Variation of echolocation pulse source levels and detection distances for bat assemblages across an environmental gradient:

"A test of the Acoustic Adaptation Hypothesis"

Candidate: David Barasa Wechuli

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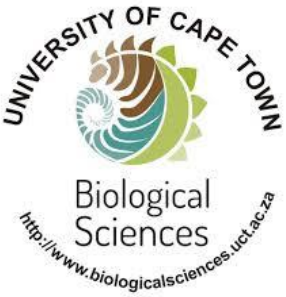
Supervisor: Prof. David Steve Jacobs

Department of Biological Sciences: University of Cape Town



Co-supervisor: Dr. Marc W. Holderied

School of Biological Sciences: University of Bristol, UK

GENERAL ABSTRACT

The study of variation in phenotypic traits of animals and its causes clarifies how evolution drives and sustains biodiversity. A sizable portion of the world's biodiversity is the product of, and maintained by, divergent adaptation to varied environments. Therefore, predicting the impacts of environmental change on dynamics of biodiversity is probably the most important concern for ecologists and evolutionary biologists to deal with. This thesis attempts to decouple the relative contribution of the physical environmental parameters to drive divergence of important traits such as acoustic signals. To improve signal transmission, individuals or populations adjust their acoustic signals to the environmental conditions of the occupied habitat. This has been confirmed in a wide range of taxa, including amphibians, birds, and mammals, including bats. Such phenotypic adjustment may be the outcome of microevolutionary processes that result in local adaptation or phenotypic flexibility. Bat echolocation is used as a test case because it is comprised of suite of parameters some or all of which may become locally adapted to atmospheric conditions and/or vegetation structure. These parameters include inter-pulse interval, duration, frequency of most energy and source levels.

In combination with climate and habitat structure, source levels of bat echolocation pulses directly influence the distance at which bats perceive targets, including prey. Habitat and prevailing climatic conditions present different challenges for sound transmission, and so the quality and content of information derived from echolocation pulse reflects these environmental challenges. Hence, echolocation pulses within or between species may vary from one habitat to the next due to variable selection pressure, resulting in local adaptation. The Acoustic Adaptation Hypothesis thus proposes that acoustic properties of the environment influence sound propagation and ultimately the evolution of echolocation pulses. If environmental factors exert an overriding influence on the echolocation pulses of bats,

detection distances in bats may be specific to particular habitats and be independent of body size and foraging strategy. In support of this, Surlykke and Kalko (2008) showed that within a bat assemblage, bats of different body sizes and foraging strategies using different frequencies emitted pulses of higher source levels and all bats had similar detection distances. This implies that bat species in their study presumably faced similar situations at the foraging habitat and were constrained to fly at comparable flight speeds and maneuverability. This suggests that detection distances may be locally adapted supporting the AAH. If as suggested by the AAH, I predicted that; (i) echolocation pulse parameters used by different species of bats within the same assemblage should result in similar detection distances and (ii) if detection distances are adapted to a particular habitat (Chapter 3) and prevailing climatic conditions (Chapter 4), the detection distances should differ among bat assemblages (Chapters 3 and 4) and, detection distances should correlate with prevailing climatic conditions (Chapter 4). Alternatively, contrary to the Acoustic Adaptation Hypothesis, detection distances may be influenced more by body size and foraging mode than by habitat under prevailing climatic conditions. If so, detection distance should (i) differ among species within assemblages, (ii) be species specific and remain the same within species between assemblages. Detection distances of species populations should correlate with environmental variables across sites in biomes. To test the Acoustic Adaptation Hypothesis, I used multiple microphone arrays to record echolocation pulses of bat species for bat assemblages across different sites in different biomes of South Africa.

Firstly, bat species were identified based on their pulse parameters used in conjunction with a reference library of pulses, distribution records of bat species and captured individuals from the areas of recording (Chapter 2). Several parameters were measured to identify a representative pulse type for each species. These initial species assignations were confirmed through Discriminant Function Analyses (DFA) and used to assign source levels of

echolocation pulses to each species. Secondly, these source levels together with frequency and weather parameters were used to calculate detection distances. General linear models were used to evaluate detection distances within and between bat assemblages and to test the predictions of the Acoustic Adaptation Hypothesis. These analyses indicated that bats in the same assemblage used different echolocation pulse source levels and frequencies resulting in different detection distances. The mean detection distances also differed among bat assemblages occupying different biomes. This was probably a consequence of detection distances being species specific and remained similar within species between assemblages. This was confirmed by the detection distances of *Miniopterus natalensis*, a species which occupied several sites. The detection distance of *M. natalensis* remained similar across biomes, hence species was a better predictor of detection distances than sites. The general linear mixed model (GLMM) was applied to evaluate the correlation between detection distances for assemblages and climatic variables. Findings indicated that the 'best' models with lowest AIC values explained the detection distances for bat assemblages and *M. natalensis* were correlated with temperature and longitude, providing partial support for the Acoustic Adaptation Hypothesis. Temperature was thus the most influential climatic variable on bat echolocation. This implies that any human-induced climate change that results in temperature change is likely to influence the foraging of bats and therefore their survival.

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DECLARATION

I, David Barasa Wechuli, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever. This thesis was submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisors have seen my Turnitin report and any concerns revealed by such have been resolved with my supervisors.

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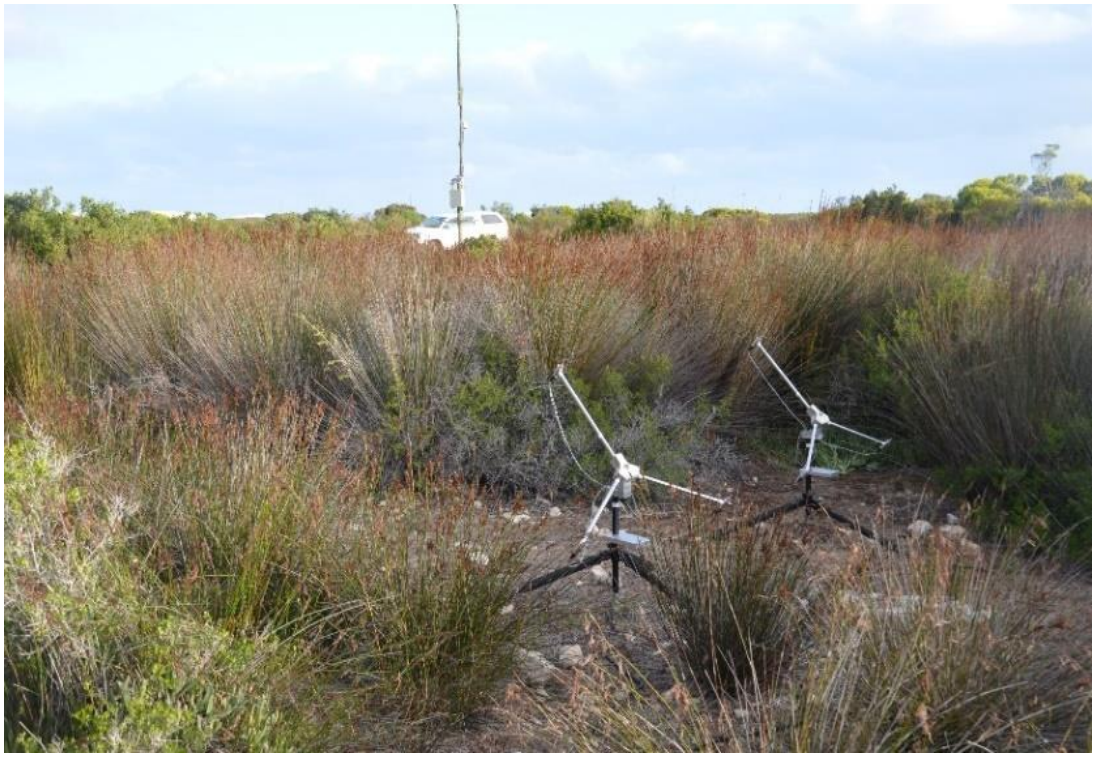

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List of abbreviations

AAH	Acoustic Adaptation Hypothesis
ALT	Albany thicket
Atm	Atmospheric pressure
BAV	Baviaanskloof
BZT	Bazley tunnel
BNV	Beneve
CBT	Coastal Belt
CF	Constant Frequencies
CSB	*Cistugo seabrae*
cl	reflection loss
dB	Decibel
dB/m	Decibel per meter
DFA	Discriminant Function Analysis
DHP	Dehoop nature reserve
DST	Desert
3D	Three dimensional
DT	Detection threshold
FFT	Fast Fourier transforms
FM	Frequency Modulated
FYN	Fynbos
FIR	Finite Impulse Response
GRL	Grassland
GLM	Generalised linear model
HCA	*Hipposideros caffer*
HDC	high duty cycle
KKK	Kalkounkrans
kHz	Kilohertz
LDC	low duty cycle
LKG	Lekkersing
LME	Linear Mixed Effect
MF	*Miniopterus fraterculus*
Mic	Microphone
MN	*Miniopterus natalensis*
MT	*Myotis tricolor*
NC	*Neoromicia capensis*
NT	*Nycteris thebaica*
NKR	Nama Karoo
PC	Principal components
PCA	Principal Component Analysis
peSPL	Peak equivalent sound pressure level
RCA	*Rhinolophus capensis*
RCL	*Rhinolophus clivosus*

RDA	*Rhinolophus damarensis*
RSM	*Rhinolophus simulator*
RSW	*Rhinolophus swinnyi*
RH	Relative humidity
SD	Standard Deviation
SDL	Sudwala
SPL	Sound Pressure Level
SAV	Savanna
SL	Source Level
TFM	Table Farm
TA	*Tadarida aegyptiaca*
TOADs	Time of arrival differences
TS	Target Strength
TLA	Transmission loss because of atmospheric absorption
TLS	Transmission loss owing to spherical spreading
V	Volts
ZPK	Zoutpansklipheuwel

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CHAPTER 1

General Introduction

1.1 Environment and acoustic adaptation

Environments are described as a combination of biotic and abiotic factors that impacts an organism and survival and development (Begon et al., 1996; Lewis et al., 2017). Information about the environment and the internal status of the organisms is essential to the fitness of organisms and is gathered by the organisms' senses. Consequently, multiple sensory systems such as the visual, gustatory, auditory, vestibular and somatosensory systems have evolved across species (Niven & Laughlin, 2008; Smith, 2008). As a result of these sensory systems, organisms have the ability to perceive their environment, to interpret it, and to respond to it (Niven & Laughlin, 2008; Torres, 2017; Weiss, 2019).

Sensory systems may be passive or active. Passive sensory systems rely on the perception of external stimuli from the environment (e.g., the electromagnetic spectrum of light and sound) and their interpretation by the nervous systems of organisms. In such instances, the physical properties of external stimuli received by the senses are not actively controlled by an organism. The active sensory system, by contrast, uses its own energy to probe its surroundings. It has complete control over the timing, intensity, spectrum, and directionality of the signals, which can be altered (Nelson & MacIver, 2006). A prominent example is bioluminescence, especially in deep-sea fish e.g., *Malacosteus niger* and insects e.g., *Arachnocampa flava,* which produce their own light, for the use in vision (Douglas & Partridge, 2011; Merritt & Patterson, 2018). Bioluminescent deep-sea fish possess photophores which produce coded light flashes, thought to serve as a means of communication within species, illuminate potential prey or avoid predators. This latter role is described as a strategy for counter illumination in deep-sea fish.

Often, predators will scan upward while swimming to detect shadows. To counter this search strategy, potential prey will counter illuminate, producing light on their bellies that matches the light coming down from above. In doing so, they become essentially invisible. Counter illumination has thus been adapted to serve as a predatory tool of camouflage. Similarly, a weak electric field can be generated by electric fish e.g., *Mormyrus rume* and dragonfish (Malacosteidae), which allows navigation, the detection of objects, i.e., electrolocation and electrocommunication with other electric fish (Nelson & MacIver, 2006; Donati et al., 2016). Some animals use acoustic signals, which is a consequence of their neurophysiology and anatomy, so that they to produce sound and detect objects e.g., obstacles or prey (Neuweiler, 2000; Madsen & Surlykke, 2013). Animal bisonar or "echolocation" is a notable example of active acoustic sense.

Echolocation is an acoustic sensory system which evolved independently, primarily for orientation and/or foraging, among toothed whales, such as porpoises and dolphins in suborder Odontoceti (Hooker, 2018), birds (Ey & Fischer, 2009) and bats, except the majority of Old World fruit bats (Thomas et al., 2004). Most of the over 1420 extant bat species (Burgin et al., 2018; Simmons & Cirranello, 2020) found in different biomes use echolocation to enable a nocturnal existence (Griffin et al., 1960). Echolocation is a prime sensory cue because it allows nocturnal bats to orient and find prey as they exploit different habitats (Schnitzler & Kalko, 2001). Through echolocation, bats can gather information about the exact position and attributes of objects in their environment, such as shape, surface structure, and orientation (Holderied & von Helversen, 2006). This information is embedded in the temporal and spectral parameters of the returning echo which the bat’s neural system compares with those in the emitted echolocation pulse to create a three-dimensional image of its surrounding (Neuweiler, 2000; Holderied et al., 2006). There is much variation in the echolocation system among bat

species attributable to several factors. They include morphological differences (Barclay, 1999; Schnitzler & Kalko, 2001) climatic conditions, particularly, temperature and humidity (Snell-Rood, 2012; Jiang et al., 2015; Mutumi et al., 2016; Chaverri & Quirós, 2017), and habitat type (Barclay, 1999; Schnitzler & Kalko, 2001). It is increasingly evident that the physical environment e.g., vegetation (Barclay, 1999) and climatic parameters such as temperature, and humidity (Jiang et al., 2015; Mutumi et al., 2016) can reflect the quality and content of information derived from echolocation pulses. In response, bat species may vary the signal design to enable efficient foraging and successful orientation in habitats which differ in vegetation structure and climatic conditions. Variation in the structure of acoustic signals from one habitat to another may be a result of variable selection pressure across different habitats resulting in local adaptation (Huey & Bennett, 1987). This was formally recognized in the ‘‘Acoustic Adaptation Hypothesis’’ (Hansen, 1979), subsequently reviewed by Boncoraglio and Saino (2007), proposing that the selection pressures associated with habitat structure have influenced the evolution of bird signals’ acoustic properties. It is presumed that various adaptations in birds' signals have evolved over time because of selection based on habitat to improve sound transmission (Boncoraglio & Saino, 2007; Bradbury & Vehrencamp, 2011a).

These predictions of the Acoustic Adaptation Hypothesis (AAH) have been tested to compare signal characteristics and their efficiency between habitats with varying vegetation density (e.g., closed verses open habitats), taking into account their variations in sound absorption trends, refraction, reflection and spreading (Wiley & Richards, 1978; Slabbekoorn & Peet, 2003). Moreover, sound can be interfered with by background sounds, for example made by wind and other animals, which may obscure the primary signal as it travels through the habitats (de la Torre & Snowdon, 2002). Since the resultant effect may constrain long-distance transmission, a shift of the acoustic signals to relatively lower frequency ranges are expected

to occur and this would help to overcome the effects from external influences (de la Torre & Snowdon, 2002). Theoretical expectations as to how acoustic signalling should change in response to habitats are theoretically justifiable, but data are insufficient and the findings are divergent (Ey & Fischer, 2009; Goutte et al., 2018).

Acoustic environments are continually changing, and it is expected that animals will be able to cope with these changes accordingly (Morton, 1975; Wilkins et al., 2013). Although long-term environmental changes are expected to lead to adaptive responses, the acoustic environment with its integrated temporal and spatial complexities are expected to select for flexibility in calling behavior (Brumm, 2013). According to the AAH, selective pressure for transmission efficiency leads to the prolonged adaptive responses of acoustic signals, guide variations in population design, and facilitate speciation through sound propagation (Morton, 1975; Wiley & Richards, 1978). Other factors such as body size can also influence the effectiveness of pulses, over long distances in addition to environmental changes, e.g., in primates, bats and birds (Barclay & Brigham, 1991; Mitani et al., 1999), genetic drift, e.g., in microhylid frogs, primates and bats (Wich et al., 2008; Yoshino et al., 2008; Stoffberg et al., 2012; Lee et al., 2016) and vocal learning/cultural drift, e.g., bats (Xie et al., 2017).

The concept of AAH has been tested either within species by comparing calls from the same species in more than two habitat types or among species residing in different habitat types, mainly birds (Ryan & Brenowitz, 1985; Ey & Fischer, 2009) and other model animals (usually specific species) such as mammals (Hedwig et al., 2015), insects (Jain & Balakrishnan, 2012) and anurans (Goutte et al., 2018). Signals produced by many of these taxa are primarily used for communication in different contexts for mate attraction and advertising territories in respective habitats. Most of the bird studies, in particular, have gathered evidence to support the AAH. For example, the songs of tinamous and passarine species that live in open habitats,

are composed of higher frequency and broad bandwidth compared to species in their lineage living in cluttered type of habitat, according to the AAH (Bertelli & Tubaro, 2002; Saunders & Slotow, 2004). However, some studies involving e.g., birds, felidae have not shown such optimal relationship (Peters & Peters, 2010; Mikula et al., 2021) or yield mixed results (Bosch & De la Riva, 2004; Ey et al., 2009).

