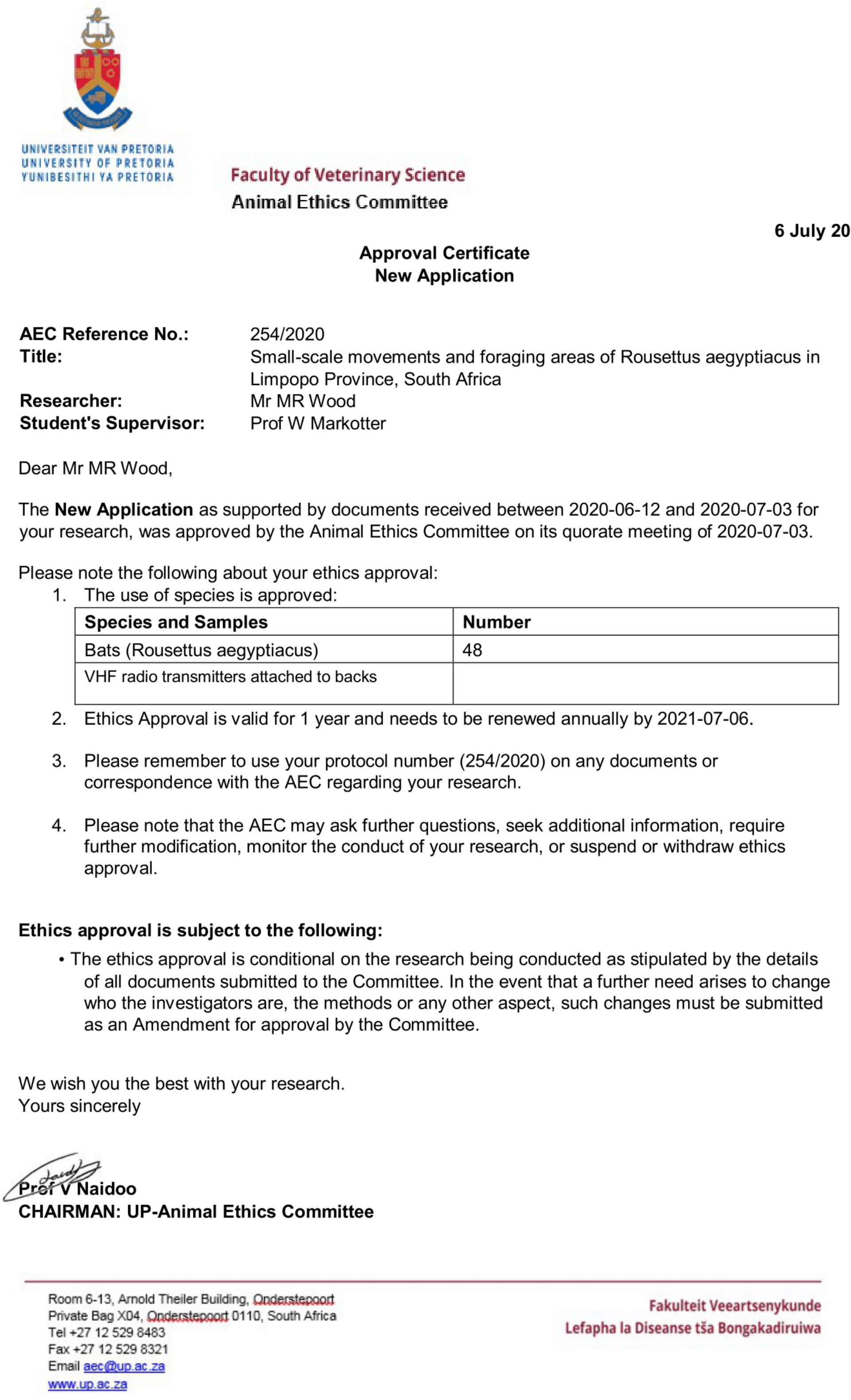
On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Appendix B6: Animal ethics certificate for 254/2020 from the AEC for 2020



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

6 July 2020

Approval Certificate
New Application

AEC Reference No.:	254/2020
Title:	Small-scale movements and foraging areas of Rousettus aegyptiacus in Limpopo Province, South Africa
Researcher:	Mr MR Wood
Student's Supervisor:	Prof W Markotter

Dear Mr MR Wood,

The **New Application** as supported by documents received between 2020-06-12 and 2020-07-03 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-07-03.

Please note the following about your ethics approval:

1. The use of species is approved:

Species and Samples	**Number**
Bats (Rousettus aegyptiacus)	48
VHF radio transmitters attached to backs	

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-07-06.
3. Please remember to use your protocol number (254/2020) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

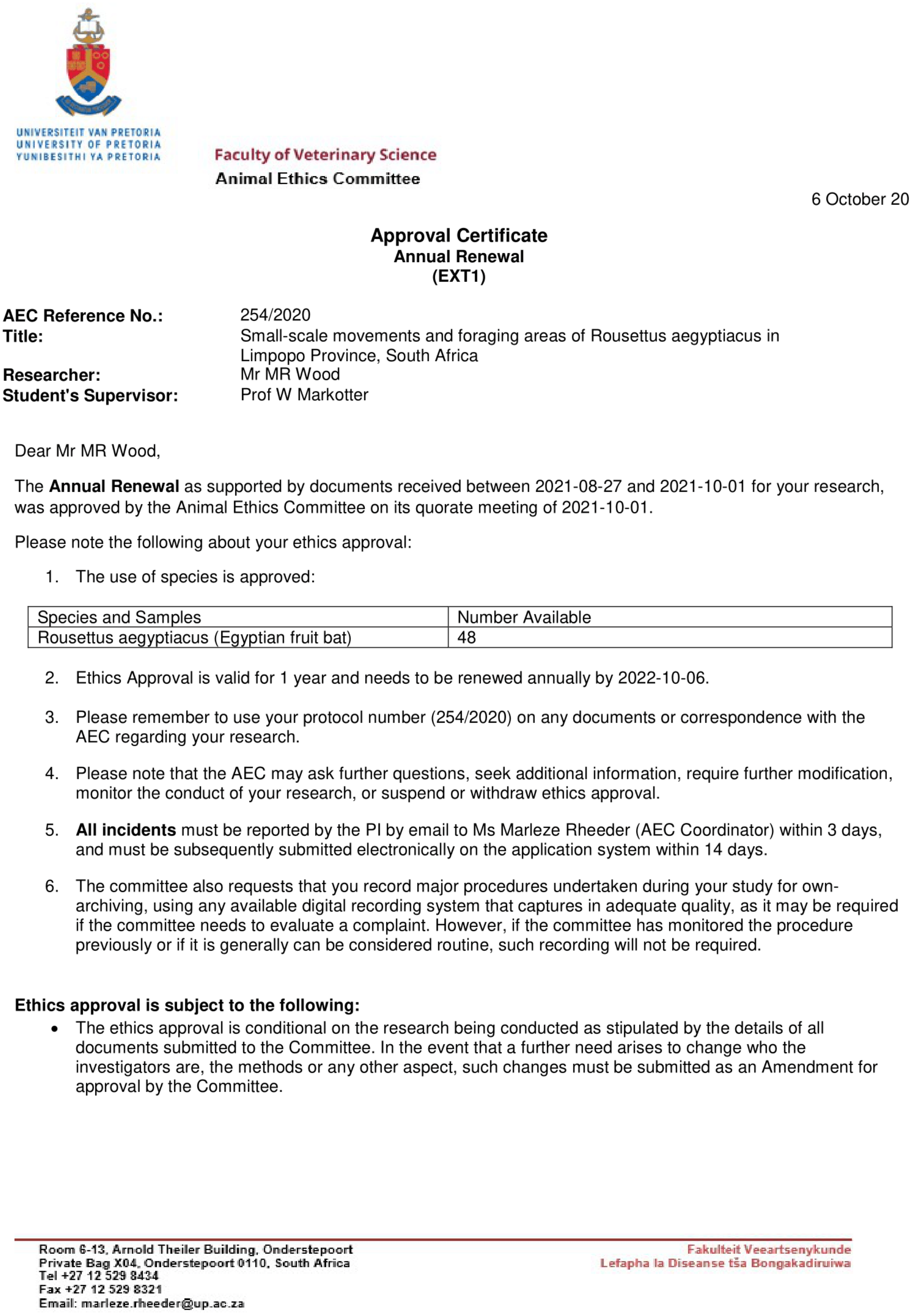
We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8483
Fax +27 12 529 8321
Email aec@up.ac.za
www.up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix B7: Animal ethics certificate for 254/2020 extension from the AEC for 2021



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

6 October 2021

Approval Certificate
Annual Renewal
(EXT1)

AEC Reference No.: 254/2020
Title: Small-scale movements and foraging areas of Rousettus aegyptiacus in Limpopo Province, South Africa
Researcher: Mr MR Wood
Student's Supervisor: Prof W Markotter

Dear Mr MR Wood,

The **Annual Renewal** as supported by documents received between 2021-08-27 and 2021-10-01 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2021-10-01.

Please note the following about your ethics approval:

1. The use of species is approved:

Species and Samples	Number Available
Rousettus aegyptiacus (Egyptian fruit bat)	48

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-10-06.
3. Please remember to use your protocol number (254/2020) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

We wish you the best with your research.

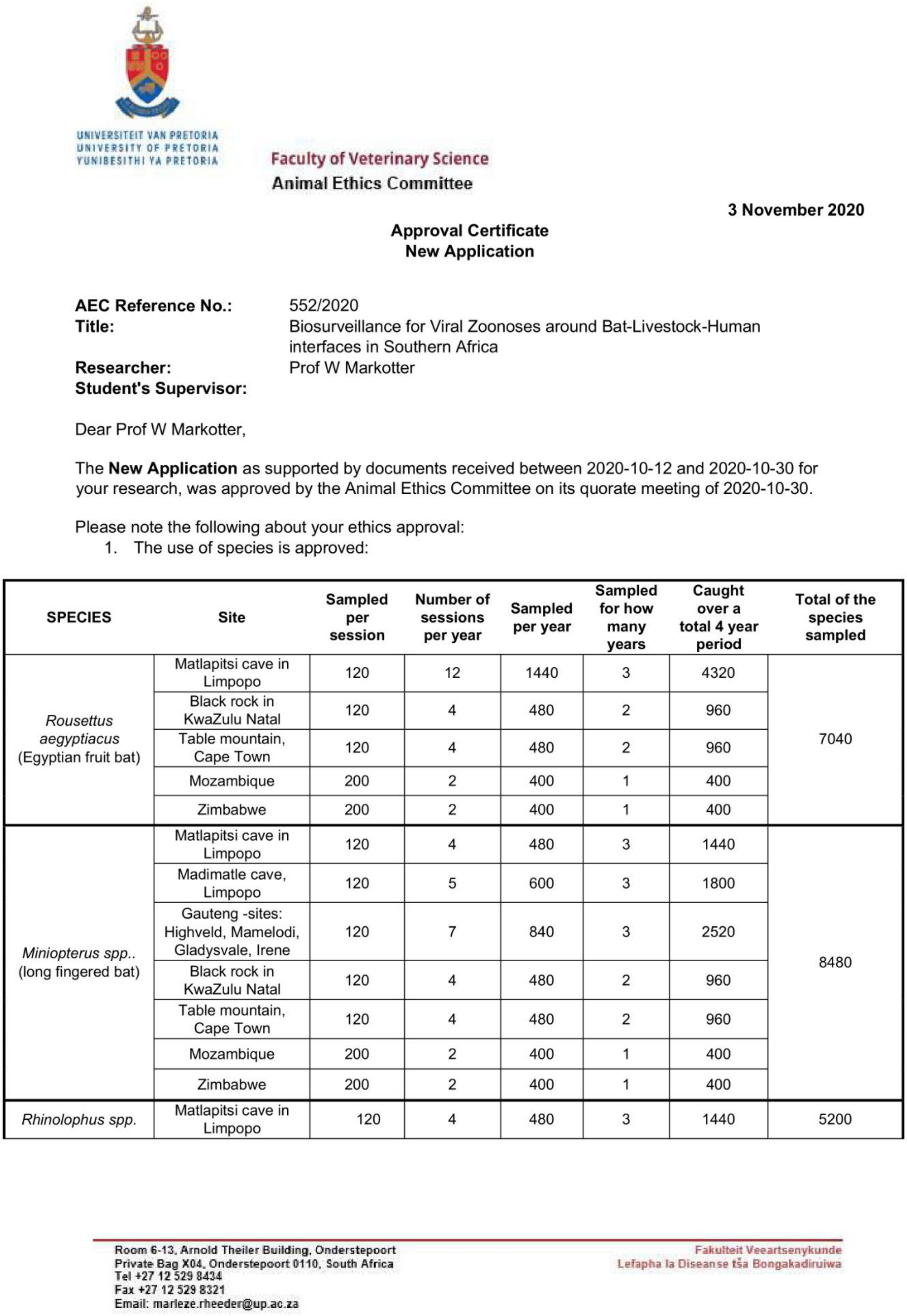
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix B8: Animal ethics certificate 552/2020 from the AEC for 2020



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

3 November 2020

Approval Certificate
New Application

AEC Reference No.: 552/2020
Title: Biosurveillance for Viral Zoonoses around Bat-Livestock-Human interfaces in Southern Africa
Researcher: Prof W Markotter
Student's Supervisor:

Dear Prof W Markotter,

The **New Application** as supported by documents received between 2020-10-12 and 2020-10-30 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-10-30.

