The Effects of Habitat, Season, and Ungulate Carcass Sites on the Chiropteran Assemblage of Telperion Nature Reserve, South Africa

By

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DECLARATION

I declare that the work: The effects of habitat, season, and ungulate carcass sites on the chiropteran assemblage of Telperion Nature Reserve, South Africa which I hereby submit for the degree of Master of Science in Nature Conservation at the University of South Africa, is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

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I further declare that I have not previously submitted this work, or part of it, for examination at UNISA for another qualification or at any other higher education institution.

Student Signature: Gregory de Jong

Date: June 2023

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ABSTRACT

Globally, insectivorous bats provide ecosystem services including pest suppression and nutrient cycling that are vital for maintaining ecosystem function and agricultural health and productivity. Sixty-two of the 67 documented South African bat species are insectivorous. Insectivorous bats form an essential component of most, if not all southern African ecosystems. Grasslands cover approximately 28.6 % of South Africa’s land area, yet the associated bat biodiversity is understudied relative to other habitats; it is reported that bat diversity may be greatest in the wetter eastern parts of the country, particularly in savanna, woodland and natural forested habitat. Telperion Nature Reserve Telperion Nature Reserve is a grassland-dominated reserve straddling the border between the Mpumalanga and Gauteng provinces of South Africa, and the grassland and savanna biomes. Using data collected by physical capture and acoustic monitoring, an insectivorous bat species inventory for Telperion Nature Reserve was compiled and a regional echolocation call library was developed. The singular and combined effects of habitat, season and bioclimatic variables on species richness, diversity, abundance, functional diversity, and relative activity of the Telperion Nature Reserve bat assemblage was assessed. Additionally, the effect of the presence of blue wildebeest (C*onnochaetes taurinus*) carcasses on bat activity was also investigated, using passive (acoustic) sampling. Bats identified on Telperion Nature Reserve were allocated to five functional groups based on morphological, ecological and behavioural traits. In total, 22 species from seven families were identified. This study developed a call library for insectivorous bats in this grassland system and provides a comprehensive baseline for future studies. A cryptic *Rhinolophus* cf. *simulator* species was recorded with characteristic frequency (Fc) of 79 kHz, significantly different to that of *Rhinolophus simulator* s.s. (~83 kHz Fc). It corroborated previous findings of sex-related echolocation call parameter variances in vespertilionid species. Habitat and season are important determinants of bat occupancy, relative abundance and activity. Bat activity varied significantly between habitat types. Watercourses were associated with the highest bat species diversity. Anthropogenic sites were associated with the highest levels of bat activity. This study demonstrates the value of watercourses and anthropogenic sites, for bats, within the relative homogeneity of grassland systems. The maintenance and conservation of watercourses in this area will enhance the conservation value of Telperion Nature Reserve for bats. Insectivorous bat activity on Telperion Nature Reserve varied significantly on a seasonal basis, with the lowest diversity and activity recorded in winter. Both bat passes and feeding buzzes were recorded over carcass sites suggesting that bats forage over such sites. Caged carcass sites exhibited elevated bat activity levels, however this result was not significant. This study should be repeated with greater sampling effort and more constrained site selection parameters to avoid potential sampling biases that may skew the data (i.e. a high intra-treatment variation). This study illustrates the importance of grasslands, even those that fall within a modified matrix, for bat ecology and conservation.

KEYWORDS

Insectivorous bats, Activity, Species diversity, Seasonal-variation, Call library, Habitat types, Microhabitats, Mixed grassland, Carcass, Diversity, Active captures, Acoustic monitoring.

CHAPTER ONE

INTRODUCTION

1.1. Introduction

Chiroptera is the only mammalian order capable of powered flight; they are a diverse group with over 1460 species worldwide (Burgin, Colella, Kahn, & Upham, 2018; Mammal Diversity Database version 1.11, 2023). They play important roles in food webs as predators and prey. They also contribute to arthropod and disease suppression, seed dispersal, pollination, and dispersal of nutrients between habitats (Kasso & Balakrishnan, 2013). The ecosystem services provided by bats are of great fiscal importance, with their value to the agricultural industry, in the United States alone, is estimated to be US$22.9 billion in 2007 (Boyles, Cryan, McCracken, & Kunz, 2011). The cost mitigation to the South African macadamia nut (*Macadamia integrifolia*) industry, through stink bug suppression by bats, is estimated at US$ 2.26 ± 1.57 million/yr (Taylor, Grass, Alberts, Joubert, and Tscharntke, 2017). Furthermore, the value of insectivorous bats is not only to agriculture; bats are useful indicators of disturbance and toxin bioaccumulation in ecosystems due to their trophic position and the manner in which their populations track arthropod prey populations (Jones, Jacobs, Kunz, Wilig, & Racey, 2009; Naidoo, Vosloo, & Schoeman, 2016).

Bat diversity in the southern African region is relatively high, approximately 130 described species, 67 of which occur in South Africa (Child, Roxburgh, Do Linh San, Raimondo, & Davies-Mostert, 2016; Monadjem, Taylor, Cotterill & Schoeman, 2020; Taylor, Grass, Alberts, Joubert, & Tscharntke, 2018). These figures may be an underestimation as chiropteran taxonomy is dynamic and subject to change as more systematics-related research is conducted. For instance, there was an 18% increase in the number of bat taxa described from the Afro-Malagasy region between 1989 and 2019 (Taylor, Denys, & Cotterill, 2019). Within South Africa, bats are unevenly distributed. The habitats associated with higher rainfall in the eastern parts of South Africa support a higher diversity of chiropterans than the more temperate and arid western regions (Gelderblom, Bronner, Lombard, & Taylor, 1995; Cooper-Bohannon, Rebelo, Jones, Cotterill, Monadjem, Schoeman, Taylor, Park, 2016). Other variables that influence bat species distribution include the presence of suitable roost sites, fresh water and prey availability (Herkt, Barnikel, Skidmore, & Fahr, 2016).

African bat species richness is highest in tropical forests, with, species richness and diversity typically higher in structurally complex habitats (Batary, Baldi, Kleijn, & Tscharntke, 2010; Fahr & Kalko, 2011; Gaujour, Amiaud, Mignolet, & Plantureux, 2012; Katunzi, Soisook, Webala, Armstrong,

& Bumrungsri, 2020; Monadjem, Conenna, Taylor, & Schoeman, 2018), and decrease in more temperate regions. Most research on South African chiropterans has focussed on the northern and eastern savanna and forest regions where there is higher species diversity (Monadjem *et al.,* 2020). Relative to savannas and forested regions, the chiropteran assemblages associated with South African grasslands are understudied. Approximately 31 to 39 species of insectivorous bats are thought to occur in South African grasslands (Gelderblom *et al.,* 1995; Seamark, 2013). However, species richness estimates predict that as many as 42 to 54 species of bats might be present in these habitats (Seamark, 2013). Fruit bats, however, seem to be absent from grassland habitats as the vital food source, fruit from fruiting trees, are rare within these areas (Monadjem *et al.,* 2020). These grasslands cover 349174 km^2, approximately 17%, of South Africa's land area (Neke & Du Plessis, 2004). Grasslands typically occur at higher altitudes and are maintained by a combination of grazing, fire and climatic conditions (Neke & Du Plessis, 2004; Skowno *et al.,* 2019).

The grassland biome is one of the most threatened landscapes in southern Africa as it has been substantially transformed for urban development, mining, and agriculture (MacFadyen, 2014). Poor grassland management practices, including uncontrolled burning and overgrazing have led to soil erosion and exotic plant invasions (Cowling, Richardson, & Pierce, 1997; Egoh, Reyers, Rouget, & Richardson, 2011; Neke & Du Plessis, 2004; Rutherford, & Westfall, 1994). Ecosystem health has been reduced as a result and ecosystem services, biodiversity and resilience have been negatively affected due to poor management practices (Egoh, Reyers, Rouget, Bode, & Richardson, 2009). Reduced ecosystem health may negatively affect the grassland bat assemblage because they are reliant of foraging, roost and water resources in grassland habitats (Chung-MacCoubrey, 1996; Herkt *et al.,* 2016).

1.2. Literature Review

1.2.1. Insectivorous bats

Bats have traditionally been divided into two groups, megachiroptera (fruit bats or pteropodids) and microchiroptera (carnivorous, insectivorous, and other nectivorous bats); they contribute approximately 14% and 86% of worldwide bat diversity respectively (Burgin *et al.,* 2018). This classification has, however, been overhauled and the current subordinal level classification of bats are the Pteropodiformes (pteropodid bats and six animalivorous or insectivorous bat families namely, Craseonycteridae, Hipposideridae, Megadermatidae, Rhinolophidae, Rhinopomatidae and Rhinonycteridae) and Vespertilioniformes (the remaining 14 bat families) (Hutcheon & Kirsch, 2006; Mammal Diversity Database, 2022).

Chiropterans are a widely distributed, diverse group that forage on fruit, insects, pollen, blood and vertebrates (Schnitzler & Kalko, 2001). The order Chiroptera includes a large diversity of foraging guilds and feeding specialisations. The majority (ca. 125 species) of southern Africa bats are aerial insectivores (Monadjem *et al.,* 2020). One species, *Nycteris grandis* forages on insects and small vertebrates (Fenton, Cumming, Hutton, & Swanepoel, 1987), Frugivorous bats (21 species) complete the southern African bat assemblage (Monadjem *et al.,* 2020). Diets of insectivorous bats vary based on bat body size, seasonality, prey availability and energetic constraints (including pregnancy or lactation) (Agosta, Morton, & Kuhn, 2003; Barclay & Brigham, 1991). Species-specific feeding strategies may also be shaped by the prey resources available to them (Norberg & Rayner, 1987). Worldwide, insectivorous bats tend to be smaller than either carnivorous or frugivorous bats (Barclay & Brigham, 1991; Norberg & Rayner, 1987).

1.2.2. Foraging ecology

Insectivorous chiropterans have developed a variety of foraging strategies including gleaning, trawling, hawking and perch hunting (Norberg & Rayner, 1987). Some species are adapted to hunting in thick clutter (an array of obstacles within the habitat which causes it to be structurally complex) (Fenton, 1990), while others are adapted to foraging in open air or within a combination of habitat types (Neuweiler, 1984). The echolocation frequencies at which bats function best is known as the frequency of best hearing (Neuweiler, 1984). This is correlated with the type of echolocation and the habitat in which these echolocation frequencies are likely to be effective (Jones & Holderied, 2007; Monadjem *et al.,* 2020). Bats that prey upon mobile animals including mammals, insects and fish have a strong need for high resolution and accurate echolocation as their prey must be tracked before capture (Neuweiler, 1984). This is in contrast to species that forage on static resources (nectar and fruit) have less need for high resolution echolocation and, in these species, echolocation ability is either reduced or completely lost (Henson & Schnitzler, 1980; Teeling *et al.,* 2005).

1.2.3. Foraging guild and habitat associations

Specific adaptations in morphology and echolocation allow bats to use a variety of habitats. Although bats are able to forage in habitats to which they are not well adapted, particular adaptations allow more successful foraging in their favoured habitats (Furlonger, Dewar, & Fenton, 1987). Species that forage in comparable habitats, using analogous foraging techniques, may be subjected to similar ecological constraints and have likely evolved convergent motor and sensory adaptations that are suited to particular habitats and prey (Norberg & Rayner, 1987; Schnitzler & Kalko, 2001) (Figure 1.1.).

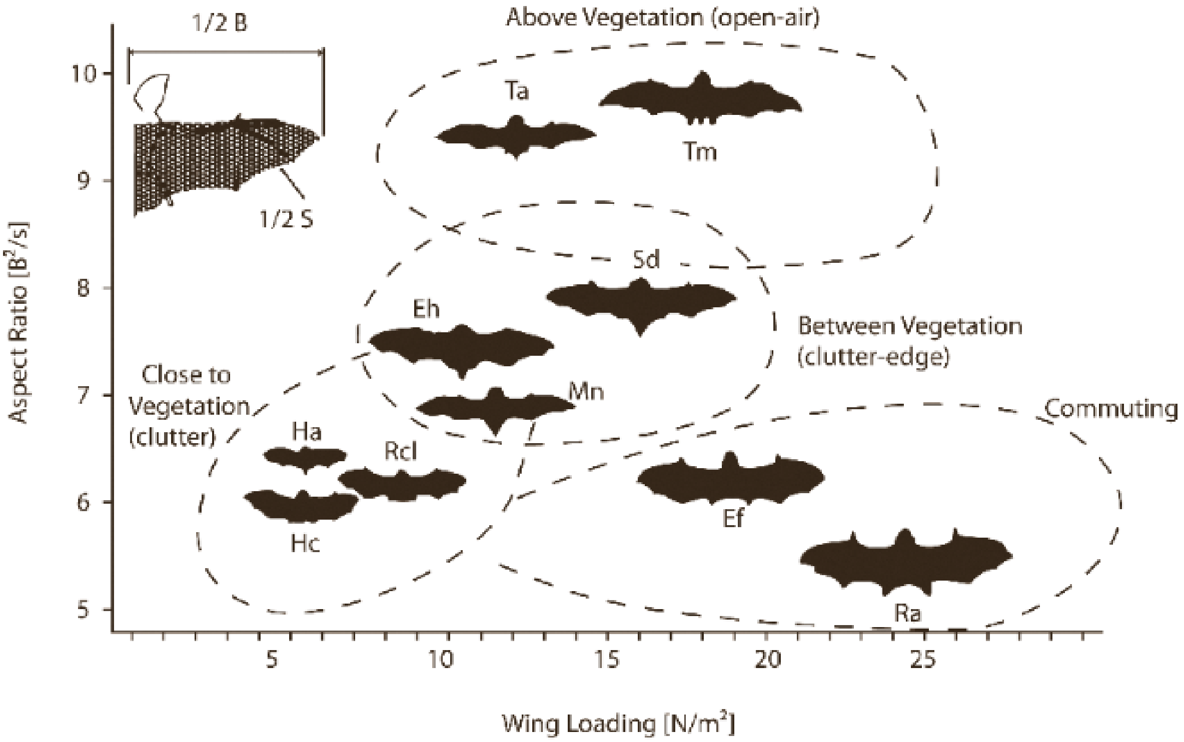

Figure 1.1. Wing adaptations of African bats in relation to forging habitat, **Ef** -*Epomops franqueti*; **Eh**-*Eptesicus hottentotus*; **Ha-***Neoromicia anchietae*; **Hc**-*Hipposideros caffer*; **Mn**-*Miniopterus natalensis*; **Ra**-*Rousettus aegyptiacus*; **Rcl-***Rhinolophus clivosus*; **Sd**-*Scotophilus dinganii*; **Ta**-*Tadarida aegyptiaca*; **Tm**-*Taphozous mauritianus*, from Monadjem *et al.,* (2010) and adapted from (Neuweiler, 2000)

Open space foragers typically have long narrow wings and are adapted to fast flight in uncluttered habitats where extreme agility is unnecessary (Saunders & Barclay, 1992; Schnitzler, Moss, & Denzinger, 2003). They have high wing loading (a measure of wing area relative to body mass) and a high aspect ratio (an index of wing shape associated with flight efficiency) (Saunders & Barclay, 1992). They tend to use lower echolocation frequencies that attenuate less over longer distances (Schnitzler *et al.,* 2003). Edge space foragers tend to forage along the edges of clutter around forest matrices or the edges of water courses, where there is a combination of open and cluttered habitats (Monadjem *et al.,* 2020; Norberg & Rayner, 1987). These bats probably forage along linear edge habitats as they offer protection from wind, predators and support abundant food resources (Downs & Racey, 2006; Schoeman & Jacobs, 2008). Edge space foragers are thought to be aerial insectivores or trawling foragers, as such they display intermediate to high wing loading and typically have more pointed wings and a higher aspect ratio than the clutter foragers (Norberg & Rayner, 1987; Schnitzler *et al.,* 2003).

Clutter foragers feed in thickly vegetated or structurally complex habitats (Fenton & Rautenbach, 1986). These species tend to have low wing loading and aspect ratio due to their short, broad wings (Norberg & Rayner, 1987). The wing morphology of clutter foragers allows them to be agile when foraging within dense, cluttered habitats.

1.2.4. Echolocation

Echolocation is the transmission of ultrasonic vocalisations and the reception of the resultant reflected echoes to produce an auditory scene for navigation and capturing prey (Moss & Surlykke, 2001). Insectivorous bats rely primarily on echolocation for foraging (Schnitzler & Kalko, 2001). Megachiroptera (historically the collective name for fruit bats), that are primarily frugivores, use olfaction and vision for foraging, and consequently have less developed echolocation capabilities than aerial insectivores and bats that capture mobile prey (Monadjem *et al.,* 2020). Initially echolocation was thought to have evolved to aid nocturnal foraging in the aerial insectivores (Neuweiler 1984), however, more recent studies have shown that echolocation first evolved to aid navigation and was subsequently modified for prey acquisition (Schnitzler, Moss, & Denzinger, 2003). Echolocating bats use the delay between their emitted call and returning echo to determine distances to obstacles and prey within their environments (Schnitzler *et al.,* 2003). Echolocation calls for orientation are typically of longer duration than calls used during foraging (Moss & Surlykke, 2001). Three call types (phases) are used when moving through feeding habitats and foraging on insect prey; 1) search phase calls are emitted during navigation and while searching for prey, 2) approach phase calls are emitted after prey detection and for initial prey tracking, and 3) terminal phase calls or feeding buzzes are rapidly produced echolocation pulses which are used for prey tracking shortly before capture (Gillam, 2007; Rydell, Entwistle, & Racey, 1996). Thus, echolocating bats can vary the timing, duration, intensity and bandwidth of calls to alter their perception of this auditory scene and improve their ability to catch prey (Moss & Surlykke, 2001).

Echolocation calls can also vary in accordance with the habitat through which the bat is navigating. Species that occur in cluttered, complex habitats, usually emit rapid high frequency calls that attenuate quickly (Griffin, 1971; Lawrence & Simmons, 1982) (Figure 1.2.). Such calls cause a high number of informative echoes that result in a high resolution auditory ‘image’ for spatial orientation in complex habitats (Norberg & Rayner, 1987). Conversely, open air fliers produce lower frequency calls of a longer duration. These calls attenuate less over distance and are more useful for orientation in open or edge habitats (Norberg & Rayner, 1987) (Figure 1.2.).

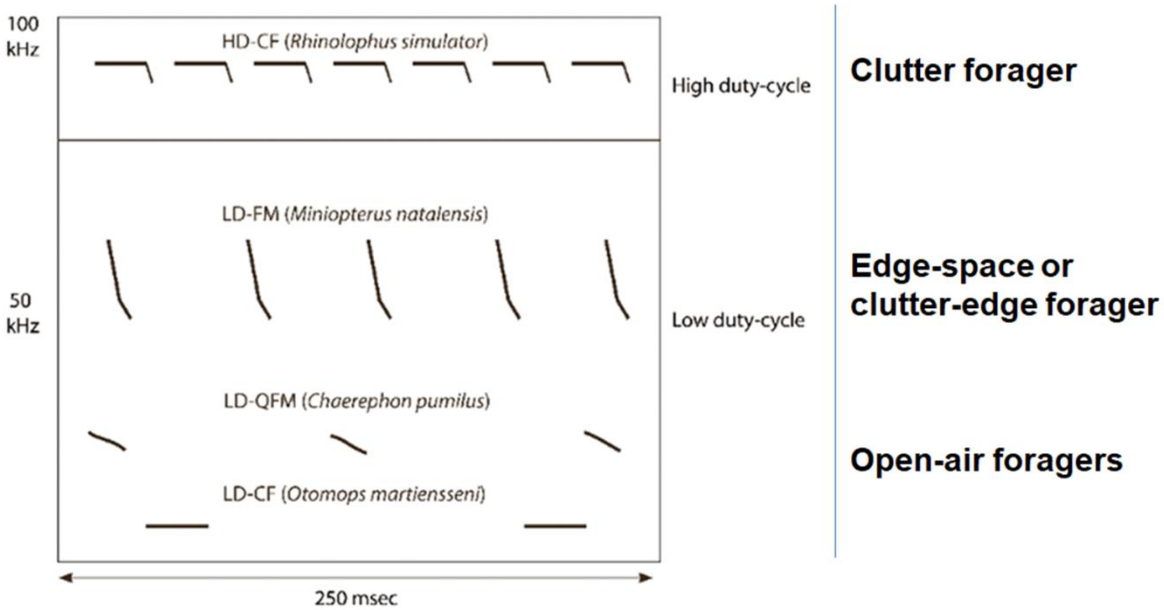

Figure 1.2. Graphical description of the frequency, duration of the main categories of echolocation calls for African chiropterans from Monadjem *et al.* (2010)

1.2.5. Echolocation parameters and foraging strategy

- **Clutter foragers** use calls with a constant frequency component, followed by a terminal, frequency modulated sweep (Schnitzler *et al.,* 2003). These tend to be high frequency, rapid, short duration calls. These calls are effective in closed and cluttered habitats as they provide a high resolution acoustic image over short distances (Fenton & Rautenbach, 1986; Siemers & Schnitzler, 2000) (Figure 1.2.).
- **Edge space foragers** use calls with a narrow band component and a steep frequency modulated (FM) sweep terminally (Schnitzler *et al.,* 2003). These calls are useful for finding fluttering insects in edge habitats (Neuweiler, 1984) (Figure 1.2.).
- **Open-air forager's** echolocation strategy is shallow frequency, narrow band, frequency modulated calls, and are the most effective for foraging in open spaces with minimal clutter (Neuweiler, 1984; Schnitzler *et al.,* 2003) (Figure 1.2.).

Body size imposes physical constraints on echolocation frequencies. Typically, smaller insectivorous bats use higher echolocation frequencies, while larger bats and aerial insectivores (open air foragers) tend to use lower frequency calls (Barclay & Brigham, 1991).

Intraspecific variation in echolocation calls may be due to variances in, sex (particularly in sexually dimorphic species), geographic location, habitat type, climatic conditions, regional bat assemblage composition, and prey diversity and availability (Barclay & Brigham, 2004; Murray, Britzke, & Robbins, 2001; Kearney, Keith, Markotter, Pretorius, & Seamark, 2019).

1.2.6. Landscape features

Keystone structures or habitats (vertical vegetation, linear landscape elements, rivers) have been shown to be important features in ecosystems as they create the structural diversity necessary for supporting an array of species within a habitat (Tews *et al.,* 2004). Bats may be attracted to topographic features and vertical landscape structures. They may use such features for commuting and as foraging habitats (Downs & Racey, 2006; Walsh & Harris, 1996; Worthington & Dickman, 2004). Bats frequently use edge and linear habitats during both commuting and foraging (Rydell, Entwistle, & Racey, 1996; Verboom & Spoelstra, 1999). Grasslands incorporate very limited vertical vegetation structure, consequently, smaller, less obvious, habitat features may play an important role as bats rarely commute through or forage over open grassland (Coleman & Barclay, 2013). There have been very few studies that have investigated spatial utilisation of grasslands by bats in the southern Africa. Kearney, Keith, Markotter, Pretorius, & Seamark (2019) suggest that caves, rocky crevices, riverine vegetation and anthropogenic structures are commonly used for roosting by bats in a grassland-dominated landscape.

1.2.7. Activity patterns in different habitats

In general, insectivorous bat activity patterns are a trade-off between prey availability and the risk of predation (Jones & Rydell, 1994). The activity patterns of bats are dictated by several factors; predation, prey abundance and availability, and bioclimatic conditions. Aerial insectivores are predominantly nocturnal, despite most insects being primarily diurnal (Erkert, 2000; Reed, 1958); Rydell *et al.,* 2014). The abundance and species diversity of arthropods differ relative to floral diversity and habitat type and, consequently, insects are not evenly distributed over landscapes (Schaffers, Raemakers, Sykora, & Ter Braak, 2008; Siemann, 1998). Bats tend to synchronise their emergence and foraging times with the activity patterns of their principle prey. Bats that prey on dipterans tend to emerge earlier in the evening when those species are most active, whereas bats that feed on lepidopterans emerge later, when their nocturnal prey emerges, or when predation risk has decreased (Fenton, Boyle, Harrison, & Oxley, 1977; Jones & Rydell, 1994; Rydell *et al.,* 1996). Frugivorous and nectarivorous bats are not reliant on peak arthropod abundance at dusk and may emerge later in the evening once predation pressure from diurnal predators has decreased (Jones & Rydell, 1994). Certain bat species forage before dusk, despite elevated threat of predation, to take advantage of abundant food resources including dipterans and winged termites (Pavey, Burwell, Grunwald, Marshall, & Neuweiler, 2001).

1.2.8. Effects of bioclimatic variables on bat species assemblages and activity

Lunar phase and bioclimatic variables such as rainfall, wind, temperature, barometric pressure and seasonality influence the activity patterns and species assemblages of bats, including those from southern Africa (Hayes, 1997; Jones *et al.,* 2009; Linden, Gaigher, Weterings, & Taylor, 2014; Paige, 1995; Taylor, Sowler, Schoeman, & Monadjem, 2013). Bats are small mammals with high metabolic rates and, consequently, have high energy demands (Erickson & West, 2002; Fenton *et al.,* 1977). Due to the high energy demands of foraging, prey availability plays a vital role in foraging success. Lower insect activity, linked to lower temperatures, may result in bats emerging from roost sites less frequently (Erickson & West, 2002). During cold periods, such as those typical of winter, lower bat species richness and abundance has been observed in other austral sites (Braun De Torrez, Wallrichs, Ober, & Mccleery, 2017; Gonsalves & Law, 2017). During cold periods or unfavourable conditions bats are known to migrate locally to more favourable roost sites or to remain in roosts (Lewis, 1995; Ortêncio-Filho, Lacher Jr, & Rodrigues, 2014). Local migrations to new roost sites by bats in temperate regions may be associated with changes in seasonality, breeding or species-specific factors (Mcguire & Boyle, 2013; Moussy *et al.,* 2012). For instance, *Miniopterus natalensis,* a South African species, is known to utilise caves as temporary roosts during maternity and whilst suckling their young (Pretorius, Van Cakenberghe, Broders, & Keith, 2020; Van Der Merwe, 1987). Bat migrations may be associated with a variety of factors including breeding and raising young, microclimate, predation levels and ectoparasite levels (Lewis, 1995). Bats tend to conserve energy by making use of night roosts for longer periods during cold or wet nights (Anthony, Stack, & Kunz, 1981). At low temperatures, bats use either homeothermic or heterothermic (torpid) strategies (Lewis, 1993). Consequently, bats need not forage when conditions are not ideal, and may curtail thermoregulatory activity when the net energy gain from foraging is low (Fenton *et al.,* 1977). Dietary breadth in insectivorous bats tends to increase during winter as insect prey is scarcer, therefore, bats may feed less selectively (Salinas-ramos, Montalvo, León-Regagnon, Arrizabalaga-Escudero, & Clare, 2015).

Insect activity and therefore bat foraging activity are negatively correlated with atmospheric pressure (Johnson, Gates, & Zegre, 2011; Paige, 1995; Turbill, 2008) It is speculated that bats use barometric pressure and changes thereof, as means of predicting the relative abundance of nocturnal flying insects beyond the roost (Paige, 1995). By ‘tracking’ barometric pressure from the roost and under perceived unfavourable conditions such as high barometric pressure, bats may implement torpor as an energy saving mechanism (Paige, 1995).

There is much debate related to the influence of lunar phase on bats (Mushabati, Eiseb, Benda, & Laverty, 2022). Observed behavioural changes include foraging closer to or in more cluttered habitats during phases of high lunar illumination (Fenton *et al.,* 1977). This is thought to be a behavioural response to reduce predation by visual predators, including various species of owls, that may be able to hunt more efficiently during periods of higher illumination (Meyer *et al.,* 2004; Rydell & Speakman, 1995). Insects reduce their activity during periods with high levels of lunar illumination, which may be a response to an increased threat of predation by aerial insectivores such as the night jars (Caprimulgidae) (Meyer, Schwarz, & Fahr, 2004). Certain South African bat species (*Laephotis capensis*, *Nycticeinops schlieffeni* and *Scotophilus viridis*) seem to suppress activity when high levels of lunar illumination prevail (Fenton *et al.,* 1977), resulting in changes in observed assemblage composition (Linden *et al.,* 2014). Lunar phobia has been documented in African chiropterans (Linden *et al.,* 2014) and has been attributed to the increased risk of predation by visual hunters such as bat hawks (*Macheiramphus alcinus*) (Fenton *et al.,* 1994, 1977). Over 20 species of diurnal birds of prey in southern Africa are known to prey on bats, with predation typically occurring during emergence from roost sites and during periods of high lunar illumination (Fenton *et al.,* 1994, 1977). In the context of African savannas, clutter foragers and edge-space/clutter-edge species appear to be the most affected by periods of increased lunar illumination (Meyer *et al.,* 2004). Large, fast-flying, open air foragers may be less susceptible to predation and may forage in brighter conditions than smaller, slower-flying species (Rydell *et al.,* 1996). Bats commute and forage less frequently over open habitats and above tree canopies during periods of high lunar illumination, during these periods they forage closer to vegetative cover and in less exposed habitats (Fenton *et al.,* 1977; Pretorius, Van Cakenberghe, Broders, & Keith, 2020; Verboom & Spoelstra, 1999).

1.2.9. Grasslands

Grasslands are productive systems and, when intact, are associated with a diverse range of fauna and flora (Egoh *et al.,* 2011). They are comprised, predominantly, of grasses (family Poaceae), and are maintained through various ecological processes including fire, frost and grazing (Mucina & Rutherford, 2006). Grasslands cover approximately 17% of South Africa's land area and lie centrally and towards the east of the country. Grasslands worldwide are threatened with transformation and overutilization (Parr, Lehmann, Bond, Hoffmann, & Andersen, 2014). Poor land use practices have damaged and altered grasslands, causing invasions by exotic organisms, soil erosion as a result of overgrazing, and reduced water quality due to mining waste (Neke & Du Plessis, 2004). In South Africa grasslands are in great need of conservation (Cadman, de Villiers, Lechmere-Oertel, & McCulloch, 2013; Cowling *et al.,* 2004; Egoh *et al.,* 2009). Over 35% of South African grassland habitats have been transformed for land uses such as cultivation and urbanisation (Egoh *et al.,* 2011). Increasingly, it is becoming necessary to monitor the effects of climate change on a broad spectrum of taxa to identify and classify threats and to develop conservation strategies and solutions (Stahlschmidt & Bruhl, 2012; Zukal, Pikula, & Bandouchova, 2015). Worldwide grasslands seem to be somewhat inhospitable to most open-air bat species. This is likely due to several factors including predation risk and susceptibility to environmental factors that affect foraging, ie. lunar illumination or high wind speeds (Fenton *et al.,* 1977). Where grasslands support diverse bat assemblages these habitats support resources that bats are able to utilise to enhance their survival and improve their fitness (Chung-MacCoubrey, 1996; Coleman & Barclay, 2013; Hodgkison, Balding, Zubaid, & Kunz, 2004; Limpens & Kapteyn, 1991).

1.2.10. Effect of ungulates on grassland assemblages

Ungulates are integral to the functioning of grassland systems. They stimulate grassland productivity, influence plant species composition and distribution through selective grazing and, seed dispersal, and they increase grassland responsiveness to altered precipitation regimes (Koerner & Collins, 2014; Mcnaughton, 1979; Miller, 1996). Ungulates affect soil mineralisation and microbial activity through the actions of trampling, urine and dung deposition and decomposition following mortality (Collins, 1970; Schrama *et al.,* 2012). Upon mortality, ungulates are subjected to scavenging and invertebrate decomposition. The presence of diverse herbivore assemblages in grasslands maintains a higher niche diversity for other organisms (including arthropod detritivores) (du Toit & Cumming, 1999). Various invertebrate decomposers, including dipterans and coleopterans, contribute substantially to the diets of South African bats (Aldridge & Rautenbach, 1987). It is possible that bats might gravitate to localised areas of dipteran abundance that result from carcasses as bats forage opportunistically on abundant prey resources (Meyer, Schwarz, & Fahr, 2004; Naidoo, Mackey, & Schoeman, 2011; Pavey *et al.,* 2001). Certain bats, notably *Myotis* species, are opportunistic feeders, taking advantage of clustered food resources (Vaughan, Jones, & Harris, 1996). Dipterans are associated with early carcass decomposition and the removal of soft tissue, while coleopterans are associated with breakdown of keratinous remains later in the decomposition process (Coe, 1978). Next generation high throughput DNA sequencing of insect remains recovered from bat guano of several African bat species, show Coleoptera and Diptera to be important prey resources (Bohmann *et al.,* 2011; Taylor, Bohmann, *et al.,* 2013).

1.2.11. Bats as bio-indicators

One method of assessing the effects of climate change and ecosystem health is through the use of bio-indicators (Jones *et al.,* 2009; Zukal, Pikula, & Bandouchova, 2015). Bio-indicators are species whose populations or activity are affected by a number of environmental factors and as such can be used as a representation of overall ecosystem health (Jones *et al.,* 2009). Bats display a number of characteristics that make them effective bio-indicators; they are variable in size, diet, mobility, longevity and morphology. They also exhibit low reproductive output and can be observed over both the short and long term (Kasso & Balakrishnan, 2013; Racey & Entwistle, 2000). Bats are sensitive to environmental stressors and populations react to changes within their immediate environment (Stahlschmidt & Bruhl, 2012). Changes in bat populations and activity patterns may be related to pollution, water quality, changes in climate and agricultural intensity as well as loss or fragmentation of natural habitats (Jones *et al.,* 2009). Insectivorous bats occupy a high trophic level and are useful indicators of bioaccumulation of heavy metals associated with mining or wastewater treatment (Naidoo, Vosloo, & Schoeman, 2016; Zocche *et al.,* 2010; Zukal, Pikula, & Bandouchova, 2015). They are particularly impacted by the use of pesticides through bioaccumulation (Jones *et al.,* 2009; Kasso & Balakrishnan, 2013). Arthropod populations affected by toxins and pesticides and changes in insect abundance will likely influence bat abundance (Jones *et al.,* 2009). Furthermore, bats are a diverse mammalian group and their species diversity is impacted by habitat changes and anthropogenic activities (Avila-Flores & Fenton, 2005; Kasso & Balakrishnan, 2013). Bats act as climatic and ecosystem indicators as changes in climate, water quality, habitat quality and pesticides induce changes in bat populations and activity (Jones *et al.,* 2009; Kasso & Balakrishnan, 2013).

1.3. Motivation and Rationale

There is a general paucity of data on the bat assemblages of grassland dominated environments in South Africa. Telperion Nature Reserve is a grassland dominated conservation area with scattered clumps of *Burkea africana* and high numbers of large African ungulates such as; eland (*Taurotragus oryx*), blue wildebeest (*Connochaetes taurinus*), Burchell’s zebra (*Equus quagga burchelli)*, greater kudu (*Tragelaphus strepsiceros*) and giraffe (*Giraffa giraffa*). Bats are more abundant in the northern and eastern parts of South Africa, however the diverse environments (rocky outcrops, river cliffs, anthropogenic buildings) present on the Telperion Nature Reserve reserve may provide suitable habitat for a variety of bat species from several functional guilds. Chiropteran research previously conducted on this reserve was limited in scope (22 individuals actively captured, all within the family Vespertilionidae) and the sampling duration did not include seasonal or habitat specific sampling (Kearney, Keith, Markotter, Pretorius, & Seamark, 2019). An unpublished acoustic study was also undertaken by Greyling and Keith (2013) in which 10 species of insectivorous bats were identified on the reserve. Prior to this study, the assemblage of insectivorous bats on Telperion Nature Reserve was thought to comprise 13 species (Greyling & Keith, 2013; Kearney *et al.,* 2019).

Of the numerous studies conducted on South African bat assemblages, very few have attempted to investigate the effects of season in relation to habitat type and its effect on bat assemblages. Similarly, little is known about the influence of bioclimatic variables (apart from temperature and altitude) on bat species richness and activity, in the southern African and grassland context.

Lastly, it is not documented that insectivorous bats utilise mammalian carcass sites (and their carcass associated arthropods) as a foraging resource patch, as there are no publications associating chiropterans and carcass sites.

1.4. Research Aim and Objectives

General study aim: To investigate the bat assemblage present in a grassland dominated landscape and determine the effects of habitat, seasonality, bioclimatic variables, and the presence of carcasses on chiropteran species richness, diversity, relative abundance and activity.

General study objectives:

- Develop a comprehensive, site specific, species inventory though active capture (mist nets, harp traps, hand nets and active searches) and passive sampling (using stationary bat detectors).
- Develop a regional call library for the area that is inclusive of grassland-dominated landscapes.
- Determine the effects of habitat type (anthropogenic, vegetated rock outcrop, vegetated water course and savanna grassland matrix) on local bat species richness, diversity (functional and taxonomic), relative abundance and activity patterns.
- Determine whether there are temporal (seasonal) differences in bat species richness, diversity, abundance, and activity.
- Determine the effect of ungulate carcasses and their associated invertebrate decomposers on the relative abundance and species richness of bats in the vicinity of the carcasses.

1.5. Research Questions and Predictions

- *What is the nature of the bat assemblage present within a grassland habitat?*

I predict that there will be species overlap with savanna habitats, as predicted by Seamark, 2013).

- *Which feeding guilds do the grassland chiropteran assemblages fall into?*

I expect the majority of grassland bats will fall into the open and edge space foraging guilds.

- *Is any within species call variation or sexual dimorphism present within species?*

It is likely that there will be some variation in echolocation calls between individuals of the same species.

- *Does relative species abundance and diversity differ seasonally and among different habitat types?*

I predict that there will be lower abundance and diversity during the colder seasons as certain roost sites may be abandoned during the colder seasons.

- *Did the presence of carcasses in the landscape influence bat activity?*

I do not expect bats to respond behaviourally to the presence of carcasses in the landscape.

1.6. Thesis Structure

- Chapter One: General Introduction and Literature Review
- Chapter Two: Study Area
- Chapter Three: Materials and Methods
- Chapter Four: Species Inventory of Bats on the Telperion Nature Reserve.
- Chapter Five: The Effects of Habitat and Season on the Chiropteran Assemblage of the Telperion Nature Reserve.
- Chapter Six: The Effects of Carcass Sites on the Chiropteran Assemblage of the Telperion Nature Reserve.
- Chapter Seven: Discussion and Conclusions
- Chapter Eight: Summary of Findings and Recommendations
- References
- Appendices

CHAPTER TWO

STUDY AREA

2.1. Study Area

Telperion Nature Reserve (-25.693111, 29.001947) is located on the border between the Gauteng and Mpumalanga provinces in South Africa (Figure 2.1.). The reserve is situated between the towns of Bronkhorstspruit and Emalahleni (formerly Witbank). Together with the Ezemvelo Nature Reserve, the Telperion / Ezemvelo complex encompasses ca. 13 000 ha, of which Telperion Nature Reserve contributes 5600 ha (O'Donoghue, Slater, & Brown, 2020). Historically, the farm Telperion was used for crop farming (Maize, *Zea mays*; Sunflower, *Helianthus annuus*; Potatoes, *Solanum tuberosum*; Lucerne, *Medicago sativa*; Peaches, *Prunus persica;* groundnuts, *Arachis hypogaea;* and various fruiting trees) and thereafter for cattle (*Bos Taurus*) farming (Coetzee, Bredenkamp, Van Der Merwe, & Mostert, 2012). Remnants of the crop fields now manifest as grazing lawns on parts of the reserve (Coetzee *et al.,* 2012; Hamunyela, Parrini, Marshal, & Louw, 2017). The reserve is currently a privately owned and managed wildlife reserve, with a focus on nature conservation and ecological research. The reserve is bounded by the Ezemvelo nature reserve on the west and cattle ranching and crop farms on the southern and eastern boundaries (Coetzee *et al.,* 2012). The reserve is bordered on the northern and western boundaries by the Wilge River, a tributary of the Olifants River, which separates the Telperion Nature Reserve from Ezemvelo.

Figure 2.1. Telperion Nature Reserve is situated in the far north of the grassland region on the border of the Mpumalanga and Gauteng provinces in South Africa

2.2. Climate

The mean temperature on Telperion Nature Reserve is 21 °C, with the mean minimum and maximum being 3 °C and 28 °C respectively (Macfadyen, 2014; Mucina & Rutherford, 2006). The nearest weather station, for which long term temperature (Figure 2.2.) and rainfall (Figure 2.3.) data are available, is Emalahleni (approximately 40 km southeast of Telperion Nature Reserve). The rainfall in the region ranges between 650 and 700 mm annually, with the majority of rain falling during the austral summer months (October – March, Figure 2.3., Climate-data.org, 2021).

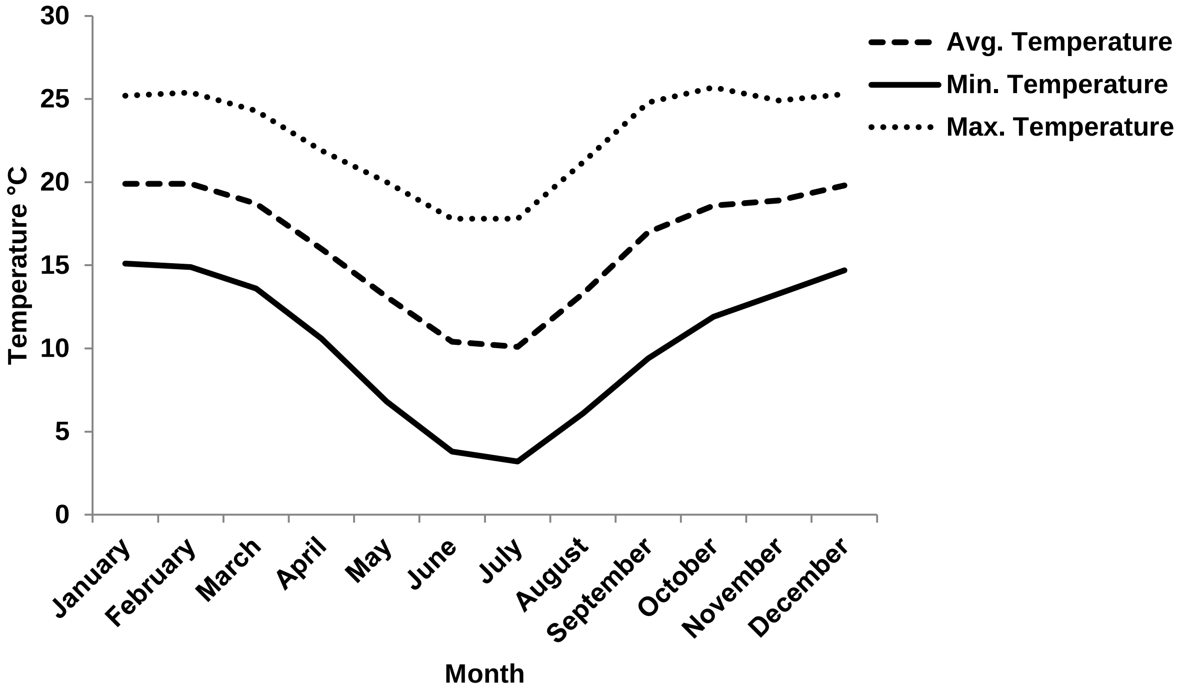

Figure 2.2. Monthly temperature variation (1999 to 2019) for the Emalahleni region of Mpumalanga, South Africa. (Climate-Data.org; URL: https://en.climate-data.org/africa/south-africa/mpumalanga/emalahleni-641/)

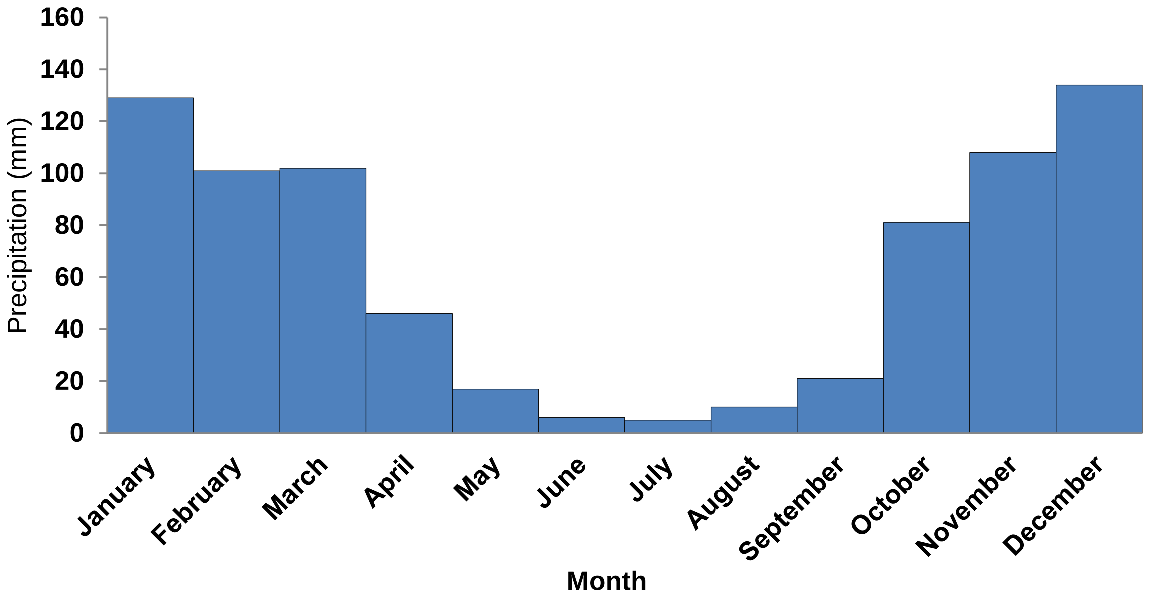

Figure 2.3. The mean monthly precipitation (1999 to 2019) for the Emalahleni region of Mpumalanga, South Africa. (Climate-Data.org; URL: https://en.climate-data.org/africa/south-africa/mpumalanga/emalahleni-641/)

2.3. Geology and land type

The geology of the reserve primarily comprise rocks of the Waterberg Group; the Waterberg sediments present on the reserve consist primarily of conglomerate, grit, sandstone, quartzite and shale (Swanepoel & Bredenkamp, 2006). Rocks of the Dwyka formation and Ecca group occur west of Telperion Nature Reserve, on the Ezemvelo reserve, however, none of the rocks from these formations are exposed in Telperion Nature Reserve (Figure 2.4.). The rocks of the area are medium to coarse-grained and their reddish colouration is a result of iron oxides (Swanepoel & Bredenkamp, 2006; Viljoen & Reimold, 1999). The mean altitude of the reserve is 1350 m above sea level.

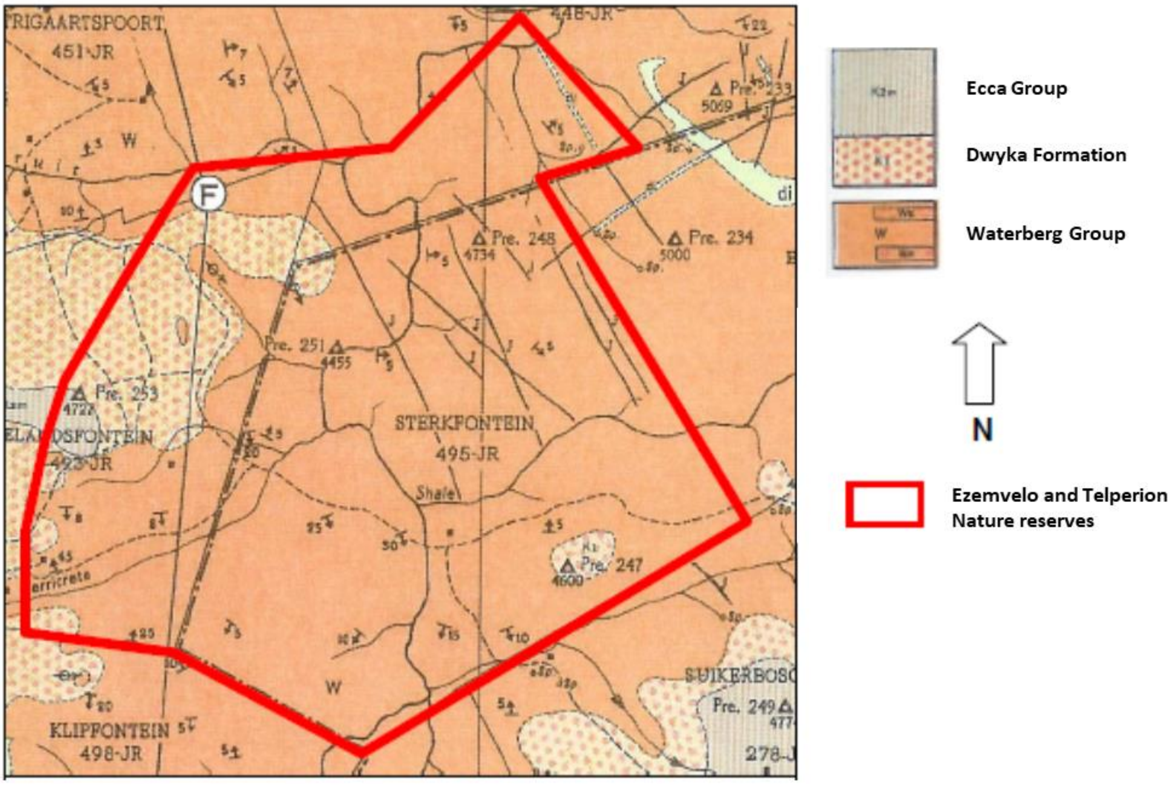

Figure 2.4. Geology of the Telperion and Ezemvelo Nature reserve complex, (from 1:125 000, 2528D and 2529C geological maps, South Africa) (Swanepoel & Bredenkamp, 2006)

The land types on the reserve are defined as Ib and Bb (Coetzee, Bredenkamp, & Van Rooyen, 1995). The Ib land type is comprised, primarily, of sandstone boulders and shallow rocky soils, with a high proportion of exposed rock, in the northern and southern areas of the reserve (Coetzee *et al.,* 1995; Swanepoel & Bredenkamp, 2006). The Bb land type is comprised of sandy loam plinthic soils, which typically occur in the low-lying areas, central and towards the eastern portions of the reserve (Job *et al.,* 2019; Swanepoel & Bredenkamp, 2006).

2.4. Flora

The dominant vegetation type on the reserve is Rand Highveld Grassland, interspersed with Loskop Mountain Bushveld on the rocky outcrops (Mucina & Rutherford, 2006); this reserve falls into the broader Bankenveld vegetation type (Acocks, 1988; Brown, Magagula, & Barrett, 2022). Telperion Nature Reserve is situated in the northern reaches of the grassland biome and is particularly diverse being situated on an ecotone between the grassland and bushveld biomes (Acocks, 1988; Macfadyen, 2014). Much of the reserve is comprised of undulating grasslands. The vegetation delineation for Telperion Nature Reserve that was used for this study followed Coetzee *et al.,* (2012) and Macfadyen (2014). The vegetation consists of seven communities. Dominant woody species include; grey poplar (*Populus* X *canescens*), river bushwillow (*Combretum erythrophyllum*), hook-thorn (*Senegalia caffra*), white stinkwood (*Celtis africana*), lavender tree (*Heteropyxis natalensis*), wild syringa (*Burkea africana*), mermaid tree (*Ochna pulchra)* and lavender croton (*Croton gratissimus*). More recently 22 plant communities were described, with five of these being primary communities widespread on the reserve (Brown *et al.* 2022). The primary plant communities are; *Combretum molle–Englerophytum magalismontanum* rocky ridge woodland*, Eragrostis curvula–Seriphium plumosum* midslope plateau grassland, *Diospyros lycioides–Combretum erythrophyllum* riparian woodland, *Paspalum urvillei–Phragmites australis* valley bottom wetland and *Cyathea dregei–Ilex mitis* ravine woodland (Brown *et al.* 2022). Several (14) sub-communities are also present (Brown *et al.,* 2022). The reserve has diverse botanical assemblages comprising 121 tree species, 207 wildflower species and 84 species of grasses (MacFadyen 2019, pers comm)*[1]. The reserve encompasses a wide variety of microhabitats of both natural and anthropogenic origin, (dams, rocky outcrops, riparian, wetlands, vegetated rocky outcrops, open grasslands, tree-grassland mosaic) throughout the reserve resulting in a heterogenous landscape. The areas along the watercourses are heavily vegetated (pers. obs.).The natural vegetation on the reserve is substantially modified by invasive species including, grey poplar (*Populus* X *canescens*) and black wattle (*Acacia mearnsii*). Portions of the grasslands on the reserve are heavily invaded by an indigenous woody shrub: bankrupt bush (*Seriphium plumosum*) (Graham, Barrett, & Brown, 2020).

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[1] Dr Duncan MacFadyen - Head of research and conservation for E Oppenheimer & Son and then Oppenheimer Generations, who oversees Management of Telperion Nature Reserve. Dr MacFadyen conducted his Doctoral ecological study on terrestrial small mammals of Telperion Nature Reserve.