Bat echolocation pulses may also have evolved in response to habitat structure that influences the propagation of signals, albeit over shorter distances because the higher frequencies of bat echolocation are attenuated to a large extent by the atmosphere the lower frequencies in bird song. Various climatic factors, particularly relative humidity and temperature may also influence several echolocation pulse parameters, in particular the frequency and source levels of echolocation pulses which permit optimization of prey detection distances in different habitats (Holderied & von Helversen, 2003; Jakobsen et al., 2013; Jacobs et al., 2017). Patterns of distribution of the considerable bat species diversity are often associated with areas where precipitation and habitat complexity is high or areas with high topographic variability (Ruggiero & Kitzberger, 2004; Milner et al., 2006). If selection improves echolocation pulse sound propagation, the distribution of bat species that emit either high frequency pulses or low-frequency pulses can be explained by a distinctive amalgamation of environmental factors, e.g., humidity, temperature and vegetation clutter. The latter are non-target objects that the bat needs to detect and avoid (Fenton, 1990).

Moreover, the diversity of insects may affect the echolocation pulse frequency of bats (Jung et al., 2014). Bats emitting pulses in the range of 20-60 kHz allow them to detect a rather large range of prey items of different sizes while maximizing response time to capture prey (Waters & Jones, 1995; Schoeman & Jacobs, 2011). This also explains why the pulse frequency of

many molossid species and vespertilionid species alternates (Kingston et al., 2003; Jung et al., 2014).

1.2 Limitations of echolocation pulse transmission in the physical environment

The greatest challenge bats may face is that the parameters of echolocation systems are impacted by the vagaries of sound transmission in different environments. How bats adapt to such challenges is dependent on phylogenetic history and life history strategies. Echolocation pulses are subject to the physical constraint such as climatic conditions (temperature and relative humidity) that lead to attenuation of sound (Luo et al., 2014; Mutumi et al., 2016; Maluleke et al., 2017; Goerlitz, 2018). Weather-induced variations in echolocation pulses can be traced to two kinds of sound attenuation: atmospheric and geometric. In geometric attenuation (also known as spreading losses), sound energy is radiated from a source in a sphere with increasing surface area, and therefore sound levels decrease by 6 dB for every doubled distance from the source. The geometric spreading of sound is independent of frequency, and it has a major effect in practically all sound propagation scenarios. In contract, atmospheric attenuation is the gradual loss of sound intensity due to multiple sources, such as sound absorption by water molecules in the air, diffraction, refraction, temperature and humidity effects, etc. (Griffin, 1971). The attenuation of sound due to these factors only impact the sound after it leaves the source. Consequently, the sound pressure level at which the sound is emitted by the source does not change.

The magnitude of the effect of atmospheric attenuation is contingent on the frequency of the sound being propagated (Lawrence & Simmons, 1982). Higher frequency sound signals from bats are subject to rapid atmospheric attenuation and to a greater level than lower frequency sounds (Guillén et al., 2000). Atmospheric attenuation is therefore a product of a complex and non-linear relationship between climatic variables and the frequency of signals (Lawrence &

Simmons, 1982; Mutumi et al., 2016). Atmospheric attenuation can have a strong influence on how far the sound produced can travel and return a relevant echo, that is, on the operational range of an echolocation pulse (Neuweiler, 2000; Stilz & Schnitzler, 2012; Goerlitz, 2018).

To overcome the impediments of echolocation pulse propagation, especially pulses emitted at high frequencies, bats can use parameters such as source levels to optimally exploit habitats (Waters & Jones, 1995; Holderied & von Helversen, 2003; Surlykke & Kalko, 2008). Both frequency and source levels (the intensity of pulse emitted, usually standardized at 10 cm from the bat) determine the echolocation range i.e., the distance at which an echolocation pulse allows a bat to detect objects or prey (Holderied et al., 2005). Like other echolocation pulse parameters such as frequency that is affected by environmental conditions (Mutumi et al., 2016; Maluleke et al., 2017; Jacobs & Mutumi, 2018), source levels may also be altered as the pulse propagates through the environment. All else being equal, signals produced at high source levels travel further than signals of low source levels because they have more energy to be dissipated by atmospheric attenuation and can increase the detection distance of objects or prey by the bat emitting them (Holderied & von Helversen, 2003).

1.3 Intensity, directionality and detection distances

The pioneering work of Griffin et al. (1960) provided vital information on bat echolocation pulse intensity as source levels (dB SPL; decibels sound pressure level) and resulted in more focus on the measurement of pulse source levels. This was initially restricted to the laboratory setting for a long while because the distance between the stationary bat and the recording microphone could be controlled and standardized in the laboratory. Such experimental studies provided vital data but with limited information on the source levels used by free flying bats or how they varied their source levels in different situations (Waters & Jones, 1995). For several decades, it was not possible to measure this important acoustic parameter for free flying

bats in the wild because of the logistical challenges that this entailed. Fortunately, with the advent of the multiple microphone arrays, it became feasible to get more information about how bats vary the pulse source levels as they fly in the wild in pursuit of prey (Holderied & von Helversen, 2003; Surlykke & Kalko, 2008). Equally, signal directionality (i.e., more pulse energy is directed in the forward path than towards the side) is crucial and is linked to source levels (Jakobsen et al., 2013). The directional emission of pulses is likely to have a few benefits for bats. Firstly, it serves as a spatial filter, minimizing the returning echoes from the edges of habitats and rear side of the bat, and thereby restricting the quantity of the input that a bat may handle. Secondly, it often introduces ingrained spatial details, that is echoes are likely to come from the forward direction, which increases the echo levels. While the echo level may cause the bat to change its source levels, it cannot directly affect the source levels. This allows bats to emit source levels by concentrating the energy of the signal in a smaller area, which allow them to detect objects farther away (Surlykke et al., 2013; Jakobsen et al., 2018). A close relationship exists between the measurement of sound source level and sound directionality in the recorded field recordings. Previous research shows that at a noticeable directionality, there is large angle-dependent source level variations between on-axis and off-axis recordings (Holderied et al., 2005; Surlykke & Kalko, 2008). In addition, the measurement of source level varies with the direction of measuring microphone. While different source levels can be determined depending on recording geometry, the actual source level does not change only its estimation by an observer. Therefore, estimating directionality in the field is incredibly difficult without the use of equipment, such as multiple microphone arrays, to track the bat's acoustic axis. Directionality estimates have been carried out not only in the field but also in the laboratory setting (e.g., Jakobsen et al., 2018). Given this background, to fully evaluate the role of echolocation in the natural environment, it is important to recognize several acoustic

characteristics of the echolocation pulses. In addition to echolocation pulse frequency and time parameters, directionality and intensity also affect the bat's perception of its acoustic space.

However, measuring intensities is not easy because the intensity at the microphone will differ from the emitted intensity as a result of atmospheric attenuation. This requires that the distance between the source of sound and the microphone be known to determine bat pulse intensity. State-of-the-art multiple microphone arrays enabled the measurement of pulse intensities at the microphones from which source level (decibels peak equivalent sound pressure level; dB peSPL) can be calculated because the position of the bat from each microphone in the array can be determined by differences in the arrival times of the pulse at each microphone (Holderied & von Helversen, 2003; Surlykke & Kalko, 2008; Koblitz, 2018). Source levels combined with the frequency of the pulse and the atmospheric conditions at the time the bat emitted the pulse can in turn be used to determine the detection distances of different species of bats comprising a community. A few studies revealed that free flying bats in their habitats had higher source levels up to 137 dB peSPL than previously known (Holderied & von Helversen, 2003; Holderied et al., 2005; Surlykke & Kalko, 2008). Brinkløv et al. (2009, 2010) found that even phyllostomids that consume fruits and animals produce more intense echolocation pulses (105-110 dB peSPL) than previously expected. Depending on where bats are flying in a habitat, their source levels among individuals of the same species could vary considerably (Schuchmann & Siemers, 2010). Variation in pulse source levels can occur also among different species in different situations (Holderied et al., 2005; Surlykke & Kalko, 2008). This flexibility allows bats to optimize the source levels for different habitats and different circumstance within the same habitat. For example, when bats emit pulses the output of signal source level and other pulse parameters differ due to varying degree of vegetation density. Bats flying near or inside thick forest habitat emit pulses mostly at lower source levels

than bats flying in open area (Brinkløv et al., 2010). The low source level emitted in a cluttered habitat may offer some benefits to some bats. Firstly, it may prohibit prey from hearing the echolocation pulses of an oncoming bat (Surlykke et al., 1993; Goerlitz et al., 2010; Corcoran & Conner, 2017). Secondly, it serves to deal with echoes from vegetation when a bat is foraging close to dense vegetation (Schnitzler & Kalko, 2001; Stidsholt et al., 2021). Because flying insects are closer to a bat than the vegetation; so by reducing their source levels, bats can filter out acoustic clutter (echoes from background vegetation) but can still detect the echo from the prey since it is nearer (Neuweiler, 1990; Jacobs & Bastian, 2016; Stidsholt et al., 2021). However, pulses emitted at higher source levels, allow the bat longer detection distances (Holderied & von Helversen, 2003; Schuchmann & Siemers, 2010). On that account, differences in source levels lead to varying prey detection distances for the bat community in different habitats and, bats would optimally use higher intense pulses to attain longer prey detection distances.

1.4 The focal system

Bat assemblages are a good system to study acoustic adaptation of species because they are widely distributed across biomes of the world and in South Africa and their echolocation pulses can be recorded (Monadjem et al., 2010). Bat assemblages consist of different families with bat species of different body sizes, foraging strategies, and pulse characteristics but occupy the same space. Therefore, for different assemblages in different biomes, the species occupy a variety of different habitats (Schoeman & Jacobs, 2003; Odendaal & Jacobs, 2011). Southern Africa is made up of a number of major biomes including Desert, Fynbos, Indian Ocean Coastal Belt, Nama Karoo, Savanna, Grassland and Albany thicket (Mucina & Rutherford, 2006). Biomes are commonly regarded as surrogates for vegetation/habitat types that differ in structure and environmental variables that affect the distribution of mammals including bat species. Some biomes such as Savanna and thicket biomes, which consists of large woody

vegetation can be more complex structurally than biomes like Fynbos, characterized by low bushy vegetation, that are not vertically complex (Cowling et al., 2004). Several authors noted the high mammalian species diversity in Savannas and suggested that this could be due to the high diversity of habitats in these biomes (Andrews & O'Brien, 2000). Biomes of the Succulent Karoo and Nama Karoo are characterized by dwarf shrubs and succulent vegetation types occurring in high temperatures above 30°C and summer rainfall ranging from 100 and 520 mm annually. In the Desert biome, a similar vegetation type occurs as in the two preceding biomes, except that the temperature is > 30°C, and the rainfall is low between 70-80 mm. A more densely impenetrable vegetated Indian Ocean Coastal Belt has a maximum temperature range of between 15° and 24°C. The neighbouring Grassland biome is dominated by varied grass species and with temperature ranges similar to Savanna biome, between 25° and 35°C, although the former has more rainfall in summer (400-2000 mm) than the later (750 mm). The Albany thicket occurs in semi-arid regions and is characterized by leafy succulents and deciduous woody shrubs. During summer, the temperature is above 40°C and rainfall varies from 200-950 mm per year. These five biomes host many bat species, and some species are present in two or more biomes. This success in the distribution of bat species across biomes is therefore attributable to various drivers. For example, precipitation is the most important factor limiting the distribution of bats that are concentrated to the wetter eastern regions of South Africa (O'Brien et al., 1998). Southern African region has a subtropical to warm temperate climate (Andrews & O'Brien, 2000) and temperature ranges are comparatively smaller in South Africa than in the tropics, i.e., average temperatures are around 7^{o}C and 11^{o}C in winter and summer, respectively (van der Merwe et al., 1994). Considering the ecological success of species distribution across a wide variety habitat type, that are capable to influencing selection pressures, differences in the echolocation signals of bat assemblages offers an excellent system to explore the AAH.

1.5 Variation in echolocation pulse structure for bat assemblages

Bats display great variation in the configuration of their echolocation pulse. While certain species in each family quite often have similar echolocation pulse structures to each other, in some cases, selective environmental pressures appear to have overridden phylogenetic constraints (Zhang et al., 2019). Indeed, that even species that are distantly related may have evolved convergent pulse features at the outset of strong selection as a result of ecologically comparable conditions (Jones & Teeling, 2006; Jacobs et al., 2016). Therefore, caution must be taken when identifying species by their echolocation only, as when multiple microphone arrays are used to measure source levels. It is nevertheless possible to classify the pulse types of bats since there are, however, several combinations of pulse parameters that characterize the pulses of bats from the same species.

Therefore, in this study, because echolocation pulses of bats in different assemblages were recorded using multiple microphone arrays, measurement of source levels was preceded by stringent identification of bat species from their echolocation pulses. Source levels were ultimately used to determine detection distances, to test the Acoustic Adaptation Hypothesis.

1.6 Thesis outline

The aim of this study was to test the Acoustic Adaptation Hypothesis using detection distances for bat assemblages, and *Miniopterus natalensis* as a focal species, across different sites in South African biomes.

Chapter 2 presents an analysis of echolocation pulses for bat species recorded with the multiple microphone arrays (where individuals emitting the pulses are unknown) to reliably assign echolocation pulse to species. Multivariate discriminant analysis, that is, DFA, of pulse parameters was used to confirm the initial assignment of the pulses to species. This was done so that source levels generated (Chapter 3) could also be correctly assigned to species.

In Chapter 3, the recorded bat echolocation pulses in different sites allowed measurement of source level of bat pulses and together with echolocation pulse frequencies, detection distances were calculated to test the predictions of the AAH. As to whether detection distances across species of bats in the same assemblage are similar as reported by Surlykke and Kalko, (2008) and, if so, whether detection distances among bat assemblages differ from one site to the next. In contrast to the AAH, which has been demonstrated by Surlykke and Kalko, (2008), detection distances may depend more on body size and/or foraging strategy than on habitat. In that case, detection distances should differ among species within assemblages, be species specific, and remain the same within species between assemblages.

In Chapter 4, I investigated (in the context of the AAH) how climatic conditions influence average detection distance for bat assemblages and *Miniopterus natalensis* across sites. I predicted that bats in the same assemblage exposed to the same climatic conditions, should have similar detection distances, which should be different across bat assemblages and a single species, *Miniopterus natalensis* occupying localities that differ in climatic conditions. Alternatively, body size, life-history strategies and phylogenetic history may have a greater influence on detection distances resulting in differences in detection distances within and across bat assemblages occupying different habitats and exposed to different climatic conditions. Detection distances should be species-specific and consistent across localities despite differences in climatic conditions.

The general synthesis and conclusion (Chapter 5) summarizes the entire thesis. This research brings the understanding of the importance species identification from echolocation pulses. It further elaborates the relative roles of acoustic adaptation in the evolution of sensory traits in the context of the AAH.

1.7 Ethics statement

The research did not require capture or handling of bat species in the field, and therefore, ethical approval was not sought. Recording of echolocation pulses was carried out on both private and public owned lands upon acquiring official consent from owners/ managers as well as the requisite permits from the respective provincial nature conservation departments of South Africa. Cape Nature (Permit No.: CN44-59-7163, 0056-AAA007-00216 and 0052-AAA007-00012), South African National Parks (SANParks; Permit No.: MUTAI1571), Northern Cape Provincial Government: Department of Environment and Nature Conservation (Permit Number: Fauna 0912/2018 and Fauna 0913/2018), Eastern Cape Provincial Government: Economic Development, Environmental Affairs and Tourism (Permit Number: CRO 178/18 CR and CRO179/18CR), Eastern Cape Parks and Tourism Agency (ECPTA; Permit Number: RA0300), Mpumalanga Tourism and Park Agency (Permit No: MPB 5590) and Ezemvelo KwaZulu-Natal Wildlife (Permit Number.: OP 3654/2017 and OP3646/2017).

