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Assessing bat (Chiroptera) diversity: determinants of assemblage and ensemble structure at Kwalata Game Ranch, Gauteng, South Africa

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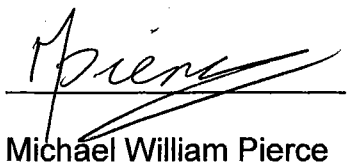
A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfilment for the degree of Master of Science.

Johannesburg, 2012

Declaration

I, the undersigned, hereby declare that this dissertation is my own unaided work. Information included derived from published or unpublished work of others has been acknowledged in the text and a list of references is provided. This dissertation is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other institution.



Michael William Pierce

12/06/2012

Abstract

In this study I assessed bat (Chiroptera) diversity on Kwalata Game Ranch (KGR) in Gauteng, South Africa. I investigated the influence of habitat heterogeneity, specifically vegetation type and level of cover, on the local bat assemblage structure. I sampled bats within three vegetation types (savanna-woodland, riparian and ecotone) on KGR and estimated percentage vegetation cover at sample sites as a proxy for vegetation structural complexity. I used passive sampling with bat detectors and active trapping with mistnets, harp-traps and roost searches to ensure as thorough an inventory as possible. Sample-based rarefaction revealed that the KGR bat assemblage is relatively species-poor and bat diversity is equivalent among the different vegetation types (confirmed with Whittaker's β diversity index). A total of only eight insectivorous species was recorded and pteropodids appear to be absent from KGR. Moreover, species richness estimators indicated sampling was exhaustive. I attributed the low bat diversity to the impacts of known land use, particularly historical grazing by cattle (during 1980's) and land clearing by humans that have resulted in a relatively fragmented savannah-woodland . In addition to the diversity assessment I evaluated effects of the deterministic processes of interspecific competition and prey defences on the ensemble structure of insectivorous bats . I measured the parameters of size, wing morphology and echolocation call structure for each species. These are the primary traits governing the habitat in which insectivorous bats can forage and the types of prey they can handle. Competition should result in size assortment of species that minimizes their similarity while defences of insect prey should result in a narrow range of effective echolocation parameters. Taking size into account is important as size can govern the type of prey able to be handled thus differently sized sympatric bat species may

have similar echolocation characteristics but do not compete for prey. I used null models to test for the effects of competition and prey defences. I compared the insectivorous bat ensemble of KGR with random ensembles constructed from regional species pools of insectivorous bats. My results suggest evidence for competition – minimum size differences were larger and more evenly distributed than expected from chance. Moreover, my results are unlikely to be reflecting the “ghost of competition past” as the majority of insectivorous bat species at KGR are generalists thus making resource overlap more likely. Prey defences, on the other hand, appear to have no influence on the KGR ensemble structure – echolocation call parameters were clumped rather than more similar than chance would expect. Evidence for competition was surprising given the species-poor nature of the ensemble. Thus alternative factors potentially contributing to assortment of size and wing morphology parameters are discussed. KGR is bordered by large peri-urban settlements with numerous street lamps and large spotlights that produce substantial light pollution. High-duty cycle bats are often the main contributors to the prey defence hypothesis as they usually echolocate outside of the hearing range of tympanate insects. However, they may actively avoid artificially lit areas as a result of the slow flight making them more susceptible to predation. Also, artificial lights can interfere with the defence mechanisms of many tympanate insects thus allowing low-duty cycle echolocating bats to take advantage of a usually unavailable resource. The lack of evidence for the influence of prey defences was thus attributed to impacts of ecological light pollution.

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Chapter 1: Introduction

Habitat heterogeneity and species diversity

At multiple spatial scales, habitat diversity is associated with greater biodiversity (Gardner, 1996; Donald *et al.*, 2001; Robinson & Sutherland, 2002). Moreover, diversity of numerous taxa has been shown to have a positive relationship with habitat diversity (e.g. birds, MacArthur & MacArthur, 1961; small mammals, Rosenzweig & Winakur, 1969; insects, Jonsen & Fahrig, 1997). This is likely a result of an interaction between taxa and the positive influences of diverse habitats (Benton *et al.*, 2003). For example, high plant diversity may promote insect diversity which in turn may attract birds that prey on them (Galbraith, 1988; Jonsen & Fahrig, 1997; Benton *et al.*, 2003). High plant diversity can also facilitate co-occurrence of many volant species through spatial niche partitioning (MacArthur *et al.*, 1962).

Indeed, the habitat heterogeneity hypothesis (Williams, 1964) predicts that higher species richness should result from greater habitat complexity. The two major facets of the habitat heterogeneity hypothesis are topographic variation and ecosystem diversity (Kerr & Packer, 1997; Kerr *et al.*, 2001; Rahbek & Graves, 2001). They are not, however, mutually exclusive and topographic heterogeneity is strongly correlated with ecosystem diversity (Rahbek & Graves, 2000; 2001). Heterogeneous topography usually leads to an increase in species richness at a regional scale as it results in a multitude of habitats along a gradient of elevations (Kerr & Packer, 1997; Ruggiero & Kitzberger, 2004). On the other hand, extreme variation in topography, such as the presence of cliffs, can facilitate local co-occurrence of ecologically and/or morphologically similar species (Saunders & Barclay, 1992). In South African savannas, topographical heterogeneity has been shown to be the most important predictor of plant species richness (Thuiller *et al.*,

2006) and should thus also be a robust predictor of animal species richness given the strong relationship between plant and animal communities (Tews *et al.*, 2004). However, while physiography is an important factor in ecosystem diversity, ecosystems can also be separated on the basis of climate, soil and vegetation thus some landscapes can have low topographic variation but maintain relatively high ecosystem diversity (Lapin & Barnes, 1995). Greater ecosystem diversity in a given area results in a higher number of niches being available per unit space through an increase in resource availability (Klopfer & MacArthur, 1960; MacArthur *et al.*, 1962; Kerr *et al.*, 2001). Indeed, habitat patches with higher rodent species richness have been shown to indicate resource levels that allow for greater niche differentiation (M'Closkey, 1976).

It follows then that the structural complexity of a habitat should provide a strong basis on which to predict species diversity. Greater structural complexity, in the form of plant biomass, can provide greater protection from predators as well as more habitable space and food resources (Heck & Wetstone, 1977; Huston, 1979; Galbraith, 1988; Weibull *et al.*, 2000) and is thus likely to result in increased species richness. At the landscape scale in southern Africa, species richness of the majority of mammals is predominantly related to woody plant species richness (Qian *et al.*, 2009). This relationship has been attributed to the fact that high plant species richness increases structural complexity potentially providing more available niches (Andrews & O'Brien, 2000; Kissling *et al.*, 2008; Qian *et al.*, 2009). At local scales too, mammal species richness has been associated with structural complexity of vegetation in Venezuela (August, 1983) and percentage plant cover in Swaziland (Monadjem, 1997). Importantly, the mammal-plant species richness associations are usually most prevalent in small-medium bodied species that utilize resources which

are distributed in three-dimensional space (e.g. arboreal and aerial mammals; Andrews & O'Brien, 2000; Qian *et al.*, 2009). Moreover, the abilities of such species to disperse and forage in three-dimensional space increase the likelihood for spatial niche partitioning in habitats with high structural complexity (Kingston *et al.*, 2000).

Deterministic processes and community ecology

Local processes, such as competition, predator-prey interactions and adaptation (Ricklefs, 1987), that influence the structure of ecological communities, can be illuminated through ecomorphological studies. Phenotypic characteristics of organisms facilitate adaptation for specific ecological functions and, being governed by natural selection, are predominantly responsible for the morphological variation among species (Wainwright & Reilly, 1994). Thus, using measurements of appropriate morphological traits, deductions can be made as to the respective ecological roles of sympatric species and the deterministic processes that allow them to co-occur (Wainwright & Reilly, 1994; Ricklefs & Schlutter, 1993; Meyer & Kalko, 2008). Specifically, Hutchinson (1959) proposed that in order for ecologically or morphologically similar taxa to co-occur they should exhibit a minimum size difference to avoid competitive interactions. Case & Sidell (1983) later postulated that if co-occurrence was indeed facilitated through character divergence then size differences among sympatric species should show relatively even distribution.

However, during the late 1970's and early 1980's competition theory received much criticism and its relevance in community ecology was fiercely contended (e.g. Strong *et al.*, 1979; Connor & Simberloff, 1979; Connell, 1980; Bowers & Brown, 1982; Lewin, 1983). The source of the contention was analyses suggesting that community assembly patterns, previously thought to show evidence of competition, were in fact no different from patterns expected from chance (Strong *et al.*, 1979;

Connor & Simberloff, 1979). Thus, it has been proposed instead that communities are randomly structured from a source pool of species as a matter of chance (Connor & Simberloff, 1979; Roughgarden, 1983). If this is the case then evidence of minimized similarity (Hutchinson, 1959) and size assortment (*sensu* Case & Sidell, 1983) should not exist in ecological communities. Importantly, the results that refuted the validity of competition were in turn rebutted as the analyses used were deemed incorrect and inappropriately applied (Grant & Abbott, 1980; Hendrickson, 1981). These rebuttals illustrate the importance of applying an appropriate null model (see below) that makes appropriate assumptions for the question at hand (Harvey *et al.*, 1983; Gotelli, 2000).

Null model analyses have been put forward as an effective means for revealing whether deterministic processes, such as competition, are influential in community structure (Gotelli, 2001). Specifically, null models generate patterns "based on randomization of ecological data or random sampling from a known or imagined distribution" and can illuminate how a community would be structured if it was only governed by stochastic events (Gotelli & Graves, 1996). Therefore, if an observed pattern differs significantly from the null model it would suggest the influence of the ecological process being tested (Gotelli, 2001). The work by Gotelli (2000; 2001) does, however, emphasise some important considerations when using null models in community ecology. He stresses that for null model analysis to be effective one must use or construct a model that excludes the mechanism being tested. Accordingly, Gotelli (2000; 2001) also highlights the importance of selecting the appropriate indices for representing co-occurrence patterns. Furthermore, appropriate algorithms, to be applied to the data matrix, must be selected and tested for their respective susceptibility to Type I and Type II errors. Also, the complexity involved in

co-existence processes require certain assumptions to be made in order to simplify the deterministic process being tested to better understand its influence/s on community ecology. Importantly though, null model analyses can provide a degree of specificity to tests of species co-occurrence patterns that is usually unobtainable with conventional statistical analyses (Gotelli, 2001). Moreover, null models can be tailored to the type of data collected and to the specific question/s being investigated (Gotelli, 2000).

Effects of anthropogenic pressure on ecological communities

It is generally accepted that the effects of anthropogenic pressure result in a loss of species richness through extreme habitat disturbance or fragmentation (Savard *et al.*, 2000; Miller & Hobbs, 2002; Shochat *et al.*, 2006). Although the density of some species may increase with certain types of land use, due to an increase in resource availability (Marzluff, 2001), these species are usually a remnant subset of native species (Shochat *et al.*, 2006). One of the main concerns associated with anthropogenic pressure on ecological communities is the potential breakdown of community-assembly processes that can result from the novel, unstable environments presented by expanding urban areas (Kozlov, 1996; Shochat *et al.*, 2006). For example, competitive exclusion may be compounded as species that readily exploit the greater productivity increase in abundance and finally dominate the community; out-competing less tolerant species resulting in their local extinction (Marzluff, 2001; McKinney, 2002; Shochat *et al.*, 2004). Anthropogenic effects such as agriculture and stock-farming are also of concern as they can alter soil resources therefore contributing to extensive habitat disturbance and fragmentation (Golodets *et al.*, 2011). Moreover, the effects of urban expansion can

further influence species interactions in surrounding natural areas through ecological light pollution and “sky glow” that alters natural light cycles (Longcore & Rich, 2004).

Fundamental to the establishment of effective conservation and management planning aimed at mitigating anthropogenic effects, is an understanding of the degree to which species and communities are sensitive to habitat disturbance and fragmentation (Bierregaard *et al.*, 1992; Sampaio *et al.*, 2003). In this context, studies focusing on relationships between specific organisms and their environment can provide valuable insight into ecosystem functioning (Hastings *et al.*, 2007). More importantly though, research aimed at the mechanistic processes that structure species assemblages should do so in the light of potential anthropogenic pressures so that alterations in community-assembly processes can be elucidated (Shochat *et al.*, 2004; 2006).

Why bats?

There are 1116 described species of bats worldwide, making Chiroptera the second largest order of mammals (Simmons, 2005). Bats are geographically ubiquitous, with the exception of a few islands they occur in every sub-Arctic and sub-Antarctic vegetated ecosystem (Medellin *et al.*, 2000; Hutson *et al.*, 2001). Furthermore, bats are one of the most diverse groups of living mammals – taxonomically and ecologically – and can form some of the largest aggregations and thus may be among some of the most abundant groups of mammals in terms of individual numbers (Kunz, 2003; O’Shea & Bogan, 2003). Bats are one of the most important components of mammalian biodiversity in both tropical and temperate regions (Hutson *et al.*, 2001; Simmons, 2005). Moreover, the tremendous trophic diversity of bats makes them useful surrogates, reflecting the status of sympatric plant and insect populations (Keddy, 1991; Jones *et al.*, 2009). This, coupled with

how quickly and easily bat inventory completeness may be reached with the correct application of a few sampling methods (Noss, 1990; Moreno & Halffter, 2000), makes bat populations effective ecological indicators (Keddy, 1991; Jones *et al.*, 2009; Monadjem *et al.*, 2010).

Additionally, recent research on the structure of bat assemblages has shown that deterministic processes can be highly influential (Kingston *et al.*, 2000; Schoeman & Jacobs, 2003; 2008; 2011). In the context of bats, interspecific competition and predator-prey interactions are the deterministic processes often proposed to be at the forefront of community ecology (Ricklefs & Schlutter, 1993; Schoeman & Jacobs, 2011). Life history traits of most insectivorous bats include long life-spans, slow growth rates and reproductive outputs, low predation risk and stable populations (Barclay & Harder, 2003; Brunet-Rossinni & Austad, 2004). Such life histories result in an increased propensity for competitive interactions because population carrying capacity is often reached under these conditions, increasing the likelihood of resources becoming limiting (Pianka, 1972; Findley, 1993).

Alternatively, predator-prey interactions may considerably influence niche partitioning and the shaping of bat assemblages (Jacobs *et al.*, 2008). Specifically, trophic niches may be greatly influenced by the defence mechanisms of insect prey, which usually refers to avoidance of echolocating bats through insects' use of tympanate organs (e.g. Rydell *et al.*, 1995). Numerous nocturnal insect species from families of moths (Lepidoptera), lacewings (Neuroptera) and mantids (Dictyoptera) have developed tympanate organs (ears) in response to predation pressure from echolocating bats (Rydell *et al.* 1995). For example, diurnal species of Notondontids are mostly deaf to the same frequencies as sympatric nocturnal species of the same family (Fullard & Dawson 1997). Moreover, few moth species produce sound so

tympanate organs are unlikely to have evolved for the purposes of intraspecific communication (Waters, 2003). Also, at local spatial scales the frequencies to which moth ears are sensitive have been shown to reflect those of sympatric bat species (Fenton & Fullard, 1979; Fullard, 1982).

Considering that most moths are unable to detect frequencies above 100 kHz (Fenton & Fullard, 1979), Novick (1977) posited that the use of high echolocation frequencies should allow bats to feed more readily on tympanate moths. Fullard (1988) termed such frequencies “allotonic” as they represent echolocation frequencies outside the hearing range of most eared insects. Since the hearing range of most tympanate insects is between 20 – 60 kHz, echolocation frequencies that fall within this range were termed “syntonic” (Fullard, 1988). The allotonic frequency hypothesis (AFH) thus relates directly to the influence of prey defences on trophic niche differentiation as it predicts that a large proportion of tympanate insects should be present in the diet of bats using echolocation frequencies above or below their hearing range (Rydell & Arlettaz, 1994; Schoeman & Jacobs, 2003; Jacobs *et al.*, 2008). On the other hand bat species using syntonic (*sensu* Fullard, 1988) echolocation frequencies generally have to hunt non-tympanate insects such as beetles, flies and some moth families (Jones, 1992; Rydell *et al.*, 1995; Schoeman & Jacobs, 2003). It follows that if both tympanate and non-tympanate families of insects are present in similar abundances within an ecosystem it should promote higher bat species richness through differential availability of prey items, provided that other key resources are not limiting.

Importantly, bat community structure can also be severely altered in response to anthropogenic pressure and the mechanisms allowing certain species to persist, rather than others, are becoming apparent (Rydell, 1992; Arlettaz *et al.*, 2000; Avila-

Flores & Fenton, 2005; Jung & Kalko, 2010; 2011). For example, the defensive mechanisms of most tympanate insects have been shown to be ineffective around different types of lights (Rydell, 1992; Svensson & Rydell, 1998). The large aggregations of tympanate insects often attracted to artificial lights may thus be exploited by many faster-flying bat species (such as Molossidae and Vespertilionidae) that are usually unable to forage on such insects(Rydell & Arlettaz, 1994; Avila-Flores & Fenton, 2005). This exploitation is often to the detriment of the usual hunters of tympante insect prey, such as Rhinolophidae and Hipposideridae, which may become competitively excluded (Arlettaz *et al.*, 2000). Moreover, these species are slow-flying which may result in an increased susceptibility to predation by visual-hunting, aerial predators, such as owls, in areas affected by artificial lights (Jung & Kalko, 2010).

Hypotheses and predictions

Considering KGR is in a subtropical savanna, I considered it likely that habitat heterogeneity – represented by vegetation structural complexity and topographic variation – should be more influential on community structure than available energy. Therefore the hypothesis of my first data chapter (Chapter 2) states that the bat assemblage within riparian and ecotone vegetation should be more species-rich than that in the savanna-woodland. This prediction is based on the habitat heterogeneity hypothesis (Williams, 1964), specifically with regard to the level of vegetation cover. I use sample-based rarefaction to test this prediction as it allows species richness comparisons to be made between habitat-types while controlling for sampling effort (Gotelli & Colwell, 2001).

In my second data chapter (Chapter 3) I use competition theory (Hutchinson, 1959; Case & Sidell, 1983) and recent research on bat community structure (e.g. Schoeman & Jacobs, 2003; 2008; Schoeman & Waddington, 2011) as a base for my predictions. My hypotheses for this chapter are 1) a minimum difference in morphological parameters, such as body size and wing morphology, will be present in the KGR insectivorous bat ensemble and 2) non-random patterns in echolocation call structure will be evident. To test these hypotheses I use null model analyses to establish whether non-random phenotypic patterns are evident within the insectivorous bat ensemble of KGR. Should I accept both hypotheses it will suggest evidence for the effects of competition (hypothesis 1) and prey defences (hypothesis 2).

Dissertation layout

Including the present chapter (Introduction) this dissertation comprises four chapters. Chapter 2 details my assessment of bat diversity at KGR. It is submitted here in the format required for submission to the *African Journal of Ecology*. In this data chapter I use multiple sampling techniques (Flaquer *et al.*, 2007; MacSwiney *et al.*, 2008) to assess the diversity of bat assemblages within three major vegetation types on KGR.

In chapter 3 I investigate the processes influencing the structure of the insectivorous bat ensemble of KGR. Chapter 3 is submitted here in the format required for submission to *African Zoology*.

Finally, in chapter 4, I discuss the overall conclusions of the present study. Because of this layout there may be repetition of some introductory material, methodology and/or discussion. Reference lists are provided at the end of each

chapter. Figures and tables are numbered sequentially in each chapter. Pages are numbered sequentially for the entire dissertation except the appendices (see below).

Two Appendices are also attached to this dissertation. These papers, namely Pierce & Keith (2011) and Pierce *et al.* (in press), are further publications that resulted directly from my Masters research project and appear in their published/submitted format.

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Chapter 2: The diversity of bat assemblages on Kwalata Game Ranch: a comparison between three vegetation types.

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Abstract

Considering the value of bats as ecological indicators, diversity assessments are critical if monitoring programs and management plans are to be successful. Furthermore, given the importance of bats from an economic and biodiversity perspective, it is important to elucidate anthropogenic effects on bat communities. We hypothesised that bat assemblages in the riparian and seep-zone (ecotone) vegetation – dominated by Tambotis (*Spirostachys africana*) – would be more species-rich than in the savanna-woodland on Kwalata Game Ranch, South Africa. Using a combination of acoustic monitoring, mistnets, harp-traps and roost searches we assessed bat diversity and assemblage structure within these three dominant vegetation types. Vegetation cover was estimated within each vegetation type and used as a proxy for habitat structural complexity. Surprisingly, we had to reject our hypothesis: sample-based rarefaction curves revealed that bat diversity was comparably equal between all vegetation types. Furthermore the bat assemblages within each vegetation type were predominantly composed of the same species. We attribute our results to the apparent absence of high-duty cycle, clutter-foraging bat species as well as pteropodids. Factors including historical land use and lack of roosts and suitable forage potentially preclude these bat species from the area.

Introduction

The habitat heterogeneity hypothesis (Williams, 1964) can potentially explain high species richness at both a regional and a local scale (August, 1983; Kerr & Packer, 1997; Kerr, Southwood & Cihlar, 2001). For example, the negative impacts of agricultural intensification, which reduces habitat heterogeneity across a landscape, on bird diversity are detectable at a continental scale (Donald, Green & Heath, 2001). Alternatively, at a local scale (~1 km) small-mammal species richness has been shown to have a strong association with the structural variation of vegetation (Williams, Marsh & Winter, 2002).

The primary factors of the habitat heterogeneity hypothesis are topographic variation and ecosystem diversity (Kerr & Packer, 1997; Kerr *et al.*, 2001; Rahbek & Graves, 2001). Topographic variation usually results in a variety of habitats along an elevational gradient which is predicted to lead to increased regional species richness (Kerr & Packer, 1997; Ruggiero & Kitzberger, 2004). Also, the more ecosystems there are present in a given area, the more readily partitioned are resources which results in a higher number of niches being available per unit space (Klopfer & MacArthur, 1960; MacArthur, MacArthur & Preer, 1962; Kerr *et al.*, 2001). For example, M'Closkey (1976) found that habitat patches with higher rodent species richness indicated resource levels that allowed for greater niche differentiation.

Being the second most specious order of mammals, bats comprise a considerable portion of the biodiversity of an area (Hutson, Mickleburgh & Racey, 2001; Simmons, 2005). They occupy a wide range of trophic levels, from primary to tertiary consumers (Kunz *et al.*, 2011), hence their critical ecological and economic importance. Without insectivorous bats, insect pest populations, both agricultural and ecological, would quickly lead to negative impacts on their respective crops (Kalka,

Smith & Kalko, 2008; Boyles *et al.*, 2011; Kunz *et al.*, 2011). Additionally, seed-dispersal and pollination of many ecologically and economically important plants are carried out almost solely by certain species of fruit bats (Fleming, Geiselman & Kress, 2009; Kunz *et al.*, 2011). Bats can form some of the largest aggregations of mammals and thus may be among the most abundant groups in terms of individual numbers (Kunz, 2003; O'Shea & Bogan, 2003).