2.5. Fauna

The reserve supports a diverse faunal assemblage, comprising 75 species of mammals (excluding chiropterans), 370 species of birds and 40 species of reptiles (MacFadyen,. 2019, pers comm)[1]. Predators, including leopard (*Panthera pardus*), brown hyena (*Hyaena brunnea*), caracal (*Caracal caracal*) and black-backed Jackal (*Lupulella mesomelas*) are present on the reserve. Several antelope species, ranging in size from the steenbok (*Raphicerus campestris*) to the large common eland (*Taurotragus oryx*) are found on the reserve. Being predominantly grassland, the reserve is suited to grazers, however, browsers such as kudu (*Tragelaphus strepsiceros*) and southern giraffe (*Giraffa giraffa*) also occur on the reserve. A number of terrestrial small mammal species (14 species from three orders; Eulipotyphla, Macroscelidea and Rodentia) (Gumbi *et al.,* 2018; MacFadyen, 2014) have been documented. Prior to this study, the chiropteran assemblage on the reserve were thought to be represented by 13 species from various families, including Emballonuridae, Miniopteridae, Molossidae, Rhinolophidae and Vespertilionidae (Greyling & Keith, 2013; Kearney *et al.,* 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Ethical clearance and permitting

All active sampling, for the purposes of live-capture of bats, was conducted in accordance with international (Sikes and Animal Care and Use Committee of the American Society of Mammalogists) (Sikes, 2016) (ASM hereafter) and national (SANS 10386, South African Bureau of Standards 2008) prescribed guidelines. In addition, the Durban Natural Science Museum animal handling standard operating procedures, inclusive of Section 20 of the Animal Disease Act: 1984, were followed. Section 20 authorisation (reference number: 12/11/1/12 (1714AC), was obtained for the latter portion (following COVID 19 regulations) of the study. The capture and handling of study animals was strictly on a catch, measure, and release basis, with animals released at their site of capture within two hours of being captured. This study was approved by the animal ethics committee of UNISA (2018/CAES/119) (Appendix 1: Figure 1.1.) and registered under project registration REC-170616-51. Permits for capture and acoustic sampling were obtained for Gauteng (CPF6 0213, CPF6 0222) (Appendix 1: Figure 1.2.) and Mpumalanga (MPB 5640, MPB 5677) (Appendix 1: Figure 1.3.) provinces. Private landowner permission was also obtained (authorisation numbers: LOP20191028, LOP20200403, LOP20210121) (Appendix 1: Figure 1.4.).

3.2. Site selection

For the purpose of this study, a habitat is defined as the environmental area (biotic and abiotic factors included) in which the species of interest lives, travels and forages (Hall, Krausman, & Morrison, 1997).

In general, bats may more frequently use linear landscape features, vegetated areas (as opposed to open habitats), rocky outcrop sites, and anthropogenic habitats for foraging, traversing and roosting (Avila-Flores & Fenton, 2005; Chung-MacCoubrey, 1996; Hagen & Sabo, 2011; Limpens & Kapteyn, 1991; Walsh & Harris, 1996). Consequently, the presence of these landscape features guided the selection of habitat specific sites for seasonal sampling (see below). Carcass and control sites (used in the carcass decomposition component of the study and discussed below) were all in open grassland habitats as the carcass component was a part of a larger carcass decomposition study.

The habitat requirements of bats vary greatly within the order, as a broad number of foraging strategies, habitat types and roost sites are used by insectivorous bats. However, there are certain elements (linear landscape elements, vegetation structure, roost and foraging sites) of a landscape that make it more attractive to particular species of bats. Structural cues to were used to guide site selection, sites that were perceived to influence bat activity (linear landscape elements, foraging sites, roost sites or relatively safe commuting routes) (Figure 3.1.) were chosen for seasonal sampling. The criteria that were identified as important for bats were, presence / proximity of anthropogenic structures, vegetated water courses, tree-grass interface, and vegetated rocky outcrops (Figure 3.1.). Four sites within each habitat type (16 sample sites) were selected. Sites that could be attributed to multiple habitat types (e.g. a rocky outcrop directly adjacent to a river) were avoided during site selection. The selection criteria for the specific habitat sites were:

- *Anthropogenic sites*: Any man-made structure that would provide adequate roost habitats and may or may not be fitted with artificial lighting (which attracts prey) and may or may not be permanently occupied by people.
- *Water course*: Any water source with permanent flowing water, typically with surrounding aquatic and terrestrial vegetation such as reeds and trees. On the reserve the main watercourse is the Wilge river and three of the watercourse sites were stationed along this river.
- *Tree grass interface/matrix*: were similar to a savanna habitat and were categorised by the presence of large clumps (over 0.5 ha in area) of indigenous *Burkea africana* trees in open grasslands. *Burkea africana* is one of the dominant tree species on the reserve (Brown *et al.,* 2022). No sampling was undertaken in the proximity of clumps of invasive trees.
- *Rocky outcrops*: were categorised by the presence of a large vegetated koppie (rock clump) with exposed rocks and crevices as well as associated vegetation between the rock clusters.

Figure 3.1. Habitat delineation used for assessing habitat selection by bats on Telperion Nature Reserve from January 2019 to April 2020

3.3. Passive acoustic sampling

Passive sampling was conducted seasonally between January 2019 and January 2020. Passive acoustic monitoring was conducted using Wildlife Acoustics® SongMeter 2 (SM2Bat+; n = 1) and SongMeter 4 Full Spectrum (SM4BAT FS; n = 3) ultrasonic recorders. The four bat detectors were set to simultaneously record at separate independent sample locations in each of the four habitat types (anthropogenic, water course, tree grass matrix, and rock outcrop) each season (Detector settings: Appendix 2: Table 3.1.). Four independent sampling stations were identified and monitored in each of the four habitat types, (16 acoustic sampling sites per season). The detectors were programmed to record for the entirety of the bat activity period: 30 minutes before sunset until 30 minutes after sunrise (Richardson, Lintott, Hosken, & Mathews, 2019).

Pseudoreplication in acoustic monitoring studies can influence abundance and activity estimates within and among habitats (Johnson *et al.,* 2011; Kingston, 2016; Wickramasinghe, Harris, Jones, & Vaughan, 2003). The Telperion Nature Reserve is a relatively small reserve and sampling constraints were imposed by both topography (restricted access to certain areas of the reserve) and the chosen study area. The distances that bats move across their habitat are species-specific and can vary from 1.1 km (*Nycteris thebaica*) to > 4 km for larger molossid species (Lehmkuhl, Dabelsteen, Bohmann, & Monadjem, 2012; Monadjem, Reside, Cornut, & Perrin, 2009). To minimise the possibility of recording the same bat during the same time interval on multiple detectors, a minimum distance of 500 m was maintained between all passive monitoring and acoustic sampling stations (Moir *et al.,* 2020a). This spatial separation was maintained for all sample sites, except for one river site where topography made it impossible to maintain this spatial criterion and remain within the specific riverine habitat type (Figure 3.2.). The distance between these two sampling sites was still >300 m (Kingston, 2016; Moir *et al.,* 2020a). This distance may be deemed suitable as its exceeds that of the ultrasonic recording device detection distance (20 – 30 m) for molossids (Monadjem, Shapiro, Mtsetfwa, Reside, & McCleery, 2017) albeit assessed for a different make and model of ultrasonic recording device. Detection distance and applicable correction factors are presently not available for Wildlife Acoustic ultrasonic bat detectors; as such, no attempts were made to applying correction factors published in Monadjem *et al.,* (2017).

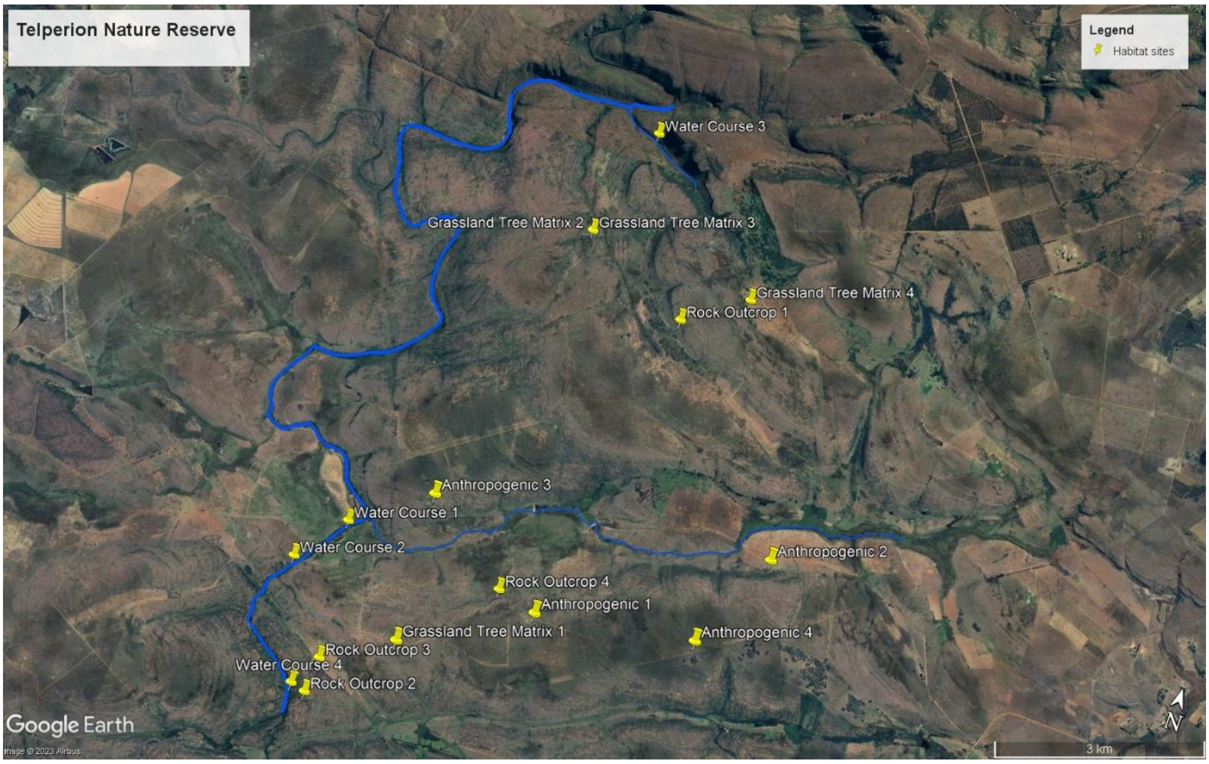

Figure 3.2. Satellite imagery identifying the anthropogenic, watercourse, rock outcrop and tree-grass matrix habitats sampled seasonally for Chiroptera on the Telperion Nature Reserve between January 2019 and April 2020. Map showing location of the Telperion Nature reserve, Mpumalanga, South Africa (2023), Google Earth, earth.google.com/web/

Omnidirectional ultrasonic microphones, connected to the bat detectors, were deployed at each sample location at a height of 2 m above the ground. During seasonal habitat specific sampling, the microphones were either attached to telescopic graphite poles or existing vegetation. Where microphones were deployed on branches, they were positioned to maximise recording space and to minimise obstruction from branches and leaves (pointed into open space). During the passive acoustic sampling at carcass locations, the detectors were positioned 2 m to the North of each carcass or control site. For the carcass experiment, recording microphones were always deployed on graphite telescopic poles (set to a height of 2 m). To prevent water ingress, while deployed, the microphones were oriented such that they pointed downwards at 45 degrees relative to the horizontal (Berry, Will, & Holderied, 2019). The microphones were always pointed away from thick clutter towards open areas or potential flyways. The ultrasonic, omnidirectional microphones sensitivity was assessed using a Wildlife Acoustics® ultrasonic calibration tool to ensure microphone sensitivity levels were within required tolerances prior to all sampling sessions (Fischer, Stott, Law, Adams, & Forrester, 2009). Two consecutive nights of acoustic data were obtained from each sampling location (32 nights overall per season), thereafter the data were downloaded, and the detectors were rotated to another sampling location within a different habitat type. To obviate any potential recording biases owing to differences in ultrasonic recorder models, detectors were routinely switched between habitat types. Simultaneous sampling in each of the four habitat types mitigated the effects of temporal or climatic influences (Fischer *et al.,* 2009; Wickramasinghe *et al.,* 2003). This sampling regime was repeated seasonally.

3.4. Active sampling - habitat and season

Active sampling using mist nets, harp traps, and hand nets was conducted seasonally (carcass sampling and summer – January 2019, autumn – May 2019, winter – July 2019, spring – October 2019, autumn – April 2021). The last active sampling session was unfortunately postponed by a year due to COVID19, the acoustic sampling was unaffected by COVID 19. Sites that were predicted to support high levels of bat diversity, including, the four habitat types (anthropogenic, watercourse, tree-grass matrix and rock outcrop), were sampled. Every habitat type was sampled each season, however the number of active sampling nights varied based on sampling constraints, (e.g. high lunar illumination, >3 mm precipitation or high river flow) that reduced sampling effectiveness. Therefore, such habitats may have been sampled multiple times during a seasonal sampling period, however never on consecutive nights. To ensure that independence was maintained between active capture sites, only one site was sampled per evening, active capture sites were ≥400 m apart and the same sites were not sampled on consecutive evenings (Krusic, Yamasaki, Neefus, & Pekins, 1996).

The most effective capture methods, according to literature, for the particular habitat types or sampling conditions were used (for instance, during high winds mist nets were not used, and at anthropogenic roosts harp traps were used). Active sampling using mist nets was conducted in each habitat type with Ecotone® monofilament mist nets (14 mm aperture, four bank) varying in length from 3 - 18 meters (site dependent). Shorter poles (2.5 - 3 m) were used most frequently, however, longer poles (3 - 5 m) were used within riverine habitats to provide adequate mist net clearance (≈300 mm) above the water surface and the proximate riparian vegetation. The longer poles were used on nets erected over rivers to ensure that any bat captures in the bottom bank of the net would be suspended above the water. Four Ecotone® monofilament mist nets were erected each evening during the capture sessions (for ≥ 6 nights per season). The net length and height of the poles used was dictated by site specific constraints, such as tall grass, aquatic vegetation or thick clutter that prevented long nets from being erected. When conditions proved unfavourable for mist netting (high winds or precipitation), the site was resampled within that season. In general, mist netting was undertaken in periods as close to new moon as possible (usually 3 days before, including, and 3 days after new moon), as bats avoid nets under higher levels of nocturnal illumination (lunar or anthropogenic), which influences and, potentially biases, capture success

(Hoyos-Díaz, Villalba-Alemán, Ramoni-Perazzi, & Muñoz-Romo, 2018). Nets were erected one hour before sunset, opened after the first flying bat had been observed and left open for 4 hours after sunset. The mist nets were checked every 10 - 15 minutes. Mist netting effort was quantified as mist net meter hours (net area (m^2)*sampling time (hours)) (Klingbeil & Willig, 2010; Moir, Richards, Rambau, Ramugondo, & Cherry, 2020b). Capture efficacy is expressed as captures/mist net meter hour, therefore any differences in sampling effort between habitat types could be evaluated (MacSwiney, Clarke, & Racey, 2008; Silkey, Nur, & Geupel, 1999).

Harp traps are designed to capture bats where they fly through narrow flight corridors such as trails or streams or through other confined areas, such as the entrances to caves and around buildings (Francis, 1989). Two Austbat™ three-bank monofilament harp traps (4.2 x 1.8 m, 3 bank, 0.35 mm nylon lines) were used to sample roof roosting bat colonies at four anthropogenic sites. On Telperion Nature Reserve, harp trapping was only effective around anthropogenic structures and in thick riverine vegetation habitats as the small trap surface area does not lend itself to sampling of large open habitats or tree-grass matrix sites. Harp trapping effort was quantified by calculating the number of hours for which the harp traps were left erected and set to allow for capture of bats (Harp trap hours (hth)) (Kingston, 2016). Harp traps were left erected from the time of first flight until four hours after sunset; traps were monitored every 10 – 15 minutes.

Hand nets were used to capture bats in night roosts associated with anthropogenic structures. Bioquip Professional Series Insect Nets (composed of fine nylon mesh (10.8 x 10.8 mesh/ cm^2, 450 mm mouth diameter)) were used for hand netting of bats on Telperion Nature Reserve. Hand netting sites were identified as any anthropogenic structure that conformed to the following criteria, 1) were not frequently disturbed by human activities, 2) bats could gain access and 3) had sufficiently rough roofs or walls to allow for roosting of bats. Hand netting sites were identified, during active searches of potential night roosts, by the presence of insect fragments and guano. These sites were checked four times per sampling night (once per hour) when sampling around anthropogenic sites. Hand netting effort was recorded as the amount of time (minutes) spent conducting active searches within and around the anthropogenic sites, and was conducted as a separate capture technique in addition to other active sampling methods.

3.5. Bat processing

After capture in nets or harp traps, bats were disentangled from the nets (in the case of mist nets and hand nets) or removed from the collection bag (in the case of harp traps, and transferred to cotton cloth bags or collapsible mesh cages (nycterid and rhinolophoid species) (each captive bat was kept in its own cotton bag or mesh cage). All personnel who handled bats had been appropriately trained in relation to the handling of bats and had been appropriately inoculated against rabies. Researchers were equipped with, and wore, appropriate PPE (nitrile gloves, N95 masks, face shields, bite-proof gloves) when handling bats. Holding bags (15 x 20 cm) and collapsible mesh cages (40 x 40 x 50 cm) were all decontaminated after each use with F10 disinfectant (O' Farrell & Gannon, 1999).

Biological data that were recorded (Appendix 1: Figure 1.5.) from captured bats included: Sex (male, female), Reproductive status (males: testes abdominal, testes semi-scrotal or testes scrotal; females: vagina imperforate, vagina perforate, pregnant, lactating, post-lactating), Relative age (free-flying sub-adult or adult). The relative age was determined based on the degree of ossification of the phalangeal joints and from forearm length measurements (Kunz & Anthony, 1982). Morphological metrics that were recorded included: Forearm length (to the nearest 0.1 mm), Total mass (g, measured using Pesola® spring balance), Nose-leaf width (To the nearest 0.1 mm, in the case of rhinolophid bats). Morphological measurements were taken using a high-quality digital caliper (Mitutoyo Series 500 Absolute Digimatic Caliper).

3.6. Wing morphology

To calculate wing area for each bat that was captured during this study, the Norberg and Rayner (1987) method was applied. The bat was held (laid on its venter) against laminated graph paper (2 x 2 mm grid squares), with the right wing extended and photographed from above, perpendicular to the outstretched wing and graph paper. A DSLR camera (Canon EOS 60D), lens (Canon EF 100mm f/2.8 Macro) and graph paper were used consistently (Figure 3.3.). The graph paper provided a scale, allowing for the calculation of wing area using the program ImageJ (Version 1.52a, Schneider, Rasband, & Eliceiri, 2012).

Figure 3.3. Measuring of bat wing morphology using 2 x 2mm graph paper and the program ImageJ

The following aspects of wing morphology; total wing area, hand wing area, arm wing area and wing tip index, were compared to animal mass to estimate wing loading (the weight of the bat (N)/ the wing area of the bat (m^2). The square root of the estimated wing loading gives an indication of likely flight speed and facilitates the inference of the foraging guild to which a bat species is associated (Norberg & Rayner, 1987) (Figure 3.4.). The sheet of laminated graph paper was sterilised with 70% ethanol after each bat was photographed.

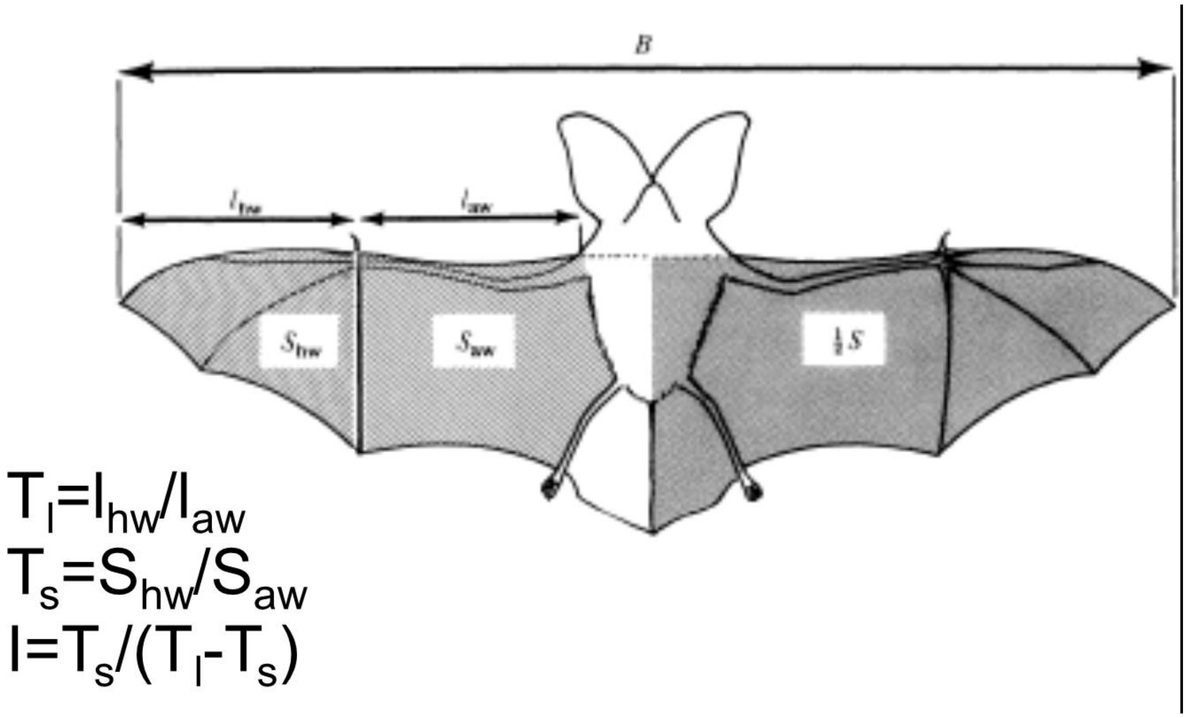

Figure 3.4. Diagrammatic representation of wing morphological characteristics used to describe bat wings (Norberg & Rayner, 1987)

B – Wingspan, length of extended wings as measured tip to tip.
S - Wing area, the wing area including the tail and body area, but not the head area
Aspect ratio (A), is the square of the wingspan divided by the wing area
Wing loading (Mg/S), weight divided by the wing area.
Hand-wing area (S_{hw}), the area of the wing distal of the 5th digit
Arm-wing area (S_{aw}), the area of the wing between the 5th digit an the legs and body
Hand-wing length (L_{hw}), the length from the tip of the outstretched wing to the 5th digit
Arm-wing length (L_{aw}), the length from the 5th digit to the body
Tip length ratio (Tl), the ratio of the length of the hand wing to the length of the arm wing.
Tip area ratio (T_s), the ratio of the hand wing area to the arm wing area.
Wingtip shape index (I), Large index values (<∞) indicate rounded to more rectangular wing tips, while smaller index values (≥1) indicates more triangular shaped wing tips. Diagram and explanation from Norberg & Rayner, (1987).

A 3 mm biopsy was taken from the wing membrane of all captured individuals, using a Kai Medical biopsy punch and following Wilmer & Barratt, (1996) (Figure 3.5.) with slight modification. The outstretched right wing of each animal was swabbed with 70 % ethanol, prior to biopsy collection. Care was taken to ensure that wing punch samples avoided wing strengthening fibres or blood vessels. The wing membrane (chiropatagium) and not the tail membrane (uropatagium) was biopsied for a genetic sample. Chiropatagia have a larger surface areas, and are less vascularised than uropatagia (Faure, Re, & Clare, 2009), and were, therefore, easier and safer to biopsy. The biopsied sites likely healed over a 3-6 week period (Weaver, Alfano, Kronquist, & Reeder, 2009); Figure 3.5.), however, the wing membrane remained discoloured, thus serving as permanent ‘marks’ on previously captured animals. Biopsied wing tissue samples were inactivated in DNAShield® and stored in 95% molecular grade ethanol for accessioning into the Durban Natural Science Museum specimen derivative bank. Data from previously captured animals were not recorded. The punch area served as a mark for previously captured animals, recaptures were not used in calculating capture success or release calls.

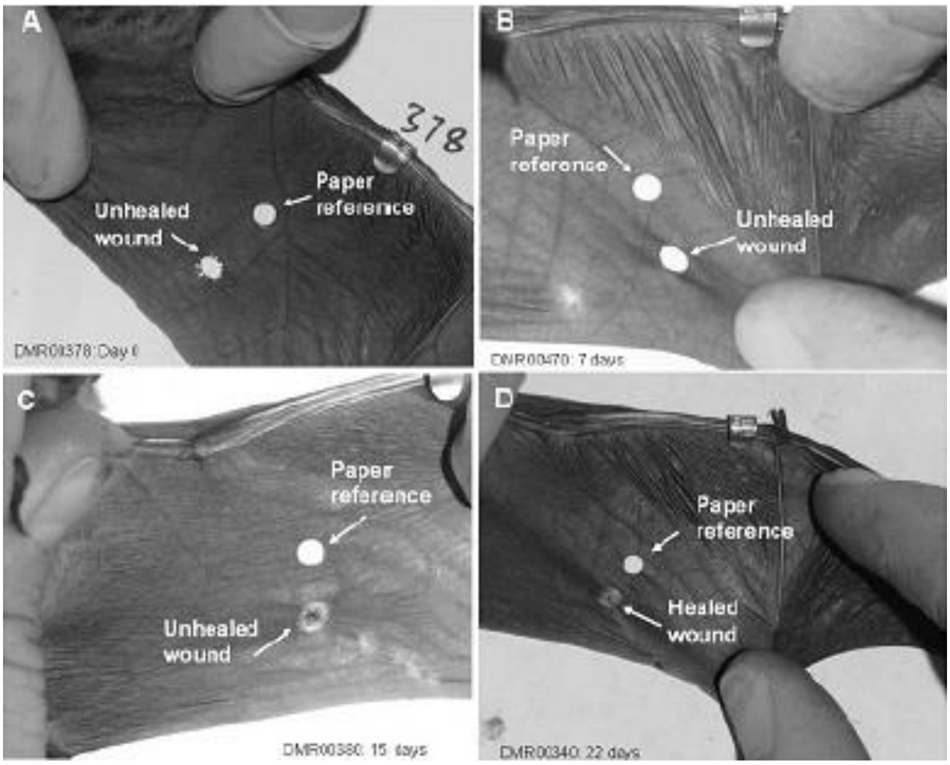

Figure 3.5. Healing of a bat wing subsequent to the collection (by biopsy) of a 3 mm genetic sample (Weaver *et al.,* 2009)

A- Immediately after genetic sample collection, B- After 7 days, C- After 15 days, D- After 22 days.

Genetic data are not reported in this dissertation; this method is included for completeness. The biopsy marks were used to identify individuals that had previously been captured during this study.

Bats that were captured were identified to species level using available identification matrices for South Africa (Monadjem, Taylor, Cotterill, & Schoeman, 2010; 2020). Following measuring and processing, the echolocation calls of hand-released bats were recorded using a Wildlife Acoustics EchoMeter 3+ (EM3+) handheld bat detector. The recordings of release calls contributed to the development of a regional call library for the species that occur on Telperion Nature Reserve.

Bats were released, one at a time, at the site of capture. In most cases prior to release the EM3+ was positioned 3 - 5 m away from the bat being released. However, it was necessary to hold the EM3+ detector 1 m from individuals of the species *Hipposideros caffer* and *Nycteris thebaica*, due to the rapidly attenuating and low intensity calls of both of these taxa (Monadjem *et al.,* 2017, 2020). Once suitably positioned, the microphone of the detector was aimed at the handheld bat and recording was initiated immediately prior to the bat being released. Contamination of release recordings by free flying bats was avoided by observing the live sonogram on the EM3+ detectors and releasing the bat of interest during lulls of bat activity, bats were also released later into the night after activity had subsided. Recording ceased once the echolocation calls of the free-flying bat were no longer detected (or observed on the screen of the EM3+) (Murray *et al.,* 2001).

3.7. Environmental monitoring and bioclimatic data

Rainfall has the potential to negatively affect data collection using bat detectors, as precipitation attenuates calls and reduces bat and insect activity (Lehmkuhl Noer *et al.,* 2012; Paige, 1995). Heavy rainfall was common in the summer (wet) season, consequently, acoustic data from any nights during which >3 mm of rainfall fell were discarded (Fischer *et al.,* 2009). In these circumstances (>3 mm of rainfall within a night), the sample sites were resampled the following evening. Microphone equipment was dried each morning following rain and before being deployed at other sample locations, as wet microphone socks or membranes reduce recorded call quality (Chenger & Tyburec, 2014).

Bioclimatic data were recorded every 10 minutes using a Kestrel® 5000 Environmental meter. The seven bioclimatic variables that were measured and recorded included: Temperature (°C), Relative Humidity (%), Barometric pressure (mb),), Wind Chill (°C), Dew Point temperature (°C), Wind Speed (M/s), and Density Altitude (M). Weather data were applied to passively recorded call data based on the nearest datum to the time of the bat pass (within 10 minutes) (Mushabati *et al.,* 2022).

3.8. Carcass experimental design

Bats utilise ephemeral patches of insect prey (Dechmann *et al.,* 2009; Egert-Berg *et al.,* 2018; Roeleke *et al.,* 2020). Bats may utilise carcass associated arthropods as ephemeral resource patches. Wildebeest (*Connochaetes taurinus*) carcasses were obtained from a management cull on Telperion Nature Reserve and used in this research project (Ethics approval UNISA (2018/CAES/119) project registration REC-170616-51 (Appendix 1: Figure 1.1.). Wildebeest carcasses were chosen as they represent a mid-sized ungulate and due to their high population numbers on the reserve. All ten blue wildebeest that were provisioned to this project were humanely killed with a single shot to the head to try to minimise arthropod ingress to the carcass via anthropogenic wounds. Five carcasses were deployed in steel mesh exclosure cages (2.5 x 1.6 x 0.7 m, 15 x 30 mm mesh) that excluded all vertebrate scavengers, five carcasses were pegged to the ground to ensure they remained in place despite scavenging activity. Five sites of similar vegetation structure and topography to those of the caged and pegged carcass sites, yet without carcasses, served as the control sites. Carcass sites were separated by a minimum of 500 m and all carcass sites were placed in open grasslands. The carcasses were deployed in two phases, phase 1 (20 January 2019) and phase 2 (5 February 2019) (separated by 14 days) to aid in logistics and data collection. Carcasses were placed in both caged and pegged configurations during both deployments to allow simultaneous comparisons. The carcasses were spread as uniformly across the reserve as logistically possible, access to the northwestern areas of the reserve was impractical due to a lack of access roads (Figure 3.6.). The carcass component of the experiment was conducted over mid-summer (January 2019 to February 2019), when insect abundance and carcass decomposition rates were likely to peak (Kelly, Van Der Linde, & Anderson, 2009).

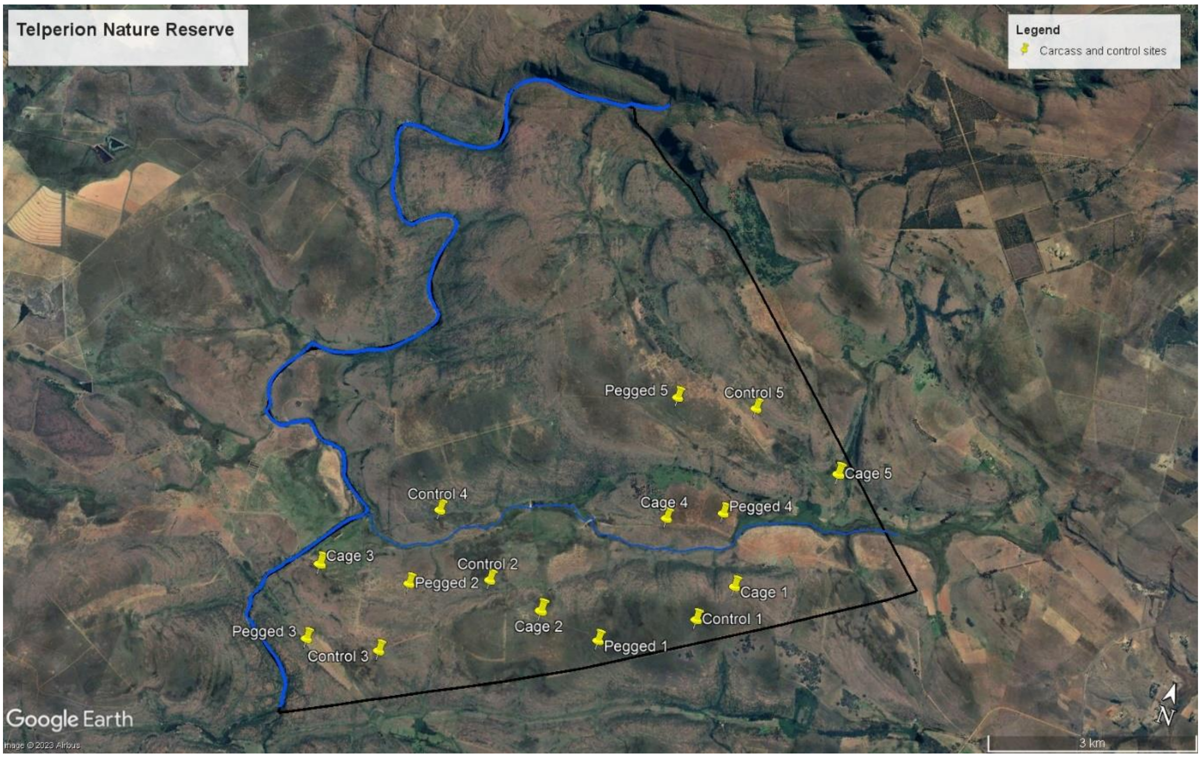

Figure 3.6. Satellite imagery showing the layout of pegged (exposed), caged (exclosure), and control (no carcass) sites on the Telperion Nature reserve between January and February 2019. Map showing location of the Telperion Nature reserve, Mpumalanga, South Africa (2023), Google Earth, earth.google.com/web/

Two passive recording bat detectors (a Wildlife Acoustics SongMeter 2 (SM2Bat+) and a SongMeter 4 Full Spectrum (SM4BAT FS) ultrasonic recorder) were used during the carcass component of the study. Both detectors were rotated through each sample site to ensure that all carcass sites had two nights of acoustic sampling with one night as an *in-situ*, pre-deployment control. Detectors were rotated throughout the sites (caged, pegged, and control) to minimise variability from inherent differences in sampling equipment (Fischer *et al.,* 2009). Ultrasonic microphones sensitivity was assessed using a Wildlife Acoustics calibration tool detector prior to deployment at the various sites. Acoustic recording was conducted for the entire night; 30 minutes before sunset until 30 minutes following sunrise (Richardson *et al.,* 2019).

The carcasses decomposed rapidly (two – three weeks), therefore only one night of acoustic recording was undertaken per site before the rotation of sampling equipment. A minimum of three independent nights (separated by at least three nights) of recording were obtained for every carcass site, while control sites had a detector stationed for a total of four different sampling nights. Sampling was conducted before (2 weeks) and during (4 weeks) the carcass deployment which allowed any within-site variation to be assessed and, if necessary, accounted for. The *in-situ* control data (before carcass placement) was compared to post carcass placement data and control site data to determine the effect of carcasses on bat activity. This design minimised within-site variation (Fischer *et al.,* 2009). Sampling was conducted at each of the 15 sites prior to carcass deployment, as an *in-situ* control.

A total of 50 nights (12 hours each) of acoustic data were recorded (600 hours of acoustic sampling total) across 3 treatment types (five caged carcass, five pegged carcasses and five control sites). Each carcass site was subject to three nights of acoustic sampling, one night was an *in situ* control (before carcass deployment) and two individual nights of acoustic sampling within 2 weeks following deployment, a maximum of two nights elapsed between consecutive recording nights at each carcass site. Control sites were subject to four nights of acoustic sampling before and during the carcass deployment.

3.9. Acoustic analyses

All acoustic data were analysed using Wildlife Acoustics Kaleidoscope Pro software (version 5.1.9g) using the ‘Bat Auto-Id’ function with the Bats of South Africa 5.1.0. classifier (Kaleidoscope recording settings: Appendix 2: Table 3.1.). Every call was manually vetted and identified to species level, due to limitations in the automatic identification tool not all species were represented in the call library classifier and calls often overlapped in parameters. Published call libraries of southern Africa were used to vet recorded calls (Moir *et al.,* 2020a; Monadjem *et al.,* 2017, 2020; Taylor, 1999; Taylor, Sowler, Schoeman, & Monadjem, 2013). Echolocation call parameters used for taxonomic identification were characteristic frequency (Fc), call duration (dur), maximum frequency (Fmax), and minimum frequency (Fmin). Other call parameters, including knee frequency (Fk) and bandwidth were detailed in the call library for each species. A minimum of three pulses with <1 second between consecutive pulses were regarded as a bat pass (Fenton, 1970; Hayes, 1997). The conditions for a bat pass were input into the trigger window settings on the detector and the signal detection parameters in Kaleidoscope Pro. Noise files were automatically identified and stored separately to call data using the noise analysis feature within the Kaleidoscope Pro analysis suite (Version 5.1.9g). A subset of noise files ≈ 25 % were manually screened for missed species, however, noise data contained < 0.2 % usable call data and were not analysed further. Noise files were analysed for usable call data in the same manner that hand released call files were analysed. As a result of the noise filter, good quality call files were uncommon in the separated noise data. Call sequences containing echoes, were manually identified and were not used in the call library or in analyses.

Echolocation calls of *Nycteris thebaica*, could not be analysed by Kaleidoscope Pro. As such, recordings of live captured animals (n = 2 animals with suitable recordings) were analysed using BatSound Pro, version 3.31b (Petterson Elektronik AB). Calls of *N. thebaica* were described by the following parameters: peak frequency (pFreq), and call duration (dur). No further parameters could be measured from the recordings.

An important and potentially confounding consideration is call overlap between bat species with similar echolocation parameters that share roosting or foraging sites. Until individual species are actively captured and their regional calls recorded and identified, it may be difficult to differentiate between species (particularly bats in the 19-20 kHz range) (Cory-Toussaint & Taylor, 2022).

3.10. Descriptive statistics and analytical approaches

The SPSS® Statistics software, version 26.0.0.0 (IMB Statistics 2019) was used to analyse the various data sets. During statistical analysis every dataset were tested for normality, non-parametric data were subject to the non-parametric tests (Kruskal-Wallis H Test, Independent-Samples Kruskal-Wallis Tests) and parametric data were subject to parametric tests (ANOVA). All the outcomes of all statistical analyses were evaluated at $\alpha \leq 0.05$.

3.11. Species inventory and regional call library

The call library developed from active captures was used in conjunction with published southern African call libraries to aid in the identification of bat calls recoded during passive acoustic monitoring at carcass and habitat sampling locations (Keraney *et al.,* 2019; Moir *et al.,* 2020a; Monadjem *et al.,* 2017, 2020; Taylor, 1999; Taylor, Sowler, Schoeman, Monadjem, 2013).

3.11.1. Echolocation call parameters

Call parameter data (Fc: characteristic frequency, Fmax: maximum frequency, Fmin: minimum frequency, Fk: frequency at knee of call and Duration: total duration of call), for each documented species, be they release calls from live captured animals or free-flights call, were calculated from all representative recorded call pulses. Sample sizes varied from 5-25 pulses, or as many pulses as feasible were used for call analysis, and were presented with mean values and standard deviations from the mean. Inter-sexual differences in echolocation call parameters were evaluated for species where ≥ 8 individuals were captured and ≥ 25 call pulses recorded. Sexual variation in call parameters was assessed using either Kolmogorov–Smirnov (KS) or Independent-Samples Kruskal-Wallis Tests based on whether the data were normally distributed (McDonald, 2014; Zar, 1999). This statistical approach was also used to assess differences in call parameters between *Rhinolophus* cf. *simulator* (R79) and *R*. *simulator* sensu stricto (s.s.).

3.11.2. Bat activity and activity indices

Bat activity was expressed as bat passes (see above) and bat passes per hour. The Miller acoustic activity index (AI) (Miller, 2001), was also calculated as follows:

$$\mathrm{AI} = \sum_{1}^{n} \mathrm{P}$$

The sum of one minute blocks in which the species of interest was detected (n) where the species was present (P), divided by the amount of effort expended during the survey gives you the acoustic index of the species of interest (AI) (Miller, 2001). Activity levels were reviewed and corrected activity levels were calculated using correction factors (after Monadjem *et al.,* 2017) for the species detected on the reserve.

Bat passes were also screened for “feeding buzzes”, defined as a sequence of pulses emitted in rapid succession during the final approach to capturing prey items (Simmons, Fenton, & Farrell, 1979). Feeding buzzes were expressed as total or cumulative counts, or reported as a proportion of bat passes.

3.11.3. Species diversity and accumulation curves

Chao 1 species richness estimates (Chao, 1987; Chao, Chazdon, Colwell, & Shen, 2004) and the Shannon-Wiener index (Magurran, 1988) of diversity were used to evaluate bat diversity on Telperion Nature Reserve. These metrics were applied to seasonal, habitat, and carcass data. The Chao 1 species richness estimate was used as the passively recorded acoustic data are abundance-based, not incidence-based (Chao, 1987). The variables used for the species estimates were the total number of calls per species, habitat type, and season. EstimateS 9.1.0 (Colwell, 2006; Colwell & Elsensohn, 2014) was used to determine the Chao 1 species richness, and the Shannon-Wiener diversity index values, and to compile species accumulation curves.

The terms ‘community’ and ‘assemblage’ were used to describe the collection of insectivorous bat species that frequented a particular area or habitat of interest. This may be used to describe the composition of bat species on smaller (microhabitat ie. water course) or larger spatial scales (reserve wide or grassland).

3.11.4. External morphological data

Species specific morphological data were recorded from captured individuals (see section 3.5 and 3.6 above). These data were presented as means and standard deviations for wing morphological data and other morphological metrics.

3.12. Habitat and Season Analyses

3.12.1. Functional diversity

The cluster analysis function in IBM® SPSS® Statistics software (version 26.0.0.0) was used to compute a dendrogram (using average linkage), to group species into functional foraging guilds based on their morphological characteristics and their habitat and roosting requirements (Moir *et al.,* 2020a). Factors used as input variables for cluster analysis were; forearm length, Fc, call type, roost sites used, foraging guild, wing aspect ratio, wing tip length ratio, wing tip area ratio, wing tip shape index and wing loading.

3.12.2. Functional group analyses

An ANOVA or Independent-Samples Kruskal-Wallis test was used to test whether functional groups, season, habitat type and a combination of these factors influenced the number of recorded bat passes. Following the ANOVA or Independent-Samples Kruskal-Wallis test, a Tukey Honestly Significantly Different (HSD) *post-hoc* test was used to assess where significant differences were present (Zar, 1999).

3.12.3. Species diversity

Chao 1 species richness, and the Shannon diversity indices were calculated using EstimateS 9.1.0 (Colwell, 2006; Colwell & Elsensohn, 2014). Data were sorted into seasonal or habitat data prior to richness estimates being run.

3.12.4. Bat activity

Bat activity (expressed as total bat passes per night and/or bat passes per hour per night) was assessed by habitat, site, and season. The Miller activity index (Miller, 2001), was calculated for both habitat and season. Statistical analyses were undertaken using relative abundance (total number of calls, as the sampling time per treatment was constant) for habitat and season, raw data were tested for normality using the Kolmogorov–Smirnov test. Independent-Samples Kruskal-Wallis Tests with adjusted significance values, using the Bonferroni correction, as multiple comparisons were performed using number of calls as the variable and season and habitat type as factors.

Species specific analyses were conducted across a seasonal and habitat gradient. The five species with the highest number of passes were analysed among seasons and habitats, as these data were likely to allow for the most rigorous statistical analyses (McDonald, 2014; Zar, 1999). Species specific data were tested for normality using a Kolmogorov-Smirnov test. The season and habitat specific activity of these species was then analysed using a Kruskal-Wallis test.

3.12.5. Effects of bioclimatic variables on bat activity

Call data were arranged into hour blocks to allow direct comparisons with bioclimatic data (recorded in 10 minute intervals) across nights (Mushabati *et al.,* 2022). A principal component analysis (PCA) based on a correlation matrix of the seven recorded variables (Peres-Neto & Jackson, 2001) was used to refine the set of bioclimatic variables and reduce collinearity amongst the variables. The principal components were named according to the groupings of variables that exhibited the highest factor loadings per respective principal component. Principal components were saved and treated as new bioclimatic variables. A Kolmogorov–Smirnov test was used to test whether bioclimatic data were normally distributed. A Spearman R test (Zar, 1999) was used to identify principal components that potentially influenced bat activity and species richness.

3.12.6. Species-specific activity

Species specific activity data were analysed for the five species with the most call files/ bat passes. The data were assessed for normality using a Kolmogorov–Smirnov test. A Kruskal-Wallis independent samples test was used to analyse species specific activity vs bioclimatic variables.

3.13. Carcass experimental analyses

3.13.1. Bat activity

Activity (calls per treatment per night) and number of feeding buzzes (FB) per treatment were calculated from carcass sampling acoustic data. The bat activity relative to each carcass treatment was calculated. Data normality was evaluated using a Kolmogorov–Smirnov test. Independent-Samples Kruskal-Wallis Tests were used as appropriate to assess whether differences existed between carcass treatments and control sites. Differences in the total number of calls and the number of feeding buzzes (indication of foraging) (Furlonger *et al.,* 1987) were investigated between carcass treatments. The percentage of calls that contained feeding buzzes was also calculated.

3.13.2. Species richness, diversity and functional groups

Species richness was presented as the number of documented taxa. The Chao 1 species richness (Chao, 1987; Chao & Chiu, 2016) and the Shannon diversity index (Shannon, 2001) were calculated for the carcass treatments and control sites. Indices were calculated using EstimateS 9.1.0 (Colwell, 2006; Colwell & Elsensohn, 2014).

A Kolmogorov–Smirnov (KS) test was used to test whether the call data of functional groups associated with carcasses were normally distributed. Where data were normal, an ANOVA was used, in the case of non-parametric equivalent (Kruskal–Wallis test) was used to determine whether any significant differences in activity or species richness existed between carcass sites.

CHAPTER FOUR

RESULTS

SPECIES INVENTORY, REGIONAL CALL LIBRARY AND CALL VARIATION

4.1. Sampling effort, species inventory and relative activity

A total of 22 species of bats from seven families were documented on Telperion Nature Reserve using passive sampling (acoustic sampling using bat detectors) methods in conjunction with a combination of active capture techniques (Table 4.1.). A rhinolophid bat, identified as *Rhinolophus* cf. *simulator*, bearing distinct echolocation characteristics (hereafter referred to as R79; see further details below), was captured on multiple occasions throughout the reserve.

A total of 128 acoustic sampling nights, from 16 sites, within four habitat types and encompassing four seasons, resulted in the identification of 21 species. Over the sampling period (January 2019 to January 2020), a total of 26133 bat passes ($\bar{X}$ = 217 ± 346, range = 2 – 2029 bat passes per night) were recorded.

Mist netting effort comprised 8654 mist net m^2 hours (including active sampling during the carcass component of this study). Harp trapping was undertaken for a total of 78 harp trap hours. An additional 12 hours of active searches and live captures using hand netting was conducted. A total of 167 bats representing 12 species were physically captured (Table 4.1.; mist netting = 10 species, harp trapping = 3 species, hand netting = 5 species). Capture success for mist netting was 0.01 bats / m^2 hour, 0.60 bats / hour for harp trapping and 0.83 bats / hour for active searches/hand netting.

Table 4.1. Bat species inventoried at Telperion Nature Reserve between January 2019 and January 2021. Sampling effort and captures per species are provided

Family and species	**Passive acoustic**	**Mist net**	**Harp trap**	**Hand net**	**Total captures per species**
Active capture effort	128 acoustic nights	8654 mist net hours	78 harp trap hours	12 hours	
Emballonuridae					
Taphozous mauritianus	*				
Hipposideridae					
Hipposideros caffer				1	1
Miniopteridae					
Miniopterus natalensis	*	2			2
Miniopterus fraterculus	*				
Molossidae					
Mops (*Chaerephon*) *pumilus*	*				
Mops midas	*				
Otomops martiensseni	*				
Tadarida aegyptiaca	*				
Tadarida ventralis	*				
Nycteridae					
Nycteris thebaica	*	4		1	5
Rhinolophidae					
Rhinolophus cf. *simulator* (R*79*)	*	2		5	7
Rhinolophus simulator	*	5			5
Rhinolophus clivosus	*			2	2
Vespertilionidae					
Eptesicus hottentotus	*				
Laephotis capensis	*	61	43	1	105
Myotis bocagii	*				
Myotis tricolor	*	9			9
Neoromicia zuluensis	*	8	1		9
Nycticeinops schlieffeni	*				
Pipistrellus hesperidus	*	6			6
Pipistrellus rusticus	*	2			2
Scotophilus dinganii	*	11	3		14
Total captures per method		110	47	10	167

An asterisk (*) denotes the species was recorded during passive acoustic monitoring and an integer denotes the number of individuals actively captured using that capture technique.

Laephotis capensis was the species most frequently recorded (passive sampling) and captured (physical capture methods), accounting for 49.1 % of acoustic detections and 62.9

% of total physical captures. Individuals of this species were detected and captured year-round in every habitat type. *Hipposideros caffer* was not detected on any passive monitoring system during the study. *Nycteris thebaica* was seldom recorded (17 passes) during passive recording, however, *N*. *thebaica* and *H*. *caffer* species were captured in mist nets and hand nets and hand nets respectively (Table 4.1.).

Activity of all the species recorded on the reserve was expressed as total number of bat passes recorded over the sampling period and ranged from 43 passes (*Myotis bocagii*) to 12674 passes (*Laephotis capensis*). Overall activity was also reported as passes per hour and varied from 0.028 passes/hour to 8.251 passes/hour. Species with rapidly attenuating or low intensity such as *Hipposideros caffer* were not detected.

Table 4.2. Bat activity, expressed as total bat passes and bat passes per hour, from passively recorded data spanning 1536 hours obtained on Telperion Nature Reserve between January 2019 and January 2020

Family and species	**Total bat passes (acoustic data)**	**Activity (bat passes/hour)**
Emballonuridae		
Taphozous mauritianus	598	0.389
Hipposideridae		
Hipposideros caffer	0	0
Miniopteridae		
Miniopterus natalensis	1645	1.071
Miniopterus fraterculus	255	0.166
Molossidae		
Mops (*Chaerephon*) *pumilus*	899	0.585
Mops midas	484	0.315
Otomops martiensseni	52	0.034
Tadarida aegyptiaca	1539	1.002
Tadarida ventralis	1300	0.846
Rhinolophidae		
Rhinolophus clivosus	103	0.067
Rhinolophus cf. *simulator* (R79)	95	0.062
Rhinolophus simulator s.s.	153	0.100

Table 4.2. continued…

Family and species	**Total bat passes (acoustic data)**	**Activity (bat passes/hour)**
Vespertilionidae		
Eptesicus hottentotus	1648	1.073
Laephotis capensis	12674	8.251
Myotis bocagii	43	0.028
Myotis tricolor	165	0.107
Nycticeinops schlieffeni	172	0.112
Neoromicia zuluensis	1547	1.007
Pipistrellus hesperidus	615	0.400
Pipistrellus rusticus	912	0.594
Scotophilus dinganii	1010	0.658

4.2. Species richness, diversity and accumulation curves

Species diversity estimators are not provided within this chapter as data were pooled for multiple habitats and seasons to determine overall richness and accumulation curves. Diversity data for habitat and season are presented in chapter five.

Twenty-one bat species were detected using passive acoustic sampling on Telperion Nature Reserve, with the species accumulation curve reaching an asymptote after approximately five nights of acoustic sampling (Figure 4.1.).

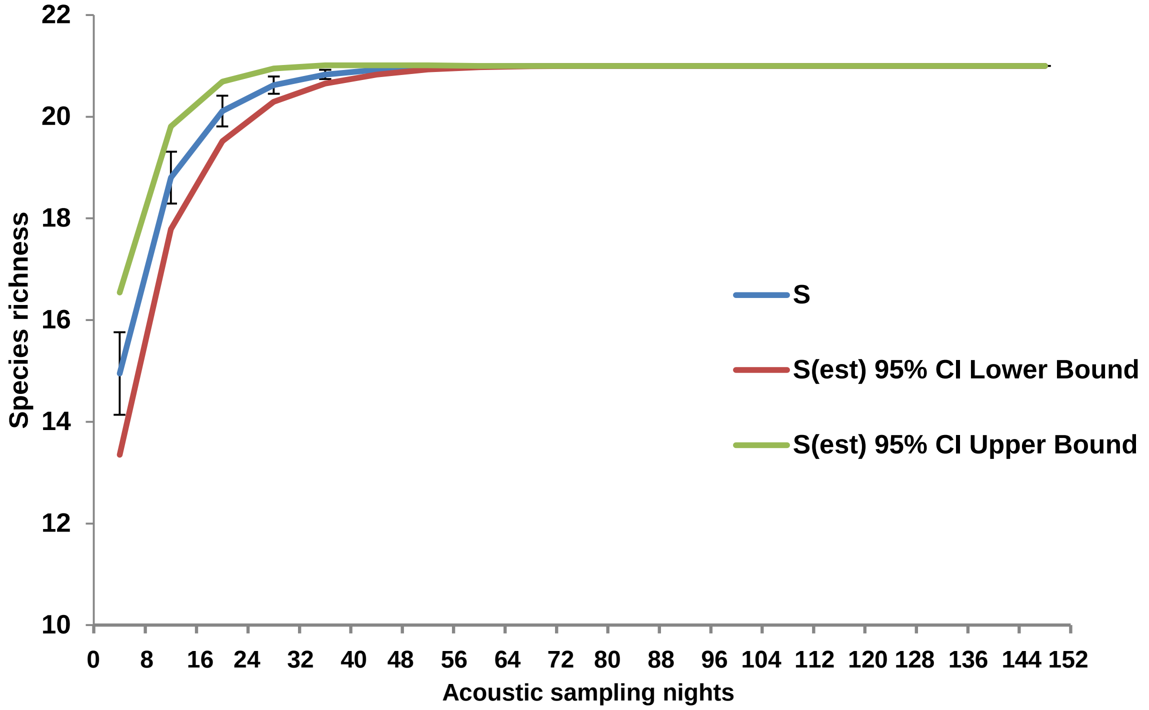

Figure 4.1. Species accumulation curve from passive sampling of bats on the Telperion Nature Reserve from January 2019 to January 2020

Note: One unit of sampling effort on the X axis is representative of 8 detector nights of recording. Each detector night equates to 12 hours of continuous recording from a single bat detector. The standard deviation of the projected numbers of species are represented by the error bars.

Active sampling resulted in the capture of 11 species (Figure 4.2.). Mist netting accounted for the capture of 10 species, the species accumulation curve for mist nets reached an asymptote at 11 species after approximately 30 sampling nights (Figure 4.3.). The projected number of species (from species accumulation curves) was four for harp trapping and five for hand netting (Appendix 3: Figure 4.1.).