CHAPTER 2

Identification of South African bat species by their echolocation pulses

Abstract

Using multiple microphone arrays to measure echolocation pulse source levels of free-flying bats does not allow one to determine the species of the bat being recorded. However, the echolocation pulses can be assigned to species based on pulse parameters used in conjunction with a reference library of pulses, the distribution records of bat species and the identification of captured individuals sampled in the area of recording. I used a combination of these sources of information to assign echolocation pulses recorded in nine localities across different biomes of South Africa, to species. Echolocation pulses of bats emerging from their own roosts were recorded, using the multiple microphone array system. From these echolocation recordings, multiple parameters were measured from pulses within each echolocation sequence to identify a representative pulse type for each species using the three sources of information. These initial species assignations were confirmed through multivariate analyses. First, uncorrelated variables were obtained from the set of measured pulse parameters using principal component analysis. Secondly, a discriminant function analysis (DFA) was applied to the new set of uncorrelated variables. Fourteen species of bats were reliably identified in this way. The DFA yielded overall correct classification rates of 83.16%. Pulses from ten species were correctly identified with > 80% accuracy. Pulses from the remaining four species, considered difficult to discern acoustically, nevertheless had classification rates, beyond 50%. Misclassified pulses and the resultant lower rates of classification was attributed to overlap parameters of pulses from species that were phylogenetically closely related. Canonical scores revealed root 1 accounted for 77.54% of the total variance and the parameter that loaded heaviest were on PC 1, PC 2 and PC 3.

Key words: acoustically, microphone array, pulse structure, reference library, source level

2.1 Introduction

The acoustic analysis of vocalizations has been applied to identify species in a wide variety of taxa, including birds (Favaro et al., 2015; Lachlan et al., 2016), mammals (Vannoni & McElligott, 2008; Burnham, 2017), insects (Wiley & Richards, 1978; Eskov, 2017) and amphibians (Narins, 2013). Most of these animals use sound to attract mates or defend territories (Amorim et al., 2016; Velásquez et al., 2018; Węgrzyn & Leniowski, 2020). Some mammals like, insectivorous bats use acoustic signals in the form of echolocation to forage (Neuweiler, 1990). Echolocating bats emit pulses that comprises several spectral (e.g., frequency) and temporal (e.g., duration) parameters (Barclay, 1982; Parsons, 2001; Schoeman & Jacobs, 2003). Depending on their foraging strategies (Fig. 2.1), bats exhibit striking variations in habitat use patterns as reflected in their echolocation pulses, which facilitates the perceptual tasks they perform while foraging. For instance, bats may adjust echolocation pulses, e.g., peak frequency, pulse duration, inter-pulse interval and source levels to cope up with the proportion (increment or reduction) of clutter in their foraging habitats (Jacobs et al., 2007; Holderied & von Helversen, 2003; Holderied et al., 2005; Schuchmann & Siemers, 2010; Fawcett & Ratcliffe, 2015).

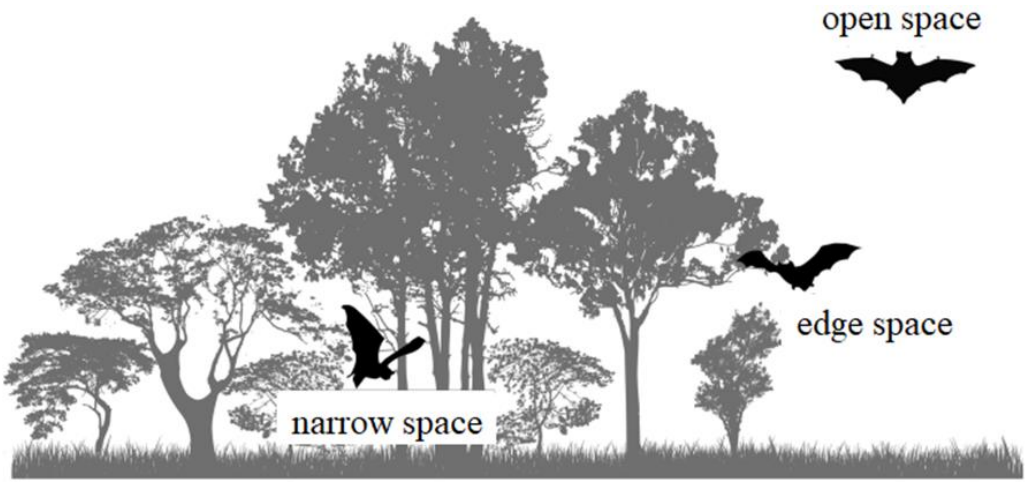

Figure 2.1. Bat species foraging modes. Open-space species fly higher to forage on the canopy of vegetation; edge-space species forage at vegetation edge; and narrow-space species forage in the interior sections of the vegetation [Adapted from Kalko et al. (2008)].

Aside from bats' foraging habitat, the adjusted echolocation pulse parameters have also been linked to differences in body size (Jones, 1999; Thiagavel et al., 2017). Patterns of species body size are ubiquitous in nature and differ greatly even among closely related species, having a major influence on echolocation pulse variation. Pulse frequency declines with body size for most species in families of bats (Hipposideridae, Rhinolophidae, Vespertilionidae, Emballonuridae and Molossidae) (Jones, 1999). However, most rhinolophids and hipposiderids emit pulses at higher frequencies for their body size than species in other families. This is because they generally channel more energy into the second, rather than the first, harmonic in their pulses. However, rhinolophids such *Rhinolophus clivosus* has higher peak frequency for their body size than expected (Jacobs et al., 2007). This deviation is associated with partitioning of foraging habitat, insect prey and the ability to communicate effectively in social interaction (Guillén et al., 2000; Jacobs et al., 2007). As a result, bats that share the similar acoustic space are able to separate their sonar frequency bands, avoiding overlap (Jacobs et al., 2007). As such, whilst echolocation pulse frequency variation may allow species identification in families of bats, it may also confound the process.

Echolocation is mainly used for orientation and foraging. Unlike bird song, for example, which is used in mate choice and is therefore species specific, identification of bats species solely by their echolocation pulses is more challenging because the echolocation pulses of species foraging in similar habitats may overlap considerably as a result of convergent evolution (Barclay et al., 1999; Barclay, 1999). Bats that forage in comparable habitats tend to have similar echolocation pulses, though being distantly related (Jones & Holderied, 2007). Similarly, closely related species occupying different habitats may also have overlapping echolocation pulses (Miller-Butterworth et al., 2005). In contrast, it is difficult to separate the contributions of phylogeny and perception to echolocation pulse designs associated with

different ecological environments. The reason being that some factors such as body size and beam shape are shaped by phylogeny and ecology and can influence echolocation pulse design considerably. Thus, both phylogeny and ecology could interact synergistically to cause pulse overlap making species identification problematical (Jones & Holderied, 2007).

Intraspecific variation in echolocation pulses as a result of ecological divergence also poses a challenge for species identification based on echolocation. Echolocation pulses of bats of the same species varied with geographic distance among localities because of ecological differences among localities (Thomas et al., 1987; Sun et al., 2013; Mutumi et al., 2016; Jacobs et al., 2017; López-Baucells et al., 2018). The identification of unknown individuals from another population based on pulse characteristics from one population can be challenging because the differences between populations are likely the result of species differences or within-species variation (Law et al., 2002).

and inter specific variation in echolocation pulse parameters is also influenced by the method and equipment Intra used for recording. O'Farrell and Gannon (1999) demonstrated that bat echolocation pulses recorded soon after hand-release and those recorded from bats emerging from roosts were no different. This is vastly severe for aerial insectivorous bats with narrow-band pulses. Previous studies proposed that the use of hand-release pulses as being the potential source of problems to successfully identifying species because these pulses may be heavily influenced by the stress caused by handling on the animal (Szewczak, 2000). It is preferable to reduce this potential bias by considering pulses recorded > 5 m far from the spot the bat is released and, if possible, only when the released bat began circling. An alternative approach is to record free-flying bats in the field with equipment such as the multichannel arrays placed at a distance > 10 m from the caves. Variability may also exist between the pulse emitted and the recorded signal by the bat detector. There is an effect of both relative position and distance of

the bat to the bat detector on the recorded pulse signal. With increasing distance, a low pass filter is applied to the signal as it travels due to its progressive attenuation, particularly at high frequencies. The result is a recorded pulse with a high-frequency that is reduced by the distance. (Goerlitz, 2018). Likewise, the received signal frequency varies depending on the angle at which the bat is facing the bat detector. In echolocation, pulses of sound are broadcasted pointing in a forward direction in an extremely narrow beam, and the bat detector primarily detects sounds that reaches the microphone within a narrow path (Jakobsen & Surlykke, 2010). Echolocation pulses will significantly suffer some form of attenuation depending on the frequency, especially pulses recorded from bats off-axis from the microphone. Lastly, echoes caused by surface reflection may degrade the recorded signal, causing it to differ significantly from the emitted bat's pulse (Schnitzler & Kalko, 2001).

Pulse variation that obfuscates species identity may also result from behavioral flexibility, developmental changes, or sexual dimorphism. Bats may adjust the parameters of their echolocation as a result of habitat heterogeneity or in the presence of and proximity to conspecifics (Obrist, 1995). For example, bats can increase or decrease their echolocation frequency in response to an increase or reduction in clutter, respectively (Fawcett & Ratcliffe, 2015). Similarly, unlike bats flying individually, bats flying in clusters emit pulses with different frequencies and/or temporal patterns (Obrist, 1995; Ulanovsky et al., 2004). Lastly, age and sex differences in echolocation pulses may also cause difficulties in species identification. For example, sex difference for *Rhinolophus hipposideros* (Jones et al., 1992) and *Rhinolophus capensis* (Odendaal & Jacobs, 2011) occurs where female bats emitted pulses at higher frequencies than males. However, *Rhinolophus euryale* is reported to have differences in sex resting frequency for adults and depending on locality of a population, females have higher pulse frequency than male and vice versa (Siemers et al., 2005). Because of these

challenges, most studies rely on a reference library which is created by recording known adults of different species and genders within a variety of habitats to get the full range of the echolocation pulse variation for each species possible (Neuweiler et al., 1987; Monadjem et al., 2010; Odendaal et al., 2014; Jacobs & Bastian, 2018). Finally, Jakobsen et al. (2013) also proposed that variations in the frequency of echolocation pulses are triggered by bats' need to adjust their signals to achieve an optimum acoustic field of view. All these factors can, to some extent, complicate the identification of species based on echolocation pulses.

Regardless of these challenges, echolocation pulses have been satisfactorily used to identify free-flying bats in range of research conducted on a global scale (O'Farrell & Miller, 1997; Parsons, 2001; Schoeman & Jacobs, 2003; Hughes et al., 2011). Bat assemblages consist of species belonging to different families, with varying body sizes, foraging strategies and pulse characteristics, but occupy the same space. The latter increases the probability that they will interact with each other rather than with bats which occupy a different space. Bats assemblages have several distinct approaches to echolocation that evolved, with each approach differing in the structure of the echolocation pulses and in how the pulses are separated from echoes. Each approach has resulted in the evolution of a unique set of auditory adaptations that enable signals to be received and processed by the brain (Neuweiler, 1990). Most echolocating bats, the low duty cycle bats (LDC) in particular, avoid forward masking, such that louder outgoing signals reduce the sensitivity of the animal to the weaker returning echoes, by separating pulse and echo in the time domain (Fenton et al., 1995). An alternative strategy is found in high duty cycle (HDC) echolocators that separate pulse and echo in the frequency domain (Schuller, 1974). The duty cycle (DC) is the percentage of time that a bat is producing sound (Fawcett et al., 2015). Duty cycles of LDC bats typically range between 5 - 20%, whereas those of HDC bats range between 25 - 70% (Fenton et al., 1995; Fenton et al., 2012). Most of the bats in

assemblages have echolocation pulses that are relatively conserved within species, but vary in spectral and temporal parameters among species (Fenton et al., 1998). The differences in pulse features are large enough in some bat assemblages to facilitate their identification based on their pulses. For example, North American species like *Euderrna maculatum* and *Lasiurus cinereus* are unmistakable in many locations because of their low-frequency echolocation pulses (Fenton & Bell, 1981; Rydell et al., 2002). Likewise, in southern Africa, *Hipposideros caffer* (133-144 kHz), *Nycteris thebaica* (83 kHz) and *Cloeotis percivali* (206 kHz) (Fenton & Bell, 1981; Jacobs, 2000) have pulses uniquely high in frequency, facilitating species identification and can be recognized. Similarly, *Tadarida aegyptiaca* has pulses uniquely low in frequency and is easy to tell apart from other low duty cycle (LDC) bats in assemblages (Schoeman & Jacobs 2003). Despite their similar pulse structures, the frequency of the constant component of the pulses of Rhinolophus species are sufficiently variable in most cases to facilitate species identification (Monadjem et al., 2007; Jacobs et al., 2007; Odendaal et al., 2014). For example, *Rhinolophus clivosus* (91.2 kHz) and *Rhinolophus eloquens* (44 kHz) are similar in size but have very constant component frequencies that are very different. Similarly, *Rhinolophus deckenii* (73.8 kHz) and *Rhinolophus fumigatus* (55.1 kHz) are similar in size but also have very different pulse frequencies (Jacobs & Bastian, 2018).

The underlying principle to correctly identify species is to compare an unknown pulse we wish to identify with a library of pulses for individuals of known age, sex and species. The unknown pulse is then attributed to the species which has the most similar reference pulse. Fortunately, such a library of references pulses exists for Southern African bat species compiled in the laboratory of David S. Jacobs at the University of Cape Town. The database continues to be supplemented by recording echolocation pulses from captured bats because of the ever-expanding research by students and researchers in the same laboratory. Captured bats are identified from their morphological characters and compared with available taxonomic keys.

Furthermore, identification of species is based on distribution records of bats (Monadjem et al., 2010). For instance, less common species such as *Cistugo seabrae* that is endemic in desert biome have overlapping echolocation parameters with *Neoromicia capensis* that is widely distributed, although the two species do not co-occur. So, it is possible to identify these species depending on their localities and habitat type.

My aim in this chapter, therefore, was to identify and assign echolocation pulses of unknown free-flying bats to species. Individuals emitting echolocation pulses were unknown because no attempt was made to capture them during the recording of their echolocation pulses so that their natural echolocation behavior was not disrupted. This was necessary to obtain natural echolocation source levels as far as possible. Accurately assigning recorded echolocation pulses to species allowed the calculated source levels to be assigned to the correct species. Multivariate statistical analysis of pulse parameters was used to correctly assign pulses to species.

2.2 Material and methods

2.2.1 Study sites

Field studies were conducted at nine different sites, each associated with a different biome in South Africa (Mucina & Rutherford, 2006). These sites (Fig. 2.2) included the following:

Senderlings drift (LKG; 28° 15' 33.012"S, 17° 1' 15.996"E)

This site is located in the desert biome along the mountainous section of the Richtersveld region linked to central north of the Namib desert (Mucina & Rutherford, 2006).

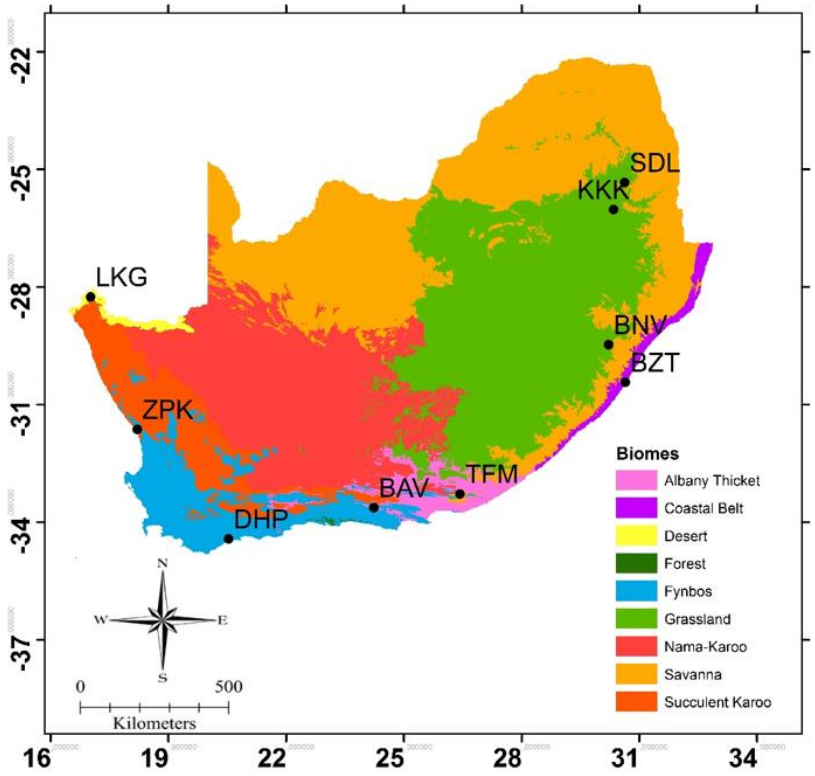

Figure 2.2. Biomes of South Africa (Mucina & Rutherford, 2006) and sampling localities for bat species. Abbreviations: Lekkersing (LKG), Zoutpansklipheuwel (ZPK), Dehoop nature reserve (DHP), Baviaanskloof (BAV), Table farm (TFM), Bazley tunnel (BZT), Beneve cave (BNV), Kalkounkrans cave (KKK) and Sudwala caves (SDL).