Please note the following about your ethics approval:

1. The use of species is approved:

SPECIES	Site	Sampled per session	Number of sessions per year	Sampled per year	Sampled for how many years	Caught over a total 4 year period	Total of the species sampled
Rousettus aegyptiacus (Egyptian fruit bat)	Matlapitsi cave in Limpopo	120	12	1440	3	4320	7040
	Black rock in KwaZulu Natal	120	4	480	2	960	
	Table mountain, Cape Town	120	4	480	2	960	
	Mozambique	200	2	400	1	400	
	Zimbabwe	200	2	400	1	400	
Miniopterus spp.. (long fingered bat)	Matlapitsi cave in Limpopo	120	4	480	3	1440	8480
	Madimatle cave, Limpopo	120	5	600	3	1800	
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	120	7	840	3	2520	
	Black rock in KwaZulu Natal	120	4	480	2	960	
	Table mountain, Cape Town	120	4	480	2	960	
	Mozambique	200	2	400	1	400	
	Zimbabwe	200	2	400	1	400	
Rhinolophus spp.	Matlapitsi cave in Limpopo	120	4	480	3	1440	5200

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

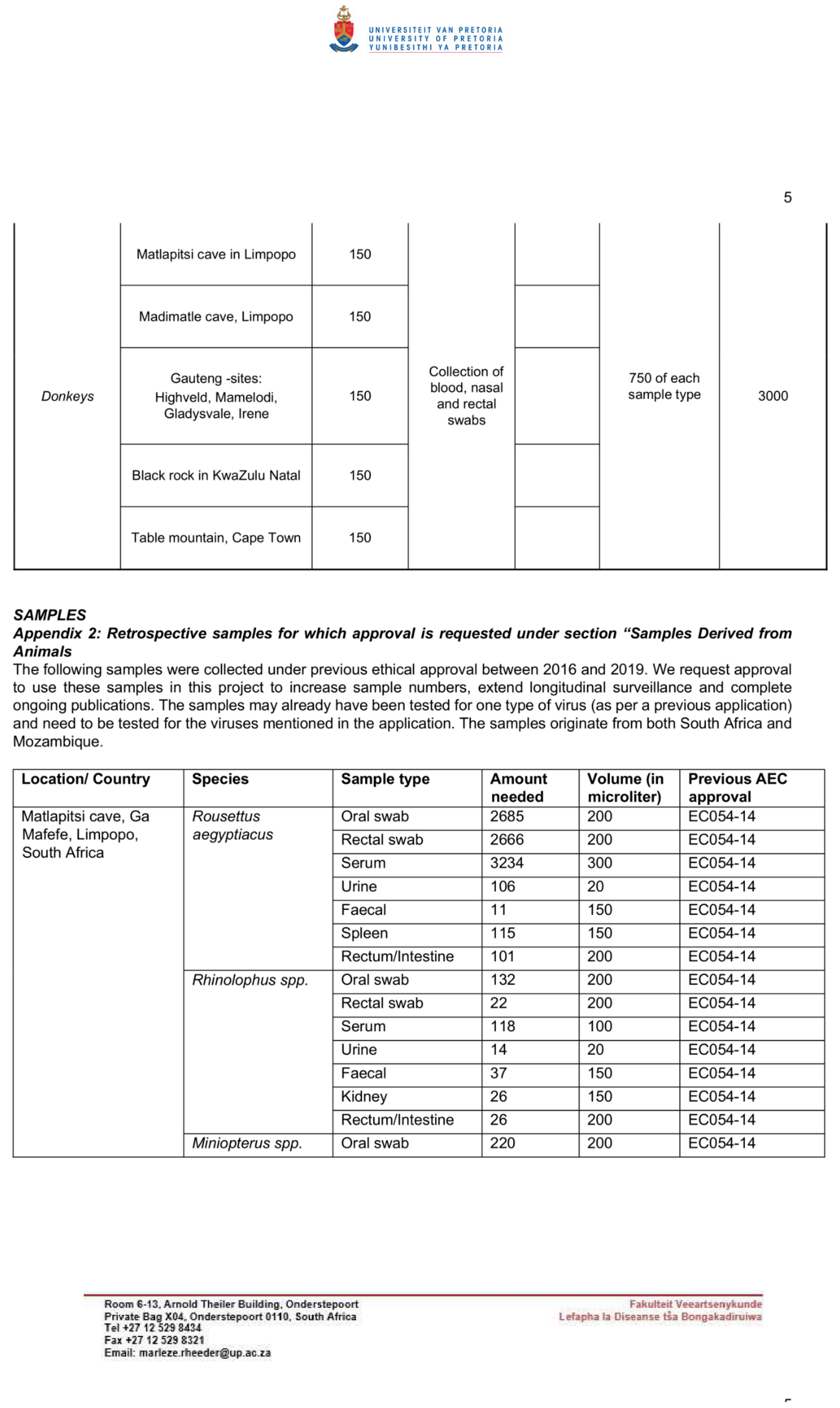
Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

(Horseshoe bat)	Madimatle cave, Limpopo	120	2	240	3	720	
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	120	2	240	3	720	
	Black rock in KwaZulu Natal	120	4	480	2	960	
	Table mountain, Cape Town	120	4	480	2	960	
	Mozambique	100	2	200	1	200	
	Zimbabwe	100	2	200	1	200	
Molossid species *(Mops spp., Chaerephon spp.etc.)*	Matlapitsi cave in Limpopo	60	4	240	3	720	2800
	Madimatle cave, Limpopo	60	2	120	3	360	
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	60	2	120	3	360	
	Black rock in KwaZulu Natal	60	4	240	2	480	
	Table mountain, Cape Town	60	4	240	2	480	
	Mozambique	100	2	200	1	200	
	Zimbabwe	100	2	200	1	200	
Opportunistically sampled species (Hipposideros spp.,Myotis spp., Nycteris spp. Scotophilus spp. Pipistrellus spp., Epomophorous spp. and other)	Matlapitsi cave in Limpopo	20	12	240	3	720	1960
	Madimatle cave, Limpopo	20	2	40	3	120	
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	20	2	40	3	120	
	Black rock in KwaZulu Natal	20	4	80	2	160	
	Table mountain, Cape Town	20	4	80	2	160	
	Mozambique	20	2	40	1	40	

	Zimbabwe	20	2	40	1	40	
Bat voucher specimens (allowance for 5 females and 5 males per species per site per season as necessary)	Matlapitsi cave in Limpopo	5	4	20	3	60	Allowance for 300 animals per species as is necessary - totalling 1200 (Rousettus, Miniopterus, Rhinolophus and Molossids) over 4 years
	Madimatle cave, Limpopo	5	4	20	3	60	
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	5	4	20	3	60	
	Black rock in KwaZulu Natal	5	4	20	2	40	
	Table mountain, Cape Town	5	4	20	2	40	
	Mozambique	5	2	20	1	20	
	Zimbabwe	5	2	20	1	20	
Population-level fecal and urine collection (sample from underneath roosting bats that will be pooled separately) – considered (60x3 individuals=180 individuals)	Matlapitsi cave in Limpopo	120	12	1440	3	4320	11,040
	Madimatle cave, Limpopo	120 (360 individuals)	5	600	3	1800	
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	120 (360 individuals)	7	840	3	2520	
	Black rock in KwaZulu Natal	120 (360 individuals)	4	480	2	960	
	Table mountain, Cape Town	120 (360 individuals)	4	480	2	960	
	Mozambique	120 (360 individuals)	2	240	1	240	
	Zimbabwe	120 (360 individuals)	2	240	1	240	

SPECIES	**Site**	**Sampled per year**	**Samples collected**	**Sampled for how many years**	**Total over a year period**	**Total samples collected**
Cattle	Matlapitsi cave in Limpopo	150	Collection of blood, nasal	1	750 of each sample type	3000

	Madimatle cave, Limpopo	150	and rectal swabs	1		
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	150		1		
	Black rock in KwaZulu Natal	150		1		
	Table mountain, Cape Town	150		1		
Goats	Matlapitsi cave in Limpopo	150	Collection of blood, nasal and rectal swabs	1	750 of each sample type	3000
	Madimatle cave, Limpopo	150		1		
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	150		1		
	Black rock in KwaZulu Natal	150		1		
	Table mountain, Cape Town	150		1		
Pigs	Matlapitsi cave in Limpopo	150	Collection of blood, nasal and rectal swabs	1	750 of each sample type	3000
	Madimatle cave, Limpopo	150		1		
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	150		1		
	Black rock in KwaZulu Natal	150		1		
	Table mountain, Cape Town	150		1		



Donkeys	Matlapitsi cave in Limpopo	150	Collection of blood, nasal and rectal swabs		750 of each sample type	3000
	Madimatle cave, Limpopo	150				
	Gauteng -sites: Highveld, Mamelodi, Gladysvale, Irene	150				
	Black rock in KwaZulu Natal	150				
	Table mountain, Cape Town	150				

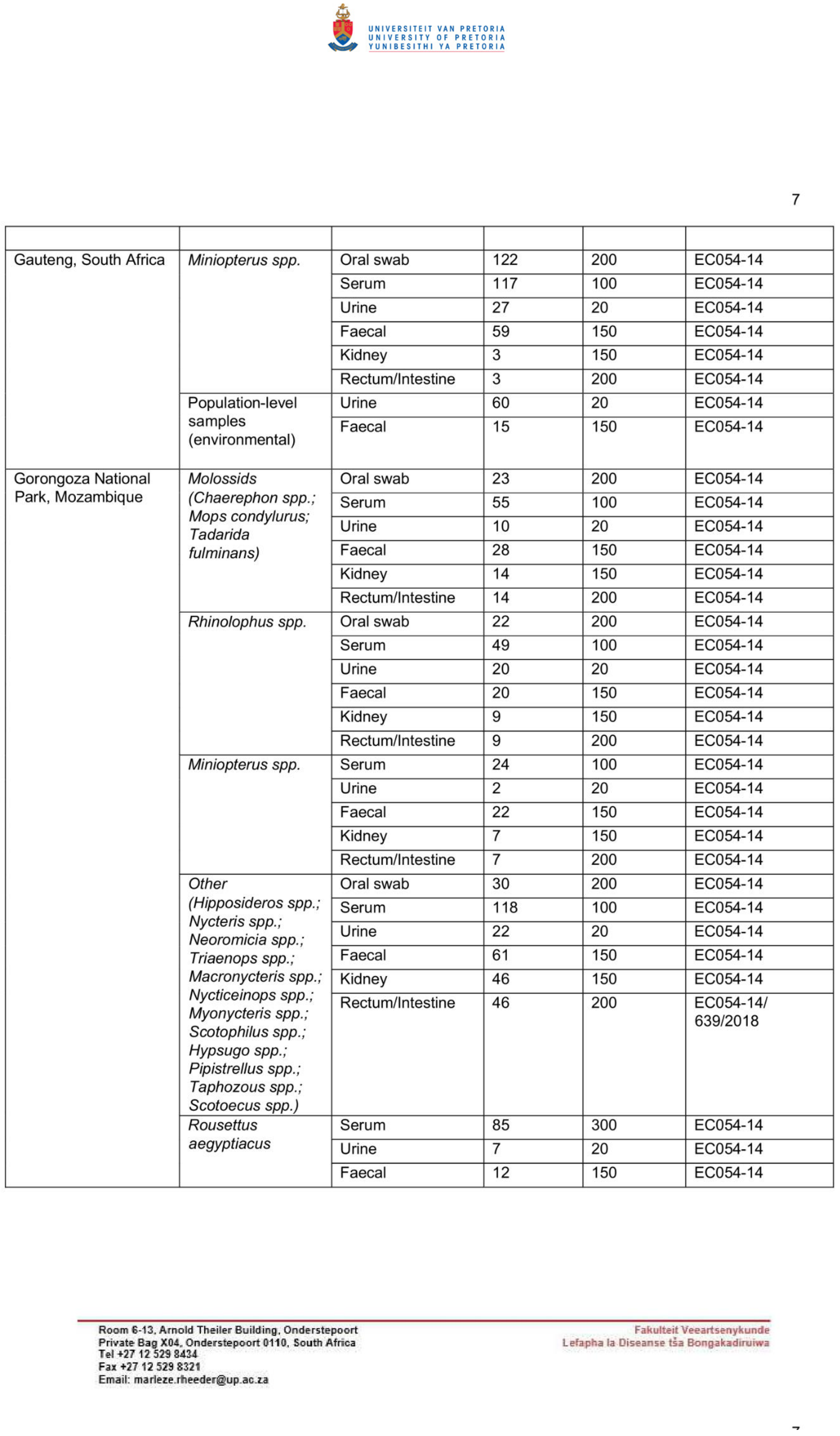
SAMPLES

Appendix 2: Retrospective samples for which approval is requested under section "Samples Derived from Animals

The following samples were collected under previous ethical approval between 2016 and 2019. We request approval to use these samples in this project to increase sample numbers, extend longitudinal surveillance and complete ongoing publications. The samples may already have been tested for one type of virus (as per a previous application) and need to be tested for the viruses mentioned in the application. The samples originate from both South Africa and Mozambique.