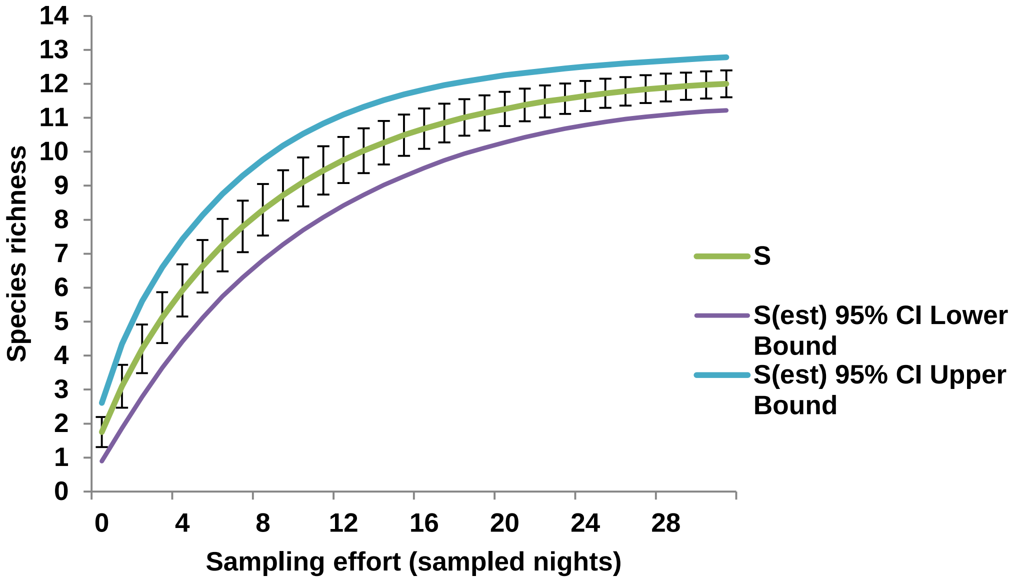

Figure 4.2. Species accumulation curve obtained from combined active sampling methods (mist netting, harp trapping and hand netting) on Telperion Nature Reserve between January 2019 and January 2021

Note: One unit of sampling effort is representative of one night of active sampling (four hours of sampling after sunset using a combination of methods). The standard deviation of the projected number of species are represented by the error bars.

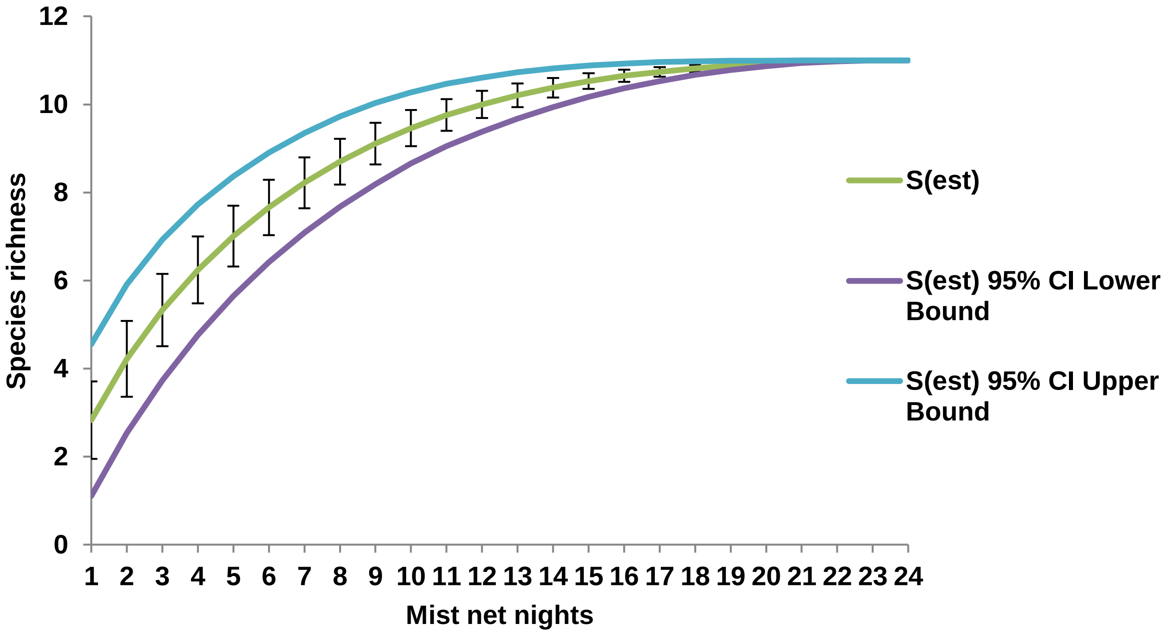

Figure 4.3. Species accumulation curves for mist netting on the Telperion Nature Reserve between January 2019 and January 2021

Note: Each unit on the x axis is representative of four hours of mist net sampling with approximately 130 m^2 of erected mist net. The standard deviation of the projected number of species are represented by the error bars.

4.3. Regional call library

A regional call library was compiled for the Telperion Nature Reserve (Table 4.3.; Figure 4.4., Figure 4.5.). Six echolocation parameters were measured from 97 individual bats belonging to 12 species that were physically captured and subsequently released. Call parameters of 10 additional bat species were recorded and identified from passive sampling data (Table 4.3.).

Table 4.3. Echolocation call parameters (mean ±SD) of the 22 bat species recorded on Telperion Nature Reserve between January 2019 and January 2021

Family and species	**n_1**	**n_2**	**Fc (kHz)**	**Fk (kHz)**	**Fmax (kHz)**	**Fmin (kHz)**	**Bandwidth (kHz)**	**Duration (ms)**	**Recording**
Emballonuridae									
Taphozous mauritianus	1	5	25.91 ±0.25	26.06 ±0.42	26.30 ±0.34	25.15 ±0.20	1.15 ±0.21	3.75 ±1.64	Free flight*
Hipposideridae									
Hipposideros caffer	1	30	138.78 ±1.33	141.14 ±1.63	143.60 ±1.19	125.04 ±9.51	18.55 ±9.79	6.21 ±1.28	Release
Miniopteridae									
Miniopterus natalensis	2	16	53.57 ±0.50	58.78 ±2.05	73.89 ±11.90	53.26 ±0.81	20.63 ±11.92	2.41 ±0.27	Release
Miniopterus fraterculus	1	5	61.70 ±4.33	88.84 ±7.34	95.14 ±3.60	38.98 ±0.56	56.16 ±3.98	3.12 ±0.37	Free flight*
Molossidae									
Mops (Chaerephon) pumilus	1	5	24.01 ±1.05	24.14 ±0.95	28.31 ±1.78	23.63 ±0.94	4.68 ±2.14	4.77 ±1.56	Free flight*
Mops midas	1	5	16.71 ±0.71	16.80 ±0.71	19.57 ±1.77	15.86 ±0.72	3.71 ±1.44	7.89 ±3.13	Free flight*
Otomops martiensseni	1	5	12.32 ±0.26	13.71 ±1.34	12.93 ±0.40	12.21 ±0.31	0.72 ±0.32	6.34 ±3.26	Free flight*
Tadarida aegyptiaca	1	5	22.46 ±0.22	23.80 ±1.48	35.56 ±1.23	21.20 ±0.26	14.36 ±1.14	8.19 ±1.26	Free flight*
Tadarida ventralis	1	5	18.53 ±0.66	18.26 ±0.84	19.20 ±0.87	17.78 ±0.66	1.42 ±0.37	4.05 ±1.77	Free flight*
Nycteridae									
Nycteris thebaica †	2	15	98.03 ±1.34 (pFreq)					3.03 ±0.33	Release
Rhinolophidae									
Rhinolophus cf. *simulator 79*	5	33	79.95 ±0.92	79.18 ±0.78	81.28 ±1.06	70.90 ±4.62	10.38 ±4.70	28.79 ±9.16	Release
Rhinolophus simulator	5	34	83.49 ±1.37	83.36 ±0.85	84.65 ±1.50	73.36 ±4.77	11.29 ±3.95	31.95 ±6.92	Release
Rhinolophus clivosus	3	18	90.63 ±0.68	89.93 ±0.98	91.85 ±0.33	74.44 ±6.15	17.41 ±6.41	34.49 ±7.33	Release
Vespertilionidae									
Eptesicus hottentotus	1	5	30.80 ±0.62	31.14 ±2.37	39.76 ±19.21	29.06 ±1.15	10.71 ±18.07	4.34 ±0.37	Free flight*

Table 4.3. continued…

Family and species	n_1	n_2	Fc (kHz)	Fk (kHz)	Fmax (kHz)	Fmin (kHz)	Bandwidth (kHz)	Duration (ms)	Recording
Laephotis capensis (Female)	27	135	37.93 ±1.32	40.47 ±2.05	73.11 ±9.73	37.61 ±1.32	35.50 ±9.97	3.94 ±1.08	Release
Laephotis capensis (Male)	25	125	38.45 ±1.34	42.57 ±2.10	74.80 ±11.47	38.06 ±1.29	36.74 ±11.51	3.64 ±1.08	Release
Myotis bocagii	1	5	43.07 ±1.50	48.09 ±2.65	75.85 ±6.79	43.11 ±1.60	32.74 ±6.90	3.27 ±0.59	Free flight*
Myotis tricolor	5	22	54.88 ±10.55	60.73 ±6.23	80.12 ±10.13	36.49 ±1.41	43.64 ±10.05	2.49 ±0.42	Release
Neoromicia zuluensis (Female)	2	29	45.93 ±1.28	48.87 ±1.98	84.90 ±15.01	45.74 ±1.31	39.15 ±15.53	2.82 ±0.60	Release
Neoromicia zuluensis (Male)	4	20	45.42 ±0.99	48.92 ±2.55	72.57 ±15.74	45.18 ±1.02	27.40 ±15.67	2.78 ±0.50	Release
Nycticeinops schlieffeni	1	5	42.74 ±0.44	43.50 ±0.63	46.48 ±1.00	41.38 ±0.49	5.10 ±1.43	4.09 ±0.54	Free flight*
Pipistrellus hesperidus	5	22	46.66 ±0.79	48.33 ±2.29	78.24 ±17.90	46.48 ±0.78	31.76 ±17.70	3.29 ±1.14	Release
Pipistrellus rusticus	2	14	48.66 ±0.83	52.76 ±2.26	79.26 ±8.95	48.26 ±1.66	31.00 ±8.26	6.40 ±1.84	Release
Scotophilus dinganii (Female)	2	40	33.06 ±1.34	37.53 ±3.30	57.44 ±9.68	32.55 ±1.31	24.89 ±9.23	2.96 ±0.55	Release
Scotophilus dinganii (Male)	4	38	32.71 ±1.48	36.87 ±2.92	63.03 ±10.10	32.20 ±1.40	30.84 ±9.61	3.06 ±0.66	Release

n_1 = number of actively captured individuals, **n_2** = number of pulses used to generate parameters, **Fc** = frequency at the flattest part of the call (characteristic frequency), **Fk** = frequency at knee of the call, **Fmax** = maximum frequency, **Fmin** = minimum frequency, **Bandwidth** = the bandwidth of the call (**Fmax - Fmin**), **Release calls** are recordings obtained post-release of actively captured individuals, **Free flight** calls are passively recorded while the animal was flying with no capture of the individuals.

*Data presented from free flight calls must be viewed with caution, until such data can be vetted by post-release recordings from live captured animals.

**Contamination of release recordings by free flying bats was avoided by observing the live sonogram on the EM3+ detectors and releasing the bat of interest during lulls of bat activity, bats were also released later into the night after the peak activity period had subsided.

† *Nycteris thebaica* calls could not be analysed using Kaleidoscope Pro. BatSound Pro was used to analyse the 15 pulses from n = 2 individuals (see page 33, Materials and methods). The peak frequency (pFreq) and duration are provided.

4.4. Variation in echolocation call parameters

A wide array of echolocation strategies are evident within the bat assemblage on Telperion Nature Reserve (Figures 4.4. and 4.5.; Table 4.3.), with call characteristic frequencies ranging from X̄ = 12.32 ±0.26 kHz (*Otomops martiensseni*) to X̄ =138.78 ±1.33 kHz (*Hipposideros caffer*). *Rhinolopus* cf. *simulator* (R79) and *Rhinolophus simulator* s.s., were morphologically similar yet differed in the structure of their connecting processes and echolocation characteristics (Figure 4.4.). The call data for these two putatively different species were not normally distributed (Appendix 3: Table 4.1.). The characteristic frequency (Fc) differed significantly between the two taxa, with R79 exhibiting a lower Fc (X̄ = 79.95 ±0.92 kHz) than *R. simulator* s.s. (X̄ = 83.49 ±1.37 kHz) (*p* < 0.001) (Figure 4.4., Table 4.3.). Significant differences were also found in frequency of the knee (Fk) (R79, X̄ = 79.18 ±0.78 kHz; *R. simulator* s.s., X̄ = 83.36 ±0.85 kHz; p <0.001), maximum frequency (Fmax) (R79, X̄ = 81.28 ±1.06 kHz; *R. simulator* s.s., X̄ = 84.65 ±1.50 kHz; p < 0.001) and minimum frequency (Fmin) (R79, X̄ = 70.90 ±4.62 kHz; *R. simulator* s.s., X̄ = 73.36 ±4.77 kHz; p < 0.001) (Appendix 3: Table 4.2.). Bandwidth (R79, X̄ = 10.38 ±4.7 kHz; *R. simulator* s.s., X̄ = 11.29 ±3.95 kHz; p = 0.521) and duration (R79, X̄ = 28.79 ±9.16 ms; *R. simulator* s.s., X̄ = 31.95 ±6.92 ms; p = 0.078) did not differ significantly between these putatively different taxa (Appendix 3: Figure 4.2.). Zero crossing spectrograms are presented in Appendix 3: Figure 4.3. and Appendix 3: Figure 4.4.

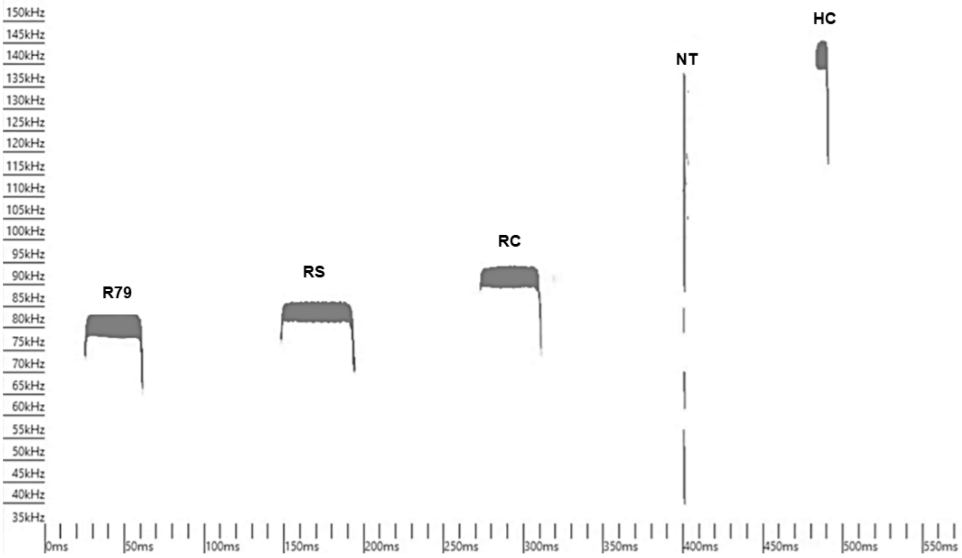

Figure 4.4. Full spectrum constant frequency (CF) echolocation calls and the frequency modulated (FM) broad bandwidth call of *Nycteris thebaica)* recorded on the Telperion nature reserve between January 2019 and January 2021. Note: **R79** = *Rhinolophus* cf. *simulator* 79, **RS** = *Rhinolophus simulator*, **RC** = *Rhinolophus clivosus*, **NT** = *Nycteris thebaica*, **HC** = *Hipposideros caffer*.

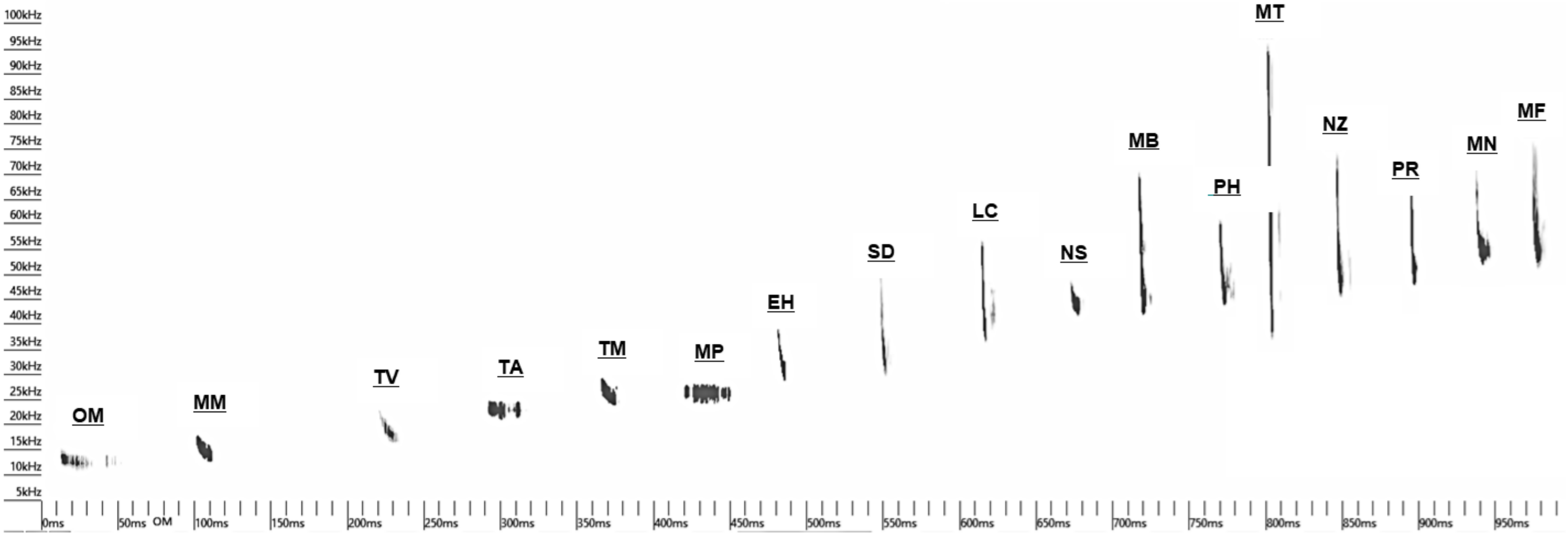

Figure 4.5. Full spectrum frequency modulated (FM) and quasi-constant frequency (QCF) echolocation calls of 17 bat species recorded on the Telperion Nature Reserve South Africa over the period of January 2019 to January 2021

Note:* = free flight call (bat not captured). **OM*** = *Otomops martiensseni*, **MM*** = *Mops midas*, **TV*** = *Tadarida ventralis*, **TA*** = *Tadarida aegyptiaca*, **TM*** = *Taphozous mauritianus*, **MP*** = Mops (*Chaerephon*) *pumilus*, **EH*** = *Eptesicus hottentotus*, **SD** = *Scotophilus dinganii*, **LC** = *Laephotis capensis*, **NS*** = *Nycticeinops schlieffeni*, **MB*** = *Myotis bocagii*, **PH** = *Pipistrellus hesperidus*, **MT** = *Myotis tricolor*, **NZ** = *Neoromicia zuluensis*, **PR** = *Pipistrellus rusticus*, **MN** = *Miniopterus natalensis*, **MF*** = *Miniopterus fraterculus*.

Generally, bat species on Telperion Nature Reserve did not exhibit notable intraspecifc variation in their call parameters (Table 4.3.). A notable exception was *Myotis tricolor*, which exhibited substantial variation in call parameters, even between individuals of the same sex (Table 4.3.). Significant intersexual variation in a number of call parameters were evident for vespertilionid species such as *L. capensis*, *Scotophilus dinganii*, *Neoromicia zuluensis* (Appendix 3: Table 4.1.).

Male and female *L. capensis* differed significantly in some of their call parameters, including characteristic frequency (Fc; n = 233, U = 102222.50, *p* = 0.003,), frequency at the knee (Fk; n = 50, U =491.00, *p* = 0.001), minimum frequency (Fmin; n = 233, U = 9309.00, *p* = 0.013) and duration (n = 233, U = 7083.00, *p* = 0.025) (Table 4.3.). Maximum frequency (Fmax) and bandwidth did not differ between sexes (Appendix 3: Table 4.3.).

In the case of *N. zuluensis* calls, males and females differed in relation to maximum frequency (Fmax; n = 49, U = 188.00, *p* = 0.038) and call bandwidth (n = 49, U = 286.00, *p* = 0.040), with females exhibiting a higher maximum frequency and greater bandwidth (Table 4.2.). The characteristic frequency (Fc), frequency at the knee (Fk), and minimum frequency (Fmin), and duration did not differ between males and females of *N. zuluensis* (Appendix 3: Table 4.4.).

The echolocation parameter data for *Scotophilus dinganii* were non-parametric (Appendix 3: Table 4.4.). Male and female calls differed significantly for maximum frequency (Fmax; n = 78, U = 1040.50, *p* = 0.005) and bandwidth (n = 78, U = 1059.00, *p* = 0.003). Males had a greater bandwidth and a higher maximum frequency (Fmax) than females. There were no sex-specific differences for the other call parameters (Fc, Fk, Fmin and Duration) for *S. dinganii* (Appendix 3: Table 4.5.).

4.5. Morphological variation

Regional species-specific, and where possible sex-specific, external morphological data for 12 taxa belonging to four families is presented in Table 4.4. Animal mass (expressed in grams) ranged from 4.00 g in the diminutive *Neoromicia zuluensis*, to approximately 30 g in *Scotophilus dinganii*. Forearm length, sometimes used as a proxy for body size, varied from 29.46 mm (*Pipistrellus rusticus*) to 56.39 ±2.00 mm (*Scotophilus dinganii* females). Clutter-foraging bats, *Hipposideros caffer and Nycteris thebaica*, exhibited the lowest wing loading values, while *Scotophilus dinganii,* a large clutter-edge foraging species, presented with the highest values. Sex-specific morphological data are presented for 10 of the 12 species captured during the study; owing to small sample sizes (apart from *L. capensis*) no further statistical analyses were applied.

Table 4.4. External morphological data (X̄ ±SD) for 12 bat taxa captured on Telperion Nature Reserve between January 2019 and January 2021.

Family and Species	**Sex and sample size**	**Mass (g)**	**Forearm length (mm)**	**1/2 Wingspan (m)**	**B Wingspan (m)**	**Length Hand wing LHW (m)**	**Length Arm wing LAW (M)**	**Area Hand Wing SHW (M²)**	**Area Arm Wing SAW (M²)**	**1/2 total wing area (1/2 S) (m²)**	**Total Wing Area S (m²)**	**Tip length ratio Tl**	**Tip area ratio TS**	**Tip Shape Index (I)**	**Wing loading (N/M²)**
Hipposideridae															
Hipposideros caffer	F(1)	8.00	49.27	0.15	0.30	0.0600	0.06	0.0023	0.0037	0.0072	0.0144	1.00	0.62	1.59	5.47
Miniopteridae															
Miniopterus natalensis	F(1)	13.50	45.50	0.16	0.31	0.0800	0.06	0.0025	0.0029	0.0069	0.0138	1.37	0.88	1.78	9.58
Miniopterus natalensis	M(1)	12.00	44.39	0.15	0.30	0.0100	0.06	0.0025	0.0031	0.0070	0.0141	0.15	0.80	1.22	8.37
Nycteridae															
Nycteris thebaica	F (1)	10.00	45.60	0.15	0.31	0.0900	0.05	0.0037	0.0035	0.0090	0.0179	1.63	1.06	1.86	5.47
Nycteris thebaica	M(4)	10.50 ±1.32	45.66 ±1.54	0.15 ±0.0082	0.30 ±0.02	0.0825 ±0.0050	0.05 ±0.01	0.0037 ±0.0001	0.0035 ±0.0003	0.0090 ±0.0004	0.0180 ±0.0009	1.80 ±0.25	1.07 ±0.10	1.68 ±0.92	5.69 ±0.69
Rhinolophidae															
Rhinolophus clivosus	M(2)	17.50 ±2.83	53.71 ±0.40	0.16 ±0.0000	0. ±0.00	0.0800 ±0.0000	0.07 ±0.00	0.0033 ±0.0004	0.0046 ±0.0008	0.0093 ±0.0015	0.0184 ±0.0030	1.10 ±0.06	0.71 ±0.06	1.95 ±0.79	9.33 ±0.01
Rhinolophus cf. simulator	F(3,2)*	9.25 ±0.35	45.51 ±0.76	0.15 ±0.0071	0.29 ±0.01	0.0700 ±0.0000	0.06 ±0.00	0.0025 ±0.0001	0.0036 ±0.0008	0.0069 ±0.0012	0.0137 ±0.0024	1.07 ±0.02	0.71 ±0.15	2.18 ±1.22	6.69 ±0.91
Rhinolophus cf. simulator	M(4)	7.17 ±2.36	42.36 ±7.95	0.14 ±0.0071	0.27 ±0.01	0.0650 ±0.0071	0.06 ±0.00	0.0023 ±0.0001	0.0037 ±0.0001	0.0068 ±0.0001	0.0136 ±0.0002	1.07 ±0.10	0.64 ±0.05	1.28 ±0.23	6.15 ±0.42
Rhinolophus simulator	F(4)	9.67 ±1.26	46.27 ±1.85	0.13 ±0.0058	0.19 ±0.12	0.0633 ±0.0058	0.06 ±0.00	0.0023 ±0.0002	0.0035 ±0.0004	0.0068 ±0.0008	0.0135 ±0.0015	1.05 ±0.06	0.67 ±0.04	1.80 ±0.30	7.06 ±1.07
Rhinolophus simulator	M(1)	9.50	47.12	0.15	0.29	0.0700	0.06	0.0026	0.0038	0.0074	0.0148	1.16	0.67	1.38	6.29
Vespertilionidae															
Laephotis capensis	F(54)	7.04 ±1.33	34.31 ±1.07	0.11 ±0.0061	0.22 ±0.01	0.0587 ±0.0035	0.04 ±0.00	0.0016 ±0.0002	0.0022 ±0.0002	0.0047 ±0.0004	0.0093 ±0.0008	1.30 ±0.07	0.72 ±0.06	1.29 ±0.27	7.11 ±1.54
Laephotis capensis	M(51)	6.57 ±0.93	32.64 ±0.86	0.11 ±0.0060	0.22 ±0.01	0.0564 ±0.0049	0.04 ±0.01	0.0019 ±0.0019	0.0033 ±0.0045	0.0043 ±0.0003	0.0086 ±0.0006	1.36 ±0.35	0.69 ±0.14	1.18 ±0.39	7.71 ±1.16
Myotis tricolor	F(7)	17.17 ±2.22	50.18 ±0.6	0.16 ±0.0049	0.32 ±0.01	0.0814 ±0.0038	0.07 ±0.01	0.0032 ±0.0002	0.0047 ±0.0004	0.0094 ±0.0004	0.0188 ±0.0008	1.19 ±0.09	0.68 ±0.09	1.38 ±0.29	9.05 ±1.11
Myotis tricolor	M(2)	15.50 ±6.36	51.16 ±2.69	0.16 ±0.0000	0.32 ±0.01	0.0750 ±0.0071	0.07 ±0.01	0.0030 ±0.0005	0.0042 ±0.0006	0.0083 ±0.0000	0.0166 ±0.0000	14.45 ±18.70	0.73 ±0.23	1.33 ±1.85	9.18 ±3.75
Neoromicia zuluensis	F(2,1)*	4.00	31.36	0.11	0.22	0.0500	0.05	0.0013	0.0019	0.0040	0.0079	1.16	0.68	1.43	4.95
Neoromicia zuluensis	M(7)	4.61 ±1.06	29.79 ±1.77	0.10 ±0.0115	0.20 ±0.02	0.2133 ±0.2743	0.04 ±0.00	0.0013 ±0.0002	0.0018 ±0.0004	0.0038 ±0.0006	0.0076 ±0.0012	5.02 ±6.35	0.71 ±0.09	0.86 ±0.70	6.05 ±2.80
Pipistrellus hesperidus	F(3)	7.67 ±3.40	32.43 ±2.50	0.11 ±0.0058	0.22 ±0.02	0.0567 ±0.0058	0.05 ±0.01	0.0014 ±0.0003	0.0019 ±0.0004	0.0041 ±0.0008	0.0082 ±0.0015	1.24 ±0.06	0.76 ±0.04	1.60 ±0.32	9.21 ±3.21
Pipistrellus hesperidus	M(3)	5.83 ±2.25	29.88 ±0.54	0.1 ±0.0100	0.20 ±0.01	0.0500 ±0.0000	0.04 ±0.00	0.0012 ±0.0002	0.0016 ±0.0002	0.0034 ±0.0005	0.0069 ±0.0011	1.17 ±0.05	0.75 ±0.03	1.80 ±0.08	8.81 ±4.48
Pipistrellus rusticus	F(1)	6.00	29.57	0.1	0.19	0.0500	0.04	0.0011	0.0018	0.0036	0.0072	1.10	0.60	1.21	8.2
Pipistrellus rusticus	M(1)	5.50	29.46	0.1	0.20	0.0500	0.04	0.0010	0.0014	0.0031	0.0063	1.16	0.69	1.45	8.62
Scotophilus dingani	F(4)	29.48 ±3.41	56.39 ±2.00	0.18 ±0.0082	0.35 ±0.02	0.0900 ±0.0000	0.08 ±0.01	0.0036 ±0.0002	0.0054 ±0.0005	0.0113 ±0.0007	0.0226 ±0.0015	1.24 ±0.06	0.67 ±0.04	1.18 ±0.20	12.81 ±0.73
Scotophilus dingani	M(10)	30.15 ±2.44	55.81 ±1.25	0.18 ±0.0074	0.36 ±0.02	0.0910 ±0.0057	0.07 ±0.00	0.0034 ±0.0002	0.0051 ±0.0004	0.0105 ±0.0007	1.27 ±0.06	0.68 ±0.04	1.16 ±0.15	14.09 ±1.42	14.09±1.42

Note:* Heavily pregnant females were not measured and handled excessively, and were released shortly following capture. This is why two sample sizes are presented in some species (total number captured, total number measured).

CHAPTER FIVE

RESULTS

THE EFFECTS OF HABITAT AND SEASON ON THE CHIROPTERAN ASSEMBLAGE OF THE TELPERION NATURE RESERVE

5.1. Sampling effort, capture success and activity according to habitat

A total of 1536 hours of acoustic sampling, comprised of 384 hours recordings in four habitat types, was undertaken during this study. Ninety-six hours (eight acoustic nights) of acoustic sampling per habitat, per season was conducted.

The number of bat passes varied in relation to habitat type (Table 5.1., Appendix 3: Table 5.1.). Significantly more bat passes were recorded at anthropogenic sites than in tree-grass sites ($\chi^2{}_3$ = 15.6, p = 0.018) and more passes were recorded at water course sites than at tree-grass sites ($\chi^2{}_3$ = -18.9, p = 0.004) (Table 5.1.). There were no significant differences in the number of bat passes between tree-grass and rock outcrop sites, between anthropogenic and water course sites, between rock outcrop and watercourse sites or between anthropogenic and water course sites (Table 5.1., Figure 5.1.).

Mist netting was found to be the most effective active capture method during the course of this study, mist nets were particularly effective in more open habitats where bats were more easily able to avoid other capture devices such as harp traps.

Table 5.1. Habitat specific activity (acoustic sampling) and mist net capture success on the Telperion Nature Reserve between January 2019 and April 2021

	Active sampling			Acoustic sampling					Indices			
Habitat	**Mist net M^2 hours**	**Mist net captures**	**Captures/ mist net hour**	**Max passes/ night**	**Min passes/ night**	**Proportion of passes (%)**	**Mean Passes per night (St dev)**	**Total passes**	**Cumulative AI (All species)**	**Shannon diversity (H)**	**Species richness (Chao 2)**	**Miller AI**
Anthropogenic	3864.4	67	0.017	1013	2	41.05	671.6 (653.8)	10746	0.16	1.89	20.31	0.163
Water	2583.7	23	0.009	254	22	30.43	507.7 (328.5)	7944	0.1	1.96	20.79	0.13
Tree Grass	2615.6	6	0.002	169	8	7.84	127.8 (69.1)	2046	0.03	2	21	0.076
Rock outcrop	536.2	14	0.02	193	28	20.68	338.8 (277.2)	5400	0.05	1.98	21	0.05
Total	9599.9	110						26136				

The total number of bat feeding buzzes did not differ significantly between habitat types ($\chi^2_3 = 2.14$, p = 0.544). The highest number of feeding buzzes (x̄ = 76 ± 37.9) was recorded in water course habitat whilst the lowest number of feeding buzzes (x̄ = 18.8 ± 15.5) was recorded in tree- grass habitats (Figure 5.1.).

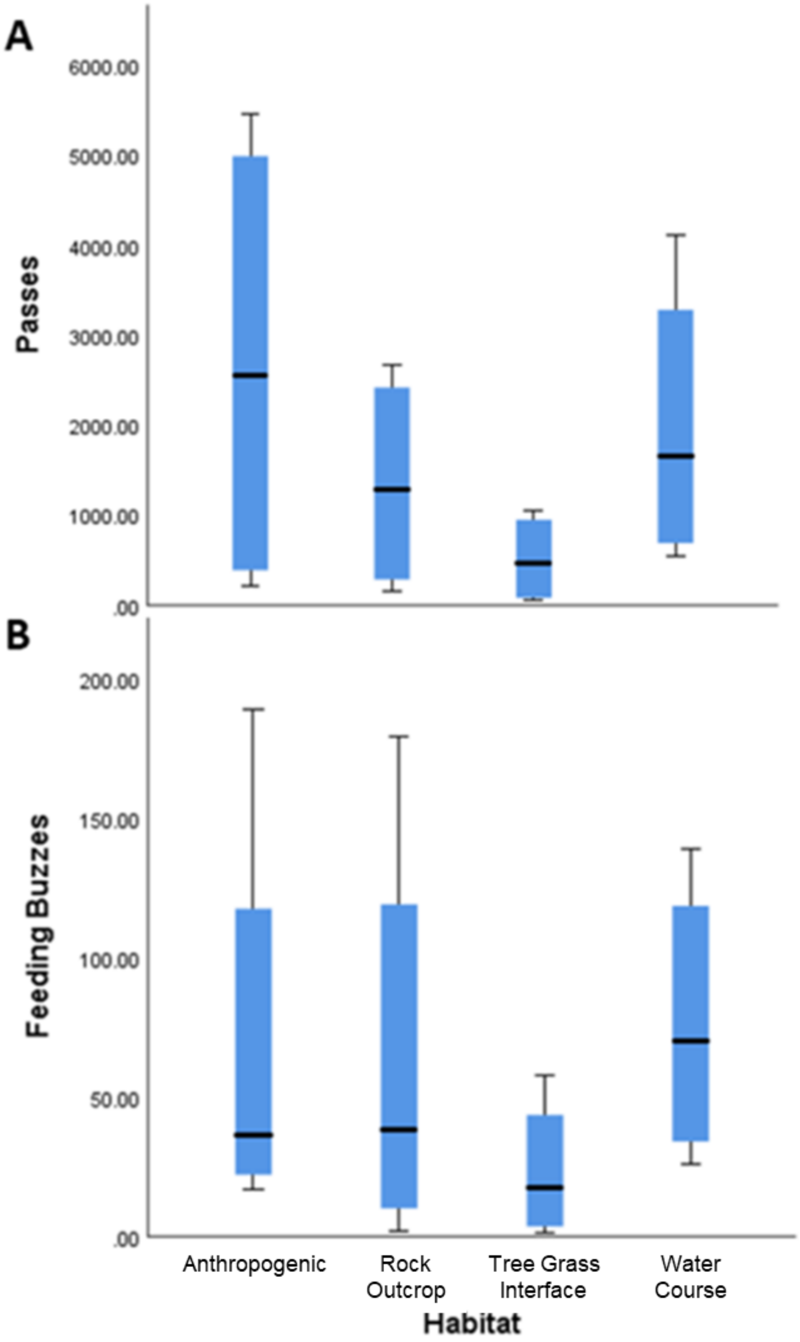

Figure 5.1. The total number of bat passes (A) and number of bat passes containing feeding buzzes (B) across different habitat types on the Telperion Nature Reserve, between January 2019 and January 2020

Higher levels of bat activity were recorded at anthropogenic sites than at sites in other habitat types (Table 5.1.). Cumulative Miller acoustic activity index (AI) values varied by habitat type (Table 5.1.). The activity index scores decreased according to habitat type in the following order; anthropogenic (AI = 0.163), water course (AI = 0.130), tree grass matrix (AI = 0.076), rock outcrop (AI = 0.050) (Table 5.1.).

The number of hourly bat passes ($\chi^2{}_3 = 61.70$, $p < 0.001$) varied significantly different among habitat types. The highest hourly activity was recorded within the anthropogenic habitat, and the lowest was in the tree-grass habitat (Figure 5.2.).

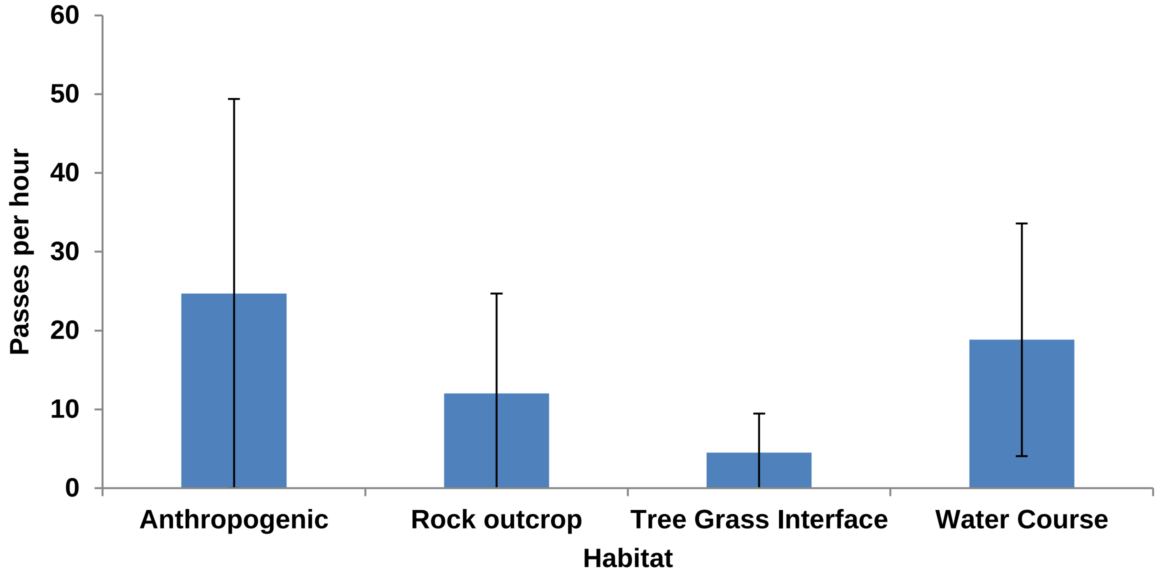

Figure 5.2. Mean bat passes per hour per habitat (x̄ ± SD) on the Telperion Nature Reserve between January 2019 and April 2021

5.2. Bat species richness and diversity according to habitat

Species richness varied according to habitat. The number of species recorded in each habitat type decreased in the following order; water course sites (total = 20, active capture = 9, acoustic recording = 17), anthropogenic sites (total = 20, active capture = 7, acoustic recording = 20), rock outcrop sites (total = 19, active capture = 4, acoustic recording =18) and tree-grass sites (total = 11, active capture = 6, acoustic recording = 9) (Appendix 3: Table 5.2.). There was no significant difference between the number of bat species recorded between anthropogenic and tree-grass interface habitats, rock outcrop and water course habitats, or tree-grass interface and rock outcrop habitats (Appendix 3: Table 5.3.). The number of species present differed significantly between anthropogenic and rock outcrop sites ($\chi^2{}_3 = -14.31$, $p = 0.029$), anthropogenic and water course sites ($\chi^2{}_3 = -19.44$, $p = 0.003$) as well as tree-grass interface and water course sites ($\chi^2{}_3 = -15.81$, p

= 0.016) (Figure 5.3.) (Appendix 3: Table 5.3.). The hourly species richness (χ^2_3 =52.82, p<0.001) was also significantly different among habitat types, with water course habitats presenting the overall highest species richness and tree-grass interface the lowest.

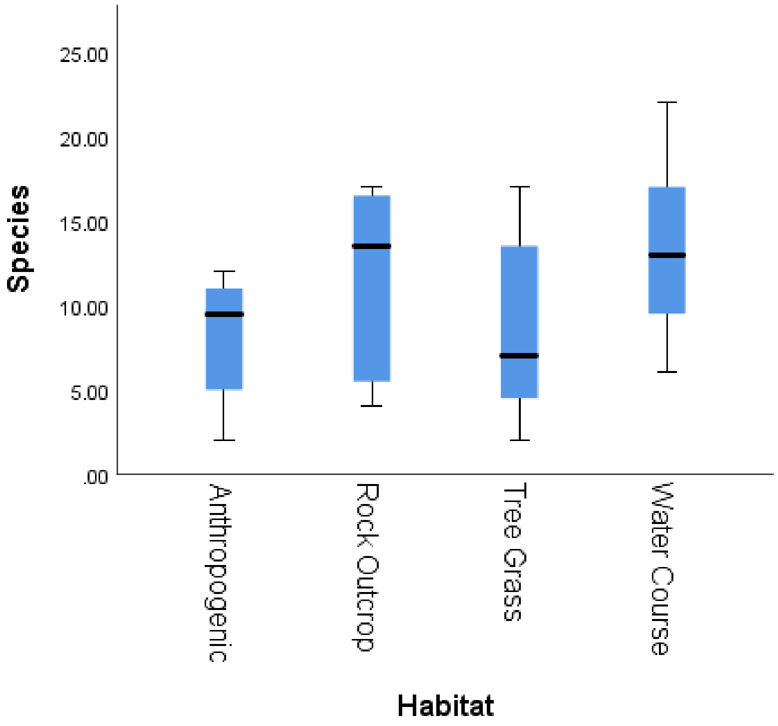

Figure 5.3. Bat species diversity recorded across habitat types on the Telperion Nature Reserve between January 2019 and January 2020

5.3. Habitat specific functional diversity

The functional diversity traits of all of the species captured on The Telperion Nature Reserve are presented (Appendix 3: Table 5.9.). Five chiropteran groupings were identified using cluster analysis (Figure 5.4.). These groupings were: Intermediate / high frequency FM clutter edge foragers (Group 1: *L. capensis*, *M. bocagii*, *M. fraterculus*, *M. natalensis*, *P. hesperidus*, *P. rusticus*, *N. schlieffeni*, *N. zuluensis*); Large, low frequency LD-QCF open air foragers (Group 2: *M. midas* and *O. martiensseni*), Intermediate and lower frequency edge and open air foragers (Group 3: *M.* (Chaerephon) *pumilus, E. hottentotus, S. dinganii, T. aegyptiaca, T. mauritianus);* HD-CF and multi-harmonic clutter foragers (Group 4: *N. thebaica, R. clivosus, Rhinolophus* cf. *simulator 79, R. simulator*) and HD-CF Very High frequency clutter foragers (Group 5: *H. caffer*) (Figure 5.4.).

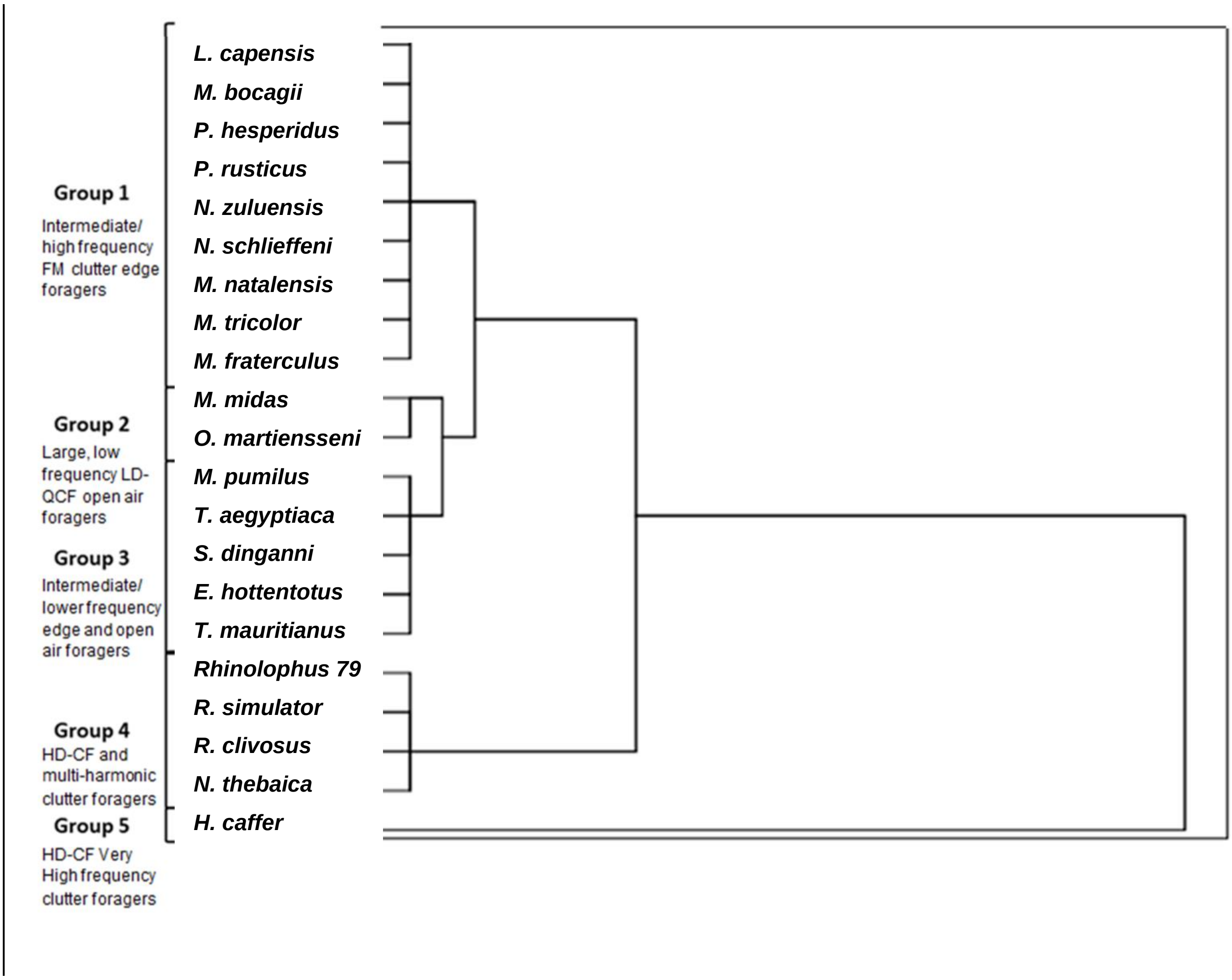

Figure 5.4. Cluster analysis derived functional groups of the bats on Telperion Nature Reserve from January 2019 to April 2021

Note: Groupings were based on species specific affinities in relation to; body size, call parameters, roosting habitat, foraging guild, and wing loading. Some ecomorphological data missing for *Tadarida ventralis*, hence not included in the analyses.

The activity of the different functional groups varied, with most of the bat passes attributable to Intermediate and High frequency clutter edge foraging species (Figure 5.5.). *Hipposideros caffer* was captured during active sampling, however it was not recorded via acoustic sampling, as such HD-CF Very high frequency clutter foragers (a monospecific group comprised exclusively of *H. caffer*) had no representation (Figure 5.5.).

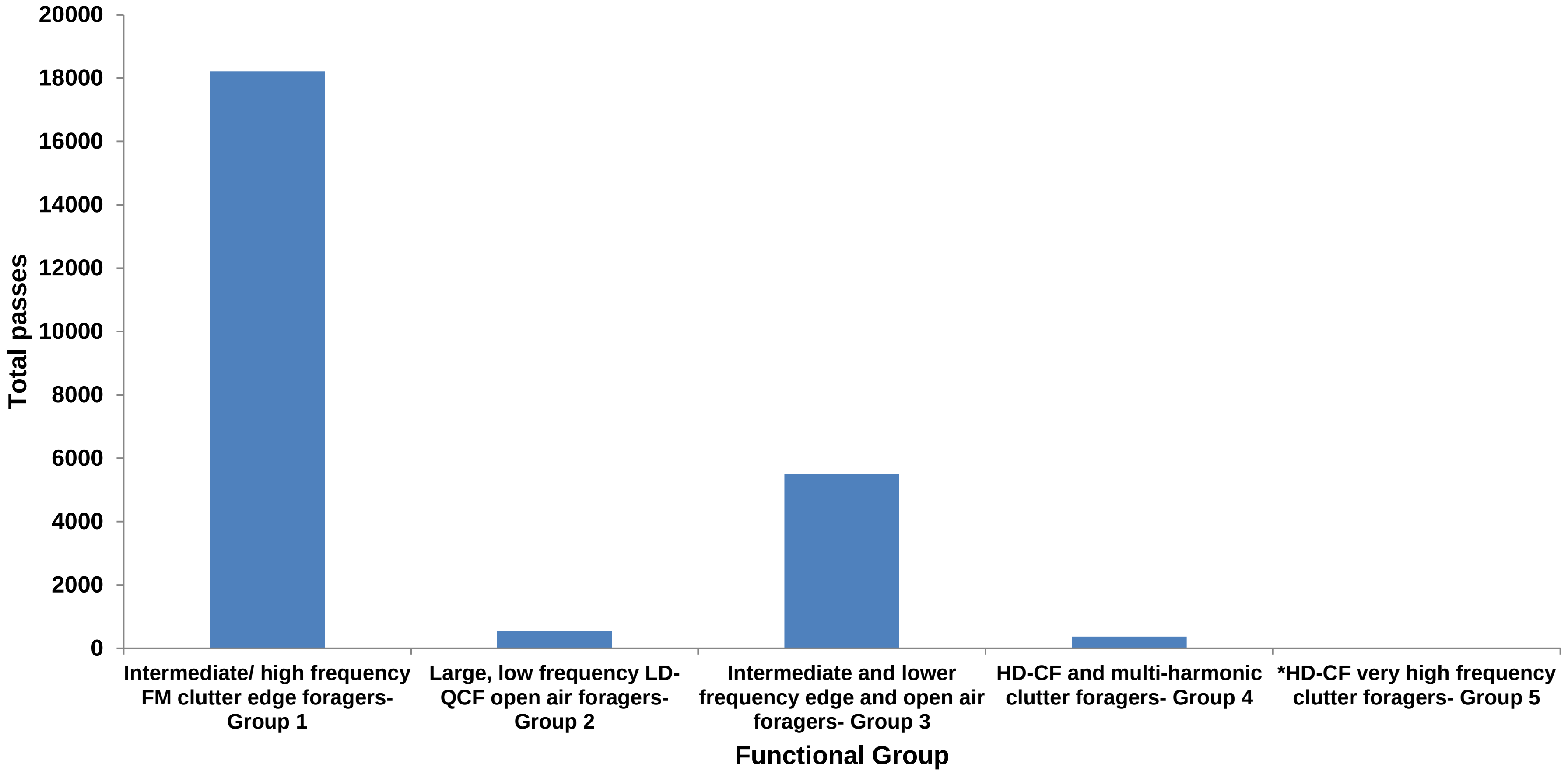

Figure 5.5. Mean number of bat passes per functional group (based on acoustic data) on the Telperion Nature Reserve between January 2019 and January 2020

*HD-CF very high frequency clutter foragers were not detected during passive acoustic sampling; therefore their total passes are not presented on this figure.

There was a significant difference in total number of functional group specific bat passes (χ^2_4 = 177.35, $p < 0.001$), (Appendix 3: Table 5.4.) Functional group 1 (Intermediate/ high frequency FM clutter edge foragers) (Figure 5.4.) differed most from the other groups ($p < 0.001$). Functional group specific bat passes vary significantly among habitat types (χ^2_3 = 2.86, $p = 0.038$).

The number of bat passes varied significantly across habitats (χ^2_3 =8.08, $p = 0.044$). Significantly more bat passes were recorded in water course habitats than in tree-grass habitats ($p = 0.010$) and at rock outcrop habitats than at tree-grass habitats ($p = 0.032$) (Appendix 3: Table 5.5.). Differences between the other pairwise habitat comparisons were non-significant (Figure 5.6.).