Summer temperatures typically reach 30°C along the inland borders. Areas in low latitude experience temperatures more than 38°C (Young & Desmet, 2016). Cold temperatures occur along the inner border of the desert. The average rainfall is 10 mm in the west, with 80 mm in the interior section of the desert and, coastal fog in the winter (Young & Desmet, 2016). The biome is characterized by dominance of dwarf, open, sparsely distributed succulent shrubbery (Young & Desmet, 2016).

Sudwala (SDL; 25° 20' 15"S, 30° 37' 57" E)

Sudwala, near to Mbombela, Mpumalanga Province, is situated in a Savanna biome which stretches from the far-northern parts of Northern Cape, the western and north-eastern parts of North-West Province (Mucina & Rutherford, 2006). The biome has a grass as ground layer

and woody vegetation forming clear-cut upper layer. Generally, the vegetation cover constitutes Shrubveld on the upper layer, the dense layer is known as Woodland, and the transitional phases commonly known as Bushveld. It is also characterized by prolonged high temperatures (18 ^{o}C - 26 ^{o}C) during summer, while the daily minimum temperature during winter is much more variable but remains above 10 ^{o}C. Most parts of the Savanna experience long and short rainfall season of 235 mm per year and the strong rainfall of 1000 mm per year occur during summer (Rutherford & Westfall, 1994).

Kalkoenkrans Caves (KKK; 25° 4' 0" S, 30° 1' 0" E)

These caves are situated in the Grassland biome which covers the high central plateau of South Africa, and the interior parts of Kwa-Zulu Natal and the Eastern Cape (Mucina & Rutherford, 2006; Okitsu, 2010). The Grassland biome is dominated by vegetation of mostly diverse grass species with some trees located on the slopes and along riverbeds. Fire plays a crucial role in revitalizing grass and most flowers species. The dominant grass species are *Themeda triandra*, *Aristida junciformis* sub sp. *galpinii*, *Elionurus muticus* and *Eragrostis plana*. Grasslands usually receive 400 mm to 2000 mm of rainfall each summer. Winters can be cold, and frost can occur (Okitsu, 2010; Mucina & Rutherford, 2006).

De Hoop Nature Reserve (DHP; 34°26′3″S, 20°32′52″E)

This reserve is in the South-Western Cape Province of South Africa. It is endowed with Fynbos biome and, is characterized mostly by plant species belonging to the families Restionaceae, Ericaceae, Fabaceae and Asteraceae (Mucina & Rutherford, 2006; Okitsu, 2010). The yearly rainfall in DHP range from 250 to 530 mm (Mucina & Rutherford, 2006), though rainfall is significantly higher throughout the time of winter and autumn (600 mm to 800 mm) than summer the months of the year when it is the lowest (Mucina & Rutherford, 2006; Okitsu,

2010). In the DPH Guano Cave, there is the largest known bat colony in South Africa, with approximately 20,000-30,000 individuals (McDonald et al., 1990).

Bazley tunnel (BZT; 30°26′0″S, 30°39′0″E) *&* ***Benave Cave*** (BNV; 29°28′0″S, 30°13′0″E)

The tunnel and the cave are located near Durban in KwaZulu-Natal province, in the Indian Ocean Coastal Belt (IOCB) biome. The biome constitutes thick impassable vegetation, monopolised by spiny succulent trees and shrubs (Mucina & Rutherford, 2006). The climate gets drier as one travels inland; however, the valleys are cooler due to their shady origins, with maximum (15°C and 24°C) and minimum (10°C and 15°C) temperatures. Coastal Forest is limited to higher rainfall zones, with more than 725 mm of rainfall in summer period and an average annual rainfall of 525 mm in winter rainfall (Rutherford & Westfall, 1994).

Zoutpansklipheuwel (ZPK; 33°37′48″S, 18°12′36″E)

This is an area that host a bat cave in the Succulent Karoo biome. The biome stretches from the south-west through the north-western areas of South Africa and into southern Namibia (Mucina & Rutherford, 2006). A dominant vegetation type in this region is dwarf, succulent shrubs, most notably the Stonecrops (Crassulaceae), Cactuses (cactacea) and the Vygies (Mesembryanthemaceae). In the spring, widespread flowering exhibits of annuals chiefly of daisy family (Asteraceae) occur, often on degraded lands. Grasses of the C3 type are uncommon, except in a few sandy areas (Low & Rebelo, 1996). The area is arid with rainfall varying between 20 and 290 mm per year. During summer, temperatures over 40 °C are common. Dehydrating, hot, and berg winds may manifest itself all year round (Low & Rebelo, 1996).

The eastern Cape has historically been identified as a transition and complex hotspot of vegetation types. It is a significant transition point for climatic, its topography, and geology,

and as a result, four major ecoregions converge in this area (Cowling, 1983). Two of the sampling sites were within biomes that converge in eastern Cape, and they include:

Table Farm (TFM; 33°17′0″S, 26°26′0″E)

Table Farm is in the Eastern Cape Provinces within the Albany Thicket biome in arid and semi-arid areas. A high diversity of plant species is widely spread and, include leaf and stem succulents, woody shrubs and, numerous grass species (Cowling, 1983). Some of the more locally uncommon and rare plants are in the families of Crassulaceae and Aizoaceae (Vlok et al., 2003). They are mainly found to be growing in dry areas on the rocky habitats. Most of the plant growth forms and taxonomic diversity in this biome are indicative of a transition phase between several vegetation types (Lubke et al., 1986; Victor & Dold, 2003). Rainfall varies between 200–950 mm pa and reaches at optimum level mostly in March but may also be reached in November. The area experience high temperatures in summer, sometimes reaching over 40 °C, while the winter months are cold and rainy.

Baaviansklоof (BAV; 33°17′60″S, 24°48′0″E)

Baaviansklоof is in the Nama Karoo biome in the Eastern Cape Province and is characterised by vegetation such as the sclerophyllous vegetation type (e.g., C_3-photosynthetic plants). Also found in the biome are succulent karroid plants, which are mainly photosynthesized by through Crassulacean Acid Metabolism (CAM). These vegetation types dominate in the eastern Cape, extending from the north-west, south-eastwards to the Grahamstown area (Lubke et al., 1986). The vegetation type is dominated by grasses and dwarf shrubs. Grasses are more prevalent in valleys and on sandy soils, and less prevalent on clay soils (Lubke et al., 1986; Victor & Dold, 2003). Summer rain varies between 100 and 520 mm annually.

2.2.2 Data collection

2.2.2.1 Recording echolocation pulses

Bat echolocation pulses were recorded using an eight-channel microphone array system. The array system was comprised of two multiple microphone arrays (described below) connected to a Dell laptop (Latitude E7240) via an eight-channel Avisoft-UltraSound Gate 816 recorder. Bat pulses were recorded across all biomes in-situ. Where possible, bat pulses were recorded at both roosting and foraging areas. Detailed descriptions of how echolocation pulses were recorded are given in Chapter 3.

The two microphone array systems each consisted of 4 microphones omnidirectional electret ultrasound microphones (Avisoft Bioacoustics, Knowles FG-O, Berlin, Germany), one positioned at the intersection of three arms that were equal in length, and 120^{o} angle between each of them. The remaining 3 microphones were placed at the ends of the three arms (Fig. 2.3).

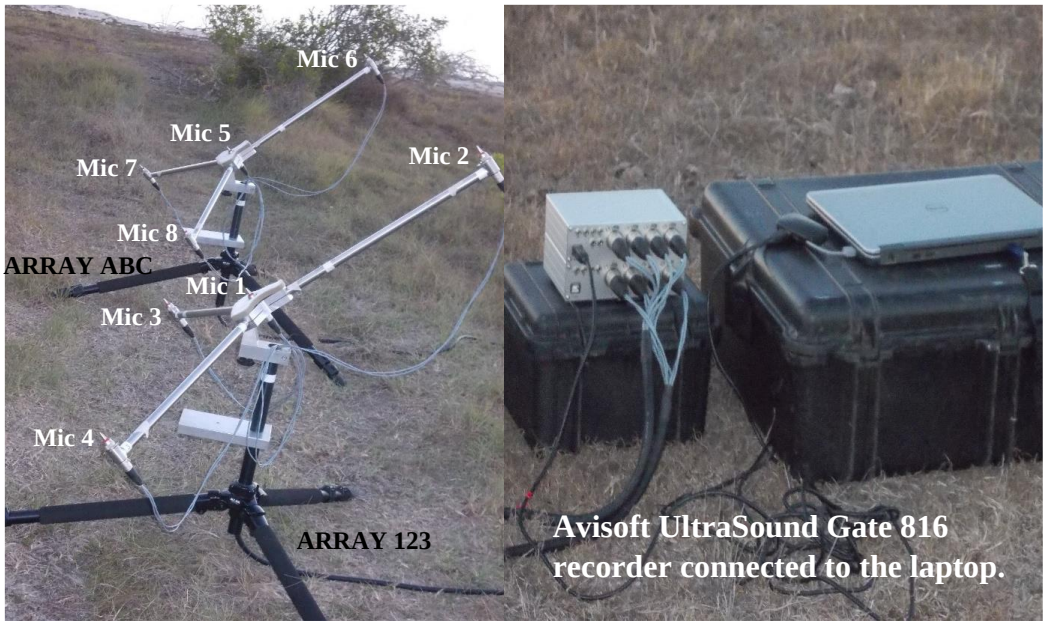

Figure 2.3. Multiple microphone array connected to the Ultrasound Gate receiver for recording echolocation pulses in the field. *Mic-Microphone.

The positioning of the arrays at each site within a biome was dependent on the species that were targeted. Thus, to record bats, that are highflyers, requires that the microphone arrays are set about 2 m high and spaced 3 m apart. While recording low flying bats, the microphone arrays were set 0.8 m high and spaced 1.9 m apart. Through trial and error, I established that these distances would allow the bats' sonar beams to impinge directly on the microphone arrays, as close to the central microphone's acoustic axis as possible. The spacing of the two arrays was particularly crucial for high duty cycle (HDC) bats that emitted highly directional pulses. For the same reason the arrays were also angled so that they were as perpendicular to the flight paths of the bats as possible.

2.2.2.2 The structure of echolocation pulses

Oscillogram, spectrogram and power spectrum for representative echolocation pulses for each species were generated using BatSoundPro software (version 3.31a, Pettersson Elektronik AB, Uppsala, 81 Sweden). Recordings were analyzed using a sampling frequency of 500 kHz, a resolution of 16 bits mono. For both spectrogram and power spectra, I generated fast Fourier transforms (FFT) of size 512 samples, Hanning window that was set at a low threshold of 500–200 ms/div to get a fine resolution, whereas the power spectrum was from an FFT size 1024, Hanning window. BatSoundPro enables the generation of high-resolution displays of the; spectrogram (a visual representation of how the frequency of a pulse changes over time), oscillogram (depicts change in the pulse amplitude over time) and power spectrum (depicts the distribution of the energy in the pulse across the frequency range of the pulse). In combination these effectively described the type of pulses emitted by LDC and HDC bats.

2.2.2.3 Selection of the echolocation recordings for analysis

Recordings of search phase echolocation pulses for bat species were selected in a range of habitats across species' distributions. The idea behind focusing only on pulses emitted during the search phase was to lessen the impacts of intra-specific variability in my analyses

(Schnitzler & Kalko 2001). A sequence of 6-10 pulses of good quality (high signal-to-noise ratio) were used from each recording. Recordings were assumed to be from different individuals. For species that used harmonics, measurements were taken from the pulse used as the main harmonic (the harmonic in which the bat put most of its pulse energy into). The integration of pulse variation in the sample data forbids biases from being generated for any specific recording scenario or recording technique. It also gives the acoustic identification tool more flexibility and generalization (Walters et al., 2012, 2013; Zamora-Gutierrez et al., 2016).

2.2.2.4 Acoustic analyses of echolocation pulses

Several acoustic parameters were reliably measured using Avisoft SASLab Pro (Avisoft Bioacoustics,Version 4.2, Glienicke, Germany) supported by USB key (dongle) device driver (MAX HL MN720427/OZRWQ/31060647-1, Berlin Germany). The acoustic measurements for HDC bats were made from the second harmonic of the pulses since this harmonic contains the considerable amount of energy. (Pye & Roberts, 1970; Jacobs & Bastian, 2016). Low duty cycle (LDC) bats usually have pulses with more than two harmonics, however, in these instances, most of the pulse energy is concentrated in the first harmonic (reviewed in Jacobs & Bastian, 2016). However, a LDC bat like *Nycteris thebaica*, the harmonic with maximum energy may vary. Therefore, these harmonics have the best signal-to-noise ratio. Both low-pass filter (removes signal components above a specified cut-off frequency) and high-pass filter (removes signal components below the specified cut-off frequency) were applied to the selected recordings (Table 2.1) using the Time Domain Filter function, Hamming window type of Avisoft. The filter has a Finite Impulse Response (FIR) which allows the removal of any background noise from recordings without phase distortion of signals. In case the background noise overlaps with the echolocation pulse signal, it was possible to completely remove that noise manually by using the command Tools/Cursors/'Standard eraser cursor'. Detailed measurements of time and frequency parameters (see below) were performed in Avisoft-

SASLAB Pro (Version 5.2.09, Avisoft Bioacoustics, Berlin, Germany) using the Automatic Parameter Measurement tool of the spectrogram window (Avisoft-SASLAB Pro). All the spectral and temporal measurements were calculated to a threshold of -20 dB, which was adjusted depending on the structure of the echolocation pulses and the background noise level. The threshold was automatically referenced to the maximum magnitude of value ranging from -9 dB to -15 dB, which changes depending on the quality of the echolocation pulses and the level of the background noise. In recordings with a high level of background noise, this threshold was not set too low. The (+) and (–) buttons were used to interactively find the best settings. In this way, the option was used to safely detect elements exhibiting varying amplitudes and was recognized more precisely.

Table 2.1. Low-pass and high-pass filters applied to the selected echolocation recordings.

Species	**Frequency Range (kHz)**	**Low-pass frequency (kHz)**	**High-pass frequency (kHz)**
Tadarida aegyptiaca	18-25	35	10
Sauromys petrophilus	26-32	45	10
Neoromicia capensis	35-45	65	25
Cistugo seabrae	35-46	65	25
Nycteris thebaica	55-83	120	50
Myotis tricolor	45-55	125	18
Miniopterus natalensis	48-57	120	35
Miniopterus fraterculus	58-63	120	35
Hipposideros caffer	133-145	150	95
Rhinolophus damarensis	82-84	95	55
Rhinolophus clivosus	89-92	97	55
Rhinolophus simulator	79-82	90	50
Rhinolophus swinnyi	99-102	115	70
Rhinolophus capensis (d)	72-75	90	50
Rhinolophus capensis (f)	82-84	90	50
Rhinolophus capensis (k)	84-86	95	50

Note: *Rhinolophus capensis* show variation in frequency across its distributional range from *d*-Desert, *f*-Fynbos and *k*-Karoo biomes.

Spectrograms for analysis of the bat species were generated using the FlatTop window, Fast Fourier Transform length (FFT length) 256 points, frame size 100% and overlap 50%. Temporal and spectral parameters measured for the LDC and HDC were pulse duration (ms), inter-pulse interval (ms), start time (ms), end time (ms), distomax (ms), peak to peak amplitude (rms). Other parameters that were measured for their start, centre, end, maximum and mean include, peak frequency (kHz), minimum frequency (kHz) and maximum frequency (kHz), band width (kHz) and peak amplitude (dB) (Table 2.2). Measuring numerous echolocation pulse parameters was to guarantee that when undertaking Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) there are enough variables to permit species to be dependably classified into independent groups.

Table 2.2. Echolocation pulse parameters measured from bat assemblages using the Automatic Parameter Measurements tool. Spectrogram (fast Fourier transform window size = 265 points, frame size 100%, overlap 50%, window flat top) of Avisoft-SASLAB Pro (Avisoft Bioacoustics, Berlin, Germany) of Avisoft-SASLAB Pro (Avisoft Bioacoustics, Berlin, Germany).

Pulse parameter	***Description of parameters***
Pulse duration (ms)	The time from the start to the end of an echolocation pulse.
Pulse Interval (ms)	The time between the start of one echolocation pulse to the start of the next echolocation pulse in a sequence.
Start time (ms)	The time at the beginning of the echolocation pulse.
End time (ms)	The time at the end of the echolocation pulse.
Distomax (ms)	The time from the start of the echolocation pulse to the point of maximum amplitude of the echolocation pulse.
Peak-to-peak amplitude (V)	The change between highest amplitude value and the lowest amplitude value of the echolocation pulse.
Peak amplitude (dB)	The maximum amplitude (magnitude-dB) of the instantaneous spectrum at the peak frequency of the entire echolocation pulse.

Peak frequency (kHz)	The frequency at the peak (maximum amplitude) in the power spectrum of the entire echolocation pulse.
Minimum frequency (kHz)	Minimum frequency reached (measured at a threshold of -20 dB) below the peak frequency of the power spectrum of the entire echolocation pulse.
Maximum frequency (kHz)	Maximum frequency reached (measured at a threshold of -20 dB) below the peak frequency of the power spectrum of the entire echolocation pulse.
Bandwidth (kHz)	The difference between the maximum and minimum frequency at -10 dB below the peak frequency.