Moreover, echolocating bats can be divided into functional groups based on their flight capabilities and echolocation call design (Schnitzler & Kalko, 2001). The three functional groups proposed by Schnitzler & Kalko (2001) are open-air, clutter-edge and clutter. Open-air bats usually have narrowband, low-duty-cycle (LDC) quasi-constant frequency (QCF) signals of low frequency (<30 kHz) and relatively long duration (8-20 ms) which allow long-range detection. Clutter-edge species use a mixture of frequency-modulated (FM) – QCF calls of medium frequencies (30-60 kHz) and short-intermediate duration (3-10 ms) that are also LDC facilitating prey detection in the presence of background clutter. Lastly, LDC clutter species (usually gleaning insectivores) use steep FM signals of short duration (1-3 ms); whereas aerial insectivores that forage in highly cluttered space, use high-duty-cycle (HDC) constant frequency (CF) calls of long duration (10-100 ms) and medium-high frequency (>30 kHz) which provide very short detection ranges but allow prey to be differentiated from background "noise" of high levels of clutter.

Flight and echolocation characteristics are part of the same adaptive complex (Aldridge & Rautenbach, 1987; Arita & Fenton, 1997). Hence, not only do they govern the habitat in which bats can hunt, they facilitate differences in prey availability even for bats hunting in the same stratum (Siemers & Schnitzler, 2004; Schoeman & Jacobs, 2008). As a result of this potential for niche partitioning, in

terms of both space-use and prey-availability, it follows that numerous bat species often live sympatrically (Medellin, Equihua & Amin, 2000; Patterson, Willig & Stevens, 2003). Also, it seems likely that habitats with greater vegetation cover should harbour higher bat species richness than those that are less cluttered, particularly since more structurally complex habitats often support a greater density of insect prey (Kalcounis & Brigham, 1995).

We compared the diversity of bat assemblages (*sensu* Fauth *et al.*, 1996) in three vegetation types (riparian, ecotone and savanna-woodland) on Kwalata Game Ranch in Gauteng, South Africa. In African savannas, strips of riparian vegetation typically comprise a more continuous canopy with a higher density of tall trees than the patchy savanna-woodland (Monadjem & Reside, 2008). Also, these linear strips of continuous canopy likely represent an expanse of edge habitat (Clark, Leslie & Carter, 1993; Grindal & Brigham, 1998; Kalcounis *et al.*, 1999; Monadjem & Reside, 2008). Thus we expected the riparian and ecotone vegetation types to represent more continuous vegetation cover for bats. Moreover, we considered the greater amount of vegetation cover and edge habitat offered by riparian and ecotone vegetation to represent a more heterogeneous three dimensional environment. Thus, based on the habitat heterogeneity hypothesis, we predicted that bat assemblages within the riparian and ecotone vegetation should be more species-rich than those in the savanna-woodland. Estimates of percentage vegetation cover at sample sites were used as a measure of habitat structural complexity.

Materials and Methods

Study site

This study was carried out on Kwalata Game Ranch (KGR) in Northern Gauteng, South Africa (main lodge 25° 23.421' S 28° 19.309' E). The 1800 hectare farm is dominated by savanna-woodland consisting of mixed Bushveld, Kalahari Thornveld and sourish mixed Bushveld veld types (Mucina & Rutherford, 2006; average elevation: 1103 m; range: 1068-1138 m). Though far from pristine, these veld types are relatively unspoilt in the context of the level of urbanisation within the Gauteng Province (Contour Project Managers cc, 2009). The study area receives predominantly summer rainfall of between 350 mm – 750 mm per year. The average night time temperature during summer is ±20°C with ±74 % relative humidity. During winter, the average night time temperature drops to ±3°C with ±76 % relative humidity. The study area also has approximately 5 km of riparian fringe vegetation (average elevation: 1080 m; range: 1068-1097 m) along the Pienaars River, a 2 km seep-zone dominated by Tamboti trees (*Spirostachys africana*) and a 2 km stream to the east. The seep-zone and stream were classified together as ecotone vegetation (associated with seasonal and/or standing water; average elevation: 1116 m; range: 1075-1136 m). Three vegetation types were thus surveyed (Fig. 2.1): savanna-woodland (31 sample sites), riparian (18 sample sites) and ecotone (14 sample sites).

Sampling techniques

The study was conducted from September 2010 – June 2011. Considering the substantial variation of bats in terms of foraging and roosting strategies it was essential to employ numerous methods to assess the composition of bat

assemblages as accurately as possible (Flaquer, Torre & Arrizabalaga, 2007; MacSwiney, Clarke & Racey, 2008). Inventories based solely on passive sampling with bat detectors can miss species using low-intensity calls (Barclay, 1999) and many fruit bats do not echolocate (Monadjem *et al.*, 2010). Alternatively, using only mistnets can bias results towards species that are readily captured with such techniques and may under-represent or completely miss many aerial insectivorous species that can detect and avoid mistnets (MacSwiney, Clarke & Racey, 2008). Although we conducted nine months of sampling, seasonal variations in bat activity are beyond the scope of our study.

Passive sampling

We passively monitored echolocation calls of bats using both Pettersson D240X time-expansion (Pettersson Elektronik AB, Sweden) and Anabat SD2 frequency division (Titley Scientific, Australia) bat detectors. The Anabat SD2 rather than the Pettersson D240X was also used to assess bat diversity along driven transects (see below). It is imperative to be aware of the potential limitations and biases of the devices used to record echolocation calls of bats (Kunz *et al.*, 1996). Hence we augmented the use of a time expansion detector with that of a frequency division detector. Time expansion detectors are not ideal for measuring diversity parameters such as relative abundance as the playback time required to record calls can result in some bat-passes being missed (Roche *et al.*, 2011). However, the resolution of the output of time expansion detectors is much higher than that of frequency division detectors. Thus we incorporated the D240X (time expansion) to ensure high quality calls for the creation of an accurate call library to aid in classification while we used the Anabat SD2 (frequency division) to provide more

appropriate relative abundance measures. Finally, driven transects were conducted primarily to provide greater coverage of bat diversity and less site-specificity.

Paired sample sites were selected using a Southern Africa Togographical and Recreational map (Garmap Africa Series 2008, March edition) on MapSource (Garmin, Ltd.). Firstly sites within riparian or ecotone vegetation were chosen such that they were both accessible and would provide as thorough coverage of the area as possible. Then corresponding savanna-woodland sites (≥ 450 m away) were chosen. At each pair, acoustic monitoring was carried out in one vegetation type while it was coupled with trapping in another. The following night the methods were switched between the two sites so that site pairs of different vegetation types were sampled with each method over consecutive nights, weather permitting. Thus, in a savanna-woodland sample site the Pettersson D240X was mounted vertically, in a weather-proof casing, on a standard camera tripod at a height of 1.5 m. A right-channel stereo patch cable connected the “tape” jack of the detector to the “microphone” jack of an iAUDIO U3 (COWON Systems Inc., Korea), also housed within the weather-proof casing. The iAUDIO U3 was set to record at 44 100 Hz, 16-bit, stereo and saved each recorded call set as a separate WMA file. Recording began shortly after sunset and continued for two hours. At a corresponding riparian or ecotone sample site the Anabat SD2 (audio div: 16; data div: 8; sensitivity: 7) was set at mistnet stations, angled at ±45°, facing the flyway in which nets were set. This allowed us to record bats not being captured which was confirmed by checking the nets each time a call was heard on the detector. Thirty-one site pairs (18 riparian/savanna-woodland; 13 ecotone/savanna-woodland) were sampled with the above protocol, 11 of which were resampled more than 3 months later (8

riparian/savanna-woodland; 3 ecotone/savanna-woodland). A further 3 sites in each vegetation type were monitored for an entire night with the Pettersson D240X.

At least 2 km of road/track ran within the riparian fringe and seep-zone vegetation. These roads allowed driven transects to be run through each vegetation type. Transects were started at least half an hour after sunset and were time-dependent so that time spent recording in each vegetation type was equal. They were started in either riparian or ecotone vegetation and driven into the savanna-woodland at 10-15 km/h. Start times for each vegetation type were noted so that bats recorded during the ±500 m commute between the different vegetation types could be excluded. Start and end points were switched so transects could be driven in both directions on consecutive nights, weather permitting. We used the frequency division detector to conduct transects because time-expansion detectors require playback time and hence would only be recording for a portion of each transect (Roche *et al.*, 2011). The Anabat SD2 was held out the window with the microphone at a 90° angle toward the sky. An eTrex Vista HCx (Garmin Ltd.) GPS unit was used to track transects, set to record a different point every 2 seconds.

WMA files, recorded using the Pettersson D240X, were converted to WAV files and analysed in BatSound Pro version 3.1b (Petersson Elektronik AB, Sweden). In BatSound Pro, with time-expansion set to 10, calls were analysed at the same settings at which they were recorded (44 100Hz, 16-bit, stereo) using a threshold of 15. The best quality calls, based on high signal-to-noise ratios and a lack of distortion or overloading (Parsons & Jones, 2000), were selected for analysis. On average, four calls were analysed per sequence so means of each call parameter for each species could be established. The time-frequency (spectrogram) and time-amplitude (oscillogram) windows were used to identify calls and to measure duration

of single calls and the interpulse interval (Jennings *et al*., 2004). Frequency information (peak, minimum and maximum frequency of the dominant harmonic) were measured from the Fast Fourier transformation (FFT) power spectrum (size 1024, using a Hanning window). The frequency with highest amplitude in on the FFT power spectrum was defined as the peak frequency of a call and ±18 dB from the peak frequency was used as the criterion for identifying the minimum and maximum frequencies (Schoeman & Jacobs, 2008).

Calls recorded with the Anabat SD2 were transferred to AnalookW version 3.7 (Chris Corben, www.hoarybat.com) for analysis. An 'all-bat' filter was applied to files to remove insect noise and call fragments. The parameters of the filter – smoothness = 30, Fc [Min] = 10 kHz, Fmin [Min] = 15 kHz and Dur [Min] = 2 ms – were such that it did not exclude any southern African bat species recordable using passive monitoring. After sorting through and deleting noisy files, those remaining were scanned with the 'all-bat' filter to produce text files containing call parameter information. All files containing one or more clear echolocation pulse were counted as bat passes (Hayes, 1997; Miller, 2001) and included in analyses.

Discriminant function analyses (DFAs) were applied to the acoustic monitoring data from the two different detectors, using XLstat (Addinsoft SARL, France – trial version). Time expansion and frequency division detectors are not directly comparable (Monadjem *et al.*, 2010) so the data could not be pooled. Parameters of release calls (see below) recorded with the two detectors were used as "knowns" in the discriminant functions, while passive monitoring and transect data were used as "unknowns". The parameters included were duration (ms), interpulse interval (ms) and peak, minimum and maximum frequency (kHz) for Pettersson recordings and Dur (duration), Fmean (mean frequency), Fknee (frequency at which there is a

dramatic change of slope), Fc (characteristic frequency), Sc (characteristic slope) and Qual (quality) for Anabat recordings. Using these parameters bats were classified into species where possible and species complexes in cases of there being numerous species using the measured call structure. The posterior classifications from the DFAs were then used to establish relative abundances of species or complexes within minute intervals for each recording session in each vegetation type.

Active sampling

Trapping sessions were undertaken in conjunction with passive monitoring, the Anabat SD2 being set at trapping sites. One to three monofilament mistnets (14 mm^2 mesh; ECOTONE, Poland) ranging from 3 – 9 m were set from canopy level down, depending on availability of appropriate branches from which to hang the nylon rope rigs and absence of potentially snagging branches between the rigs. Otherwise, nets were set from ground level up on 4 m aluminium poles. Nets were opened at dusk when the first bat had either been seen or heard on the Anabat SD2 detector and were monitored continuously for approximately two hours. Where possible, nets were also set across the potential entrances of crevice roosts identified within a granite quarry in the study area; nets were used as the possible paths of exit were too dispersed to cover with a harp-trap. Additional trapping attempts were undertaken using harp-traps or hand-nets. A large harp-trap (±1.2 m X 1.7 m) was set for a week at a time in a potential flyway within each vegetation-type, consecutively, for a period of two months. This resulted in three sites per vegetation-type being trapped for more than 250 harp-trap hours each. Traps were checked at least once every night and every morning. A small harp-trap (±1.2 m x 0.9 m) was also set before sunset in front of potential roosts in crevices in lodge buildings and

cavities in trees. The harp-trap was checked at least once during the night collected the following morning. Finally, a hand-net was used to capture bats from roosts in the apex of roofs that were impossible to trap with conventional methods. The locations of such roosts were communicated to us by the public.

Captured bats were placed, individually, into numbered cotton bags. The morning after capture, bats were identified to species (where possible) based on morphological characteristics including body size (see below), pelage, the presence and shape of nose-leaves, the shape of ears, presence and shape of the tragus and the relationship between the tail and tail-membrane (following Taylor, 2000 and Monadjem *et al.*, 2010). Each captured individual was photographed to document morphology. Body mass (measured to the nearest 0.1 g with a Pesola 30 g spring balance), forearm length, head length and tibia length (measured to the nearest 0.1 mm using a dial calliper) were measured. Sub-adults and adults were categorised according to the degree of ossification of the epiphyses (Anthony, 1988) and reproductive condition of individuals was noted. Bats not taken as vouchers were released in open areas near their site of capture just prior to sunset the evening after capture so they could be followed for as long as possible. In so doing, search phase calls of released bats were recorded with both detectors. .

Two individuals per sex per species were taken as vouchers from each sample site and deposited in the Ditsong National Museum of Natural History (DNMNH) in Pretoria. The vouchers were used for cranial, dental and bacular measurements for accurate identification to species level. Vouchers were taken in accordance with the Guidelines of the American Society of Mammalogists (Gannon *et al.*, 2007). All procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (ethics no. 2010/39/2A) and research was authorised

by the Gauteng Department of Agriculture, Conservation and Environment (permit no. CPF6-N0028).

Vegetation measurements

The structural complexity of vegetation at all Passive/Active sampling sites was measured by estimating the percentage vegetation cover at five different height classes. From a central point at each site, percentage cover was estimated along four 15 m long transects (north, east, south & west). Estimates were taken at the start and end points of each transect giving two estimates per height class (<1 m; 1-2 m; 2-5 m; 5-10 m & >10 m) per transect. This method resulted in eight estimates of percentage cover per height class at each site. We considered the grain of the vegetation height classes appropriate as it follows Lloyd, Law & Goldingay (2006) closely, yet our vegetation estimates are at a much finer grain. The coarser grain of Lloyd, Law & Goldingay (2006) still yielded meaningful estimates of vegetation cover along a gradient of disturbed forests. The size of their sample plots (20 m X 30 m; Lloyd, Law & Goldingay, 2006) was also similar to ours.

Sample based rarefaction and species richness estimators

Using EstimateS version 8.2 (Colwell, 2009), sample-based rarefaction curves were produced to compare species richness among the different vegetation types while controlling for sampling effort. Rarefaction curves were rescaled by individuals rather than number of sampling nights. This was done so that species richness rather than species density could be compared (Colwell, Mao & Chang, 2004). Both Chao 1 and Chao 2 species richness estimators were used to verify sampling completeness as they take into account unequal detection probabilities and are effective even when very few, if any, individuals are recaptured (Chao, 1984; 1987). These estimators differ in the way they tally rare species to correct S_{obs} (observed

species; Coddington, Young & Coyle, 1996; Gimaret-Carpentier *et al.*, 1998). Chao 1 corrects S_{obs} by including the number of species represented by only one individual (singletons) and two individuals (doubletons; Chao, 1984). Whereas Chao 2 replaces singletons and doubletons with the number of species present in only one sample (uniques) and those present in exactly two samples (duplicates; Chao, 1987). Shannon and Simpson diversity indices were calculated for each sampling method within each vegetation type. Finally, using Biodiversity Pro (McAleece *et al.*, 1997), Simpson's evenness index and Whittaker's measure of beta diversity (β_w) was calculated for each sampling method in each vegetation type.

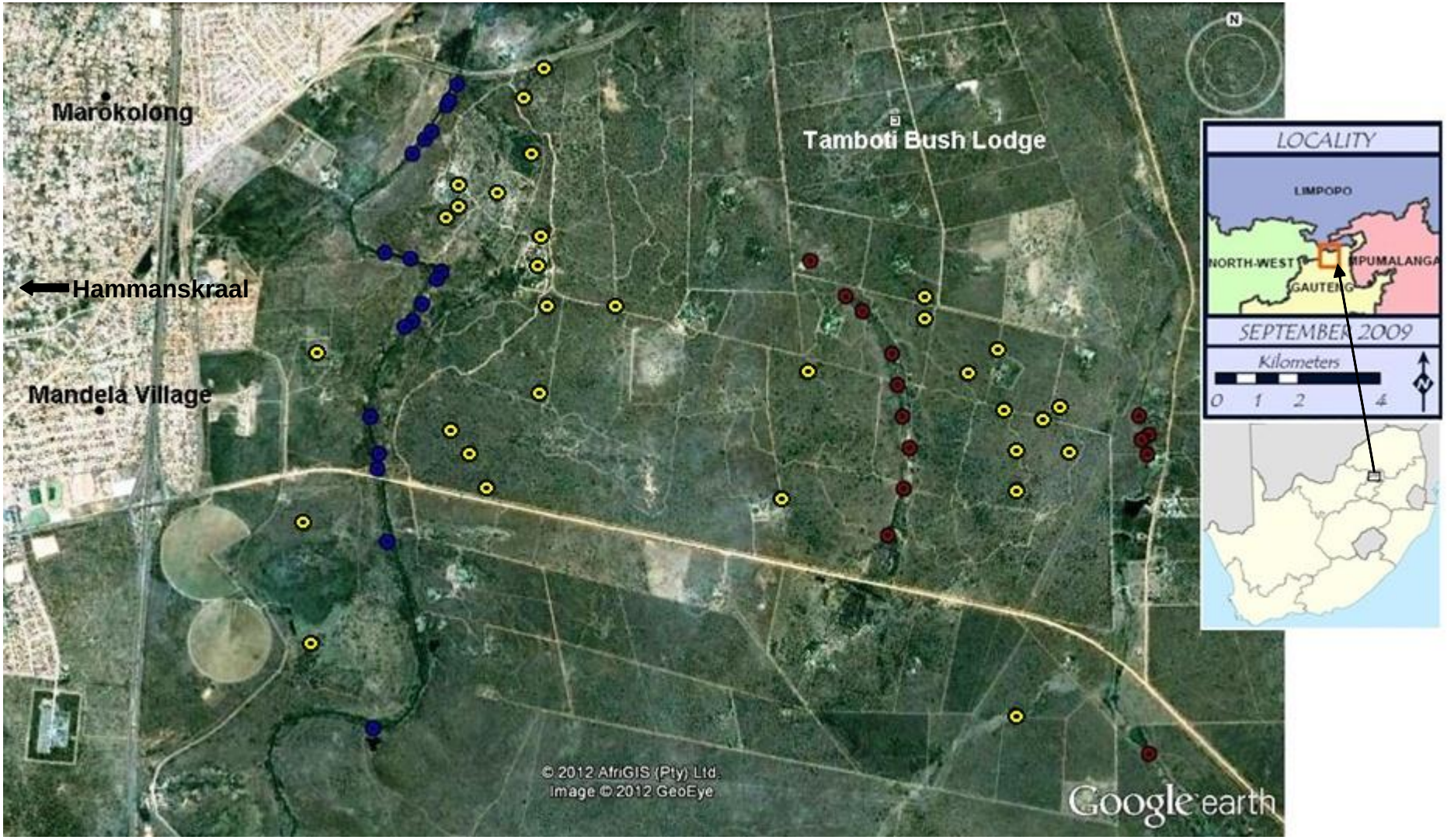

Figure 2.1. Google earth image of Kwalata Game Ranch, bordering settlements and sample sites. Savanna-woodland sample sites = yellow dots; Riparian sample sites = blue dots and Ecotone sample sites = red dots.

Results

Vegetation measurements revealed a surprising lack of difference in the structural complexity between the three vegetation types (Fig. 2.2). However, it should be noted that the ecotone category had the fewest number of sample sites (n = 14) and the stream included in this category was in virtually open grassland with negligible canopy cover. Also, vegetation measurements were limited to sites where we sampled for bats. It was impossible to set canopy nets in the large open areas of the savanna-woodland areas and macro-mistnets were not used in our study thus we had no sample sites within such areas and hence lack the related vegetation measurements.

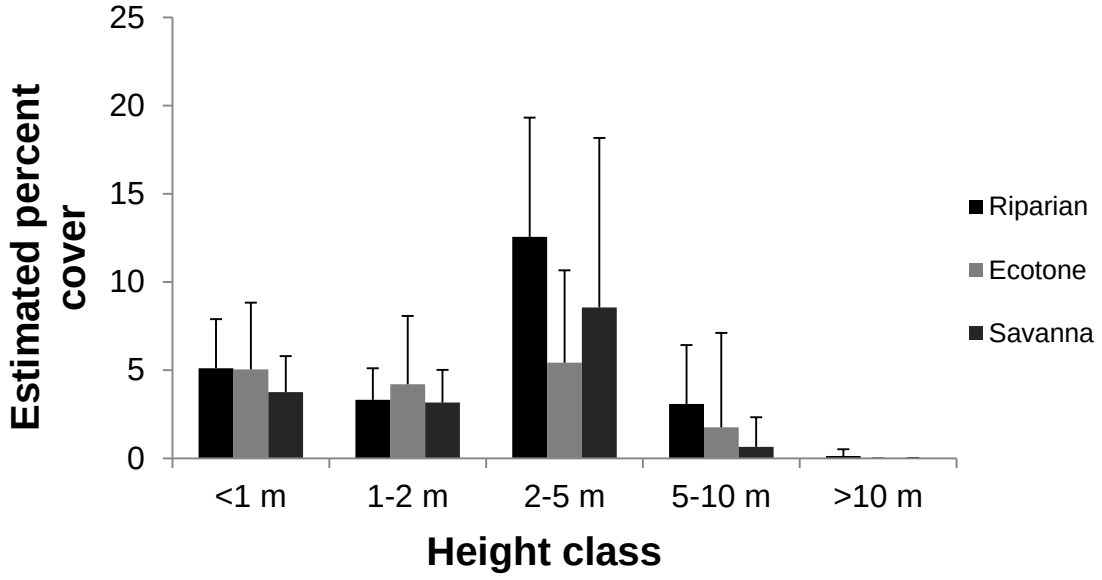

Figure 2.2. Structural complexity of vegetation measured as estimated percentage vegetation cover at five height classes (+ 1 standard deviation). Though the vegetation types appear relatively similar, riparian vegetation, and to some extent ecotone vegetation, was on average taller.

Since vegetation measurements were taken only at sites where we conducted sampling with mistnets and static passive monitoring, the percentage cover of the

savanna-woodland is potentially an overestimation. Furthermore, figure 2.2 shows that riparian vegetation, and to some extent ecotone vegetation, did in fact represent greater structural complexity of vegetation above 5 m.

A total of 3220 calls, recorded with the Pettersson D240X, from 71 sampling nights and more than 85 000 calls, recorded with the Anabat SD2, from 79 nights were analysed. A further 7025 calls, recorded with the Anabat SD2 during a total of 38 driven transects, were also analysed. Only 10 of these transects sampled the riparian vegetation. Most of the roads within the riparian zone were not driveable due to flooding for a large portion of the rainy season.

Two hundred and forty eight bats representing three families and at least six different species and genera, were captured and 80 vouchers taken. Release calls of 57 bats were recorded with the Pettersson D240X and 161 with the Anabat SD2.

Based on the posterior classifications of the DFAs of echolocation call parameters for both recording methods, at least six species were recorded during passive monitoring sessions and driven transects. The posterior classification error rate (8%) was within the range deemed acceptable in other studies (e.g. Nicieza, 1995; Webb *et al.,* 2002; Kerns *et al.*, 2005),, thus species classification based on echolocation call parameters was deemed accurate. Frequency characteristics were the most important classifying factors in the first function of both Pettersson and Anabat DFAs (Fig. 2.3), whereas time characteristics contributed more to the second function of the Pettersson DFA (Fig. 2.3a) and time and shape characteristics contributed more to the Anabat DFA (Fig. 2.3b).