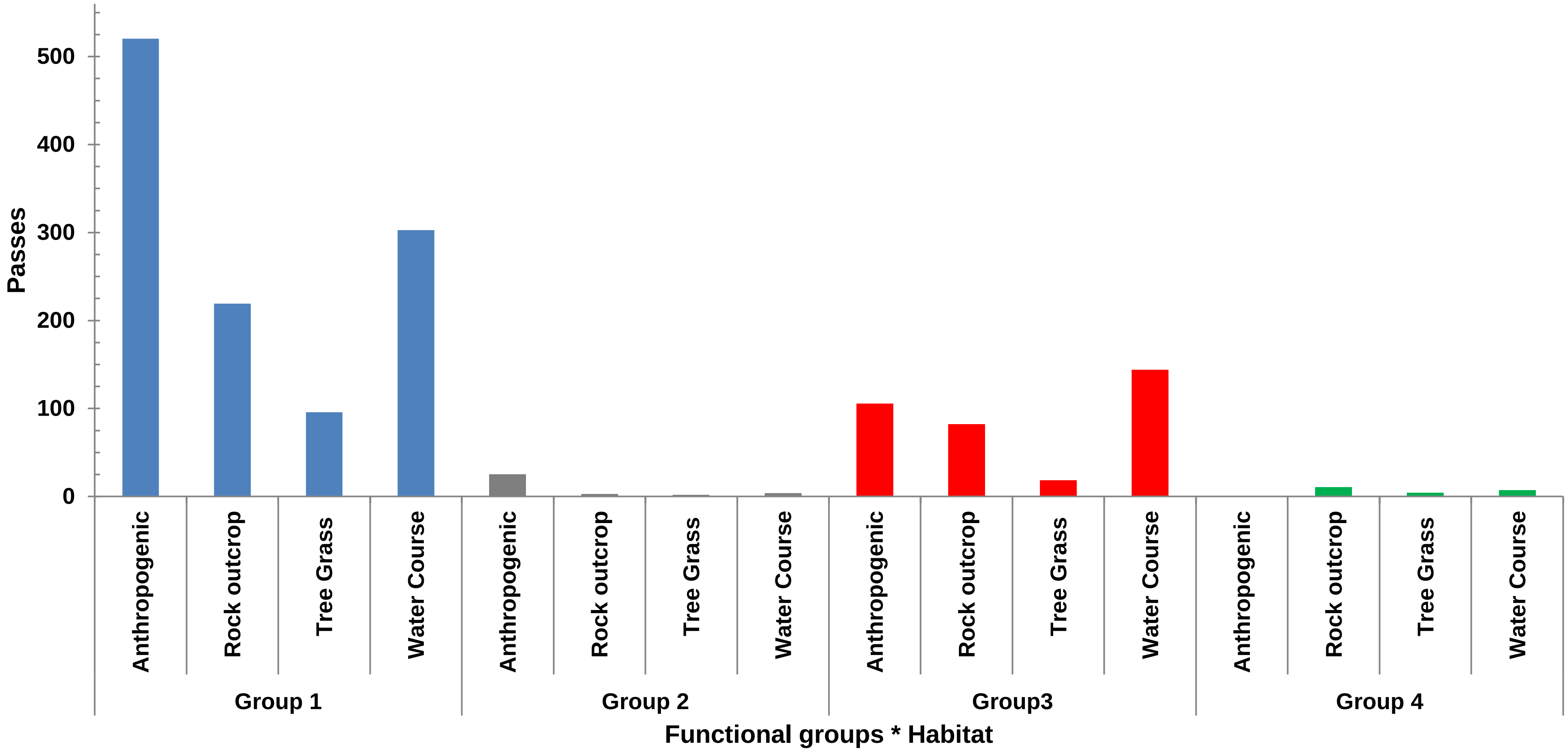

Figure 5.6. The number of functional group specific bat passes relative to habitat types on the Telperion Nature Reserve between January 2019 and April 2021

Note: Intermediate / high frequency FM clutter edge foragers (Group 1), Large, low frequency LD-QCF open air foragers (Group 2), Intermediate and lower frequency edge and open-air foragers (Group 3), HD-CF and multi-harmonic clutter foragers (Group 4) and HD-CF Very High frequency clutter foragers (Group 5). No passes for Group 5 were recorded using acoustic sampling.

5.4. Species specific habitat associations

Some species were more closely associated with certain habitats than others. Certain species were only recorded in specific habitats, such as *Miniopterus fraterculus* was only recorded in rock outcrop and water course habitats (Table 5.2.). Species such *as Laephotis capensis* were extremely widespread, being captured during all seasons and in every habitat, whereas some other species were absent from certain habitats, notably *Myotis spp.* and *Miniopterus spp.*, whereas *H. caffer* was not documented during acoustic sampling due to the rapidly attenuated, high frequency calls produced. Species specific biology and foraging, as well as roosting requirements likely have an effect on the habitat types utilised by species.

Table 5.2. Habitat specific bat detections recorded using passive acoustic sampling on the Telperion Nature Reserve between January 2019 and January 2020, with the exception of *Hipposideros caffer*

Family and Species	**Anthropogenic**	**Rock Outcrop**	**Tree Grass Interface**	**Water Course**	**Total**
Emballonuridae					
Taphozous mauritianus	102	157	13	326	598
Hipposideridae					
Hipposideros caffer	0	0	0	0	0
Miniopteridae					
Miniopterus fraterculus	0	8	0	247	255
Miniopterus natalensis	120	559	237	729	1645
Molossidae					
Mops (*Chaerephon*) *pumilus*	26	291	45	537	899
Mops midas	393	31	17	43	484
Myotis bocagii	3	2	0	38	43
Otomops martiensseni	11	15	8	18	52
Tadarida aegyptiaca	478	328	124	609	1539
Tadarida ventralis	208	252	91	749	1300
Nycteridae					
Nycteris thebaica	1	3	4	9	17
Rhinolophidae					
Rhinolophus clivosus	5	36	54	8	103
Rhinolophus cf. *simulator 79*	5	44	2	44	95
Rhinolophus simulator	7	86	9	52	154
Vespertilionidae					
Eptesicus hottentotus	530	455	87	576	1648
Laephotis capensis	7948	2262	820	1668	12698
Myotis tricolor	5	79	74	7	165
Neoromicia zuluensis	89	425	247	787	1548
Nycticeinops schlieffeni	14	16	11	131	172
Pipistrellus hesperidus	123	37	113	243	516
Pipistrellus rusticus	24	117	29	742	912
Scotophilus dinganii	645	92	22	251	1010
Total	10737	5295	2007	7814	25853

Species specific activity differed among habitat types for *Tadarida aegyptiaca* (p = 0.029, χ^2_3 = 9.05), no other species exhibited significant differences in activity among habitat types (Table 5.4.). When overall activity was assessed per habitat type, bat activity varied significantly (p = 0.040, t = 3.483, df = 3) among habitat types (Table 5.2.).

Miniopterus natalensis, T. aegyptiaca and *N. zuluensis* were associated with water course habitats, while *E. hottentotus* was strongly associated with most habitats except tree-grass interface sites and *L. capensis* was strongly associated with anthropogenic habitats.

Species specific activity (Miller AI) was notably higher around anthropogenic and water course sites for most species (Table 5.3.).

Table 5.3. Total bat passes and the Miller Index of acoustic activity (AI) (Miller, 2001) across habitat types on the Telperion Nature Reserve between January 2019 and January 2020

Family and species	**Total passes**	**Miller AI**			
		Anthropogenic	**Water Course**	**Tree-Grass**	**Rock outcrop**
Emballonuridae					
Taphozous mauritianus	598	0.00269	0.00378	0.00048	0.00369
Hipposideridae					
Hipposideros caffer	0	0	0	0	0
Miniopteridae					
Miniopterus fraterculus	255	0	0.002	0	0.0003
Miniopterus natalensis	1645	0.00265	0.0076	0.00091	0.00056
Molossidae					
Chaerephon (*Mops*) *pumilus*	899	0.00074	0.00777	0.00113	0.00208
Mops midas	484	0.00239	0.00087	0.00052	0.00074
Otomops martiensseni	52	0.00039	0.00048	0.00017	0.00052
Tadarida aegyptiaca	1539	0.01011	0.01081	0.00339	0.0043
Tadarida ventralis	1300	0.00473	0.01063	0.00256	0.00499
Nycteris thebaica	17	0.00004	0.0003	0.00004	0.00013
Rhinolophidae					
Rhinolophus clivosus	103	0.00022	0.0003	0.00065	0.00039
Rhinolophus simulator	154	0.00022	0.00087	0.00026	0.00069
Rhinolophus cf. *simulator 79*	95	0.00017	0.00074	0.00009	0.00122
Vespertilionidae					
Eptesicus hottentotus	1648	0.01042	0.01011	0.00165	0.00326
Laephotis capensis	12698	0.10807	0.0115	0.00851	0.01228
Myotis bocagii	43	0.00004	0.00065	0	0.00009
Myotis tricolor	165	0.00013	0.00026	0.00048	0.00017
Neoromicia zuluensis	1548	0.00278	0.01385	0.00326	0.00894
Nycticeinops schlieffeni	172	0.00061	0.0033	0.00035	0.00056
Pipistrellus hesperidus	616	0.00295	0.0036	0.00091	0.00087
Pipistrellus rusticus	912	0.001	0.00985	0.00095	0.00243
Scotophilus dinganii	1011	0.01293	0.00408	0.00043	0.00143

Note: A total of 23040 minutes of sampling was conducted in each habitat type.

Habitat type had a significant effect on a one of the five most frequently detected species on the Telperion Nature reserve (Table 5.4.).

Table 5.4. The association of habitat type with activity for the five most frequently detected bat species on the Telperion Nature Reserve between January 2019 and January 2020

Family and species	**Total bat passes per habitat type**			
	Anthropogenic	**Rock outcrop**	**Tree-grass matrix**	**Watercourse**
Miniopteridae				
Miniopterus natalensis	120	559	237	729
χ^2_3 = 7.75; KS = 0.000; p = 0.051				
Molossidae				
Tadarida aegyptiaca	478	328	124	609
χ^2_3 = 9.05; KS = 0.000; p = 0.029*				
Vespertilionidae				
Eptesicus hottentotus	530	455	87	576
χ^2_2 = 5.71; KS = 0.200; p = 0.057				
Laephotis capensis	7948	2262	820	1668
χ^2_3 = 3.75; KS = 0.002; p = 0.290				
Neoromicia zuluensis	89	425	247	787
χ^2_3 = 3.50; KS = 0.003; p = 0.316				

*A p value of <0.05 indicates significant differences in activity between habitat sites.

5.5. Seasonal variation in capture success and activity

The most bat passes were recorded in spring, and the fewest bat passes were recorded during winter (Table 5.5.). The number of bat passes (χ^2_3 = 10.65, p = 0.014) and the number of feeding buzzes (χ^2_3 = 9.82, p = 0.02) varied significantly among seasons.

Table 5.5. Active and passive sampling effort expended seasonally on the Telperion Nature Reserve. Passive acoustic sampling was conducted between January 2019 and January 2020, active sampling was conducted between January 2019 and April 2021

Season	Active captures	Mist net hours (m^2*hours)	Harp trap (hours)	Hand net active search (hours)	Passive acoustic recording (hours)	Passively recorded call sequences	Total activity - four detectors (passes/ hour)
Autumn	52*	1460.6	11.1	2.5	384	1599	4.2
Winter	12	2122.5	8.0	2.5	384	1274	3.3
Spring	32	1670.6	10.5	3.0	384	12795	33.3
Summer	28	2756.3	0.0	4.0	384	10468	27.3
Total	124	8010.0	29.6	12	1536	26136	17.0

*High autumn active capture values were due to sampling being undertaken in the proximity of anthropogenic sites containing *Laephotis capensis* roosts.

More bat passes were recorded during the warmer months than during the cooler months. Significant seasonal differences in the number of bat passes ($\chi^2_3 = 7.04$, $p < 0.001$) (Appendix 3: Table 5.6.) were evident between autumn and spring ($p < 0.001$), autumn and summer ($p = 0.001$), winter and spring ($p < 0.001$) and winter and summer ($p < 0.001$). No significant differences in the number of bat passes were evident between spring and summer or autumn and winter (Figure 5.7.).

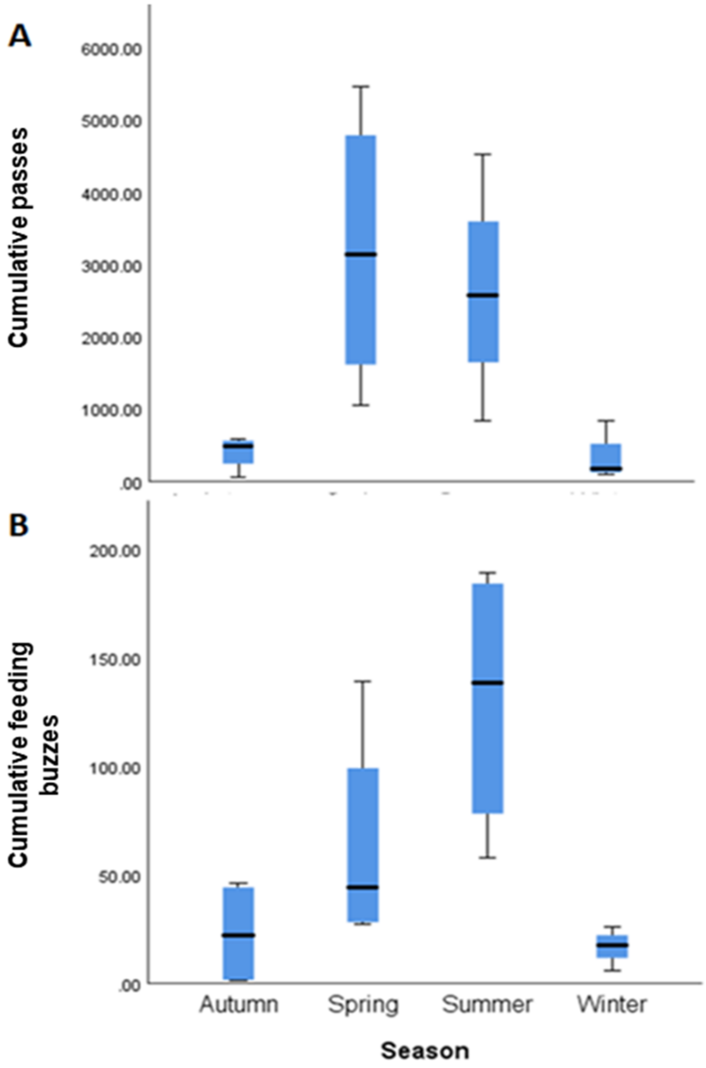

Figure 5.7. Variability in bat passes (A) and bat passes containing feeding buzzes (B) among different seasons on the Telperion nature reserve between January 2019 and January 2020

Significantly more bat passes were recorded in the summer than in the winter ($\chi^2_3 = 27.19$, $p < 0.001$), in the summer than in autumn ($\chi^2_3 = 21.69$, $p = 0.001$), in the spring than in the winter ($\chi^2_3 = 31.94$, $p < 0.001$), and in spring than in autumn ($\chi^2_3 = 26.44$, $p < 0.001$) (Table 5.6., Figure 5.7.). No significant differences in the total number of bat passes were evident when comparing autumn and winter and spring and summer (Appendix 3: Table 5.6.). The highest bat activity was recorded during the spring and summer (Figure 5.7., Appendix 3: Table 5.7.). The number of hourly bat passes also differed significantly among seasons (χ^2_3 = 497.05, p<0.001), with spring showing the highest number of hourly bat passes ($\bar{x}$ =33.3) and winter the lowest ($\bar{x}$ = 3.3). All bat species were more active (more bat passes recorded) in spring and summer (warm months) than in the autumn and winter (cold months) (Table 5.6.). The number of hourly bat passes (χ^2_3 = 497.05, p<0.001) and the hourly species richness (χ^2_3 = 531.75, p < 0.001) differed significantly among seasons (Figure 5.7.).

Table 5.6. Species specific seasonal variation in acoustically recorded bat passes on the Telperion Nature Reserve between January 2019 and January 2020

Family and Species	**Autumn Passes**	**Spring Passes**	**Summer Passes**	**Winter Passes**
Emballonuridae				
Taphozous mauritianus	25	309	257	7
Hipposideridae				
*Hipposideros caffer**	0	0	0	0
Miniopteridae				
Miniopterus fraterculus	7	20	6	222
Miniopterus natalensis	8	539	1091	7
Molossidae				
Mops (Chaerephon) pumilus	23	455	422	0
Mops midas	17	57	395	15
Otomops martiensseni	8	33	7	4
Nycteridae				
Nycteris thebaica	5	5	7	0
Rhinolophidae				
Rhinolophus cf. *simulator 79*	25	23	44	3
Rhinolophus clivosus	2	44	54	3
Rhinolophus simulator	12	22	115	5
Vespertilionidae				
Eptesicus hottentotus	74	826	748	0
Laephotis capensis	623	5854	5908	313
Myotis bocagii	2	23	18	0
Myotis tricolor	2	14	149	0
Neoromicia zuluensis	231	857	258	202
Nycticeinops schlieffeni	15	94	29	34
Pipistrellus hesperidus	14	165	418	19
Pipistrellus rusticus	337	273	7	295
Scotophilus dinganii	30	553	424	3
Tadarida aegyptiaca	32	1421	50	36
Tadarida ventralis	45	1152	23	81
Total	1512	12430	10173	1242

**Hipposideros caffer* was only detected during active sampling.

5.6. Seasonal variation in bat species richness and diversity

Similar numbers of species were recorded in spring (total = 21, active capture = 8, acoustic recording = 20), summer (total = 21, active capture = 12, acoustic recording = 20) and autumn (total = 21, active capture = 4, acoustic recording = 20). However, comparatively fewer species were recorded in winter (total = 16, active capture = 4, acoustic recording = 16). The hourly species richness (χ^2_{3} = 531.75, $p<0.001$) differed significantly among seasons, with the highest hourly diversity in spring (14 species) and the lowest hourly diversity in winter (0 species) (Table 5.7.).

The effective number of species (ENS) varied seasonally, with a higher effective number of species being recorded during the spring (H = 1.98, Chao 1 = 21, ENS =7.24) and summer (H = 2.01, Chao 1 = 21, ENS =7.46) months than in autumn (H = 1.88, Chao 1 = 19.69, ENS =6.55) and winter (H = 1.94, Chao 1 = 21, ENS =6.96). The number of species detected on acoustic detectors and physically captured during active sampling also varied seasonally (Table 5.7.).

Table 5.7. Bat species recorded and / or captured on the Telperion Nature Reserve from January 2019 to April 2021

Family and species	Autumn		Winter		Spring		Summer		Overall	
	AS	**AC**	**AS**	**AC**	**AS**	**AC**	**AS**	**AC**	**AS**	**AC**
Emaballonuridae										
Taphozous mauritianus	Y	0	Y	0	Y	0	Y	0	Y	0
Hipposideridae										
Hipposideros caffer	N	0	N	0	N	0	Y*	1	Y	1
Miniopteridae										
Miniopterus natalensis	Y	0	Y	0	Y	1	Y	1	Y	2
Miniopterus fraterculus	Y	0	Y	0	Y	0	Y	0	Y	0
Molossidae										
Mops (Chaerephon) pumilus	Y	0	N	0	Y	0	Y	0	Y	0
Mops midas	Y	0	Y	0	Y	0	Y	0	Y	0
Otomops martiensseni	Y	0	Y	0	Y	0	Y	0	Y	0
Tadarida aegyptiaca	Y	0	Y	0	Y	0	Y	0	Y	0
Tadarida ventralis	Y	0	Y	0	Y	0	Y	0	Y	0
Nycteridae										
Nycteris thebaica	Y	0	2	0	Y	1	Y	1	Y	4
Rhinolophidae										
Rhinolophus cf. simulator 79	Y	1	Y	0	Y	1	Y	5	Y	7
Rhinolophus simulator	Y	0	Y	3	Y	1	Y	1	Y	5
Rhinolophus clivosus	Y	0	Y	0	Y	0	Y	2	Y	2
Vespertilionidae										
Eptesicus hottentotus	Y	0	N	0	Y	0	Y	0	Y	0
Myotis bocagii	Y	0	N	0	Y	0	Y	0	Y	0
Myotis tricolor	Y	0	N	0	Y	7	Y	2	Y	9
Laephotis capensis	Y	38	Y	3	Y	17	Y	7	Y	65
Neoromicia zuluensis	Y	3	Y	4	Y	0	Y	1	Y	8
Nycticeinops schlieffeni	Y	0	Y	0	Y	0	Y	0	Y	0
Pipistrellus hesperidus	Y	0	Y	0	Y	1	Y	5	Y	6
Pipistrellus rusticus	Y	0	Y	0	Y	0	Y	2	Y	2
Scotophilus dinganii	Y	10	Y	0	Y	3	Y	0	Y	13

**Hipposideros caffer* was only recorded upon release after active capture. The low intensity call of this species was not recorded during passive acoustic monitoring. Y denotes a species recorded acoustically during that season. N denotes a species not recorded acoustically during that season. An integer indicates the number of individuals of that species captured by active sampling methods (mist nets, harp traps, hand nets). AS = acoustic sampling; AC = active capture.

Species diversity per site was higher in summer and in spring than in autumn and winter (Figure 5.8.). The number of bat passes ($\chi^2_3 = 10.65$, $p = 0.014$) and the number of feeding buzzes ($\chi^2_3 = 9.82$, $p = 0.02$) varied seasonally. There were significant differences in bat species diversity between winter and summer (χ^2_3 = 29.53, p < 0.001), winter and spring (χ^2_3 = 33.16, p < 0.001), autumn and summer (χ^2_3 = 18.09, p = 0.006) and autumn and spring (χ^2_3 = 21.72, p = 0.001), with species diversity being higher in the warmer months (spring and summer) and lower in the winter months (autumn and winter) in all cases. Species diversity did not differ significantly between autumn and winter or spring and summer (Figure 5.8., Appendix 3: Table 5.8., Appendix 3: Figure 5.1.).

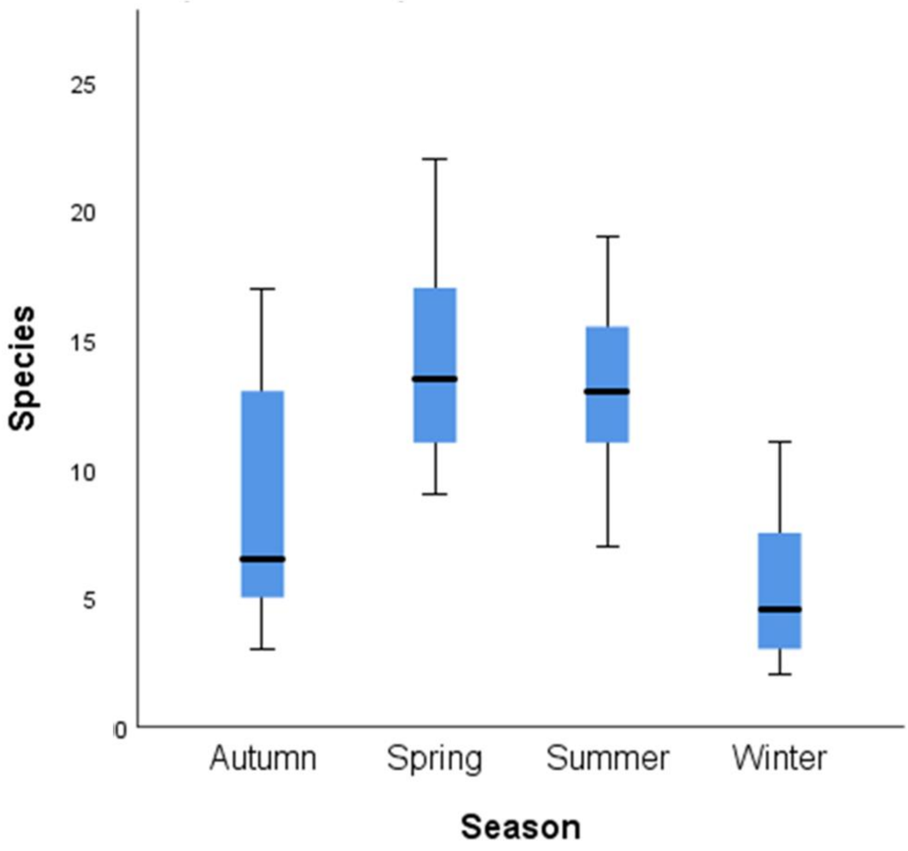

Figure 5.8. Seasonal bat species diversity from acoustic monitoring on the Telperion Nature Reserve between January 2019 and January 2020

5.7. Seasonal functional diversity

The highest total number of bat passes were recorded during the spring and summer months (Figure 5.9., Figure 5.10.). The highest number of passes for intermediate / high frequency FM clutter edge foragers (Group 1) and intermediate and lower frequency edge and open-air foragers (Group 3) were also recorded during these seasons. Large, low frequency LD-QCF open air foragers (Group 2) displayed the highest activity during spring and HD-CF very high frequency clutter foragers (Group 4) displayed the highest activity during summer (Figure 5.9., Figure 5.10.).

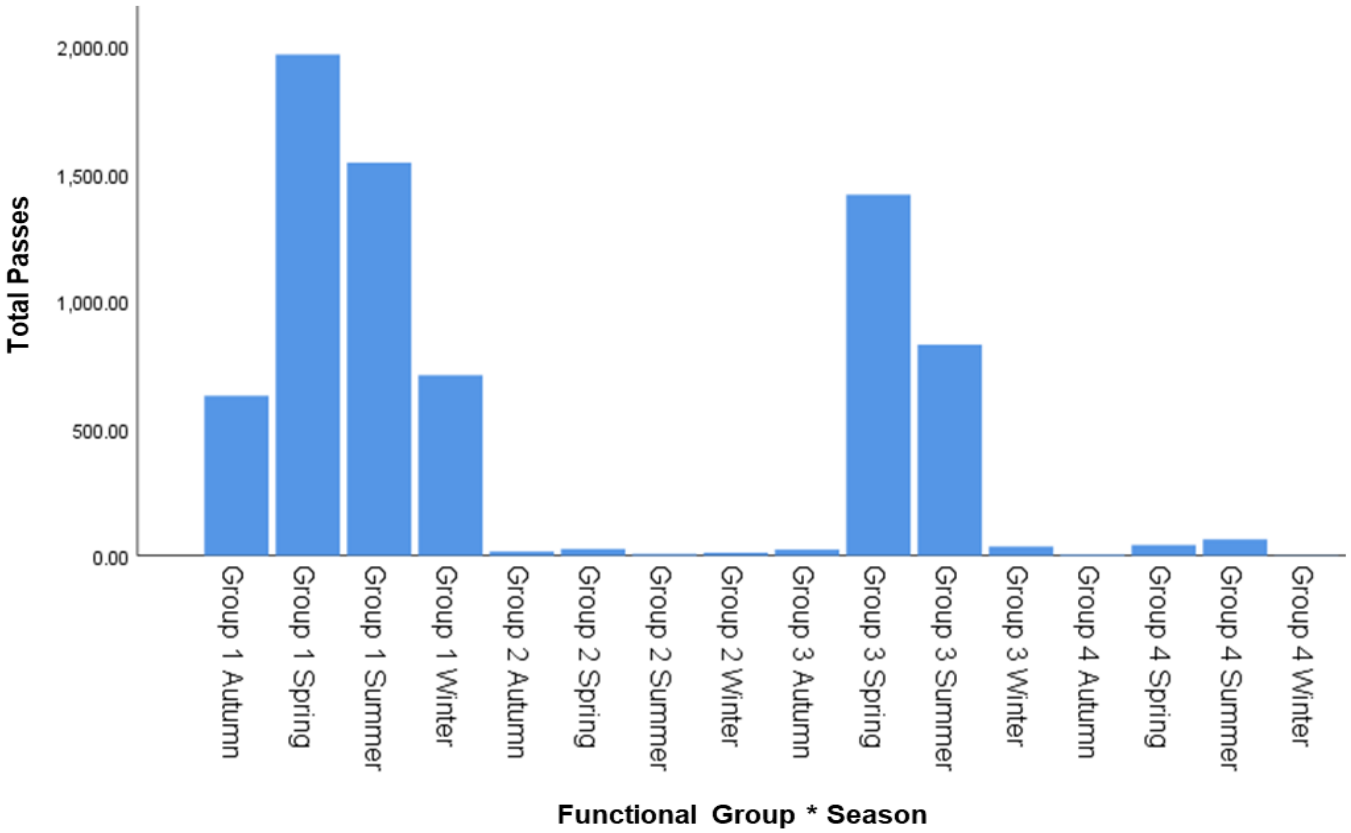

Figure 5.9. Seasonal variation in the total number of bat passes per functional group on the Telperion Nature Reserve between January 2019 and January 2020

Group 1: Intermediate / high frequency FM clutter edge foragers
Group 2: Large, low frequency LD-QCF open air foragers
Group 3: Intermediate and lower frequency edge and open-air foragers
Group 4: HD-CF and multi-harmonic clutter foragers
Note: Group 5 (*H. caffer*) was not included as it was only recorded during active captures

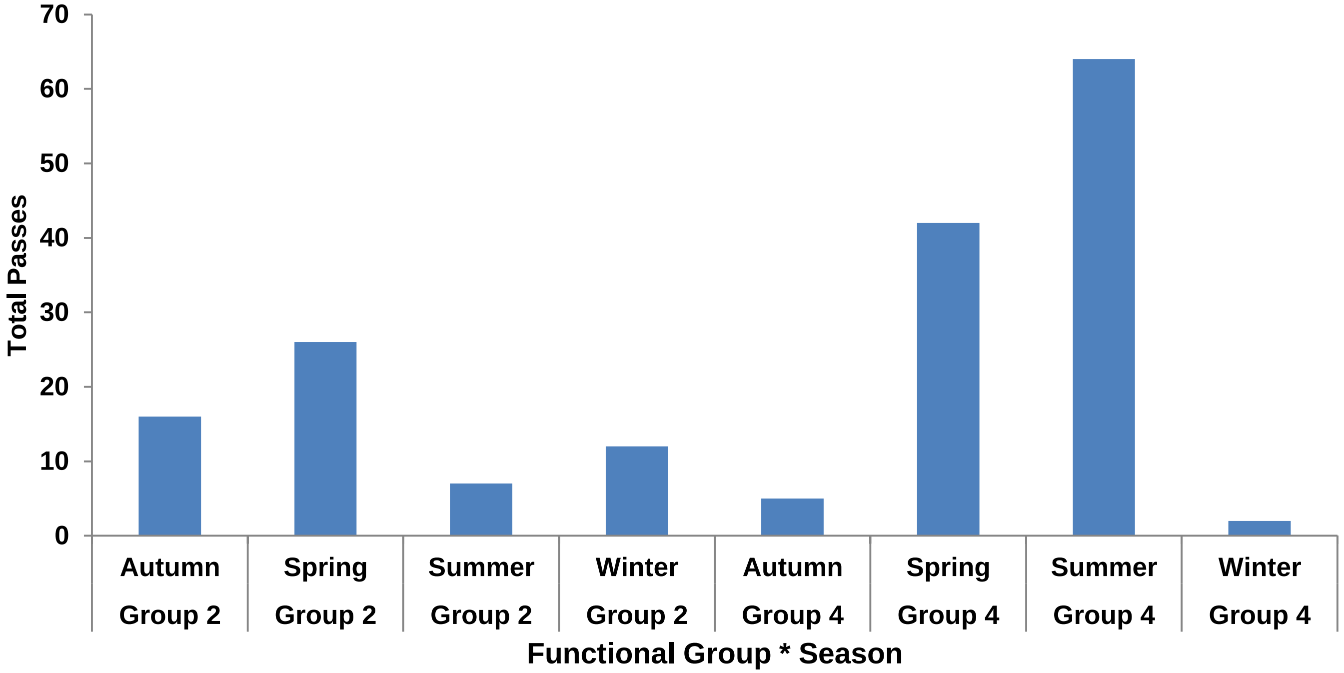

Figure 5.10. Seasonal variation in the total number of bat passes for functional groups 2 and 4 on the Telperion Nature Reserve between January 2019 and January 2020

Group 1: Intermediate / high frequency FM clutter edge foragers
Group 2: Large, low frequency LD-QCF open air foragers
Group 3: Intermediate and lower frequency edge and open-air foragers
Group 4: HD-CF and multi-harmonic clutter foragers
Note: Group 5 (*H. caffer*) was not included as it was only recorded during active captures

5.8. Seasonal variation in species specific biology

Reproductive condition (secondary sexual characteristics) varied seasonally in both male and female bats, most female bats were pregnant or lactating during the spring months (Table 5.8.). Males were usually scrotal during the spring and summer months; however some were also scrotal during autumn. Males with abdominal testes were captured year round (Table 5.8.).

Table 5.8. Seasonal variation in sexual morphology of bats captured on the Telperion Nature Reserve between January 2019 and April 2021

			Females					**Males**		
Family	**Species**	**Season**	**Pregnant**	**Lactating**	**Perforate**	**Imperforate**	**Undocumented**	**Scrotal**	**Semi-scrotal**	**Abdominal**
Rhinolophidae	*Rhinolophus* cf. *simulator* 79	Autumn 2019								1
Vespertilionidae	*Laephotis capensis*	Autumn 2019	1		7	10		2	10	9
Vespertilionidae	*Neoromicia zuluensis*	Autumn 2019						3		
Vespertilionidae	*Scotophilus dinganii*	Autumn 2019			2	2		3	3	1
Miniopteridae	*Miniopterus natalensis*	Spring 2019						1		
Nycteridae	*Nycteris thebaica*	Spring 2019								1
Rhinolophidae	*Rhinolophus* cf. *simulator* 79	Spring 2019								1
Rhinolophidae	*Rhinolophus simulator*	Spring 2019	1							
Vespertilionidae	*Laephotis (capensis*	Spring 2019	5	2				4	6	
Vespertilionidae	*Myotis tricolor*	Spring 2019	4	1				2		
Vespertilionidae	*Pipistrellus hesperidus*	Spring 2019	1							
Vespertilionidae	*Scotophilus dinganii*	Spring 2019						3		
Vespertilionidae	*Laephotis capensis*	Summer 2019			1	1	23	5		10
Vespertilionidae	*Scotophilus dinganii*	Summer 2019					1			
Hipposideridae	*Hipposideros caffer*	Summer 2021			1					
Miniopteridae	*Miniopterus natalensis*	Summer 2021			1					
Nycteridae	*Nycteris thebaica*	Summer 2021			1					
Rhinolophidae	*Rhinolophus clivosus*	Summer 2021						2		
Rhinolophidae	*Rhinolophus* cf. *simulator* 79	Summer 2021			2	1		1		2
Rhinolophidae	*Rhinolophus simulator*	Summer 2021				1				
Vespertilionidae	*Laephotis capensis*	Summer 2021	1			1		3	2	
Vespertilionidae	*Myotis tricolor*	Summer 2021			1	1				
Vespertilionidae	*Neoromicia zuluensis*	Summer 2021			1					
Vespertilionidae	*Pipistrellus hesperidus*	Summer 2021				1	1	2	1	
Vespertilionidae	*Pipistrellus rusticus*	Summer 2021	1							

Table 5.8. continued...

			Females					Males		
Family	**Species**	**Season**	**Pregnant**	**Lactating**	**Perforate**	**Imperforate**	**Undocumented**	**Scrotal**	**Semi-scrotal**	**Abdominal**
Nycteridae	*Nycteris thebaica*	Winter 2019								2
Rhinolophidae	*Rhinolophus simulator*	Winter 2019			2					1
Vespertilionidae	*Laephotis capensis*	Winter 2019								2
Vespertilionidae	*Neoromicia zuluensis*	Winter 2019								3

Note: During the first capture season (January 2019) female reproductive status was not recorded, thereafter, each season reproductive aspects were noted for each captured individual. Sampling periods- Autumn 2019: May 2019, Spring 2019: October 2019, Summer 2019: January 2019, Summer 2021: April 2021, Winter 2019: July 2019.

5.9. Combined effects of habitat and season on activity and species richness

A total of 1536 hours of acoustic sampling was undertaken during this study. Activity (mean bat passes and mean bat passes per hour) varied relative to habitat and season, with anthropogenic sites exhibiting the highest number of bat passes per hour, irrespective of season (Tables 5.9., 5.10.). More bat passes were recorded during the spring and summer months (November to March), with the highest number of bat passes being recorded during summer at anthropogenic habitat sites (Tables 5.9., 5.10.).

Table 5.9. Seasonal, habitat specific bat activity (mean number of bat passes) recorded from Telperion Nature Reserve between January 2019 and January 2020

Season	**Anthropogenic**		**Rock Outcrop**		**Tree-Grass**		**Watercourse**	
	X̄	**SD**	**X̄**	**SD**	**X̄**	**SD**	**X̄**	**SD**
Autumn	142.8	70.3	110.3	74.2	15.3	5.9	181.0	106.2
Spring	1365.8	1797.5	541.0	248.1	265.0	79.5	1027.0	399.7
Summer	1127.8	685.3	667.3	763.7	208.0	163.7	614.0	525.5
Winter	50.0	61.9	36.5	22.6	23.0	27.4	209.0	282.8

Table 5.10. Seasonal, habitat specific bat activity (bat passes / hour) recorded from Telperion Nature Reserve between January 2019 and January 2020

Season	**Anthropogenic**		**Rock Outcrop**		**Tree-Grass**		**Watercourse**	
	X̄	**SD**	**X̄**	**SD**	**X̄**	**SD**	**X̄**	**SD**
Autumn	5.94	2.93	4.59	3.09	0.63	0.25	7.54	4.42
Spring	56.90	74.90	22.54	10.34	11.04	3.31	42.79	16.65
Summer	46.98	28.55	27.80	31.82	8.66	6.82	25.58	21.90
Winter	2.08	2.58	1.52	0.94	0.96	1.14	8.71	11.78

Hourly activity was relatively uniform in rock outcrop and tree-grass sites (Figure 5.10.). There was a distinct increase in bat activity in the early evening (between 18:00 and 20:00) at water course sites (Figure 5.10.). At anthropogenic sites there was an increase in bat activity later in the night (between 23:00 and 01:00) (Figure 5.10.).

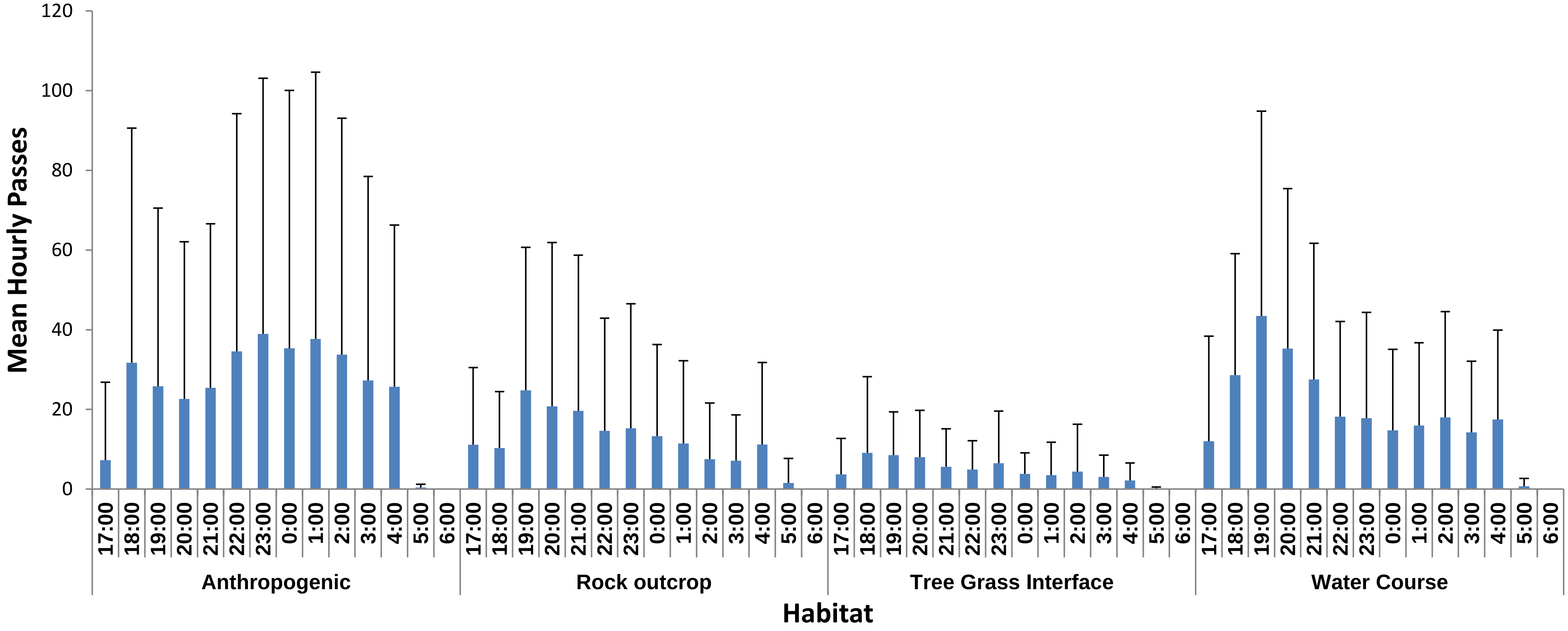

Figure 5.11. Habitat specific activity vs time of bats recorded on the Telperion Nature Reserve between January 2019 and January 2020

The highest levels of bat species diversity were recorded in spring and summer in all habitat types (Table 5.11., Appendix 3: Figure 5.1.). Rock outcrop and water course sites supported the most diverse bat assemblages (Table 5.11., Appendix 3: Figure 5.1.).

Table 5.11. Seasonal variation in the number of bat species recorded using passive acoustic monitoring on the Telperion Nature Reserve between January 2019 and January 2020

Habitat type	**Autumn**		**Winter**		**Spring**		**Summer**	
	X̄	**SD**	**X̄**	**SD**	**X̄**	**SD**	**X̄**	**SD**
Anthropogenic	5.75	0.96	4.25	3.86	10.50	1.00	11.25	0.96
Water Course	11.25	3.50	9.00	1.83	18.50	4.04	14.75	4.19
Tree Grass Interface	5.00	1.83	3.75	1.50	13.75	2.99	12.00	3.56
Rock Outcrop	11.75	5.12	4.50	1.00	15.25	2.06	14.50	2.65

Species specific variation in seasonal activity was only evident for *Laephotis capensis* (χ^2_3 = 10.74, p = 0.013) and *Neoromicia zuluensis* (χ^2_3 = 7.90, p = 0.049), the other species investigated did not show significant variation in seasonal activity (Table 5.12.)

Table 5.12. Seasonal variation in species specific activity of the five most frequently detected bat species on Telperion Nature Reserve, between January 2019 and January 2020

Family and species	**Season**			
	dKS	**DF**	**χ^2**	**p**
Miniopteridae				
Miniopterus natalensis	0.000	3	1.30	0.730
Molossidae				
Tadarida aegyptiaca	0.000	3	4.20	0.241
Vespertilionidae				
Eptesicus hottentotus	0.200	3	3.18	0.365
Lephotis capensis	0.002	3	10.74	0.013*
Neoromicia zuluensis	0.003	3	7.90	0.049*

*A p value of <0.05 indicates significant changes in activity among seasons.

5.10. Effects of bioclimatic variables on bat activity

A principal component analysis based on correlation matrix derived from seven bioclimatic variables (Temperature (°C), Relative Humidity (%), Barometric pressure (mb),), Wind Chill (°C), Dew Point temperature (°C), Wind Speed (M/s), and Density Altitude (M)), revealed that six principal components explained 100% of sample variation (Table 5.13).

Table 5.13. Sample variance and six principal component contributions derived from a principal component analysis of seven bioclimatic factors recorded at Telperion Nature Reserve between January 2019 and January 2020

Principal component	**Initial Eigenvalues**		
	Total	**% of Variance**	**Cumulative %**
1	3.99	57.05	57.05
2	1.47	21.06	78.11
3	0.97	13.83	91.94
4	0.55	7.81	99.74
5	0.02	0.25	99.99
6	0.00	0.01	100.00
7	0.00	0.00	100.00

Principal component 1 (Temperature and pressure), Principal component 2 (Humidity), Principal component 3 (Wind speed) explained most of the variation in activity – see Table 5.14.

The first three principal components (PC) explained 91.94 % of the variation (Table 5.13.) and, based on factor loadings (Table 5.14), were classified as temperature + pressure (PC1), humidity (PC2) and wind speed (PC3) variables.

Table 5.14. Factor loadings of seven bioclimatic variables on the first three principal components

Bioclimatic variables	**Principal component 1 (Temperature + Pressure)**	**Principal component 2 (Humidity)**	**Principal component 3 (Wind Speed)**
Temperature (° C)	**0,961**	-0,120	-0,181
Relative humidity (%)	-0,133	**0,983**	0,083
Barometric pressure (kPa)	**-0,719**	0,068	-0,282
Wind chill (° C)	**0,959**	-0,120	-0,190
Dew point (° C)	**0,710**	0,684	-0,107
Wind speed (m/s)	0,350	-0,044	**0,892**
Density altitude (m)	**0,995**	-0,061	-0,073

Note: Values in bold explained most of the variation in bat activity for each principal component.

Temperature, wind chill, dew point and density altitude were positively and strongly correlated with PC1, while barometric pressure was interacted negatively with PC1. Dew point temperature showed a strong positive interaction with PC2 (Humidity). Wind speed (PC3) was strongly and negatively interaction with PC3.

Bat passes were positively and significantly influenced by PC1 (temperature and pressure variable) ($r = 0.293$, $p < 0.001$), indicating that bat passes increased with increasing temperature and decreasing barometric pressure. Similarly, bat passes were positively and significantly influenced by PC 2 (humidity) ($r = 0.079$, $p < 0.001$); bat passes increased with increasing humidity. Unlike PC1 and PC2, bat passes were negatively and significantly influenced by PC3 (wind speed) ($r = -0.077$, $p < 0.001$). The number of bat passes decreased with increasing wind speed.

Principal component analysis was used to test for the effect of the bioclimatic variables on species richness. PC 1 (Temperature) interacted positively with species richness ($r = 0.468$, $p < 0.001$). Relative humidity (PC2) interacted positively with species richness ($r = 0.153$, $p < 0.001$). Wind speed interacted negatively with species richness (PC3) ($r = -0.159$, $p < 0.001$), (Figure 5.12.). The above suggests that species richness increases with temperature and relative humidity yet is reduced with increasing barometric pressure and wind speed.

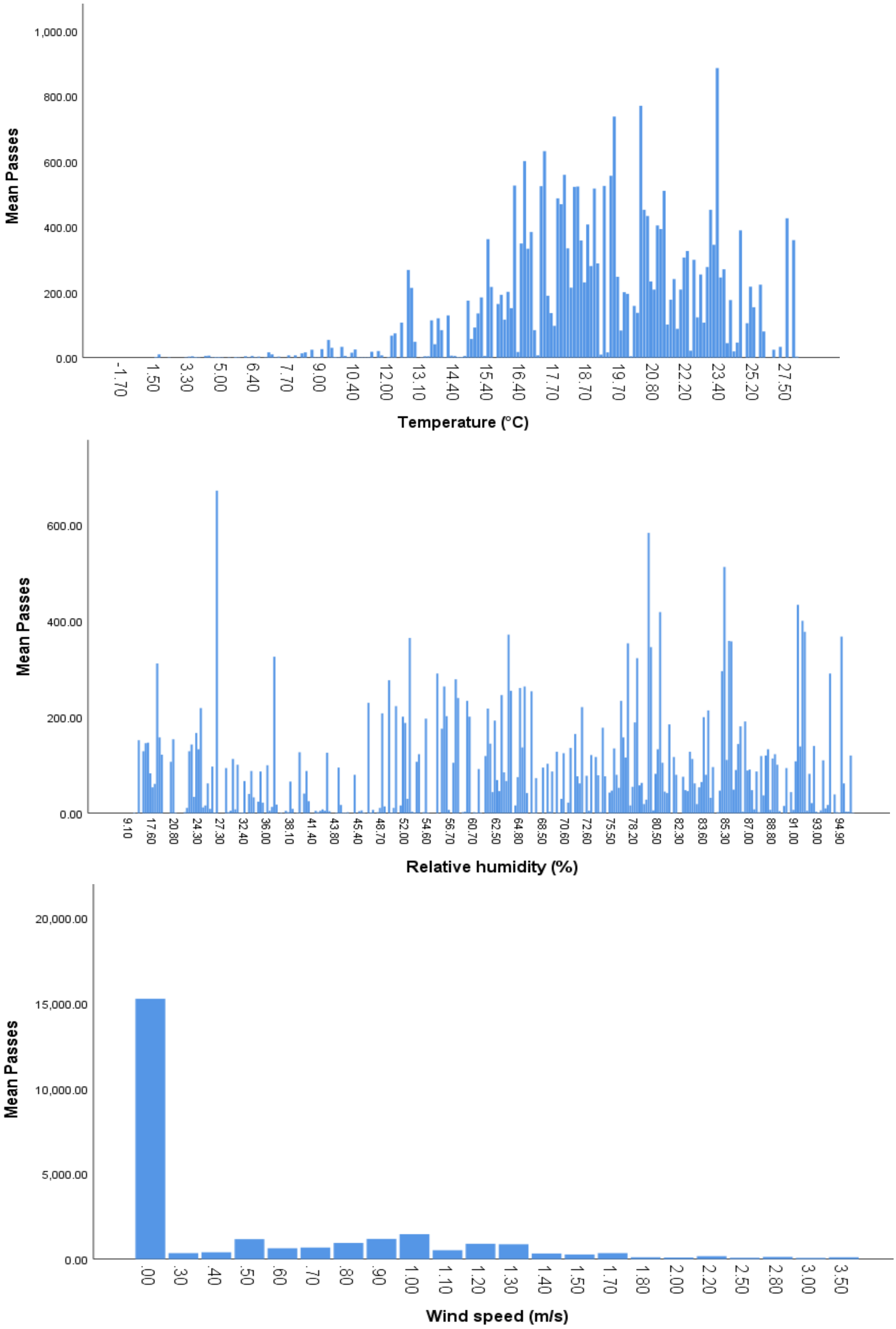

Figure 5.12. Total bat passes relative to the bioclimatic variables, temperature, relative humidity, and wind speed on the Telperion Nature Reserve between January 2019 and January 2020

The activity of the bat species on the Telperion Nature Reserve was influenced by the three primary bioclimatic variables; activity was positively correlated with temperature and humidity and negatively correlated with wind speed (Figure 5.12.). The species-specific analyses did not yield significant results (Table 5.15.), however when all species, seasons and habitats were considered, the impact of bioclimatic variables on bat activity was notable (Figure 5.12.). The effect of barometric pressure on overall bat activity was found to be elevated during lower and more normal (central) barometric pressures, however activity was much reduced when barometric pressures were higher (Appendix 3: Figure 5.2.).

Table 5.15. The effect of bioclimatic conditions on species specific activity tested using a principal component analysis and a Spearman R test on the Telperion Nature Reserve between January 2019 and January 2020

Family and species	**KW Test Values**								
	Temperature			**Humidity**			**Wind Speed**		
	DF	χ^2	**p**	**DF**	χ^2	**p**	**DF**	χ^2	**p**
Miniopteridae									
Miniopterus natalensis	76	76	0.478	124	124	0.483	21	21	0.459
Molossidae									
Tadarida aegyptiaca	93	93	0.48	129	129	0.483	21	21	0.459
Vespertilionidae									
Eptesicus hottentotus	80	80	0.479	138	138	0.484	19	19	0.457
Lephotis capensis	121	121	0.483	206	206	0.487	206	206	0.487
Neoromicia zuluensis	102	102	0.481	154	154	0.485	21	21	0.459

Bat activity of the five species with the highest number of bat passes, was analysed against bioclimatic data using non-parametric correlation analyses.

CHAPTER SIX

THE EFFECTS OF BLUE WILDEBEEST (*CONNOCHAETES TAURINUS*) CARCASS SITES ON THE CHIROPTERAN ASSEMBLAGE OF THE TELPERION NATURE RESERVE

6.1. Sampling effort and overall bat activity

A total of 50 nights (from 17:00 to 05:00) of acoustic data were recorded during the carcass decomposition experiment, both around carcass and control sites. Four nights of passive acoustic recording was conducted at control sites. One night of before sampling, at each of the treatment sites, was conducted prior to carcass deployment. Two separate nights of acoustic recording was conducted at carcass treatment and control sites following placement of carcasses. The number of nightly bat passes recorded over each of the treatments was; caged ($\bar{X}$ = 119.5 ±109.6), pegged ($\bar{X}$ = 50.7 ±46.0), control ($\bar{X}$ = 66.0 ±43.3). The mean number of recorded nightly feeding buzzes at each of the treatments; caged ($\bar{X}$= 8.9 ±14.9), pegged ($\bar{X}$= 1.9 ±3.1), control ($\bar{X}$= 2.4 ±3.0).

6.1.1 Bat activity at carcass sites before vs after carcass deployment

No significant differences in bat activity (number of recorded bat passes at carcass sites) were evident before ($\bar{X}$ = 69.1 ±48.7) and after carcass placement ($\bar{X}$ = 93.2 ±104.7) although the mean number of bat passes did increase after carcass deployment (Figure 6.1., Appendix 3: Table 6.1.). There was no significant difference in the number of bat passes at caged carcass sites before ($\bar{X}$ = 94.4 ±46.0) and after carcass placement ($\bar{X}$ = 132.1 ±131.2) (χ^2_{15} = 28.0, p= 0.768), nor was there a significant change in the number of bat passes at pegged carcass sites before ($\bar{X}$ = 43.8 ±40.3) and after carcass placement ($\bar{X}$ = 54.2 ±50.3) (χ^2_{15} = 28.0, p= 0.768).