2.2.2.5 Initial species identification of echolocation pulses

Based on their temporal and spectral parameters (see Table 2.1 & 2.2) including the harmonic with maximum energy (1st harmonic for most LDC and 2nd harmonic for all HDC species), pulses were first assigned to LDC or HDC echolocation. Among HDC bats, populations of *Rhinolophus capensis* differ in Constant Frequency (CF) frequencies in different geographical localities. Despite the desert populations having CF frequencies of 75 and 84 kHz, I classified these as *Rhinolophus capensis* and *Rhinolophus damarensis*, respectively, because genetic analyses indicated that these were their species affiliations (Jacobs et al., 2013; Odendaal et al., 2014). Pulses in each of these two groups were then assigned to species and the structure of the pulses using an echolocation reference library (compiled by D. S. Jacobs), and the distribution of records of the species**.** The pulses in the echolocation reference library were recorded from bats (from hand-held individuals for HDC bats or hand released individuals for LDC bats) whose species identity was later confirmed from genetic analyses on skin biopsies taken from bats when still in hand. This initial identification of species was possible because of the already available reference echolocation library for the bats of southern Africa. This involved extensive fieldwork in different localities all over southern Africa, to record echolocation pulses using time expansion bat detectors. Potential roost sites (caves, sinkholes, disused mineshafts, old

buildings) within the sampling locations were explored. Bats within the roost site were captured by using hand nets and where roosts were inaccessible, harp traps and mist nets put at the entrance or exits of the roost. Captured bats, particularly HDC bats were recorded while being held in the hand. Low duty cycle bats were recorded using hand-release, but the clutter foragers recorded in a flight room. Both LDC and HDC were at first identified in-hand using taxonomic keys and confirmed these identifications with genetic markers (Eick et al., 2005; Miller-Butterworth et al., 2007; Stoffberg et al., 2010; Jacobs et al., 2014; Odendaal et al., 2014; Foley et al., 2015; Dool et al., 2016). In this way, geographic intraspecific difference in the resting frequency of HDC and the peak frequencies of LDC species became known giving a sense of how specific the pulses of each species were. These confirmed species were assembled as part of the extensive database of echolocation reference library available in David Jacobs laboratory at the University of Cape Town.

2.2.3 Statistical analysis

Based on the measured parameters the echolocation pulses were initially assigned to species by using the parameters of the pulses jointly with a reference library and the distribution records of bat species, as well as the species identification of captured bats caught at some of the localities sampled in this study. This initial assignment was tested using Discriminant Function Analysis (DFA) (Russo & Jones, 2002; Fukui et al., 2004). Considering that strong correlations are common between echolocation pulse spectral features (Hughes et al., 2011), a Principal Component Analysis (PCA) was conducted on the echolocation pulse parameters to extract uncorrelated principal components because DFA assumes that variables are uncorrelated. A DFA was performed on these extracted principal components with eigen values >1 (Kaiser, 1960). PCA and DFA analyses were preceded by log-transformation of echolocation pulse parameters to ensure linearity. Wilk's lambda values were obtained with a multivariate analysis

of variance (MANOVA) to test for statistical significance of DFA models. The standardized discriminant function coefficients were used to determine the contribution each variable made to the classification of pulse parameters to species. Mahalanobis–squared distance between species groupings generated by the DFA was used as a quantitative estimate of the relative separation of species in acoustic space. For each species, descriptive statistics (mean ±SE) and ranges were calculated. All tests were carried out with STATISTICA 13.0 (StatSoft, Inc).

2.3 Results

2.3.1 Structure of echolocation pulse

A single representative pulse for each species was selected and used to generate oscilloscopes, spectrograms and power spectra to display the typical temporal, spectral and power structure, respectively, of the pulses for each species (Figs. 2.4, 2.5 & 2.6). Echolocation pulses of all LDC bat species were composed primarily of a frequency modulated component followed by a quasi-constant frequency component (FM/QCF) except for *M. tricolor* which has only a steeply modulated FM component and *N. thebaica* which was characterized by frequency modulated component and multiple harmonics pulses. In contrast, *T. aegyptiaca* and *S. petrophilus* had narrowband quasi-constant frequency pulses (Fig. 2.4). Hipposiderid (*H. caffer*) emitted CF/FM echolocation pulse type, that is, pulses with a short, constant-frequency component (CF) followed by a short, terminal frequency-modulated sweep (FM) (Fig. 2.6). Rhinolophid species (*R. capensis*, *R. damarensis, R. clivosus, R. simulator* and *R. swinnyi)* emitted typical FM/CF/FM echolocation pulses, that is, pulses with a long, purely constant-frequency component (CF) before and after a short, frequency-modulated sweep (FM) (Fig. 2.5). Echolocation pulses from *H. caffer* showed higher values for all frequency parameters, which did not overlap with Rhinolophid species.

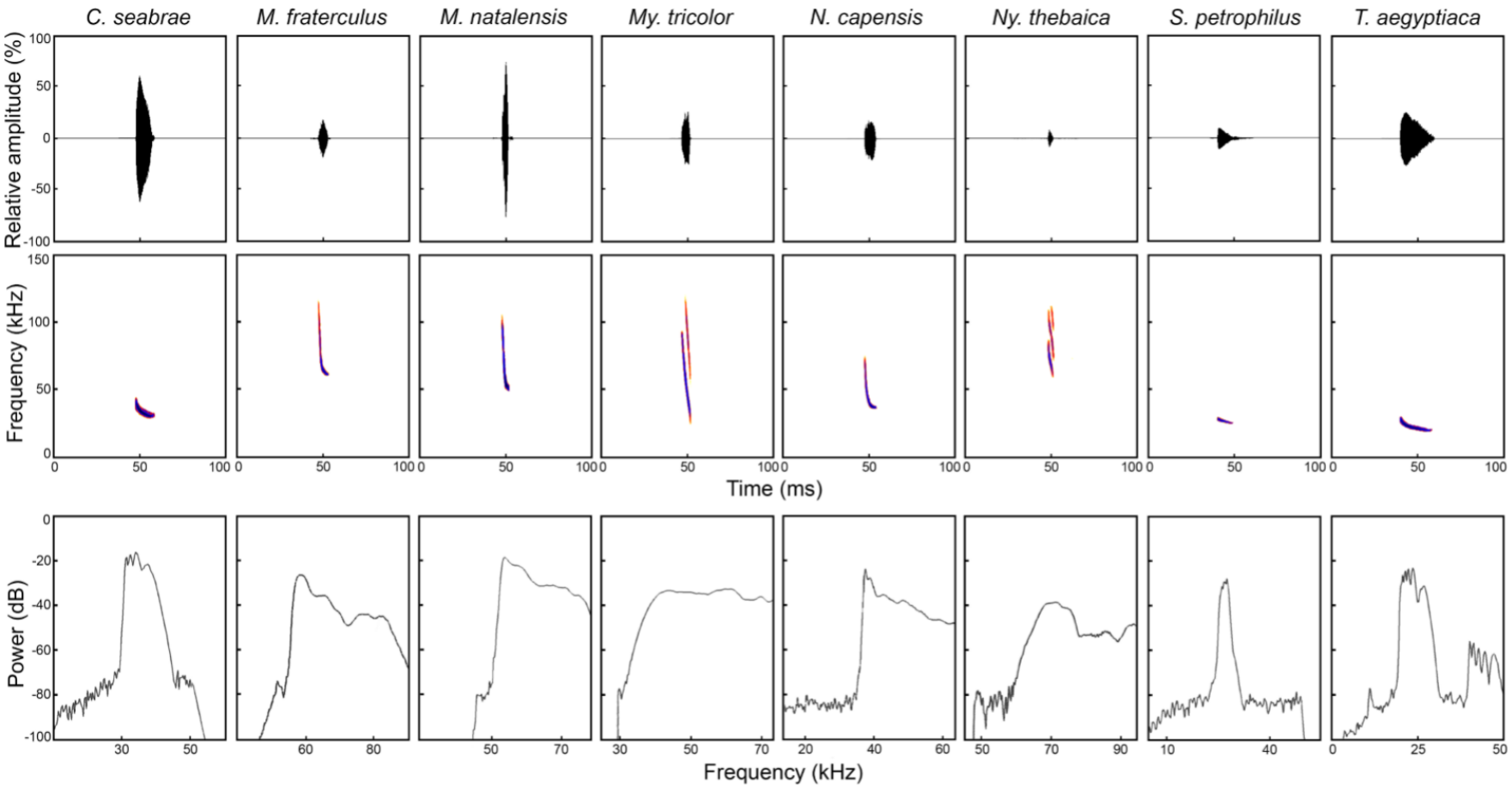

Figure 2.4. Oscillogram (top), spectrograms (middle) and power spectra (bottom) of representative echolocation pulse for LDC bats

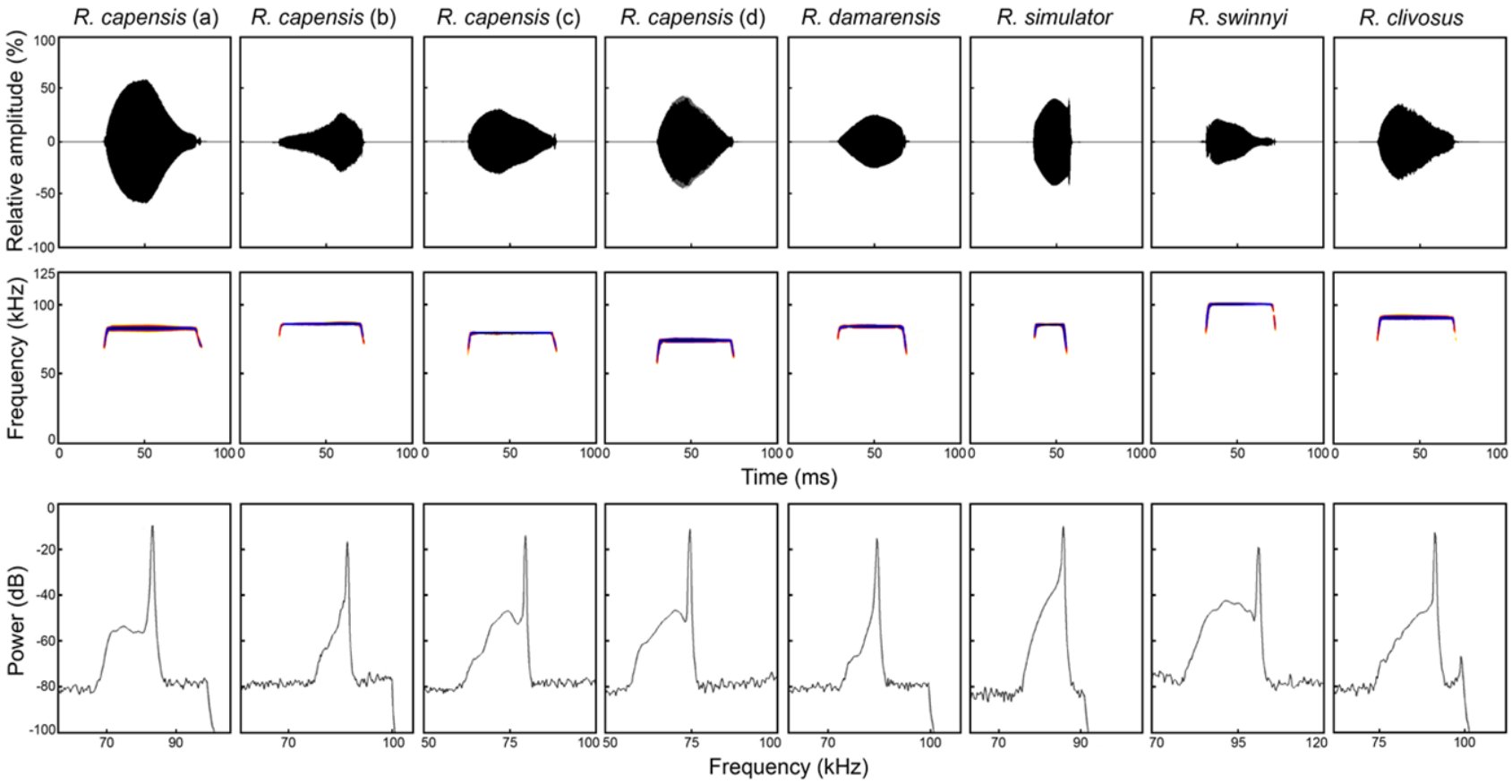

Figure 2.5. Oscillogram (top), spectrograms (middle) and power spectra (bottom) of representative echolocation pulse for HDC bats: *R. capensis* (a)-Fynbos; *R. capensis* (b)-Albany thicket; *R. capensis* (c)-Succulent Karoo; *R. capensis* (d)-Desert

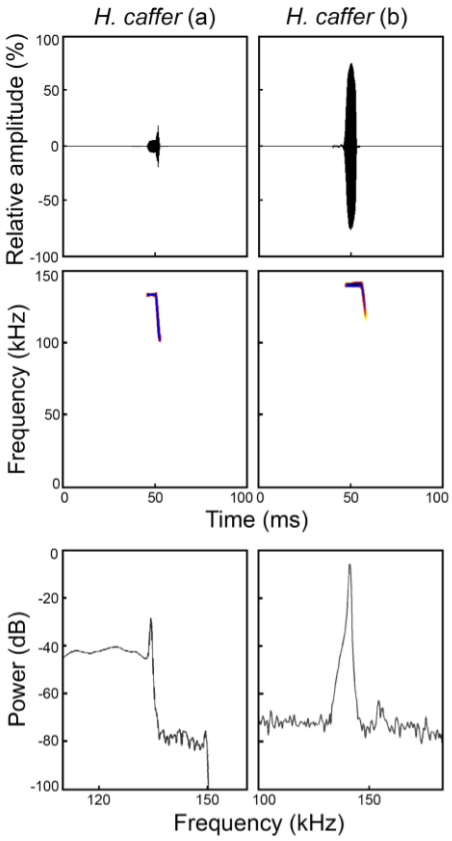

Figure 2.6. Oscillogram (top), spectrograms (middle) and power spectrum (bottom) of representative echolocation pulse for *H. caffer* that occur in Desert (a) and Savanna (b) biomes.

2.3.2 Identification of species

The initial classification yielded six species of HDC bats which included *R. capensis, R. clivosus, R. damarensis, R. simulator, R. swinnyi* and *H. caffer* (Table 2.3). Species such as *R. damarensis, R. capensis* and *R. simulator* overlap in their peak frequencies as indicated in the probability table (Table S2.1) by the classification matrix of pulses for each species. However, since populations of *R. capensis* differ in frequencies along a gradient of geographical distribution (Odendaal & Jacobs, 2011), it was possible to single out which population would possibly be problematic when assigning peak frequency to species. In that case, *R. capensis* in Fynbos biome overlap in peak frequency with *R. damarensis* in desert biome. Whereas *R. simulator*s overlap in their peak frequency with *R. capensis* in Succulent Karoo, it occurs in a separate location. So, I relied on previous records to confidently assign peak frequency to

species at the initial stage. Initially, I had eight LDC species which included, *N. thebaica, M. natalensis, M. fraterculus, N. capensis, C. seabrae, T. aegyptiaca, M. tricolor* and *S. petrophilus* (Table 2.4 & Table S2. 1). Both *M. natalensis* and *M. fraterculus* co-occur in some localities and known to have similar echolocation pulse structure (Miller-Butterworth et al., 2005). However, their peak frequencies may overlap because of their behavioral response during foraging. I therefore opted to classify them based on a minimum and maximum cut off frequencies; the former was assigned 48-57 kHz and the latter 58-64 kHz. Although *M. natalensis* and *M. tricolor* depicted overlap in peak frequencies, I could visually differentiate their pulses because the later has a distinct vertical shape from other recorded species (Fig. 2.4). Also, *N. capensis* and *C. seabrae* showed slightly overlapping peak frequencies and since the latter species is endemic in desert area and they also don't co-occur as indicated in the distribution record (e.g., Monadjem et al., 2010), simplified the classification of the two species. The first four principal components (PC) had Eigen values > 1 and accounted for approximately 89.23% of the variation among species (Table 2.5 & Fig. S2.2). PC1 which accounted for 48.26 % of the variation contained variables associated with frequency including peak frequency (maximum), maximum frequency (maximum) and maximum frequency (centre) (Table 2.5 & Table S2.2). PC2 which accounted for 19.91 % of the variation described differences in time/frequency structure. Variables which loaded high on PC2 included bandwidth (center), bandwidth (maximum) and bandwidth (mean) (Table 2.5 & Table S2. 2). Variables which loaded high on PC3 which accounted for 16.94% included peak amplitude (start), peak amplitude (center) and peak amplitude (maximum). Finally, PC4 which accounted for 4.12% of the variation with bandwidth (end) as a parameter that was loaded high (Table 2.5 & Table S2. 2).