Location/ Country	**Species**	**Sample type**	**Amount needed**	**Volume (in microliter)**	**Previous AEC approval**
Matlapitsi cave, Ga Mafefe, Limpopo, South Africa	*Rousettus aegyptiacus*	Oral swab	2685	200	EC054-14
		Rectal swab	2666	200	EC054-14
		Serum	3234	300	EC054-14
		Urine	106	20	EC054-14
		Faecal	11	150	EC054-14
		Spleen	115	150	EC054-14
		Rectum/Intestine	101	200	EC054-14
	Rhinolophus spp.	Oral swab	132	200	EC054-14
		Rectal swab	22	200	EC054-14
		Serum	118	100	EC054-14
		Urine	14	20	EC054-14
		Faecal	37	150	EC054-14
		Kidney	26	150	EC054-14
		Rectum/Intestine	26	200	EC054-14
	Miniopterus spp.	Oral swab	220	200	EC054-14

		Rectal swab	17	200	EC054-14
		Serum	229	100	EC054-14
		Urine	13	20	EC054-14
		Faecal	123	150	EC054-14
		Kidney	15	150	EC054-14
		Rectum/Intestine	15	200	EC054-14
	Other (Hipposideros caffer; Myotis tricolor; Nycteris thebaica)	Oral swab	86	200	EC054-14
		Rectal swab	1	200	EC054-14
		Serum	72	300	EC054-14
		Urine	4	20	EC054-14
		Faecal	14	150	EC054-14
		Kidney	1	150	EC054-14
		Rectum/Intestine	1	200	EC054-14
	Population-level samples (environmental)	Urine	1311	20	639/2018
		Faecal	1093	150	639/2018
Madimatle cave, Meletse, Limpopo, South Africa	*Miniopterus spp.*	Oral swab	530	200	EC054-14
		Rectal swab	19	200	EC054-14
		Serum	430	100	EC054-14
		Urine	209	20	EC054-14
		Faecal	334	150	EC054-14
		Kidney	19	150	EC054-14
		Rectum/Intestine	19	200	EC054-14
	Rhinolophus spp.	Oral swab	345	200	EC054-14
		Rectal swab	42	200	EC054-14
		Serum	123	100	EC054-14
		Urine	78	20	EC054-14
		Faecal	99	150	EC054-14
		Kidney	32	150	EC054-14
		Rectum/Intestine	32	200	EC054-14
	Other (Hipposideros caffer; Myotis tricolor; Nycteris thebaica; Neoromicia spp.; Scotophilus spp.)	Oral swab	216	200	EC054-14
		Rectal swab	20	200	EC054-14
		Serum	186	100	EC054-14
		Urine	50	20	EC054-14
		Faecal	70	150	EC054-14
		Kidney	11	150	EC054-14
		Rectum/Intestine	11	200	EC054-14/ 639/2018
	Population-level samples (environmental)	Urine	66	20	EC054-14
		Faecal	20	150	EC054-14



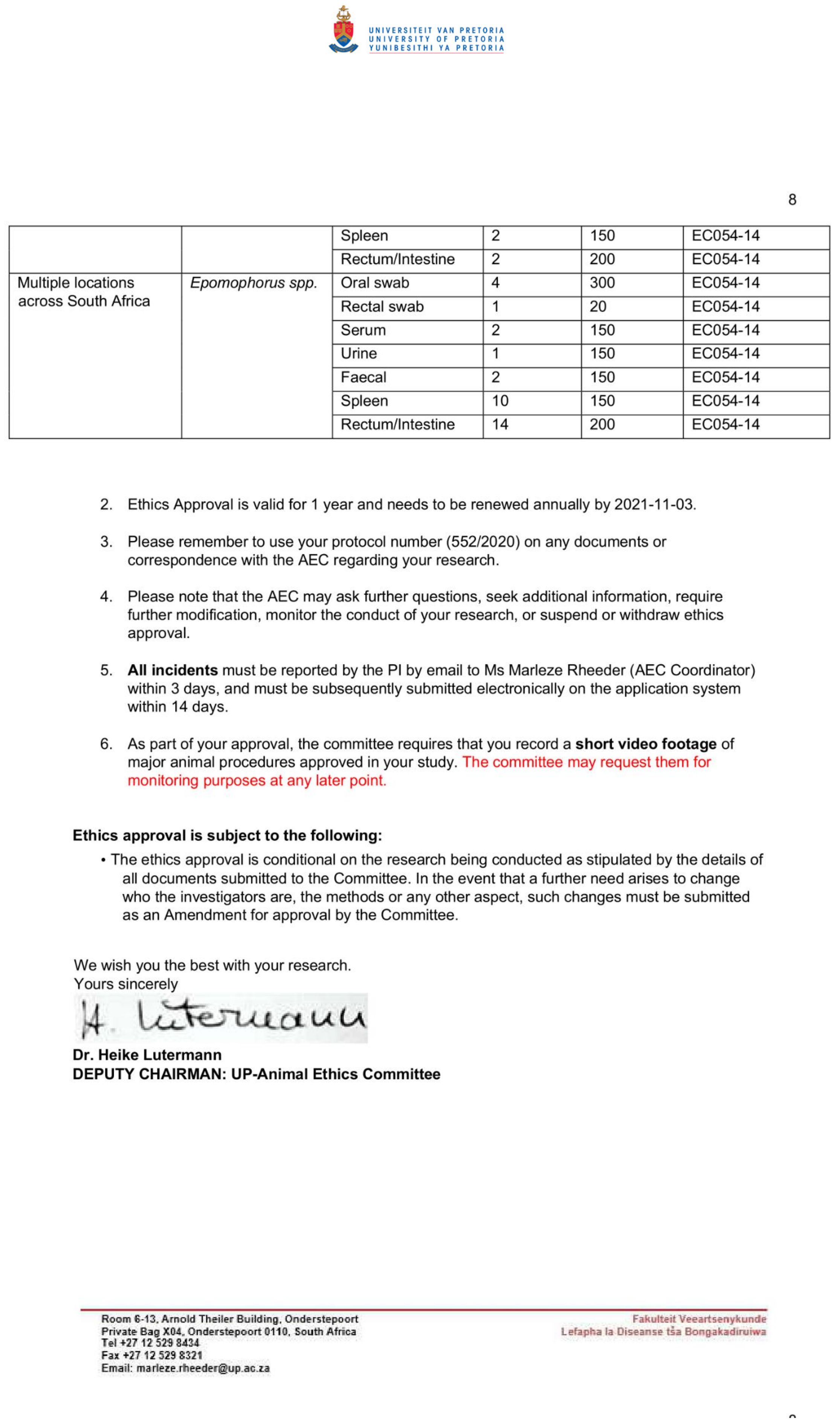
UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

7

Gauteng, South Africa	*Miniopterus spp.*	Oral swab	122	200	EC054-14
		Serum	117	100	EC054-14
		Urine	27	20	EC054-14
		Faecal	59	150	EC054-14
		Kidney	3	150	EC054-14
		Rectum/Intestine	3	200	EC054-14
	Population-level samples (environmental)	Urine	60	20	EC054-14
		Faecal	15	150	EC054-14
Gorongoza National Park, Mozambique	*Molossids (Chaerephon spp.; Mops condylurus; Tadarida fulminans)*	Oral swab	23	200	EC054-14
		Serum	55	100	EC054-14
		Urine	10	20	EC054-14
		Faecal	28	150	EC054-14
		Kidney	14	150	EC054-14
		Rectum/Intestine	14	200	EC054-14
	Rhinolophus spp.	Oral swab	22	200	EC054-14
		Serum	49	100	EC054-14
		Urine	20	20	EC054-14
		Faecal	20	150	EC054-14
		Kidney	9	150	EC054-14
		Rectum/Intestine	9	200	EC054-14
	Miniopterus spp.	Serum	24	100	EC054-14
		Urine	2	20	EC054-14
		Faecal	22	150	EC054-14
		Kidney	7	150	EC054-14
		Rectum/Intestine	7	200	EC054-14
	Other (Hipposideros spp.; Nycteris spp.; Neoromicia spp.; Triaenops spp.; Macronycteris spp.; Nycticeinops spp.; Myonycteris spp.; Scotophilus spp.; Hypsugo spp.; Pipistrellus spp.; Taphozous spp.; Scotoecus spp.)	Oral swab	30	200	EC054-14
		Serum	118	100	EC054-14
		Urine	22	20	EC054-14
		Faecal	61	150	EC054-14
		Kidney	46	150	EC054-14
		Rectum/Intestine	46	200	EC054-14/ 639/2018
	Rousettus aegyptiacus	Serum	85	300	EC054-14
		Urine	7	20	EC054-14
		Faecal	12	150	EC054-14

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Disenase tša Bongakadiruiwa



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

		Spleen	2	150	EC054-14
		Rectum/Intestine	2	200	EC054-14
Multiple locations across South Africa	*Epomophorus spp.*	Oral swab	4	300	EC054-14
		Rectal swab	1	20	EC054-14
		Serum	2	150	EC054-14
		Urine	1	150	EC054-14
		Faecal	2	150	EC054-14
		Spleen	10	150	EC054-14
		Rectum/Intestine	14	200	EC054-14

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-11-03.