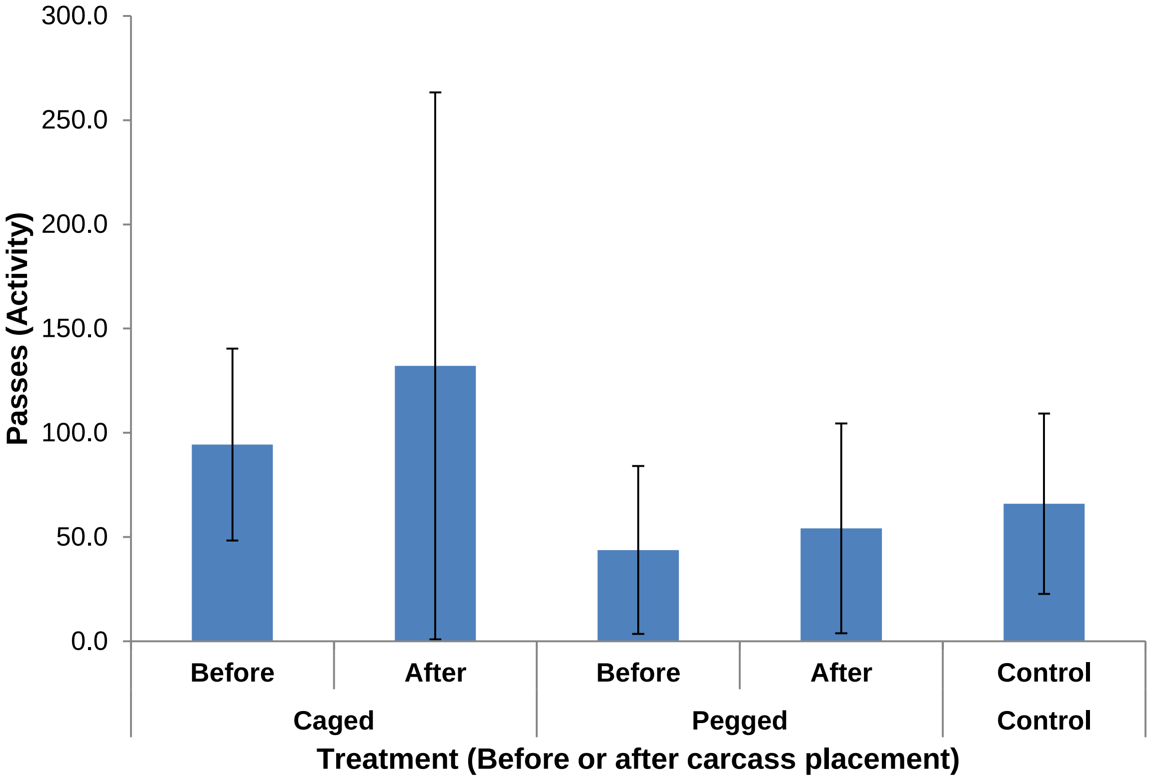

Figure 6.1. Bat activity (mean bat passes) at carcass sites before vs after blue wildebeest (*Connochaetes taurinus*) carcass deployment on The Telperion Nature Reserve during January and February 2019

There was no significant change in the foraging activity (number of feeding buzzes) before and after carcass deployment, this despite an almost doubling of mean feeding buzz events recorded subsequent to carcass deployment at caged and pegged treatment sites (Figure 6.2., Appendix 3: Table 6.1.). No significant differences in the number of feeding buzzes were recorded at caged carcass sites before ($\bar{X}$ = 5.2 ±4.0) and after (X= 10.8 ±18.1) carcass deployment (χ^2_{15} = 27.0, p= 0.859). No significant differences in feeding buzzes were recorded at pegged carcass sites before ($\bar{X}$ = 1.4 ±1.7) and after ($\bar{X}$= 2.2 ±3.7) carcass deployment (χ^2_{15} = 22.0, p = 0.768).

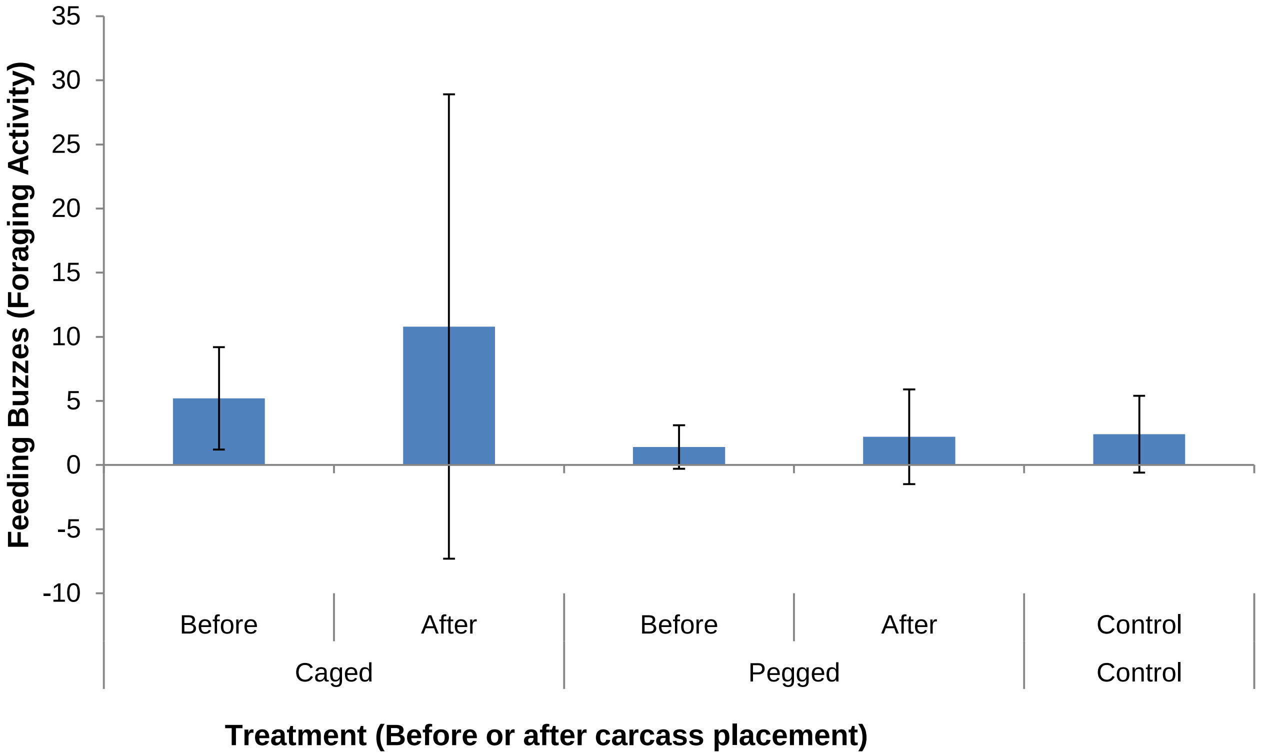

Figure 6.2. Foraging activity (number of feeding buzzes) of bats at carcass sites before vs after blue wildebeest (*Connochaetes taurinus*) carcass deployment on The Telperion Nature Reserve during January and February 2019

6.1.2 Hourly bat activity at carcass treatment and control sites.

Hourly bat activity (bat passes per hour block) data were non-normally distributed; ($\chi^2_{700} = 0.293$, $p < 0.001$). Activity varied throughout the night, on an hourly basis, ($\chi^2_{13} = 256.201$, $p < 0.001$), with activity typically being highest in the early evening hours, between 18:00 and 20:00 (hourly passes $\bar{X} = 8.2 \pm 7.2$) and lowest in the early morning between 03:00 and 05:00 (hourly bat passes $\bar{X} = 3.4 \pm 2.1$) (Figure 6.3.) (Appendix 3: Table 6.2.).

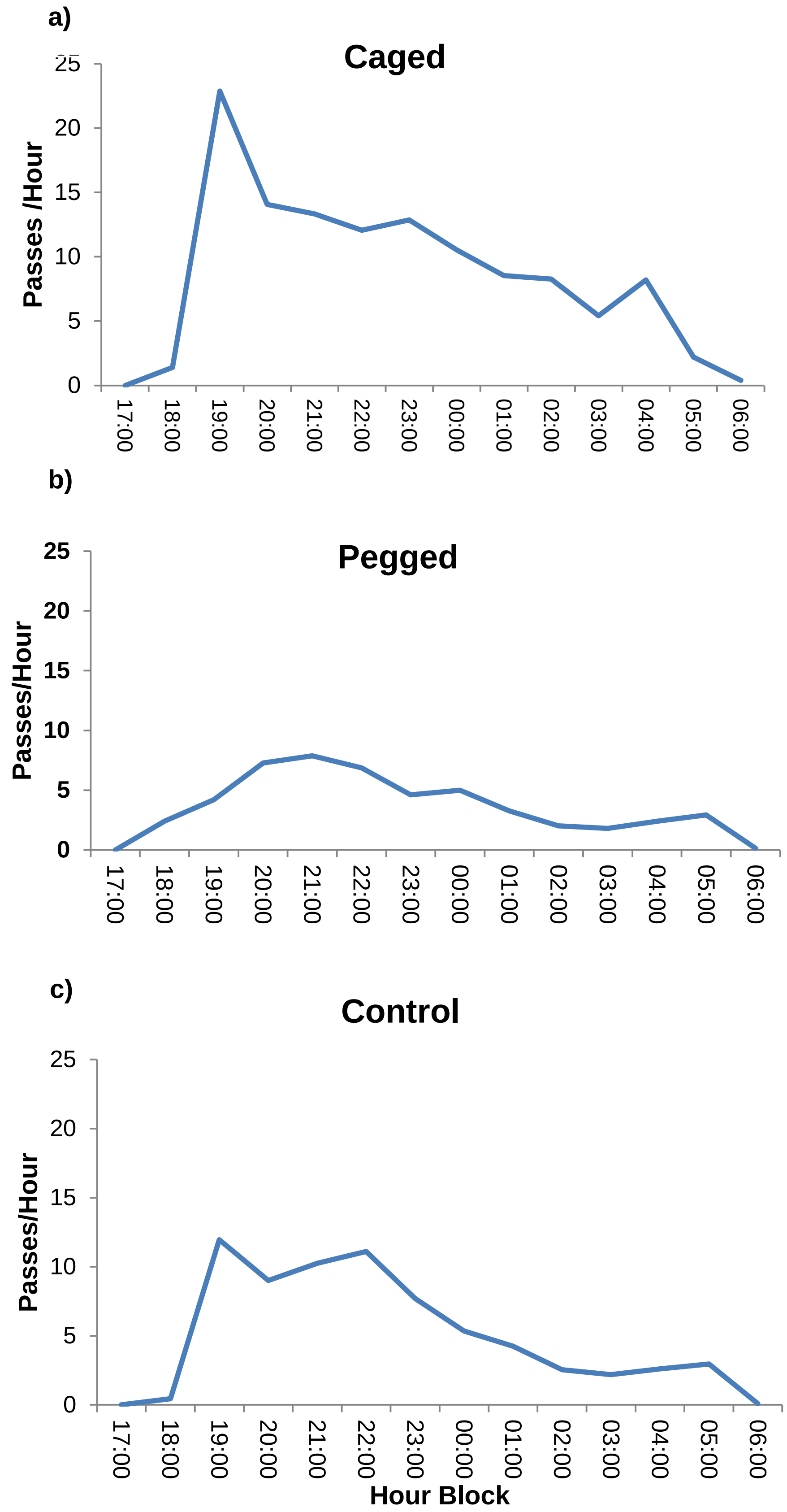

Figure 6.3. Mean hourly bat passes per carcass treatment type (a) caged, b) pegged and c) control) on the Telperion Nature Reserve during January and February 2019

6.1.3 Differences in bat activity between carcass treatment and control sites

Bat activity data were non-normally distributed (Appendix 3: Table 6.1.). Almost twice as many bat passes were recorded over caged carcass sites (X̄ = 132 ±131 per night) when compared to pegged (X̄= 54 ±50 per night) and control (X̄= 66 ±43 per night) sites (Figure 6.4.), however within site variation was high and there was no significant difference in the total number of bat passes between caged, pegged, and control sites ($\chi^2{}_2$ = 4.768, p = 0.092). The percentage of bat passes that contained feeding buzzes (an indication of foraging) was substantially higher at caged carcasses (8.18 %) than at either pegged carcasses (4.06 %) or control sites (3.64 %). However, there was no significant difference in the number of feeding buzzes between the two treatments and control sites ($\chi^2{}_2$ = 4.850, p = 0.088) (Appendix 3: Figure 6.1.). The overall number of bat passes and feeding buzzes did not differ significantly between control sites and carcass sites before carcass placement (Appendix 3: Table 6.1.).

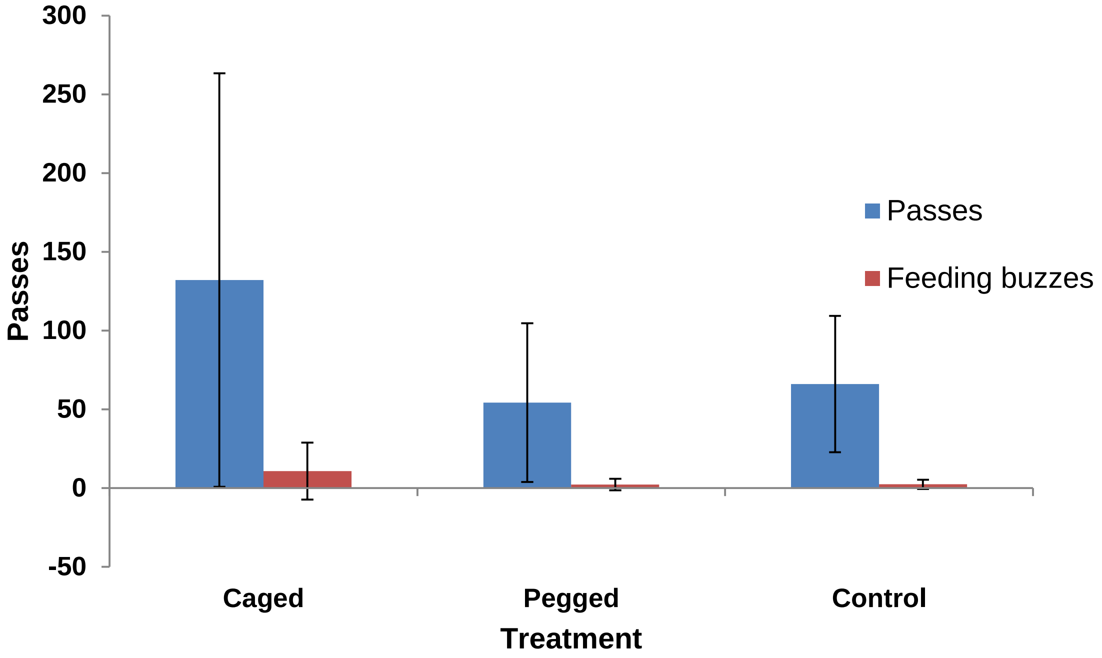

Figure 6.4. Treatment specific (X̄ ±SD) chiropteran passes and feeding buzzes at blue wildebeest (*Connochaetes taurinus*) carcass sites on Telperion Nature Reserve during January and February 2019

6.1.4 Intra-site variation in activity at carcass treatment and control sites

The number of bat passes per treatment post carcass deployment varied significantly within sites: caged ($\chi^2{}_3$ = -31.96, p < 0.001), (X̄= 81.98 ±78.79) per evening, pegged ($\chi^2{}_3$ = -15.13, p = 0.030), (X̄ = 50.73 ±46.01) per evening, control ($\chi^2{}_3$ = -23.00, p < 0.001), (X̄ = 65.95 ±43.31) per evening.

6.2. Species richness and diversity at carcass treatment and control sites

Species richness data post carcass deployment were non-normally distributed (χ^2 = 0.207, DF = 699, p< 0.001). Mean species richness was significantly (χ^2 = 50.424, p = 0.008) higher at Caged ($\bar{X}$ = 15.00 ±3.61) than at Pegged ($\bar{X}$ = 13.60 ±2.88) carcass sites. Species richness was significantly higher (χ^2= 48.894, p = 0.006) at Control ($\bar{X}$ = 17.40 ±3.58) sites than at caged and pegged carcass sites. There was no significant difference (χ^2 = 1.530, p = 0.932) in species richness between caged and control sites (Figure 6.5.). Pegged sites were associated with the lowest species richness of all of the carcass treatments (Figure 6.5.).

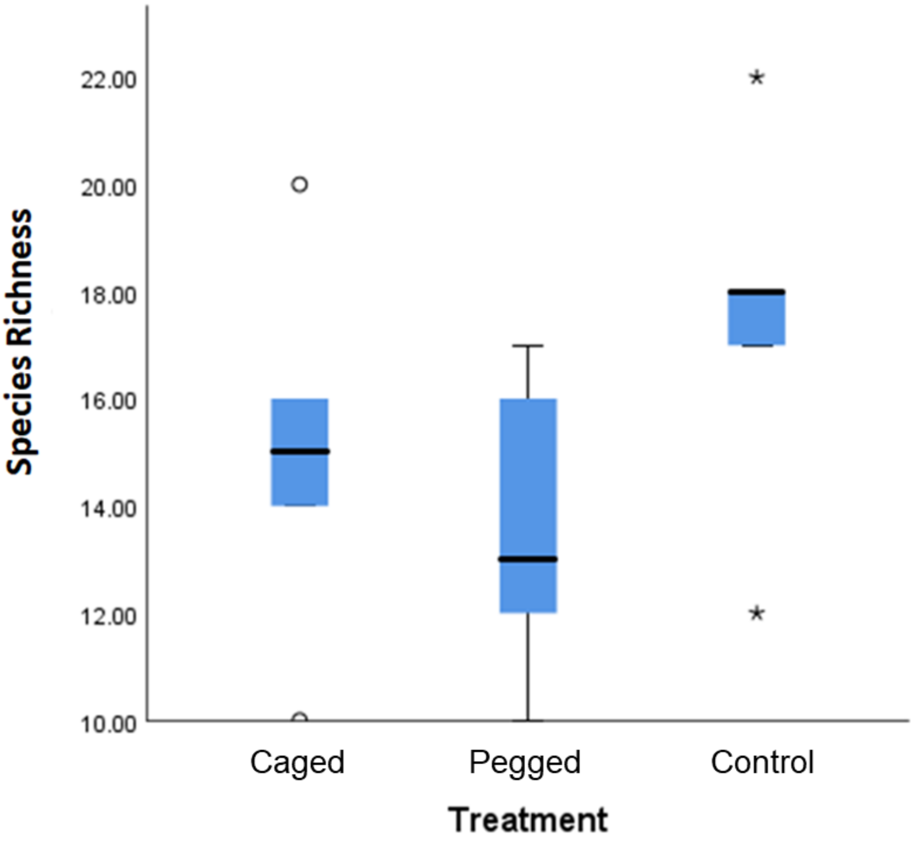

Figure 6.5. Treatment specific variation in chiropteran species richness for blue wildebeest (*Connochaetes taurinus*) carcasses deployed on the Telperion Nature Reserve between January and February 2019

Outliers were likely due to favourable or unfavourable foraging conditions or proximity to roost sites.

Hourly species richness was non-normally distributed (Kolmogorov-Smirnov) (χ^2_{699} = 0.286, p< 0.001). Species richness varied significantly according to the hour of the night (χ^2_{13} = 244.839, p< 0.001), with species richness being highest in the early evening between 18:00 and 20:00 ($\bar{X}$= 2.9 ±0.9) and lowest in the morning hours between 03:00 and 05:00 ($\bar{x}$ 2.3 ±0.5) (Figure 6.5., Figure 6.6.). Sunrise and sunset times are presented, should activity levels after sunset be required (Appendix 3: Table 6.5.) Pairwise comparisons between hour blocks and species richness also yielded significant results these comparisons exhibited the most species documented in the early evening with the species richness tapering off into the night, with a small peak in species richness sometimes evident before dawn (Figure 6.6., Appendix 3: Table 6.3.).

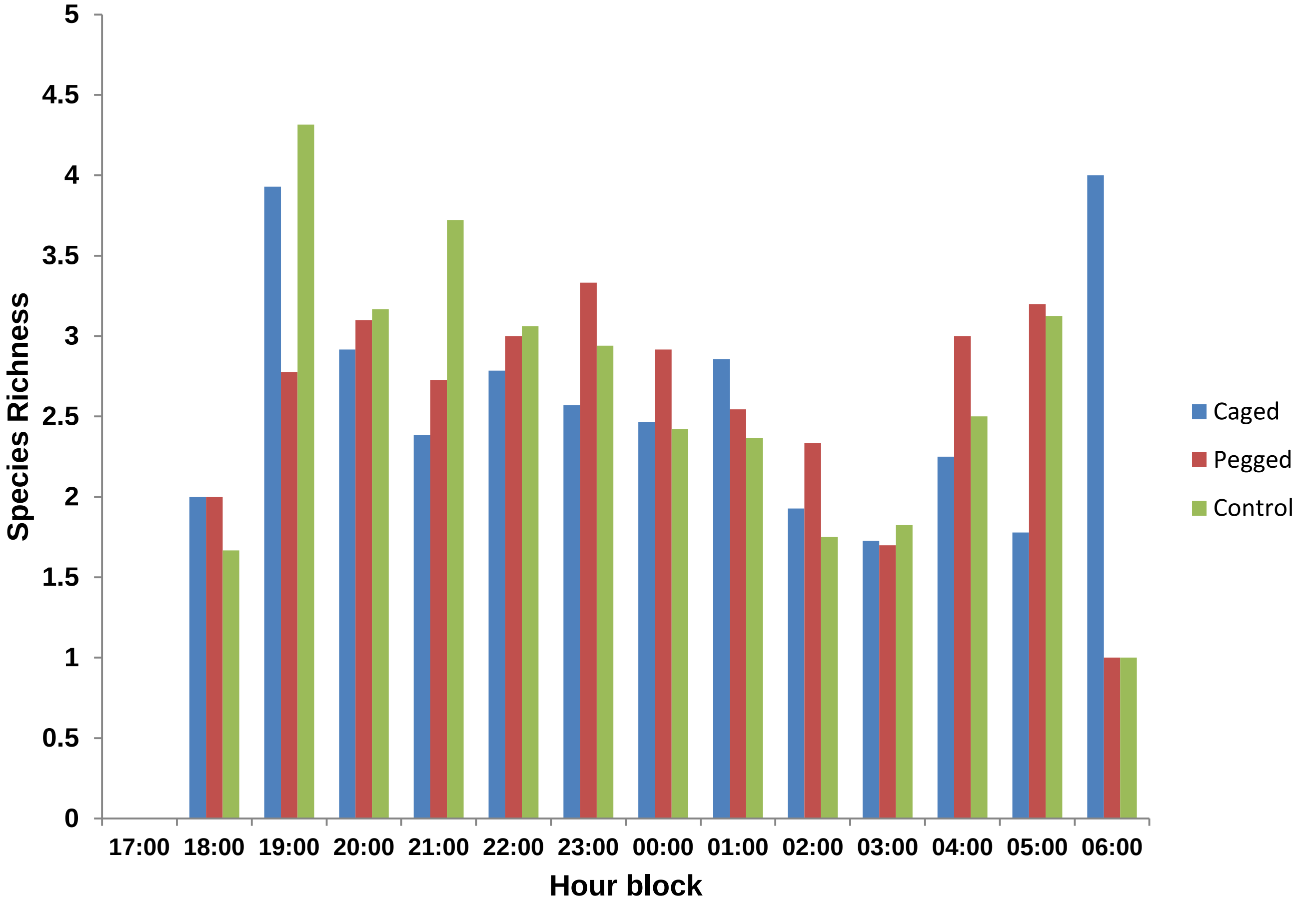

Figure 6.6. Hourly, treatment specific bat species richness after blue wildebeest (*Connochaetes taurinus*) carcass placement on the Telperion Nature Reserve during January and February 2019

The species diversity was relatively uniform across the sites, caged (Chao 1 = 18.21, H = 1.50), pegged (Chao 1 = 19.29, H = 1.45) and control (Chao 1 = 20.00, H = 1.44).

6.3. Species specific associations and functional guilds present at carcass treatment and control sites

Twenty bat species representing five foraging guilds were detected over both carcass and control sites (Table 6.1.). *Hipposideros caffer* was not identified during acoustic sampling around carcass sites and was not included in table 6.1. (however, it was actively captured on the reserve).

Table 6.1. Chiropteran species detected at blue wildebeest (*Connochaetes taurinus*) carcass and control sites on the Telperion Nature Reserve during January and February 2019

Family/ Species	**Functional group**	**Cage**	**Pegged**	**Control**
Emballonuridae				
Taphozous mauritianus	Group 3	*	*	*
Miniopteridae				
Miniopterus natalensis	Group 1	*	0	0
Miniopterus fraterculus	Group 1	*	*	*
Molossidae				
Mops (Chaerephon) pumilus	Group 3	*	*	*
Mops midas	Group 2	*	*	*
Otomops martiensseni	Group 2	*	*	*
Tadarida aegyptiaca	Group 3	*	*	*
Tadarida ventralis	Group 2	*	*	*
Nycteridae				
Nycteris thebaica	Group 4	*	0	*
Rhinolophidae				
Rhinolophus simulator	Group 4	*	*	*
Rhinolophus clivosus	Group 4	0	0	*
Vespertilionidae				
Eptesicus hottentotus	Group 3	*	*	*
Myotis bocagii	Group 1	*	*	*
Myotis tricolor	Group 1	*	*	*
Laephotis capensis	Group 1	*	*	*
Neoromicia zuluensis	Group 1	*	*	*
Nycticeinops schlieffeni	Group 1	0	*	*
Pipistrellus hesperidus	Group 1	*	*	*
Pipistrellus rusticus	Group 1	*	*	*
Scotophilus dinganii	Group 3	*	*	*

Functional groups are discussed in more detail in chapter 5

Group 1: Intermediate / high frequency FM clutter edge foragers
Group 2: Large, low frequency LD-QCF open air foragers
Group 3: Intermediate and lower frequency edge and open-air foragers
Group 4: HD-CF and multi-harmonic clutter foragers
Group 5 (*H. caffer*) was not included as it was only recorded during active captures.
indicates species detected, 0 indicates species not detected.

All functional groups, except for Group 5 (a monospecific group that only included *H. caffer*), were detected during the carcass sampling component (Table 6.2.). Intermediate / high frequency FM clutter edge foragers (Group 1) were most active around all of the treatment sites, all guilds except HD-CF Very High frequency clutter foragers (Group 5) were recorded over carcass and control sites. Feeding buzzes were recorded at all treatments and during all sampling nights.

Table 6.2. Total Number of foraging guild specific bat passes per blue wildebeest (*Connochaetes taurinus*) carcass treatment on the Telperion Nature Reserve during January and February 2019

Treatment	Group 1	Group 2	Group 3	Group 4	Group 5
Caged	1488	23	162	1	0
Pegged	482	37	162	5	0
Control	755	29	306	4	0

Note: All carcass and control sites were situated in open grassland habitats.
Group 1: Intermediate / high frequency FM clutter edge foragers
Group 2: Large, low frequency LD-QCF open air foragers
Group 3: Intermediate and lower frequency edge and open air foragers
Group 4: HD-CF and multi-harmonic clutter foragers
Group 5 (*H. caffer*) was not included as it was only recorded during active captures.

No significant differences were observed in the number of bat passes between carcass treatment sites for any of the foraging guilds (Appendix 3: Table 6.4.).

Species specific activity over carcass and control sites is best described using an activity index, this corrects the activity of species not often recorded or those that are difficult to record using acoustic sampling methods (Table 6.3.).

Table 6.3. Comparison of Miller AI values between control sites and carcass sites (caged and pegged combined) on the Telperion Nature Reserve during January and February 2019

	Species specific activity index	
	Carcasses	**Control**
Family/ Species	**(Caged and Pegged Merged)**	
Emballonuridae		
Taphozous mauritianus	0.0042	0.0042
Miniopteridae		
Miniopterus natalensis	0.0006	0.0012
Miniopterus fraterculus	0.0001	0.0000
Molossidae		
Mops (Chaerephon) pumilus	0.0032	0.0022
Mops midas	0.0019	0.0017
Otomops martiensseni	0.0022	0.0003
Tadarida aegyptiaca	0.0033	0.0026
Tadarida ventralis	0.0053	0.0069
Nycteridae		
Nycteris thebaica	0.0006	0.0001
Rhinolophidae		
Rhinolophus clivosus	0.0000	0.0001
Rhinolophus simulator	0.0004	0.0001
Vespertilionidae		
Eptesicus hottentotus	0.0057	0.0060
Myotis bocagii	0.0015	0.0009
Myotis tricolor	0.0020	0.0015
Laephotis capensis	0.1266	0.0438
Neoromicia zuluensis	0.0028	0.0019
Nycticeinops schlieffeni	0.0002	0.0003
Pipistrellus hesperidus	0.0010	0.0010
Pipistrellus rusticus	0.0013	0.0019
Scotophilus dinganii	0.0061	0.0063

CHAPTER SEVEN

DISCUSSION

7.1. Overview

Worldwide, grasslands are one of the most extensive biomes as they cover a considerable area, over several continents including North America (prairies), Europe and Asia (steppes), South America (pampas) and Africa (grasslands or veld) (Zhao, Liu, & Wu, 2020). The considerable land area (40.5% of total unfrozen land area) covered by grasslands (Zhao *et al.,* 2020) contributes to agricultural crop, dairy and meat production, flood control, carbon storage, soil erosion mitigation, nutrient cycling, nutrient retention, biomass production and terrestrial cooling during the night (Peciña *et al.,* 2019; Zhao *et al.,* 2020). Grasslands with higher landscape heterogeneity tend to support greater species richness and diversity as they comprise a broader habitat variability (Gaujour *et al.,* 2012). Chiropteran biodiversity in grasslands is typically understudied, with relatively few publications compared to more tropical and structurally (vegetation) complex habitats (Anderson, Norton, & Mathews, 2020). Worldwide, bats tend to be more prevalent around structurally complex grasslands or grasslands containing vertical vegetation elements or water sources (Barros, Pessoa, & Rui, 2014; Braun de Torrez, Ober, & McCleery, 2018; Ewert, Knörnschild, Jung, & Frommolt, 2023; Heim, Treitler, Tschapka, Knörnschild, & Jung, 2015). When assessing bat diversity, there may be utility in documenting areas of complex vertical structure within grasslands, particularly within close proximity to water or roost resources as such areas typically harbour higher bat species diversity in otherwise structurally homogeneous grassland habitats (Braun de Torrez *et al.,* 2018; Gaujour *et al.,* 2012; Jung, Kaiser, Bohm, Nieschulze, & Kalko, 2012). There is good reason to focus field-based biodiversity studies on structurally diverse grasslands, particularly those containing islands or microhabitats with complex vegetation structure, vertical landscape elements, or other resources (prey, roost, linear landscape elements) (Heim *et al.,* 2015), as these areas support high levels of bat diversity and activity, and may serve as source populations for forays of species into homogenous grasslands (Walsh & Harris, 1996)

7.2. Species Inventory, Regional Call Library and Call Variation

There is a gradient of increasing bat diversity from southern Africa, north into equatorial Africa (Monadjem *et al.,* 2018). This is due to the increase in vegetation structure (associated with savanna and forest habitats) and higher rainfall towards the equator (Monadjem *et al.,* 2018). The mixed habitats in north-west Africa seem to exhibit the same trends as Telperion Nature Reserve, with increasing bat species richness being correlated with increasing habitat heterogeneity (Fahr & Kalko, 2011), likely due to increasing resource availability. Throughout Africa bat assemblages are most diverse in heterogeneous habitat patches containing resources such as, roost sites, prey, water, and linear landscape features (as travel corridors), particularly in areas with diverse vertical vegetation structure and edge habitats (Batary *et al.,* 2010; Fahr & Kalko, 2011; Gaujour *et al.,* 2012; Katunzi *et al.,* 2020; Monadjem *et al.,* 2018). The comparative study of Seamark (2013) indicated that southern African grasslands harbour fewer bat species (n = 39) than the savanna biome (n=56). Telperion Nature Reserve offers a unique opportunity to study and understand grassland-associated bat assemblages as it is situated in a transitional zone between the grassland and savanna biomes (Mucina & Rutherford, 2006).

The grassland mosaic of Telperion Nature Reserve seems to support a relatively rich and diverse assemblage of bats. Previous studies have recorded 13 species from four families (Greyling & Keith, 2013; Kearney *et al.,* 2019). The present study has nearly doubled the bat species list for the reserve, and has recorded 22 species representing seven families. This high diversity is unexpected considering that the reserve is relatively small (5600 ha). Not only was the bat diversity on Telperion Nature Reserve high for a relatively small grassland-dominated reserve, but also in comparison with other habitats that are more structurally diverse, and typically associated with high levels of bat diversity. The high bat biodiversity documented on Telperion Nature Reserve may not be surprising as this reserve is located on an ecotone between the grassland and savanna habitats, ecotones typically exhibit higher diversity than the surrounding biomes due to their structural complexity and species diversity (Kark, 2013).

The subtropical, moist northern (e.g. Pafuri), north-eastern and eastern parts of South Africa, encompassing the Limpopo, KwaZulu-Natal and Eastern Cape provinces, and Eswatini (previously Swaziland), comprise Afromontane, coastal forest mosaic, and dry savanna ecoregions (Olson *et al.,* 2001), and are some of the most diverse and bat species rich areas of South Africa (Brinkley, Parker, & Taylor, 2018; Cooper-Bohannon *et al.,* 2016; Monadjem *et al.,* 2020). The Pafuri region supports at least 59 of the 67 bat species recorded in South Africa (Monadjem *et al.,* 2020; O'Donoghue *et al.,* 2020; Taylor *et al.,* 2022). The southern KwaZulu-Natal and Eastern Cape forests harbour 25 species from eight families (Moir *et al.,* 2020b), the Soutpansberg mountain range, within Limpopo, supports approximately one third of South African bat taxa (Linden *et al.,* 2014; Taylor, Sowler, Schoeman, & Monadjem, 2013; Weier, Linden, Gaigher, White, & Taylor, 2016), while 42 species have been recorded from the Kruger National Park (Brinkley *et al.,* 2021; Rautenbach, Fenton, & Braack, 1985). The small country of Eswatini harbours 32 species from nine families (Monadjem *et al.,* 2021). The high diversity associated with the region is likely due to the subtropical climate, the vegetation diversity and structure, the availability of a diversity of microhabitats, and the high rainfall (Cooper-Bohannon *et al.,* 2016; Gelderblom *et al.,* 1995; Monadjem, Schoeman, Reside, Stoffberg, *et al.,* 2010; Schoeman & Monadjem, 2018; Venter & Gertenbach, 1986). In contrast, studies by Howard, Monadjem, MacFadyen, & Chimimba, (2022) and Power *et al.,* (2019) sampled grassland mosaic habitats in the North West and KwaZulu-Natal provinces, respectively, recorded only 11 bat species. This relatively low species richness may be explained by sampling limitations. In the South African context, Telperion Nature Reserve seems to be a node of bat diversity. On closer assessment, this is unsurprising as this heterogenous, grassland-dominated, reserve comprises a variety of habitats with 22 distinct plant communities (Brown *et al.,* 2022), each with ecotonal microhabitats that species from a variety of feeding guilds, apart from frugivores, might be able to exploit (Downs & Racey, 2006). The apparent absence of fruit bats may be due to the lack of appropriate foraging and roosting resources for such species in this mixed grassland.

The success of this study, in terms of the number of species identified, is likely attributable to two factors, the duration of the study and the diversity of methods employed. Seasonal sampling was employed and, predictably, some species were not recorded during the colder periods of the year. High sampling effort (both in terms of active captures and passive recordings) was necessary to detect and identify species with low intensity echolocation parameters and those that avoided capture devices.

During this study a combination of both active and passive sampling techniques were used. This approach has been adopted by several researchers in South Africa, including Moir *et al.,* (2020a) in the Eastern Cape and southern KwaZulu-Natal regions, (Howard *et al.,* 2022) in the KwaZulu-Natal Drakensberg foothills, Taylor, Sowler, *et al.,* (2013) in the northern Limpopo region and Brinkley *et al.,* (2021) in the Kruger National Park. Using multiple sampling techniques to inventory animals is ubiquitous in animal ecology (Appel *et al.,* 2021; Blake & Loiselle, 2001; Redding & Apple, 2019). The use of multiple techniques to sample the same group enhances the accuracy of any inventory as elusive or cryptic species that are difficult to detect using one method may be detected using alternative or complimentary methods (Howard *et al.,* 2022; Kingston, 2016). Thus, using the combination of active and acoustic sampling maximised bat species detection on Telperion Nature Reserve.

Both active (hand-netting, mist netting and harp trapping) and passive (acoustic recording) sampling methods were effective, however, there are pros and cons to both approaches when used exclusively, however when used in unison they are complimentary and allow a wider assemblage to be documented (Kingston, 2016). Passive detectors allow for continuous sampling with minimal disturbance to a large assemblage of species (Jones, Vaughan, & Parsons, 2000; Kingston, 2016). Acoustic recording does however, require a large amount of time to comb through the data and make accurate species identifications, particularly where species with overlapping parameters are present. Active sampling, particularly mist netting and hand netting (that in the case of Telperion Nature Reserve yielded a varied species haul) allowed positive identification of species from morphometrics and afforded the opportunity to record release calls. Harp trapping was less effective on Telperion Nature Reserve due to the large open areas associated with grassland habitats, and, as a result, was only used at roost sites and in dense, vegetated flyways. Active trapping is however subject to bioclimatic constraints and seldom results in the capture of certain bat taxa (particularly high flying molossids), unless this is undertaken at roost sites (Brinkley *et al.,* 2021; Fenton & Rautenbach, 1986).

The combination of these methods allowed the identification of 22 species on the Telperion Nature Reserve. Twenty-one species were identified using passive acoustic sampling, whereas active sampling methods recorded 12 species: 10 by mist-netting, five by hand netting / active searches and four by harp-trapping. Some overlap in haul, between methods, is typical (Kingston, 2016), as was the case in this study. Ubiquitous species such *Laephotis capensis* and *Scotophilus dinganii* were recorded using multiple sampling techniques. Using this combination of methods allowed the documentation of various species, in habitats, where they may not have been detected using acoustic monitoring alone. Species with rapidly attenuating calls (ie. *Hipposideros* spp.), or species with low call intensity (ie. *Nycteris* spp.) are seldom detected using acoustic sampling. Furthermore, some groups such as rhinolophoid bats (Wechuli, Webala, Patterson, & Ochieng, 2016) and fast, high-flying molossid species (i.e. *Tadarida aegyptiaca*) (Monadjem *et al.,* 2020) are adept at avoiding active capture devices.

The value of using both active capture and passive recording methods, when sampling bat assemblages, is emphasised by the case of *Hipposideros caffer* on Telperion Nature Reserve. This species was neither detected nor identified during passive acoustic sampling, however, it was captured using hand netting. The failure to detect this species using passive acoustic methods is likely due to the high frequency, and resultant rapid attenuation, of its echolocation calls (Griffin, 1971; Lawrence & Simmons, 1982). It seems that, in the case of Anabat detectors, the maximum detection distance for *Hipposideros caffer* is ca. 0.3 m (Monadjem, *et al.,* 2017), consequently, the likelihood of detecting this species, using acoustic monitoring with these devices, is low. *Hipposideros caffer* is adept at avoiding capture devices (Wechuli *et al.,* 2016), and was only detected as a result of the use of hand netting. *Nycteris thebaica* was seldom recorded during passive monitoring, probably due to the low intensity of the calls that it produces (Monadjem *et al.,* 2017, 2020), however, individuals of this species were regularly captured in mist nets and hand nets.

This study revealed relatively high molossid diversity, five species (*Mops (Chaerephon) pumilus, Mops midas, Otomops martiensseni, Tadarida aegyptiaca and T. ventralis*) likely present on the reserve. While these five species were regularly recorded using acoustic sampling, no individuals were ever physically captured. Their fast and high-flying behaviour likely contributed to poor capture success (Monadjem *et al.,* 2020). Active searches of anthropogenic structures, both in use and abandoned, did not yield evidence of their presence. This suggests that synathropic molossid species such as *Mops* (*Chaerephon*) *pumilus*, may make use of naturally occurring roosts on Telperion Nature Reserve, such as rocky crevices and crevasses (Monadjem *et al.,* 2020). In addition, molossid social calls were recorded proximate to boulders and in rock crevices, along the steep sandstone-granite gorge faces, however, accessible, active roosting sites for the afore-mentioned species could not be identified, hence the reliance on acoustic data, alone, to document their presence on Telperion Nature Reserve.

In addition to there being variation between species detection by acoustic and active methods, there was variability in detectability using acoustic sampling. Southern African bats emit calls across a broad spectrum of frequencies (10 - 210 kHz; Monadjem *et al.,* 2020). On Telperion Nature Reserve, *Otomops martiensseni* emit calls in the range of ca. 12 kHz while *Hipposideros caffer* call at ca. 139 kHz. Furthermore, there are a variety or organisms that emit sounds within the lower frequency ranges (10 – 12 KHz) of bat calls. This can result in some of the bat vocalisations being designated as noise files when using automated call vetting. Therefore, the value of using bat detectors that are designed to detect calls within a wide frequency range, and analysis programs that are calibrated for the analysis of calls throughout this broad frequency range, cannot be over emphasised. It is clear, from this, that there are many specific considerations associated with biodiversity monitoring utilising passive acoustic monitoring (Kingston, 2016).

During this study the species accumulation curve, for acoustic sampling, approached an asymptote after approximately 10 nights. However, four detectors were continually rotated throughout the reserve, consequently, one night equated to four bat-detector nights of sampling. It seems, therefore, that at least forty detector nights of acoustic sampling provides a good baseline data set for the development of a site-specific species inventory. In a study with similar sampling effort, Moir *et al.* (2020a) employed six SongMeter ultrasonic detectors over six nights (36 detector nights) within 17 forests, assigned to seven different forest types, in KwaZulu-Natal and the Eastern Cape. In their study Moir *et al.* (2020a) found that species richness estimates from acoustic data, plateaued after 7 to 10 sampling nights, except in the Transkei Coastal Scarp forest where the species accumulation curve did not reach an asymptote, even after 22 nights. The habitat type, amount of bat activity, and the species' distribution influences the sampling effort required to develop what might approximate a ‘complete’ inventory (Kingston, 2016; MacSwiney, Clarke, & Racey, 2008; Moir *et al.,* 2020a).

Accurate taxonomic assignment is important for gaining a sound understanding of relative, or species-specific activity levels (Nadin-davis, Guerrero, Knowles, & Feng, 2012). There are various published bat call libraries for South Africa. Most of these are for the northern and eastern parts of the country, with habitats comprising complex vertical vegetation structure (forests and savanna habitats) and high rainfall (Moir *et al.,* 2020; Monadjem *et al.,* 2017, 2020; Parker & Bernard, 2018; Shapiro & Monadjem, 2016; Taylor, Sowler, Schoeman, & Monadjem, 2013)**,** where bat diversity is highest**.** These call libraries have utility, as they allow for the quantification of a baseline bat assemblage, the assessment of foraging guilds, habitat use, and activity patterns using acoustic sampling alone (Sherwin, Gannon, & Haymond, 2014).

Apart from Kearney *et al.,* (2019), who reported four call parameters (Fc - characteristic frequency, Sc – slope at start of the call, Tc – time from start of call to Fc, and duration of call) for three species, and Howard *et al.,* (2022) who provided six parameters (Fc, Sc, Fmin – minimum frequency, Fmx – maximum frequency, Fmean – mean frequency and duration) for 10 species, few attempts have been made to develop call libraries for open grassland habitats, particularly in eastern Gauteng / western Mpumalanga region of South Africa. It is apparent from this study that there is a need for the development of call libraries for grassland habitats, such as those represented by Telperion Nature Reserve, that have been neglected due to historical biogeographical sampling biases (Gelderblom *et al.,* 1995; Howard *et al.,* 2022) or the perception that the region has a low species richness (Howard *et al.,* 2022). Furthermore, documenting call variation across a diversity of habitat types and regions allows for the description of regional variation in bat calls which will enhance the ability of automated identification protocols to accurately identify bats to species level (Parker & Bernard, 2018; Stahlschmidt & Bruhl, 2012).

The development of the call library for this study followed the methodology of Brinkley *et al.,* (2021) and Moir *et al.,* (2020a) using time expansion data gathered by a handheld detector EchoMeter 3 (EM3), in combination with SongMeter (SM2 Bat+ and SM4 FS) detectors. The different makes and models of bat detectors vary in their detectable recording spaces, thus acoustic data may not be directly comparable between recording stations or studies (Darras, Pütz, Fahrurrozi, Rembold, & Tscharntke, 2016). Most other South African studies have used AnaBat detectors (Howard *et al.,* 2022; Linden *et al.,* 2014; Monadjem *et al.,* 2017; Taylor *et al.,* 2013) or Avisoft recorders (Schoeman & Jacobs, 2008).This study therefore expands the available call data derived from Song meter detectors in South Africa. Call libraries have utility in documenting known species and assemblages, however, when new species (Taylor *et al.,* 2022), cryptic species (e.g. Howard *et al.,* 2022)*,* or species with overlapping call parameters (that are difficult to discern (Linden *et al.,* 2014; Moir *et al.,* 2020a)) are involved, active captures make species identification more reliable. Consequently, there is value in continually updating and improving the baseline call libraries, to add new species as they are detected and update and supplement data for existing species libraries. Acoustic monitoring should be continued in areas with existing call libraries to allow for the validation of those libraries and to detect and document elusive species that may have been missed in the past, or whose ranges have expanded into new areas (Braun De Torrez *et al.,* 2017).

Species assignments based on acoustic data alone, in the absence of a comprehensive, site-specific, call library, are likely to carry some level of error (Linden *et al.,* 2014). *Mops* (*Chaerephon*) *pumilus* and *Taphozous mauritianus* generally have overlapping call parameters; however, the former can be distinguished from the latter by the general absence of call harmonics, a broader call bandwidth and longer call duration (Monadjem & Reside, 2008; Schoeman & Jacobs, 2008). There can be some confusion when identifying species based on acoustic parameters alone. (Greyling & Keith, 2013) identified *Rhinolophus darlingi* on Telperion Nature Reserve based on its call at approximately 82 kHz. However, when active captures were used in conjunction with acoustic sampling (as in this study) it was apparent that the species was likely *Rhinolophus simulator* rather than *Rhinolophus darlingi,* reinforcing the necessity of complimentary sampling methods to confirm species assignment from acoustic parameters. Further acoustic sampling of *Hipposideros caffer* and *Nycteris thebaica*, for the purposes of bolstering call library data for these species within the region, is necessary.

During this study a bat species (with Fc of ~79 Khz)(*Rhinolophus* cf. *simulator*) that was morphologically similar to *Rhinolophus simulator* was recorded, yet it differed in two important ways from the other two *Rhinolophus* species (*Rhinolophus simulator* s.s. and *Rhinolophus clivosus)* found on Telperion Nature Reserve. *Rhinolophus* cf. *simulator* 79 was captured in tree-grass interface habitats and nearby anthropogenic structures. *Rhinolophus* cf. *simulator* 79 has a different nose-leaf connecting process morphology and emitted calls with a lower mean characteristic frequency (*Rhinolophus* cf. *simulator* 79, Fc = 79.95 ±0.92 kHz) than *Rhinolophus simulator* s.s. (Fc = 83.49 ±1.37 kHz) and *Rhinolophus clivosus* (Fc = 90.63 ±0.68 kHz) that were also recorded from the reserve during this study. In the southern African context, most rhinolophid species emit echolocation calls with Fc ranging between 82 – 111 kHz, with the exception of the *R*. *hildebrandti* complex (~33, 38, 40, 46 kHz), *R*. *fumigatus* (~54 kHz) and *R*. *deckeni* (~72 kHz) (Monadjem *et al.,* 2020). For the purposes of this study most comparisons were made with *R. simulator* s.s. as it seemed to emit calls with the most similar echolocation parameters. The call parameters of *R.* cf. *simulator* (R79) and *R*. *simulator* s.s. differed significantly in several parameters; mean characteristic frequency (Fc) (79.95 vs 83.49 kHz), frequency at the knee of the call (Fk) (79.18 vs

83.36 kHz), maximum frequency (Fmax) (81.28 vs 84.65 kHz) and minimum frequency (Fmin) (70.90 vs 73.36 kHz). This variability may be a consequence of intraspecific call plasticity in the call of *R. simulator*, alternatively it may be that *R*. cf. *simulator* (R79) is a separate species. To verify whether *R*. cf. *simulator* (R79) is a distinct species more active sampling will be required on Telperion Nature Reserve. The morphology of the nose leaves of *R.* cf. *simulator* (R79) should be accurately described (Monadjem *et al.,* 2020), voucher specimens should be collected and genetic analysis should be performed (Maree & Grant, 1997; Monadjem *et al.,* 2020). In addition, special attention should be paid to the release and free flight calls of rhinolophid species on the reserve to determine whether *Rhinolophus.* cf. *simulator* (R79) is indeed a distinct rhinolophid species.

This study corroborated several bat species call parameters associated with the eastern and northeastern regions of South Africa. It also substantiated the sex-specific variation in *Laephotis capensis* calls first presented by Kearney *et al.* (2019). Findings from the current study suggest that such variation is not limited to *L. capensis* but extends to the other vespertilionids, *Neoromicia zuluensis* and *Scotophilus dinganii*. Vespertilionid species are known to display sexual dimorphism (Monadjem, *et al.,* 2020). It is therefore, unsurprising that certain vespertilionid species have the potential for intersexual, intraspecific call variation as call frequency, for example, is known to negatively scale with body size (Jones, 1999). Morphological data, including body mass and forearm length (proxies for overall body size) recorded during this study, indicated female-biased sexual dimorphism in most vespertilionid, whereas rhinolophids showed male-biased sexual dimorphism. Due to limited sample sizes, intraspecific call variation could not be tested among all species captured during this study, ideally, ≥ 10 individuals of each sex should be captured and tested for intraspecific call variation.

In the case of *Myotis tricolor*, high individual call variation (irrespective of sex) resulted in a high degree of intraspecific call variation, confounding the assessment of any potential sex-linked variation. Other authors have presented similar findings for this species (Moir *et al.,* 2020a; Monadjem *et al.,* 2017). This study also tentatively reported call parameters (free flight calls, not based on catch-mark-release) for other species for which there are limited call data, in the South African literature, including *Eptesicus hottentotus* and *Tadarida ventralis*.

A further important justification for, and benefit of, physically capturing bats when assessing their diversity is the ability to record site and species specific morphometrics. Wing morphology in African and southern African bats has been well documented (Monadjem *et al.,* 2020; Norberg & Rayner, 1987; Schoeman & Jacobs, 2008), however, it is not fully understood whether it varies based on sex, geographic location or foraging strategy (Aspetsberger, Brandsen, & Jacobs, 2003). Site-specific wing morphology data is important as these data allow inferences to be made about the foraging habits and strategies of bats within specific habitats, and may provide insights into the microhabitats used as foraging patches. This study supplements the morphological data collated in Monadjem *et al.* (2020), by providing, in most instances, more comprehensive sex specific measurements (including hand wing area, arm wing area, total wing surface area) for the bats on Telperion Nature Reserve. These ecomorphological traits were used to delineate functional guilds among the Telperion Nature Reserve bat assemblage (see below).

7.3. The Effects of Habitat and Season on the Chiropteran Assemblage of Telperion Nature Reserve

7.3.1. The effects of habitat on bat species richness, diversity, and activity

Worldwide, open grasslands seem to be unfavourable to all but the largest open air bat species (Coleman & Barclay, 2013; Monadjem *et al.,* 2020). This is likely due to factors including predation risk and environmental factors that affect foraging, such as lack of vertical structure, increased lunar illumination or high wind speeds (Fenton, Boyle, Harrison, & Oxley, 1977; Meyer, Schwarz, & Fahr, 2004). Where grasslands support diverse bat assemblages there are typically associated microhabitats and/or resources (e.g. food, commuting or roost sites) that enhance bat survival and improve their fitness (Katunzi *et al.,* 2020).

Bats are typically associated with habitats that comprise varied vertical vegetation structure (Jung *et al.,* 2012). Grasslands usually lack complex vegetation structure and, consequently, are seldom thought to be associated with diverse bat assemblages. This preconception, coupled with historical biogeographical sampling biases (Anderson *et al.,* 2020; Coleman & Barclay, 2013; Fahr & Kalko, 2011; Monadjem *et al.,* 2020), has likely resulted in grassland bat assemblages being poorly studied and, consequently, poorly represented in literature (Howard *et al.,* 2022). Two bat-related studies have been conducted on the grassland-dominated Telperion Nature Reserve; however, both were limited in scope and sampling duration (Greyling & Keith, 2013; Kearney *et al.,* 2019). The study by Greyling & Keith, (2013) reported 10 species based only on acoustic data, but was limited in duration, acoustic sampling effort (only one recording system was utilised), and only two microhabitats that were sampled. The study by Kearney *et al.* (2019) predominantly involved active sampling, particularly around anthropogenic structures, resulting in the capture of eight species and the development of call library of echolocation parameters for three species. Kearney *et al.* (2019), however, did not include long-term, or wide-scale acoustic monitoring, seasonal monitoring or documention of the near complete assemblage on the reserve and how these species are distributed. This study addressed many of these shortcomings using a comprehensive and robust sampling strategy that incorporated multiple active captures methods and recording systems, across four different habitats, over four seasons. The value of this approach is self-evident in the amplification of the species haul for this site from 13 to 22 species.

Telperion Nature Reserve comprises a number of vegetation types with substantial variation in vegetation structure (grasslands, groves of invasive poplar and black wattle, and tree-grass interface habitats) and topography, (steep cliffs, undulating homogenous plains, vegetated rocky outcrops, vegetated, unvegetated and rocky water courses), (Brown *et al.,* 2022; Kearney *et al.,* 2019). This structural diversity likely influences the diversity of bats on the reserve (Ewert *et al.,* 2023; Heim *et al.,* 2015). The Telperion Nature Reserve is a gradual ecotone between the grassland and savanna habitats, with a complex mosaic of structurally diverse habitats or vegetation types (Brown *et al.,* 2022).

Gradual ecotones promote complex habitat mosaics able to support a large diversity of insectivorous bats (Broken-Brow, Armstrong, & Leung, 2019). The high species richness due to microhabitats or fragments of structurally complex habitat is well documented (Batary *et al.,* 2010; Fahr & Kalko, 2011; Gaujour *et al.,* 2012; Katunzi *et al.,* 2020; Monadjem *et al.,* 2018). In southeast Asia, forest fragments held greater diversity than the 'oceans' of altered habitat separating them, species diversity was also directly proportional to the size of the forested 'islands' (Struebig, Kingston, Zubaid, Mohd-Adnan, & Rossiter, 2008).