Table 2.3. (Mean ± SD) for echolocation pulse temporal (ms) and spectral (kHz) parameters of six high duty cycle (HDC) bat species recorded in different biomes of South Africa.

Acoustic Parameters	***R. capensis* *(n=68)***	***R. damarensis* *(n=16)***	***R. clivosus* *(n =29)***	***R. swinnyi* *(n=14)***	***R. simulator* *(n=13)***	***H. caffer* *(n=16)***
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Pulse duration	40.4±9.3 (21.2-63.8)	43.8±9.3 (24.7-59.7)	41.6±8.4 (21.1-59.8)	44.1±5.7 (37.0-53.1)	40.8±8.5 (20.7-49.8)	14.6±20.6 (4.7-64.4)
Pulse interval	77.1±19.7 (36.4-131.1)	101.2±18.8 (60.5-138.7)	84.7±22.3 (42.9-146.0)	79.6±9.3 (64.5-94.7)	78.1±16.6 (44.7-98.9)	66.6±117.5 (16.5-405.6)
Disttomax	19.3±5.4 (10.1-36.6)	20.4±6.5 (9.1-33.6)	18.8±3.8 (11.0-25.7)	19.4±4.1 (12.0-27.8)	17.2±5.7 (7.4-32.5)	8.8±9.7 (3.7-32.7)
Peak-to-peak ampl	259.1±143.6 (30.9-813.3)	225.0±133.9 (62.8-591.8)	232.4±147.1 (40.9-531.9)	135.6±70.8 (47.6-293.2)	204.5±110.6 (29.2-418.8)	154.4±232.2 (28.0-723.4)
Peak freq (max)	80.2±4.5 (72.4-86.5)	83.4±0.5 (82.1-84.3)	90.7±0.8 (89.1-92.6)	99.9±0.6 (98.6-100.9)	82.1±1.0 (80.8-83.5)	131.6±5.2 (124.6140.1)
Peak ampl (max)	20.6±4.9 (37.3-8.9)	21.6±4.4 (31.3-13.3)	22.6±6.1 (35.2-12.3)	27.0±5.8 (38.2-19.7)	26.2±6.2 (39.1-18.3)	30.3±9.3 (41.5-10.2)
Min freq (max)	76.8±4.5 (69.1-83.0)	80.2±0.7 (78.7-80.9)	87.4±0.7 (85.5-89.1)	96.5±0.7 (95.1-97.8)	78.6±0.9 (77.3-79.8)	127.6±5.4 (119.8-136.8)
Peak freq (mean)	80.2±4.4 (72.1-86.3)	83.2±0.4 (82.1-84.1)	90.6±0.8 (89.1-92.6)	100.0±0.9 (98.6-101.9)	81.8±1.0 (80.8-83.2)	134.7±3.2 (131.8-140.8)
Peak ampl (mean)	24.8±5.3 (43.4-13.2)	25.5±4.6 (37.4-17.3)	26.8±6.4 (40.2-16.2)	31.5±5.8 (42.6-24.4)	31.5±6.5 (43.1-21.2)	33.9±9.7 (44.7-12.6)
Bandw (mean)	8.0±0.3 (7.3-9.2)	7.8±0.4 (7.0-8.2)	8.3±0.7 (7.5-10.9)	8.4±0.7 (8.5±0.8)	8.5±0.8 (7.2-9.4)	10.7±3.3 (8.0-17.6)

n = number of trajectories; () = range. Spectrogram setting (FFT window size=265 points, frame size 100%, overlap 50%, window flat top) of Avisoft-SASLab Pro

Table 2.4. (Mean ± SD) for echolocation pulse temporal (ms) and spectral (kHz) parameters of eight low duty cycle (LDC) bat species recorded in different biomes of South Africa.

Acoustic Parameters	*M. natalensis (n=80)*	*M. fraterculus (n=28)*	*N. capensis (n=41)*	*M. tricolor (n=32)*	*T. aegyptiaca (n=13)*	*C. seabrae (n=17)*	*S. petrophilus (n=14)*	*N. thebaica (n=12)*
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Pulse duration	3.9±1.1 (2.1-7.0)	2.8±0.9 (1.3-5.2)	6.1±2.1 (2.9-11.3)	2.4±0.8 (1.2-4.3)	14.9±1.7 (12.2-17.4)	6.5±1.9 (4.0-10.1)	6.8±2.5 (1.3-11.8)	1.54±0.7 (0.80-2.9)
Pulse interval	65.9±19.5 (27.7-121.9)	56.2±24.0 (25.0-153.6)	114.6±37.4 (58.0-213.5)	54.3±11.0 (38.7-90.3)	311.6±95.1 (197.1-488.0)	118.5±58.8 (60.7-269.9)	114.5±51.1 (8.5-221.1)	27.8±3.7 (20.7-33.1)
Distomax	1.83±0.5 (0.9-3.6)	1.3±0.5 (0.7-2.8)	2.9±1.3 (1.0-7.1)	1.6±0.7 (0.5-3.3)	5.5±1.9 (2.3-9.2)	2.5±1.1 (1.0-5.1)	3.7±1.7 (0.7-7.1)	0.8±0.4 (0.4-1.6)
Peak-to-peak ampl	205.1±133.9 (24.9-727.3)	153.7±101.8 (30.4-420)	130.0±74.7 (24.4-297.3)	322.4±213.2 (38.8-1079.1)	77.1±34.5 (33.0-130.4)	270.5±139.0 (46.4-580.2)	142.0±104.8 (26.7-341.6)	92.6±36.3 (71.0-130.9)
Peak freq (max)	55.5±5.3 (45.1-66.2)	63.1±3.0 (57.5-69.5)	39.8±4.8 (30.6-51.6)	55.8±9.6 (39.6-85.3)	21.3±1.2 (19.7-22.9)	36.0±3.3 (29.6-41.1)	30.4±1.1 (27.7-32.1)	86.7±6.3 (85.9-96.8)
Min freq (max)	51.5±5.0 (41.5-61.7)	58.9±2.7 (53.8-64.3)	36.2±4.6 (26.9-46.4)	49.5±8.9 (35.5-78.3)	17.9±1.1 (16.4-19.4)	32.4±3.2 (26.2-37.3)	26.8±1.2 (24.0-28.8)	81.7±6.2 (72.4-90.7)
Max freq (max)	61.9±5.7 (51.2-73.6)	69.5±3.7 (62.6-77.9)	45.5±5.2 (36.8-59.7)	63.0±10.1 (45.9-93.4)	25.8±1.1 (24.2-27.3)	40.9±3.3 (34.3-46.2)	35.7±1.4 (33.1-38.1)	93.2±7.4 (81.4-105.4)
Peak freq (mean)	52.6±4.7 (43.5-64.0)	60.7±3.0 (56.2-66.4)	38.3±3.6 (30.9-44.7)	56.2±9.5 (40.2-85.3)	21.1±1.1 (19.4-22.6)	32.5±2.2 (28.6-36.5)	30.2±1.3 (28.0-32.3)	39.2±3.0 (75.0-97.1)
Peak ampl (mean)	34.7±5.2 (47.7-24.3)	36.1±7.1 (47.6-24.3)	36.4±5.7 (48.0-27.0)	34.1±6.6 (52.1-21.8)	34.8±4.3 (42.7-28.9)	29.3±4.7 (40.1-21.3)	36.0±7.1 (47.9-26.4)	86.2±7.3 (43.4-35.1)
Min freq (mean)	47.8±4.6 (38.8-59.0)	55.6±2.7 (51.8-60.0)	33.9±3.5 (26.0-40.6)	41.7±6.1 (30.0-56.9)	17.3±0.9 (16.3-18.7)	27.1±2.0 (23.4-30.4)	25.4±1.3 (23.6-27.9)	72.5±7.9 (63.5-84.5)
Max freq (mean)	76.0±10.6 (60.1-99.0)	85.4±12.6 (69.1-110.7)	54.2±10.7 (39.6-84.6)	93.6±8.6 (76.0-110.2)	26.5±1.9 (24.1-29.6)	49.9±8.7 (37.6-71.6)	43.2±5.9 (35.0-57.9)	105.7±3.6 (102.5-110.6)
Bandw (mean)	28.2±9.14 (14.2-54.6)	29.8±11.5 (12.9-51.2)	20.3±8.6 (11.5-47.1)	51.9±8.7 (35.6-66.3)	9.1±1.1 (7.8-11.2)	22.7±8.1 (12.1-42.6)	17.9±5.7 (11.5-31.9)	33.2±7.2 (18.9-40.1)

n = number of trajectories; () = range; Spectrogram setting (FFT window size = 265 points, frame size 100%, overlap 50%, window flat top) of Avisoft-SASLab Pro.

Table 2.5. Results of the PCA and DFA on echolocation variables. Only factors with eigenvalues >1 and only variable with the highest factor loadings are shown.

Variable	**PCA Factor Loadings**			
	PC 1	**PC 2**	**PC 3**	**PC 4**
Peak freq (max)	0.98			
Max freq (max)	0.99			
Max freq (centre)	0.99			
Bandw (centre)		-0.85		
Bandw (max)		-0.87		
Bandw (mean)		-0.86		
Peak ampl (start)			0.91	
Peak ampl(centre)			0.95	
Peak ampl (max)			0.95	
Bandw (end)				-0.74
Eigenvalues	14.96	6.17	5.25	1.27
Cumulative %	48.26	68.17	85.11	89.22

Variable	**DFA Results**					
	Root 1	**Root 2**	**Root 3**	**Wilks' λ**	***F*-Remove**	***p*-value**
PC 1	2.32	-0.24	0.44	0.00018	2059.22	< 0.001
PC 2	2.32	0.44	-0.59	0.00013	1456.70	< 0.001
PC 3	-0.34	-0.82	-0.04	0.00001	75.89	< 0.001
PC 4	-0.51	1.28	-0.85	0.00003	245.57	< 0.001
Eigen values	63.60	7.53	3.37			
Wilks' λ	0.000005	0.0003	0.0026			
χ2	9045.59	5975.66	4397.24			
Df	403	360	319			
Cumulative %	77.54	86.71	90.83			
p-value	< 0.001	< 0.001	< 0.001			

A Multivariate DFA on four principal components correctly classified 83.16 % of the pulses to species (Wilks's lambda = 0.000004, $F_{416, 4089} = 23.482$, $p < 0.001$) even though overlaps in echolocation parameter occurred between some species. Analysis of canonical scores revealed root 1 accounted for 89.68%, of the total variance of LDC and HDC bats. Both LDC and HDC

bats along root 1 were discriminated by variables from PC 1, PC 2 and PC 3. But because LDC bats had negative loadings while HDC bats had positive loadings on the PCs, it resulted to a conspicuous divergence between the two clusters of bats (Fig. 2.7 & 2.8; Table 2.5). *H. caffer* considerably separated out from a cluster of rhinolophids on root 2 associated with PC 1, PC 2 and PC 4 which corresponds to variables peak frequency (maximum), maximum frequency (maximum), max frequency (centre), bandwidth (center), bandwidth (maximum), bandwidth (mean) and bandwidth (end) (Fig. 2.7 & Table 2.5). Similarly, both *M. tricolor* and *N. thebaica* markedly separated out from other LDC bats because of the difference in parameters on root1 and root 2 and the latter again separating on root 3 (Fig. 2.7 & 2.8). Also, *T. aegyptiaca* was distinctly separated from other LDC bats on root 3 because of higher discriminatory power contained in PC 3 and PC 4 which corresponds to variables peak amplitude (start), peak amplitude (center), peak amplitude (maximum) and bandwidth (end) (Fig. 2.8 & Table 2.5).

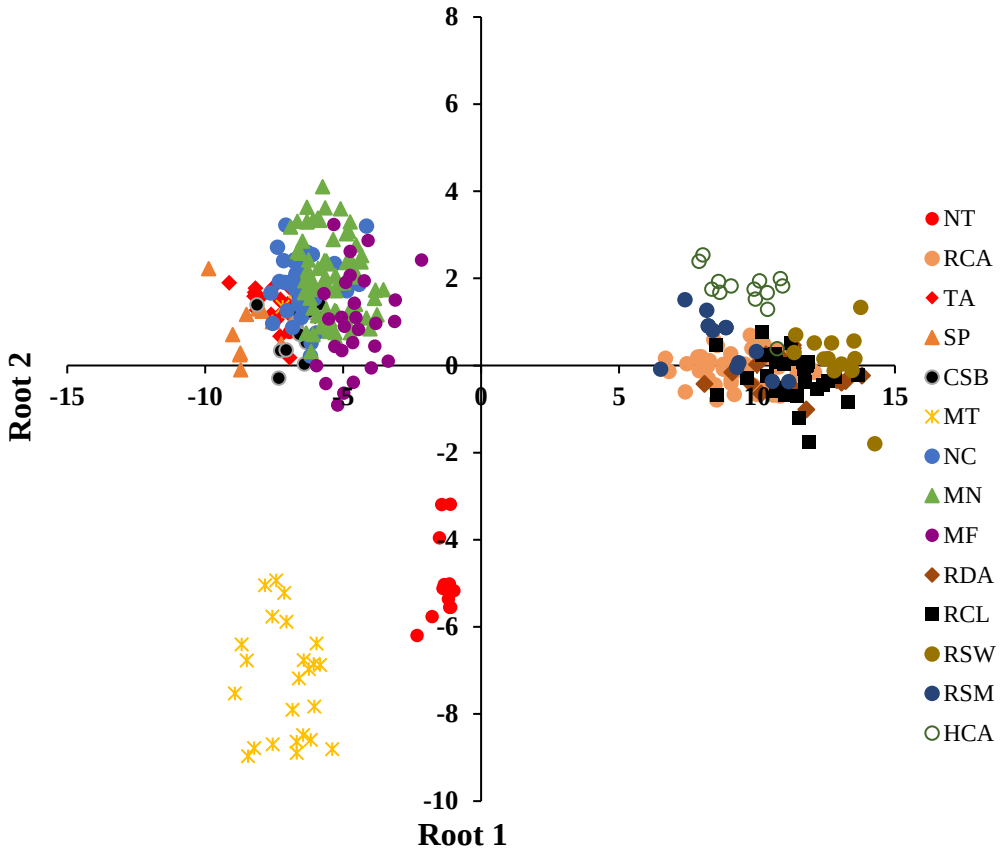

Figure 2.7: Plot of canonical scores (root 1 vs root 2) extracted by Discriminant Function Analysis from 22 echolocation pulse parameters of bat species: TA-*Tadarida aegyptiaca*, NT-*Nycteris thebaica*, MN-*Miniopterus natalensis*, MF-*Miniopterus fraterculus*, NC-*Neoromicia capensis*, CSB-*Cistugo seabrae*, MT-*Myotis tricolor*, SP-*Sauromys petrophilus*, RCA-*Rhinolophus capensis*, RCL-*Rhinolophus clivosus*, RDA-*Rhinolophus damarensis*, RSM-*Rhinolophus simulator*, RSW- *Rhinolophus swinnyi* and HCA-*Hipposideros caffer*.