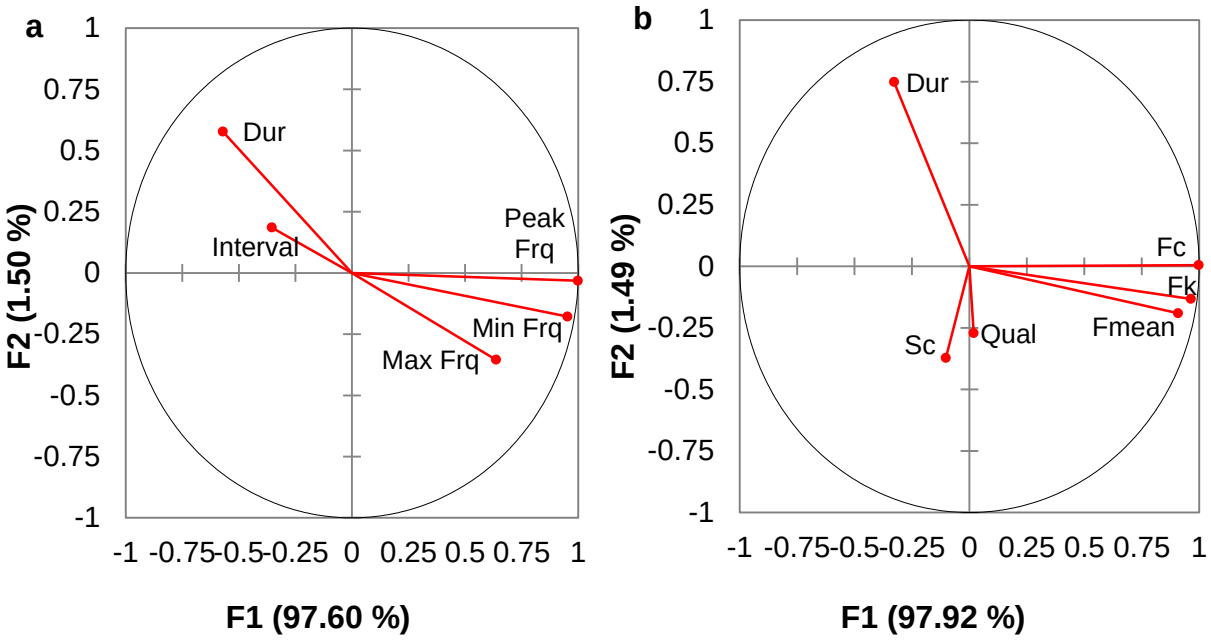

Figure 2.3. Discriminant function analysis biplots of Pettersson and Anabat passive recordings: (a) The first two functions of the Pettersson DFA explained 99.10% of the variation in echolocation parameters; with F1 basing classifications primarily on frequency characteristics (Max Frq = maximum frequency, Min Frq = minimum frequency and Peak Frq = peak frequency) and F2 based more on time characteristics of calls (Dur = duration and Interval = interpulse interval). (b) The first two functions of the Anabat DFA explained 99.41% of echolocation parameter variation; F1 based classifications primarily on frequency characteristics (Fc = characteristic frequency, Fk = frequency at the knee and Fmean = mean frequency) while F2 classifications were based on time and shape (Dur = duration, Sc = characteristic slope and Q = quality).

The bat species captured/recorded at KGR are presented in Table 2.1. The corresponding vegetation types in which each species was recorded are presented with the passive monitoring and transect data. Capture data were not included in statistical analyses thus no associated vegetation data are presented (Table 2.1).

Table 2.1. Bat species list of Kwalata Game Ranch based on multiple sampling methods. Letters represent vegetation types (R – Riparian, E – Ecotone, S-W – Savanna-Woodland) in which bats were recorded during passive monitoring and transects. No associated vegetation data is presented for capture methods as they were excluded from statistical analyses. "^" = ~55 kHz species complex. "X?" = identification unconfirmed as no bats of these species were captured, thus no release calls could be obtained.

Species	Sampling Method				
	Pettersson	**Anabat**	**Transect**	**Mist netting**	**Harp-trapping/Roost searches**
Neoromicia capensis	**X** (R, E & S-W)	**X** (R, E & S-W)	**X** (R, E & S-W)	**X**	**X**
Neoromicia zuluensis^	**X?** (R, E & S-W)	**X?** (R)	**X?** (S-W)	**-**	**-**
Pipistrellus rusticus	**X** (R, E & S-W)	**X** (R, E & S-W)	**X** (R, E & S-W)	**X**	**X**
Pipistrellus hesperidus^	**X?** (R, E & S-W)	**X?** (R)	**X?** (S-W)	**X?**	**-**
Hypsugo anchietae^	**X?** (R, E & S-W)	**X?** (R)	**X?** (S-W)	**-**	**-**
Scotophilus dinganii	**X** (R, E & S-W)	**X** (R, E & S-W)	**X** (R, E & S-W)	**X**	**X**
Laephotis botswanae	**-**	**-**	**-**	**X**	**-**
Tadarida aegyptiaca	**X** (R, E & S-W)	**X** (R, E & S-W)	**X** (R, E & S-W)	**-**	**X**
Mops midas	**X?** (S-W)	**X?** (R, E & S-W)	**X?** (E & S-W)	**-**	**-**
Nycteris thebaica	**-**	**-**	**-**	**-**	**X**

It is noteworthy that each passive sampling method (Pettersson recording, Anabat recording and Transects) did not record certain species in some vegetation types. However, altogether the combined sampling methods illustrated the presence of all species, recorded acoustically, in all vegetation types (Table 2.1).

The time expansion calls of the six passively recorded species, and the single *Laephotis botswanae* individual captured, are shown in Figure 2.4. However, the *L. botswanae* calls (Fig. 2.4G) were recorded in a room-flown environment and thus may not represent search-phase calls for this species. Also, no *Mops midas* individuals were captured so the identification of calls for this species (Fig. 2.4A) is based on the literature (Taylor, 2000; Monadjem *et al.*, 2010). The corresponding frequency division calls are shown in Figure 2.5.

In the field, 55 individuals taken as vouchers were identified into a *Neoromicia – Pipistrellus – Hypsugo complex* based on external measurements and morphology. Of these, 22 were identified as *Pipistrellus rusticus*, 32 as *Neoromicia capensis* and 1 as *P. hesperidus*, based on cranial and bacula measurements (identified by T. Kearney of DNMNH). Substantial variation in size, based on cranial measures, was found in *N. capensis* and it is likewise unclear whether the individual identified as *P. hesperidus* is merely a larger morph of *P. rusticus* (T. Kearney, pers. comm.).

All release calls from bats with body measurements and morphology equivalent to those of the voucher specimens identified as *P. rusticus* were found to have a peak frequency of ~45 kHz (Fig. 2.4F & Fig. 2.5E). *Pipistrellus rusticus* has been widely reported to have a peak echolocation frequency of between 50 – 55 kHz (Taylor, 1999 & 2000; Schoeman & Jacobs, 2008). Thus a new phonic type of *P. rusticus* (with a peak frequency ~45 kHz) appears to exist on KGR. Although echolocation calls with a peak frequency of ~55 kHz were recorded in this study (Fig.

2.4E & Fig. 2.5F), all such calls were recorded passively. It was thus necessary to classify these calls into a species complex containing *Hypsugo anchietae*, *P. hesperidus* and *N. zuluensis*. This complex was based on reported echolocation parameters and geographical distributions (Taylor, 2000; Monadjem *et al.*, 2010) as no *H. anchietae* or *N. zuluensis* individuals were identified from captured bats (Table 2.1) and no release calls of ~55 kHz were recorded.

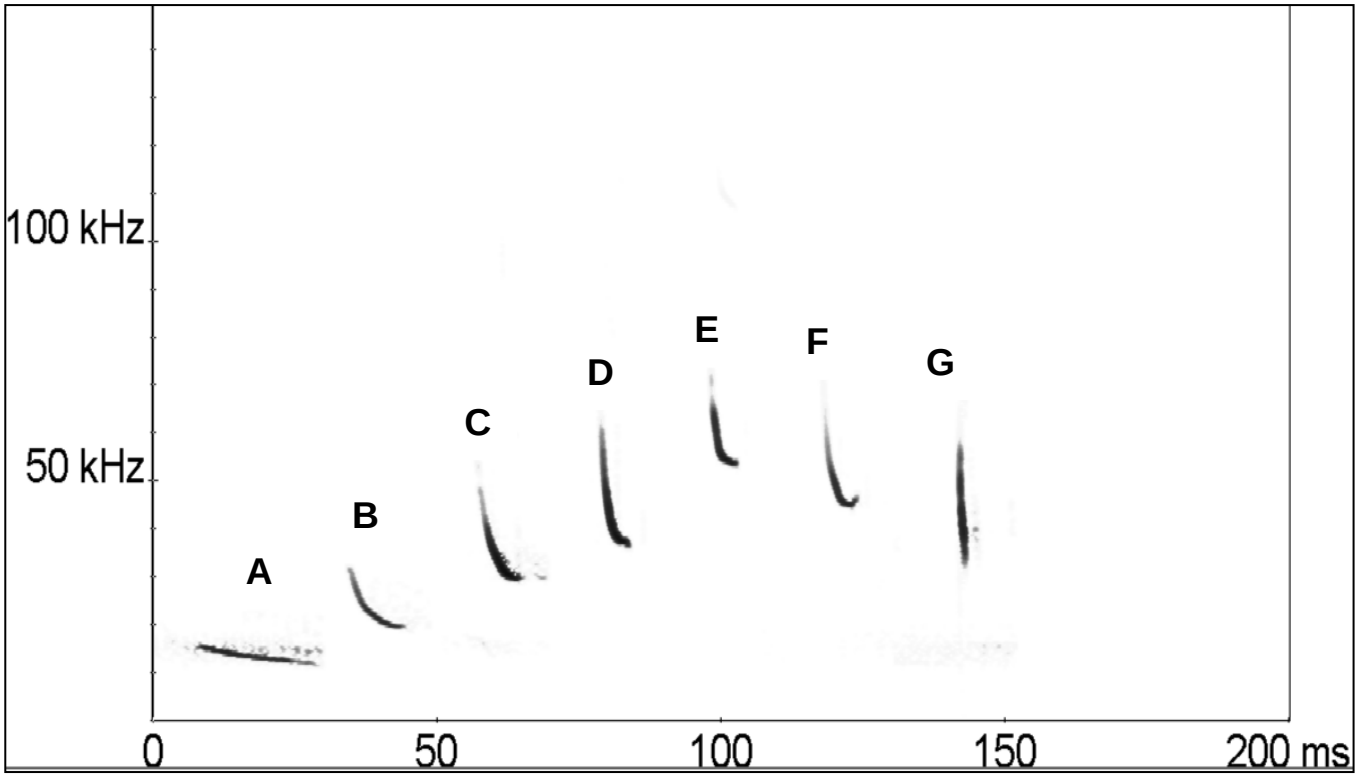

Figure 2.4. Characteristic echolocation calls recorded with a Pettersson D240X time expansion bat detector as represented in Batsound Pro. A) *Mops midas*, B) *Tadarida aegyptiaca*, C) *Scotophilus dinganii*, D) *Neoromicia capensis*, E) *Hypsugo – Pipistrellus – N. zuluensis complex*, F) *Pipistrellus rusticus*, G) *Laephotis botswanae*. The classification of *M. midas* calls was based on literature as no individuals were captured during this study. The single *L. botswanae* individual was room-flown thus this call may not represent a characteristic search-phase call for the species.

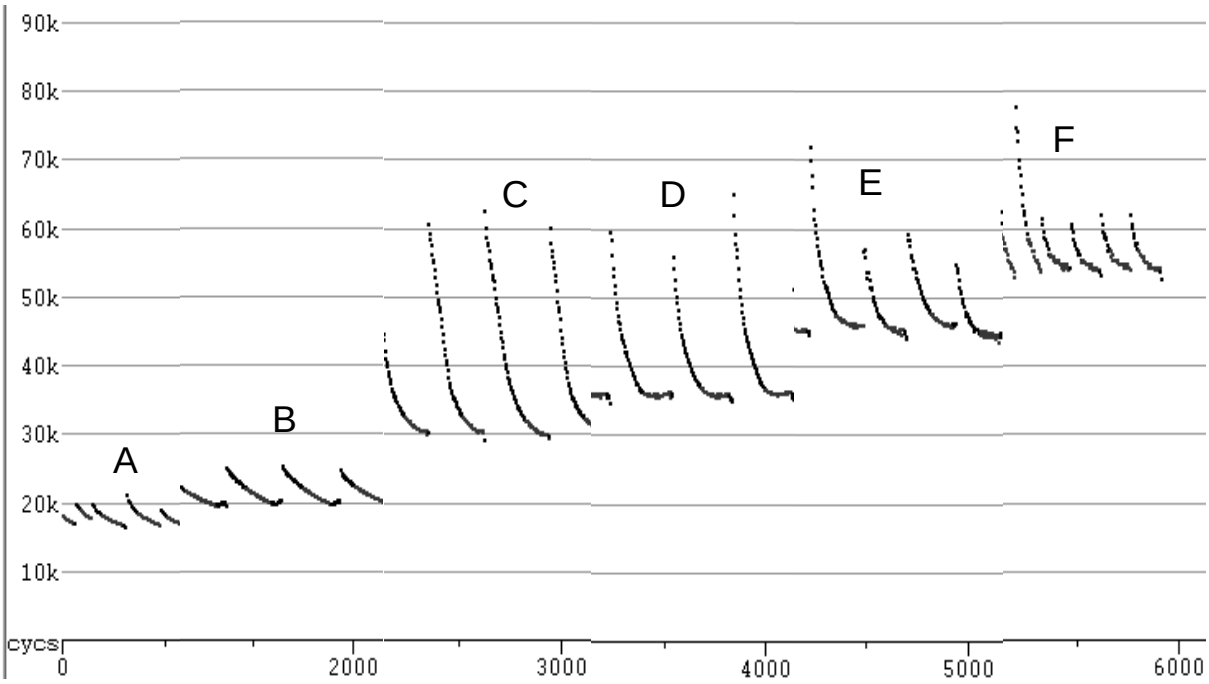

Figure 2.5. Characteristic frequency division calls recorded with the Anabat SD2 and represented in AnalookW, corresponding to those in Figure 2.4. A) *Mops midas*, B) *Tadarida aegyptiaca*, C) *Scotophilus dinganii*, D) *Neoromicia capensis*, E) *Pipistrellus rusticus*, F) *Hypsugo – Pipistrellus – N. zuluensis complex*. No room-flown calls were recorded for the *L. botswanae* individual.

Based on rarefaction curves, bat species richness was found to be equivalent between the different vegetation types (Figures 2.6-2.8). The inflection points of the curves are very similar for each of the different sampling methods. This indicates equivalent bat diversity because it shows that in each vegetation type the species inventory was reached after sampling a similar number of individuals. It is interesting to note that for each sampling method the rarefaction curves suggest slight differences in species richness between the different vegetation types. This is merely a result of some species not being recorded in certain vegetation types by each method. During Pettersson passive monitoring sessions one additional species (*M.*

midas) was only recorded in the savanna-woodland (Table 2.1). Whereas the *Hypsugo – Pipistrellus – N. zuluensis complex* was only recorded in the riparian vegetation during Anabat passive monitoring sessions and only in the savanna-woodland during transects (Table 2.1). Also, *M. midas* was not recorded in the riparian vegetation during transects.

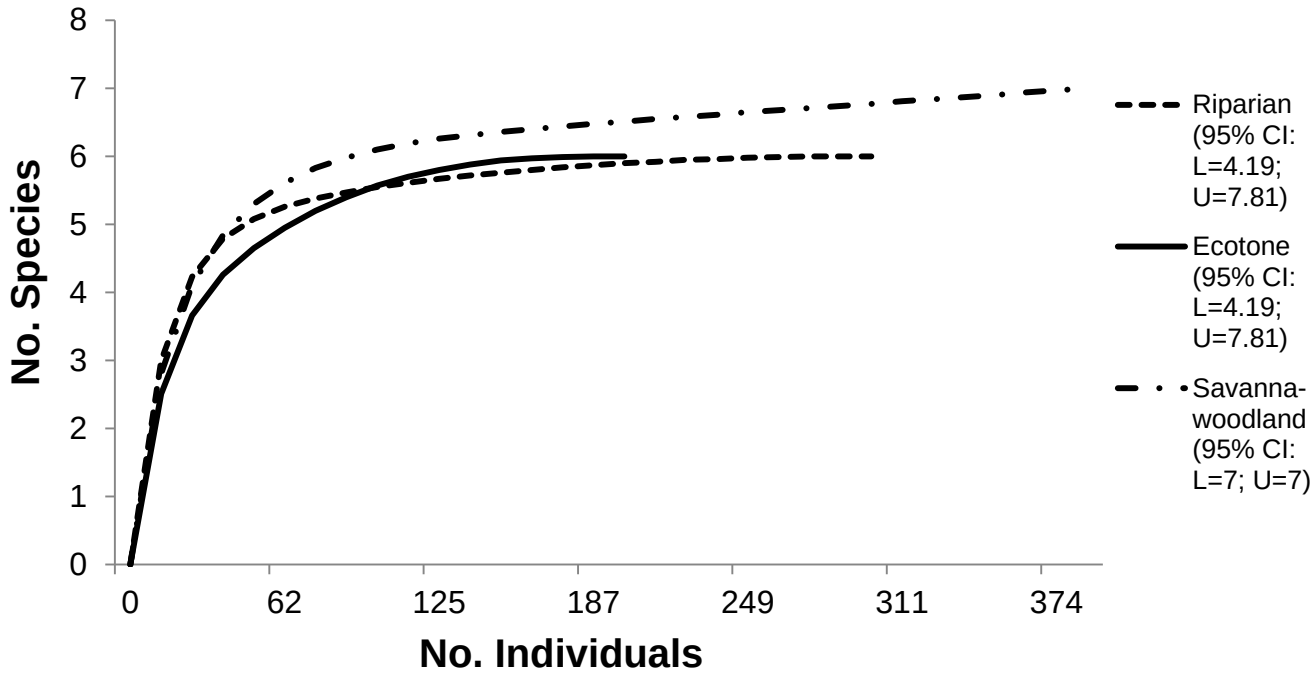

Figure 2.6. Sample-based rarefaction curves of passive monitoring data (Pettersson D240X) in three vegetation types. Curves have been rescaled by individuals to allow for diversity comparisons based on species richness rather than density. Lower (L) and upper (U) 95% confidence intervals (CI) for each vegetation type are shown in the legend.

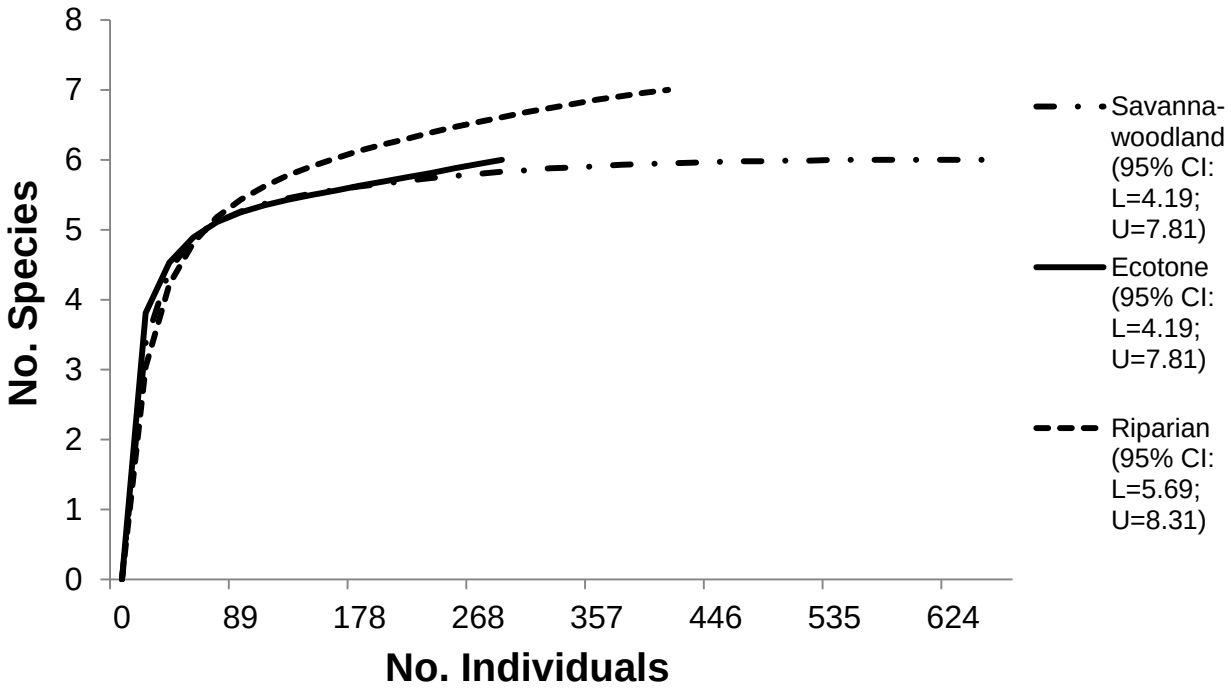

Figure 2.7. Sample-based rarefaction curves of passive monitoring data (Anabat SD2) in three vegetation types. Curves have been rescaled by individuals to allow for diversity comparisons based on species richness rather than density. Lower (L) and upper (U) 95% confidence intervals (CI) for each vegetation type are shown in the legend.

Chao species richness estimators (Tables 2.2-2.4) for each sampling method indicate that sampling was exhaustive in our study. Chao 1 and 2 estimate total species richness including species not present in any sample (Chao, 1984; 1987). The observed number of species was the same as the estimated number in every case. The recorded number of species can thus be regarded as an accurate representation of the bat species richness in the three vegetation types.