3. Please remember to use your protocol number (552/2020) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.

6. As part of your approval, the committee requires that you record a **short video footage** of major animal procedures approved in your study. The committee may request them for monitoring purposes at any later point.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.
Yours sincerely

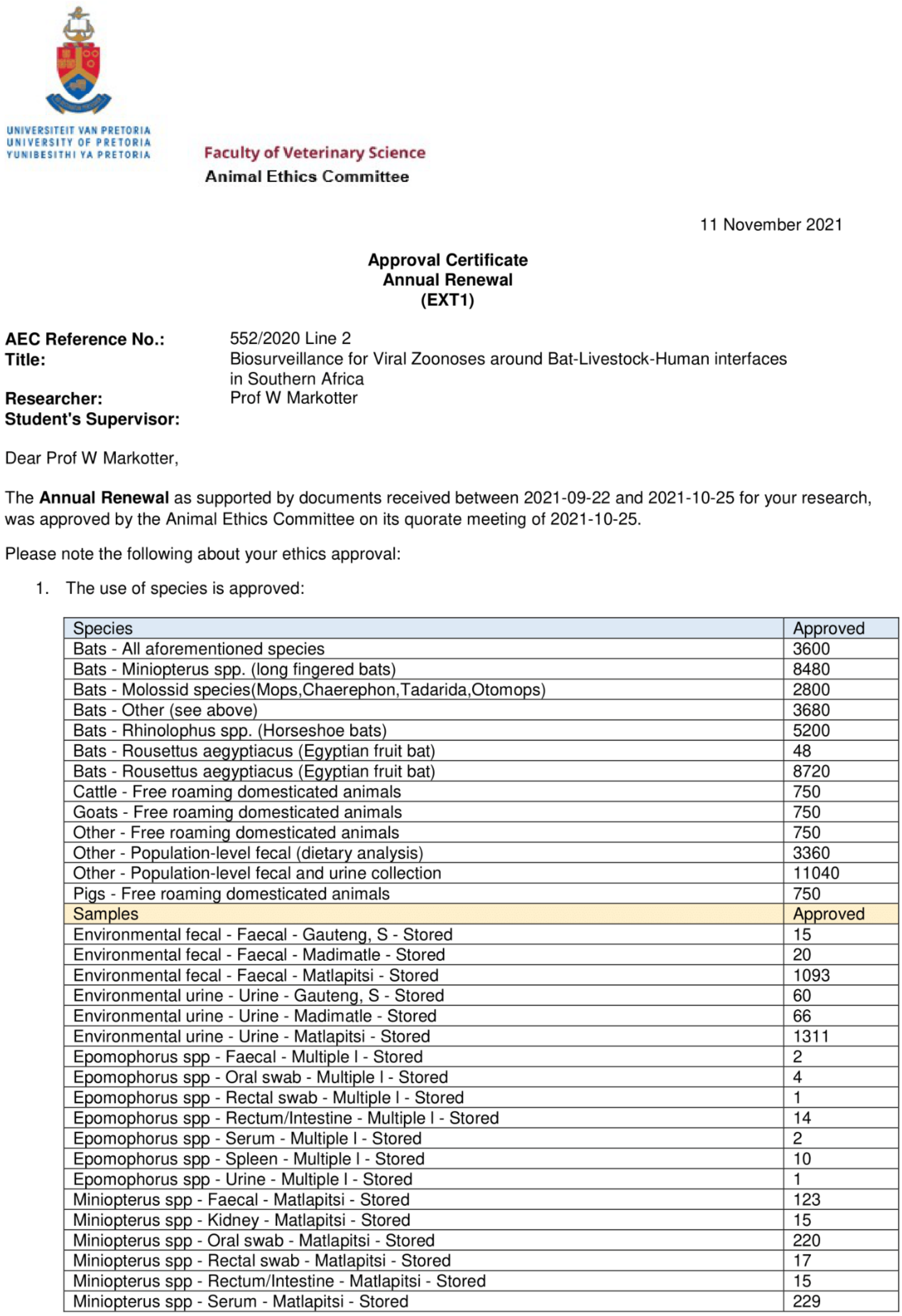
H. Lutermann

Dr. Heike Lutermann
DEPUTY CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Disenase tša Bongakadiruiwa

Appendix B9: Animal ethics certificate for 552/2020 extension from the AEC for 2021



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

11 November 2021

Approval Certificate
Annual Renewal
(EXT1)

AEC Reference No.: 552/2020 Line 2
Title: Biosurveillance for Viral Zoonoses around Bat-Livestock-Human interfaces in Southern Africa
Researcher: Prof W Markotter
Student's Supervisor:

Dear Prof W Markotter,

The **Annual Renewal** as supported by documents received between 2021-09-22 and 2021-10-25 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2021-10-25.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Approved
Bats - All aforementioned species	3600
Bats - Miniopterus spp. (long fingered bats)	8480
Bats - Molossid species(Mops,Chaerephon,Tadarida,Otomops)	2800
Bats - Other (see above)	3680
Bats - Rhinolophus spp. (Horseshoe bats)	5200
Bats - Rousettus aegyptiacus (Egyptian fruit bat)	48
Bats - Rousettus aegyptiacus (Egyptian fruit bat)	8720
Cattle - Free roaming domesticated animals	750
Goats - Free roaming domesticated animals	750
Other - Free roaming domesticated animals	750
Other - Population-level fecal (dietary analysis)	3360
Other - Population-level fecal and urine collection	11040
Pigs - Free roaming domesticated animals	750
Samples	Approved
Environmental fecal - Faecal - Gauteng, S - Stored	15
Environmental fecal - Faecal - Madimatle - Stored	20
Environmental fecal - Faecal - Matlapitsi - Stored	1093
Environmental urine - Urine - Gauteng, S - Stored	60
Environmental urine - Urine - Madimatle - Stored	66
Environmental urine - Urine - Matlapitsi - Stored	1311
Epomophorus spp - Faecal - Multiple I - Stored	2
Epomophorus spp - Oral swab - Multiple I - Stored	4
Epomophorus spp - Rectal swab - Multiple I - Stored	1
Epomophorus spp - Rectum/Intestine - Multiple I - Stored	14
Epomophorus spp - Serum - Multiple I - Stored	2
Epomophorus spp - Spleen - Multiple I - Stored	10
Epomophorus spp - Urine - Multiple I - Stored	1
Miniopterus spp - Faecal - Matlapitsi - Stored	123
Miniopterus spp - Kidney - Matlapitsi - Stored	15
Miniopterus spp - Oral swab - Matlapitsi - Stored	220
Miniopterus spp - Rectal swab - Matlapitsi - Stored	17
Miniopterus spp - Rectum/Intestine - Matlapitsi - Stored	15
Miniopterus spp - Serum - Matlapitsi - Stored	229

Miniopterus spp - Urine - Matlapitsi - Stored	13
Miniopterus spp. - Faecal - Gauteng, S - Stored	59
Miniopterus spp. - Faecal - Gorongoza - Stored	22
Miniopterus spp. - Faecal - Madimatle - Stored	334
Miniopterus spp. - Kidney - Gauteng, S - Stored	3
Miniopterus spp. - Kidney - Gorongoza - Stored	7
Miniopterus spp. - Kidney - Madimatle - Stored	19
Miniopterus spp. - Oral swab - Gauteng, S - Stored	122
Miniopterus spp. - Oral swab - Madimatle - Stored	530
Miniopterus spp. - Rectal swab - Madimatle - Stored	19
Miniopterus spp. - Rectum/Intestine - Gauteng, S - Stored	3
Miniopterus spp. - Rectum/Intestine - Gorongoza - Stored	7
Miniopterus spp. - Rectum/Intestine - Madimatle - Stored	19
Miniopterus spp. - Serum - Gauteng, S - Stored	117
Miniopterus spp. - Serum - Gorongoza - Stored	24
Miniopterus spp. - Serum - Madimatle - Stored	430
Miniopterus spp. - Urine - Gauteng, S - Stored	27
Miniopterus spp. - Urine - Gorongoza - Stored	2
Miniopterus spp. - Urine - Madimatle - Stored	209
Molossids - Faecal - Gorongoza - Stored	28
Molossids - Kidney - Gorongoza - Stored	14
Molossids - Oral swab - Gorongoza - Stored	23
Molossids - Rectum/Intestine - Gorongoza - Stored	14
Molossids - Serum - Gorongoza - Stored	55
Molossids - Urine - Gorongoza - Stored	10
Other species - Faecal - Gorongoza - Stored	61
Other species - Faecal - Madimatle - Stored	70
Other species - Faecal - Matlapitsi - Stored	14
Other species - Kidney - Gorongoza - Stored	46
Other species - Kidney - Madimatle - Stored	11
Other species - Kidney - Matlapitsi - Stored	1
Other species - Oral swab - Gorongoza - Stored	30
Other species - Oral swab - Madimatle - Stored	216
Other species - Oral swab - Matlapitsi - Stored	86
Other species - Rectal swab - Madimatle - Stored	20
Other species - Rectal swab - Matlapitsi - Stored	1
Other species - Rectum/Intestine - Gorongoza - Stored	46
Other species - Rectum/Intestine - Madimatle - Stored	11
Other species - Rectum/Intestine - Matlapitsi - Stored	1
Other species - Serum - Gorongoza - Stored	118
Other species - Serum - Madimatle - Stored	186
Other species - Serum - Matlapitsi - Stored	72
Other species - Urine - Gorongoza - Stored	22
Other species - Urine - Madimatle - Stored	50
Other species - Urine - Matlapitsi - Stored	4
Rhinolophus spp - Faecal - Gorongoza - Stored	20
Rhinolophus spp - Kidney - Gorongoza - Stored	9
Rhinolophus spp - Oral swab - Gorongoza - Stored	22
Rhinolophus spp - Rectum/Intestine - Gorongoza - Stored	9
Rhinolophus spp - Serum - Gorongoza - Stored	49
Rhinolophus spp - Urine - Gorongoza - Stored	20
Rhinolophus spp. - Faecal - Madimatle - Stored	99
Rhinolophus spp. - Faecal - Matlapitsi - Stored	37
Rhinolophus spp. - Kidney - Madimatle - Stored	32
Rhinolophus spp. - Kidney - Matlapitsi - Stored	26
Rhinolophus spp. - Oral swab - Madimatle - Stored	345
Rhinolophus spp. - Oral swab - Matlapitsi - Stored	132
Rhinolophus spp. - Rectal swab - Madimatle - Stored	42
Rhinolophus spp. - Rectal swab - Matlapitsi - Stored	22
Rhinolophus spp. - Rectum/Intestine - Madimatle - Stored	32
Rhinolophus spp. - Rectum/Intestine - Matlapitsi - Stored	26
Rhinolophus spp. - Serum - Madimatle - Stored	123
Rhinolophus spp. - Serum - Matlapitsi - Stored	118

Rhinolophus spp. - Urine - Madimatle - Stored	78
Rhinolophus spp. - Urine - Matlapitsi - Stored	14
Rousettus aegyptiacus - Faecal - Gorongoza - Stored	12
Rousettus aegyptiacus - Faecal - Matlapitsi - Stored	11
Rousettus aegyptiacus - Oral swab - Matlapitsi - Stored	2685
Rousettus aegyptiacus - Rectal swab - Matlapitsi - Stored	2666
Rousettus aegyptiacus - Rectum/Intestine - Gorongoza - Stored	2
Rousettus aegyptiacus - Rectum/Intestine - Matlapitsi - Stored	101
Rousettus aegyptiacus - Serum - Gorongoza - Stored	85
Rousettus aegyptiacus - Serum - Matlapitsi - Stored	3234
Rousettus aegyptiacus - Spleen - Gorongoza - Stored	2
Rousettus aegyptiacus - Spleen - Matlapitsi - Stored	115
Rousettus aegyptiacus - Urine - Gorongoza - Stored	7
Rousettus aegyptiacus - Urine - Matlapitsi - Stored	106

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-11-11.
3. Please remember to use your protocol number (552/2020) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. All incidents must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