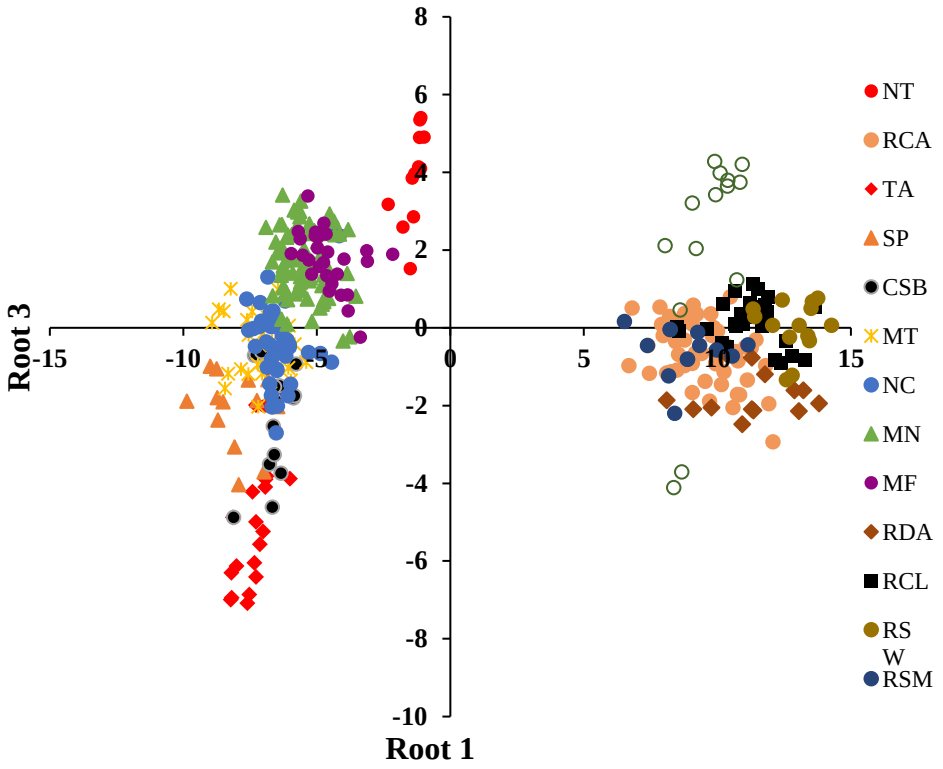

Figure 2.8: Plot of canonical scores (root 1 vs root 3) extracted by Discriminant Function Analysis from 22 echolocation pulse parameters of bat species: TA-*Tadarida aegyptiaca*, NT-*Nycteris thebaica*, MN-*Miniopterus natalensis*, MF-*Miniopterus fraterculus*, NC-*Neoromicia capensis*, CSB-*Cistugo seabrae*, MT-*Myotis tricolor*, SP-*Sauromys petrophilus*, RCA-*Rhinolophus capensis*, RCL-*Rhinolophus clivosus*, RDA-*Rhinolophus damarensis*, RSM-*Rhinolophus simulator*, RSW-*Rhinolophus swinnyi* and HCA-*Hipposideros caffer*

Classification success of HDC and LDC species varied (Table S2.1). Among Rhinolophids, *R. swinnyi* had the highest classification success of 92.86%. Other species (*R. clivosus*, *R. capensis*, *R. simulator* and *R. damarensis*) had > 50% of their pulses identified correctly. *R. clivosus* was most classified as *R. capensis* and *R. swinnyi* possibly because their duration, distomax, peak amplitude and bandwidth overlapped (Table 2.4). A similar trend is notable where *R. simulator* is misclassified to *R. capensis* due to overlap of their duration, interval, peak frequency and bandwidth (Table 2.4). Conversely, the *Hipposideros caffer*, is quite distinctive among other HDC bats with highest 85.71% classification success. All Squared Mahalanobis distances (Table S2.3) were significantly different despite all but one of them being in the same genus.

The classification rate for all LDC bats (Table S2.1) was determined and the highest correct discrimination was achieved for *M. tricolor* with 100.00% of pulses correctly identified. Identification rate for other species with pulses were also correctly identified at high % (*M. natalensis* 87.00%, *T. aegyptiaca* 83.33% *N. thebaica* 83.33% *C. seabrae,* 76.47%, *N. capensis,* 82.93%, *S. petrophilus,* 85.71% and *M. fraterculus* 71.43%). However, some species had low classification success, probably as a result of parameter overlap. For example, the echolocation pulses for *M. fraterculus* had the lowest classification success; seven out of twenty-eight pulses of *M. fraterculus* were classified as *M. natalensis*. A few pulses of the *N. thebaica* were classified as the former. Similarly, 3 of the thirteen pulses of *S. petrophilus* were classified as *T. aegyptiaca*. However, 3 of the fifteen pulses of *C. seabrae* were classified as *N. capensis* and 2 pulses were classified as *S. petrophilus*. All three of these species are from different families but they are similar in size and may forage in similar habitats (Monadjem et al., 2018; Schoeman & Jacobs, 2008).

As with the classification success, the Squared Mahalanobis distances for LDC bats were also generally higher than those for HDC species, and they were all significantly different (Table S2.4). LDC constitutes a cluster with more degree of separation of species in the acoustic space. The lowest Squared Mahalanobis distances, albeit still significantly different, was between the closely related *M. natalensis* and *M. fraterculus*.

2.4. Discussion

The results of this study revealed that echolocation pulses can be adopted to accurately identify bat species. Although the initial species classifications were based on more than just echolocation parameters, species could be reliably distinguished by their echolocation pulses alone, provided multiple parameters are used. The most important of these are peak frequency (maximum), maximum frequency (maximum), bandwidth (maximum, center, mean, end) and

peak amplitude (start, center, maximum). However, acoustic identification of species was facilitated by the low levels of phylogenetic relatedness in the bat assemblages investigated in different biomes of Southern Africa. The assemblages generally consisted of several families with one to three species per family (see Table S2.5); possibly due to species that are rare or acoustically more cryptic not being recorded. For example, species in the Savanna (e.g., *Cloeotis percivali, Scotophilus dinganii, Rhinolophus hildebrandti*), in the Nama Karoo (e.g., *Chaerephon pumilus, Rhinolophus fumigatus*) and in the desert biomes (e.g., *Taphozous mauritianus, Kerivoula lanosa*) (Schoeman & Jacobs, 2003, 2008; Monadjem et al., 2010) were not recorded. Even though these species were missed, they would not have posed a great challenge to species identification considering that they belong to different families (e.g., Hipposideridae Rhinolophidae Emballonuridae and genera (e.g., Scotophilus, Chaerephon, Kerivoula). However, even with the missed species, the numbers of species in the assemblages were low, simplifying their identification. This would not be true for all bat assemblages in other regions of southern Africa or elsewhere in Africa. Bat assemblages in tropical regions like West Africa (Fahr & Kalko, 2011), South America (Walters et al., 2013), Southeast Asia (Hughes et al., 2011; Walters et al., 2013) and East Africa (Webala et al., 2009; Wechuli et al., 2017; Musila et al., 2020) have high bat species diversity. In such situations a mixture of acoustic surveys and various capture techniques would be necessary to reliably identify species. This difference is driven by lack of comprehensive echolocation reference libraries for resident species in these tropical regions (Walters et al., 2013). Moreover, tropical regions contain many genetically similar bat species and a stronger sense of pulse resemblance which makes species identification difficult. (Hughes et al., 2011) and in many cases genetic analyses have to be used. In the cluttered situations of tropical rain forests, for instance, it is sometimes difficult to identify similar species based on acoustic characteristics because many similar species have similar pulse structures (Kingston et al., 2003).

Bat assemblages comprise different bat families that exhibit variability in echolocation pulses due to various factors that may allow or confound species identification. This study revealed pulse variations of species in the bat family Rhinolophidae. The variations could be attributable to frequency in relation to age, sex, body size and geographic location (Chen et al., 2009; Odendaal et al., 2014; Jacobs & Bastian, 2018). For example, *H. caffer*, from different localities differed in pulse frequency by more than 10 kHz (133-145 kHz). It is unlikely that such a frequency range is indicative of cryptic species because similar trends have been found in southern Africa and other parts of the world (Monadjem et al., 2010; Monadjem et al., 2020). For example, *Hipposideros fulvus* exhibits similarly large geographic differences in echolocation frequency (Heller & Helversen, 1989). A similar trend of pulse variation in relation to species locality occurs among Rhinolophidae. Despite the fact that *R. simulator* and *R. capensis* have overlapping frequencies, they do not occur in the same geographic areas; therefore, their pulses can be reliably distinguished by the locality at which the pulses were recorded. Thus, species could be distinguished virtually with certainty once their distribution pattern is taken into account.

This study indicated variability in echolocation pulses among LDC bats in families Molossidae and Vespertilionidae. For example, there were variations in echolocation pulse structure between *T. aegyptiaca* and *S. petrophilus*. Molossids are a diversified community of bats, with so many species encountered in tropical regions and far fewer in temperate regions. (Simmons, 2005). Other molossids, apart from *Molossops temminckii*, have been observed hunting in open spaces and above vegetation cover, posing a similar sensorial challenge in manevering away from obstacles and detecting spaceborne insect prey in broad horizons (Jung et al., 2014; Hintze et al., 2020). Many molossids possess a high plasticity in the structure of their search pulses, which includes frequency shifts, variations of peak frequencies within pulse sequences (Guillen-Servent & Ibanez 2007). Vespertilionids like the *M. tricolor* revealed a large

bandwidth with the first harmonic of their echolocation pulses than *N. capensis* with a relatively narrow bandwidth. This pattern is supported in Europe, where some species within the family Vespertilionidae, especially bats of the genus *Myotis* have evolved intricate echolocation behaviour to detect prey near to the vegetation by using broadband, frequency modulated (FM) pulses of short duration. Broad bandwidths are used to classify targets and discriminate background echoes, while short pulse duration reduces overlap of echoes from target and background (Schoeman & Jacobs, 2003; Siemers & Schnitzler, 2004).

The differences in phylogeny within each assemblage are also reflected in differences in foraging mode which contributed to the differences in echolocation pulses that facilitated species classification. Because species in the same habitat experience similar sensory problems during orientation and foraging, their echolocation pulses show a high degree of similarity, as well as a typical design reflecting foraging strategy. For instance, open-air high-flying foragers (such as molossids; *T. aegyptica, S. petrophilus*) in the present study had overlapping parameters. Since the main echolocation requirement for these two species is long-range detection, their echolocation pulses are at relatively low frequencies, with similar echolocation characteristics, including long pulse duration and pulse intervals, and low bandwidth (Barclay & Brigham, 1991; Schnitzler & Kalko, 2001; Kingston et al., 2003). Clutter-edge foraging species e.g., Miniopterus; *M. natalensis, M. fraterculus* species often emitted echolocation pulses of similarly high frequencies, with a wider in frequency sweep, and shorter duration (Barclay, 1999; Siemers & Schnitzler, 2000; Schnitzler & Kalko, 2001). Bats do this in order to avoid overlapping of the departing echolocation pulse with the incoming echo (Kalko & Schnitzler, 1993) that warrant the use shorter pulses when bats are nearer to targets. Narrow space gleaning foragers in families Nycteridae, e.g., *N. thebaica* and gleaning/trawling in family Verspertilionidae, e.g., *M. tricolor* (Moyo & Jacobs, 2020) are characterized by short, multi-harmonic and broadband echolocation signals (Neuweiler, 1989). They have high

frequencies and low source levels to minimize from the background echoes from the clutter (Neuweiler, 1989). Known clutter foragers such as the HDC species in the genus Rhinolophus and Hipposideros use clutter resistant pulses dominated by a constant frequency component (Neuweiler, 1989; Pavey et al., 2001) which are more stereotypical allowing species identification using the frequency of the constant frequency component alone (Russo & Jones, 2002; Odendaal et al., 2014; Jacobs & Bastian, 2018; Webala et al., 2019). The combination of low phylogenetic relatedness combined with the resultant differences in foraging modes and in echolocation pulses facilitated species identification in this study.

Despite the success of acoustic identification of some species, e.g., *M. natalensis*, *M. tricolor*, *R. simulator* were rather partly confounded because of overlapping echolocation parameters with other species. Low duty cycle bats show pulse alternation (Kingston et al., 2003) as they forage which leads to high levels of intraspecific variation in echolocation pulses. This was true, particularly for *M. natalensis* and *M. fraterculus,* whether they co-occur or not, they tend to use similar echolocation pulse structure and their peak frequencies may overlap as a behavioral response during foraging (Miller-Butterworth et al., 2005). I, therefore, opted to classify their pulses based on a minimum and maximum cut-off frequencies as indicated in (Monadjem et al., 2010). *M. natalensis* and *M. tricolor* depict minor overlaps in peak frequencies, but because the structures of their echolocation pulses are so distinctive (Fig. 2.4), detected in the current analyses using multiple parameters (e.g., distomax), it was possible to differentiate their pulses; the pulses of *M. tricolor* are more steeply frequency modulated without a quasi-constant frequency component (Fig. 2.4).

Although most rhinolophids can be distinguished by the frequency of their constant frequency components, there is some overlap among species and species identification based on this component alone must be done with caution. For example, the frequency of the constant

component of *R. damarensis* was congruent to that of Fynbos and Albany thicket populations of *R. capensis* (Fig. 2.5). Such overlap also occurs between these populations of *R. capensis* and populations of *R. simulator* in the Indian Ocean Coastal Belt biome. In such cases, the use of the frequency of the constant frequency component alone would lead to erroneous identifications. To avoid such misidentifications in the initial species classifications, I relied on available bat species distribution records (Schoeman & Jacobs, 2003, 2008; Monadjem et al., 2010) and a study on the population genetics of *R. capensis* which shows that its peak frequency differs across a geographical gradient (Odendaal et al., 2014). The initial species classifications were confirmed through the multivariate analyses of echolocation pulse parameters reported here.

Overall, the study showed the possibility of identifying bat species hinged on echolocation alone provided multiple pulse parameters are used and the bat assemblages under investigation consist of a few distantly related species. Most species emit species-specific echolocation pulses that are distinguishable, e.g., *H. caffer, R. swinnyi, T. aegyptica, N. thebaica.* On the other hand, the potential to identify some species, e.g., *N. capensis, C. seabrae* may also be hampered because sometimes they alternate their pulse shapes and frequencies during flight (Schoeman & Jacobs, 2003, 2008). In open habitats during flight, they often alternate between two pulse structures in the same way. In turn, pulse parameters completely overlap, and when such pulses are selected for species identification, they may lead to confusion. So, the alternative way to reliably tell them apart is by using complementary techniques such as taxonomic keys and/or genetics on captured bats and accurate distribution records (Taylor, 1999; Monadjem et al., 2010). South Africa has comprehensive bat distribution records with valuable information about habitat associations of different species in different localities (Monadjem et al., 2010). This record incorporated the already documented species mainly from

extensive field work done over many years by (e.g., Jacobs, 1999a, 2000; Taylor, 1999; Schoeman & Jacobs, 2003; Stoffberg et al., 2004; Miller-Butterworth et al., 2005; Jacobs et al., 2005; Monadjem et al., 2007; Schoeman & Jacobs, 2008) in different habitats in Southern Africa. From their work, bats were captured and identified from their morphometric measurements and echolocation pulses recorded from both room-flown and hand-held individuals to both low duty cycle and high duty cycle bats. Naturally foraging bats were recorded in some studies as well and where possible, genetic confirmation of the species status was carried out. Therefore, the current distribution record is adequate to aid species identity across geographical locations in South Africa. Owing to well documented distribution records, the potential of acoustic survey of bats in the different localities can also be enhanced.

Finally, this study may have a bearing on acoustic monitoring of bats needed for environmental impact assessment in the alternative energy industry, particularly wind energy sector. Studies indicate that a significant number of bats are killed at wind energy facilities in USA (Baerwald et al., 2009), Europe (Rydell et al., 2010), Africa (Doty & Martin, 2013) as a result of direct collision with rotating blades. Many species in South Africa that are adapted to fly high up in open space and are likely to fly at heights within the sweep zone of rotor blades during migration or when foraging. Species like *Rousettus aegyptiacus, Otomops martiensseni, Tadarida ventralis, Kerivoula argentata, T. aegyptiaca, C. seabrae, M. fraterculus, M. tricolor* and *M. natalensis* have been killed at wind energy installations in South Africa (Sowler et al., 2017; MacEwan et al., 2018). It, therefore, implies that during the development of wind energy turbines, the assessment of bat activity at proposed sites is crucial and must factor in all mitigation measures against bat fatalities. The first undertaking is to identify bat species assemblages in that area and because capture methods alone are ineffective for detecting bat species, for example, species adapted to flying in open spaces (Schnitzler & Kalko, 2001),

identification can effectively be achieved from their echolocation pulses. It is the most accurate approach to bat identification and efficient for long-term monitoring of, e.g., species declines in those areas (Brooks, 2011). However, as this study indicates, such acoustic monitoring must proceed with caution.

CHAPTER 3

Variation of echolocation pulse source levels and detection distances for bat assemblages within and across sites in biomes of South Africa:

"A test of the Acoustic Adaptation Hypothesis"

Abstract

Echolocation pulses used by bats comprise several parameters and one of them is the source level. This is a vital parameter as it can directly impact the distance at which bats perceive targets in their environment and, most importantly, distances at which they detect prey. Different habitats present different challenges for echolocation systems, and so the quality and content of information derived from echolocation pulses reflects these environmental challenges. As such, echolocation pulses within or between species may vary from one habitat to the next due to variable selection pressure, resulting in local adaptation. Habitat is, therefore, a key component in shaping the evolution of echolocation. The Acoustic Adaptation Hypothesis (AAH) proposes that acoustic properties of the environment influence sound propagation and therefore the evolution of echolocation pulses. It predicts that; (i) echolocation pulse parameters used by different species of bats within the same assemblage should result in similar detection distances, (ii) if detection distances are adapted to a particular biome, the detection distances should differ among bat assemblages, and (iii) detection distances should be different for a single species, occupying localities that differ habitat structure. Alternatively, contrary to the AAH, detection distances may be influenced more by body size and foraging mode than by habitat. If so, detection distance should; (i) differ among species within assemblages, (ii) be species specific and remain the same within species between assemblages. Here, I used multiple microphone arrays to measure the source levels of echolocation pulses of 14 bat species in several bat assemblages across sites in six biomes in South Africa. Contrary to the AAH, my results revealed that bats in the same assemblage used different echolocation

pulse source levels, frequencies and duration resulting in different detection distances, which differ among bat assemblages occupying different sites. Furthermore, detection distance was species specific and remained similar within species between assemblages; suggesting that species is a better predictor of detection distances compared to habitat as indicated by *Miniopterus natalensis* across all seven sites.