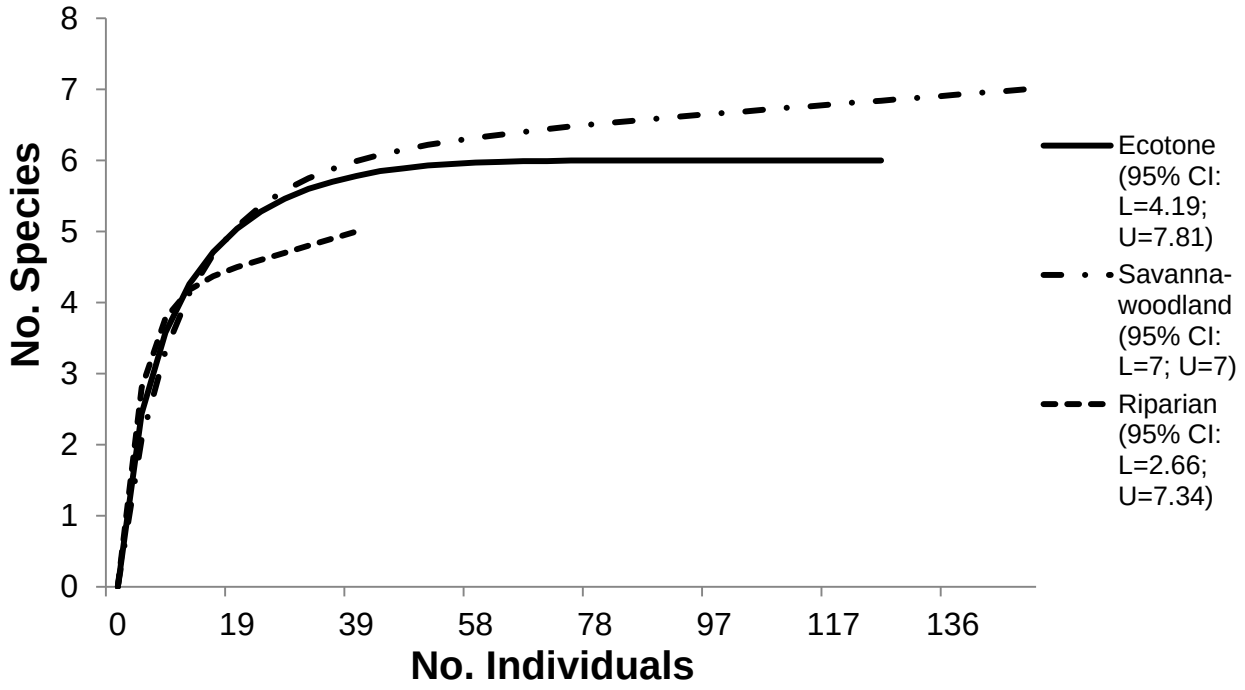

Figure 2.8. Sample-based rarefaction curves of driven transect data (Anabat SD2) in three vegetation types. Curves have been rescaled by individuals to allow for diversity comparisons based on species richness rather than density. Only the seep-zone vegetation could be sampled to represent the Ecotone category during driven transects as the stream on the eastern perimeter of KGR had no track access. Lower (L) and upper (U) 95% confidence intervals (CI) for each vegetation type are shown in the legend.

Table 2.2. Chao 1 and 2 species richness estimators (Chao, 1984; 1987) of Anabat passive monitoring data. Both estimators indicate exhaustive sampling in all vegetation types. Sobs = observed number of species.

Vegetation type	Sobs (Mao Tau)	Chao 1 Mean	Chao 2 Mean
Seep-zone/ecotone	6	6	6
Riparian	7	7	7.5
Savanna-woodland	6	6	6

Table 2.3. Chao 1 and 2 species richness estimators (Chao, 1984; 1987) of Pettersson passive monitoring data. Both estimators indicate exhaustive sampling in all vegetation types. Sobs = observed number of species.

Vegetation type	Sobs (Mao Tau)	Chao 1 Mean	Chao 2 Mean
Seep-zone/ecotone	6	6	6
Riparian	6	6	6
Savanna-woodland	7	7	7

Table 2.4. Chao 1 and 2 species richness estimators (Chao, 1984; 1987) of transect passive monitoring data (Anabat SD2). Both estimators indicate exhaustive sampling in all vegetation types. Sobs = observed number of species.

Vegetation type	Sobs (Mao Tau)	Chao 1 Mean	Chao 2 Mean
Seep-zone/ecotone	6	6	6
Riparian	5	5	5
Savanna-woodland	7	7	7

Shannon and Simpson indices corroborate the lack of difference in diversity between vegetation types (Table 2.5). While the Simpson values show some slight variance, the Shannon values are similar across sampling methods and vegetation types. The variance in the Simpson values suggests a slight gradient of decreasing diversity from the riparian vegetation into the savanna-woodland. This may be an artefact of bats utilizing the river to drink hence resulting in a slightly elevated level of diversity within riparian vegetation. However, there are numerous water bodies (e.g. pools, dams) within the savannah-woodland (at lodges) and within the ecotone vegetation at KGR. Thus bats that are active within these vegetation types need not commute to and from the river just to drink. Pools are also a more stable source of

water as the Pienaars river is often fast-flowing and bats are unlikely to drink from water with much surface disturbance (Greif & Siemers, 2010). Furthermore, the Simpson index is sensitive to abundances and the savanna-woodland is a much larger patch than the other two vegetation types on KGR. The suggested gradient is therefore a likely result of the dilution effect.

Table 2.5. Shannon and (Simpson) diversity indices calculated separately for each sampling method within each vegetation type. Minor differences in values suggest that bat diversity is similar between the three vegetation types.

Method	**Vegetation type**		
	Riparian	**Savanna-woodland**	**Ecotone**
Pettersson	1.49 (3.97)	1.24 (2.61)	1.35 (2.95)
Anabat	1.53 (4.22)	1.34 (2.96)	1.37 (3.22)
Transect	1.45 (4.1)	1.6 (4.1)	1.62 (4.69)

Further support for the similarity in bat diversity between the three vegetation types assessed was found in the Simpson evenness measures (Table 2.6) and Whittaker's beta diversity indices (Table 2.7). These results show that the dominant bat species within each vegetation type also have very similar relative abundances.

Table 2.6. Simpson evenness measures calculated separately for each sampling method within each vegetation type. Very little difference in evenness was evident, corroborating the similarity in bat diversity between the vegetation types.

Method	Vegetation type		
	Riparian	Savanna-woodland	Ecotone
Pettersson	0.567	0.401	0.421
Anabat	0.603	0.441	0.460
Transects	0.586	0.586	0.669

Table 2.7. Whittaker's beta diversity index (β_w) calculated separately for each sampling method within each vegetation type. Similar beta diversity values for each sampling method further support the lack of difference in bat diversity between the three vegetation types.

Method	Vegetation type		
	Riparian	Savanna-woodland	Ecotone
Pettersson	2.80	2.33	2.56
Anabat	2.30	2.13	1.84
Transects	2.50	3.37	2.87

Discussion

Our results oblige us to reject the hypothesis that structural complexity of vegetation is the primary factor influencing local bat species richness at KGR. In contrast August (1983) found that local mammal assemblages were associated with the structural complexity of different habitat types. On the other hand, our results seem to corroborate those of Ricklefs & Lovette (1999) who found bat species richness was poorly related with habitat diversity and rather correlated best with area. These authors surveyed a tropical archipelago and found that small islands set an upper limit on bat and bird population size – hence the strong correlation between area and bat species richness. Their results are interesting though, considering the vagility of bats and birds and their likely ability to disperse. Moreover, bat species richness was also poorly correlated with elevation (Ricklefs & Lovette, 1999).

However, topographical heterogeneity, an important factor in the habitat heterogeneity hypothesis, is the most important predictor of plant species richness in South African savannas (Thuiller *et al.*, 2006). Considering the strong influence plant communities have on the animal species present (Tews *et al.*, 2004), topographical heterogeneity should therefore also be a robust predictor of animal species richness. Indeed, Thuiller *et al.* (2006) found that topographic heterogeneity spatially afforded many ecological niches and facilitated temporal niche persistence. However, KGR has low topographic relief (~70 m: minimum elevation=1068 m; maximum elevation=1138 m). The relatively flat landscape is thus unlikely to promote increased between-habitat diversity that is associated with high topographic variability (Kerr & Packer, 1997; Rahbek & Graves, 2000).

Furthermore, habitat heterogeneity as a predictor of species richness may be more important at regional levels (Rahbek & Graves, 2000; 2001). For example, at a landscape scale the species richness of all but fossorial and aquatic mammals in

southern Africa is most strongly related to woody plant species richness since higher plant species richness provides greater structural complexity and thus potentially increased niche availability (Andrews & O'Brien, 2000; Qian *et al.*, 2009). Habitat heterogeneity is also a more powerful predictor of species richness at lower latitudes because energy availability is less likely to be limiting (Kerr & Packer, 1997). Conversely, Field *et al.* (2008) found climate and productivity to have the highest primacy in explaining species richness, and environmental heterogeneity had the lowest. However, these results were at a global scale.

Direct measures of productivity and energy, such as potential evapotranspiration (PET) or actual evapotranspiration (AET), were not measured in our study. However, significant positive correlations between productivity and energy availability and numerous animal taxa, including volant taxa such as birds, have been widely recorded (Currie, 1991; Ding, 2001; Haddad *et al.*, 2001). The most important of these being in terms of insect diversity since our study focuses on insectivorous bat ensembles. Productivity is a fundamental explanatory variable of insect diversity (Haddad *et al.*, 2001). So too is energy availability, though to a lesser degree as insect diversity is likely more limited by the amount of energy flowing through a food web rather than the total energy available in an area (Hawkins *et al.*, 2003). The similarity of insect communities between the three vegetation types assessed in our study (Moreton, unpublished data), suggests that neither productivity nor energy availability are limiting factors of insect diversity. Both species richness and abundance of insects are relatively homogenous across the different vegetation types at KGR. Moreover, the majority of insectivorous species at KGR are generalists, with only the clutter-forager *Nycteris thebaica* likely to show significant specialisation in its diet. Therefore, the relatively even distribution and

abundance of a range of prey, such as Coleoptera, Diptera, Hemiptera and Lepidoptera, across KGR (Moreton, unpublished data) could potentially be a causal factor in the relatively uniform bat diversity.

The total bat species richness at KGR is surprisingly low when compared with expected species richness based on extrapolated distributions of southern African bats (Monadjem *et al.*, 2010). With these species distributions as a basis and considering that the savanna is one of the most species-rich biomes in southern Africa (Schoeman & Jacobs, 2008), a minimum richness of 10–15 species could be expected at KGR. However, using both active and passive sampling, only eight species of three families (Vespertilionidae, Molossidae and Nycteridae) were recorded. The predominance of widespread generalists at KGR (i.e. most of the recorded vespertilionids and molossids) may explain our lack of evidence for a link between bat diversity and habitat heterogeneity. Importantly, however, the presence of the clutter-specialist species *N. thebaica* suggests that the apparent lack of other clutter-specialist species is not a result of there being insufficient vegetation cover.

This study illustrates the importance of employing multiple sampling techniques when assessing bat diversity of an area. Each method can uncover a different aspect of bat communities (Sedlock, 2001; MacSwiney *et al.*, 2008). For example, the species identified as *Mops midas* and the *Hypsugo – Pipistrellus – N. zuluensis complex* was sampled only through passive monitoring. Although we used two different types of bat detector, direct comparisons are beyond the scope of our study. However, rarefaction suggested that both systems yielded similar species saturations after sampling a similar number of individuals (Figs 2.6 & 2.7). The same can also be said for the driven transects conducted with the Anabat SD2 (Fig. 2.8). Since driven transects allowed bat diversity to be measured rapidly over a greater

area, and sampling efficacy appears similar to that of passive monitoring, it can be recommended that future studies need only incorporate transects if monitoring bat diversity is the primary goal. However, the sporadic application of capture methods is also recommended as they can reveal the presence of species not readily sampled through echolocation (see below).

Capture methods were used mostly to supplement identification through voucher specimens and release calls, thus capture data were not included in statistical analyses. The two species sampled only through capture methods were *N. thebaica* and *Laephotis botswanae.* A colony (23 individuals) of *N. thebaica* was captured from a roost in one of the lodges in the study area and bats of this species were also seen at feeding stations (night roosts) in the bathrooms of a second lodge ~3 km away (Pierce pers. obs.). This distance being more than double the distance *N. thebaica* is expected to travel to hunt, based on wing morphology (Monadjem *et al.*, 2009), suggests that at least one other colony of this species is present at KGR. Additionally, a single male *L. botswanae* was mist-netted within the riparian fringe vegetation (see Pierce *et al.*, in press for voucher information). It is not surprising that these two species were only recorded through captures as they emit very low amplitude calls (“whispering bats”) so are seldom surveyed with bat detectors (Griffin, 1958; Fenton & Bell, 1981; Monadjem *et al*., 2010).

While the bat species richness at KGR is surprisingly low, the similar bat diversity within the different vegetation types at KGR is not novel. At the Sengwa Wildlife Research Area in Zimbabwe, Fenton & Thomas (1980) recorded thirteen species representing seven families of insectivorous bats; more than double the number of species in our study. However, their results showed very little habitat preference with virtually every species utilizing a range of habitat-types from open

floodplains, miombo and mopane woodlands to riparian fringe habitats. Conversely, in Swaziland, 19 insectivorous bats were recorded in riparian habitat while only 11 species were recorded in the adjacent savanna (Monadjem & Reside, 2008). Numerous bat species rely heavily on riparian zones often resulting in higher species richness in these areas (Wickramasinghe *et al*., 2003; Ford *et al.*, 2005). High insectivorous bat species richness in riparian zones has been attributed to high densities of prey insects (e.g. Barclay, 1991; Hayes, 1997). Although the difference in species richness reported by Monadjem & Reside (2008) was attributed to some species favouring riparian habitats, this was not a result of bats responding to the riparian vegetation structure, and the species composition of the savanna habitat was found to be a subset of that in the riparian zone suggesting that numerous species readily utilized both habitat-types.

Though the species richness at KGR is substantially lower than recorded in Swaziland (Monadjem & Reside, 2008), it appears that a similar pattern may be present. The majority of species at KGR are generalists and thus seem readily able to utilize numerous vegetation types. Similar results have been reported from the tropical savanna-woodlands of West Africa. The species richness of insectivorous bats in the Guinea savanna of Ivory Coast is upwards of 50 species representing every major family except Miniopteridae (Meyer, Schwarz & Fahr, 2004; Fahr & Kalko, 2010). While both these studies found that local bat diversity was prominently influenced by habitat-characteristics, many of the species were recorded within both savanna vegetation and more heavily wooded gallery forests. Because bats are volant they are able to disperse fairly large distances when foraging (McCracken, 1996; Rainey *et al.*, 2009). Accordingly, the overlap of habitat-utilization has been

thought to result from a “spillover” effect with species expanding from their central habitat into neighbouring habitats (Fahr & Kalko, 2010).

Important factors to consider with regards to the ability to disperse are the echolocation and flight capabilities of the various species. The species found to extend their activity beyond their core habitats were those that echolocate with constant – quasi–constant frequency (CF-QCF) or frequency modulated – quasi–constant frequency (FM-QCF) calls i.e. molossids, emballonurids and many vespertilionids (Meyer *et al.*, 2004; Fahr & Kalko, 2010). These types of echolocation are effective in open habitats as well as clutter-edge environments as they are less susceptible to attenuation than steep FM and HDC-CF calls of high frequencies. Bats using HDC-CF calls, such as rhinolophids and hipposiderids, are usually restricted to highly cluttered habitats. Such clutter-specialist species are usually more manoeuvrable than QCF and FM-QCF bats because echolocation and wing morphology are part of the same adaptive complex (Aldridge & Rautenbach, 1987; Arita & Fenton, 1997). The manoeuvrability of clutter-specialist bats is a result of their short, broad wings that provide low wing loading and thus low flight speeds, whereas the longer, narrower wings of open-air and clutter-edge bats give higher wing loadings that allow for faster, more agile flight (Aldridge & Rautenbach, 1987; Norberg & Rayner, 1987).

Although many species of HDC-CF bats are closely associated with savanna-woodland as well as riparian vegetation, some may be limited by the availability of cave roosts (e.g. *Cloeotis percivali*, *Hipposideros vittatus*) (Monadjem *et al.*, 2010). The lack of appropriate caves at or near our study site may thus exclude these species from the area, particularly since Hipposideridae and Rhinolophidae (the two families of southern African CF bats) seldom forage >3 km from their day roosts

(Fenton & Rautenbach, 1986; Fenton 1997; Pavey, Grunwald & Neuweiler, 2001; Reiter, 2004). However, a number of rhinolophids or hipposiderids have been reported roosting in hollow trees, small rocky caverns or even man-made structures (LaVal & LaVal, 1980; Fenton & Rautenbach, 1986; Russo, Jones & Migliozzi, 2002; Wickramasinghe *et al.*, 2003; Reiter, 2004). Nevertheless, the trees in which these species have been reported roosting have usually been hollow Baobabs (*Adansonia digitata*) (e.g. *R. hildebrandti* – Fenton & Rautenbach, 1986; *H. caffer* – Dawn Cory Toussaint, pers. comm.). It is unlikely that most of the tree species at Kwalata provide cavities large enough to be suitable roosts for these bats. Additionally, recording free-flying rhinolophids or hipposiderids can be difficult as they would need to pass very close to the microphone of a detector because the calls of most HDC-CF bats are relatively low amplitude, highly directional and often of high frequency and so are extremely susceptible to atmospheric attenuation (Fenton, 1986; Russo *et al.*, 2002 and references therein; Monadjem, Reside & Lumsden, 2007).

The only potentially suitable roosts at KGR for the many cave-dependent rhinolophid and hipposiderid species are two small, water-filled, red granite quarries <100 m from the riparian fringe vegetation. The exposed faces of these contain myriad crevices and small openings that could provide roosting sites for a number of species. However, no additional species were recorded at the quarry roosts. These crevice roosts appear to be dominated by large numbers of *T. aegyptiaca* as numerous trapping and recording attempts made within the quarries only yielded individuals of this species. The number and inaccessibility of many of the crevices made capturing bats from the numerous roosts virtually impossible.

The lack of any mistnet captures of rhinolophids or hipposiderids in our study is not entirely surprising since their manoeuvrability and high echolocation frequencies

makes catching them with this method difficult (Francis, 1989; Kunz, Hodkison & Weise, 2009). It has been suggested though that these species may be more readily sampled using harp-traps (Schoeman & Waddington, 2011). However, over 250 harp-trap hours in each vegetation type yielded not a single capture. This result may suggest that these families of bats are absent from the area. However, no bats, even of the predominant clutter-edge species (e.g. *N. capensis, S. dinganii, P. rusticus*), were captured during the above-mentioned harp-trapping. Based solely on this data, it is thus unreasonable to rule out the presence of HDC-CF bats completely. Furthermore, it may be that their abundances are particularly low and, coupled with their low-amplitude echolocation calls, could lead to them seldom being recorded.

Clutter-specialist species (e.g. rhinolophids and hipposiderids) are, however, generally more sensitive to habitat disturbance (Danielsen & Heegaard, 1995; Kingston *et al.*, 2003). Even though some species such as *H. caffer* and *R. clivosus* are known to utilize roosts in man-made structures, their susceptibility to landscape fragmentation could explain their absence. The apparent absence of these species at KGR may thus be a result of historical grazing pressure (up until the late 1980's) KGR experienced through livestock farming, which often results in dense clumps of woody vegetation through bush encroachment (Scholes & Archer, 1997). We took no direct measures of land use during our study, relying predominantly on local knowledge of impacted areas. However, given that habitat fragmentation has negative qualitative and quantitative effects on the persistence of clutter-specialists (Kapos, 1989; Saunders, Hobbs & Margules, 1991), we considered our inferences justified. Furthermore, grazing by large herbivores can result in patchy soil resources (Golodets, Kigel & Sternberg, 2011) hence savanna-woodlands are often very patchy (August, 1983). Since most clutter-specialist species are also ecologically

constrained to environments of continuous clutter by their wing and echolocation specializations (Kingston *et al.*, 2003) it is likely that the patchy savanna-woodland of KGR is unfavourable habitat for rhinolophids and hipposiderids. Moreover, the ability of LDC-QCF and FM-QCF bats – the predominant species recorded in our study – to move readily between patches of open land and relative clutter could explain the homogenous distribution of the bat assemblages at KGR.

Although an individual pteropodid (likely *Eidolon helvum*) was reportedly removed from a house in a neighbouring settlement in 2009 (Charl Pretorius, pers. comm.), no fruit bats were captured or observed during this study. The factor limiting this family of bats from the area may be a lack of food. The pteropodid species likely to occur in the area (*Eidolon helvum*, *Epomophorus crypturus*, *E. wahlbergi* & *Rousettus aegyptiacus*), based on geographic distribution, appear to prefer *Ficus* species for forage (Fenton *et al.*, 1985; Kalko, Herre & Handley, 1996; Monadjem *et al.*, 2010). However, no *Ficus* trees are found within the riparian fringe of the Pienaars River or the mixed Bushveld vegetation of the savanna-woodland at KGR. Nonetheless, these fruit bats have been reported to forage on a relatively wide variety of fruits and flowers (Thomas & Fenton, 1978; Smithers, 1983; Fujita & Tuttle, 1991; Korine, Izhaki & Arad, 1999). However, very few of the genera of fruit trees mentioned are present at KGR and of those that are (e.g. *Terminalia*), the species present (*Terminalia sericea*) are not known as forage for pteropodids (Fujita & Tuttle, 1991). Furthermore, *R. aegyptiacus* is probably more limited by roost availability (caves) than appropriate forage (Monadjem *et al.*, 2010), further restricting this species from the area. The single record of a pteropodid mentioned above was thus likely a vagrant foraging on the fruits of anthropogenically introduced tree species.

Although the availability of suitable roost sites is likely to be a major limiting factor for clutter-specialist bats, the fragmentation of the savanna-woodland as a result of historical land impacts cannot be disregarded. The proximity of KGR to a major highway (<2km) means there are numerous bridges and culverts in the area that could potentially accommodate small numbers of clutter species. However, their sensitivity to habitat fragmentation (Danielsen & Heegaard, 1995; Kingston *et al.*, 2003) likely results in clutter-specialist bat species rapidly becoming absent from a community that has been impacted by land use. A similar assessment of bat diversity at a broader landscape scale could shed light on what specifically are the predominantly limiting factors for both clutter species and pteropodids. Such an assessment could include the large tracts of mostly undisturbed savanna-woodland to the southeast of our study area and so allow for a comparison between areas that have undergone historic land impacts such as overgrazing and areas whose ecosystem functioning remains almost pristine. Also, our study site falls within the boundary of the Dinokeng Game Reserve (DGR) which has recently seen the reintroduction of a small herd of elephants. The foraging impacts of these megaherbivores are well known to reduce the structural diversity of vegetation of a landscape (Dublin & Niskanen, 2003; Wiseman, Page & O'Connor, 2004; Guldemond & van Aarde, 2010). A landscape-scale assessment of bat communities at DGR could thus provide a unique opportunity to monitor the responses of bat populations to regional habitat disturbance as the effects take shape.

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Chapter 3: The insectivorous bats of Kwalata Game Ranch: influences of competition and prey defences on phenotypic structure.

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ABSTRACT

The deterministic processes of competition and prey defences have been shown to be influential in structuring insectivorous bat assemblages at the ensemble and functional group level; even in environments impacted by urbanization. We investigated the influences of these deterministic processes on a relatively species-poor insectivorous bat ensemble at a game ranch bordering large peri-urban settlements. Using null models we compared observed phenotypic patterns of size, wing morphology and echolocation call parameters with patterns expected from chance. Non-random patterns, suggesting the influence of competition, were found for size and wing morphology. However, no evidence for the influence of prey defences was found – phenotypic patterns of echolocation call structure were clumped. The effects of ecological light pollution are probably counter-acting the defence mechanisms of the tympanate insects in the area. Access to these prey

items by the syntonic bat species at the study site may have contributed to competitive exclusion of rhinolophid and hipposiderid bats.

Key words: insectivorous bats, competition, prey defences, null models.

INTRODUCTION

The question whether ecological communities are structured through deterministic or random processes has been debated for decades (Lawton 2000). Deterministic processes that are often proposed to be at the forefront of community ecology are interspecific competition and predator-prey interactions (Ricklefs & Schlutter 1993; Schoeman & Jacobs 2011). Following the assumption that the phenotypic characteristics of an organism facilitate adaptation for specific ecological functions and, being governed by natural selection, are predominantly responsible for the morphological variation among species (Wainwright & Reilly 1994), ecomorphological studies can help illuminate the processes that structure ecological communities. Such studies are based on the use of measurements of certain morphological traits of sympatric species to deduce their respective ecological features (Wainwright & Reilly 1994). In so doing they can provide insight into co-occurrence patterns (Ricklefs & Schlutter 1993; Meyer & Kalko 2008).