Terrestrial small mammal studies have shown Telperion Nature Reserve to support an abundance of small mammals, with a variety of microhabitats being associated with different species assemblages and densities (MacFadyen, 2014). The location of Telperion Nature Reserve within a matrix of farmlands, with both crop and stock farming being prevalent in the area may also have an effect on species diversity. However, in the case of terrestrial small mammals Gumbi *et al.* (2018) demonstrated similar outcomes in species richness, diversity and abundance in both natural and modified (agricultural) landscapes. It is well documented that bats utilise agricultural habitats, particularly when these areas are interspersed with structurally complex islands of natural habitat (Noer, Dabelsteen, Bohmann, & Monadjem, 2012; Struebig *et al.,* 2008). The position of the reserve and the diversity of microhabitats likely amplify its conservation value when compared to extensive homogenous undulating grassland habitats (Ewert *et al.,* 2023). Maintaining the conservation status of Telperion Nature Reserve will likely enhance connectivity between habitat islands and the preservation of such areas is important to reduce edge and island effects (Moir, Richards, Rambau, & Cherry, 2020a). This study demonstrates that Telperion Nature Reserve is likely of high bat conservation value within the grasslands of Gauteng and Mpumalanga.

Certain capture methods are more effective for specific bat species or within certain habitats (Kearney *et al.,* 2019; Kingston, 2016). For example, harp traps may be more effective in capturing clutter specialists such rhinolophoid bats, which are adept at detecting and avoiding mist nets

(Kingston, 2016). Mist nets and harp traps are typically erected perpendicular to the flight paths of bats within natural, semi-enclosed flyways, and between vegetation, rock faces or other vertical structures within the environment (Kunz, Hodgkison, & Weise, 2009). The physical capture of bats within grassland habitats is often compromised by the lack of vegetation structure. However, physical capture is necessary to positively identify species and confirm acoustic parameters.

Standard mist-netting was not conducted throughout the reserve as such sampling is dependent on the presence of suitable structure within the environment to erect the equipment. Habitat specific sampling effort ranged from 536.20 (rocky outcrop) to 3864.40 (anthropogenic) mist net hours; this increased sampling effort undoubtedly contributed to the increased number of total captures (n = 67) at anthropogenic sites vs at rocky outcrops (n = 14). *Laephotis capensis* roosts in most anthropogenic structures on the reserve (Kearney *et al.,* 2019), and capture efforts proximate to such structures elevated the number of total captures in this habitat. Harp trapping proved to be effective, particularly around anthropogenic sites (n = 47 captures) that contained medium to large-sized *L. capensis* roosts. It was also effective for capturing *Scotophilus dinganii.* However, it was ineffective in the more open habitats on the reserve. Harp-trapping is preferable to mist-netting where the capture of high numbers of individuals is anticipated (such as at large roost sites), as bats can be easily extricated from the collecting bag and processed quickly (Whitaker, McCracken, & Siemers, 2009), unlike the delicate and time consuming process of removing bats from mist nets.

Species richness, based on the combined results from active capture and acoustic detection methods, was relatively uniform with 19 species recorded from tree-grass interface and 21 species recorded from anthropogenic, rocky outcrops and water course sites. Species richness derived from acoustic detections was more uniform and greater than that based on active captures across the four habitats. This reinforces the value of using multiple sampling methods to inventory bat communities across different habitats (Howard *et al.,* 2022; Moir, Richards, Rambau, Ramugondo, & Cherry, 2020a).

Bat activity was not uniform throughout the reserve and certain habitats were associated with higher levels of activity. Bat activity was highest in the proximity of anthropogenic sites, likely due to the presence of *L. capensis* roosts (Kearney *et al.,* 2019). *Laephotis capensis* is the most common bat species on the reserve and was detected and captured in every habitat type and during every season. Their ubiquity is likely due to their adaptability, and habitat and roost plasticity (Moir & Durand, 2014). In addition, acoustic data may contain multiple detections of the same individual during the same recording session (Miller, 2001). This could apply to the synanthropic species, *L. capensis* and *T. mauritianus* previously documented from anthropogenic sites at Telperion Nature Reserve (Kearney *et al.,* 2019), leaving and returning to their roosts during nocturnal foraging bouts.

Hence, activity levels at anthropogenic sites, based on overall numbers of bat passes, are likely to be inflated. The anthropogenic structures on Telperion Nature Reserve likely function similarly to farmland matrices elsewhere (buildings surrounded by invasive and introduced vegetation within a natural/ modified grassland matrix). These structures often contained large roosts of *Laephotis capensis*, a generalist species well known for roosting in man-made structures (Kearney *et al.,* 2019; Shackleton, 2005). Other metropolitan species were also documented roosting in anthropogenic structures, an abandoned structure on the east of the reserve contained a roost of *Scotophilus dinganii*. Such structures provided valuable roosting habitat in the rooves and ceilings and synthesized foraging habitat around the anthropogenic light sources. The most behaviorally plastic and adaptable species, (typically generalist species) are known to adapt best to the presence of anthropogenic disturbance and habitat alteration (Moir & Durand, 2014; Weier, 2019). These phenotypically plastic species were the species typically found in anthropogenic structures on the Telperion Nature Reserve.

Bat activity was elevated around watercourse sites. This is likely due to three factors, the presence of prey resources (including water associated flying insects), the riverine vegetation and the watercourse that forms a linear landscape feature that allows safe commuting, and the presence of roost sites in riverside cliffs (Fenton *et al.,* 1977; Greyling & Keith, 2013). Lower activity levels were measured at rocky outcrop sites in comparison to anthropogenic sites or water causeways, with the lowest activity being present around tree-grass interface sites. The lower activity levels within the tree-grass interface may be due to lower food resources and roost availability relative to watercourse and rocky outcrop habitats (Hagen & Sabo, 2011; Monadjem *et al.,* 2020). This is expected as bat activity, species richness, diversity and abundance is higher in riparian sites, than in the adjacent savanna (Monadjem & Reside, 2008). Similar findings have been reported for the Kruger National Park, where capture success was significantly greater in riparian habitats than in the proximate dry savanna woodland, despite this, there was no significant difference in species diversity or evenness between the two habitat types (Rautenbach, Fenton, & Whiting, 2014). This study demonstrates that Telperion Nature Reserve is likely of high bat conservation value within the grasslands of Gauteng and Mpumalanga.

The highest relative proportion of feeding buzzes (5.0%) were recorded around vegetated rock outcrop sites, these sites provided potential roosts (personal observation) as well as potential foraging habitat for bats on the reserve (Kearney *et al.,* 2019). The rocky outcrops, where soils are shallow (MacFadyen, 2014), also provided ephemeral water sources (rock pools and ephemeral streams) which provided variable seasonal habitat for aquatic invertebrates (personal observation). The relative proportion of feeding buzzes were similar across other habitats, apart from anthropogenic sites. This study found 4.2% of calls recorded at water courses contained feeding buzzes. Many bat species used the river channel as a flyway for commuting, (see Kearney *et al.,* 2019) as it provides linear vegetation along the river banks and few obstructions along the river channel itself (Downs & Racey, 2006; Limpens & Kapteyn, 1991). Similarly, a high proportion of feeding buzzes (4.8%) were recorded at the tree-grass interface sites, however, the overall level of bat activity was relatively low (AI = 0.076). The congregation of prey (flying insects) around anthropogenic light sources serve as a high-density prey sources for insectivorous bats such as molossid and vespertilionid species (Schoeman, 2015). However, despite an expected increase in prey availability, the lowest proportion (2.6%) of feeding buzzes was recorded at anthropogenic sites. This phenomenon may be a consequence of the presence of medium-to-large sized roof roosts leading to higher, yet species-skewed, activity levels at these sites with corresponding lower number of feeding buzzes relative to the number of bat passes. Consequently, it is recommended that cumulative number of feeding buzzes be used as a metric to estimate feeding activity instead of reporting feeding activity as the proportion of feeding buzzes to total bat passes.

There were clear instances of habitat specific bat species associations. *Miniopterus fraterculus* and *Myotis bocagii* were associated more frequently with water course habitats. *Myotis bocagii* is known to depend on wetland habitats, foraging close to the surface of water (Monadjem *et al.,* 2020) and reeded river margins (Moir *et al.,* 2020a). *Myotis tricolor* was most often detected (acoustically) in vegetated habitats (tree grass interface and vegetated rock outcrops) and is classified as a habitat generalist (Moir *et al.,* 2020a) yet was only actively captured at water courses. Similarly, *M. bocagii* and *M. tricolor* frequents water courses to glean winged aquatic invertebrates near the surface of the water (Monadjem *et al.,* 2020). *Laephotis capensis* was frequently detected, and captured, in anthropogenic habitats. This species is well adapted to urbanisation and uses man-made structures for roosting, and it uses the associated anthropogenic light sources as foraging sites (Schoeman, 2015).

7.3.2. The effects of season on bat species richness, diversity, and activity

There are few chiropteran studies that are conducted over sufficient time to assess seasonal differences in assemblage and activity across seasons. Some interesting results were obtained by extending the sampling of this study to a four season approach and not only sampling during the wet and dry seasons, which is the norm (Klingbeil & Willig, 2010; Ortêncio-Filho *et al.,* 2014; Salinas-ramos *et al.,* 2015). Seasonal studies (spanning at least a year) provide more information regarding annual seasonal migrations, assemblage changes and seasonal activity variations and therefore have utility and mitigate seasonal biases typically associated with bat sampling (Weller, Cryan, & O’Shea, 2009).

Bat activity and species richness were elevated during the warm months similar to other austral sites in Australia (Gonsalves & Law, 2017) and Brazil (Barros *et al.,* 2014). Parker & Bernard, (2018) documented significantly higher activity during the warmer months than in colder periods in the Mapungubwe National Park. This higher activity during the warmer months may, in part, be due to young bats taking flight (Erickson & West, 2002). However, this does not explain the difference of one order of magnitude higher activity during the warmer months than during the colder months at Telperion Nature Reserve. A contributing factor to the lower activity during the colder months is likely also the relatively harsh environmental conditions (low temperatures, high wind speed), lower abundance of insect prey and the use of torpor to conserve energy. Similar trends have been observed in the northern hemisphere (Braun De Torrez *et al.,* 2017). However, in the northern hemisphere bats use hibernation and torpor to conserve energy to a larger extent than on Telperion Nature Reserve due to the harsher climate of boreal winter in higher latitudes (Blomberg *et al.,* 2021). In lower latitudes (latitudes roughly equivalent to those on Telperion Nature Reserve), in the United States, bats are known to be more active during the winter months than their counterparts at higher latitudes, however these bats still exhibit lower activity in the autumn and winter than during the spring and summer months (Bernard & McCracken, 2017; Geluso, 2007).

The fact that seasonality influences bat activity is reinforced by the results from the active capture efforts during this study. A total of 124 individual bats were captured during the course of this study. Active captures were highest during the austral autumn months (n = 52) and lowest during the winter months (n = 12), this despite an almost 1.5 times greater mist net hour effort during winter. The number of individuals captured were similar in spring and summer, with 32 and 28 individuals captured in each of those seasons, respectively.

In contrast to active captures, overall bat activity (total bat passes and bat passes per hour), was an order of magnitude lower during the autumn and winter than in the spring and summer. In addition, the cumulative number of feeding buzzes increased dramatically in summer. The highest percentages of feeding buzzes per bat pass was present in winter and summer, with feeding buzzes being present in 5.6% of calls during both winter and summer. In autumn feeding calls comprised 4.6% of all bat passes and in spring feeding buzzes comprised 2.0% of total passes. An important consideration is that the overall number of passes during summer and spring were higher than during autumn and winter by a substantial margin, despite the proportion of feeding buzzes not being higher. The relatively high proportion of feeding buzzes during the winter and autumn months makes sense as due to poor foraging conditions it is likely that bats would try to maximise foraging efficiency and limit the foraging period (due to higher energy expenditure), and as such may be less selective of prey and likely would forage as efficiently as possible (Agosta *et al.,* 2003; Salinas-ramos *et al.,* 2015). These constraints would be lower during spring and summer as foraging conditions are less harsh and prey availability is higher (Coleman & Barclay, 2013; Meyer, Schwarz, & Fahr, 2004; Munhenga *et al.,* 2014).This may be one of the reasons that the proportion of feeding buzzes per bat pass is lower during spring than during autumn and winter.

Insectivorous bats are known to forage heavily during the productive seasons (spring and summer) to build up fat reserves to allow torpor to be employed during the unfavourable, colder seasons, when the metabolic cost of thermoregulation is higher and prey resources are less abundant (Monadjem *et al.,* 2020). In a mixed agroecosystem in the subtropical Limpopo province, bat passes peaked in the warmer months, with the highest activity recorded during autumn when peaks in bat activity coincided with increased seasonal abundance of insect crop pests including stinkbugs and plant-boring moths (Taylor, Bohmann, *et al.,* 2013). The difference in peaks of activity between the present study and that of Taylor, *et al.,* (2013) may be a consequence of differential climatic conditions between the two sites. The Limpopo province is characterised by a subtropical climate with warmer temperatures persisting into the autumn months, unlike on Telperion Nature Reserve where there is a marked drop in temperature from April onwards (Climate-Data.org; URL: https://en.climate-data.org/africa/south-africa/mpumalanga/emalahleni-641/).

The apparent absence of certain bat species during winter passive sampling may, in part, be attributed to torpor and local migration (Moussy *et al.,* 2012). *Myotis tricolor*, whilst captured and recorded from passive sampling systems, appeared to be absent during the colder months (not captured, nor recorded). The absence of recordings of certain species (*Eptesicus hottentotus, Myotis bocagii, Myotis tricolor*) during winter could be due to extremely low levels of activity during the autumn and winter months (Schoeman & Monadjem, 2018). This reduced activity, or the use of torpor is likely an attempt to conserve energy when low temperatures prevail and arthropod prey availability is dramatically reduced (MacFadyen, 2014). This absence may also be attributable to seasonal migrations by this species (McDonald, Rautenbach, & Nel, 1990; Schoeman & Monadjem, 2018). Similar trends were found for *Miniopterus natalensis*, another species known to undertake local migrations within South Africa (Pretorius *et al.,* 2021). Interestingly, *Laephotis capensis* activity decreased by an order of magnitude during autumn and winter relative to spring and summer. This synanthropic species is known to utilise buildings and roof spaces as communal maternity roost (Kearney *et al.,* 2019; Monadjem *et al.,* 2020; Taylor, Cheney, & Sapsford, 1999); it is likely that the decreased activity during the winter months may be due to individuals dispersing from these maternity roosts once young have left the natal roost (Lino, Fonseca, Mendes, João, & Pereira, 2015). *Miniopterus fraterculus* was the only species where an increase in activity was noted during the winter months. This species is known to make use of hibernacula, particularly caves (Taylor, Cheney, & Sapsford, 1999), and may be able to use the more stable temperatures associated with their hibernacula to conserve energy and maintain activity during the winter months (Monadjem *et al.,* 2020).

Species richness, based on combined sampling effort, was similar in spring, summer and autumn. Mean species richness was higher during the spring and summer months than during autumn and winter, which may be due to local seasonal migrations (McDonald *et al.,* 1990; Monadjem *et al.,* 2020; Mushabati, 2019), or reduced activity and resultant reduction in detections during passive recording (Moir *et al.,* 2020a; Monadjem *et al.,* 2020). The significant differences in species richness between the warm (spring and summer) and the cold (autumn and winter) months is likely due to reduced prey availability and activity, poor foraging conditions (cold/ windy) and the employment of torpor to conserve energy during unfavourable conditions (McDonald *et al.,* 1990; Rydell, 1991; Turbill, 2008).

Functional group 1 (Intermediate / high frequency FM clutter edge foragers) exhibited the highest levels of activity over all seasons. However, this functional group is comprised of the most species and was likely the most active because a number of these species used well insulated roost sites in anthropogenic structures and roost in large numbers (lower metabolic cost of thermoregulation) (Menzies *et al.,* 2016) and foraged around anthropogenic light sources (concentrated, abundant prey resources throughout the year)(Schoeman, 2015). This is likely a behavioural adaptation to anthropogenic developments that can improve foraging success by exploiting concentrated prey resources (Cory-toussaint & Taylor, 2022). Furthermore, this group was disproportionately more active over most habitat types, as they are able to utilise edge habitats in most of the habitat types sampled, whereas other foraging groups had more specific requirements (such as thick clutter in the case of group 5) and may be more limited by other foraging constraints such as exposure to predation during nights of high lunar illumination in the case of edge / open air foragers such as *Scotophilus* or molossid species (Fenton *et al.,* 1977).

The highest proportion of the actively captured female bats (all species) were pregnant during spring on Telperion Nature Reserve. Most of the actively captured males (all species) were scrotal when captured, however due to fewer individuals being captured during the winter, more sampling should be conducted before final conclusions regarding variation in species specific reproductive condition are drawn. It is known that South African microchiroptera tend to give birth approximately one month before maximum rainfall, this has positive implications for the young bats, as the period immediately after weaning coincides with the period of maximum prey (arthropod) density (Cumming & Bernard, 1997; Happold & Happold, 1990). Eight reproductive chronologies have been documented in insectivorous bat species south of the Sahara, however, there is significant variation among species due to external factors such as seasonality and prey availability (Happold &

Happold, 1990). Little is known about the reproductive biology of South African bats (Happold & Happold, 1990; Monadjem *et al.,* 2020). Knowledge of the reproductive phenology of bats has important conservation implications, as their breeding success is likely to be higher if disturbance and the amount of anthropogenically induced stress that they are exposed to is reduced (Jones *et al.,* 2009; Kasso & Balakrishnan, 2013) during that critical period.

7.3.3. Combined effects of habitat and season

The Combined effects of habitat and season were as expected with the highest bat activity being observed during the warmer months (Spring and Summer) at the sites most regularly frequented by bats (anthropogenic and watercourse habitats). The highest species diversity was also documented during the Spring and Summer seasons; however species diversity was highest in watercourse and rock outcrop habitats. These results point to the importance of preserving of habitat diversity in conservation areas.

7.3.4. Effects of Bioclimatic Variables on Bat activity

Bioclimatic conditions have a substantial influence on bat activity. Any environmental conditions that reduce thermoregulatory ability or increase the energetic cost of maintenance of metabolism (ie. higher wind speeds, rainfall, or lower temperatures) reduce / depress bat activity (Mushabati, 2019; Turbill, 2008). Relative humidity was positively correlated with bat activity at Telperion Nature Reserve, with more bat passes being recorded during conditions of higher humidity. This is likely related to invertebrate prey availability as insects are more active during periods of high humidity (Bursell, 1974).

Bats exhibited increased levels of activity during periods of lower and more moderate barometric pressures, with low activity during periods of high barometric pressure. These conditions of lower pressures are typically associated with the onset of rain (Cooke, 1888; Paige, 1995), and the amplified activity may be due to bats needing to provide a nutritional buffer against a period of reduced resource availability during cold weather and rain. The period of lower barometric pressure also coincides with maximum insect (prey) availability which would allow for efficient foraging by insectivorous bats (Paige, 1995).

Temperature also had a direct positive correlation with bat activity. Very few bat passes were recorded when the temperature was < 10°C. This is likely due to bats' large surface area to volume ratio resulting in rapid heat and energy loss (Parker & Bernard, 2018), and high energy requirements (Agosta *et al.,* 2003; Salinas-ramos *et al.,* 2015). During cold periods it would be more efficient to utilise torpor than to forage in cold conditions when arthropod abundance is depressed (Erickson & West, 2002; Mushabati *et al.,* 2022; Taylor *et al.,* 2022).

7.4. The Effects of Blue Wildebeest (*Connachaetes taurinus*) Carcass Sites on The Chiropteran Assemblages of Telperion Nature Reserve

Vertebrate carcass sites in open grassland habitats may act as ephemeral resource patches for insectivorous bats as this study has demonstrated that insectivorous bats forage over carcasses. Carcass associated dipteran activity dropped substantially during the evening and into the cooler nights (personal observation). Consequently, it seemed that there might be a degree of temporal separation between peaks of arthropod activity and peaks of bat activity at carcass sites. The presence of, and the evidence for, feeding behaviour by insectivorous bats over the carcass sites suggests that the bats may have been foraging on nocturnal, carcass associated, invertebrates, or were foraging on non-carcass associated nocturnal arthropods, or were hawking roosting dipterans off structural elements (both natural and anthropogenic) associated with the carcasses.

Bats forage opportunistically on arthropod prey (Egert-Berg *et al.,* 2018; Fenton, Boyle, Harrison, & Oxley, 1977; Klingbeil & Willig, 2010). For instance, insectivorous bats are known to forage over herds of cattle (and likely other herbivores) as they are attractive to parasitic insects such as mosquitoes and dipterans (flies) that are prey for bats (Ancillotto *et al.,* 2017). Should conditions prevail that allow temporal overlap between the activity of insectivorous bats and carcass-associated arthropods, it is likely that the bats would make use of this ephemeral prey resources. The majority of carcass associated dipterans are primarily active during the day, however some carcass associated arthropods are nocturnal and their activity would overlap considerably with periods of bat activity (Oliveira & Vasconcelos, 2017). Although there seems to be a mismatch between the activity pattern of many dipterans and bats, it is documented that that bats take advantage of insect pulses before sunset (Lima & O'Keefe, 2013; Mise, De Souza, Campos, Keppler, & de Almeida, 2010). The presence of concentrated prey and their temporal overlap with the onset of the bat activity period may provide sufficient incentive for bats to forage over carcass sites. The data collected during this study suggests that this may have been the case. Using rich ephemeral prey patches can increase survival in otherwise resource poor environments (Egert-Berg *et al.,* 2018; Prat & Yovel, 2020). This phenomenon is not only documented in insectivorous bats but also in fruit bats and pollen feeders, where food resources may ripen synchronously and only be available for short periods of time before they become unsuitable or depleted (Egert-Berg *et al.,* 2018; Hurme *et al.,* 2022; Klingbeil & Willig, 2010).

The carcasses, during this experiment, were deployed in open, structurally depauperate grassland habitats. The absence of *Hipposideros*, *Nycteris* and *Rhinolophus* species at some sites during the carcass sampling period was likely either due to rapid call attenuation, low intensity calls or due to open grassland habitats lacking any of the vertical vegetation structure or flyways selected for by these bats (Fenton & Rautenbach, 1986).

The bat functional groups that likely benefited from the placement of these carcasses were the species best adapted to foraging in open, relatively featureless habitats. The species that might conform to this prerequisite are large, fast fliers adapted to foraging in open or edge habitats such as functional group 2 (Large, low frequency LD-QCF open air foragers) and functional group 3 (Intermediate and lower frequency edge and open-air foragers). Had the carcasses been deployed to more cluttered or structurally complex habitats the functional groups likely to use the ephemeral resources associated with the carcasses might have been different. It seems plausible that despite the relatively restricted resource, insectivorous bats may forage on carcass associated dipterans when temporal overlap in activity occurs. It is likely that carcass sites act as an equivalent resource patch to insect emergences or other events where arthropod prey is concentrated e.g. around street lamps (Furlonger *et al.,* 1987; Naidoo *et al.,* 2011). There are many examples of insectivorous bats utilising ephemeral resource patches such as insect emergences, highly nutrified water bodies and areas containing high insect densities (ie anthropogenic light sources) (Dechmann *et al.,* 2009; Egert-Berg *et al.,* 2018; Naidoo *et al.,* 2016; Roeleke *et al.,* 2020).

During this study it was noted that insectivorous bats were both active and feeding over experimental carcass sites, however dietary analysis would improve the robustness of such a study. This activity is unsurprising as the carcasses were a concentrated hub of arthropod activity. As there are no other studies of this type, to our knowledge, some of the inferences regarding the bat responses to carcasses may be speculative. Surprisingly, albeit not statistically significant, there was more bat activity over carcasses enclosed within steel exclosure cages than over pegged carcasses. There is no documentary evidence of why there might be a difference in bat activity relative to these treatments. However, it is theoretically possible that the microclimate around caged carcasses was subject to slightly elevated temperatures due to residual heat within the steel structure of exclosure cages (Baggs & Mortensen, 2006). If this were the case, the arthropods (prey taxa) may have extended their activity longer into the cooler evenings (Baker *et al.,* 2003), extending the period of increased resource availability for bats. The prey being active later into the evening might allow more temporal overlap between dipterans and chiropterans, hence more available food resources and higher activity (Campobasso, Di Vella, & Introna, 2001). It is also likely that bats hawked nocturnal insects over the carcass sites in the same fashion as swallows do during the day (personal observation).

Species richness was relatively low around both carcass and control sites, the lower diversity makes sense as carcass sites were placed only in open grassland habitats without linear landscape elements or vertical vegetation structure (both elements important to most foraging and commuting bats) (Coleman & Barclay, 2013; Hodgkison *et al.,* 2004; Limpens & Kapteyn, 1991). The marginally higher bat species diversity at control sites may be related to the higher sampling effort at those sites (one extra night of sampling per control site), and less disturbance (no carcass placement or associated mesh grids or cages) at the control sites. Species richness was higher during the seasonal sampling in the grassland habitat than over carcasses deployed during summer in the grassland habitat. This may be due to the increased sampling effort (twice as many detectors, and more sampling nights, as well as sampling more structurally complex microhabitats during the seasonal and habitat specific component of this study. There was little in common between the open grassland sites used for the carcass component of this study and the highly diverse, heavily vegetated, and structurally complex microhabitats tested during the habitat component. This is likely one of the reasons that mean bat activity over (open grassland) carcass sites was substantially lower than mean activity over the more structurally complex habitat sites. It is likely that these open grasslands act as a homogeneous matrix with islands of productive habitat for all but the largest open air bat species. The massive discrepancy in activity and foraging activity between open grassland sites (with and without carcasses) and microhabitats with vertical vegetation structure and access to important resources (water, foraging habitat, roost sites) emphasises the importance of microhabitats to insectivorous bats. This is likely one of the first, if not the first, study to assess whether insectivorous bats respond behaviourally to the presence of decaying carcasses in the landscape. The evidence of insectivorous bat feeding activity in the proximity of decaying carcasses lends weight to the hypothesis that carcasses may act as ephemeral resource patches for insectivorous bats and this phenomenon requires further investigation.

The grassland habitat on the Telperion Nature reserve exhibited a surprising diversity of bat species and activity, particularly for a grassland dominated reserve. The variety of microhabitats supported a diversity of discrete assemblages of bats. Data on the bats that were captured, identified, and recorded, resulted in a call library and an improved species inventory for this reserve. The novel effects of vertebrate carcass sites on chiropteran activity and diversity demonstrated that bats do indeed forage over carcass sites. This emphasises the potential of further study into this type of ephemeral resource, and it influence on the chiropteran assemblage. Telperion Nature Reserve likely acts as an important conservation region in the ecotone between the grassland and savanna biomes of South Africa

CHAPTER EIGHT

SUMMARY OF FINDINGS AND RECOMMENDATIONS

8.1. Overview

This study addressed several deficits in the body of knowledge on the bat community associated with the grassland dominated eastern region of Mpumalanga, South Africa. The objectives of the study were to a) develop a comprehensive, site specific, species inventory through active capture and passive sampling, b) to develop a regional call library, c) to determine the effects of habitat type on local bat species diversity (functional and taxonomic), relative abundance and activity patterns, d) determine whether there are temporal differences in bat diversity, abundance, and activity within and among habitats and e) to determine the effect of ungulate carcasses and their associated invertebrate decomposers on the relative abundance and species richness of bats. This chapter provides a synthesis of important findings and contribution to the literature, and offers suggested recommendations in respects of future studies, conservation practices and possible management interventions.

8.2. Species Inventory, Regional Call Library and Call Variation

This study provided information on species richness, activity, echolocation parameters and external morphology (including wing morphology), for a bat assemblage within an area where the bat assemblage has been understudied. This study has almost doubled (from 13 to 22 species) the list of known bat species on Telperion Nature Reserve.

8.2.1. Multi-method sampling approach and duration of sampling

It is important to ensure that sufficient effort is committed to obtaining as accurate a representation of the bat species assemblage, within a study area, as possible. The duration and intensity of sampling is important, as insufficient sampling likely results in an underestimation of the species richness, diversity, and relative species abundance of a chiropteran assemblage. For example, (Greyling & Keith, 2013) limited their investigation solely to the use of acoustic methods, with relatively low sampling effort (one detector used over one sampling session), resulting in the identification of 10 bat species on Telperion Nature Reserve. Contrast this with the 22 species from six families that were identified using the variety of methods in the present study. It is apparent that increased effort using a variety of methods is required to assess the bat diversity on a site such as Telperion Nature Reserve. Similarly, this study corroborates the findings of Moir *et al.* (2020a) that recommends an acoustic sampling period of at least 36 nights. Based on the above, and the species accumulation curves for acoustic sampling on Telperion Nature Reserve, it is recommended that future studies on southern African bats follow a multi-method sampling approach and that acoustic sampling be conducted over a minimum of 36 - 40 sampling nights to gain an accurate estimate of the bat faunal assemblage.

8.2.2. Species inventory and sampling considerations

This study addressed a notable knowledge gap in relation to bats in the Mpumalanga province, where wind energy developments have recently been proposed (https://sawea.org.za/seriti-to-construct-155-mw-wind-farm-in-mpumalanga/;accessed online 18 May 2023). Twenty-two species of bats belonging to the families Hipposideridae, Miniopteridae, Molossidae, Nycteridae, Rhinolophidae and Vespertilionidae were recorded on the reserve, including several species categorised as 'of conservation importance' (Child *et al.* 2016). Most of the species documented on the Telperion Nature Reserve (with the exception of clutter foragers) are at high risk for wind turbine collision (Sowler, MacEwan, Aronson, & Lötter, 2020). The Telperion Nature Reserve bat assemblage closely resembles that of eastern and north-eastern regions of South Africa (Linden, Gaigher, Weterings, & Taylor, 2014; Taylor, Sowler, Schoeman, & Monadjem, 2013). Species richness seemed to be similar to that reported for the forested regions of the Eastern Cape and southern KwaZulu-Natal regions (Moir *et al.,* 2020a) and the Soutpansberg Mountain area (Taylor *et al.,* 2013; Linden *et al.,* 2014; Weier *et al.,* 2016). The inclusion of multiple habitat types in the study design likely contributed to the amplification of the documented bat biota on the Reserve. While five species of molossid bats were documented on the reserve, none were physically captured. Acoustic data suggests that they frequent rocky outcrop regions and water course-ways. It is recommended that extensive molossid roost searches be conducted in under-sampled areas of the reserve, especially in sandstone-granite rocky gorges. Increased sampling effort with tall mist nets (> 6 m) across water course-ways may result in captures of these elusive species, and may further expand the bat species list for Telperion Nature Reserve. Crevice and roost searches should be undertaken to potentially expand the bat species list for the Reserve. Furthermore, a bat monitoring programme should be implemented on the reserve to monitor variations in the bat species assemblage over time.

The Near Threatened *Otomops martiensseni* (Richards *et al.,* 2016), was also detected on the reserve. Because of its sparse distribution and low echolocation call frequency (Adams, Bonaccorso, & Winkelmann, 2015), acoustic studies should make provision for bat acoustic monitoring with detector frequencies as low as 12 kHz to potentially detect this species.

8.2.3. Regional call library and interspecific and intraspecific call variation

This study addressed a gap in the published record of southern African bat call libraries. It provided echolocation call parameters for 12 taxa from post release free flight call recordings. A further 10 species were assigned based exclusively on passive acoustic data. During future studies individuals that are captured, particularly from species that may not previously have been captured and documented on the reserve, should have their post release echolocation calls recorded. This will aid in vetting species assignments made during this study and provide more reliable species identification than free flight calls (Monadjem & Reside, 2008).

This study provides evidence indicative of the presence of a cryptic *Rhinolophus* species morphologically similar to *Rhinolophus simulator* s.s., yet with different connecting process morphology and echolocation call parameters. To determine whether this putatively different species is a different species, genetic analysis, in addition to morphological analysis will be required (Monadjem *et al.,* 2020). Genetic material (wing biopsies) were collected from most captured individuals during this study, to serve as a means of identifying previously captured individuals (Sikes, 2016). As genetic analyses, based on material including wing biopsies, have been successfully used for identifying cryptic *Rhinolophus* taxa from the southern African region (Taylor *et al.,* 2012, 2018), it is proposed that the existing samples be subject to genetic analysis to evaluate whether *R.* cf. *simulator* (R79) and *R*. *simulator* s.s. are distinct species.

This study confirmed intraspecific sex-related echolocation call variation, primarily among vespertilionid species. Studies investigating echolocation call characteristics of southern African bats ought to account for potential inter-sex call variation and treat male and female individuals separately in descriptive analyses.

8.3. The effects of habitat on the chiropteran assemblage of Telperion Nature Reserve

Habitat type had a strong effect on bat species diversity and activity. Habitats that provided resources (prey, water and roost sites) were the areas most frequented by bats on the Telperion Nature Reserve. Water courses and anthropogenic habitats were associated with the highest diversity and highest activity respectively.

8.3.1. Bat community composition and activity in relation to habitat and suggestions for future studies

When selecting sampling sites for this study, structural habitat cues (linear landscape elements, foraging sites, roost sites or relatively safe commuting routes) that are thought to influence bat activity were prioritised. Open and exposed habitats were avoided. A detailed analysis of the vegetation types on the Telperion Nature reserve has recently been published (Brown *et al.,* 2022); future studies should use these vegetation delineations to identify areas likely to support high levels of bat diversity and guide sample site selection on the reserve. Similar habitat sites should be identified and considered for active and acoustic sampling in other reserves to facilitate comparability among studies.

8.3.2. Important bat roosting and utilisation features- their preservation and future sampling considerations

Anthropogenic sites

This study showed that 20 species were associated with anthropogenic habitats. Active sampling of anthropogenic sites demonstrated that at least six taxa (*Hipposideros caffer*, *Laephotis capensis*, *Nycteris thebaica*, *Rhinolophus* cf. *simulator*, *R*. *simulator* s.s., and *Scotophilus dinganii*) use such areas as day or night roosts. Roof spaces of all actively sampled building contained medium to large colonies of *Laephotis capensis*, thus skewing bat activity metrics (bat passes and activity indices) in this habitat type. The use of Miller (2001) activity index for future studies will facilitate improved and direct comparisons between studies of a similar type or in similar habitats. A disused cold room, constructed from natural rock, was identified as an important roost site for *Hipposideros, Laephotis*, *Nycteris*, and *Rhinolophus* bats on the reserve. The cold room, situated away from frequent disturbance and close to woody vegetation, provided night roosts for five (possibly six, *Rhinolophus* cf. *simulator* R79) species of bats. This site was used simultaneously, by multiple species, throughout the year. It may be that the combination of characteristics (constructed of natural rock with rough cement ceiling and steel ceiling support beams) of the cold room may have made it particularly appealing to bats as a night roost and possibly a cave proxy. The construction of similar cave proxies, particularly if they are constructed of natural stone and provide areas for crevice and cave roosting species, might increase the amount of roost habitat available and this could be used as a strategy to attract more bats to the site, or to increase the bat species diversity of specific areas on the reserve. This strategy would have direct conservation benefits and aid in the management of arthropod pest species, particularly in the surrounding agricultural areas.

Riparian areas

Riparian sampling sites along the Wilge river were associated with high levels of bat activity, and with the highest species diversity on the reserve. This is likely due to the diverse structural and biotic composition of this habitat type (see Monadjem & Reside, 2008). The riverine habitat comprises thick riverine vegetation, including substantial reed beds, and likely attracts abundant river associated invertebrate prey. The high level of bat diversity (21 species) and activity associated with the riparian areas highlights the importance of river channels for bats and emphasises the need for river channel conservation. Bats are important bioindicators in habitats such as this (Jones *et al.,* 2009), as they use rivers for a variety of reasons including, commuting, roosting (in river valley caves etc.) drinking, and foraging. Some evidence exists that this river is under threat. Invasive aquatic plants such as parrot's feather (*Myriophyllum aquaticum*) and water hyacinth (*Pontederia crassipes*) are present in the channel. Mining, industry, and agriculture are present in the catchment area of the Wilge river, consequently, high nutrient inputs into the river have been documented and eutrophic conditions are likely to manifest in the future (Farrell, Van Vuuren, & Ferreira, 2015). Bats are able to forage on aquatic invertebrates associated with contaminated or highly nitrified waterways, however they may suffer negative health consequences (Naidoo *et al.,* 2011, 2016). The conservation of this river is important as it provides habitat for at least 20 species of bats, all of which could be threatened by further alteration or pollution of this resource. Furthermore, this river is a component of a larger riparian system flowing through major conservation areas, the conservation of which is important to biodiversity in general.

Tree-grass matrix

Eleven species were recorded from tree-grass matrix habitats. Bat activity and diversity in tree-grass habitats was lower than most of the other sites on the reserve. This habitat was used by a sub-assemblage of the bats on Telperion Nature Reserve including more specialist species such as *Nycteris thebaica* and *Rhinolophus simulator.* Patch sizes of the tree-grass fragments likely influenced the species composition (Perry, Thill, & Leslie, 2006), as such larger fragments were selected as sample sites during this study. Future studies should focus on large tree-grass habitat patches and utilise active captures and passive acoustic monitoring to determine how these patches are used by specialist bat species such as *Nycteris thebaica* and *Hipposideros caffer*.

Rocky outcrops

Nineteen bat species were associated with rocky outcrop areas. Activity in this habitat was lower than anthropogenic, tree-grass and water course habitats, however, species diversity was still high within rocky outcrop habitats. Most of this habitat, both sampled and unsampled, comprises inaccessible, sheer cliff areas in the southern region of the reserve and bordering the Wilge River.

Increased sampling effort should be implemented in these areas as they are likely roosting places for the crevice-roosting molossids on the reserve. Sampling methods such ‘bicycle traps’ or harp traps suspended from the summit of these cliffs (Cotterill & Fergusson, 1993), may prove useful in capturing molossids and other bat species that utilise such areas.

8.4. The effects of season on the chiropteran assemblage of Telperion Nature Reserve

Due to their small body size with large surface areas, most South African bats are influenced by seasonality (Parker & Bernard, 2018). Their activity and foraging strategies are affected by cold and windy conditions. During periods where low temperatures or high winds prevail it may be more energetically viable to utilise torpid strategies and forgo foraging bouts until bioclimatic conditions are more favourable (McDonald *et al.,* 1990; Mushabati, 2019). Studies on southern African bat communities have typically followed a once-off sampling approach (Greyling & Keith, 2013; Kearney *et al.,* 2019; Linden *et al.,* 2014; Moir *et al.,* 2020a; Rautenbach *et al.,* 2014; Taylor, Geiselman, Kabochi, Agwanda, & Turner, 2005) or have included a seasonal component limited to a comparison between “wet’ and ‘dry’ seasons (Fenton & Thomas, 1980; Mtsetfwa, Mccleery, & Monadjem, 2018; Shapiro, Monadjem, Röder, & Mccleery, 2019). This study sought to address the paucity in data on bat species richness, diversity, and activity in relation to the four distinct astronomical seasons with specific reference to the bat assemblage on Telperion Nature Reserve.

8.4.1. Seasonal species richness and activity and sampling recommendations

Unsurprisingly, bat species richness, diversity and activity decreased during the winter months (activity reduced by a factor of 10) when compared to the summer and spring. The highveld grassland region of South Africa experiences dramatic fluctuations in temperature between the warmer spring and summer months (average temperatures = 20 °C) and the cooler, temperate autumn, and winter months (average temperatures = 13 °C). This study identified an apparent seasonal change in assemblage composition with *Myotis bocagii* and *Mops (Chaerephon) pumilus*, seemingly absent during winter. Furthermore, during winter there was a reduction in *Laephotis capensis* activity at anthropogenic sites on the reserve suggesting that such roosts may constitute maternity roosts. This apparent absence may be linked to seasonal, local migrations, however further sampling will be required to confirm this. In future studies, depending on the questions being posed, should seasonal sampling not be required, active sampling during the warmer months would likely yield more captures than sampling during colder months.

8.4.2. Seasonal trends in bat demographics on Telperion Nature Reserve and sampling considerations

Both activity and species diversity were significantly affected by season. Bat activity peaked during the warm months of spring and summer and declined considerably during the cold months of autumn and winter. Certain species were not recorded during the coldest period of the year (winter season), this may be attributable to small scale seasonal migrations, or the employment of torpor (Happold & Happold, 1990; McDonald *et al.,* 1990; Mushabati, 2019). These phenomena have been well documented in other studies (McDonald *et al.,* 1990; Monadjem *et al.,* 2020; Turbill, 2008). It is likely that the duration of foraging bouts is reduced during the winter months to reduce energy expenditure, there is also the possibility of less selectivity of prey during this period, to maximise foraging efficiency, however, this would need to be confirmed with dietary analysis studies (Brinkley *et al.,* 2018; Shively, Barboza, Doak, & Jung, 2018).

Most females that were captured during the spring were pregnant. This should be considered when planning future bat research on the reserve. Perhaps scheduling of future research so that pregnant female are not subject to the stress of capture would be advisable. Males exhibited scrotal testes throughout the year, except during winter.

8.5 The effects of Blue Wildebeest (*Connachaetes taurinus*) carcass site on the chiropteran assemblage of Telperion Nature Reserve

There are no previous records of bat responses to the decomposition of large African ungulates such as blue wildebeest (*Connachaetes taurinus*). This study is the first to experimentally investigate the effects of ungulate carcass sites on bat activity, species diversity and foraging. The use of wild, unmedicated, animals in such decomposition experiments is of importance as domestic stock (such as those that might be used as proxies for wild animals or humans in decomposition studies) are often exposed to medications and antibiotics during life (and possibly during euthanasia) which might have effects on the decomposer insect and bacterial assemblages (Herrero-villar, Sánchez-Barbudo, Camarero, Taggart, & Mateo, 2021; Tomberlin, Barton, Lashley, & Jordan, 2017).

8.5.1. Study area and microhabitat

The carcass sites in this study were all in exposed grassland habitats that are associated with relatively low levels of bat activity, and potentially high levels of predation risk (Fenton *et al.,* 1977; Jones & Rydell, 1994). The carcass sites in this study were all in open grassland habitats that supported relatively low levels of bat activity, in comparison to other habitats (e.g. anthropogenic and water course-ways). Overall 20 species were detected using acoustic sampling over carcass and control sites, *Rhinolophus* cf. *simulator* (79) and *Hipposideros caffer* were not recorded in open grassland habitats. Clutter specialists such as the *Nycteris* and *Rhinolophus* species also exhibited very low activity levels in open grassland habitats during the carcass component of this study. This low activity was probably a consequence of their habitat selection,- these species are typically associated with cluttered and clutter edge (vegetated) habitats (Monadjem *et al.,* 2020). High levels of bat activity were recorded around anthropogenic sites, water courses, and around tree-grass matrix habitats within the reserve, therefore, there may be value in conducting further carcass experiments in each of the component habitat types on Telperion Nature Reserve. To distil the any influence of the decomposition of carcasses on bats, future studies should incorporate sampling across a variety of habitat types and seasons, they should also incorporate different carcass sizes and carcasses in different microhabitats to allow comparisons of activity across microhabitats.

8.5.2. Study design

This study used a three-treatment experimental design, with reference sites, caged carcass sites, and pegged (exposed) carcass sites. The grid and cage system worked well for pegged and caged carcasses, however, should similar projects be initiated, it would be valuable to deploy grids and exclosure cages at control sites to replicate more accurately the circumstances at the treatment sites. The experimental sites were subject to high levels of human activity (multiple investigations were conducted simultaneously). This may have influenced not just chiropteran species, but other vertebrates, particularly scavengers that have been heavily persecuted by stock farmers in the surrounding areas (pers comm, 2020, Dr Duncan MacFadyen). Due to the ephemeral nature of carcass sites, it is essential that control sites be used to monitor baseline activity of bats within the area.

8.5.3. Sampling effort and duration

This study suffered from too small a sample size, to make robust inferences (McDonald, 2014) in relation to the influence of the presence of carcasses on bat behaviour and activity. One of the shortcomings of this portion of the study was our limited access to bat-detectors with the consequence that the simultaneous monitoring of all carcass sites was not possible. It is recommended that, should future studies of this nature be implemented, at least one bat detector be deployed at each sample site for the duration of the active decomposition process. This will obviate the possible effects of temporal variation in deployment and data and allow for direct comparisons between treatment and control sites.

8.6. Conclusion

Bats are an important taxonomic group and they are of ecological, conservation and economic value in grasslands. This study provided important insights into bat ecology of the grassland biome. It resulted in a comprehensive species list and baseline call library of bat taxa for an under-sampled grassland region and associated habitats. The study demonstrated that grassland habitats provide important resources for chiropterans and are utilised by a surprising number of bat taxa. Habitat had a significant effect on species diversity and activity of grassland bats. Seasonality influenced bat activity and diversity on Telperion Nature Reserve. The presence of carcasses influences bat activity as it was demonstrated that bats foraged over ungulate carcass sites.

Recommendations

- A minimum of 36 - 40 nights of acoustic sampling should be undertaken to obtain a comprehensive species inventory in grassland habitats.
- Acoustic studies should make provision for bat acoustic monitoring with detector frequencies set as low as 10-12 kHz and as high as 150 kHz to maximise detection success.
- Structural features (linear landscape elements, foraging sites, roost sites or relatively safe commuting routes) should be selected as sampling sites to improve active and acoustic capture success in grassland habitats.
- Further studies should investigate the molossid diversity in the south and other gorge areas of the reserve.
- The Telperion Nature reserve and the Wilge river should be conserved as it contains a rich chiropteran assemblage and acts as an ecotone between the grassland and savanna biomes of South Africa.
- Active sampling for biodiversity studies should be conducted in spring or summer to maximise detection and capture success.
- The carcass decomposition study should be repeated with carcasses in different microhabitats and with simultaneously recording bat detectors.

REFERENCES

Acocks, J. P. H. (1988). *Veld types of South Africa* (3rd ed.). Pretoria: Botanical Research Institute, Mem. Bot. Surv. South Africa *57*, 1-146.

Adams, R. A., Bonaccorso, F. J., & Winkelmann, J. R. (2015). Revised distribution for *Otomops martiensseni* (Chiroptera: Molossidae) in southern Africa. *Global Ecology and Conservation*, *3*, 707–714. http://doi.org/10.1016/j.gecco.2015.03.006

Agosta, S. J., Morton, D., & Kuhn, K. M. (2003). Feeding ecology of the bat *Eptesicus fuscus*:preferred prey abundance as one factor influencing prey selection and diet breadth. *Journal of Zoology*, *260*, 169–177. http://doi.org/10.1017/S0952836903003601

Aldridge, H. D. J. N., & Rautenbach, I. L. (1987). Morphology, echolocation and resource partitioning in insectivorous bats. *The Journal of Animal Ecology*, *56*(3), 763–778.

American Society of Mammalogists. (2020). Mammal diversity database (Version 1.10.). Retrieved from www.mammaldiversity.org.

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 and Environment*, *241*, 54–61. http://doi.org/10.1016/j.agee.2017.03.001

Anderson, M., Norton, L., & Mathews, F. (2020). Grassland management affects vegetation structure, bats and their beetle prey. *Diversity*, *12*(10), 1–18.

Anthony, E. L. P., Stack, M. H., & Kunz, T. H. (1981). Night roosting and the nocturnal time budget of the little brown bat, *Myotis lucifugus*: effects of reproductive status, prey density, and environmental conditions. *Oecologia*, *51*, 151–156.

Appel G., Capaverde U.D., De Oliveira L.Q., do Amaral Pereira L.G., da Cunha Tavares V., López-Baucells A., Magnusson W.E., Baccaro F.B., & Bobrowiec P.E. (2021). Use of complementary methods to sample bats in the Amazon. *Acta Chiropterologica*, *23*(2), 499–511. http://doi.org/https://doi.org/10.3161/15081109ACC2021.23.2.017

Aspetsberger, F., Brandsen, D., & Jacobs, D. S. (2003). Geographic variation in the morphology , echolocation and diet of the little free-tailed bat, *Chaerephon pumilus* (Molossidae). *African Zoology*, *38*(2), 245–254. http://doi.org/10.1080/15627020.2003.11407278

Avila-Flores, R., & Fenton, M. B. (2005). Use of spatial features by foraging insectivorous bats in a large urban landscape. *Journal of Mammalogy*, *86*(6), 1193–1204. http://doi.org/10.1644/04-MAMM-A-085R1.1

Baggs, D., & Mortensen, N. (2006). Thermal mass in building design. *Environment Design Guide*, 1–9.

Baker, L. A., Brazel, A. J., Selover, N., Martin, C., McIntyre, N., Steiner, F. R., & Musacchio, L. (2003). Urbanization and warming of Phoenix (Arizona , USA): Impacts , feedbacks and mitigation. *Urban Ecosystems*, *6*, 183–203.

Barclay, R. M., & Brigham, R. M. (2004). Geographic variation in the echolocation calls of bats: a complication for identifying species by their calls. In R. M. Brigham, E. K. V Kalko, G. Jones, S. Parsons, & H. J. G. A. Limpens (Eds.), *Bat echolocation research: tools, techniques, and analysis.* (pp. 144–149). Austin, Texas: Bat Conservation International.

Barclay, R. M. R., & Brigham, R. M. (1991). Prey detection , dietary niche breadth , and body size in bats: Why are aerial insectivorous bats so small? *The American Naturalist*, *137*(5), 693–703.

Barros, M. A. S., Pessoa, D. M. A., & Rui, A. M. (2014). Habitat use and seasonal activity of insectivorous bats (Mammalia: Chiroptera) in the grasslands of southern Brazil. *Zoologia*, *31*(2), 153–161.

Batary, P., Baldi, A., Kleijn, D., & Tscharntke, T. (2010). Landscape-moderated biodiversity effects of agri-environmental management: a meta-analysis. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1713), 1894–1902. http://doi.org/10.1098/rspb.2010.1923

Bernard, R. F., & Mccracken, G. F. (2017). Winter behavior of bats and the progression of white-nose syndrome in the southeastern United States. *Ecology and Evolution*, *7*(5), 1487–1496. http://doi.org/10.1002/ece3.2772

Berry, A. N., Will, O., & Holderied, M. W. (2019). Detection and avoidance of harp traps by echolocating bats. *Acta Chiropterologica*, *6*(2), 335–346.

Blake, J. G., & Loiselle, B. A. (2001). Bird assemblages in second-growth and old-growth forests, Costa Rica: perspectives from mist nets and point counts. *The Auk*, *118*(2), 304–326.

Blomberg, A. S., Vasko, V., Meierhofer, M. B., Johnson, J. S., Eeva, T., & Lilley, T. M. (2021). Winter activity of boreal bats. *Mammalian Biology*, *101*, 609–618. http://doi.org/10.1007/s42991-021-00111-8

Bohmann, K., Monadjem, A., Noer, C. L., Rasmussen, M., Zeale, M. R. K., Clare, E., & Gilbert, M. T. P. (2011). Molecular diet analysis of two African free-tailed bats (Molossidae) using high throughput sequencing. *PLoS One*, *6*(6), 1–11. http://doi.org/10.1371/journal.pone.0021441

Boyles, J. G., Cryan, P. M., McCracken, G. F., & Kunz, T. H. (2011). Economic importance of bats in agriculture. *Science*, *332*(6025), 41–42. http://doi.org/10.1126/science.1201366

Braun de Torrez, E. C., Ober, H. K., & McCleery, R. A. (2018). Critically imperiled forest fragment supports bat diversity and activity within a subtropical grassland. *Journal of Mammalogy*, *99*, 273–282. http://doi.org/10.1093/jmammal/gyx169

Braun De Torrez, E. C., Wallrichs, M. A., Ober, H. K., & Mccleery, R. A. (2017). Mobile acoustic transects miss rare bat species: implications of survey method and spatio-temporal sampling for monitoring bats. *PeerJ*, *5*. http://doi.org/10.7717/peerj.3940

Brinkley, E. R., Parker, D. M., & Taylor, P. J. (2018). *Re-surveying the insectivorous bats of northern Kruger National Park, South Africa*. Master of Science Thesis, Rhodes University.

Brinkley, E. R., Weier, S. M., Parker, D. M., & Taylor, P. J. (2021). Three decades later in the northern Kruger National Park: multiple acoustic and capture surveys may underestimate the true local richness of bats based on historical collections. *Hystrix, the Italian Journal of Mammalogy*, *32*(2), 109–117. http://doi.org/10.4404/hystrix

Broken-Brow, J., Armstrong, K. N., & Leung, L. K. P. (2019). The importance of grassland patches and their associated rainforest ecotones to insectivorous bats in a fire-managed tropical landscape. *Wildlife Research*, *46*(8), 649–656.

Brown, L. R., Magagula, I. P., & Barrett, A. S. (2022). A vegetation classification and description of Telperion Nature Reserve, Mpumalanga , South Africa. *Vegetation Classification and Survey*, *3*, 199–219. http://doi.org/10.3897/VCS.85209

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), 1–14. http://doi.org/10.1093/jmammal/gyx147

Bursell, E. (1974). Chapter 2: Environmental Aspects - Humidity. In M. Rockstein (Ed.), *The Physiology of Insecta* (2nd ed., pp. 43–84). Academic Press, Miami.

Cadman, M., de Villiers, C., Lechmere-Oertel, R., & McCulloch, D. (2013). *Grasslands ecosystem guidelines: interpretation for planners and managers*. South African National Biodiversity Institute, Pretoria.

Campobasso, C. Pietro, Di Vella, G., & Introna, F. (2001). Factors affecting decomposition and Diptera colonization. *Forensic Science International*, *120*, 18–27.

Chao, A. (1987). Estimating the Population Size for Capture-Recapture Data with Unequal Catchability. *Biometrics*, *43*(4), 783–791.

Chao, A., Chazdon, R. L., Colwell, R. K., & Shen, T. (2004). A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecology Letters*, *8*(2), 148–159. http://doi.org/10.1111/j.1461-0248.2004.00707.x

Chao, A., & Chiu, C.-H. (2016). *Species Richness : Estimation and Comparison*.

Chenger, J. D., & Tyburec, J. D. (2014). Bat detectors and microphones comparing: height above ground level, orientations and microphone types. Poster presentation at the Southeast Bat Diversity Network Meeting, Nacogdoches, Texas.