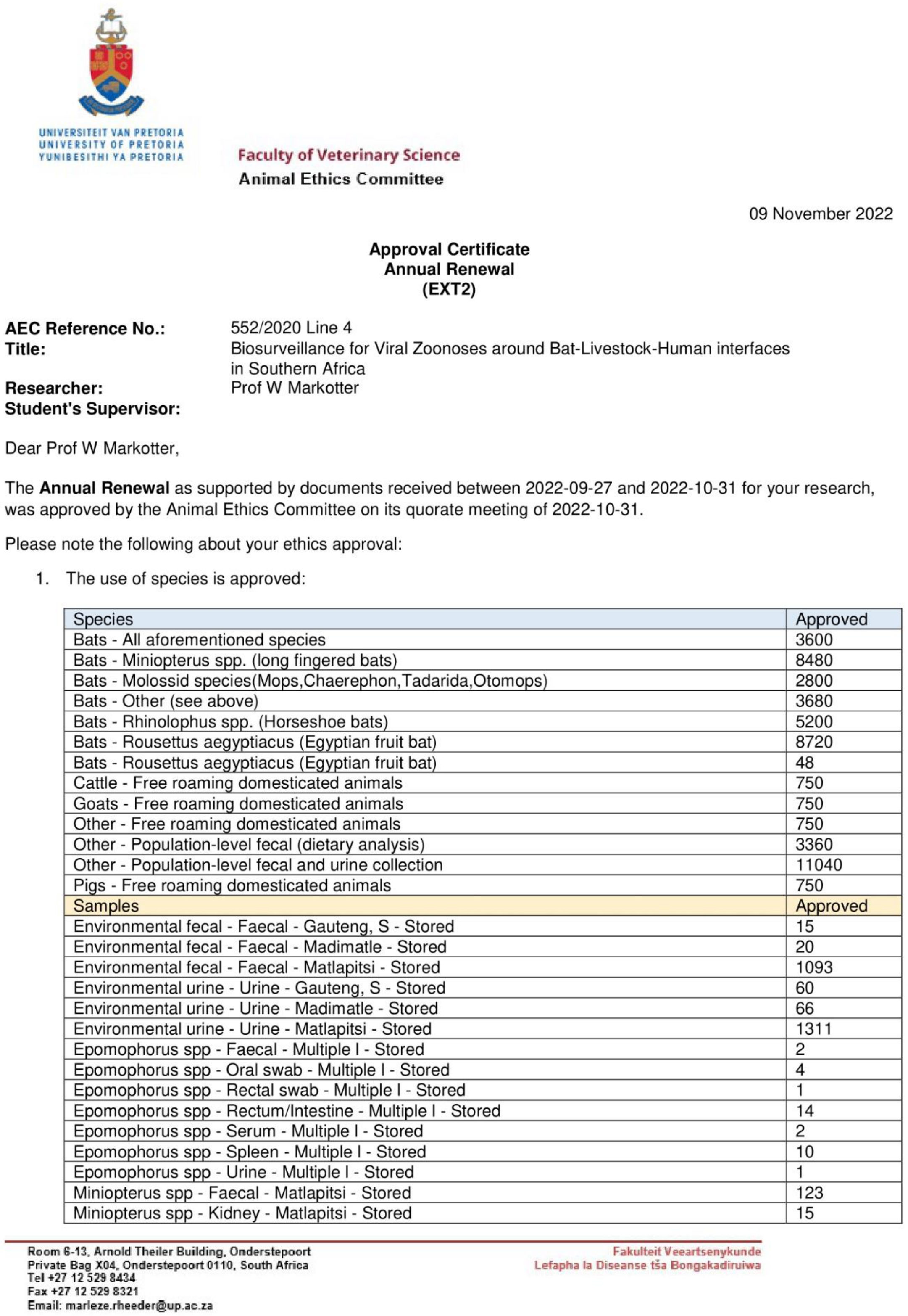
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Appendix B10: Animal ethics certificate for 552/2020 extension from the AEC for 2022



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

09 November 2022

Approval Certificate
Annual Renewal
(EXT2)

AEC Reference No.:	552/2020 Line 4
Title:	Biosurveillance for Viral Zoonoses around Bat-Livestock-Human interfaces in Southern Africa
Researcher:	Prof W Markotter
Student's Supervisor:	

Dear Prof W Markotter,

The **Annual Renewal** as supported by documents received between 2022-09-27 and 2022-10-31 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-10-31.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Approved
Bats - All aforementioned species	3600
Bats - Miniopterus spp. (long fingered bats)	8480
Bats - Molossid species(Mops,Chaerephon,Tadarida,Otomops)	2800
Bats - Other (see above)	3680
Bats - Rhinolophus spp. (Horseshoe bats)	5200
Bats - Rousettus aegyptiacus (Egyptian fruit bat)	8720
Bats - Rousettus aegyptiacus (Egyptian fruit bat)	48
Cattle - Free roaming domesticated animals	750
Goats - Free roaming domesticated animals	750
Other - Free roaming domesticated animals	750
Other - Population-level fecal (dietary analysis)	3360
Other - Population-level fecal and urine collection	11040
Pigs - Free roaming domesticated animals	750
Samples	Approved
Environmental fecal - Faecal - Gauteng, S - Stored	15
Environmental fecal - Faecal - Madimatle - Stored	20
Environmental fecal - Faecal - Matlapitsi - Stored	1093
Environmental urine - Urine - Gauteng, S - Stored	60
Environmental urine - Urine - Madimatle - Stored	66
Environmental urine - Urine - Matlapitsi - Stored	1311
Epomophorus spp - Faecal - Multiple I - Stored	2
Epomophorus spp - Oral swab - Multiple I - Stored	4
Epomophorus spp - Rectal swab - Multiple I - Stored	1
Epomophorus spp - Rectum/Intestine - Multiple I - Stored	14
Epomophorus spp - Serum - Multiple I - Stored	2
Epomophorus spp - Spleen - Multiple I - Stored	10
Epomophorus spp - Urine - Multiple I - Stored	1
Miniopterus spp - Faecal - Matlapitsi - Stored	123
Miniopterus spp - Kidney - Matlapitsi - Stored	15

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Disenase tša Bongakadiruiwa

Miniopterus spp - Oral swab - Matlapitsi - Stored	220
Miniopterus spp - Rectal swab - Matlapitsi - Stored	17
Miniopterus spp - Rectum/Intestine - Matlapitsi - Stored	15
Miniopterus spp - Serum - Matlapitsi - Stored	229
Miniopterus spp - Urine - Matlapitsi - Stored	13
Miniopterus spp. - Faecal - Gauteng, S - Stored	59
Miniopterus spp. - Faecal - Gorongoza - Stored	22
Miniopterus spp. - Faecal - Madimatle - Stored	334
Miniopterus spp. - Kidney - Gauteng, S - Stored	3
Miniopterus spp. - Kidney - Gorongoza - Stored	7
Miniopterus spp. - Kidney - Madimatle - Stored	19
Miniopterus spp. - Oral swab - Gauteng, S - Stored	122
Miniopterus spp. - Oral swab - Madimatle - Stored	530
Miniopterus spp. - Rectal swab - Madimatle - Stored	19
Miniopterus spp. - Rectum/Intestine - Gauteng, S - Stored	3
Miniopterus spp. - Rectum/Intestine - Gorongoza - Stored	7
Miniopterus spp. - Rectum/Intestine - Madimatle - Stored	19
Miniopterus spp. - Serum - Gauteng, S - Stored	117
Miniopterus spp. - Serum - Gorongoza - Stored	24
Miniopterus spp. - Serum - Madimatle - Stored	430
Miniopterus spp. - Urine - Gauteng, S - Stored	27
Miniopterus spp. - Urine - Gorongoza - Stored	2
Miniopterus spp. - Urine - Madimatle - Stored	209
Molossids - Faecal - Gorongoza - Stored	28
Molossids - Kidney - Gorongoza - Stored	14
Molossids - Oral swab - Gorongoza - Stored	23
Molossids - Rectum/Intestine - Gorongoza - Stored	14
Molossids - Serum - Gorongoza - Stored	55
Molossids - Urine - Gorongoza - Stored	10
Other species - Faecal - Gorongoza - Stored	61
Other species - Faecal - Madimatle - Stored	70
Other species - Faecal - Matlapitsi - Stored	14
Other species - Kidney - Gorongoza - Stored	46
Other species - Kidney - Madimatle - Stored	11
Other species - Kidney - Matlapitsi - Stored	1
Other species - Oral swab - Gorongoza - Stored	30
Other species - Oral swab - Madimatle - Stored	216
Other species - Oral swab - Matlapitsi - Stored	86
Other species - Rectal swab - Madimatle - Stored	20
Other species - Rectal swab - Matlapitsi - Stored	1
Other species - Rectum/Intestine - Gorongoza - Stored	46
Other species - Rectum/Intestine - Madimatle - Stored	11
Other species - Rectum/Intestine - Matlapitsi - Stored	1
Other species - Serum - Gorongoza - Stored	118
Other species - Serum - Madimatle - Stored	186
Other species - Serum - Matlapitsi - Stored	72
Other species - Urine - Gorongoza - Stored	22
Other species - Urine - Madimatle - Stored	50
Other species - Urine - Matlapitsi - Stored	4
Rhinolophus spp - Faecal - Gorongoza - Stored	20
Rhinolophus spp - Kidney - Gorongoza - Stored	9
Rhinolophus spp - Oral swab - Gorongoza - Stored	22
Rhinolophus spp - Rectum/Intestine - Gorongoza - Stored	9
Rhinolophus spp - Serum - Gorongoza - Stored	49
Rhinolophus spp - Urine - Gorongoza - Stored	20
Rhinolophus spp. - Faecal - Madimatle - Stored	99
Rhinolophus spp. - Faecal - Matlapitsi - Stored	37
Rhinolophus spp. - Kidney - Madimatle - Stored	32
Rhinolophus spp. - Kidney - Matlapitsi - Stored	26
Rhinolophus spp. - Oral swab - Madimatle - Stored	345

Rhinolophus spp. - Oral swab - Matlapitsi - Stored	132
Rhinolophus spp. - Rectal swab - Madimatle - Stored	42
Rhinolophus spp. - Rectal swab - Matlapitsi - Stored	22
Rhinolophus spp. - Rectum/Intestine - Madimatle - Stored	32
Rhinolophus spp. - Rectum/Intestine - Matlapitsi - Stored	26
Rhinolophus spp. - Serum - Madimatle - Stored	123
Rhinolophus spp. - Serum - Matlapitsi - Stored	118
Rhinolophus spp. - Urine - Madimatle - Stored	78
Rhinolophus spp. - Urine - Matlapitsi - Stored	14
Rousettus aegyptiacus - Faecal - Gorongoza - Stored	12
Rousettus aegyptiacus - Faecal - Matlapitsi - Stored	11
Rousettus aegyptiacus - Oral swab - Matlapitsi - Stored	2685
Rousettus aegyptiacus - Rectal swab - Matlapitsi - Stored	2666
Rousettus aegyptiacus - Rectum/Intestine - Gorongoza - Stored	2
Rousettus aegyptiacus - Rectum/Intestine - Matlapitsi - Stored	101
Rousettus aegyptiacus - Serum - Gorongoza - Stored	85
Rousettus aegyptiacus - Serum - Matlapitsi - Stored	3234
Rousettus aegyptiacus - Spleen - Gorongoza - Stored	2
Rousettus aegyptiacus - Spleen - Matlapitsi - Stored	115
Rousettus aegyptiacus - Urine - Gorongoza - Stored	7
Rousettus aegyptiacus - Urine - Matlapitsi - Stored	106

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-11-09.

3. Please remember to use your protocol number (552/2020) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

5. All incidents must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.

6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

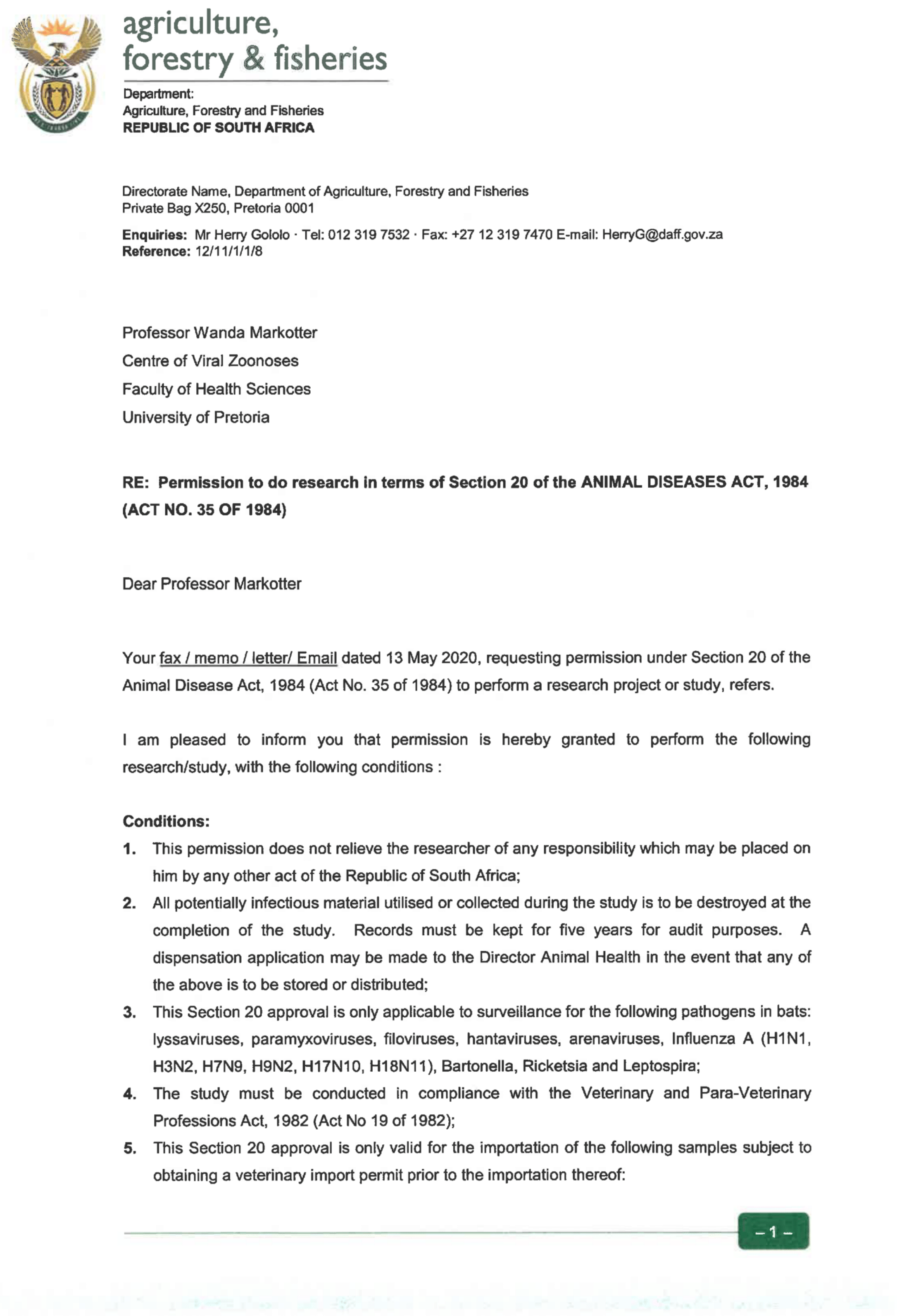
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Prof A Tordiffe
DEPUTY CHAIRMAN: UP-Animal Ethics Committee