A seminal paper in this regard proposed that in order for closely related or morphologically similar taxa to co-occur, they should exhibit a minimum size difference to avoid competitive interactions (Hutchinson 1959). Case & Sidell (1983) later purported that character divergence should indeed occur between morphologically similar species, otherwise competitive exclusion will lead to local extinction of certain species. Assuming that character divergence rather than local extinction takes place, size differences among sympatric species should then show

relatively even distribution (Case & Sidell 1983). The alternative to these predictions is that sympatric species co-occur simply as a matter of chance (Roughgarden 1983). While it may be possible for certain species-sets to show a minimum size difference if this is so, it is unlikely that it would hold for all species in the community. Moreover, the difference in body sizes of species-sets in communities structured by chance should be highly variable.

The long life-spans, slow growth rates and reproductive outputs, low predation risk and stable populations that characterise the life histories of most insectivorous bats (Barclay & Harder 2003; Brunet-Rossinni & Austad 2004) increase their propensity for interspecific competition. Given these life-history circumstances, population carrying capacity is often reached and resources may become limiting hence assemblage patterns are shaped by deterministic processes such as competition and/or prey defences (Pianka 1972; Findley 1993; Schoeman & Jacobs 2008; Shoeman & Waddington 2011). Although the influence of interspecific competition on assemblage patterns has been a point of contention (Strong *et al.* 1979; Connor & Simberloff 1979; Bowers & Brown 1982; Lewin 1983; Lawton 2000), the direct link between morphology and ecology of insectivorous bats (Norberg 1994; Swartz *et al.* 2003) makes them an ideal study group for investigations into this aspect of community ecology.

A number of studies have purported that competition is only a minor contributor to the assemblage patterns of bat communities or is not consistent across a landscape (Willig & Moulton 1989; Arita 1997; Stevens & Willig 1999). However, these studies looked for the influences of competition at regional scales (i.e. gamma diversity), whereas the predictions of Hutchinson (1959) and Case & Sidell (1983) are more likely to be evident at local scales (Moreno *et al.* 2006). Therefore, in the

present study, we examine the potential processes that may have influenced the structure of a local insectivorous bat ensemble (*sensu* Fauth *et al.* 1996) at Kwalata Game Ranch (hereafter KGR) in South Africa.

The information gathered on species co-occurrence processes through ecomorphological studies can have profound implications for conservation and management efforts. This is particularly true in areas that are impacted to some degree, by the effects of urbanization (Shochat *et al.* 2006) since anthropogenic effects such as habitat fragmentation and ecological light pollution (*sensu* Longcore & Rich 2004) can compound the influences of deterministic processes, like competition, on sympatric bat species (Arlettaz *et al.* 2000). Therefore, we conducted our investigation with close regard to the potential effects of anthropogenic pressure as KGR is bordered (within 3 km) by a large peri-urban settlement which contains numerous large spotlights interspersed with many street lamps.

We focused our study at the level of an insectivorous bat ensemble because competitive interactions between members of an ensemble are more likely than between members of, for example, a bat assemblage (which would include frugivores and insectivores), thus influences of deterministic processes on morphological structure of coexisting species should be more evident (Moreno *et al.* 2006). Furthermore, since size, wing morphology and echolocation parameters are part of the same adaptive complex and govern bats' foraging strategies (Aldridge & Rautenbach 1987; Arita & Fenton 1997), we used these morphological measures to test for influences of competition and prey defences on phenotypic patterns.

Prey defences, in the context of bat ecology, refer to avoidance of echolocating bats by tympanate insects. It is widely accepted that tympanate insects have coevolved with echolocating bats in a classic arms race between predator and prey

(Bogdanowicz *et al.* 1999; Jones & Waters 2000; Schoeman & Jacobs 2003; Jacobs *et al.* 2008). Many nocturnal insects from families of moths (Lepidoptera), lacewings (Neuroptera) and mantids (Dictyoptera) have developed tympanate organs (ears) in response to predation pressure from echolocating bats (Rydell *et al.* 1995). Indeed, much evidence suggests that ears in insects have evolved for the sole purpose of allowing tympanate insects to detect and avoid echolocating bats (Fullard 1988). For example, very few moths produce sound so their ears are unlikely to have an intraspecific communication role (Waters 2003) and diurnal species of Notondontids have been shown to be mostly deaf to the same frequencies as sympatric nocturnal species of the same family (Fullard & Dawson 1997).

In accordance with Hutchinson's (1959) prediction of minimizing similarity, if interspecific competition influenced ensemble structure we predicted that phenotypic differences between species would be larger than expected from chance. Also, following Case & Sidell (1983), phenotypic differences among species should be less variable than those expected from chance.

Finally, if prey defences rather than interspecific competition shaped ensemble structure then we predicted that phenotypic differences, particulary in echolocation parameters, would be smaller than expected from chance. In response to the influences of predator avoidance by tympanate insects, a number of bat species, particularly rhinolophids and hipposiderids, have coevolved echolocation calls that utilize frequencies outside of the hearing range of most tympanate insects (Jacobs *et al.* 2008). Thus if phenotypic distances between the echolocation characteristics of insectivorous bats in the KGR ensemble are smaller than expected from chance, it would suggest that they have adapted in response to prey defence rather than interspecific competition.

We tested our predictions by comparing any observed patterns in phenotypic structure against the null hypothesis that species co-occur randomly using null model analyses (Gotelli & Graves 1996). Null models are effective for ascertaining whether deterministic processes are indeed evident as they incorporate stochastic environmental effects and allow for multiple outcomes including one of “no effect” (Gotelli & Graves 1996).

MATERIALS & METHODS

Study site and sampling

An insectivorous bat ensemble was surveyed on Kwalata Game Ranch (KGR) in Northern Gauteng, South Africa (main lodge 25° 23.421’ S 28° 19.309’ E). The 1800 hectare farm is dominated by savanna-woodland consisting of mixed Bushveld, Kalahari Thornveld and sourish mixed Bushveld veld types (Mucina & Rutherford 2006; average elevation: 1110 m; range: 1077-1138 m).

A combination of trapping methods was used to sample bats during both wet and dry seasons from September 2010 – June 2011. Free-flying bats were captured using a combination of ground-level and canopy mistnets while identified roosts were trapped using either a harp-trap or hand net.

One to three monofilament mistnets (14 mm^2 mesh; ECOTONE, Poland) ranging from 3 – 9 m were set from canopy level down, depending on availability of appropriate branches from which to hang the nylon rope rigs and lack of potentially snagging branches between the rigs. Otherwise, nets were set from ground level up on 4 m aluminium poles. Nets were opened at dusk and were monitored continuously for approximately two hours.

The harp-trap (dimensions: ±1.2 m x 0.9 m) was placed at the entrance of an identified roost before dusk and trapped bats were collected the following morning. Cotton capture bags were placed at the bottom of the PVC trough to provide captured bats some shelter. Where roosts were too open to for harp-trapping to be effective (e.g. a roost of *Nycteris thebaica* in the apex of a thatched roof), a hand-net was used.

Size, wing morphology and echolocation parameters

All captured bats were placed, individually, into numbered cotton bags. Body mass of each bat was measured to the nearest 0.1 g using a Pesola 30 g spring balance. This was done the morning after capture to ensure their digestive tracts had been voided so mass could be used as a measure of body size (Schoeman & Jacobs 2011). Sex and reproductive status was also assessed and sub-adult bats were identified by the degree of ossification of the epiphyses (Anthony 1988). Only measurements from adult male bats were used in subsequent analyses to eliminate any potential biases resulting from sexual dimorphism or immaturity (Schoeman & Waddington 2011). Measurements of wing span (WS) and wing area (WA) for all species in the regional pool (see below), including the species captured at KGR, were taken from the literature (Norberg & Rayner 1987; Aldridge & Rautenbach 1987; Schoeman & Jacobs 2008). Based on these measurements and following Schnitzler & Kalko (2001) we classified bats into three functional groups namely clutter (C), clutter-edge (CE) and open-air (O).

Echolocation calls of free-flying bats were recorded using a Pettersson D240X bat detector (Pettersson Elektronik AB, Sweden) linked to an iAUDIO U3 mp3 recorder (COWON Systems Inc., Korea). The detector was mounted vertically, in a

weather-proof casing, on a standard camera tripod at a height of 1.5 m. The iAUDIO U3 was set to record at 44 100 Hz, 16-bit, stereo. Recording began shortly after sunset and continued for two hours. Files were converted to WAV files using COWON Media Center (COWON Systems Inc., Korea) and analysed in BatSound Pro version 3.1b (Petersson Elektronik AB, Sweden). With time-expansion set to 10, calls were analysed at the same settings at which they were recorded (44 100Hz, 16-bit, stereo) using a threshold of 15. The best quality calls, based on high signal-to-noise ratios and a lack of distortion or overloading (Parsons & Jones 2000), were selected for analysis. The time-frequency (spectrogram) and time-amplitude (oscillogram) windows were used to identify calls and to determine duration (Dur) of single calls. Peak frequency (PF) of the dominant harmonic was measured from the Fast Fourier transformation (FFT) power spectrum (size 1024, using a Hanning window). Minimum and maximum frequencies of individual calls were measured at ±18 dB from the PF on the FFT power spectrum (Schoeman & Jacobs, 2008). Echolocation parameters of species captured during active trapping were based on search phase calls recorded when the bats were released. Reference libraries were used for echolocation parameters of species that were not sampled in active trapping (Monadjem *et al.* 2010; Pierce *et al*. in press).

Flight capabilities and echolocation characteristics are part of the same adaptive complex (Aldridge & Rautenbach 1987; Arita & Fenton 1997). We thus used principle components analysis (PCA) in XLstat (Addinsoft SARL, France – trial version) to create two new variables (PC1 & PC2) from the multivariate data on species' size (mass), wing morphology (WS, WA) and two echolocation parameters (PF, Dur). PCA removes the redundancy of the highly correlated variables of size, wing and echolocation parameters but maintains morphological distances between

species. The values of all parameters were first Log_{10} transformed to ensure the data were normally distributed with similar variances.

Testing for influences of competition or prey defence on phenotypic patterns in morpho-space

Using the size overlap module of EcoSim (Version 7.72; Gotelli & Entsminger 2011) we quantified morphological differences between coexisting bats of the KGR ensemble with two indices: minimum segment length ratio (MSL) and variance in segment length ratios (VSL). Indices of size ratios of adjacent species rather than absolute distances are more appropriate when testing the predictions of Hutchinson's (1959) and Case & Sidell's (1983) competition hypotheses (Gotelli & Entsminger 2011). After log-transforming the morphological parameters of each species, $log(A/B) = log(A) – log(B)$ where A and B are values of the same morphological parameter for adjacent species. For n species $n – 1$ segment lengths were calculated and sorted in descending order. MSL was the smallest segment length ratio out of the set of segment length ratios calculated. This index was used to test the prediction of a minimum size difference between adjacent species in morpho-space to avoid competitive interactions. It also allowed us to test the contrasting prediction from the prey defences hypothesis that phenotypic parameters of adjacent species should show convergence (Schoeman & Waddington 2011).

If the observed MSL value was significantly larger than 95% of the simulated MSL values, we concluded that coexisting species were further apart in morphological space than expected from chance. On the other hand, if the observed MSL was significantly smaller than 95% of the simulated MSL values, we concluded coexisting species were more similar in morphological space than expected by

chance. If the observed VSL value was significantly smaller than 95% of simulated values, we concluded that phenotypic patterns were more evenly spaced than expected by chance.

The observed MSL and VSL values calculated for the bats of the KGR ensemble were compared with the values of simulated ensembles randomly drawn from two source pools. The first biological source pool included 42 insectivorous bat species recorded in the savanna biome of South Africa that could potentially occur in our study area and was derived from species distribution records of Monadjem *et al.* (2010). The second source pool comprised a log-uniform null distribution. There are approximately equal numbers of species in each segment-length ratio class in this distribution and it is less prone to Type II errors than log-normal distributions (Schoeman & Jacobs 2008; Gotelli & Entsminger 2011).The minimum and maximum values of each parameter in the biological source pool were used as the limits for the log-uniform null distribution. Observed indices of the KGR ensemble were compared with the distribution of index values for 1000 randomly generated ensembles drawn from both source pools. The same number of species as in the observed ensemble was drawn at random from the source pools. Each species in the source pools had the same probability of being drawn for each simulated ensemble and once drawn, a species could not be drawn again for that ensemble.

RESULTS

The insectivorous bat ensemble of KGR comprised 7 species of 7 different genera representing 3 families. Based on wing measurements, four species were classified into the CE functional group (*Laephotis botswanae*, *Neoromicia capensis*, *Pipistrellus rusticus* and *Scotophilus dinganii*), two into the O functional group (*Mops*

midas and *Tadarida aegyptiaca*) and only one into the C functional group (*Nycteris thebaica*). Only one of the species (*M. midas*) was recorded solely through echolocation calls compared with a reference library. *Nycteris thebaica* and a single *L. botswanae* individual were sampled only through captures. The accuracy of the observed species richness of the insectivorous bat ensemble of KGR was confirmed with sample-based rarefaction and species richness estimators (see Chapter 2).

Principal component analysis

The first two principle components explained 83.5% of the variance of size, wing morphology and echolocation parameters (PC1 explained 62.88% and PC2 explained 20.62%; Fig. 3.1) for the 42 species of insectivorous bats in the biological source pool. The bivariate plot of PC1 and PC2 generally grouped species into family and/or functional groups. However, there was overlap in species belonging to different functional groups (Fig. 3.1a). Considering the variables used for the PCA, three open-air foragers (*Chaerephon pumilus, Sauromys petrophilus* and *Tadarida aegyptiaca*) and three clutter foragers (*Nycteris thebaica, N. macrotis* and *Kerivoula lanosa*) grouped amongst the clutter-edge foragers. Also, two of the larger-bodied clutter-edge foragers (*Scotophilus dinganii* and *Myotis welwitschii*) overlapped with two medium-sized open-air foragers (*Mops condylurus* and *M. niveiventer*).

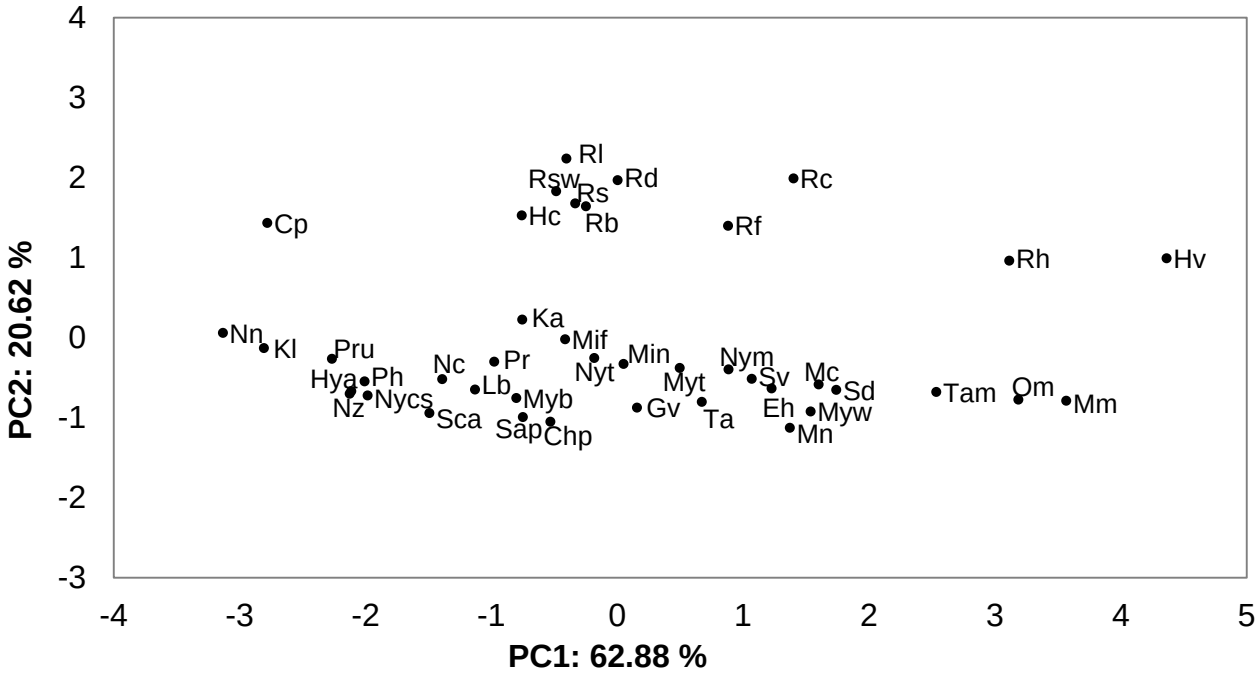
Plotting the factor loadings showed the clear separation of size and wing morphology parameters from call variables (Fig. 3.1b). We interpreted the principal components as follows. PC1 was a representation of size and wing morphology (i.e. mass, WS and WA; Table 3.1). Species with high PC1 scores were larger bats with greater WS and WA (e.g. *Hipposideros vitatus, M. midas, Rhinolophus hildebrantii* and *Otomops martiensseni*; Fig. 3.1a) than species with low PC1 scores (e.g.

Neoromicia nana, Cloeotis percivali and *K. lanosa*; Table 3.2). PC2 was a representation of echolocation (i.e. PF and Dur; Table 3.1). Bat species that loaded high on PC2 had echolocation calls with higher PF (e.g. *C. percivali* and *H. caffer*) and greater Dur (e.g. *R. landeri, R. clivosus* and *R. darling*) than species with low PC2 scores (e.g. *M. niveiventer, C. pumilus* and *S. petrophilus*; Table 3.2).

Table 3.1. Factor loadings of the first two principal components (PC1 & PC2) created with principal component analysis of size (mass), wing (WS = wing span and WA = wing area) and echolocation (PF = peak frequency and Dur = duration) parameters of 42 insectivorous bat species (values in bold indicate the parameters contributing most to the principal components).

Parameter	PC1	PC2
Mass (g)	**0.954**	-0.151
WS (cm)	**0.975**	0.035
WA (cm^2)	**0.921**	0.182
PF (kHz)	-0.492	**0.719**
Dur (ms)	0.438	**0.676**

(**a**)



(**b**)

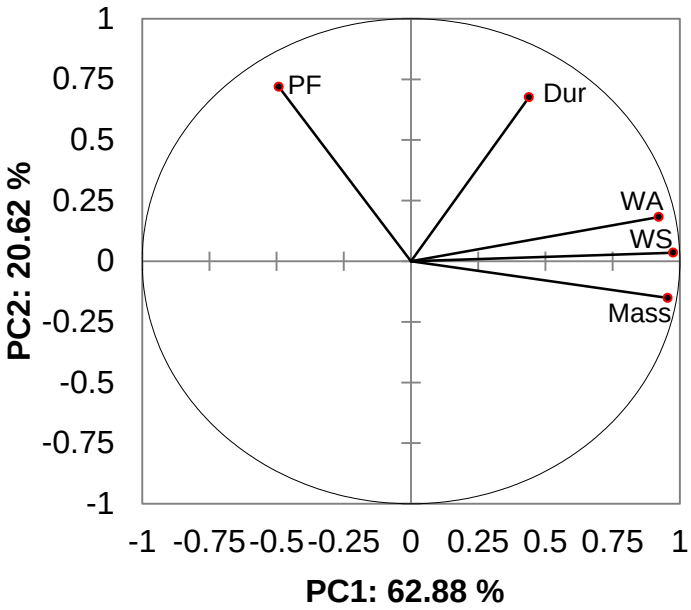


Figure 3.1. Principal component analysis of mass, wing morphology (WS and WA) and echolocation (PF and Dur) parameters of the 42 insectivorous bat species in the biological source pool: (a) Plot of factor scores of the species for the first two principal components (PC1 & PC2). (b) Plot of the factor loadings for size, wing morphology and echolocation parameters on the first two principal components. Lines indicate distance to the origin (0,0). See Table 3.2 for species abbreviations and functional groups.

Predictions of non-random phenotypic patterns

We found evidence that competition shaped phenotypic patterns with respect to size and wing morphology (Table 3.3). Observed MSL ratios of WS and PC1 (a measure of size and wing morphology) were significantly larger than expected from chance based on the biological and log-uniform source pools, respectively. Also, observed VSL of WA was significantly smaller than the expected values in the log-uniform source pool. Furthermore, observed MSL of WA and WS were larger than 90% of expected values drawn from the biological and log-uniform source pools, respectively, albeit not statistically significant at the 95% level.

Conversely, we found no evidence that prey defences influenced the phenotypic patterns of echolocation parameters (Table 3.3). Contrary to the predictions of the prey defence hypothesis, the observed MSLs of PC2 (a measure of echolocation parameters) were not significantly smaller than expected from chance irrespective of source pool.

The observed VSL of PC2 was significantly larger than expected from chance irrespective of source pool (Table 3.3). However, this neither corroborates nor refutes predictions of either hypothesis; it merely indicates that species in the KGR ensemble are clumped with respect to echolocation characteristics (Gotelli & Entsminger 2011). This result is thus a reflection of most of the KGR species being part of the CE functional group and having similar echolocation characteristics.

Table 3.2. Phenotypic characters, functional groups and species abbreviations of 42 insectivorous bat species included in principal component analysis. These species formed a biological source pool of insectivorous bat species recorded in the savanna biome of South Africa that could potentially occur in our study area and was derived from species distribution records of Monadjem *et al.* (2010). Measures of mass, wing span (WS), wing area (WA) and echolocation parameters (PF = peak frequency, Dur = duration) for species not recorded in the KGR ensemble (^) were taken from the literature (Norberg & Rayner 1986; Aldridge & Rautenbach 1987; Schoeman & Jacobs 2008). Functional groups: O = open-air, CE = clutter-edge and C = clutter.