Child, M. F., Roxburgh, L., Do Linh San, E., Raimondo, D., & Davies-Mostert, H. T. (2016). *The 2016 red list of mammals of South Africa, Swaziland and Lesotho. "The 2016 Red List of Mammals of South Africa, Swaziland and Lesotho." South African National Biodiversity Institute and Endangered Wildlife Trust,* South Africa*.*

Chung-MacCoubrey, A. L. (1996). *Grassland bats and land management in the Southwest*. *Ecosystem disturbance and wildlife conservation in western grassland - A symposium proceedings. September 22-26, 1994; Albuquerque, NM. General Technical Report RM-GTR-285.* Fort Collins, CO*: USDA Forest Service, Rocky Mountain Forest and Range Experiment*.

Climate-data.org. (2021). Climate Emalahleni (South Africa). Retrieved August 11, 2021, from https://en.climate-data.org/africa/south-africa/mpumalanga/emalahleni-641/

Coe, M. (1978). The decomposition of elephant carcases in the Tsavo (East) National Park, Kenya. *African Journal of Ecology*, *15*(1), 49–55. http://doi.org/10.1111/j.1365-2028.1977.tb00377.x

Coetzee, C., Bredenkamp, G. J., Van Der Merwe, M., & Mostert, T. H. C. (2012). *The effect of vegetation on the behaviour and movements of Burchell ' s Zebra, Equus burchelli (Gray 1824) in the Telperion Nature Reserve, Mpumalanga, South Africa.* Master of Science Thesis*, University of Pretoria*

Coetzee, J. P., Bredenkamp, G. J., & Van Rooyen, N. (1995). The phytosociology of the grasslands of the Ba and Ib land types in the Pretoria–Witbank–Heidelberg area. *South African Journal of Botany*, *61*(3), 123–133. http://doi.org/10.1016/S0254-6299(15)30498-1

Coleman, J. L., & Barclay, R. M. R. (2013). Prey availability and foraging activity of grassland bats in relation to urbanization. *Journal of Mammalogy*, *94*(5), 1111–1122. http://doi.org/10.1644/12-mamm-a-217.1

Collins, M. (1970). *Studies on the decomposition of carrion and its relationship with its surrounding ecosystem.* Doctoral Thesis, University of Reading, Reading, UK.

Colwell, R. K. (2006). EstimateS: statistical estimation of species richness and shared species from simples, version 8.0.

Colwell, R. K., & Elsensohn, J. E. (2014). EstimateS turns 20: statistical estimation of species richness and shared species from samples, with non-parametric extrapolation. *Ecography*, *37*(6), 609–613.

Cooke, W. W. (1888). *Report on Bird Migration in the Mississippi Valley in the Years 1884 and 1885*. US Department of Agriculture, Division of Economic Ornithology, Washington, D.C.

Cooper-Bohannon, R., Rebelo, H., Jones, G., Cotterill, F., Monadjem, A., Schoeman, M. C., & Park, K. (2016). Predicting bat distributions and diversity hotspots in southern Africa. *Hystrix, the Italian Journal of Mammalogy*, *27*(1), 1–11. http://doi.org/10.4404/hystrix-27.1-11722

Cory-toussaint, D., & Taylor, P. J. (2022). Anthropogenic light, noise, and vegetation cover differentially impact different foraging guilds of bat on an opencast mine in South Africa. *Frontiers in Ecology and Evolution*, *10*, 1–12. http://doi.org/10.3389/fevo.2022.752665

Cotterill, F. P. D., & Fergusson, R. A. (1993). Seasonally Polyestrous Reproduction in a Free-Tailed Bat *Tadarida fulminans* (Microchiroptera: Molossidae) in Zimbabwe. *Biotropica*, *25*(4), 487–492.

Cowling, R. M., Richardson, D. M., & Pierce, S. M. (2004). *Vegetation of southern Africa*. Cambridge: Cambridge University Press.

Cumming, G. S., & Bernard, R. T. F. (1997). Rainfall, food abundance and timing of parturition in African bats. *Oceologia*, *111*, 309–317.

Darras, K., Pütz, P., Fahrurrozi, Rembold, K., & Tscharntke, T. (2016). Measuring sound detection spaces for acoustic animal sampling and monitoring. *Biological Conservation*, *201*, 29–37. http://doi.org/10.1016/j.biocon.2016.06.021

Dechmann, D. K. N., Heucke, S. L., Giuggioli, L., Safi, K., Voigt, C. C., & Wikelski, M. (2009). Experimental evidence for group hunting via eavesdropping in echolocating bats. *Proceedings of the Royal Society B: Biological Sciences*, *276*, 2721–2728. http://doi.org/10.1098/rspb.2009.0473

Downs, N. C., & Racey, P. A. (2006). The use by bats of habitat features in mixed farmland in Scotland. *Acta Chiropterologica*, *8*(1), 169–185. http://doi.org/10.3161/1733-5329(2006)8[169:TUBBOH]2.0.CO;2

du Toit, J. T., & Cumming, D. H. M. (1999). Functional significance of ungulate diversity in African savannas and the ecological implications of the spread of pastoralism. *Biodiversity and Conservation*, *8*, 1643–1661. http://doi.org/10.1023/A

Egert-Berg, K., Hurme, E. R., Greif, S., Goldstein, A., Harten, L., Herrera, L. G. M., Flores-Martınez, J. J., Valdes, A. T., Johnston, D. S., Eitan, O., Borissov, I., Shipley, J. R., Medellin, R. A., Wilkinson, G. S., Goerlitz, H. R., & Yovel, Y. (2018). Resource ephemerality drives social foraging in bats. *Current Biology*, *28*(22), 3667–3673. http://doi.org/10.1016/j.cub.2018.09.064

Egoh, B. N., Reyers, B., Rouget, M., & Richardson, D. M. (2011). Identifying priority areas for ecosystem service management in South African grasslands. *Journal of Environmental Management*, *92*(6), 1642–1650. http://doi.org/10.1016/j.jenvman.2011.01.019

Egoh, B., Reyers, B., Rouget, M., Bode, M., & Richardson, D. M. (2009). Spatial congruence between biodiversity and ecosystem services in South Africa. *Biological Conservation*, *142*(3), 553–562. http://doi.org/10.1016/j.biocon.2008.11.009

Erickson, J. L., & West, S. D. (2002). The influence of regional climate and nightly weather conditions on activity patterns of insectivorous bats. *Acta Chiropterologica*, *4*(1), 17–24.

Erkert, H. G. (2000). Bats- flying nocturnal mammals. In S. Halle & N. C. Stenseth (Eds.), *Activity patterns in small mammals* (pp. 253–272). Berlin, Heidelberg: Springer.

Ewert, S. P., Knörnschild, M., Jung, K., & Frommolt, K. H. (2023). Structurally rich dry grasslands–Potential stepping stones for bats in open farmland. *Frontiers in Ecology and Evolution*, *11*(995133). http://doi.org/10.3389/fevo.2023.995133

Fahr, J., & Kalko, E. K. V. (2011). Biome transitions as centres of diversity: habitat heterogeneity and diversity patterns of West African bat assemblages across spatial scales. *Ecography*, *2*(34), 177–195. http://doi.org/10.1111/j.1600-0587.2010.05510.x

Farrell, K., Van Vuren, J. H. J., & Ferreira, M. (2015). Do aquatic macroinvertebrate communities respond to land-use effects in the Wilge River , Mpumalanga , South Africa? *African Journal of Aquatic Science*, (May 2015), 37–41. http://doi.org/10.2989/16085914.2015.1025220

Faure, P. A., Re, D. E., & Clare, E. L. (2009). Wound healing in the flight membranes of big brown bats. *Journal of Mammalogy*, *90*(5), 1148–1156.

Fenton, M. B. (1970). A technique for monitoring bat activity with results obtained from different environments in southern Ontario. *Canadian Journal of Zoology*, *48*(4), 847–851.

Fenton, M. B. (1990). The foraging behaviour and ecology of animal-eating bats. *Canadian Journal of Zoology*, *1*(68), 411–422.

Fenton, M. B., Boyle, N. G. H., Harrison, T. M., & Oxley, D. J. (1977). Activity patterns, habit use, and prey selection by some African insectivorous bats. *Biotropica*, *9*(2), 73–85. http://doi.org/10.2307/2387662

Fenton, M. B., Cumming, D. H. M., Hutton, J. M., & Swanepoel, C. M. (1987). Foraging and habitat use by *Nycteris grandis* (Chiroptera: Nycteridae) in Zimbabwe. *Journal of Zoology*, *211*(4), 709–716.

Fenton, M. B., & Rautenbach, I. L. (1986). A comparison of the roosting and foraging behaviour of three species of African insectivorous bats (Rhinolophidae, Vespertilionidae, and Molossidae). *Canadian Journal of Zoology*, *64*(12), 2860–2867. http://doi.org/10.1139/z86-412

Fenton, M. B., Rautenbach, I. L., Smith, S. E., Swanepoel, C. M., Grosell, J., & van Jaarsveld, J. (1994). Raptors and bats: threats and opportunities. *Animal Behaviour*, *48*(1), 9–18.

Fenton, M. B., & Thomas, D. W. (1980). Dry-season overlap in activity patterns, habitat use, and prey selection by sympatric African insectivorous bats. *Biotropica*, *12*(2), 81–90.

Fischer, J., Stott, J., Law, B. S., Adams, M. D., & Forrester, R. I. (2009). Designing effective habitat studies: quantifying multiple sources of variability in bat activity. *Acta Chiropterologica*, *11*(1), 127–137. http://doi.org/10.3161/150811009X465749

Francis, C. M. (1989). A comparison of mist nets and two designs of harp traps for capturing bats. *Journal of Mammalogy*, *70*(4), 865–870.

Furlonger, C. L., Dewar, H. J., & Fenton, M. B. (1987). Habitat use by foraging insectivorous bats. *Canadian Journal of Zoology*, *65*(2), 284–288. http://doi.org/10.1139/z87-044

Gaujour, E., Amiaud, B., Mignolet, C., & Plantureux, S. (2012). Factors and processes affecting plant biodiversity in permanent grasslands. A review. *Agronomy for Sustainable Development*, *32*, 133–160. http://doi.org/10.1007/s13593-011-0015-3

Gelderblom, C. M., Bronner, G. N., Lombard, A. T., & Taylor, P. J. (1995). Patterns of distribution and current protection status of the Carnivora, Chiroptera and Insectivora in South Africa. *South African Journal of Zoology*, *30*(3), 103–114.

Geluso, K. (2007). Winter activity of bats over water and along flyways in New Mexico. *The Southwestern Naturalist*, *52*(4), 482–492.

Gillam, E. H. (2007). Eavesdropping by bats on the feeding buzzes of conspecifics. *Canadian Journal of Zoology*, *85*(7), 795–801.

Gonsalves, L., & Law, B. (2017). Seasonal activity patterns of bats in North Sydney, New South Wales: implications for urban bat monitoring programs. *Australian Mammalogy*, *40*(2), 220–229.

Graham, S. C., Barrett, A. S., & Brown, L. R. (2020). Impact of Seriphium plumosum densification on Mesic Highveld Grassland biodiversity in South Africa. *Royal Society Open Science*, *7*(4), 192025.

Greyling, C., & Keith, M. (2013). *Assessment of species richness and community structure of Chiprotera in the Bankenveld ecotone*. Honours Thesis, University of Pretoria.

Griffin, D. (1971). The importance of atmospheric attenuation for the echolocation of bats (chiroptera). *Animal Behaviour*, *19*(1), 55–61.

Gumbi, B. C., Shapiro, J. T., Mahlaba, T., Mccleery, R., Macfadyen, D., & Monadjem, A. (2018). Assessing the impacts of domesticated versus wild ungulates on terrestrial small mammal assemblages at Telperion Nature Reserve, South Africa. *African Zoology*, *51*(3), 23–29. http://doi.org/10.1080/15627020.2018.1449665

Hagen, E. M., & Sabo, J. L. (2011). A landscape perspective on bat foraging ecology along rivers: does channel confinement and insect availability influence the response of bats to aquatic resources in riverine landscapes? *Oecologia*, *166*, 751–760. http://doi.org/10.1007/s00442-011-1913-4

Hall, L. S., Krausman, P. R., & Morrison, M. L. (1997). The habitat concept and a plea for standard terminology. *Wildlife Society Bulletin*, *25*(1), 173–182.

Hamunyela, N., Parrini, F., Marshal, J., & Louw, H. (2017). Foraging behabiour of ruminant and non-ruminant grazers as a function of habitat heterogeneity in Telperion and Ezemvelo Nature Reserves (Ezemelo section). Master of Science Thesis, University of the Witwatersrand.

Happold, D. C., & Happold, M. (1990). Reproductive strategies of bats in Africa. *Journal of Zoology*, *4*(220), 557–583.

Hayes, J. P. (1997). Temporal variation in activity of bats and the design of echolocation-monitoring studies. *American Society of Mammalogists*, *78*(2), 514–524.

Heim, O., Treitler, J. T., Tschapka, M., Knörnschild, M., & Jung, K. (2015). The importance of landscape elements for bat activity and species richness in agricultural areas. *PLoS One*, *10*(7), 1–13. http://doi.org/10.1371/journal.pone.0134443

Henson, O. W., & Schnitzler, H. (1980). Performance of airborne animal sonar systems, II: vertebrates other than microchiroptera. In *Animal Sonar Systems* (pp. 183–195). New York: Plenum Press.

Herkt, K. M. B., Barnikel, G., Skidmore, A. K., & Fahr, J. (2016). A high-resolution model of bat diversity and endemism for continental Africa. *Ecological Modelling*, *320* (October 2015), 9–28. http://doi.org/10.1016/j.ecolmodel.2015.09.009

Herrero-villar, M., Sánchez-Barbudo, I. S., Camarero, P. R., Taggart, M. A., & Mateo, R. (2021).

Increasing incidence of barbiturate intoxication in avian scavengers and mammals in Spain. *Environmental Pollution*, *1*(284), 117452. http://doi.org/10.1016/j.envpol.2021.117452

Hodgkison, R., Balding, S. T., Zubaid, A., & Kunz, T. H. (2004). Habitat structure, wing morphology, and the vertical stratification of Malaysian fruit bats (Megachiroptera: Pteropodidae). *Journal of Tropical Ecology*, *20*, 667–673. http://doi.org/10.1017/S0266467404001737

Howard, A., Monadjem, A., Macfadyen, D. N., & Chimimba, C. T. (2022). Testing the efficacy of bat monitoring methods for identification and species surveys in KwaZulu-Natal province. *African Zoology*, *57*(4), 180–194.

Hoyos-Díaz, J. M., Villalba-Alemán, E., Ramoni-Perazzi, P., & Muñoz-Romo, M. (2018). Impact of artificial lighting on capture success in two species of frugivorous bats (Chiroptera: Phyllostomidae) in an urban locality from the Venezuelan Andes. *Mastozoologia Neotropica*, *25*(2), 473–478.

Hurme, E., Fahr, J., Monitoring network, E., Bakwo Fils Eric-Moise, C., Hash, T., O' Mara, T., Dechmann, D. K. N. (2022). Fruit bat migration matches green wave in seasonal landscapes. *Functional Ecology*, *36*(8), 2043–2055. http://doi.org/10.1111/1365-2435.14097

Hutcheon, J. M., & Kirsch, J. A. W. (2006). A moveable face: deconstructing the Microchiroptera and a new classification of extant bats. *Acta Chiropterologica*, *8*(1), 1–10. http://doi.org/10.3161/1733-5329(2006)8[1:AMFDTM]2.0.CO;2

Job, N. M., le Roux, P. A. L., Turner, D. P., van der Waals, J. H., Grundling, A. T., van der Walt, M., de Nysschen, G. P. M., & Paterson, D. G. (2019). Developing wetland distribution and transfer functions from land type data as a basis for the critical evaluation of wetland delineation guidelines by inclusion of soil water flow dynamics in catchment areas. Water Research Commission Report No. 2461/1/18. Johannesburg.

Johnson, J. B., Gates, J. E., & Zegre, N. P. (2011). Monitoring seasonal bat activity on a coastal barrier island in Maryland , USA. *Environmental Monitoring and Assessment*, *173*, 685–699. http://doi.org/10.1007/s10661-010-1415-6

Jones, G. (1999). Scaling of Echolocation Call Parameters in Bats. *The Journal of Experimental Biology*, *202*, 3359–3367.

Jones, G., & Holderied, M. W. (2007). Bat echolocation calls: adaptation and convergent evolution. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1612), 905–912. http://doi.org/10.1098/rspb.2006.0200

Jones, G., Jacobs, D. S., Kunz, T. H., Wilig, M. R., & Racey, P. A. (2009). Carpe noctem: The importance of bats as bioindicators. *Endangered Species Research*, *8*, 93–115. http://doi.org/10.3354/esr00182

Jones, G., & Rydell, J. (1994). Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *346(1318)*, 445–455.

Jones, G., Vaughan, N., & Parsons, S. (2000). Acoustic identification of bats from directly sampled and time expanded recordings of vocalizations. *Acta Chiropterologica*, *2*(2), 155–170.

José Zocche, J., Dimer Leffa, D., Paganini Damiani, A., Carvalho, F., Ávila Mendonça, R., dos Santos, C. E. I., Appel Boufleur, L., Ferraz Dias, J., & de Andrade, V. M. (2010). Heavy metals and DNA damage in blood cells of insectivore bats in coal mining areas of Catarinense coal basin, Brazil. *Environmental Research*, *110*(7), 684–691. http://doi.org/10.1016/j.envres.2010.06.003

Jung, K., Kaiser, S., Bohm, S., Nieschulze, J., & Kalko, E. K. V. (2012). Moving in three dimensions: effects of structural complexity on occurrence and activity of insectivorous bats in managed forest stands. *Journal of Applied Ecology*, *49*(2), 523–531. http://doi.org/10.1111/j.1365-2664.2012.02116.x

Kark, S. (2013). Ecotones and ecological gradients. In *Ecological systems: selected entries from the encyclopedia of sustainability science and technology* (pp. 147–160). Springer, New York.

Kasso, M., & Balakrishnan, M. (2013). Ecological and economic importance of bats (Order Chiroptera). *ISRN Biodiversity*, 1–9. http://doi.org/10.1155/2013/187415

Katunzi, T., Soisook, P., Webala, P. W., Armstrong, K. N., & Bumrungsri, S. (2020). Bat activity and species richness in different land-use types in and around Chome Nature Forest Reserve, Tanzania. *African Journal of Ecology*, *59*(1), 117–131. http://doi.org/10.1111/aje.12783

Kearney, T. C., Keith, M., Markotter, W., Pretorius, M., & Seamark, E. C. J. (2019). Bat species (Mammalia: Chiroptera) occurring at Telperion nature reserve. *Annals of the Ditsong National Museum of Natural History*, *8*, 30–42.

Kearney, T. C., & Seamark, E. C. J. (2014). *Report on EOS funded bat survey of the Telperion Nature Reserve*.

Kingston, T. (2016). Bats (Rapid Assessment). *Core standardized method for rapid biological field assessment. Arlington, Virginia: Conservation International*, pg 60-82. Retrieved from https://www.researchgate.net/publication/299892020

Klingbeil, B. T., & Willig, M. R. (2010). Seasonal differences in population- , ensemble- and community-level responses of bats to landscape structure in Amazonia. *Oikos*, *119*(10), 1654–1664. http://doi.org/10.1111/j.1600-0706.2010.18328.x

Koerner, S. E., & Collins, S. L. (2014). Interactive effects of grazing, drought, and fire on grassland plant communities in North America and South Africa. *Ecology*, *95*(1), 98–109.

Krusic, R. A., Yamasaki, M., Neefus, C. D., & Pekins, P. J. (1996). Bat habitat use in white mountain national forest. *The Journal of Wildlife Management*, *60*(3), 625–631.

Kunz, T. H., & Anthony, E. L. P. (1982). Age estimation and post-natal growth in the bat *Myotis lucifugus*. *Journal of Mammalogy*, *63*(1), 23–32.

Kunz, T. H., & Parsons, S. (2009). Methods of capturing and handling bats. In S. Parsons (Ed.), *Ecological and Behavioral Methods for the Study of Bats* (2nd ed.). John Hopkins University Press, Baltimore.

Lawrence, B. D., & Simmons, J. A. (1982). Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *The Journal of the Acoustical Society of America*, *71*(3), 585–590.

Lehmkuhl Noer, C., Dabelsteen, T., Bohmann, K., & Monadjem, A. (2012). Molossid bats in an African agro-ecosystem select sugarcane fields as foraging habitat. *African Zoology*, *47*(1), 1–11.

Lewis, E. (1993). Effect of climatic variation on reproduction by pallid bats (*Antrozous pallidus*). *Canadian Journal of Zoology*, *71*(7), 1429–1433.

Lewis, S. E. (1995). Roost fidelity of bats: a review. *Journal of Mammalogy*, *76*(2), 481–496.

Lima, S. L., & O'Keefe, J. M. (2013). Do predators influence the behaviour of bats? *Biological Reviews*, *88*(3), 626–644.

Limpens, H. J. G. A., & Kapteyn, K. (1991). Bats, their behaviour and linear landscape elements. *Myotis*, *29*(6), 63–71.

Linden, V. M. G., Gaigher, I., Weterings, M. J. A., & Taylor, P. J. (2014). Changes of bat activity , species richness, diversity and community composition over an altitudinal gradient in the Soutpansberg Range, South Africa. *Acta Chiropterologica*, *16*(1), 27–40. http://doi.org/10.3161/150811014X683246

Lino, A., Fonseca, C., Mendes, G., João, M., & Pereira, R. (2015). Roosting behaviour and phenology of the Lesser horseshoe bat (*Rhinolophus hipposideros*) in a breeding colony in Sintra , Portugal. Galemys: Boletín informativo de la Sociedad Española para la conservación y estudio de los mamíferos, 27(1), 1–12. http://doi.org/10.7325/Galemys.2015.A1

Macfadyen, D. N. (2014). *The dynamics of small mammal populations in Rocky Highveld Grassland, Telperion, South Africa*. Doctoral Thesis, University of Pretoria.

MacSwiney G, M, C., Clarke, F. M., & Racey, P. A. (2008). What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages. *Journal of Applied Ecology*, *45*(5), 1364–1371. http://doi.org/10.1111/j.1365-2664.2008.01531.x

Magurran, A. E. (1988). *Ecological diversity and its measurement.* Princeton University Press.

Maree, S., & Grant, W. S. (1997). Origins of horseshoe bats (*Rhinolophus*, Rhinolophidae) in southern Africa: evidence from allozyme variability. *Journal of Mammalian Evolution*, *4*(3), 195–215. http://doi.org/10.1023/A

Mcdonald, J. A. J., Rautenbach, I. L., & Nel. (1990). Roosting requirements and behaviour of five bat species at De Hoop Guano Cave, Southern Cape Province of South Africa. *South African Journal of Wildlife Research*, *20*(4), 157–161.

McDonald, J. H. (2014). *Handbook of biological statistics.* (2nd ed.). Sparky house publishing, Baltimore.

Mcguire, L. P., & Boyle, W. A. (2013). Altitudinal migration in bats: evidence, patterns, and drivers. *Biological Reviews*, *88*, 767–786. http://doi.org/10.1111/brv.12024

Mcnaughton, S. J. (1979). Grazing as an optimization process: grass-ungulate relationships in the serengeti. *The American Naturalist*, *113*(5), 691–703.

Menzies, A. K., Webber, Q. M. R., Baloun, D. E., McGuire, L. P., Muise, K. A., Cote, D., & Willis, C. K. R. (2016). Metabolic rate, latitude, and thermal stability of roosts, but not phylogeny, affect rewarming rates of bats. *Physiology & Behavior*, *164*, 361–368. http://doi.org/10.1016/j.physbeh.2016.06.015

Meyer, C. F. J., Schwarz, C. J., & Fahr, J. (2004). Activity patterns and habitat preferences of insectivorous bats in a West African forest- savanna mosaic. *Journal of Tropical Ecology*, *20*(4), 397–407. http://doi.org/10.1017/S0266467404001373

Miller, B. W. (2001). A method for determining relative activity of free flying bats using a new activity index for acoustic monitoring. *Acta Chiropterologica*, *3*(1), 93–105.

Miller, M. F. (1996). Dispersal of *Acacia* seeds by ungulates and ostriches in an African savanna. *Journal of Tropical Ecology*, *12*(3), 345–356.

Mise, K. M., De Souza, A. S. B., Campos, C. D. M., Keppler, R. L. F., & de Almeida, L. M. (2010). Coleoptera associated with pig carcass exposed in a forest reserve. *Biota Neotropica*, *10*(1), 321–324.

Moir, M. I., & Durand, F. (2014). *The habitat use, temporal distribution and preferred weather conditions of* Tadarida aegyptiaca *and* Neoromicia capensis*, and its application to wind farm development in South Africa*. Master of Science Thesis, University of Johannesburg.

Moir, M. I., Richards, L. R., Rambau, Ramugondo, V., & Cherry, M. I. (2020). Bats of Eastern Cape and southern KwaZulu-Natal forests, South Africa: diversity, call library and range extensions. *Acta Chiropterologica*, *22*(2), 365–381. http://doi.org/10.3161/15081109ACC2020.22.2.011

Moir, M., Richards, L. R., Rambau, R. V., & Cherry, M. I. (2020). *Evaluating the effects of biogeography and fragmentation on the taxonomic , functional , and genetic diversity of forest-utilising bats in a South African biodiversity hotspot*. Doctoral Thesis, Stellenbosch University.

Monadjem, A., Conenna, I., Taylor, P. J., & Schoeman, M. C. (2018). Species richness patterns and functional traits of the bat fauna of arid southern Africa. *Hystrix, the Italian Journal of Mammalogy*, *29*(1), 19–24. http://doi.org/10.4404/hystrix

Monadjem, A., & Reside, A. (2008). The influence of riparian vegetation on the distribution and abundance of bats in an African savanna. *Acta Chiropterologica*, *10*(2), 339–348. http://doi.org/10.3161/150811008X414917

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. http://doi.org/10.1515/MAMM.2009.056

Monadjem, A., Schoeman, M. C., Reside, A., Pio, D. V, Stoffberg, S., Bayliss, J., Taylor, P. J. (2010). A recent inventory of the bats of Mozambique with documentation of seven new species for the country. *Acta Chiropterologica*, *12*(2), 371–391. http://doi.org/10.3161/150811010X537963

Monadjem, A., Shapiro, J. T., Mtsetfwa, F., Reside, A. E., & McCleery, R. A. (2017). Acoustic call library and detection distances for bats of Swaziland. *Acta Chiropterologica*, *19*(1), 175–187. http://doi.org/10.3161/15081109ACC2017.19.1.014

Monadjem, A., Simelane, F., Shapiro, J. T., Gumbi, B. C., Mamba, M. L., Sibiya, M. D., & Mahlaba, T. A. M. (2021). Using species distribution models to gauge the completeness of the bat checklist of Eswatini. *European Journal of Wildlife Research*, *67*(2), 1–34.

Monadjem, A., Taylor, P. J., Cotterill, W., & Schoeman, M. C. (2020). *Bats of southern and central Africa: a biogeographic and taxonomic synthesis.* Wits University Press, Johannesburg. http://doi.org/http://dx.doi.org/10.1644/12-MAMM-R-184.1

Moss, C. F., & Surlykke, A. (2001). Auditory scene analysis by echolocation in bats. *The Journal of the Acoustical Society of America*, *110*(4), 2207–2226. http://doi.org/10.1121/1.1398051

Moussy, C., Hosken, D. J., Mathews, F., Smith, G. C., Aegerter, J. N., & Bearhop, S. (2012). Migration and dispersal patterns of bats and their influence on genetic structure. *Mammal Review*, *43*(3), 183–195. http://doi.org/10.1111/j.1365-2907.2012.00218.x

Mtsetfwa, F., Mccleery, R. A., & Monadjem, A. (2018). Changes in bat community composition and activity patterns across a conservation-agriculture boundary. *African Zoology*, *53*(3), 99–106. http://doi.org/10.1080/15627020.2018.1531726

Mucina, L., & Rutherford, M. (2006). *The vegetation of South Africa, Lesotho and Swaziland.* (L. Mucina & M. Rutherford, Eds.). South African National Biodiversity Institute, Pretoria.

Munhenga, G., Brooke, B. D., Spillings, B., Essop, L., Hunt, R. H., Midzi, S., & Koekemoer, L. L. (2014). Field study site selection, species abundance and monthly distribution of anopheline mosquitoes in the northern Kruger National Park, South Africa. *Malaria Journal*, *13*(27), 1–11.

Murray, K. L., Britzke, E. R., & Robbins, L. W. (2001). Variation in search-phase calls of bats. *Journal of Mammalogy*, *82*(3), 728–737.

Mushabati, L. M. (2019). Changes in the seasonal activity of bats occuring in the Kunene region, Namibia: Influence of environmental factors. Master of Science Thesis, University of Namibia. Retrieved from http://hdl.handle.net/11070/2511

Mushabati, L. M., Eiseb, S. J., Benda, P., & Laverty, T. M. (2022). Effects of lunar phase and temperature on bat activity and species richness at varying altitudes in the Kunene Region , Namibia. *African Journal of Ecology*, *60*(3), 467–480.

Nadin-davis, S. A., Guerrero, E., Knowles, M. K., & Feng, Y. (2012). DNA barcoding facilitates bat species identification for improved surveillance of bat-associated rabies across Canada. *The Open Zoology Journal*, *5*(1), 27–37.

Naidoo, S., Mackey, R. L., & Schoeman, C. (2011). Foraging ecology of insectivorous bats (Chiroptera) at a polluted and an unpolluted river in an urban landscape. *Durban Natural Science Museum Novitates 34*, (34), 21–28.

Naidoo, S., Vosloo, D., & Schoeman, M. C. (2016). Pollutant exposure at wastewater treatment works affects the detoxification organs of an urban adapter, the banana bat. *Environmental Pollution*, *208*, 830–839. http://doi.org/10.1016/j.envpol.2015.09.056

Neke, K., & Du Plessis, M. (2004). The threat of transformation: quantifying the vulnerability of grasslands in South Africa. *Conservation Biology*, *18*(2), 466–477.

http://doi.org/10.1111/j.1523-1739.2004.00157.x

Neuweiler, G. (1984). Foraging, echolocation and audition in bats. *Naturwissenschaften*, 71(9), 446–455.

Neuweiler, G. (2000). *The biology of bats.* (1st ed.). Oxford University Press, New York.

Noer, C. L., Dabelsteen, T., Bohmann, K., & Monadjem, A. (2012). Molossid bats in an African agro-ecosystem select sugarcane fields as foraging habitat. *African Zoology*, *47*(1), 1–11. http://doi.org/10.3377/004.047.0120

Norberg, U. M. L., & Rayner, J. (1987). Ecological morphology and flight in bats (Mammalia ; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of The Royal Society B Biological Sciences*, *316*(1179), 335–427. http://doi.org/10.1098/rstb.1987.0030

O' Farrell, M. J., & Gannon, W. L. (1999). A comparison of acoustic versus capture techniques for the inventory of bats. *Journal of Mammalogy*, *80*(1), 24–30.

O'Donoghue, T. L., Slater, K., & Brown, L. R. (2020). Seasonal habitat utilisation and morphological characteristics of *Chamaeleo dilepis* on Telperion Nature Reserve, Mpumalanga, South Africa. *African Journal of Herpetology*, *69*(2), 182–199. http://doi.org/10.1080/21564574.2020.1798290

Oliveira, D. L., & Vasconcelos, S. D. (2017). Diversity, daily flight activity and temporal occurrence of necrophagous diptera associated with decomposing carcasses in a semi-arid environment. *Neotropical Entomology*, *47*(4), 470–477. http://doi.org/10.1007/s13744-017-0540-0

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., D'Amico, J. A., Itoua, I., & Loucks, C. J. (2001). Terrestrial ecoregions of the world: a new map of life on Earth. *BioScience, 51(11), 933-938.*

Ortêncio-Filho, H., Lacher Jr, T. E., & Rodrigues, L. C. (2014). Seasonal patterns in community composition of bats in forest fragments of the Alto Rio Paraná, southern Brazil. *Studies on Neotropical Fauna and Environment*, 169-179. http://doi.org/10.1080/01650521.2014.950834

Paige, K. N. (1995). Bats and barometric pressure: conserving limited and tracking energy insects from the roost. *Functional Ecology*, *9*(3), 463–467.

Parker, D. M., & Bernard, R. T. F. (2018). The use of acoustic detectors for assessing bat species richness and functional activity in a South African national park. *Mammalia*, *83*(1), 53–63.

Parr, C. L., Lehmann, C. E. R., Bond, W. J., Hoffmann, W. A., & Andersen, A. N. (2014). Tropical grassy biomes: misunderstood, neglected, and under threat. *Trends in Ecology & Evolution*, *29*(4), 205–213. http://doi.org/10.1016/j.tree.2014.02.004

Pavey, C. R., Burwell, C. J., Grunwald, J.-E., Marshall, C. J., & Neuweiler, G. (2001). Dietary benefits of twilight foraging by the insectivorous bat *Hipposideros speoris*. *Biotropica*, *33*(4), 670–681. http://doi.org/10.1111/j.1744-7429.2001.tb00224.x

Peciña, M. V., Ward, R. D., Bunce, R. G. H., Sepp, K., Kuusemets, V., & Luuk, O. (2019). Science of the total environment country-scale mapping of ecosystem services provided by semi-natural grasslands. *Science of the Total Environment*, *661*, 212–225. http://doi.org/10.1016/j.scitotenv.2019.01.174

Peres-Neto, P. R., & Jackson, D. A. (2001). The importance of scaling of multivariate analysis in ecological studies. *Ecoscience*, *8*(4), 522–526.

Perry, R. W., Thill, R. E., & Leslie, D. M. J. (2006). Scale-dependent effects of landscape structure and composition on diurnal roost selection by forest bats. *Journal of Wildlife Management*, *72*(4), 913–925. http://doi.org/10.2193/2006-435

Power, R. J., Van Straaten, A., Schaller, R., Mooke, M., Boshoff, T., & Nel, H. P. (2019). An inventory of mammals of the North West Province, South Africa. *Annals of the Ditsong National Museum of Natural History*, *8*(1), 6–29.

Prat, Y., & Yovel, Y. (2020). Decision making in foraging bats. *Current Opinion in Neurobiology*, *60*, 169–175. http://doi.org/10.1016/j.conb.2019.12.006

Pretorius, M., Markotter, W., Kearney, T., Seamark, E., Broders, H., & Keith, M. (2021). No evidence of pre-hibernation or pre-migratory body mass gain in *Miniopterus natalensis* in north-eastern South Africa. *Journal of Vertebrate Biology*, *70*(1), 1–13. http://doi.org/10.25225/jvb.20088

Pretorius, M., Van Cakenberghe, V. Broders, H., & Keith, M. (2020). Temperature explains variation in seasonal temporal activity of *Miniopterus natalensis* more than moonlight or humidity. *Mammal Research*, *65*(4), 755–765.

Racey, P. A., & Entwistle, A. (2000). *Reproductive biology of bats*. (E. G. Crichton & P. H. Krutzsch, Eds.). Academic Press, Tucson.

Rautenbach, I. L., Fenton, M. B., & Braack, L. E. O. (1985). First records of five species of insectivorous bats from the Kruger National Park. *Koedoe: African Protected Area Conservation and Science*, *28*, 73–80.

Rautenbach, I. L., Fenton, M. B., & Whiting, M. J. (2014). Bats in riverine forests and woodlands : a latitudinal transect in southern Africa. *Canadian Journal of Zoology*, *74*(2), 312–322.

http://doi.org/10.1139/z96-039

Redding, M., & Apple, J. (2019). *Mammal species inventory using various trapping methods in Zone 4 of Billy Barquedier National Park, Belize during rainy season*. Bachelor of Science Thesis, University of Arkansas.

Reed, H. B. (1958). A study of dog carcass communities in Tennessee, with special reference to the insects. *American Midland Naturalist*, *59*(1), 213–245.

Richards, L., Schoeman, C., Taylor, P., White, W., Cohen, L., Jacobs, D., & Monadjem, A. (2016). A conservation assessment of *Otomops martiensseni*. In *The Red List of Mammals of South Africa, Swaziland and Lesotho. South African National Biodiversity Institute and Endangered Wildlife Trust, South Africa*.

Richardson, S. M., Lintott, P. R., Hosken, D. J., & Mathews, F. (2019). An evidence-based approach to specifying survey effort in ecological assessments of bat activity. *Biological Conservation*, *231*, 98–102. http://doi.org/10.1016/j.biocon.2018.12.014

Roeleke, M., Blohm, T., Hoffmeister, U., Marggraf, L., Schlägel, U. E., Teige, T., & Voigt, C. C. (2020). Landscape structure influences the use of social information in an insectivorous bat. *Oikos*, *129*(6), 912–923. http://doi.org/10.1111/oik.07158

Rutherford, M. C. & Westfall, R. H. (1994). *Biomes of southern Africa: an objective categorization*. National Botanical Institute, Cape Town.

Rydell, J. (1991). Seasonal use of illuminated areas by foraging northern bats *Eptesicus nilssoni*. *Ecography*, *14*(3), 203–207.

Rydell, J., Entwistle, A., & Racey, P. A. (1996). Timing of foraging flights of three species of bats in relation to insect activity and predation risk. *Oikos*, *76*(2), 243–252.

Rydell, J., & Speakman, J. R. (1995). Evolution of nocturnality in bats: potential competitors and predators during their early history. *Biological Journal of the Linnean Soceity*, *54*, 183–191.

Salinas-ramos, V. B., Montalvo, L. G. H., León-Regagnon, V., Arrizabalaga-Escudero, A., & Clare, E. L. (2015). Dietary overlap and seasonality in three species of mormoopid bats from a tropical dry forest. *Molecular Ecology*, *24*, 5296–5307. http://doi.org/10.1111/mec.13386

Saunders, M. B., & Barclay, R. M. R. (1992). Ecomorphology of insectivorous bats: a test of predictions using two morphologically similar species. *Ecology*, *73*(4), 1335–1345.

Schaffers, A. P., Raemakers, I. P., Sykora, K. V, & Ter Braak, C. J. F. (2008). Arthropod assemblages are best predicted by plant species composition. *Ecology*, *89*(3), 782–794.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675.

Schnitzler, H., & Kalko, E. K. V. (2001). Echolocation by insect-eating bats. *BioScience*, *51*(7), 557–569.

Schnitzler, H., Moss, C. F., & Denzinger, A. (2003). From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology & Evolution*, *18*(8), 386–394. http://doi.org/10.1016/S0169-5347(03)00185-X

Schoeman, M. C. (2015). Light pollution at stadiums favors urban exploiter bats. *Animal Conservation*, *19*(2), 120–130. http://doi.org/10.1111/acv.12220

Schoeman, M. C., & Jacobs, D. S. (2008). The relative influence of competition and prey defenses on the phenotypic structure of insectivorous bat ensembles in southern Africa. *PLoS One*, *3*(11), 1–10. http://doi.org/10.1371/journal.pone.0003715

Schoeman, M. C., & Monadjem, A. (2018). Community structure of bats in the savannas of southern Africa : influence of scale and human land-use. *Hystrix, the Italian Journal of Mammalogy*, *29*(1), 3–10. http://doi.org/10.4404/hystrix

Schrama, M., Heijning, P., Bakker, J. P., Wijnen, H. J. Van, Berg, M. P., & Olff, H. (2012). Herbivore trampling as an alternative pathway for explaining differences in nitrogen mineralization in moist grasslands. *Oecologia*, *172*(1), 231–243. http://doi.org/10.1007/s00442-012-2484-8

Seamark, E. C. J. (2013). The use of incidence data to estimate bat (Mammalia: Chiroptera) species richness and taxonomic diversity and distinctness within and between the biomes of South Africa, Lesotho and Swaziland. Master of Science Thesis, University of the Witwatersrand.

Shackleton, A. L. (2005). *Population genetics of the Cape serotine bat (Neoromicia capensis) in South Africa*. Master of Science Thesis, University of Cape Town.

Shannon, C. E. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing and Communications Review*, *5*(1), 3–55.

Shapiro, J. T., & Monadjem, A. (2016). Two new bat species for Swaziland and a revised list for the country. *Mammalia*, *80*(3), 353–357. http://doi.org/10.1515/mammalia-2014-0174

Shapiro, J. T., Monadjem, A., Röder, T., & Mccleery, R. A. (2019). Response of bat activity to land cover and land use in savannas is scale- , season- , and guild-specific. *Biological Conservation*, *(241),* 108245*.* http://doi.org/10.1016/j.biocon.2019.108245

Sherwin, R. E., Gannon, W. L., & Haymond, S. (2014). The efficacy of acoustic techniques to infer differential use of habitat by bats. *Acta Chiropterologica*, *2*(2), 145–153.

Shively, R., Barboza, P., Doak, P., & Jung, T. S. (2018). Increased diet breadth of little brown bats (*Myotis lucifugus*) at their northern range limit: a multimethod approach. *Canadian Journal of Zoology*, *96*(1), 31–38.

Siemann, E. (1998). Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology*, *79*(6), 2057–2070.

Siemers, B. J., & Schnitzler, H. (2000). Natterer's bat (*Myotis nattereri* Kuhl , 1818) hawks prey close to vegetation using echolocation signals of very broad bandwidth. *Behavioral Ecology and Sociobiology*, *47*(6), 400–412. http://doi.org/10.1007/s002650050683

Sikes, R. S. (2016). 2016 Guidelines of the American society of mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy*, *97*(3), 663–688.

Silkey, M., Nur, N., & Geupel, G. R. (1999). The use of mist-net capture rates to monitor annual variation in abundance: a validation study. *The Condor*, *101*(2), 288–298.

Simmons, J. A., Fenton, M. B., & Farrell, M. J. O. (1979). Echolocation and pursuit of prey by bats. *Science*, *203*, 16–21.

Skowno, A. L., Poole, Carol, J., Raimondo, Domitilla, J., Sink, Kerry, K., Van Deventer, H., Van Niekerk, L., & Driver, A. (2019). *National Biodiversity Assessment 2018: The status of South Africa's ecosystems and biodiversity: Synthesis report.* Pretoria.

Sowler, S., MacEwan, K., Aronson, J., & Lötter, C. (2020). South African best practice guidelines for pre-construction monitoring of bats at wind energy facilities. South African Bat Assessment Association.

Stahlschmidt, P., & Bruhl, C. A. (2012). Bats as bioindicators – the need of a standardized method for acoustic bat activity surveys. *Methods in Ecology and Evolution*, *3*(3), 503–508. http://doi.org/10.1111/j.2041-210X.2012.00188.x

Struebig, M. J., Kingston, T., Zubaid, A., Mohd-Adnan, A., & Rossiter, S. J. (2008). Conservation value of forest fragments to Palaeotropical bats. *Biological Conservation*, *141*(8), 2112–2126. http://doi.org/10.1007/s10592-007-9384-1

Swanepoel, B. A., & Bredenkamp, G. J. (2006). *The vegetation ecology of Ezemvelo Nature Reserve, Bronkhorstspruit, South Africa*. Master of Science Thesis, University of Pretoria.

Taylor, P. J. (1999). Echolocation calls of twenty southern African bat species. *South African Journal of Zoology*, *34*(3), 114–124.

Taylor, P. J., Bohmann, K., Steyn, J. N., Schoeman, C., Matamba, E., Zepeda-Mendoza, M L Nangammbi, T., & Gilbert, M. T. P. (2013). Bats eat pest green vegetable stink bugs (*Nezara* *viridula*): diet analyses of seven insectivorous species of bats roosting and foraging in macadamia orchards. *Southern African Macadamia Growers Association Yearbook*, *21*, 37-43.

Taylor, P. J., Cheney, C., & Sapsford, C. (1999). Roost habitat evaluation and distribution of bats (Chiroptera) in the Durban Metropolitan Region. *Durban Museum Novitates*, *24*(1), 62–71.

Taylor, P. J., Denys, C., & Cotterill, F. P. D. (2019). Taxonomic anarchy or an inconvenient truth for conservation? Accelerated species discovery reveals evolutionary patterns and heightened extinction threat in Afro-Malagasy small mammals. *Mammalia*, *83*(4), 313–329.

Taylor, P. J., Geiselman, C., Kabochi, P., Agwanda, B., & Turner, S. (2005). Intraspecific variation in the calls of some African bats (Order Chiroptera). *Durban Museum Novitates*, *30*(1), 24–37.

Taylor, P. J., Grass, I., Alberts, A. J., Joubert, E., & Tscharntke, T. (2017). Economic value of bat predation services– A review and new estimates from macadamia orchards. *Ecosystem Services*, *30*, 372–381. http://doi.org/10.1016/j.ecoser.2017.11.015

Taylor, P. J., Macdonald, A., Goodman, S. M., Kearney, T., Cotterill, F. P. D., Stoffberg, S. A. M., & Richards, L. R. (2018). Integrative taxonomy resolves three new cryptic species of small southern African horseshoe bats (*Rhinolophus*). *Zoological Journal of the Linnean Society*, *184*, 1249–1276.

Taylor, P. J., Sowler, S., Schoeman, M. C., & Monadjem, A. (2013). Diversity of bats in the Soutpansberg and Blouberg Mountains of northern South Africa: complementarity of acoustic and non-acoustic survey methods. *South African Journal of Wildlife Research*, *43*(1), 12–26.

Taylor, P. J., Stoffberg, S., Monadjem, A., Schoeman, M. C., Bayliss, J., & Cotterill, F. P. D. (2012). Four New Bat Species (*Rhinolophus hildebrandtii* Complex) Reflect Plio-Pleistocene Divergence of Dwarfs and Giants across an Afromontane Archipelago. *PLoS One*, *7*(9). http://doi.org/10.1371/journal.pone.0041744

Taylor, P. J., Strydom, E., Richards, L., Markotter, W., Toussaint, D. C., Kearney, T., Mamba, M. L. Magagula, S. & Monadjem, A. (2022). Integrative taxonomic analysis of new collections from the central Angolan highlands resolves the taxonomy of African pipistrelloid bats on a continental scale. *Zoological Journal of the Linnean Society*, *196*(4), 1570–1590.

Teeling, E. C., Springer, M. S., Madsen, O., Bates, P., O’brien, S. J., & Murphy, W. J. (2005). A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science*, *307*(5709), 580–584. http://doi.org/10.1126/science.1105113

Tews, J., Brose, U., Grimm, V., Tielborger, K., Wichmann, M. C., Schwager, M., & Jeltsch, F. (2004). Animal species diversity driven by habitat heterogeneity/ diversity: the importance of keystone structures. *Journal of Biogeography*, *31*(1), 79–92.

Tomberlin, J. K., Barton, B. T., Lashley, M. A., & Jordan, H. R. (2017). Mass mortality events and the role of necrophagous invertebrates. *Current Opinion in Insect Science*, *23*, 7–12.

Turbill, C. (2008). Winter activity of Australian tree-roosting bats: influence of temperature and climatic patterns. *Journal of Zoology*, *276*(3), 285–290. http://doi.org/10.1111/j.1469-7998.2008.00487.x

Van Der Merwe, M. (1987). Other bat species in maternity caves occupied by *Miniopterus schreibersii natalensis*. *South African Journal of Wildlife Research*, *17*(1), 25–27.

Vaughan, N., Jones, G., & Harris, S. (1996). Effects of sewage effluent on the activity of bats (Chiroptera: Vespertilionidae) foraging along rivers. *Biodiversity and Conservation*, *78*, 337–343.

Venter, F. J., & Gertenbach, W. P. D. (1986). A cursory review of the climate and vegetation of the Kruger National Park. *Koedoe: African Protected Area Conservation and Science*, *29*(1), 139-148.

Verboom, B., & Spoelstra, K. (1999). Effects of food abundance and wind on the use of tree lines by an insectivorous bat, *Pipistrellus pipistrellus*. *Canadian Journal of Zoology*, *77*(9), 1393–1401.

Viljoen, M. J., & Reimold, W. U. (1999). *An introduction to South Africa's geological and mining heritage*. Mintek, Randburg.

Walsh, A. L., & Harris, S. (1996). Foraging habitat preferences of vespertilionid bats in Britain. *The Journal of Applied Ecology*, *33*(3), 508. http://doi.org/10.2307/2404980

Weaver, K. N., Alfano, S. E., Kronquist, A. R., & Reeder, D. M. (2009). Healing rates of wing punch wounds in free-ranging little brown myotis (*Myotis lucifugus*). *Acta Chiropterologica*, *11*(1), 220–223. http://doi.org/10.3161/150811009X465866

Wechuli, D. B., Webala, P. W., Patterson, B. D., & Ochieng, R. S. (2016). Bat species diversity and distribution in a disturbed regime at the Lake Bogoria National Reserve , Kenya. *African Journal of Ecology*, *55*(4), 465–476. http://doi.org/10.1111/aje.12376

Weier, S. M. (2019). *Ecology and management of bat communities to increase pest control in macadamia orchards, Limpopo, South Africa*. Doctoral Thesis, University of Venda.

Weier, S. M., Linden, V. M. G., Gaigher, I., White, P. J. C., & Taylor, P. J. (2016). Changes of bat species composition over altitudinal gradients on northern and southern aspects of the Soutpansberg mountain range, South Africa. *Mammalia*, *81*(1), 49–60. http://doi.org/10.1515/mammalia-2015-0055

Weller, T. J., Cryan, P. M., & O’Shea, T. J. (2009). Broadening the focus of bat conservation and research in the USA for the 21st century. *Endangered Species Research*, *8* (1-2), 129–145. http://doi.org/10.3354/esr00149

Whitaker, J. O., McCracken, G. F., & Siemers, B. M. (2009). Food habits analysis of insectivorous bats. In T. H. Kunz & S. Parsons (Eds.), *Ecological and Behavioral Methods for the Study of Bats* (2nd ed., pp. 567–592). Johns Hopkins University Press, 2009.

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.

Williams, A. J., & Dickman, C. R. (2004). The ecology of insectivorous bats in the Simpson Desert central Australia: habitat use. *Australian Mammology*, *26*(2), 205–214.

Worthington Wilmer, J., & Barratt, E. (1996). A non-lethal method of tissue sampling for genetic studies of chiropterans. *Bat Research News.*, *37*(1) 1-5.

Zar, J. H. (1999). *Biostatistical analysis* (5th ed.). Pearson Education, India.