Appendix C: DALRRD section 20 Disease ecology of zoonotic pathogens in bat species 2020



agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Name, Department of Agriculture, Forestry and Fisheries
Private Bag X250, Pretoria 0001

Enquiries: Mr Herry Gololo · Tel: 012 319 7532 · Fax: +27 12 319 7470 E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/8

Professor Wanda Markotter
Centre of Viral Zoonoses
Faculty of Health Sciences
University of Pretoria

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

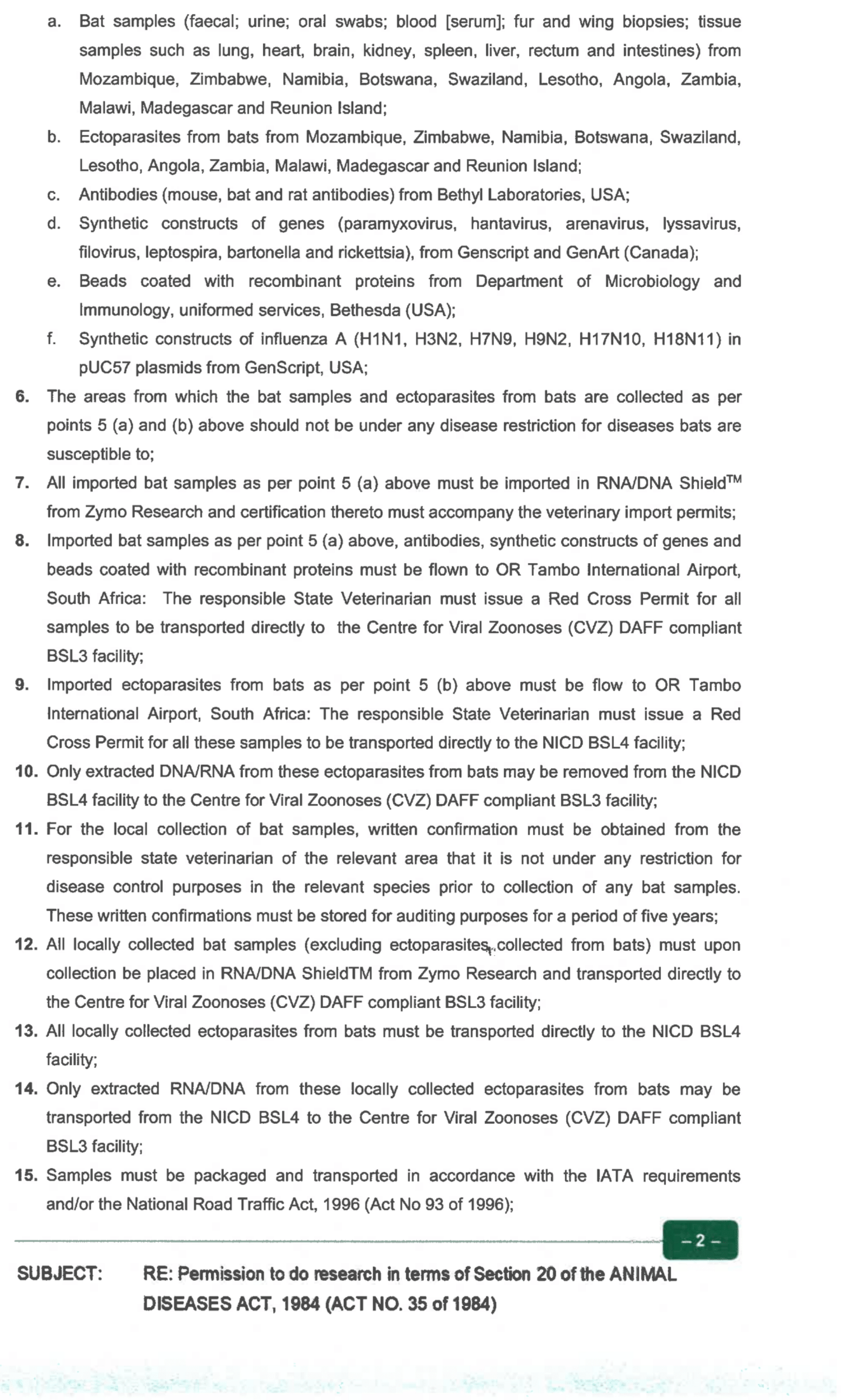
Dear Professor Markotter

Your fax / memo / letter/ Email dated 13 May 2020, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions :

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. This Section 20 approval is only applicable to surveillance for the following pathogens in bats: lyssaviruses, paramyxoviruses, filoviruses, hantaviruses, arenaviruses, Influenza A (H1N1, H3N2, H7N9, H9N2, H17N10, H18N11), Bartonella, Ricketsia and Leptospira;
4. The study must be conducted in compliance with the Veterinary and Para-Veterinary Professions Act, 1982 (Act No 19 of 1982);
5. This Section 20 approval is only valid for the importation of the following samples subject to obtaining a veterinary import permit prior to the importation thereof:



   a. Bat samples (faecal; urine; oral swabs; blood [serum]; fur and wing biopsies; tissue samples such as lung, heart, brain, kidney, spleen, liver, rectum and intestines) from Mozambique, Zimbabwe, Namibia, Botswana, Swaziland, Lesotho, Angola, Zambia, Malawi, Madegascar and Reunion Island;
   b. Ectoparasites from bats from Mozambique, Zimbabwe, Namibia, Botswana, Swaziland, Lesotho, Angola, Zambia, Malawi, Madegascar and Reunion Island;
   c. Antibodies (mouse, bat and rat antibodies) from Bethyl Laboratories, USA;
   d. Synthetic constructs of genes (paramyxovirus, hantavirus, arenavirus, lyssavirus, filovirus, leptospira, bartonella and rickettsia), from Genscript and GenArt (Canada);
   e. Beads coated with recombinant proteins from Department of Microbiology and Immunology, uniformed services, Bethesda (USA);
   f. Synthetic constructs of influenza A (H1N1, H3N2, H7N9, H9N2, H17N10, H18N11) in pUC57 plasmids from GenScript, USA;

6. The areas from which the bat samples and ectoparasites from bats are collected as per points 5 (a) and (b) above should not be under any disease restriction for diseases bats are susceptible to;

7. All imported bat samples as per point 5 (a) above must be imported in RNA/DNA Shield^{TM} from Zymo Research and certification thereto must accompany the veterinary import permits;

8. Imported bat samples as per point 5 (a) above, antibodies, synthetic constructs of genes and beads coated with recombinant proteins must be flown to OR Tambo International Airport, South Africa: The responsible State Veterinarian must issue a Red Cross Permit for all samples to be transported directly to the Centre for Viral Zoonoses (CVZ) DAFF compliant BSL3 facility;

9. Imported ectoparasites from bats as per point 5 (b) above must be flow to OR Tambo International Airport, South Africa: The responsible State Veterinarian must issue a Red Cross Permit for all these samples to be transported directly to the NICD BSL4 facility;

10. Only extracted DNA/RNA from these ectoparasites from bats may be removed from the NICD BSL4 facility to the Centre for Viral Zoonoses (CVZ) DAFF compliant BSL3 facility;

11. For the local collection of bat samples, written confirmation must be obtained from the responsible state veterinarian of the relevant area that it is not under any restriction for disease control purposes in the relevant species prior to collection of any bat samples. These written confirmations must be stored for auditing purposes for a period of five years;

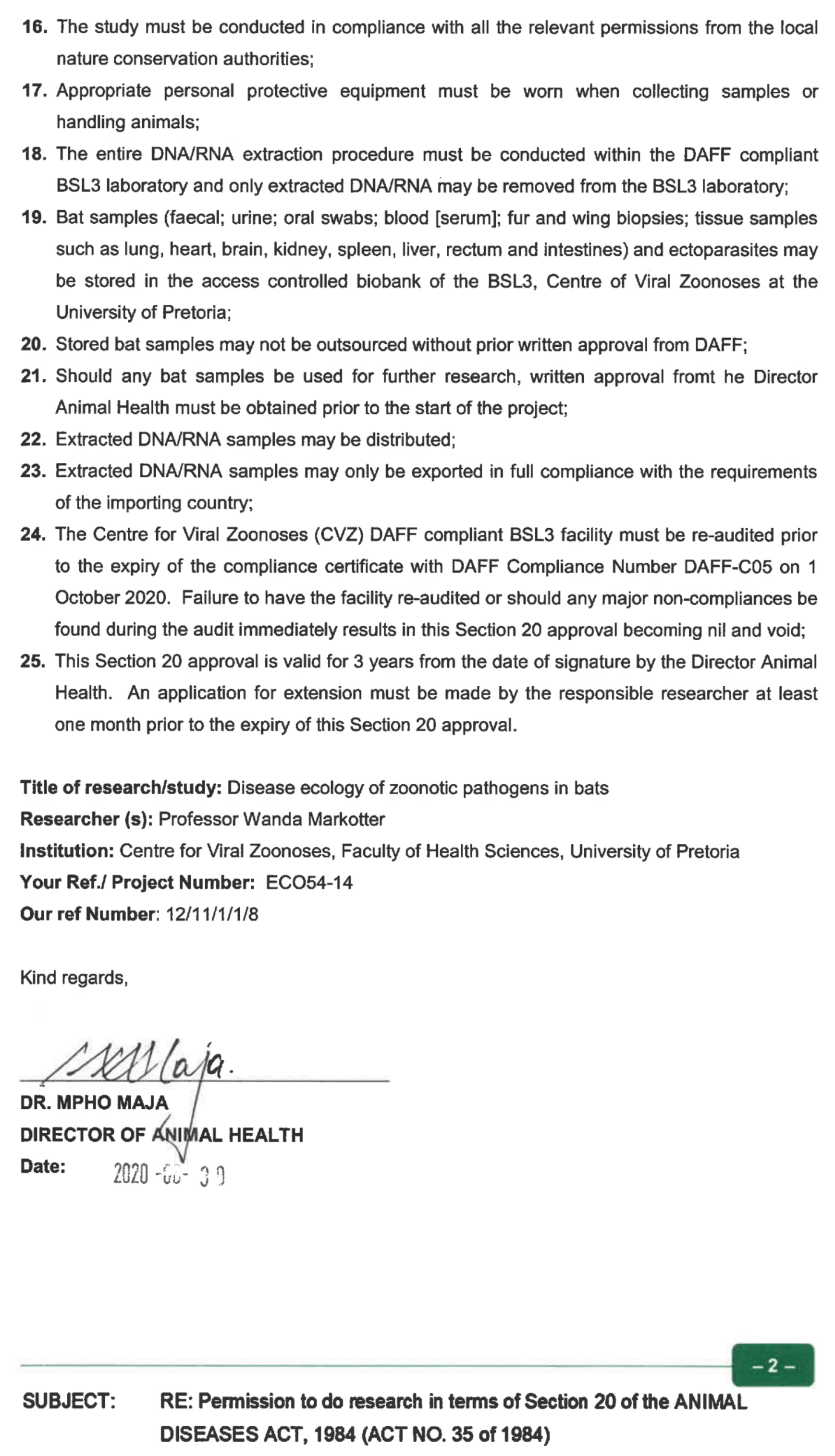
12. All locally collected bat samples (excluding ectoparasites collected from bats) must upon collection be placed in RNA/DNA ShieldTM from Zymo Research and transported directly to the Centre for Viral Zoonoses (CVZ) DAFF compliant BSL3 facility;