Species	Functional group	Abbreviation	Mass (g)	WS (cm)	WA (cm^2)	PF (kHz)	Dur (ms)
Chaerephon pumilus^	O	Chp	11.2	26.6	96.2	29.9	4.1
Cloeotis percivali^	C	Cp	5.3	20.9	83.7	207.8	4.6
Eptesicus hottentotus^	CE	Eh	18.1	33.1	180.8	30.6	5.5
Glauconycteris variegatus^	CE	Gv	13	30.4	148.4	41.1	2.3
Hipposideros caffer^	C	Hc	8.5	27.7	139.6	142.3	8.4
Hipposideros vittatus^	C	Hv	68.7	52.4	438.2	65	13.1
Hypsugo anchietai^	CE	Hya	6	21.7	86.9	55.9	2.1
Kerivoula argentata^	C	Ka	7.6	30.1	148.6	100	2
Kerivoula lanosa^	C	Kl	4.8	20.4	78.4	90	2
Laephotis botswanae	CE	Lb	6	24.84	101.71	33	5
Miniopterus fraterculus^	CE	Mif	8.9	29.4	136.5	62.1	3.7
Miniopterus natalensis^	CE	Min	11.6	30.6	146	51.4	3.4
Mops condylurus^	O	Mc	28.3	33.6	149.7	26.7	10
Mops midas	O	Mm	39.6	45.7	235	15	16.5
Mops niveiventer^	O	Mn	28.7	31.7	129.7	20.3	8.1
Myotis bocagii^	CE	Myb	9.1	25.8	120.7	44.6	2.5
Myotis tricolor^	CE	Myt	13.1	31.8	175.7	47.8	3.3

Table 3.2. *Continued*

Species	Functional group	Abbreviation	Mass (g)	WS (cm)	WA (cm^2)	PF (kHz)	Dur (ms)
Myotis welwitschii^	CE	Myw	18	36.6	234.9	34	2.4
Neoromicia capensis	CE	Nc	7.3	22.6	93	39.4	5.1
Neoromicia nana^	CE	Nn	3.3	20.6	58	69.4	4.6
Neoromicia zuluensis^	CE	Nz	5.8	21.4	81.4	48.4	2.7
Nycteris macrotis^	C	Nym	17.8	35.1	237.3	76.7	1.2
Nycteris thebaica	C	Nyt	12.6	29.3	167.3	77.5	1.7
Nycticeinops schlieffeni^	CE	Nycs	6.3	21.4	78.2	42.5	3.5
Otomops martiensseni^	O	Om	32	40.4	209.2	11.8	27
Pipistrellus hesperidus^	CE	Ph	7.3	21.4	84.4	59.1	2.5
Pipistrellus rueppelli^	CE	Pr	6.8	25.2	100.9	37.3	7.1
Pipistrellus rusticus	CE	Pru	6.3	20.1	74.2	55.7	4.5
Rhinolophus blasii^	C	Rb	10	27	130.3	86.5	27.7
Rhinolophus clivosus^	C	Rc	19	33.6	204.3	91.7	37.4
Rhinolophus darlingi^	C	Rd	8.8	28.5	146.1	87.1	39.5
Rhinolophus fumigatus^	C	Rf	12.3	30.8	169.6	53.7	40.3
Rhinolophus hildebrandti^	C	Rh	28.9	40.1	287.8	33.2	44.8
Rhinolophus landeri^	C	Rl	7.5	27	140.1	107.3	40
Rhinolophus simulator^	C	Rs	8.2	26.9	132.1	80.1	31.3
Rhinolophus swinnyi^	C	Rsw	7.7	27.9	135.3	106.6	22.3
Sauromys petrophilus^	O	Sap	9.8	26	88.9	29.3	4.7
Scotoecus albofuscus^	CE	Sca	10.5	21.1	84	39.3	3.3
Scotophilus dinganii	CE	Sd	28.7	34.3	200.4	33.6	4.9
Scotophilus viridis^	CE	Sv	27.8	30.8	172.1	45.3	3.9
Tadarida aegyptiaca	O	Ta	16	30.7	116	22.7	9.6
Taphozous mauritianus^	O	Tam	34	39.5	216.2	25.9	7.4

Table 3.3. Comparison of observed (Obs) and expected (Exp) minimum segment length (MSL) and variance in segment length (VSL) ratios of mass, wing span (WS), wing area (WA), peak frequency (PF), duration (Dur) and principal component (PC1 & PC2) parameters of the KGR insectivorous bat ensemble. Expected values were calculated from two source pools – biological and log-uniform. Boldface values represent indices that are statistically significantly different at the 95% level.

Parameter	Source Pool	Index	Obs	Exp
Mass (g)	Biological	MSL	0.00349	0.01792
WS (cm)	Biological	MSL	**0.02027****	0.00764
WA (cm^2)	Biological	MSL	0.03888*	0.01371
PF (kHz)	Biological	MSL	0.01366	0.024
Dur (ms)	Biological	MSL	0.04885	0.01811
PC1	Biological	MSL	0.03511	0.01525
PC2	Biological	MSL	0.01078	0.01075
Mass (g)	Biological	VSL	0.00919	0.01863
WS (cm)	Biological	VSL	0.00129	0.00192
WA (cm^2)	Biological	VSL	0.00177	0.0075
PF (kHz)	Biological	VSL	0.00618	0.0109
Dur (ms)	Biological	VSL	0.01943	0.04299
PC1	Biological	VSL	0.00847	0.01257
PC2	Biological	VSL	**0.02455˜**	0.00404
Mass (g)	Log-uniform	MSL	0.00349	0.0271
WS (cm)	Log-uniform	MSL	0.02027*	0.0082
WA (cm^2)	Log-uniform	MSL	0.03888	0.01807
PF (kHz)	Log-uniform	MSL	0.01366	0.02542
Dur (ms)	Log-uniform	MSL	0.04885	0.03173
PC1	Log-uniform	MSL	**0.03511****	0.00008
PC2	Log-uniform	MSL	0.01078	0.00875
Mass (g)	Log-uniform	VSL	0.00919	0.02323
WS (cm)	Log-uniform	VSL	0.00129	0.00232
WA (cm^2)	Log-uniform	VSL	**0.00177****	0.01052
PF (kHz)	Log-uniform	VSL	0.00618	0.02215
Dur (ms)	Log-uniform	VSL	0.01943	0.03351
PC1	Log-uniform	VSL	0.00847	0.0329
PC2	Log-uniform	VSL	**0.02455˜**	0.00244

**Obs MSL ratios larger than, or Obs VSL ratios smaller than 95% of expected values.

˜Obs VSL ratios larger than 95% of expected values.

*Obs MSL ratios larger than 90% of expected values.

DISCUSSION

Through null model analysis only our predictions based on the hypotheses of competition theory (Hutchinson 1959; Case & Sidell 1983) were confirmed. Non-random phenotypic patterns of size and wing morphology were evident within the insectivorous bat ensemble of KGR. However, we found no evidence to support any effect of prey defences on the phenotypic patterns of echolocation parameters. Variances of PC2, a measure of echolocation call structure, were significantly larger than those expected from chance (Table 3.3). Non-random patterns would be evident from significantly small MSLs of echolocation parameters. Instead, our data suggest a clumped distribution of phenotypic patterns in echolocation call structure.

It is interesting that our analyses yielded evidence for the competition hypothesis even though it was conducted at the ensemble level rather than at a finer scale of organisation such as functional group. The ensemble level of organisation only includes insectivorous bat species within the local habitat of KGR (i.e. phylogenetically related species that use a similar set of resources *sensu* Fauth *et al.* 1996). Although they may utilize similar food resources, species from the O (open-air) and C (clutter) functional groups (Schnitzler & Kalko 2001) within the ensemble, are morphologically restricted to hunt separately and are thus unlikely to interact competitively with regards to their trophic niches (Kingston *et al.* 2000; Schoeman & Jacobs 2011). However, at least three species within each functional group are required to calculate the MSL and VSL indices we used (Gotelli & Entsminger, 2011), thus the relatively species-poor insectivorous bat ensemble of KGR precluded the possibility to conduct our analyses at a finer scale of organisation. Yet of the seven species in the ensemble, four are in the CE (clutter-edge; Schnitzler & Kalko 2001) functional group and two in the O functional group. Considering that CE

and O species can forage away from clutter, overlap in resource-utilization is more likely between, and certainly within, these two functional groups. Competitive interactions are thus still probable amongst the majority of KGR species. Indeed our results suggest that interactions to minimise similarity in body size (Hutchinson 1959) are present.

Conversely, because of the species-poor nature of the KGR ensemble, our evidence for the influence of competition on the phenotypic patterns of size and wing morphology seems surprising. Competitive interactions are thought to be more likely in species-rich environments as niche availability is more restricted (Schoener 1974; Schall & Pianka 1978). Our results may instead be an artefact of differential use of micro-habitats by morphologically and ecologically similar species (e.g. *N. capensis* and *P. rusticus*, or *T. aegyptiaca* and *M. midas*). Indeed, there is even evidence that the co-occurrence of con-generic, morphologically similar *Myotis* species is predominantly a result of spatial partitioning of foraging habitat (Saunders & Barclay 1992; Arlettaz *et al.*, 1997). Furthermore, temporal differences in the times of peak activity of favoured prey items allow temporal variation in emergence and foraging time of sympatric bat species (Rydell *et al.* 1996) thus impeding competitive interactions. Alternatively, small differences in echolocation characteristics of potentially competitive species can facilitate niche-separation through sensory bias (Siemers & Schnitzler 2004). Also, the availability of a range of prey sizes may facilitate niche partitioning (Schoener 1974; Schoeman & Jacobs 2011). Thus the minimum size differences between the apparently competitive species mentioned above could be maintained through differential prey-accessibility or access to equally abundant but differently sized prey. However, we did not assess prey availability in this study and so its influence on the KGR ensemble structure remains unclear.

It could also be argued that our evidence for a minimum size difference between the insectivorous bat species of KGR could merely be illustrating the “ghost of competition past” (Connell, 1980). However, instead of being used as an explanation for the evidence of competitive interactions that have already come to a conclusion, the “ghost of competition past” is more often cited as a reason for a lack of evidence of competition (Connell, 1980). Moreover, most of the species in the KGR ensemble are generalists thus there is a greater likelihood of resource overlap resulting in competitive exchanges.

The lack of evidence for the influence of prey defences on ensemble structure in our study is particularly surprising. A number of recent studies of insectivorous bat assemblages in southern Africa have suggested that prey defences have a significant effect on phenotypic patterns of echolocation parameters in bat ensembles and functional groups (Jacobs *et al.* 2008; Schoeman & Jacobs 2003, 2008, 2011). Furthermore, Lepidoptera appear the most abundant order of nocturnal insects and the most abundant Lepidopteran family at KGR is the Geometridae (Moreton, unpublished data) of which numerous species are tympanate. Thus it is likely that tympanate prey species are present at KGR. However, from the current study, the KGR ensemble appears entirely devoid of rhinolophids and hipposiderids. The presence of these clutter foragers is usually a major factor contributing to evidence of the prey defence hypothesis. Their use of high duty-cycle constant frequency (HDC-CF) calls with high PFs that are generally allotonic to the range of frequencies maximally detectable by tympanate insects (Fullard 1988), is thought to be a coevolutionary response to insect auditory systems (Waters 2003; Jacobs *et al.* 2008). Indeed, there is substantial evidence showing a high proportion of tympanate

moths in the diet of bats using high PFs (Jones 1992; Bogdanowicz *et al.* 1999; Schoeman & Jacobs 2003).

The predominance of syntonic (*sensu* Fullard 1988) species in the KGR ensemble could thus be an explanation for our lack of support for the prey defence hypothesis. Only two of the seven species in the KGR ensemble (*N. thebaica* ~ 70 kHz and *M. midas* ~ 15 kHz) use PFs outside the optimum hearing range of most tympanate insects (20-60 kHz; Fullard 1988; Jones 1992; Schoeman & Jacobs 2003). The remaining five species use PFs within this range and are likely unable to forage on tympanate prey. However, Schoeman & Waddington (2011) suggested that their evidence for the effects of prey defences may have been a reflection of "the narrow but optimal range of echolocation frequencies that can be used by sympatric insectivorous bats to exploit an abundant but widely distributed resource". An expectation for our null model analyses to yield similar results (i.e. non-random patterns of echolocation parameters) could thus be justified, but the KGR ensemble may be too species-poor to render the morphological distances between species' echolocation parameters statistically significant.

The effects of ecological light pollution (*sensu* Longcore & Rich 2004), however, could be the most appropriate explanation for the structure of the insectivorous bat ensemble of KGR. The presence of high-intensity spotlights at the gates of KGR lodges and KGR's proximity (~2 km) to the intensely lit peri-urban settlements of Mandela Village, Marokolong and Hammanskraal, may have contributed to the lack of rhinolophid and hipposiderid bats. Bat species from the CE and O functional groups have been shown to take advantage of the abundance of insects that congregate around light sources (Rydell 1992). Moreover, the defensive mechanisms of tympanate moths are rendered ineffective when flying around street

lights (Svensson & Rydell 1998). Thus HDC bat species, such as rhinolophids and hipposiderids, may become competitively excluded from areas affected by lights because low duty-cycle (LDC) bats are able to exploit the prey items usually unavailable to them (Arlettaz *et al.* 2000). Rhinolophids have also been shown to avoid regular commuting routes if they are altered by artificial lighting (Stone *et al.* 2009). Additionally, the low mobility of HDC bats may negatively affect their ability to persist in areas affected by urbanization in a two-fold manner. Food resources and roost sites may become too scattered for them to exploit (Jung & Kalko 2011) and, coupled with the effects of ecological light pollution such as “sky glow” (Longcore & Rich 2004), their slow flight may render them vulnerable to predation by visual hunting predators such as owls (Jung & Kalko 2010).

The proportion of species from the three functional groups of insectivorous bats (i.e. O, CE & C; Schnitzler & Kalko 2001) within an ensemble has been proposed as a good indication of the level of habitat disturbance and useful for exposing areas that are in need of management (Jung & Kalko 2011). Considering then that the insectivorous bat ensemble of KGR is species-poor and dominated by CE bats, it seems clear that this area is in dire need of management plans and efforts tasked with mitigating the effects of ecological light pollution. While lack of appropriate roost sites may be severely limiting the presence of C bat species it is unlikely to be the sole reason for their absence. A major highway, with numerous bridges that could accommodate small colonies of C bats, lies only ~2km west of KGR. However, the effects of ecological light pollution have also been shown to significantly alter invertebrate community composition (Davies *et al.*, 2012). Thus clutter-specialist bats may also be indirectly excluded from KGR as a result of shifts in composition of prey populations. Future research should incorporate measures of trophic

interactions and insect diversity so that a clearer picture of the processes structuring the KGR ensemble can be realised and the goals of the above-mentioned management plans refined.

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Chapter 4: Conclusion

My study focused on assessing the diversity of bats (Chiroptera) in an area where anthropogenic pressures may be mediating the ecological processes involved in assemblage structure. Specifically, I investigated how habitat heterogeneity may have influenced the structure of the local bat assemblage of Kwalata Game Ranch (KGR) in northern Gauteng, South Africa. Furthermore, I examined the extent to which interspecific competition and the effects of prey defences may have influenced the structure of the local ensemble of insectivorous bats.

The 1800ha extent of KGR falls within the subtropical savanna biome (Mucina & Rutherford, 2006). Considering the high species richness of insectivorous bats within the savanna biome recorded by Schoeman & Jacobs (2008), an appropriate estimate of 15–20 insectivorous species could be considered for KGR. Moreover, species distribution records for southern African pteropodids (Monadjem *et al.*, 2010) suggest at least two species of frugivorous bats should also be present. However, my results suggest that KGR is relatively species-poor with only eight insectivorous species being recorded and a complete absence of frugivores (Chapter 2). Species richness estimators confirmed that sampling was exhaustive suggesting my results are an accurate representation of the bat assemblage at KGR. Moreover, sample-based rarefaction analyses suggested that the species richness is mostly homogenous across KGR with no apparent species-associations to particular vegetation types.

I proposed a number of factors that could potentially limit the bat species richness of KGR. A lack of appropriate forage is the most likely reason for the apparent dearth of frugivorous species. Several species adapted to forage in highly cluttered environments (Schnitzler & Kalko, 2001), from the bat families

Rhinolophidae and Hipposideridae, have been recorded in the savannas of southern Africa (Simmons, 2005; Monadjem & Reside, 2008; Schoeman & Jacobs, 2008; Monadjem *et al.*, 2010). However, during nine months of active and passive sampling, no evidence of the presence of these families at KGR was recorded. Many rhinolophids and hipposiderids are dependent on roost sites, such as caves, wherein they can congregate in large numbers (Monadjem *et al.*, 2010). The lack of such roost sites is thus a probable limiting factor in the area of my study.

Furthermore, the low levels of habitat heterogeneity recorded at KGR may account for the low bat species richness. Mammal species richness has been shown to be strongly related to structural heterogeneity of vegetation (August, 1983; Monadjem, 1997; Andrews & O'Brien, 2000; Qian *et al.*, 2009). However, historical grazing pressure and land use by humans (e.g. fire suppression or tree clearing) has resulted in bush encroachment which increases woody plant density and shifts savanna to the forest end of the environmental spectrum (Scholes & Archer, 1997; Ward, 2005). Thus, coupled with the low topographic relief of the area, anthropogenic effects may have decreased the habitat heterogeneity of KGR by creating a relatively homogenous savanna-woodland matrix.

It is important to note, however, the extent of vegetation measurements in my study was limited to sites where I sampled for bats. Thus, considering sample sites were areas with potential fly-ways where mistnet trapping was more likely to be effective, more open areas of the savanna-woodland were probably under-represented. As a result, the structural complexity of the savanna-woodland is possibly an overestimation. Moreover, since no direct measures of land use were taken, the effects thereof were mostly inferred.

In contrast to my study, Medellin *et al* (2000) found a significant relationship between bat species richness and structure and diversity of vegetation along a disturbance gradient. Importantly, theses authors quantified species diversity as well as vegetation structure at sample sites and created fuzzy-set descriptions of vegetation. Such an approach afforded a greater level of detail of habitat complexity parameters at a scale that appears appropriate for bats. However, there were upwards of forty species recorded in the Selva Lacandona, Mexico (Medellin *et al.*, 2000). The high species richness of the area when compared with KGR may result in stronger bat-habitat associations and the presence of higher numbers of specialist species. The presence of numerous specialist species may in turn yield more robust results on the effects of disturbance as such species are unlikely to persist.

Nevertheless, if management plans are to be successful it is critical to elucidate specific bat-habitat associations and at what scale different species partition the environment. Importantly, although Monadjem & Reside (2008) found differences in the bat community structure between riparian and adjacent savannah-woodlands, their results showed that bats readily discriminate between microhabitats but do not illustrate strong responses to large-scale habitat characteristics. Thus in order to understand more accurately the state of environmental flux which KGR is in and its effects on local bat community structure, future studies would be advised to conduct comparative surveys in the savanna southeast of KGR, such as the Wallmanntsthal SANDF base, which is mostly undisturbed. Extensive vegetation transects (e.g. Druce *et al.*, 2008) could also be conducted and coupled with densiometer measurements within each vegetation type to provide a more in-depth representation of habitat complexity across the landscape. Additionally, if landscape-scale surveys are initiated in the future, climatic and productivity-related variables of the habitat

heterogeneity hypothesis (e.g. potential/actual evapotranspiration) should be incorporated (Field *et al.*, 2008).

In chapter 3 I found evidence that competition has been influential in structuring the insectivorous bat ensemble of KGR. Species within an ensemble (*sensu* Fauth *et al.*, 1996) are considered to be more likely to interact resulting in a greater likelihood for ecological processes to influence ensemble structure (Moreno *et al.*, 2006). My results seem to corroborate this. However, testing for phenotypic patterns at a finer level of organisation (e.g. functional group) may have yielded even stronger evidence for competition. Schnitzler & Kalko (2001) proposed dividing insectivorous bat species into functional groups based on their flight and echolocation capabilities, which influence the habitat strata wherein they can hunt (e.g. open-air, clutter-edge or within highly cluttered space). Testing for influences on phenotypic patterns at the functional group level is thus favourable as competitive interactions between species that hunt within the same stratum (e.g. in amongst the clutter of sub-canopy vegetation) are highly probable. However, since at least three species within each functional group are required to calculate the MSL and VSL indices I used (Gotelli & Entsminger, 2011), the species-poor ensemble of KGR precluded such an in-depth analysis. Significantly large MSL values of size and wing morphology, suggesting competition in the species-poor KGR ensemble, is surprising as competitive interactions are considered more probable in species-rich environments where niche availability is likely to be more restricted (Schoener, 1974; Schall & Pianka, 1978). Conversely though, the assortment of size and wing morphology parameters of insectivorous bats at KGR (Chapter 3) might in fact be a result of the low species richness which may allow differential access to micro-habitats and prey size-classes for each species. Future assessment of the diet of the

insectivorous bats at KGR would illuminate whether trophic niche partitioning is more influential in structuring the ensemble.

Regardless of the likely presence of tympanate prey (Moreton, unpublished data), I did not find any evidence for the effects of prey defences on ensemble structure. Evidence for the influence of prey defences on ensemble structure is usually a result of the presence of rhinolophids and hipposiderids, as these bats generally use echolocation signals that allow them to capture tympanate insects (Schoeman & Jacobs, 2003; Jacobs *et al.*, 2008). However, there is much evidence suggesting that high duty-cycle echolocating bats such as rhinolophids and hipposiderids, may be absent from areas affected by artificial light (e.g. Arlettaz *et al.*, 2000; Jung & Kalko, 2010; 2011). Thus, though not directly measured in my study, the effects of ecological light pollution (*sensu* Longcore & Rich, 2004) have possibly contributed to the competitive exclusion of rhinolophid and hipposiderid species from KGR. In addition to trophic niche patterns, incorporating dietary analysis in future studies should also clarify whether rhinolophids and hipposiderids are being competitively excluded from KGR by revealing whether a large proportion of tympanate insects are present in the diet of the clutter-edge bat species.

My study describes a bat assemblage potentially impacted by anthropogenic pressures. It also provides evidence that competition has been influential in structuring an insectivorous ensemble and that the influence of prey defences may be absent as a result of the adverse impacts of ecological light pollution. However, the exact extent of the anthropogenic impacts proposed in my study still needs to be quantified. As does the extent to which anthropogenic impacts may be compounding other limiting factors, such as lack of appropriate roost sites. The efficacy of management plans depends on proper understanding of population- and community-

level responses to disturbances (Gorresen & Willig, 2004). My study presents a platform from which comparative studies can be conducted to elucidate what rehabilitation processes may be effective in encouraging increased bat diversity at KGR. For example, focusing future investigations on specific bat-habitat associations could help shed light on whether management processes, such as controlled burning to counteract the effects of bush encroachment, will be effective in encouraging greater bat species richness in a less patchy savannah-woodland. Such studies should include sampling bats from nearby but less impacted savannas. Moreover, incorporating environmental variables should illuminate the abiotic factors governing assemblage structure and to what extent anthropogenic pressures may be having a mediating effect.

Furthermore, future research should consider the types of lighting used at lodges and in the peri-urban settlements neighbouring KGR and how it affects the invertebrate community. Certain artificial light can significantly alter the surrounding invertebrate community composition (Davies *et al.*, 2012) which may have important implications for local bat communities. Hence further knowledge of the potential impacts of the artificial lighting around KGR will help guide decisions to help control the extent of ecological light pollution. I am hopeful that I have provided insight that may guide conservation efforts tasked with promoting and maintaining healthy bat populations in the densely human-populated province of Gauteng.

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Appendices

The first appendix, Pierce & Keith (2011), is a short communication on the healing rates of the wing membranes of two Vespertilionid species (*Neoromicia capensis* and *Vesper sp.*). Since publication, the voucher specimens mentioned therein have been identified and the individuals identified as *Vesper sp.* have been confirmed to be *Pipistrellus rusticus* (T. Kearney, pers. comm.). The purpose of that short study was to determine any negative effects of small (3 mm) tissue biopsies from the wing membranes of small insectivorous bats. Such tissue samples are of great value when developing reference call libraries as they can ensure accurate species identification, of released bats, based on genetic analysis. However, the intention to biopsy wing membranes can delay studies unnecessarily, due to contentions with ethics committees. We thus set out to elucidate whether there are in fact any negative ramifications of the procedure. The paper is presented here in its published format from *African Bat Conservation News*.

The second, Pierce *et al.* (in press), is the result of a new species record for the province of Gauteng being captured during this Masters research. *Laephotis botswanae* (Vespertilionidae) is near-endemic to southern Africa and has a wide but sparse distribution in the region (Monadjem *et al.*, 2010). Since the closest locality record to the capture associated with the present research is ~140 km north, this new locality warranted publication. Furthermore, it represents the first record of *L. botswanae* in Gauteng province. However, only a single individual was captured. We therefore deemed it appropriate to combine this new locality record with others across southern Africa so that the updated distribution records could be captured in a single publication. As this paper is still currently in press it is presented here in the format in which it was submitted to *Durban Museum Novitates*.