Zhao, Y., Liu, Z., & Wu, J. (2020). Grassland ecosystem services: a systematic review of research advances and future directions. *Landscape Ecology*, *3*(35), 793–814. http://doi.org/10.1007/s10980-020-00980-3

Zukal, J., Pikula, J., & Bandouchova, H. (2015). Bats as bioindicators of heavy metal pollution: History and prospect. *Mammalian Biology*, *80*(3), 220–227. http://doi.org/10.1016/j.mambio.2015.01.001

APPENDICES

APPENDIX 1 - ETHICAL CLEARANCE AND PERMITTING

Appendix 1: Figure 1.1. Ethical clearance from The University of South Africa for approval of the ‘Death in the long grass project, issued 2019 and renewed annually until 2021. Registration number REC-170616-051 and reference numbers 2018_CAES_119 and 2021/CAES_AREC/009

Ref no 2018_CAES_119.pdf

2021_CAES_AREC_009 RS2 upd.pdf

Appendix 1: Figure 1.2. Scientific permits issued by Gauteng Provincial Government Department of Agriculture and Rural Development for the ‘Death in the long grass project (2020); reference numbers CPF6_ 0213 and CPF6_0222

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Appendix 1: Figure 1.3. Scientific permits issued by Mpumalanga Parks and Tourism agency for the ‘Death in the long grass project (2019 to 2021); reference number MPB 5640

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Appendix 1: Figure 1.4. Landowner permission letters to conduct chiropteran research on the Telperion Private Nature Reserve

Acrobat Document

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Appendix 1: Figure 1.5. Small mammal active capture data sheets used during mist netting, hand netting and harp trapping on the Telperion Nature Reserve in 2019 and 2020

Adobe Acrobat Document

APPENDIX 2 – PASSIVE ACOUSTIC SAMPLING

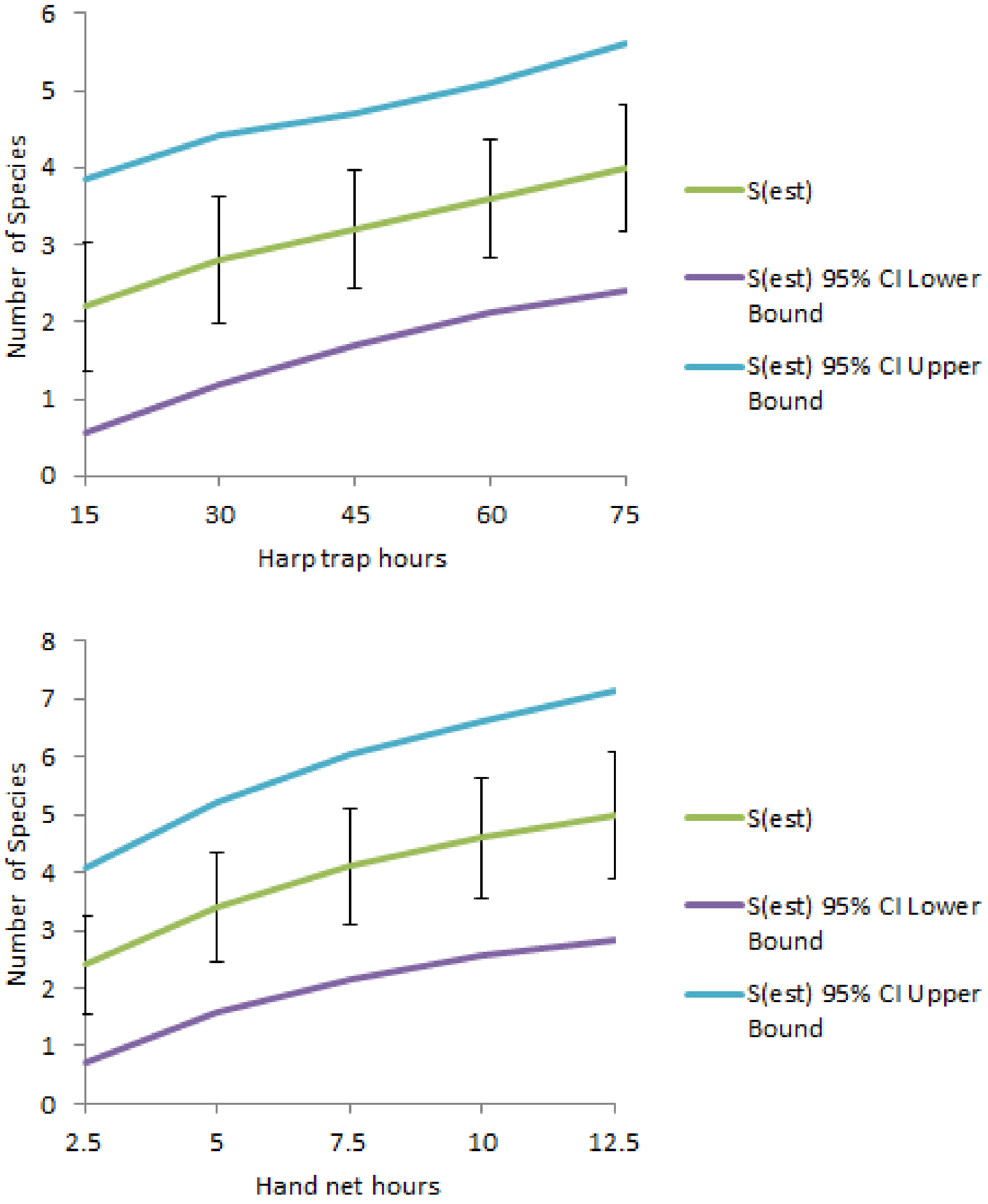
Appendix 2: Table 3.1. Detector settings used in this study

Recording device	**Setting**	**Value**
SongMeter 4 Full Spectrum (SM4BAT FS)	Gain	0dB
	High filter	10 kHz
	Sample rate	384 kHz
	Minimum duration	1.5 ms
	Maximum duration	None
	Minimum trigger frequency	12 kHz
	Maximum trigger frequency	6 dB
	Trigger level window	1 s
	Maximum length	15 s
	Compression	None
	Sunrise/ Sunset type	Solar sunrise
	Delay start	No
	LED Indicator	5 min
	Schedule mode	Daily
	Sleep	Do not sleep
	Start	Set -00:30
	Duty	Always
	End	Rise +00:30
	Expansion factor	10
	Buffer time	1000 ms
	Start monitoring	Armed
	Time zone	UTC +2:00
SongMeter 2Bat+ (SM2Bat)	Sample rate	384000
	Channels	Mono-L
	File format	WACO
	Gain left	+0.0 dB
	Gain right	+0.0 dB
	DIG HPF left	Fs/24
	DIG HPF right	Fs/24
	DIG LPF left	Off
	DIG LPF right	Off
	Trigger level left	6 SNR
	Trigger level right	16 SNR
	Trigger window left	1 s
	Trigger window right	1 s
	Trigger max length	15 s
	Bits	16
	Division ratio	16
	Nap trigger level	Off
	At Sset	-00:30
	Schedule 02	Do
	Record	00:30:00
	Goto line	03:00X
	Unt srise	+00:30
	Goto line	01:00X
	Timezone	UTC +2:00

APPENDIX 3 – SUPPLEMENTARY RESULTS

CHAPTER FOUR

SPECIES INVENTORY, REGIONAL CALL LIBRARY AND CALL VARIATION



Appendix 3: Figure 4.1. Species accumulation curves for hand netting and harp trapping of bats on Telperion Nature Reserve between January 2019 and January 2020

Note: Standard deviation is depicted as error bars.

Appendix 3: Table 4.1. Kolmogorov–Smirnov (KS) test statistics for call parameters between male and female bats of the same species captured on the Telperion Nature Reserve between January 2019 and January 2020.

Call parameter	KS value LCA Female	KS value LCA Male
Fc	0.200	0.200
Fk	0.003	0.200
Fmax	0.013	0.001
Fmin	0.034	0.200
Duration	0.017	0.000
Bandwidth	0.005	0.001
Call parameter	**KS value SDI Female**	**KS value SDI Male**
Fc	0.103	0.113
Fk	0.112	0.130
Fmax	0.134	0.191
Fmin	0.099	0.118
Duration	0.098	0.102
Bandwidth	0.119	0.200
Call parameter	**KS value R79**	**KS value RSI**
Fc	0.000	0.000
Fk	0.001	0.000
Fmax	0.000	0.000
Fmin	0.001	0.002
Duration	0.013	0.200
Bandwidth	0.012	0.001
Call parameter	**KS value NZU Female**	**KS value NZU Male**
Fc	0.200	0.158
Fk	0.200	0.007
Fmax	0.001	0.200
Fmin	0.200	0.200
Duration	0.076	0.200
Bandwidth	0.004	0.200

Abbreviations: LCA - *Laephotis capensis*; SDI – *Scotophilus dinganii*; NZU – *Neoromicia zuluensis*; R79 – *Rhinolophus* cf. *simulator* 79; RSI – *Rhinolophus simulator*; Fc - characteristic frequency; Fk - knee frequency call duration (dur), Fmax - maximum frequency; Fmin - minimum frequency.

Appendix 3: Table 4.2. Mann-Whitney U Test for *Rhinolophus simulator* and *Rhinolophus cf. simulator 79* echolocation call parameters recorded on the Telperion Nature Reserve between January 2019 and January 2020

Parameter	**U**	**p**
Fc	1828.5	<0.001
Fk	1829.0	<0.001
Fmax	1829.0	<0.001
Fmin	1408.5	<0.001
Duration	1122.0	0.078
Bandwidth	839.0	0.521

Appendix 3: Table 4.3. Mann-Whitney U Test for *Laephotis capensis echolocation call parameters recorded* on the Telperion Nature Reserve between January 2019 and January 2020

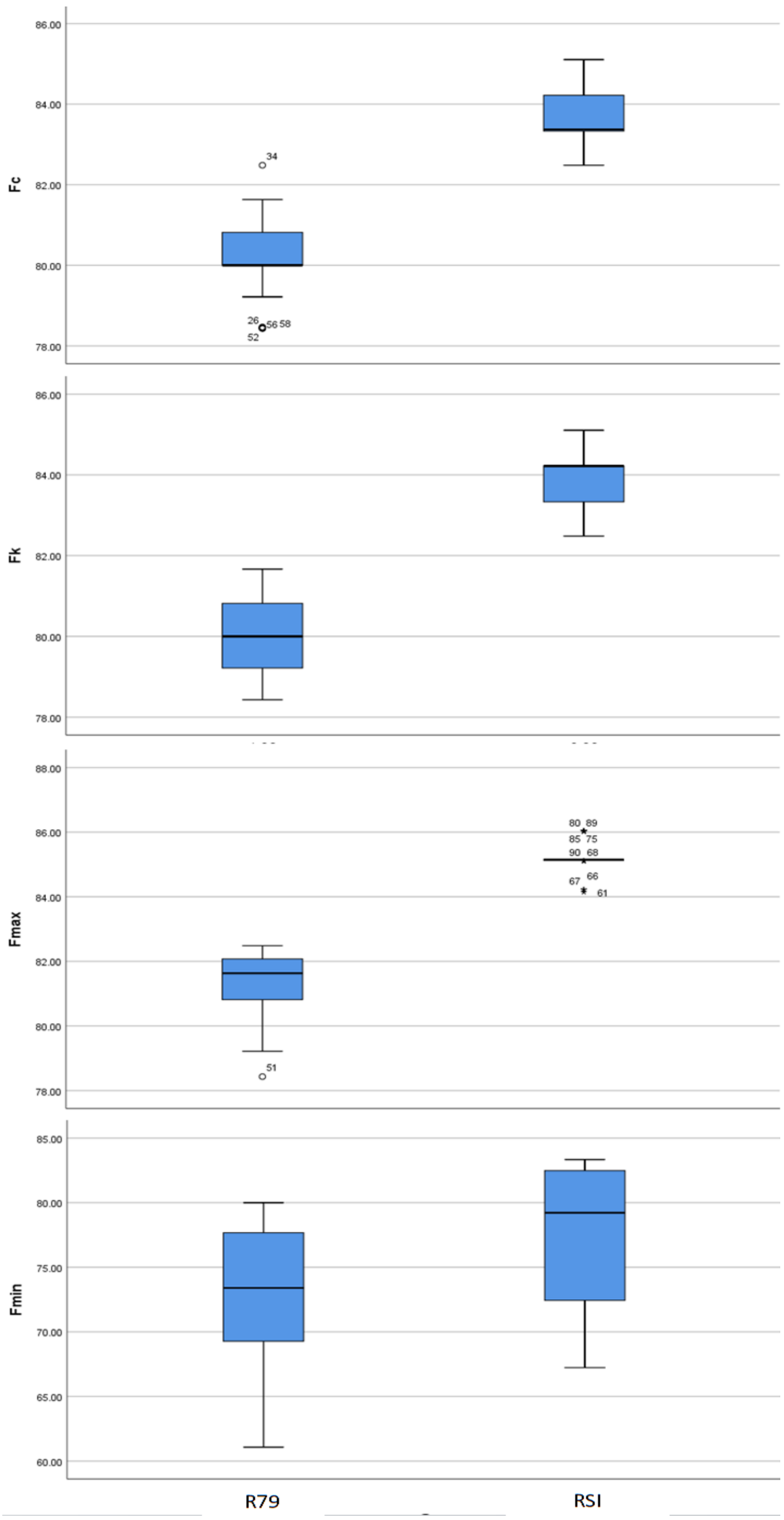
Parameter	**U**	**p**
Fc	6652.5	0.003
Fk	134.0	0.001
Fmax	7329.0	0.067
Fmin	6927.5	0.013
Duration	9792.0	0.025
Bandwidth	7566.0	0.150

Appendix 3: Table 4.4. Mann-Whitney U Test for *Neoromicia zuluensis* echolocation call parameters recorded on the Telperion Nature Reserve between January 2019 and January 2020

Parameter	**U**	**p**
Fc	218.5	0.145
Fk	283.5	0.895
Fmax	188.0	0.038
Fmin	209.0	0.099
Duration	286.0	0.935
Bandwidth	189.0	0.040

Appendix 3: Table 4.5. Mann-Whitney U Test for *Scotophilus dinganii* echolocation call parameters recorded on the Telperion Nature Reserve between January 2019 and January 2020

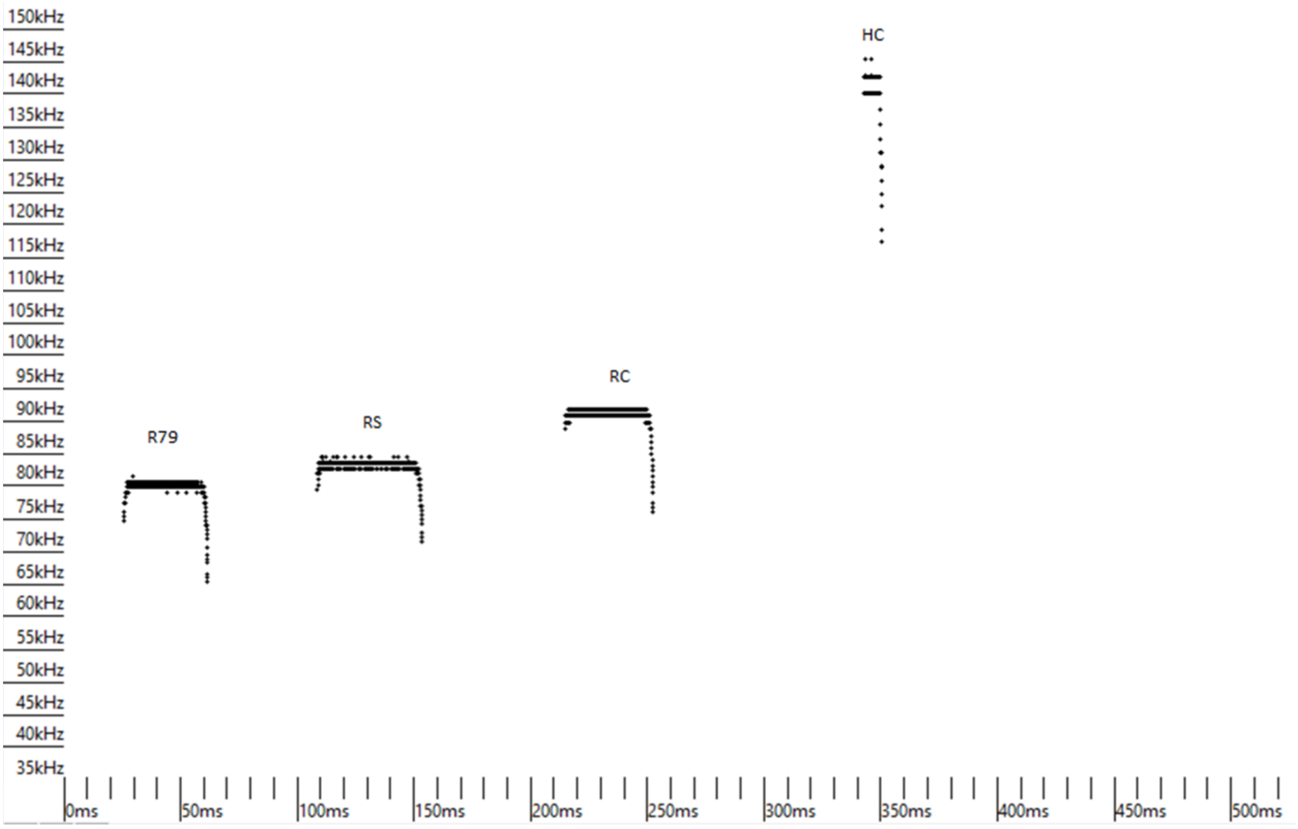
Parameter	U	p
Fc	810.5	0.614
Fk	814.0	0.589
Fmax	479.5	0.005
Fmin	845.0	0.395
Duration	713.5	0.642
Bandwidth	461.0	0.003



Appendix 3: Figure 4.2. Box and whisker plots, presenting the median and data spread, for constant frequency (Fc), maximum frequency (Fmax) and minimum frequency (Fmin) echolocation call parameters of *Rhinolophus* cf. *simulator* 79 (R79) and *Rhinolophus simulator* s.s. (RSI)

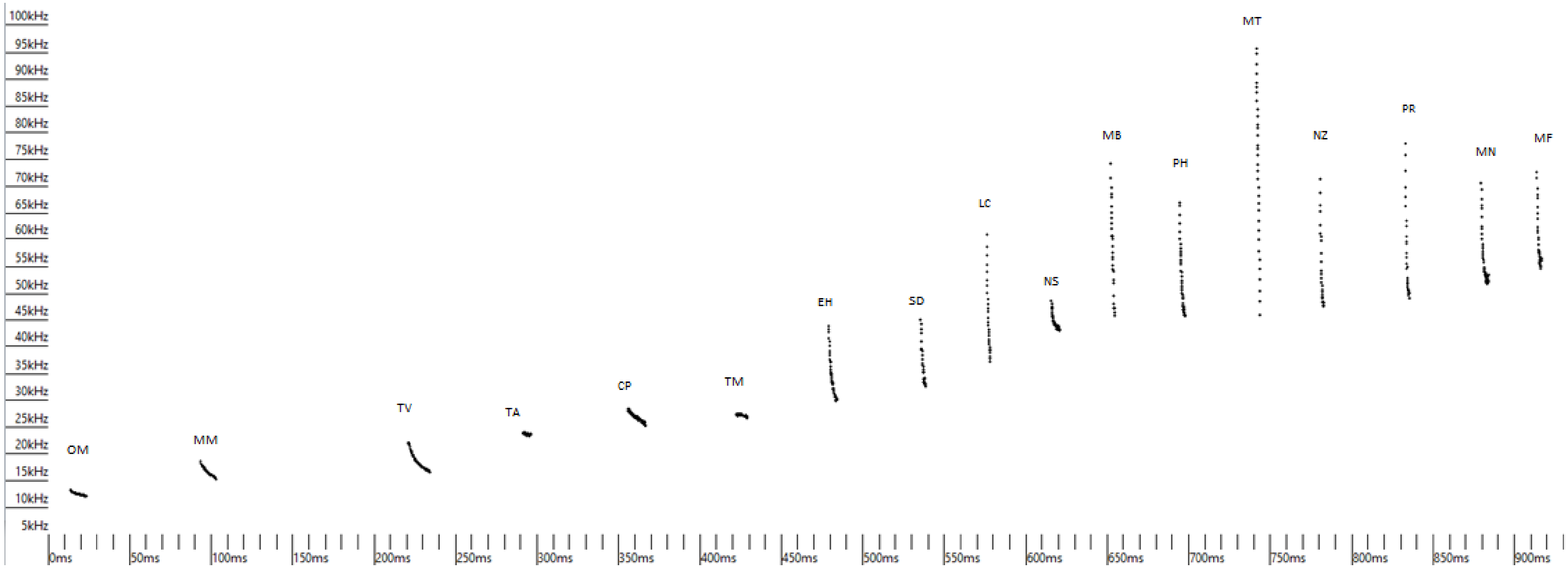
Appendix 3: Table 4.6. Results of a Kruskal-Wallis test conducted on bat functional foraging groups on the Telperion Nature Reserve between January 2019 and January 2020.

Functional foraging group	Independent-samples Kruskal-Wallis Test	*N*
Group 1	0.368	3
Group 2	0.368	3
Group 3	0.368	3
Group 4	0.368	3
Group 5	1	3



Appendix 3: Figure 4.3. Zero crossing constant frequency echolocation calls of bats captured on the Telperion Nature Reserve between January 2019 and January 2021

Notes: A) R79 = *Rhinolophus* cf. *simulator*, RS = *Rhinolophus simulator*, RC = *Rhinolophus clivosus*, HC = *Hipposideros caffer*.



Appendix 3: Figure 4.4. Zero crossing frequency modulated and quasi-constant frequency echolocation calls of bats on the Telperion Nature Reserve between January 2019 and January 2021.

Notes: * = free flight call (bat not captured). CP* = *Mops* (*Chaerephon*) *pumilus*, EH* = *Eptesicus hottentotus*, LC = *Laephotis capensis* (Monadjem, *et al.,* 2020), MF* = *Miniopterus fraterculus*, MN = *Miniopterus natalensis*, MM* = *Mops midas*, MB* = *Myotis bocagii*, MT = *Myotis tricolor*, NZ = *Neoromicia zuluensis*, NS* = *Nycticeinops schlieffeni*, OM* = *Otomops martiensseni*, PH = *Pipistrellus hesperidus*, PR = *Pipistrellus rusticus*, SD = *Scotophilus dinganii*, TA* = *Tadarida aegyptiaca*, TV* = *Tadarida ventralis*, TM* = *Taphozous mauritianus*

CHAPTER FIVE

THE EFFECTS OF HABITAT AND SEASON ON THE CHIROPTERAN ASSEMBLAGE OF THE TELPERION NATURE RESERVE

Appendix 3: Table 5.1. Kruskal-Wallis Test comparing the number of bat passes between habitat on the Telperion Nature Reserve between January 2019 and January 2020

Habitat comparison	DF	H	p
Tree grass vs Rock outcrop	3	10.9	0.099
Tree grass vs Anthropogenic	3	15.6	0.018*
Tree grass vs Water course	3	-18.9	0.004*
Rock outcrop vs Anthropogenic	3	4.7	0.473
Rock outcrop vs Water course	3	-8.0	0.222
Anthropogenic vs Water course	3	-3.3	0.615

Significance values have been adjusted by the Bonferroni correction due to multiple tests being run.
* indicates significance at $p \leq 0.05$

Appendix 3: Table 5.2. Species captured using active and passive sampling on the Telperion Nature reserve between January 2019 and April 2021

Anthropogenic	Rock outcrop	Tree grass	Water course
Rhinolophus cf. simulator 79	_*Nycteris thebaica_	_*Nycteris thebaica_	_*Myotis tricolor_
Mops (Chaerephon) pumilus	_Rhinolophus cf. simulator 79_	_*Rhinolophus cf. simulator 79_	_*Rhinolophus cf. simulator 79_
Eptesicus hottentotus	_Mops (Chaerephon) pumilus_	_Eptesicus hottentotus_	_*Rhinolophus simulator_
Miniopterus natalensis	_Eptesicus hottentotus_	_Miniopterus natalensis_	_Mops (Chaerephon) pumilus_
Mops midas	_Miniopterus fraterculus_	_Laephotis capensis_	_Eptesicus hottentotus_
Myotis bocagii	_Miniopterus natalensis_	_Neoromicia zuluensis_	_Miniopterus fraterculus_
Myotis tricolor	_Mops midas_	_Pipistrellus hesperidus_	_Miniopterus natalensis_
Laephotis capensis	_Myotis bocagii_	_Pipistrellus rusticus_	_Mops midas_
Neoromicia zuluensis	_Laephotis capensis_	_Rhinolophus simulator_	_Myotis bocagii_
Nycteris thebaica	_Neoromicia zuluensis_	_Rhinolophus clivosus_	_Laephotis capensis_
Nycticeinops schlieffeni	_Nycticeinops schlieffeni_	_Tadarida ventralis_	_Neoromicia zuluensis_
Otomops martiensseni	_Otomops martiensseni_		_Nycteris thebaica_
Pipistrellus hesperidus	_Pipistrellus hesperidus_		_Nycticeinops schlieffeni_
Pipistrellus rusticus	_Pipistrellus rusticus_		_Otomops martiensseni_
Rhinolophus simulator	_Rhinolophus simulator_		_Pipistrellus hesperidus_
Rhinolophus clivosus	_Scotophilus dinganii_		_Pipistrellus rusticus_
Scotophilus dinganii	_Tadarida ventralis_		_Scotophilus dinganii_
Tadarida ventralis	_Tadarida aegyptiaca_		_Tadarida ventralis_
Tadarida aegyptiaca	_Taphozous mauritianus_		_Tadarida aegyptiaca_
Taphozous mauritianus			_Taphozous mauritianus_

Species only captured using active sampling in each habitat are labelled with an *

Appendix 3: Table 5.3. Kruskal-Wallis test comparing number bat species recorded during seasonal acoustic sampling on the Telperion Nature Reserve between January 2019 and January 2020

Habitat comparison	DF	H	p
Anthropogenic vs Tree grass	3	-3.6	0.581
Anthropogenic vs Rock outcrop	3	-14.3	0.029*
Anthropogenic vs Water course	3	-19.4	0.003*
Tree grass vs Rock outcrop	3	10.7	0.104
Tree grass vs Water course	3	-15.8	0.016*
Rock outcrop vs Water course	3	-5.1	0.435

.* indicates significance at $p \leq 0.05$

Appendix 3: Table 5.4. Kruskal-Wallis test comparing number bat passes recorded for each functional foraging guild during acoustic sampling on the Telperion Nature Reserve between January 2019 and January 2020

Functional Group	χ^2_4	Std. Error	p
Group 5-Group 2	69.031	15.748	0.000
Group 5-Group 4	77.344	15.748	0.000
Group 5-Group 3	130.579	15.810	0.000
Group 5-Group 1	198.602	15.748	0.000
Group 2-Group 4	-8.312	15.748	0.598
Group 2-Group 3	-61.548	15.810	0.000
Group 2-Group 1	129.570	15.748	0.000
Group 4-Group 3	53.236	15.810	0.001
Group 4-Group 1	121.258	15.748	0.000
Group 3-Group 1	68.022	15.810	0.000

Intermediate / high frequency FM clutter edge foragers (Group 1), Large, low frequency LD-QCF open air foragers (Group 2), Intermediate and lower frequency edge and open air foragers (Group 3), HD-CF and multi-harmonic clutter foragers (Group 4) and HD-CF Very High frequency clutter foragers (Group 5). No passes for group 5 were recorded using acoustic sampling.

Appendix 3: Table 5.5. Kruskal-Wallis test comparing number bat passes among habitats sites on the Telperion Nature Reserve between January 2019 and January 2020.

Habitat type	χ^2_3	Std. Error	p
Tree-Grass-Anthropogenic	13.453	12.917	0.298
Tree-Grass-Rock Outcrop	27.808	12.969	0.032*
Tree-Grass-Water Course	-33.383	12.917	0.010*
Anthropogenic-Rock Outcrop	-14.354	12.969	0.268
Anthropogenic-Water Course	-19.930	12.917	0.123
Rock Outcrop-Water Course	-5.575	12.969	0.667

.* indicates significance at $p \leq 0.05$

Appendix 3: Table 5.6. Kruskal-Wallis test comparing number bat passes recorded across seasonal sampling on the Telperion Nature Reserve between January 2019 and January 2020

Season comparison	DF	H	p
Winter vs Autumn	3	5.50	0.403
Winter vs Summer	3	27.19	<0.001*
Winter vs Spring	3	31.94	<0.001*
Autumn vs Summer	3	21.69	0.001*
Autumn vs Spring	3	26.44	<0.001*
Summer vs Spring	3	-0.475	0.471

The Bonferroni correction was used as multiple tests were run on the data simultaneously.
* indicates significance at p ≤ 0.05

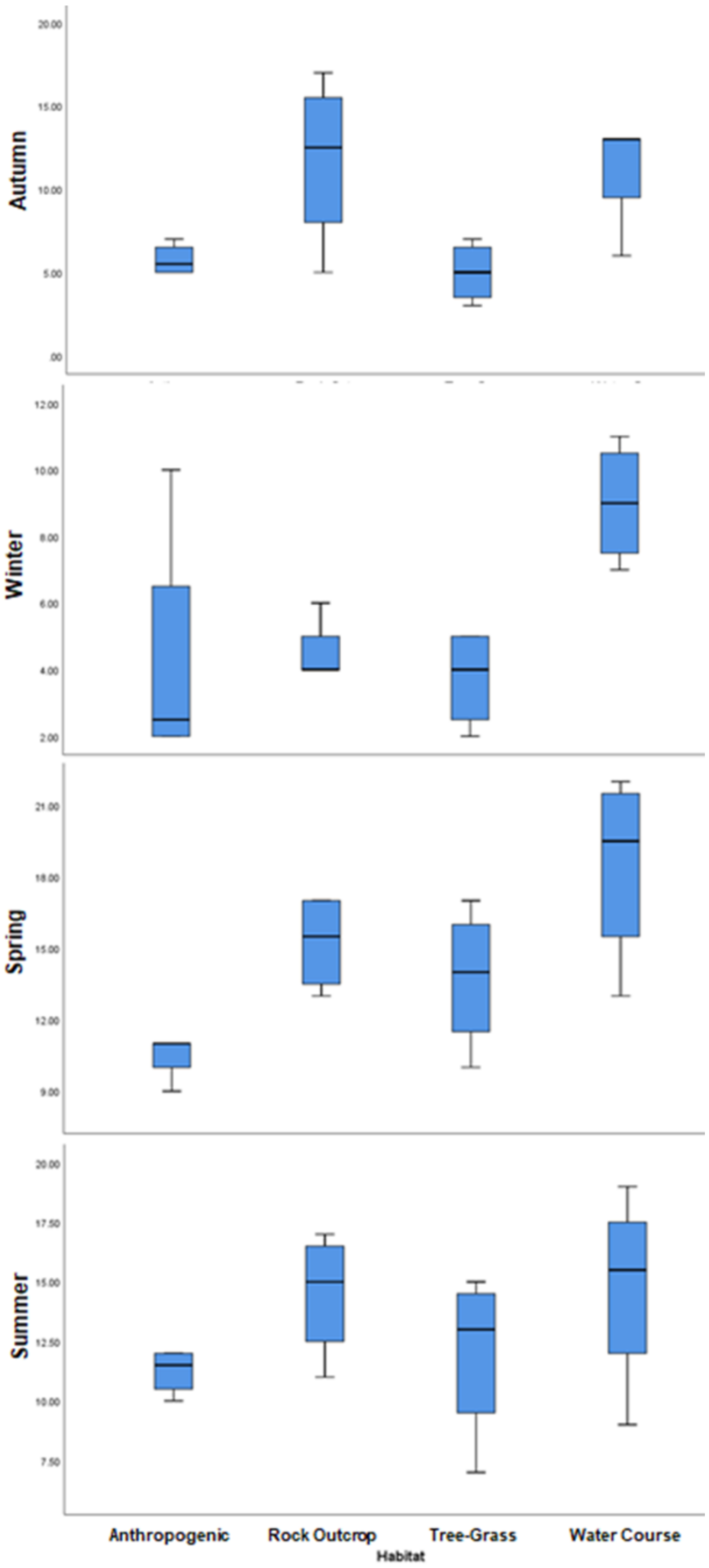
Appendix 3: Table 5.7. Seasonal variation in chiropteran activity levels (calls/ acoustic night) on the Telperion Nature Reserve between January 2019 and January 2020.

Season	x̄ passes/ night	SD	Max passes	Min passes
Autumn	58.78	45.44	131	4
Spring	382.72	479.80	2029	10
Summer	381.25	383.79	1274	9
Winter	39.81	75.92	317	2

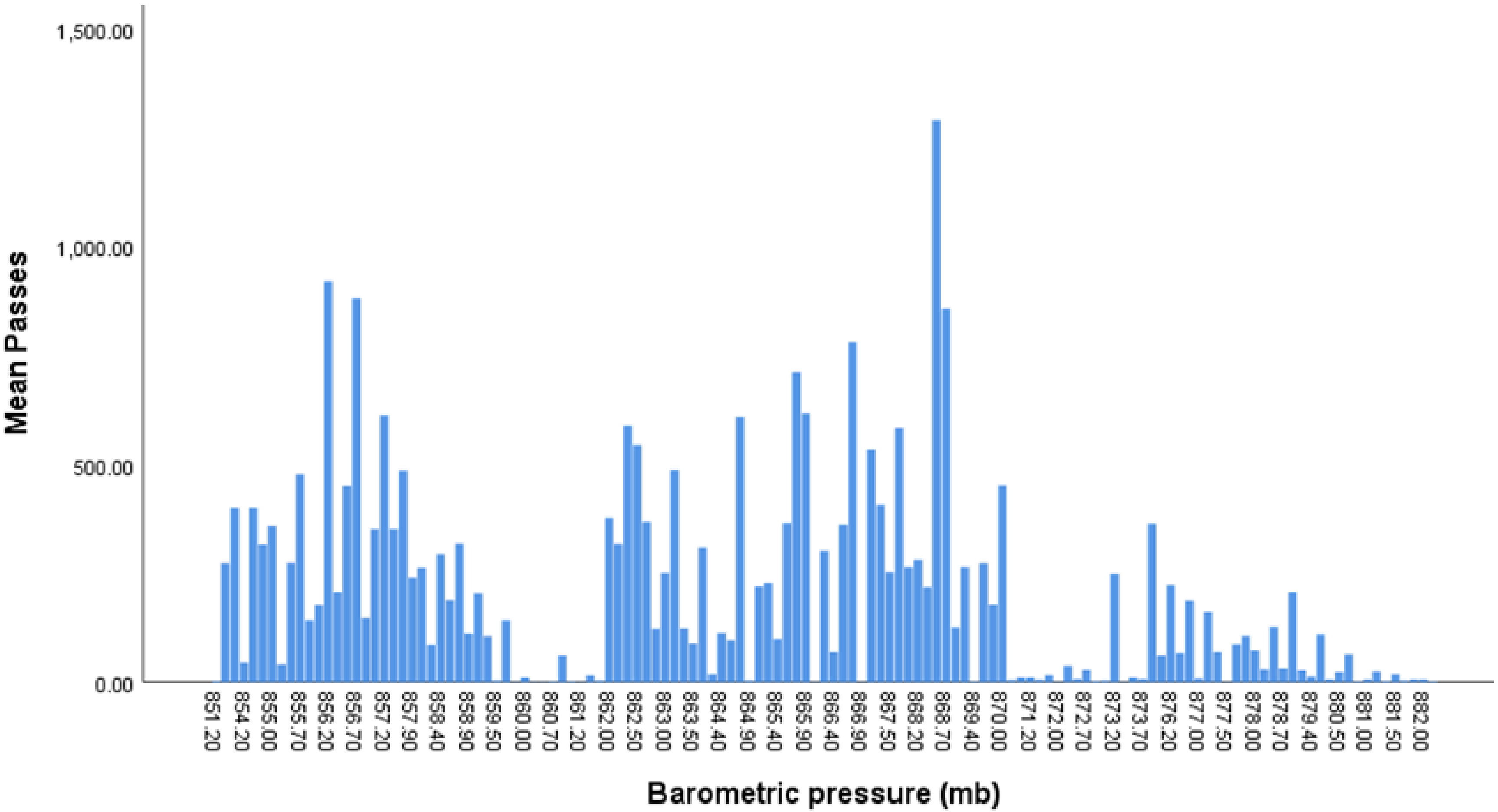
Appendix 3: Table 5.8. Kruskal-Wallis test comparing the seasonal occurrence of bat species on the Telperion Nature Reserve between January 2019 and January 2020

Season comparison	DF	H	p
Winter vs Autumn	3	11.44	0.082
Winter vs Summer	3	29.53	0.000*
Winter vs Spring	3	33.16	0.000*
Autumn vs Summer	3	18.09	0.006*
Autumn vs Spring	3	21.72	0.001*
Summer vs Spring	3	3.63	0.581

The Bonferroni correction was used as multiple tests were run.
* indicates significance at p ≤ 0.05



Appendix 3: Figure 5.1. The number of species captured among different habitat types during seasonal sampling on the Telperion Nature Reserve between January 2019 and January 2020



Appendix 3: Figure 5.2. The effect of barometric pressure on bat activity on the Telperion Nature Reserve between January 2019 and January 2020

Appendix 3: Table 5.9. Functional diversity of the bats on the Telperion Nature Reserve between January 2019 and April 2021

Species	n	FA (mm)	Diet	Fc	Call type	Roost site	Foraging guild	Aspect ratio	Wing loading (N/M²)
Hipposideros cafer	1	49.27	Insectivore	138.7	HD-CF	Caves	Clutter	6.20	5.47
Miniopterus natalensis	1	44.95	Insectivore	53.57	LD-FM	Caves	Clutter-Edge	6.76	8.98
Myotis tricolor	9	47.11	Insectivore	54.88	LD-FM	Caves	Edge	5.67	9.07
Laephotis capensis-Female	54	34.31	Insectivore	37.93	LD-FM	Anthropogenic	Edge	5.34	6.87
Laephotis capensis-Male	51	32.68	Insectivore	38.45	LD-FM	Anthropogenic	Edge	5.58	7.71
Neoromicia zuluensis	9	29.18	Insectivore	45.82	LD-FM	Vegetation (aloes)	Clutter-Edge	5.68	5.81
Nycteris thebaica	5	36.83	Insectivore	90	LD-FM	Caves, burrows, culverts	Clutter	4.97	5.63
Pipistrellus hesperidus	6	31.16	Insectivore	46.66	LD-FM	Tree crevices, rock crevices	Edge	6.10	9.01
Pipistrellus rusticus	2	33.99	Insectivore	48.66	LD-FM	Tree crevices	Clutter-Edge	5.66	8.41
Rhinolophus cf. simulator 79	7	45.62	Insectivore	79.95	HD-CF	Caves	Clutter	5.60	6.42
Rhinolophus clivosus	2	53.71	Insectivore	90.63	HD-CF	Caves	Clutter	5.89	9.32
Rhinolophus simulator	5	46.44	Insectivore	83.49	HD-CF	Caves	Clutter	5.37	7.76
Scotophilus dingani	14	55.97	Insectivore	31.82	LD-FM	Anthropogenic	Edge	6.03	13.73
Mops (Chaerephon) pumilus	0	37.60	Insectivore	25.6	LD-QCF	Rock crevices, tree crevices, anthropogenic	Open	8.60	11.80
Eptesicus hottentotus	0	49.90	Insectivore	30.6	LD-FM and LD-QCF	Caves and rock crevices	Clutter-Edge	6.30	10.30
Miniopterus fraterculus	0	43.75	Insectivore	58.59	LD-FM	Caves	Edge	6.64	6.35
Mops midas	0	62.65	Insectivore	16.1	LD-QCF and LD-CF	Rocks crevices, tree crevices, anthropogenic	Open	8.90	11.40
Myotis bocagii	0	38.49	Insectivore	39.68	LD-FM	Tree crevices	Clutter-Edge	5.84	6.76
Nycticeinops schlieffeni	0	31.20	Insectivore	42.5	LD-FM	Tree crevices, anthropogenic	Clutter-Edge	6.90	6.70
Otomops martiensseni	0	63.00	Insectivore	11	LD-QCF and LD-CF	Anthropogenic	Open	9.30	14.90
Tadarida aegyptiaca	0	46.50	Insectivore	22.7	LD-QCF	Caves, rock crevices and tree crevices	Open	8.10	13.10
Tadarida ventralis	0	63.20	Insectivore	19.3	LD-QCF and LD-CF	Rock crevices, anthropogenic	Unknown	Unknown	Unknown
Taphozous mauritianus	0	62.00	Insectivore	25.9	HD-QCF	Tree crevices	Open	10.10	15.40

Gaps in data from this research were filled in from published resources (Moir *et al.,* 2020; Monadjem *et al.,* 2020). Abbreviations are as follows: (HD - high duty-cycle; LD - low duty-cycle; CF - constant frequency; QCF- quasi-constant frequency; FM - frequency modulated; Fc - characteristic frequency; n – total number of captured individuals; FA – forearm length)

CHAPTER SIX

THE EFFECTS OF BLUE WILDEBEEST (*CONNOCHAETES TAURINUS*) CARCASS SITES ON THE CHIROPTERAN ASSEMBLAGE OF THE TELPERION NATURE RESERVE

Appendix 3: Table 6.1. Kolmogorov-Smirnov test of normality for total bat passes and feeding buzzes at blue wildebeest (*Connochaetes taurinus*) carcass treatment sites on the Telperion Nature Reserve during January 2019 and February 2019

Treatment	χ^2 (Passes)	DF (Passes)	p (Passes)	$\bar{x}$ Passes ± SD	Statistic (FB)	DF (FB)	p (FB)	Mean FB
Caged	0.321	15	0.000	119.5 ± 109.6	0.312	15	0.000	9.0
Pegged	0.222	15	0.044	50.7 ± 46.0	0.333	15	0.000	2.0
Control	0.211	20	0.020	66.0 ± 43.3	0.282	20	0.000	2.4

Note 1: FB- Feeding buzz
Note 2: None of these data conform to a normal distribution

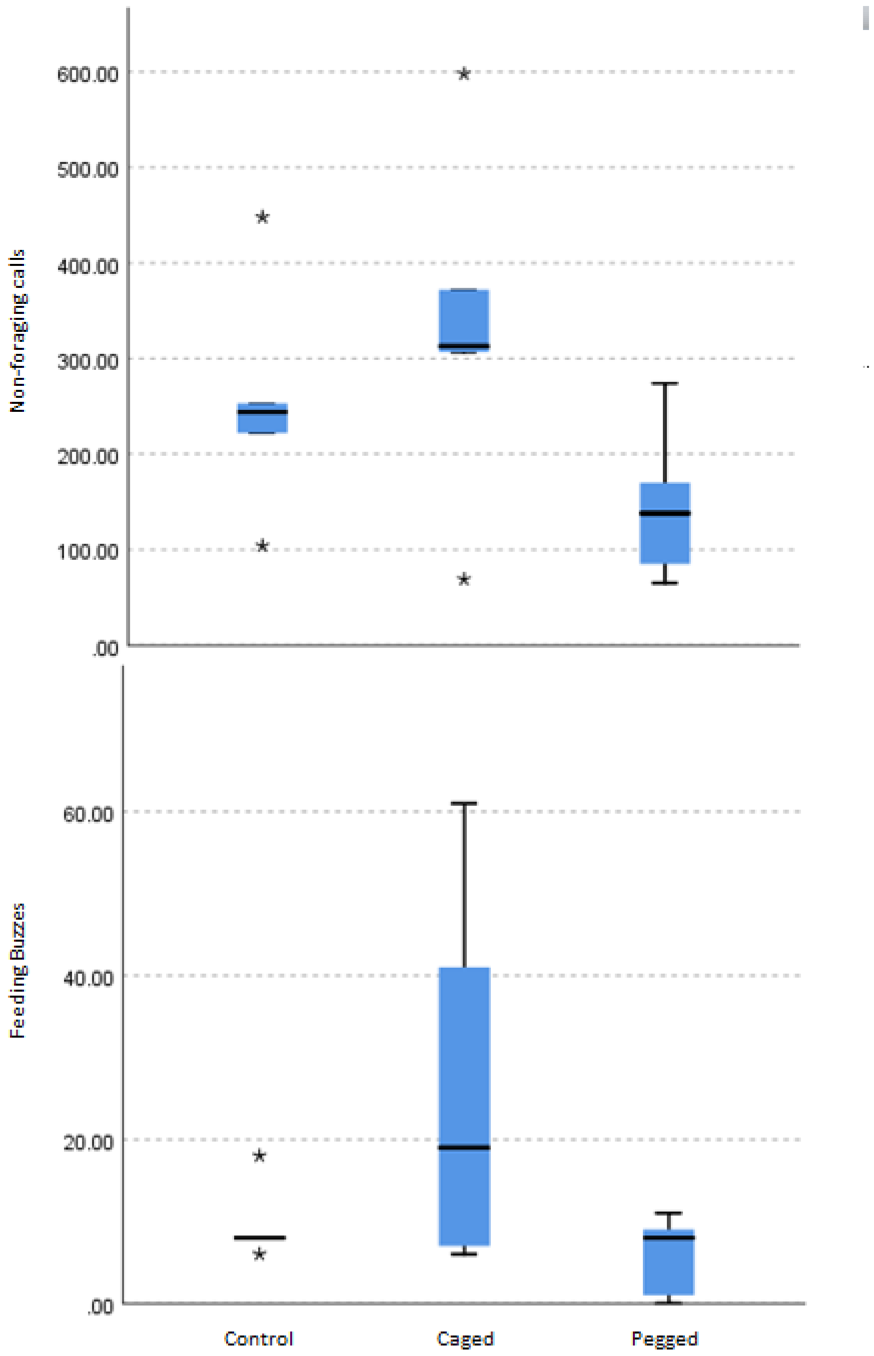
Appendix 3: Table 6.2. Pairwise Comparisons of hourly bat passes (activity) over blue wildebeest (*Connochaetes taurinus*) carcass treatment sites on the Telperion Nature Reserve between January and February 2019

Hour block comparison	χ^2	Std. Error	p
17:00-06:00	18.48	39.428	0.639
17:00-18:00	-73.56	39.428	0.062
17:00-05:00	130.92	39.428	0.001
17:00-04:00	208.28	39.428	< 0.001
17:00-03:00	208.50	39.428	< 0.001
17:00-02:00	216.30	39.428	< 0.001
17:00-01:00	274.32	39.428	< 0.001
17:00-00:00	324.27	39.428	< 0.001
17:00-22:00	-325.18	39.428	< 0.001
17:00-20:00	-326.03	39.428	< 0.001
17:00-23:00	-330.83	39.428	< 0.001
17:00-21:00	-332.24	39.428	< 0.001
17:00-19:00	-360.09	39.428	< 0.001
06:00-18:00	-55.08	39.428	0.162
06:00-05:00	112.44	39.428	0.004
06:00-04:00	189.80	39.428	< 0.001
06:00-03:00	190.02	39.428	< 0.001
06:00-02:00	197.82	39.428	< 0.001
06:00-01:00	255.84	39.428	< 0.001
06:00-00:00	305.79	39.428	< 0.001
06:00-22:00	-306.70	39.428	< 0.001
06:00-20:00	-307.55	39.428	< 0.001
06:00-23:00	-312.35	39.428	< 0.001
06:00-21:00	-313.76	39.428	< 0.001
06:00-19:00	-341.61	39.428	< 0.001
18:00-05:00	57.36	39.428	0.146
18:00-04:00	134.72	39.428	0.001
18:00-03:00	134.94	39.428	0.001

Appendix 3: Table 6.2 continued…

Hour block comparison	χ^2	Std. Error	p
18:00-02:00	142.74	39.428	< 0.001
18:00-01:00	200.76	39.428	< 0.001
18:00-00:00	250.71	39.428	< 0.001
18:00-22:00	-251.62	39.428	< 0.001
18:00-20:00	-252.47	39.428	< 0.001
18:00-23:00	-257.27	39.428	< 0.001
18:00-21:00	-258.68	39.428	< 0.001
18:00-19:00	-286.53	39.428	< 0.001
05:00-04:00	77.36	39.428	0.05
05:00-03:00	77.58	39.428	0.049
05:00-02:00	85.38	39.428	0.030
05:00-01:00	143.4	39.428	< 0.001
05:00-00:00	193.35	39.428	< 0.001
05:00-22:00	-194.26	39.428	< 0.001
05:00-20:00	-195.11	39.428	< 0.001
05:00-23:00	-199.91	39.428	< 0.001
05:00-21:00	-201.32	39.428	< 0.001
05:00-19:00	-229.17	39.428	< 0.001
04:00-03:00	0.22	39.428	0.996
04:00-02:00	8.02	39.428	0.839
04:00-01:00	66.04	39.428	0.094
04:00-00:00	115.99	39.428	0.003
04:00-22:00	-116.9	39.428	0.003
04:00-20:00	-117.75	39.428	0.003
04:00-23:00	-122.55	39.428	0.002
04:00-21:00	-123.96	39.428	0.002
04:00-19:00	-151.81	39.428	< 0.001
03:00-02:00	7.80	39.428	0.843
03:00-01:00	65.82	39.428	0.095
03:00-00:00	115.77	39.428	0.003
03:00-22:00	-116.68	39.428	0.003
03:00-20:00	-117.53	39.428	0.003
03:00-23:00	-122.33	39.428	0.002
03:00-21:00	-123.74	39.428	0.002
03:00-19:00	-151.59	39.428	< 0.001
02:00-01:00	58.02	39.428	0.141
02:00-00:00	107.97	39.428	0.006
02:00-22:00	-108.88	39.428	0.006
02:00-20:00	-109.73	39.428	0.005
02:00-23:00	-114.53	39.428	0.004
02:00-21:00	-115.94	39.428	0.003
02:00-19:00	-143.79	39.428	< 0.001
01:00-00:00	49.95	39.428	0.205
01:00-22:00	-50.86	39.428	0.197
01:00-20:00	-51.71	39.428	0.190
01:00-23:00	-56.51	39.428	0.152
01:00-21:00	-57.92	39.428	0.142
01:00-19:00	-85.77	39.428	0.030
00:00-22:00	-0.91	39.428	0.982
00:00-20:00	-1.76	39.428	0.964
00:00-23:00	-6.56	39.428	0.868
00:00-21:00	-7.97	39.428	0.840
00:00-19:00	-35.82	39.428	0.364
22:00-20:00	0.85	39.428	0.983
22:00-23:00	-5.65	39.428	0.886
22:00-21:00	7.06	39.428	0.858
22:00-19:00	34.91	39.428	0.376
20:00-23:00	-4.80	39.428	0.903
20:00-21:00	-6.21	39.428	0.875
20:00-19:00	34.06	39.428	0.388
23:00-21:00	1.41	39.428	0.971
23:00-19:00	29.26	39.428	0.458
21:00-19:00	27.85	39.428	0.480

Note: The data presented here are for all treatments combined.



Appendix 3: Figure 6.1. Box and whisker plots of the total number of bat passes that did not contain feeding buzzes, and the number of passes that did contain feeding buzzes (foraging calls) for three blue wildebeest (*Connochaetes taurinus*) carcass site treatments on the Telperion Nature Reserve between January and February 2019

Note: The control sites were monitored for four nights, three nights of which had eight feeding buzzes recorded, hence an accurate box and whisker plot could not be compiled for control sites.

Appendix 3: Table 6.3. Pairwise Comparisons of Time block vs species richness on the Telperion Nature Reserve during January and February 2019.

Hour block comparison	χ^2	Std. Error	p
17:00-06:00	21.40	39.119	0.584
17:00-18:00	-69.67	39.119	0.075
17:00-05:00	144.76	39.119	< 0.001
17:00-04:00	205.82	39.119	< 0.001
17:00-03:00	218.18	39.119	< 0.001
17:00-02:00	228.25	39.119	< 0.001
17:00-20:00	-305.58	39.119	< 0.001
17:00-01:00	306.47	39.119	< 0.001
17:00-00:00	307.36	39.119	< 0.001
17:00-21:00	-313.68	39.119	< 0.001
17:00-22:00	-317.21	39.318	< 0.001
17:00-23:00	-327.23	39.119	< 0.001
17:00-19:00	-358.27	39.119	< 0.001
06:00-18:00	-48.27	39.119	0.217
06:00-05:00	123.36	39.119	0.002
06:00-04:00	184.42	39.119	< 0.001
06:00-03:00	196.78	39.119	< 0.001
06:00-02:00	206.85	39.119	< 0.001
06:00-20:00	-284.18	39.119	< 0.001
06:00-01:00	285.07	39.119	< 0.001
06:00-00:00	285.96	39.119	< 0.001
06:00-21:00	-292.28	39.119	< 0.001
06:00-22:00	-295.81	39.318	< 0.001
06:00-23:00	-305.83	39.119	< 0.001
06:00-19:00	-336.87	39.119	< 0.001
18:00-05:00	75.09	39.119	0.055
18:00-04:00	136.15	39.119	0.001
18:00-03:00	148.51	39.119	< 0.001
18:00-02:00	158.58	39.119	< 0.001
18:00-20:00	-235.91	39.119	< 0.001
18:00-01:00	236.80	39.119	< 0.001
18:00-00:00	237.69	39.119	< 0.001
18:00-21:00	-244.01	39.119	< 0.001
18:00-22:00	-247.54	39.318	< 0.001
18:00-23:00	-257.56	39.119	< 0.001
18:00-19:00	-288.60	39.119	< 0.001
05:00-04:00	61.06	39.119	0.119
05:00-03:00	73.42	39.119	0.061
05:00-02:00	83.49	39.119	0.033
05:00-20:00	-160.82	39.119	< 0.001
05:00-01:00	161.71	39.119	< 0.001
05:00-00:00	162.60	39.119	< 0.001
05:00-21:00	-168.92	39.119	< 0.001
05:00-22:00	-172.454	39.318	< 0.001
05:00-23:00	-182.47	39.119	< 0.001
05:00-19:00	-213.51	39.119	< 0.001
04:00-03:00	12.36	39.119	0.752
04:00-02:00	22.43	39.119	0.566
04:00-20:00	-99.76	39.119	0.011
04:00-01:00	100.65	39.119	0.010
04:00-00:00	101.54	39.119	0.009
04:00-21:00	-107.86	39.119	0.006
04:00-22:00	-111.39	39.318	0.005
04:00-23:00	-121.41	39.119	0.002
04:00-19:00	-152.45	39.119	< 0.001
03:00-02:00	10.07	39.119	0.797
03:00-20:00	-87.40	39.119	0.025
03:00-01:00	88.29	39.119	0.024
03:00-00:00	89.18	39.119	0.023
03:00-21:00	-95.50	39.119	0.015
03:00-22:00	-99.03	39.318	0.012
03:00-23:00	-109.05	39.119	0.005
03:00-19:00	-140.09	39.119	< 0.001
02:00-20:00	-77.33	39.119	0.048
02:00-01:00	78.22	39.119	0.046
02:00-00:00	79.11	39.119	0.043
02:00-21:00	-85.43	39.119	0.029
02:00-22:00	-88.96	39.318	0.024
02:00-23:00	-98.98	39.119	0.011
02:00-19:00	-130.02	39.119	0.001
20:00-01:00	0.89	39.119	0.982
20:00-00:00	1.78	39.119	0.964
20:00-21:00	-8.10	39.119	0.836
20:00-22:00	-11.63	39.318	0.767

Appendix 3: Table 6.3. continued…

Hour block comparison	χ^2	Std. Error	p
20:00-23:00	-21.65	39.119	0.580
20:00-19:00	52.69	39.119	0.178
01:00-00:00	0.89	39.119	0.982
01:00-21:00	-7.21	39.119	0.854
01:00-22:00	-10.74	39.318	0.785
01:00-23:00	-20.76	39.119	0.596
01:00-19:00	-51.80	39.119	0.185
00:00-21:00	-6.32	39.119	0.872
00:00-22:00	-9.85	39.318	0.802
00:00-23:00	-19.87	39.119	0.611
00:00-19:00	-50.91	39.119	0.193
21:00-22:00	-3.53	39.318	0.928
21:00-23:00	-13.55	39.119	0.729
21:00-19:00	44.59	39.119	0.254
22:00-23:00	-10.02	39.318	0.799
22:00-19:00	41.06	39.318	0.296
23:00-19:00	31.04	39.119	0.427

Appendix 3: Table 6.4. Kruskal-Wallis Test comparing the number of passes for each functional group between carcass treatments and control sites on the Telperion Nature Reserve during January and February 2019

Functional group	p	N	χ^2
Group 1	0.368	3	2.00
Group 2	0.368	3	2.00
Group 3	0.368	3	2.00
Group 4	0.368	3	2.00
Group 5	1	3	2.00

Group 1: Intermediate / high frequency FM clutter edge foragers
Group 2: Large, low frequency LD-QCF open air foragers
Group 3: Intermediate and lower frequency edge and open air foragers
Group 4: HD-CF and multi-harmonic clutter foragers
Group 5 (*H. caffer*) was not included as it was only recorded during active captures.

Appendix 3: Table 6.5. Table showing seasonal sunrise and sunset times on the Telperion Nature Reserve between 2019 and 2020.

Month	Season	Sunset time	Sunrise time
January 2019	Summer	19:00	5:25
May 2019	Autumn	17:25	6:34
July 2019	Winter	17:29	6:49
October 2019	Spring	18:09	5:29
January 2020	Summer	19:00	5:24

https://www.timeanddate.com/calendar/?year=2019&country=62