13. All locally collected ectoparasites from bats must be transported directly to the NICD BSL4 facility;

14. Only extracted RNA/DNA from these locally collected ectoparasites from bats may be transported from the NICD BSL4 to the Centre for Viral Zoonoses (CVZ) DAFF compliant BSL3 facility;

15. Samples must be packaged and transported in accordance with the IATA requirements and/or the National Road Traffic Act, 1996 (Act No 93 of 1996);

SUBJECT: **RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)**



16. The study must be conducted in compliance with all the relevant permissions from the local nature conservation authorities;
17. Appropriate personal protective equipment must be worn when collecting samples or handling animals;
18. The entire DNA/RNA extraction procedure must be conducted within the DAFF compliant BSL3 laboratory and only extracted DNA/RNA may be removed from the BSL3 laboratory;
19. Bat samples (faecal; urine; oral swabs; blood [serum]; fur and wing biopsies; tissue samples such as lung, heart, brain, kidney, spleen, liver, rectum and intestines) and ectoparasites may be stored in the access controlled biobank of the BSL3, Centre of Viral Zoonoses at the University of Pretoria;
20. Stored bat samples may not be outsourced without prior written approval from DAFF;
21. Should any bat samples be used for further research, written approval fromt he Director Animal Health must be obtained prior to the start of the project;
22. Extracted DNA/RNA samples may be distributed;
23. Extracted DNA/RNA samples may only be exported in full compliance with the requirements of the importing country;
24. The Centre for Viral Zoonoses (CVZ) DAFF compliant BSL3 facility must be re-audited prior to the expiry of the compliance certificate with DAFF Compliance Number DAFF-C05 on 1 October 2020. Failure to have the facility re-audited or should any major non-compliances be found during the audit immediately results in this Section 20 approval becoming nil and void;
25. This Section 20 approval is valid for 3 years from the date of signature by the Director Animal Health. An application for extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 approval.

Title of research/study: Disease ecology of zoonotic pathogens in bats
Researcher (s): Professor Wanda Markotter
Institution: Centre for Viral Zoonoses, Faculty of Health Sciences, University of Pretoria
Your Ref./ Project Number: ECO54-14
Our ref Number: 12/11/1/1/8

Kind regards,

Maja.

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2020 -06- 30

– 2 –

SUBJECT: **RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)**

Appendix D1: Limpopo bats and non-volant mammal permit (ZA/LP/110489) for 2021-2022

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
ECONOMIC DEVELOPMENT, ENVIRONMENT & TOURISM

DO SCIENTIFIC RESEARCH ON MAMMALS

(Issued in terms of the provisions of the Limpopo Environmental Management Act 2003, Act no.7 of 2003).
In terms of and subject to the provisions of the abovementioned legislation and the regulation framed thereunder, the holder of this permit is hereby authorized to catch and/or collect the species and number of mammals specified on the table below for scientific purpose in the property mentioned on this permit.

Permit Holder

Name PROF. WANDA MARKOTTER
Trade Name N/A
ID/Passport Number 7406270122080

Address [Physical | Postal]		
	ROOM 2-72, PATHOLOGY BUILDING, 5 BOPHELO ROAD, PRINSHOF CAMPUS, UNIVERSITY OF PRETORIA, CORNER OF STEVE BIKO AND DR SAVAGE ST, PRETORIA 0001	ROOM 2-72, PATHOLOGY BUILDING, PRINSHOF CAMPUS,UNIVERSITY OF PRETORIA, UNIVERSITY OF PRETORIA, P.O.BOX 323, ARCADIA,0007, RSA

Permit Details

Permit No : ZA/LP/110489
Reference No : CPM/41645/2021
Date Issued : 2021-10-06
Valid until : 2022-10-05
Paid (ZAR): R 60.00
Receipt No : 1153385

Stamp: CITES & PERMIT MANAGEMENT
ENVIRONMENTAL AFFAIRS
LIMPOPO PROVINCE

Farm Name / Organization	**District**	**Province**	**Country**
	N/A	Limpopo	South Africa

See Special Condition

Species Name	**Scientific Name**	**Quantity**	**Note**

Printed by: Seakamela TJ
Printed Date: 2021-10-06
Effective Date: 2021-10-06

Signature of Permit Holder
I acknowledge, accept and understand fully the permit conditions as described.

WILDLIFE TRADE & REGULATION

Cnr Dorp and Suid Street, Polokwane, 0699 P.O. Box 55464, Polokwane, 0700
Tel: +27 15 290 7171/7173-78 Fax: +27 15 295 5018 Website:www.ledet.gov.za Email: permits@ledet.gov.za

The heartland of southern Africa - development is about people!

Appendix D2: Limpopo bats and non-volant mammal permit (ZA/LP/106216) for 2020-2021

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
ECONOMIC DEVELOPMENT, ENVIRONMENT & TOURISM

DO SCIENTIFIC RESEARCH ON MAMMALS

(Issued in terms of the provisions of the Limpopo Environmental Management Act 2003, Act no.7 of 2003).
In terms of and subject to the provisions of the abovementioned legislation and the regulation framed thereunder, the holder of this permit is hereby authorized to catch and/or collect the species and number of mammals specified on the table below for scientific purpose in the property mentioned on this permit.

Permit Holder		
Name	PROF. WANDA MARKOTTER	
Trade Name	N/A	
ID/Passport Number	7406270122080	
Address [Physical | Postal]	ROOM 2-72, PATHOLOGY BUILDING, 5 BOPHELO ROAD, PRINSHOF CAMPUS, UNIVERSITY OF PRETORIA, CORNER OF STEVE BIKO AND DR SAVAGE ST, PRETORIA 0001	ROOM 2-72, PATHOLOGY BUILDING, PRINSHOF

Permit Details			
Permit No :	ZA/LP/106216		
Reference No :	CPM/36719/2020		
Date Issued :	2020-09-23	Stamp:	CITES & PERMIT MANAGEMENT ENVIRONMENTAL AFFAIRS LIMPOPO PROVINCE
Valid until :	2021-09-22		
Paid (ZAR):	R 60.00		
Receipt No :	1138954		

Farm Name / Organization	District	Province	Country
	N/A	Limpopo	South Africa

See Special Condition

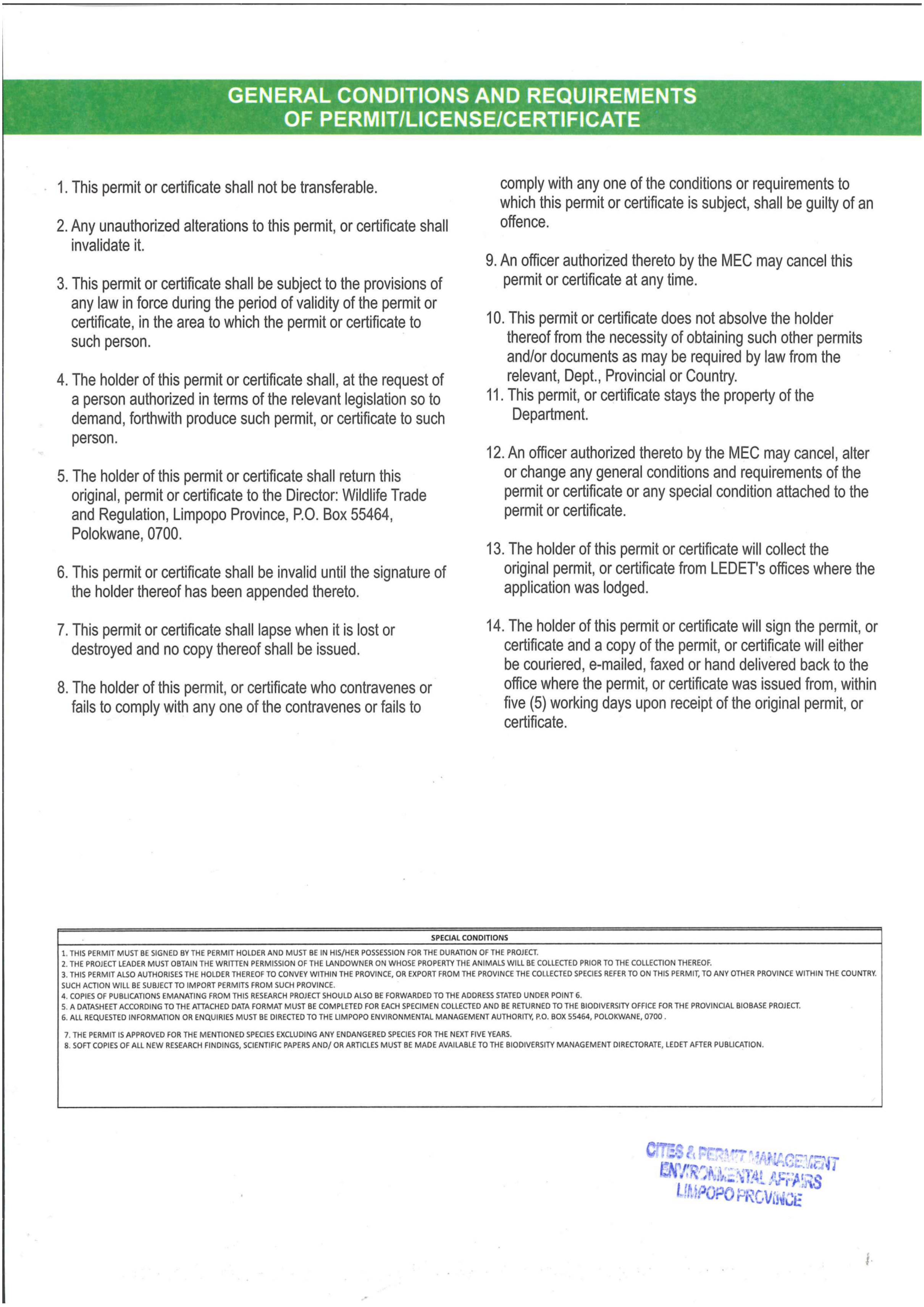
Species Name	Scientific Name	Quantity	Note

	2020-09-23	2020-09-23	
Printed by: Seakamela TJ	**Printed Date:**	**Effective Date:**	**Signature of Permit Holder** I acknowledge, accept and understand fully the permit conditions as described.

WILDLIFE TRADE & REGULATION

Cnr Dorp and Suid Street, Polokwane, 0699 P.O. Box 55464, Polokwane, 0700
Tel: +27 15 290 7171/7173-78 Fax: +27 15 295 5018 Website:www.ledet.gov.za Email: permits@ledet.gov.za

The heartland of southern Africa - development is about people!



GENERAL CONDITIONS AND REQUIREMENTS OF PERMIT/LICENSE/CERTIFICATE

1. This permit or certificate shall not be transferable.

2. Any unauthorized alterations to this permit, or certificate shall invalidate it.

3. This permit or certificate shall be subject to the provisions of any law in force during the period of validity of the permit or certificate, in the area to which the permit or certificate to such person.

4. The holder of this permit or certificate shall, at the request of a person authorized in terms of the relevant legislation so to demand, forthwith produce such permit, or certificate to such person.

5. The holder of this permit or certificate shall return this original, permit or certificate to the Director: Wildlife Trade and Regulation, Limpopo Province, P.O. Box 55464, Polokwane, 0700.

6. This permit or certificate shall be invalid until the signature of the holder thereof has been appended thereto.

7. This permit or certificate shall lapse when it is lost or destroyed and no copy thereof shall be issued.

8. The holder of this permit, or certificate who contravenes or fails to comply with any one of the contravenes or fails to comply with any one of the conditions or requirements to which this permit or certificate is subject, shall be guilty of an offence.

9. An officer authorized thereto by the MEC may cancel this permit or certificate at any time.

10. This permit or certificate does not absolve the holder thereof from the necessity of obtaining such other permits and/or documents as may be required by law from the relevant, Dept., Provincial or Country.
11. This permit, or certificate stays the property of the Department.

12. An officer authorized thereto by the MEC may cancel, alter or change any general conditions and requirements of the permit or certificate or any special condition attached to the permit or certificate.

13. The holder of this permit or certificate will collect the original permit, or certificate from LEDET's offices where the application was lodged.