Scientific contributions

Healing rates of wing membranes in two species of Vespertilionid bats



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Researchers often take tissue samples from bats in the form of biopsies from the wing membrane (patagia). Genetic studies based on DNA analysis of tissue samples are extremely valuable (WIMSATT *et al*., 2005 and references therein). For example relatedness and reproductive strategies can be ascertained (KARUPPUDURAI and SRIPATHI, 2010). Molecular analyses on tissue samples can also greatly augment discoveries of range expansions (MORRIS *et al*., 2009) and cryptic species (JACOBS *et al*., 2006). Furthermore, isotope analysis of tissue samples can be used to assess critical ecological traits such as feeding patterns and habitat use (SULLIVAN *et al*., 2006). Apart from molecular data from wing biopsies, the punch marks are an effective alternative to banding for mark-recapture studies (BONACCORSO and SMYTHE, 1972). The scars and unpigmented tissue that result from punches persist for an extended period and can be used to identify previously captured individuals (FAURE *et al*., 2009; WEAVER *et al*., 2009). Biopsy scars remain fully unpigmented for at least 6 months (Pierce, pers. obs.). However, it is unclear exactly how long it takes for scars to fully regain pigment and so for how long such marks are useful to identify recaptures.

A number of researchers have reported healing rates of wing membranes (FAURE *et al*., 2009: 1151; WEAVER *et al*., 2009: 221; KARUPPUDURAI and SRIPATHI, 2010: 044). However, there is still very little known about the effects wing punches may have on survivability in African Vespers. Here we report on the healing rates of wing punches of 13 free-ranging individuals of two Vesper species. We predicted that biopsies taken from the wing membranes would fully heal within 3 – 4 weeks and show no effect on the survivability of biopsied bats. Individuals of one of the species are still awaiting species confirmation based on molecular data, as external characters can only narrow identification down to three potential species. For the purposes of this paper these bats have been categorized into a species complex, "Vesper sp.", comprised of *Hypsugo anchietae* (Seabra, 1900); *Neoromicia zuluensis* (Roberts, 1924) and *Pipistrellus rusticus* (Tomes, 1861). *Neoromicia capensis* (Smith, 1829) individuals were distinguished from the species complex based on their larger size, specifically head lengths more than 16 mm (Seamark, *pers. comm*.). Voucher specimens of both species were taken and deposited at the Ditsong National Museum of Natural History in Pretoria, South Africa.

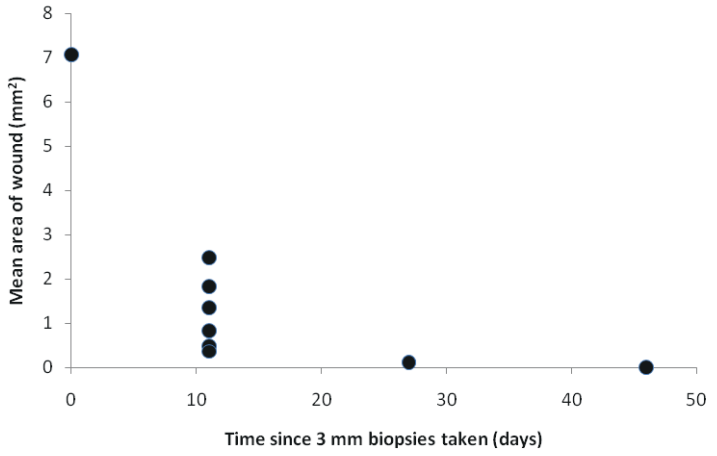

Figure 1: Averaged wound areas of each bat at each recapture indicating distinct variation in healing rates in the first 2 weeks. The point illustrating healed wounds (area=0 mm²) is represented by 5 individuals (Table 1). The point on the y-axis indicates the size of initial wounds for all bats (7.07 mm²).

Using a harp trap, we trapped bats exiting from two known roost-sites in the buildings of lodges at Kwalata Game Ranch, Gauteng, South Africa (25° 23.421' S 28° 19.309' E). The morning after capture a 3 mm circular biopsy was taken from both wing membranes of each bat. Three millimetre biopsy punches are generally accepted for use on smaller bat species (WEAVER *et al*., 2009: 220). Bats were then released the evening after capture. The roost sites were re-trapped 11, 27 and 46 days later. The head and forearm lengths of recaptured bats were used to identify individuals and compare healing rates to previous observations. However, mass was not used to identify individual bats as it is naturally variable (Seamark, *pers. comm*.) thus bats were not re-weighed. Recaptured bats were photographed with an Olympus µ850 SW digital camera set to macro (8 MPixel resolution, Olympus, Tokyo, Japan). Bats were positioned at a standard distance from the lens and their wings spread to the same degree to ensure minimal contortion of the wounds. A standard ruler was then positioned at the same distance from the lens and photographed with the same settings. This image was used to calibrate a global scale in ImageJ (Broken Symmetry Software) which was used to gauge healing rates from the images. In ImageJ the area of each wound was measured in mm² (WEAVER

Table 1: Time in days between recaptures and average percentage of biopsy wound still unhealed in 13 free-ranging individuals of two Vesper species. The unhealed surface area was measured in ImageJ. Values were divided by 7.07 mm² (original wound surface area) and multiplied by 100 giving a percentage of each wound remaining unhealed. Percentages were then averaged for each bat.

Field #	Sex	Species	Days between captures	% of wound unhealed
MWP290111_B3	♂	Vesper sp.	11	12
MWP290111_B4	♂	Vesper sp.	11	26
MWP290111_B8	♂	Vesper sp.	11	19
MWP290111_B9	♂	Vesper sp.	11	7
MWP290111_B10	♂	Vesper sp.	11	35
MWP290111_B12	♀	Vesper sp.	11	5
MWP270211_B1	♂	*N. capensis*	27	2
MWP270211_B3	♂	*N. capensis*	27	2
MWP180111_B3	♂	Vesper sp.	46	0
MWP180111_B4	♀	Vesper sp.	46	0
MWP180111_B6	♀	Vesper sp.	46	0
MWP180111_B9	♀	Vesper sp.	46	0
MWP180111_B12	♀	Vesper sp.	46	0

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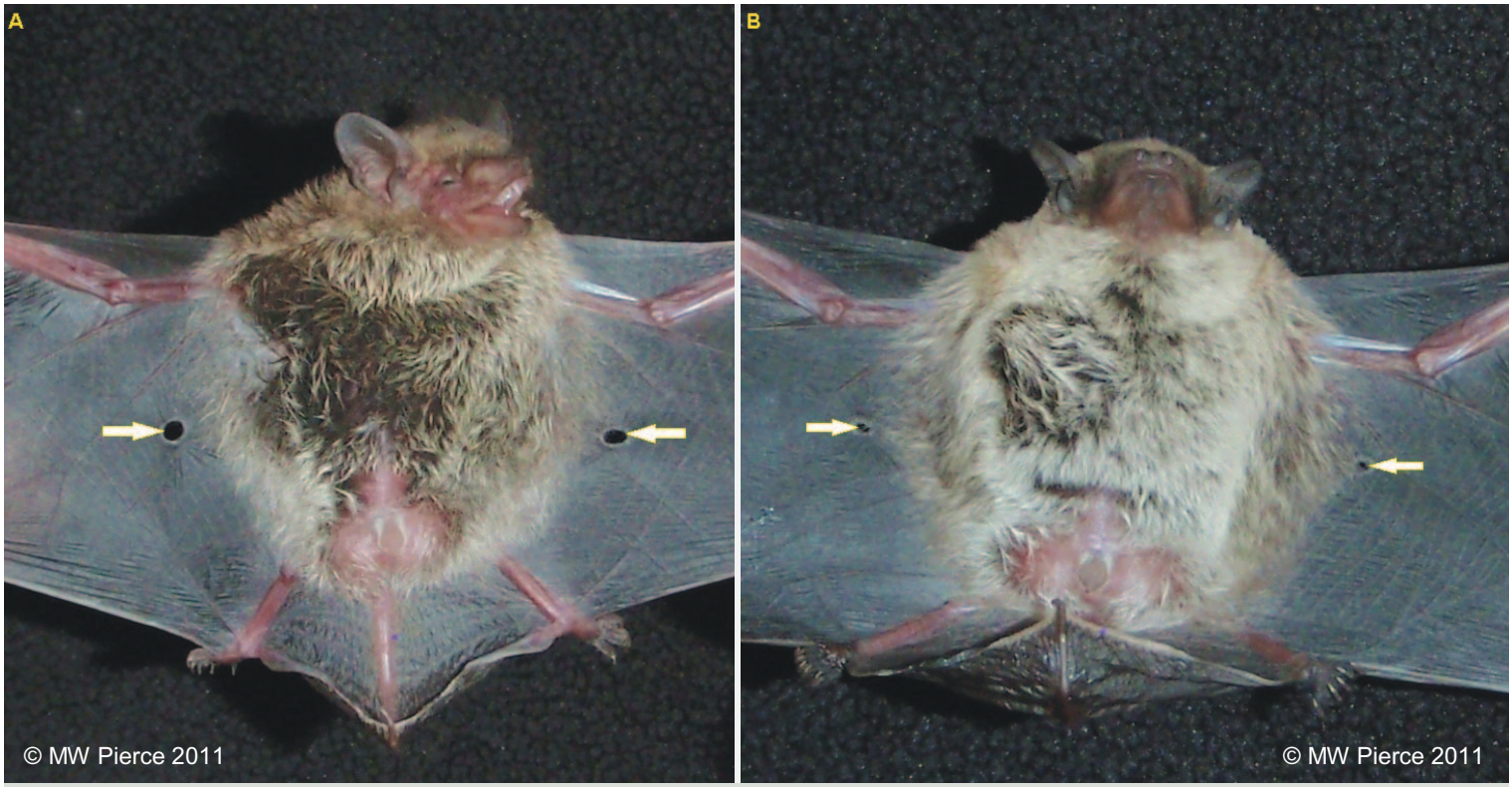

Figure 2: Variation in healing rates of wing punches. Both Vesper sp. individuals show 11 days worth of wound healing: A) MWP290111_B10, average hole size = 2.48 mm^2; B) MWP290111_B9, average hole size = 0.49 mm^2 (Table 1). Initial wound size = 7.07 mm^2. Arrows indicate unhealed biopsy wounds.



Figure 3: A) An individual of *N. capensis* showing 27 days worth of wound healing, MWP270211_B1 (Table 1). Arrows indicate small patches of unhealed skin at the centre of closed wounds. B) Completely healed wounds of a Vesper sp. individual; MWP180111_B3 (Table 1). Arrows indicate unpigmented tissue of biopsy scar that can be used to identify recaptured individuals.

et al., 2009: 221). In cases where the wound was completely closed but a small patch of unhealed skin remained, the area of such patches was measured. Each measurement was divided by 7.07 mm^2 (the surface area of a single wound) and multiplied by 100 to give a percentage of each wound that remained unhealed. These percentages were then averaged for each bat.

Although the relative healing rates of wing membranes varied among individuals, all recaptured bats showed more than 50% wound healing in the first 11 days (Figure 1, 2). This result is somewhat contrary to the results of WEAVER *et al*. (2009: 221) who found that *Myotis lucifugus* (LeConte, in McMurtrie, 1831) showed very little healing during the first week after biopsies were taken. Furthermore, the wounds of *M. lucifugus* had all closed after only 16 days (WEAVER *et al*., 2009: 221) whereas in our study slight wounds were still present on bats captured 27 days after being biopsied (Figure 3A). These differences may, however, be a result of different parts of the wing membrane being biopsied in the two studies. FAURE *et al.* (2009) observed that biopsies from the tail membrane (uropatagium) of *Eptesicus fuscus* (Beauvois, 1796) healed faster than those on the wing membrane. It is thus possible that more distal regions of the wing membrane may show higher healing rates than those nearer the body, or vice versa.

In terms of time taken for wounds to heal completely our results are more similar to those for *Cynopterus sphinx* (Vahl, 1797) of 3-4 weeks (KARUPPUDURAI and SRIPATHI, 2010: 044). It is important to note that only *N. capensis* individuals were recaptured at 27 days (Table 1). The wounds of Vesper sp. individuals may thus have followed a pattern closer to that reported by WEAVER *et al*. (2009).

Furthermore, few females were recaptured after 27 days (Table 1). Further study is thus needed to establish any differences in healing rates between sexes. Additionally, since none of the females were either pregnant or lactating during this study, further information is required to discern whether the energy demands of different reproductive states may affect wound healing rates.

Although our results are in parallel with the current literature on the ability of wing membranes to recover from damage rapidly, we cannot be 100% certain that no bats were adversely affected as not all marked bats were recaptured during the study. This is not unusual though (WEAVER *et al*., 2009) and the regular fission and fusion of subgroups within a bat colony between numerous roosts in the same general area (KERTH and KÖNIG, 1999) could account for not all bats being recaptured. Moreover since body mass was deemed too variable a measure for identifying recaptured individuals, body mass index (BMI) was not calculated for recaptured bats. Since healing rates are directly linked with BMI (D, Reeder, *pers. comm*.) it will be an important measure to include in future studies.

Since stable isotope analyses have shown relationships between diet and tissue turnover (FLEMING *et al*., 1993; VOIGT *et al*., 2003; VOIGT and KELM, 2006), it is likely that seasonal variations in diet may compromise energy availability for wound healing. In the present study, however, all bats were captured in late summer (January-February) thus it is improbable that food availability constrained healing rates. Interestingly, healing rates of wing membranes may not be representative of the turnover rate of the entire wing tissue, in that biopsies heal at a much higher rate than stable isotope turnover (VOIGT *et al*., 2003). The low rate of isotope turnover in bat wing membranes has been attributed to high concentrations of slowly regenerating collagen and elastin fibres (VOIGT *et al*., 2003) which are predominantly in the middle dermal layer (HOLBROOK and ODLAND, 1978). This suggests that rapid regeneration of the two outer epidermal layers becomes a physiological priority for bats when a wing membrane is wounded.

REICHARD and KUNZ (2009: 458) suggest that wounds on the wing membranes of bats have the potential to negatively affect foraging success. However, given the context of their study, they likely refer to relatively extensive wounding such as that caused by White-nose syndrome (WNS) (REICHARD and KUNZ, 2009). Our results suggest that wing biopsies show little impact on survivability. This is supported in the literature and further corroborated by evidence for bats sustaining similar wounds from natural obstacles (DAVIS, 1968). Indeed REICHARD and KUNZ (2009: 462) state that foraging success of bats showing light wing damage resulting from WNS is unlikely to be affected and elaborate that light damage "may reflect 'normal' wing conditions".

Considering the value of wing biopsies for rapidly obtaining field-samples for important molecular analyses as well as an effective method of marking individuals it should thus be encouraged as part of bat surveys. Furthermore, as long as it is to be carried out by a person trained in the procedure, wing biopsies should no longer be a point of contention for ethics committees.

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Above: Michael Pierce (right) showing an Egyptian Free-tailed bat (*Tadarida aegyptiaca*) to Kwalata director, Charl Pretorious (left), Charl's children and Wits Masters student, Marco Vierra (centre). Michael is currently finishing his Masters field-work, assessing bat diversity and community composition on Kwalata Game Ranch, north of Pretoria.

New records and echolocation information of *Laephotis botswanae* (Chiroptera, Vespertilionidae) from Southern Africa

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Summary

The Botswana Long-eared Bat (*Laephotis botswanae*) has been recorded from localities across central and southern Africa; in the Democratic Republic of Congo, Tanzania, Angola, Zambia, Malawi, Zimbabwe, Namibia, Botswana, and South Africa. *Laephotis botswanae* is currently listed as Least Concern, in view of its wide distribution, presumed large population, and because it is unlikely to be declining fast enough to qualify for a listing in a more threatened category. Records of new localities, information about additional specimens from previously published localities, updated distribution maps, and new echolocation call information are important contributions to our understanding of this rarely caught species. This contribution reports twelve new locality records for *L. botswanae* in southern Africa, three in Botswana, two in Namibia, five in Malawi and one in South Africa. Additionally, ten previously unpublished specimens, one from Malawi, one from Mozambique, three from

Namibia and five from Zimbabwe are reported. Finally, we also report echolocation call parameters for the individual caught in South Africa and for seven of the individuals caught in Malawi.

Key words: Laephotis botswanae, new locality records, echolocation parameters

New records

The new locality and specimen records for Botswana and South Africa were from captures in mist nets during fieldwork by the authors (ES and TK for Botswana, and MP, ES and TK for South Africa), while those for Namibia were based on specimens in the collection of the State Museum of Namibia in Windhoek examined by one of the authors (TK). The new locality and specimen records for Malawi and the unpublished specimen record for Mozambique were also from captures in mist nets by the authors MC and M. Kopp (hereafter MK). Identifications were made based on external and cranial measurements as indicated in Kearney and Seamark (2005). Based on the degree of ossification of the epiphyses (Anthony 1988) all vouchers, except one (TMSA 48307), were adults.

The three new specimens representing two new locality records for *L. botswanae* in Botswana, from the Chitabe area next to the Gomoti River and Kwetsani Island (see Appendix I for locality and specimen details), are the fourth and fifth recorded localities of this species in the country (see Fig. 1). The specimens from the Chitabe area were mist netted in riverine woodland vegetation next to the Gomoti River, while that from Kwetsani Island was mist netted within woody vegetation on the 'island'. These new localities are east and west of the closest localities for previously known records of *L. botswanae*, at Kurunxaraga and Xugana. Kearney and Seamark (2005) incorrectly synonymized these distinct localities; Kurunxaraga (see Cotterill 1996) and Xugana (see Archer 1977), which was corrected in Monadjem *et al.* (2010b). The Gomoti River locality is 50 km SE of Xugana, while Kwetsani Island is 54 km NW Kurunxaraga.

The two previously unpublished localities for *L. botswanae* in Namibia, are based on three specimens: two collected by Sue Churchill and Rogan Draper at Lianshulu Lodge,

Mudumu National Park in the eastern Caprivi, and one collected by C.G. 'Neels' Coetzee, 85 km from Tsumkwe (see Appendix I for locality and specimen details). These represent the third and fourth recorded localities of the species in the country (see Fig. 1). Lianshulu Lodge is 29 km S of the closest previously known record of *L. botswanae* from San Michelle (Monadjem *et al.*, 2010b), while the locality 85 km from Tsumkwe is 191 km SW of the closest previously known record of the holotype of *L. botswanae* from 19 km. S of Shakawe in Botswana (Setzer 1971).

The five *L. botswanae* individuals (see Table 2 and Appendix I for measurements and locality details) captured in Malawi represent two new localities at Mt Mulanje that were referred to in Monadjem *et al*. (2010a). These localities at the Tea Research Foundation Forest in the foothills of Mt. Mulanje and the Hydroelectric dam at the opening of the Ruo Gorge are roughly 15 km SE of the closest previously known record of *L. botswanae* from Likabula Mission at Mt Mulanje in Malawi (see Fig. 1; Kearney and Seamark 2005).

The single, previously unpublished, specimen record from Mozambique, mentioned in Monadjem *et al.* (2010a), represents the first recorded locality of the species in the country (see Fig.1; African Chiroptera Report 2011; Monadjem *et al.* 2010b). The specimen was captured at Mt. Mabu, roughly 100 km ESE of the Mt. Mulanje localities in Malawi. This specimen was mist netted in remnant natural vegetation bordering an abandoned tea plantation at the base of the mountain at an altitude of 550 m. Forest type at this altitude represented a transition zone between lowland miombo woodland and montane evergreen forest. The biome class of Olson *et al.* (2001) is tropical/sub-tropical broadleaved forest.

The new locality record for *L. botswanae* in South Africa, from Kwalata Game Farm (see Appendix I for locality and specimen details), is the fifth recorded locality for the occurrence of this species in the country, and the first record of the species in Gauteng Province (see Fig. 1). Kwalata Game Farm is 138 km south of the closest previously known record of *L. botswanae* from Klipfontein 54 JS in the Waterberg in Limpopo Province (Herholdt 1989). This specimen was mist netted within riverine vegetation along the Pienaars River.

Information for nine new (previously unreported) specimens of *L. botswanae* from Malawi, Namibia and Zimbabwe are also included in Appendix I. However, these specimens were captured at previously reported localities.

Echolocation call parameters

Echolocation call data was obtained from eight individuals of *L. botswanae*. Seven animals captured in Malawi were hand-released and a 1.7 second call sequence recorded from each. Calls were recorded using a Petterson D240x ultrasound detector (Pettersson Electronik AB Uppsala Sweden), expanded 10 times, and transferred onto a Cowon-iAudio X5L mp3 player (Cowon Electronics http://www.cowon.ch/index.html). The iAudio had a sampling rate of 48 kHz and bit rate of 320 kbps ("lossless mp3"). Calls were analysed using the sound analysis software Raven Pro 1.3 (Cornell Lab of Ornithology, Bioacoustics Research Program, http://birds.cornell.edu/brp/raven). Audio data was transferred to Raven Pro with a sampling frequency of 44 kHz, which translated to an effective sampling rate of 440 kHz, and 8 bits per sample, when calculating frequency grid spacing or resolution (because Raven Pro accounts for the 10x time expansion factor embedded in the recording from the D240x). This corresponded to a frequency grid spacing (i.e. the frequency resolution of the spectrogram) of 431 Hz, or roughly half a kHz. Spectrograms were generated by a Fast Fourier Transformation (FFT) using 1024 samples, a 3 dB Filter Bandwidth of 1239 Hz and a Hanning-Window. Call parameters represent means from a call sequence from a single individual (up to 10 calls per sequence). To ensure that the identity of hand-released individuals could be traced back to a voucher specimen from the same location, biopsies of wing tissue were collected from all but one of the seven individuals hand-released in Malawi. While a full analysis has not yet been conducted, biopsies from three of the seven individuals were sequenced yielding two 16S RNA sequences and one cytB sequence (D. Pio, University of Lausanne; unpublished data). Sequence data (cytB) was additionally extracted from all collected specimens from Malawi and Mozambique (F. Mayer, Museum für Naturkunde; unpublished data), but these datasets have not yet been linked together.

Therefore the hand-release calls from Malawi are primarily derived from species identifications in the field. The remaining biopsies are preserved in 90% alcohol, under the custody of MC at the ETH Zurich. The material is thus available for future analysis.

The single animal captured at Kwalata Game Farm was recorded, using a Tranquility III (Courtpan Design Limited, UK) time expansion detector as it was flying in a room (± 5 x 6 x 2.5 m). The recordings were transferred from the detector to a Mecer Xpression Notebook and analysed in Batsound Pro (Pettersson Elektronik AB Sweden), at a sampling rate of 44.1 kHz, 16 bit stereo, time expansion set at 10, and a threshold of 15. Frequency information was ascertained using the Fast Fourier transformation (FFT) power spectrum (size 1024, using a Hanning Window). A minimum threshold of -55 dB was used in the assessment of frequencies to improve the signal-to-noise ratio. From a set of 20 calls, six calls were analysed.

Using the above data, five call parameters were measured 1) call duration (ms duration from the onset of the call to the end of the call), 2) peak frequency (the frequency that contains the maximum energy/intensity measured in kHz), 3) minimum frequency (of the call measured in kHz), and 4) maximum frequency (of the call measured in kHz) and 5) bandwidth (calculated in kHz by subtracting minimum frequency from maximum frequency for each call). Call duration was measured from the oscillogram, while the other measures were made from the power spectrum.