14. The holder of this permit or certificate will sign the permit, or certificate and a copy of the permit, or certificate will either be couriered, e-mailed, faxed or hand delivered back to the office where the permit, or certificate was issued from, within five (5) working days upon receipt of the original permit, or certificate.

SPECIAL CONDITIONS

1. THIS PERMIT MUST BE SIGNED BY THE PERMIT HOLDER AND MUST BE IN HIS/HER POSSESSION FOR THE DURATION OF THE PROJECT.
2. THE PROJECT LEADER MUST OBTAIN THE WRITTEN PERMISSION OF THE LANDOWNER ON WHOSE PROPERTY THE ANIMALS WILL BE COLLECTED PRIOR TO THE COLLECTION THEREOF.
3. THIS PERMIT ALSO AUTHORISES THE HOLDER THEREOF TO CONVEY WITHIN THE PROVINCE, OR EXPORT FROM THE PROVINCE THE COLLECTED SPECIES REFER TO ON THIS PERMIT, TO ANY OTHER PROVINCE WITHIN THE COUNTRY. SUCH ACTION WILL BE SUBJECT TO IMPORT PERMITS FROM SUCH PROVINCE.
4. COPIES OF PUBLICATIONS EMANATING FROM THIS RESEARCH PROJECT SHOULD ALSO BE FORWARDED TO THE ADDRESS STATED UNDER POINT 6.
5. A DATASHEET ACCORDING TO THE ATTACHED DATA FORMAT MUST BE COMPLETED FOR EACH SPECIMEN COLLECTED AND BE RETURNED TO THE BIODIVERSITY OFFICE FOR THE PROVINCIAL BIOBASE PROJECT.
6. ALL REQUESTED INFORMATION OR ENQUIRIES MUST BE DIRECTED TO THE LIMPOPO ENVIRONMENTAL MANAGEMENT AUTHORITY, P.O. BOX 55464, POLOKWANE, 0700 .
7. THE PERMIT IS APPROVED FOR THE MENTIONED SPECIES EXCLUDING ANY ENDANGERED SPECIES FOR THE NEXT FIVE YEARS.
8. SOFT COPIES OF ALL NEW RESEARCH FINDINGS, SCIENTIFIC PAPERS AND/ OR ARTICLES MUST BE MADE AVAILABLE TO THE BIODIVERSITY MANAGEMENT DIRECTORATE, LEDET AFTER PUBLICATION.

CITES & PERMIT MANAGEMENT
ENVIRONMENTAL AFFAIRS
LIMPOPO PROVINCE

Appendix D3: Limpopo bats and non-volant mammal permit (ZA/LP/100499) for 2019-2020

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
ECONOMIC DEVELOPMENT, ENVIRONMENT & TOURISM

DO SCIENTIFIC RESEARCH ON MAMMALS

(Issued in terms of the provisions of the Limpopo Environmental Management Act 2003, Act no.7 of 2003).
In terms of and subject to the provisions of the abovementioned legislation and the regulation framed thereunder, the holder of this permit is hereby authorized to catch and/or collect the species and number of mammals specified on the table below for scientific purpose in the property mentioned on this permit.

Permit Holder

Name	PROF. WANDA MARKOTTER	
Trade Name	N/A	
ID/Passport Number	7406270122080	
Address [Physical | Postal]	ROOM 2-72, PATHOLOGY BUILDING, 5 BOPHELO ROAD, PRINSHOF CAMPUS,UNIVERSITY OF PRETORIA, CORNER OF STEVE BIKO AND DR SAVAGE ST, PRETORIA 0001	ROOM 2-72, PATHOLOGY BUILDING, PRINSHOF CAMPUS,UNIVERSITY OF PRETORIA, UNIVERSITY OF PRETORIA, P.O.BOX 323, ARCADIA,0007, RSA

Permit Details

Permit No :	ZA/LP/100499	
Reference No :	CPM/29253/2019	
Date Issued :	2019-10-04	Stamp: CITES & PERMIT MANAGEMENT ENVIRONMENTAL AFFAIRS LIMPOPO PROVINCE
Valid until :	2020-10-04	
Paid (ZAR):	R 55.00	
Receipt No :	1130327	

Farm Name / Organization	District	Province	Country
	N/A	Limpopo	South Africa

See Special Condition

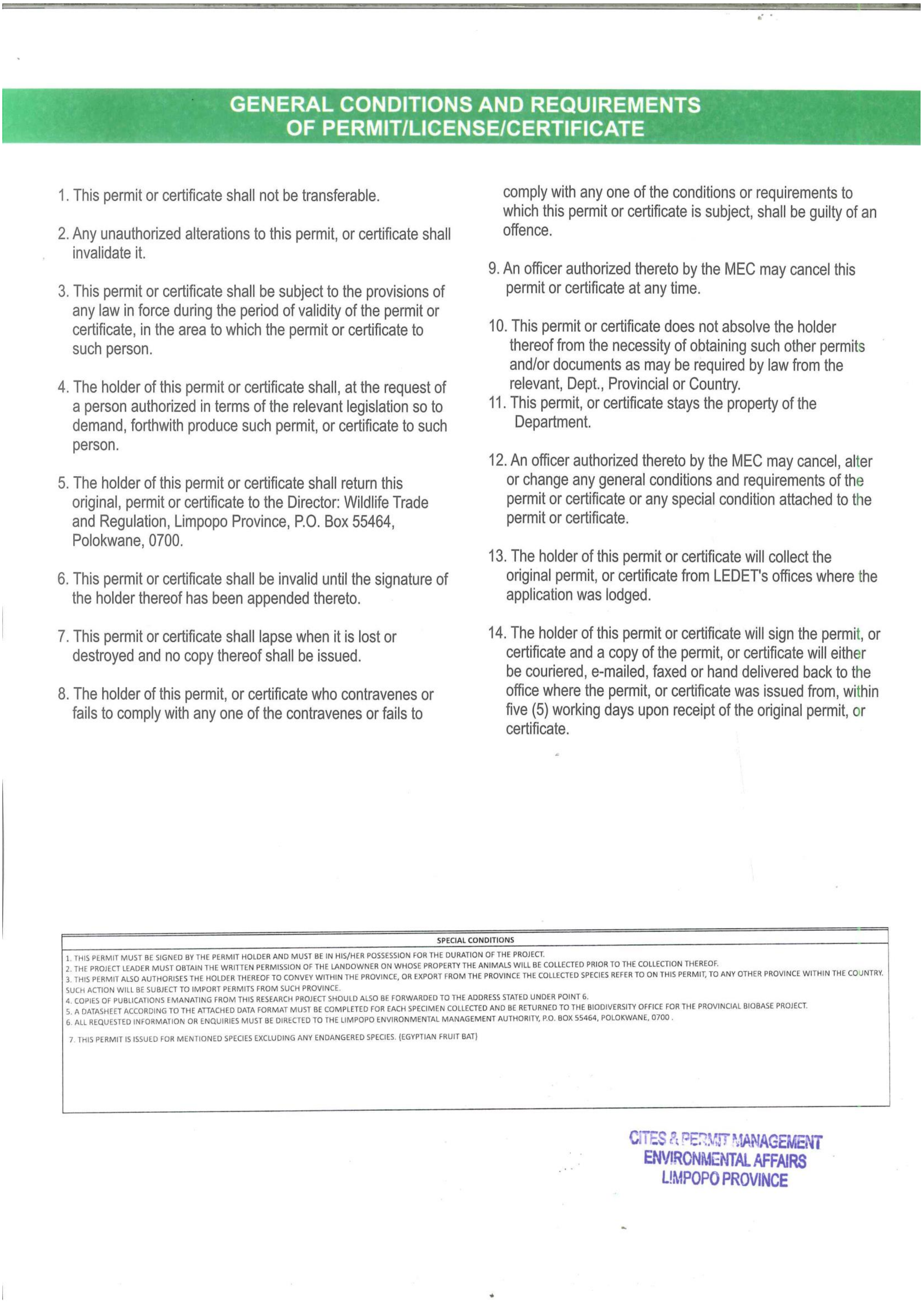
Species Name	Scientific Name	Quantity	Note

	2019-10-04	2019-10-04	
Printed by:Seakamela TJ	Printed Date:	Effective Date:	Signature of Permit Holder

I acknowledge, accept and understand fully the permit conditions as described.

WILDLIFE TRADE & REGULATION
Cnr Dorp and Suid Street, Polokwane, 0699 P.O. Box 55464, Polokwane, 0700
Tel: +27 15 290 7171/7173-78 Fax: +27 15 295 5018 Website:www.ledet.gov.za Email: permits@ledet.gov.za

The heartland of southern Africa - development is about people!



GENERAL CONDITIONS AND REQUIREMENTS OF PERMIT/LICENSE/CERTIFICATE

1. This permit or certificate shall not be transferable.

2. Any unauthorized alterations to this permit, or certificate shall invalidate it.

3. This permit or certificate shall be subject to the provisions of any law in force during the period of validity of the permit or certificate, in the area to which the permit or certificate to such person.

4. The holder of this permit or certificate shall, at the request of a person authorized in terms of the relevant legislation so to demand, forthwith produce such permit, or certificate to such person.

5. The holder of this permit or certificate shall return this original, permit or certificate to the Director: Wildlife Trade and Regulation, Limpopo Province, P.O. Box 55464, Polokwane, 0700.

6. This permit or certificate shall be invalid until the signature of the holder thereof has been appended thereto.

7. This permit or certificate shall lapse when it is lost or destroyed and no copy thereof shall be issued.

8. The holder of this permit, or certificate who contravenes or fails to comply with any one of the contravenes or fails to comply with any one of the conditions or requirements to which this permit or certificate is subject, shall be guilty of an offence.

9. An officer authorized thereto by the MEC may cancel this permit or certificate at any time.

10. This permit or certificate does not absolve the holder thereof from the necessity of obtaining such other permits and/or documents as may be required by law from the relevant, Dept., Provincial or Country.

11. This permit, or certificate stays the property of the Department.

12. An officer authorized thereto by the MEC may cancel, alter or change any general conditions and requirements of the permit or certificate or any special condition attached to the permit or certificate.

13. The holder of this permit or certificate will collect the original permit, or certificate from LEDET's offices where the application was lodged.

14. The holder of this permit or certificate will sign the permit, or certificate and a copy of the permit, or certificate will either be couriered, e-mailed, faxed or hand delivered back to the office where the permit, or certificate was issued from, within five (5) working days upon receipt of the original permit, or certificate.

SPECIAL CONDITIONS

1. THIS PERMIT MUST BE SIGNED BY THE PERMIT HOLDER AND MUST BE IN HIS/HER POSSESSION FOR THE DURATION OF THE PROJECT.
2. THE PROJECT LEADER MUST OBTAIN THE WRITTEN PERMISSION OF THE LANDOWNER ON WHOSE PROPERTY THE ANIMALS WILL BE COLLECTED PRIOR TO THE COLLECTION THEREOF.
3. THIS PERMIT ALSO AUTHORISES THE HOLDER THEREOF TO CONVEY WITHIN THE PROVINCE, OR EXPORT FROM THE PROVINCE THE COLLECTED SPECIES REFER TO ON THIS PERMIT, TO ANY OTHER PROVINCE WITHIN THE COUNTRY. SUCH ACTION WILL BE SUBJECT TO IMPORT PERMITS FROM SUCH PROVINCE.
4. COPIES OF PUBLICATIONS EMANATING FROM THIS RESEARCH PROJECT SHOULD ALSO BE FORWARDED TO THE ADDRESS STATED UNDER POINT 6.
5. A DATASHEET ACCORDING TO THE ATTACHED DATA FORMAT MUST BE COMPLETED FOR EACH SPECIMEN COLLECTED AND BE RETURNED TO THE BIODIVERSITY OFFICE FOR THE PROVINCIAL BIOBASE PROJECT.
6. ALL REQUESTED INFORMATION OR ENQUIRIES MUST BE DIRECTED TO THE LIMPOPO ENVIRONMENTAL MANAGEMENT AUTHORITY, P.O. BOX 55464, POLOKWANE, 0700 .

7. THIS PERMIT IS ISSUED FOR MENTIONED SPECIES EXCLUDING ANY ENDANGERED SPECIES. {EGYPTIAN FRUIT BAT}

CITES & PERMIT MANAGEMENT
ENVIRONMENTAL AFFAIRS
LIMPOPO PROVINCE