The echolocation calls of the room-flown *L. botswanae* were low duty-cycle, frequency modulated (FM), with a short mean duration of 2.68 ms (±1.09 ms), a mean peak frequency of 37.13 kHz (±2.37 kHz), and a mean bandwidth of 26.43 kHz (±11.49 kHz) (see Fig. 2). In Malawi, hand-released bats produced slightly longer FM-QCF (quasi-constant frequency) calls that had similar frequency parameters: mean duration = 4.73 ms (±1.10 ms), mean peak frequency = 32.34 kHz (±1.42 kHz) and mean bandwidth = 29.72 kHz (±9.62 kHz). The calls of six of the individuals recorded in Malawi were conducted in open space habitat, whereas one of the individuals was recorded in dense clutter in the forest understory. The call sequences from cluttered habitat exhibit a smaller bandwidth in comparison to open

space (20.60 kHz ± 4.18 kHz in clutter versus 31.8 kHz ± 9.3 kHz in open space) which can be explained by a lower maximum frequency (48.94 kHz ± 4.6 kHz in clutter versus 60.78 kHz ± 9.33 kHz in open space). However, the lack of comparative sample sizes restricts interpretation, therefore in Table 1 means are calculated from calls in both habitats.

Table 1 indicates how these parameters differ slightly to those previously reported by Fenton (1975), Fenton and Thomas (1980), Fenton and Bell (1981), Taylor (2000), and Monadjem *et al.* (2010b). The work by Fenton (1975), Fenton and Thomas (1980), and Fenton and Bell (1981), was conducted at the Hostes Nicolle Institute of Wildlife Research, in the Sengwa Wildlife Research Area, in Zimbabwe in January 1975 and June 1977, and they reported information for *Laephotis* that were identified as *L. angolensis*. Although the species relationship between *L. angolensis* and *L. botswanae* remains unresolved (Kearney and Seamark 2005), and should be tested by additional information, such as molecular sequences, specimens of *Laephotis* from Sengwa in the Bulawayo Museum collection that were included in the analyses in Kearney and Seamark (2005), plotted with other individuals in a group identified as *L. botswanae* and separate from two individuals identified as *L.* cf. *angolensis*. Subsequently, another five specimens from Sengwa, identified in the Royal Ontario Museum collection as *L. angolensis* have been measured by one of the authors (TK), following Kearney and Seamark (2005), and identified as *L. botswanae* (see Appendix I). Hence, it is now assumed, also by Monadjem *et al.* (2010b: 432), that the species reported by Fenton (1975), Fenton and Thomas (1980), and Fenton and Bell (1981), was *L. botswanae*.

Unfortunately, the number of individuals recorded, the context of the calls, and the geographic location were not stipulated for the call data of *L. botswanae* reported in Taylor (2000) and Monadjem *et al.* (2010b). Variation in the echolocation call of individuals within a species, as indicated in Table 1, may have one, or several explanations. Hayes (2000: 227) suggests differences in equipment may confound recordings, whereas Kalko and Schnitzler (1993), Obrist (1995), Guillen *et al.* (2000), and Sedlock (2001) indicate that call variation may also be associated with variation in the environmental context of the bat, variation in

environmental variables, and subtle morphological changes within a species, which may or may not be associated with variation across the geographic range of a species. Fenton and Thomas (1980: 83) indicated the calls of bats flown in a lit room, including those of *Laephotis*, produced double orientation pulses that were not seen in calls of bats flown under natural conditions. Fig. 2A and Fig. 2B illustrates the echolocation pulses of the individual caught in South Africa (TMSA 48323), showing the possible double orientation pulses also recorded in this study. More importantly, the slight variations between the call parameters of this study as well as previously published records (Table 1) corroborate the importance of the context in which a bat is flying. Accordingly, call recordings were taken a few seconds after bats were released by hand to allow the bat time to orientate, and initial calls were excluded from analysis as they are often shorter, steeper, orientation calls and not representative of search phase echolocation parameters. Fig. 2C represents a call from Malawi, recorded from a hand-released bat (collectors: MC & MK, field number: 2007-586).

Remarks

Prior to Monadjem *et al.* (2010b) reporting the first records of *L. botswanae* from two localities in northern Namibia and the third locality in Botswana, Kearney and Seamark (2005) had calculated, using Rutherford and Westfall (1994) and Olson *et al*. (2001), that 75 % of the *L. botswanae* records were associated with the savanna biome, while 25 % of the localities, which were most of the localities of *L. botswanae* in Malawi, fell within the grassland biome. According to the classifications by Olson *et al*. (2001) the new localities in Botswana at Gomoti River and Kwetsani Island, and in Namibia at Lianshulu Lodge fall within the 'Flooded grassland and savanna' biome and the 'Zambezian flooded grasslands' ecoregion, while the other locality in Namibia, 85 km from Tsumkwe, as well as Kwalata Game Farm in South Africa, and Mt. Mulanje in Malawi, all fall within the 'Tropical and subtropical grasslands, savannas and shrubland' biome, but in different ecoregions of 'Kalahari Acacia-Baikiaea Woodland', 'Southern African Bushveld', and 'Eastern Miombo Woodland' respectively. The Rutherford and Westfall (1994) data available for South Africa,

Namibia and Botswana, also have all these new localities falling within the 'Savanna' biome. The exception to this association, of *L. botswanae* with the savanna biome over most of its distribution, other than some grassland associated specimens in Malawi, is the record in Monadjem *et al*. (2010a) of *L. botswanae* from Mozambique. According to the classifications by Olson *et al*. (2001) this locality in Mozambique is within the 'Tropical and Subtropical Moist, Broadleaf Forest' biome, and the 'Southern Inhambane Coastal Forest Mosaic' ecoregion.

Although there seems to be some disparity as to the specific habitat associations of *L. botswanae*, biome and ecoregion delineations are very coarse spatial units with considerable habitat heterogeneity within each unit (Olson et al. 2001). Recent advances in macroecology have facilitated the development of finer-scaled distribution maps for many species of African Chiroptera through ecological niche modelling of species occurrence data (e.g. Elith *et al.* 2006).

Monadjem *et al*. (2010b) provides a starting point by which habitat associations (either to discreet habitat classes such as biomes and ecoregions, or to continuous environmental data and vegetation cover) may be analysed across the entire predicted range of a species rather than to individual collection localities which may be strongly influenced by logistic limitations and sampling bias. It is expected that new distribution data will be available across the majority of African Chiroptera in the near future (J. Fahr, pers. comm.) which could provide an excellent setting to test theories of macroecological habitat associations and to highlight collection gaps. At the same time, the paucity of records of *L. botswanae* (as with many other African bat species) requires that the validity of predictive distribution maps be tested with new locality information collected in the field in order to improve their accuracy and precision.

At a local level, consultation of the primary literature relating to the collection of specimens could yield new information regarding *L. botswanae'*s habitat preferences. For example, although the Malawi specimens and other individuals recorded by MC and MK fall under the biome of 'Tropical and subtropical grasslands, savannas and shrubland', the

species was recorded in relatively large numbers (ca. 12 % of a total of 56 individuals) in lowland evergreen forest at the base of the mountain. Therefore, although the locality records of *L. botswanae* may be sparse across its range, and its habitat associations largely biased towards more open habitats, finer scale associations may yield new information about the species' use of habitats across a landscape. The five new specimen records of *L. botswanae* reported here from the Hostes Nicolle Institute also identify another locality where this species appears to be more abundant than at other localities. Thus the new records reported here show support for the species' listing of Least Concern (LC, Schlitter 2008) and suggest more extensive fieldwork will continue to improve our understanding of the species' distribution, habits, and habitat associations.

Since *L. botswanae* is a rarely caught species with very few prior echolocation call recordings (Stanley and Kock 2004; Kearney and Seamark 2005; Monadjem *et al.* 2010b), the call information presented here that is associated with voucher specimens, is informative in the context of where and how it was recorded. The recordings of the individual from South Africa might be seen to be compromised, in being from a room-flown individual (Fenton *et al*. 2004) and made with a fairly inexpensive time expansion bat detector and thus may not be entirely representative of search phase calls of *L. botswanae*. Given the variation that can potentially be found in the recorded echolocation calls of a species (Obrist 1995; Guillen *et al.* 2000; Sedlock 2001) it is important that call reference libraries attempt to include information from calls that would cover as much of this variation as possible. In the present study the call data from the individuals recorded in Malawi does well to cover different flight contexts. Interestingly, variation of call parameters was negligible between individuals even though levels of clutter at the release sites ranged from open space to densely-cluttered. Furthermore, distance of bats from the microphone (close or distant) also did not seem to result in notable differences between calls. Finally, the identity of most of the hand-released individuals recorded in Malawi can be verified by genetic comparison to collected specimens (through wing punches collected from most of the hand-released individuals). The coupling

of genetic data with call data that covers different flight contexts greatly improves the usefulness of such reference calls.

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Appendix I

Details of voucher specimens of *Laephotis botswanae*: [*]= new locality records, [+] = new specimen records from previously published localities. Following Ruedas *et al*. (2000) all these specimens have been examined and the species identification confirmed by either TK or MC.

Museum accession abbreviations: BMNH – The Natural History Museum, London; MoM – Museum of Malawi; MHNG -Museum of Natural History, Geneva; ROM – Royal Ontario Museum, Ontario; SMW – State Museum, Windhoek, Namibia; TMSA – Ditsong National Museum of Natural History (formerly Transvaal Museum), Pretoria.

Collector's number abbreviation: ECJS – Ernest C. J. Seamark (for specimens still to be lodged with a museum in Botswana); MW – Mirjam Kopp & Michael Curran (for a specimen lodged with the Museum of Malawi, but not yet accessioned).

BOTSWANA: Gomoti River, Chitabe (19.42585ºS, 23.41696ºE): ECJS-6/2009[*], ECJS-11/2009[*]. Kwetsani Island (19.24625ºS, 22.53737ºE): ECJS-159/2009[*].

NAMIBIA: 85 km Tsumkwe, Grootfontein (19.41667ºS, 19.73333ºE): SMW 10661[*]. Lianshulu Lodge, Mudum National Park, Caprivi (18.13333ºS, 23.41667ºE): SMW 14539[*]-14540[*]. San Michelle, Kwando River, Caprivi (17.86 ºS, 23.36 ºE): SMW 13587[+], SMW 13610[+]. Dose Pan, Kavango, Dumusfie Water (18.23333ºS, 20.88333ºE): SMW 10677[+]

MALAWI: Tea Research Foundation Forest, Mt. Mulanje foothills, Mulanje District (16.0992ºS, 35.6245ºE): TMSA 48307[*]-48308[*], MoM: MW-192*. Ruo Gorge, Hydro Station, Mulanje District (15.9715°S, 35.6548°E): MHNG 1971.010[*]-1971.011[*]. Zomba (15.38333ºS, 35.31667ºE): BMNH 87.1137[+]

MOZAMBIQUE: Mount Mabu base camp, Zambesia Province (16.305806°S, 36.424222°E): MHNG 1971.009[+].

SOUTH AFRICA: Kwalata Game Farm, Gauteng (25.3822ºS, 28.31542ºE): TMSA 48323[*].

ZIMBABWE: Hostes Nicolle Institute (18.16667ºS, 28.21667ºE): ROM 70984[+], 70988-70990[+], 88414[+].

Table 1

Echolocation call parameters recorded from *L. botswanae* specimens at Kwalata Game Ranch (South Africa) and Mt. Mulanje (Malawi), compared with information from Fenton (1975), Fenton and Thomas (1980), Fenton and Bell (1981), Taylor (2000) and Monadjem *et al.* (2010b).* = calculated for this table. – = not specified in publication.

	# individuals	Context	Max (kHz)	Min (kHz)	Bandwidth (kHz)	Power (kHz)	Duration (ms)
This study (South Africa)	1	Flown in naturally lit room	55	29	26	37.13	2.68
This study (Malawi)	7	Hand-released	58.63	28.91	29.72	32.34	4.73
Fenton (1975)	3	Flown in lit room	65	35	30*	-	-
Fenton and Thomas (1980)	-	Free flying	65	35	30*	-	2-7
Fenton and Bell (1981)	4	Free flying	55	32	23	33	5
Taylor (2000)	-	-	55	32	23*	33	5
Monadjem *et al.* (2010)	-	-	-	-	22	33	5

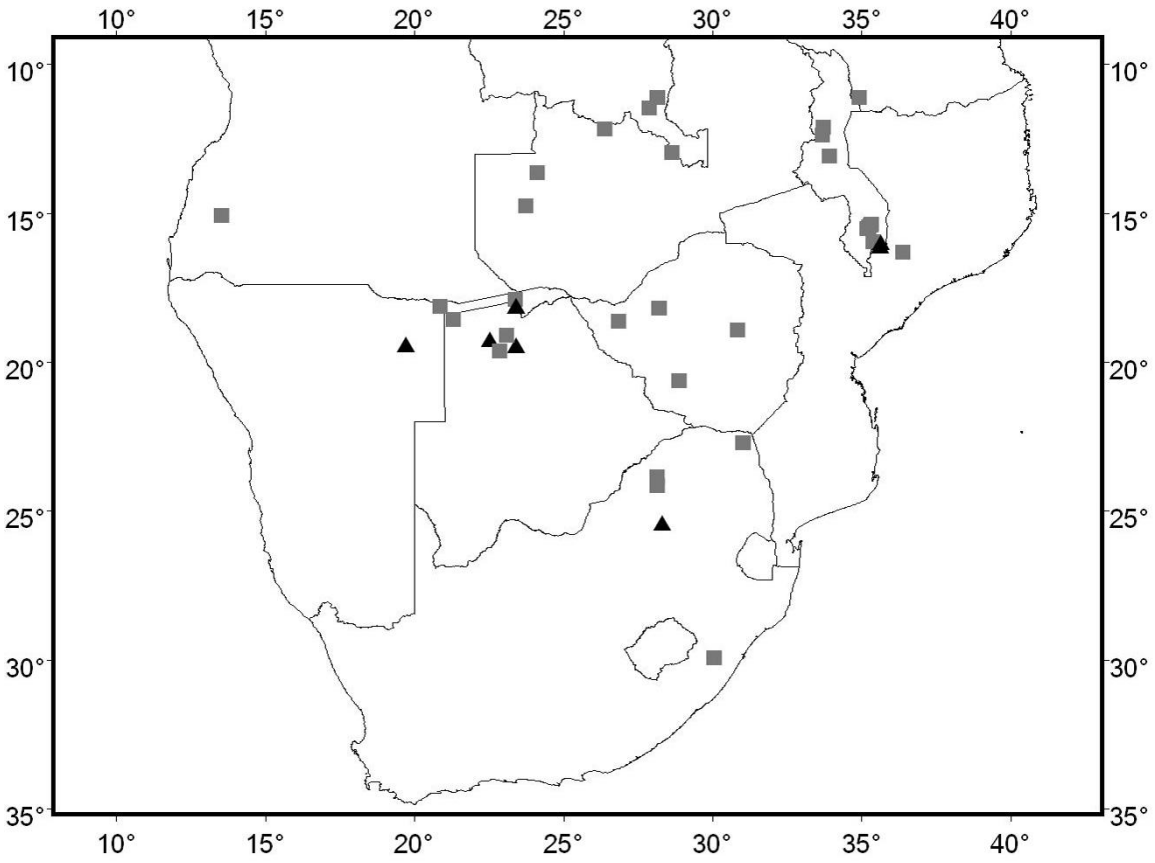
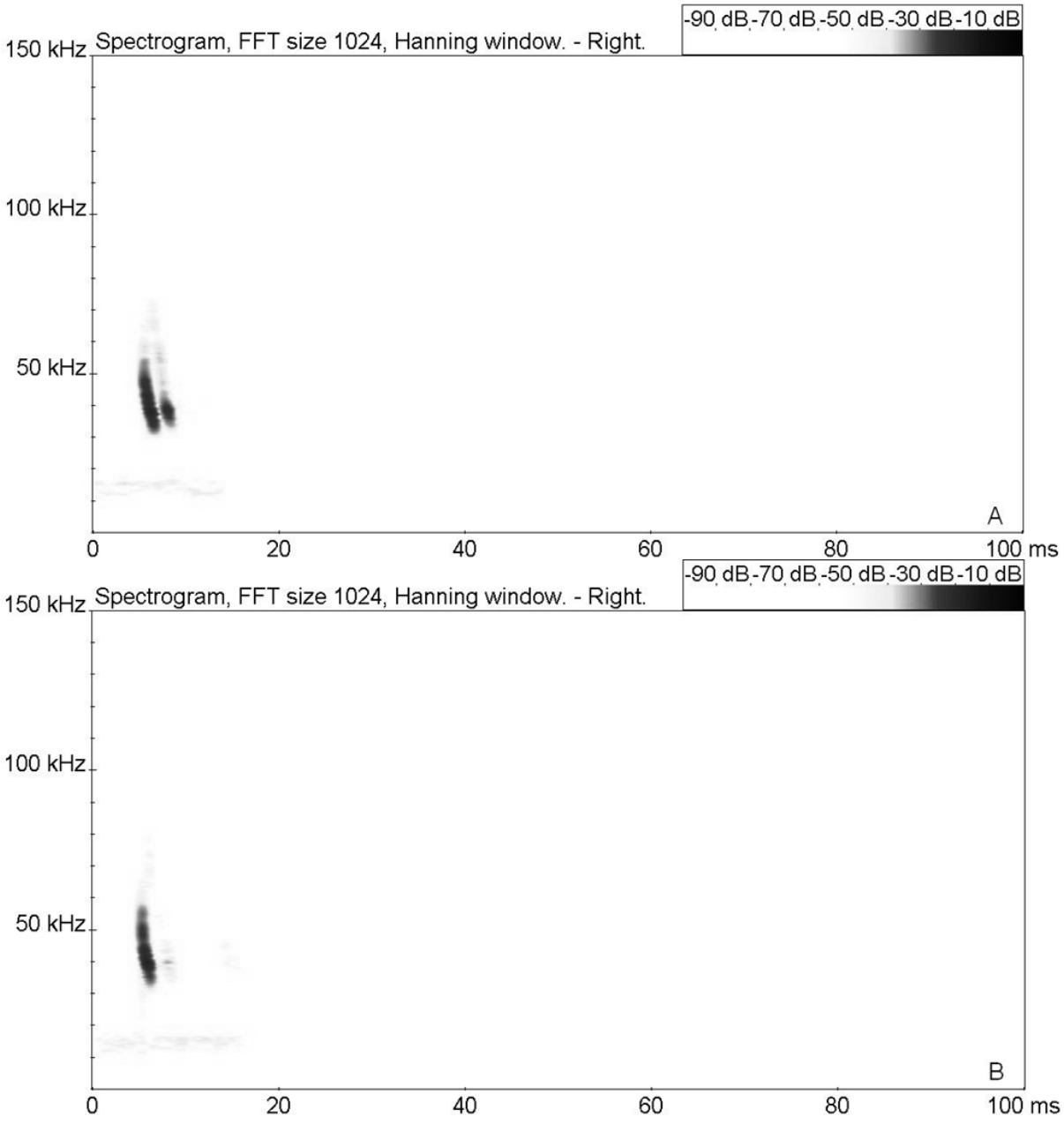


Fig. 1. Updated distribution of *Laephotis botswanae*. Grey squares = previously published records, black triangles = new records. See Appendix I for locality and specimen details.


-90 dB -70 dB -50 dB -30 dB -10 dB
Spectrogram, FFT size 1024, Hanning window. - Right.
150 kHz
100 kHz
50 kHz
A
0
20
40
60
80
100 ms
-90 dB -70 dB -50 dB -30 dB -10 dB
Spectrogram, FFT size 1024, Hanning window. - Right.
150 kHz
100 kHz
50 kHz
B
0
20
40
60
80
100 ms

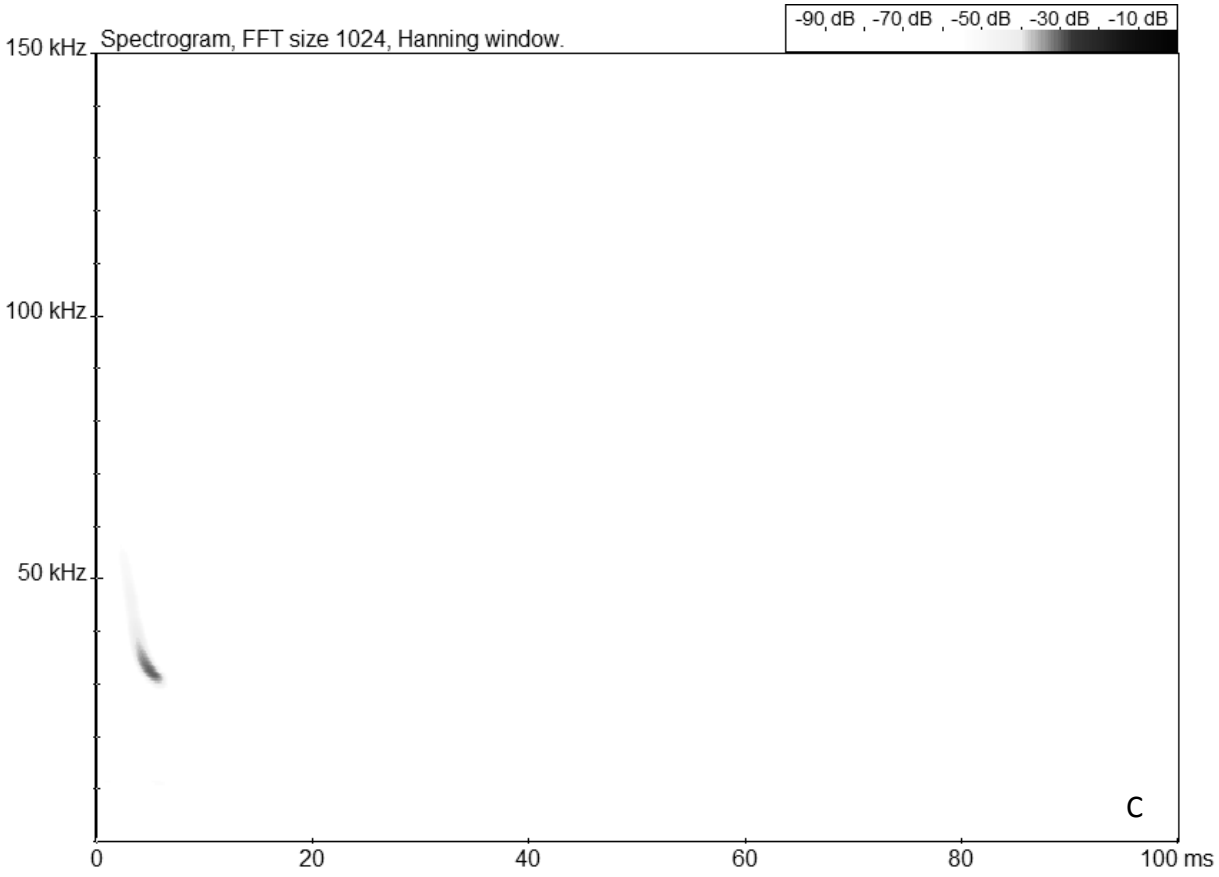


Fig. 2. Echolocation calls of *Laephotis botswanae*: A) & B) from TMSA 48323 in South Africa, which was room-flown, and C) from an individual (field number 2007-586) released by MC and M. Kopp in Malawi in an uncluttered environment. A) shows a potential double orientation pulse induced by the room-flown environment (e.g. Fenton and Thomas, 1980), while B) shows a more representative echolocation pulse with minor echo. However, C) is likely most representative of a search phase call for the species.