Key words: acoustic properties, echolocation systems, habitats, local adaptation, microphone array, selection pressure

3.1 Introduction

Bioacoustic signals primarily serve a communicative role (including mate attraction and territory defence; (Wilkins et al., 2013) in wide range of animals; including insects, anurans, birds, fish and mammals (Klump & Shalter, 1984; Nowicki & Searcy, 2005; Pinto-Juma et al., 2005; Campbell et al., 2010; Oliver & Lobel, 2013; Chaverri et al., 2018; Ryan et al., 2019). In some animals, such as dolphins, bats, rodents and some birds, acoustic signals play critical roles in orientation and food acquisition. Such acoustic signals are the basis of echolocation, which is a sophisticated acoustic sensory system that allows animals, particularly bats, to operate in complete darkness (Griffin, 1958; Au, 1993). Although echolocation is used primarily for orientation and foraging, there is increasing evidence that it also serves in communication (Gillam & Fenton, 2016; Finger et al., 2017). The acoustic signals utilized by animals vary both within and between species and the variation is associated with a number of factors. A correspondence between signal variation and measures of ideal signal transmission is a function of habitat structure (complexity), climatic factors (humidity and temperature), community composition (e.g., frogs and birds emit signals to avoid masking interference), background noise levels and the phylogenetic history of species (Ryan & Brenowitz, 1985; Chek et al., 2003; Patten et al., 2004; Grant & Grant, 2010; Luther & Derryberry, 2012; Snell-Rood, 2012).

The structure of habitats influences how acoustic signals evolve. Often, the characteristics of acoustic signals are correlated with habitat structure (Brumm & Naguib, 2009; Wilkins et al., 2013). For instance, acoustic signals traveling through a cluttered environment are subject to transmission challenges due to inevitable degradation of signals caused by the acoustic clutter (Dabelsteen et al., 1993; Badyaev & Leaf, 1997; Bradbury & Vehrencamp, 2011a). Signal degradation occurs when processes like scattering and attenuation occur in the atmosphere, and the designs of sound signals are affected (Lawrence & Simmons, 1982; Dabelsteen et al., 1993; Slabbekoorn, 2004). Across all habitats, scattering increases as the frequency of sound increases, whereas at lower frequencies, it attenuates less. In open environments sound transmission properties typically induce high amplitude fluctuations that largely mask low frequencies of amplitude modulation. Forested habitats also show a high amplitude fluctuation, and tends to mask high frequencies of amplitude modulation (Wiley & Richards, 1982). The aforementioned factors are all due to the stronger influence of environmental conditions leading to variation in the propagation of signals. Thus, the acoustic properties of habitats are crucial in that they influence the transmission of acoustic signals, allowing for some to travel over longer distances, while other signals may not (Naguib & Wiley, 2001; Slabbekoorn & Smith, 2002). This widely supported fact has been formalized in the ‘‘Acoustic Adaptation Hypothesis’’ (Hansen, 1979) and was subsequently reviewed to examine how widespread such adaptations are across taxa (Boncoraglio & Saino, 2007; Ey & Fischer, 2009).

The Acoustic Adaptation Hypothesis (AAH) proposes that the transmission of animal signals over long distances are affected by the sound propagation properties of the environment, which thus exerts an influence on the evolution of the properties of signals (Morton, 1975; Hansen, 1979; Ey & Fischer, 2009). Although originally formulated in the context of long-distance

transmission of bird song, this hypothesis should be broadly applicable to bat echolocation albeit of higher frequency than bird song makes it operational over shorter distances.

The AAH has been tested on different model species mostly on birds, anurans and mammals, which include bats (Ryan & Brenowitz, 1985; Tanaka et al., 2006; Ey & Fischer, 2009; Hedwig et al., 2015; Velásquez et al., 2018; Goutte et al., 2018). Most studies support the AAH although some do not, and others yield mixed results. For example, several species of birds optimize their signal transmission in respective habitats (Waas, 1988; Bertelli & Tubaro, 2002; Saunders & Slotow, 2004; Nicholls & Goldizen, 2006; Ey & Fischer, 2009). Other bird species show no correlation of their signals with habitat type (Lemon & Date, 1993; Naguib & Wiley, 2001; Dabelsteen & Mathevon, 2002; Ey & Fischer, 2009). For example, the Eurasian blackcap *Sylvia atricapilla* occupying temperate deciduous forest at times do not show diurnal variation in signal clarity and long-range communication when background noise and transmission conditions (e.g., temperature, relative humidity and wind speed) are optimal (Dabelsteen & Mathevon, 2002). This proof for an absence of a habitat effect does not certainly refute the AAH, but instead indicates that other key factors, such as phylogeny and morphology, influence the structure of vocalizations (Ryan & Brenowitz, 1985; Brumm & Naguib, 2009).

Similarly, while some anurans' call transmission supports the AAH, other studies provide contradictory evidence or mixed support for the AAH (Zimmerman, 1983; Ryan et al., 1990; Bosch & De la Riva, 2004; Ey & Fischer, 2009; Goutte et al., 2018; Velásquez et al., 2018). For example, the spectral characteristics (dominant frequency) of torrent frog calls differ from those in different habitats because of the differences in atmospheric noise levels, in line with AAH, though call bandwidth does not follow this pattern. However, temporal characteristics such as duration and amplitude modulation do not appear to be influenced by the calling habitats of frogs. (Goutte et al., 2018). Previous studies on mammalian taxa have revealed

ambiguous results with some supporting the AAH (Schleich & Busch, 2002), but others contradicting the AAH (Brown et al., 1995; Schleich & Busch, 2002; Hedwig et al., 2015). In the solitary subterranean rodent *Ctenomys talarum* acoustic signals are shifted to lower frequencies which transmit better in underground burrows (Schleich & Busch, 2002). In contrast, in the gorilla (*Gorrila* spp.), the spatial and visual separation of the caller from other group members explains variation fundamental of frequency and calling rate in short distance vocalizations better than environmental influences (Hedwig et al., 2015). These contradictory results might be more dependent on other factors that influence the evolution of acoustic signals including phylogeny, morphology, call context and caller arousal (Forrest, 1994; Wilkins et al., 2013). Among bat species, AAH is linked to the function of echolocation pulses used during foraging (Barclay, 1999; Neuweiler, 2000). Bats may face perceptive impediments, associated with different habitats, and thus, flexibility in foraging behaviour often necessitates specific sensory adaptations. Habitats determine which echolocation frequencies operate effectively based on habitat characteristics. For instance, low frequency pulses are ideal for detecting prey at a longer distance, so they are a good for bats that forage in open spaces (Neuweiler, 1990). However, high-frequency pulses have enhanced directionality, offer appreciable resolution of targets, and are thus particularly fit for detection at short distances in very cluttered environment (Norberg & Rayner, 1987; Schnitzler & Kalko, 2001; Jacobs et al., 2007). Based on the assumptions of the AAH, bats may be locally adapted to a particular habitat and, habitat clutter selects for a gradient of echolocation pulse increasing frequency.

Bat assemblages are a good focal system to study acoustic adaptation of species because bats are widely distributed in different localities across biomes of South Africa (Monadjem et al., 2010). Bat assemblages typically consist of species belonging to different families and vary in body sizes and foraging strategies; they typically exhibit a variety of different adaptations to

their habitats (Schoeman & Jacobs, 2003, 2008; Odendaal & Jacobs, 2011). Because of the ecological success of species distribution across a variety of habitats, that may to exert varying levels of selection pressures, variation in the echolocation signals of bat assemblages present a good paradigm for investigating of Acoustic Adaptation Hypothesis.

Several environmental factors are likely to play a role in the propagation of bat echolocation pulses and which in turn will influence bat echolocation evolution. These include reflection and scattering of sound by vegetation and attenuation by the atmosphere as the sound is propagated through it (Padgham, 2004; Luo et al., 2014; Mutumi et al., 2016; Maluleke et al., 2017; Goerlitz, 2018). Attenuation is in turn influenced by climatic factors particularly temperature and humidity. More often, bats use various ways to enhance the propagation of their pulses in habitats to facilitate prey detection. Of much importance is how bats overcome atmospheric attenuation of their pulses, especially pulses emitted at high frequencies (Griffin, 1971; Hartley, 1989). Source levels (i.e., the energy the bat places in its emitted pulses) is one of the parameters that bats can utilize to optimally exploit different habitats (Waters & Jones, 1995; Holderied & von Helversen, 2003; Surlykke & Kalko, 2008). Together with frequency of pulses, source levels determine the operational range of bat echolocation (Holderied et al., 2005; Jakobsen et al., 2013). However, because of atmospheric attenuation, one must know the position of the bat in relation to the microphones used to record its pulse to measure source levels. This is because atmospheric attenuation dissipates the energy in the pulse as the pulse travels between the bat and the microphone. Multiple microphone arrays are therefore used to reconstruct the bat's flight path so that the distance between bat and microphone are known for each pulse. In turn source levels for pulses generated together with echolocation pulse frequency are used to calculate the detection distances (Waters & Jones, 1995; Holderied & von Helversen, 2003).

If environmental factors exert an overriding influence on the echolocation pulses of bats, detection distances in bats may be specific to particular habitats and be independent of other factors (body size, size of the ear pinnae or foraging strategy). In support of this, Surlykke and Kalko (2008) showed that within a tropical bat assemblage, small bats using high frequencies emitted pulses of higher source levels than larger bats using lower frequencies. Bats in the assemblage belonged to the guild of edge-space aerial hawkers, edge-space trawling hawkers and open-space aerial hawkers. Regardless of their echolocation pulse parameters, body sizes or foraging strategy, all bats had similar detection distances. The implication is that bat species in their study presumably encountered similar circumstances at the foraging habitat and were constrained to fly at comparable flight speeds and maneuverability. This suggests that detection distances may be locally adapted supporting the AAH. If so, bat species in the same habitat may experience the same detection distances though detection distances may vary across different habitats to optimize detection distances in each habitat in accordance with the different prevailing environmental conditions. However, Surlykke and Kalko (2008) tested only one bat assemblage and confirmation of the AAH would require that several bat assemblages across a diverse range of habitats be tested.

Alternatively, and contrary to the AAH, body size (which has an effect on flight speed and maneuverability) and foraging mode (gleaning versus aerial hawking which require shorter and longer detection distances, respectively) may impact the evolution of detection distances more than environmental factors. If so, detection distances within the same assemblage should differ from species to species because of differences in body size (Jones & Purvis, 1997; Jacobs et al., 2007), flight speeds (Fawcett & Ratcliffe, 2015) and differences in foraging mode and prey (Denzinger & Schnitzler, 2013; Segura-Trujillo et al., 2016). These factors could result in differences in detection distance both within and between habitats over and above differences expected due to local adaptation. To test the AAH and this alternative hypothesis, bat detection

distances should be compared across species and across biomes, an approach that complements and extends that followed by Surlykke & Kalko (2008). Studies in birds (e.g., Blumstein & Tuner, 2005; Sebastián-González, 2018; Mikula et al., 2021) and a tropical cricket assemblage (Jain & Balakrishnan, 2012), acknowledges testing the AAH using the entire assemblage/s. However, in my study, a limitation is that not all species are found in all assemblages across biomes. Thus, in addition to the general comparisons across species and assemblages, I also compared populations of one species, that is, *Miniopterus natalensis* known to occur in different biomes of South Africa (Schoeman & Jacobs 2008; Monadjem et al., 2010, 2020).

The aim of this study was therefore to test the Acoustic Adaptation Hypothesis by recording bat echolocation pulses in different biomes, measuring the source level of bat pulses and using both to calculate detection distances. This in order to test the following predictions of the AAH: (i) Echolocation pulses frequency and source levels used by different species of bats in the same assemblage result in similar detection distances (see Surlykke & Kalko 2008), (ii) If so, detection distances among bat assemblages should differ from one site to the next, (iii) detection distances should be different for a single species, *Miniopterus natalensis* occupying sites in different habitats.

Alternatively, and contrary to the AAH, detection distances may be influenced more by body size and /foraging strategy than by habitat. In this case, detection distances should; (i) differ among species within assemblages and, (ii) be species specific and remain the same within species between assemblages.

3.2 Material and methods

3.2.1 Study animals

The echolocation pulses of several insectivorous bat assemblages were recorded from a wide range of biomes in South Africa (Table S3.1). The echolocation pulses of a total of 14 species

of free-flying bats were recorded to measure their source levels and prey detection distances. The recordings were also used to assign echolocation sequences to species as described in Chapter 2. Some species were restricted to one or two biomes only, but others were common to more than two biomes (Mucina & Rutherford, 2006) (Table S3.1). The foraging modes of the various species identified were obtained from the literature (Schoeman & Jacobs, 2003).

3.2.2 Study sites

Free flying bats were recorded at six sites, each associated with a different biome (Table S3.1). Detailed descriptions of the sites are provided in Chapter 2.

3.2.3 Description of equipment set up

Bat echolocation pulses were recorded during bats' emergence from their day roosts using multiple microphone arrays assembled at the University of Cape Town, South Africa (Fig. 3.1). The microphone arrays consisted of two arrays labelled as array ABC and array 123 (partly described in Chapter 2), each having four omnidirectional condenser microphones. Three microphones are fixed at the corners of an equilateral triangle with sides 0.94 m in length. The microphones were attached to three arms 1.2 m in length that bisected each corner of the triangle and intersected in the centre of the triangle. There was a fourth microphone at the intersection of the three arms in the centre of the triangle. The centre pieces that held the central microphones (microphones 1 and 5) of each array was covered with ultrasound absorbing foam. After the arrays were positioned relative to each other in the field, angular measurement in degrees (^{o}) of the two arrays relative to each other were recorded using a transparent plastic protractor. A Leica Disto D2 laser device (Leica Geosystems, Berlin, Germany) was used to take measurements of distance (m) between the arrays and the height (m) of each array from the ground to the central microphones (microphones 1 and 5).

Figure 3.1. The setup of the multiple microphone array system to record echolocation pulses.

The positioning of the arrays was dependent on the species of interest at each site within a biome at both roosting and foraging areas. Thus, to record high-flying bats, the microphone array was set approximately 2 m high and spaced 3 m apart. When recording low flying bats, the microphone array was set 0.8 m high and 1.9 m apart. Each site was inspected to find a suitable flight route using the available landmarks because echolocating bats may learn to rely on these acoustic landmarks to direct their spatial orientation. Knowing these flight routes and the established distances between the arrays would allow the bats sonar beam to impinge directly on the microphones, as close to the central microphone's acoustic axis as possible. Spacing of the two arrays was particularly crucial for some HDC bats that emitted highly directional pulses. For the same reason, the arrays were also angled so that they were as perpendicular as possible to the flight paths of bats. After every recording session of echolocation pulse in the field, microphones were calibrated at 25 cm (as per product recommendations) using a 40 kHz (84 dB SPL) signal generator (Avisoft Bioacoustics, Berlin, Germany). The calibrator emits a calibration tone of known frequency and intensity at a given distance. This signal is then transmitted to the microphones and the intensity and frequency of the reference tone as recorded by each microphone is compared with the known intensity and

frequency of the reference tone. This provided a correction factor by which the parameters of the echolocation pulses recorded by the same microphones were adjusted to obtain the parameters as emitted by the bat. Microphones were not calibrated before recording echolocation pulses so as not to interfere with the microphone array once it was set up. Prior to use in the field, the microphones were calibrated for polar sensitivity in the acoustics laboratory of Marc Holderied at the University of Bristol. The calibration was performed at various temperature and relative humidity levels. Calibration in the laboratory considered signal attenuation that occurs because of variation in microphone sensitivity to different frequencies, achieved by reference to the equipment's frequency response curve.

3.2.4 Recording of echolocation pulses and weather parameters

Echolocation pulses were recorded on-site with an 8-channel recorder (Avisoft-UltraSound Gate 816H (Avisoft Bioacoustics, Berlin, Germany) in real time. The recorder was linked to a PC-hard disk recording software (Avisoft-Recorder USGH, version 4.2.23, Berlin, Germany) at a sampling frequency of 300 kHz while the resolution was 16 bits. This software is a versatile multichannel triggering hard-disk recording system. The UltraSound Gate 816H with a gain adjustment potentiometer set at 50% was hooked up to the omnidirectional electret ultrasound microphones (Avisoft Bioacoustics, Knowles FG-O; sensitivity of approximately 300 mV/pa) and, also connected to a Dell Laptop (Model: Latitude E7240, Johannesburg, South Africa). The laptop supplied power to the UltraSoundGate during the recording. Spectrogram displays of the laptop were observed in real time to trigger recordings by hand when a series of best-quality pulse appearing on eight active channels were observed. The recorded echolocation pulses were stored securely in the laptop's hard drive during recording. They were later transferred on separate external hard drives with high storage capacity.

A portable weather station (Model WMR89/WMR89A, Oregon Scientific Inc., Tualatin, Oregon, USA) was erected on each location during the recording session of echolocation pulses to continuously record weather parameters. Temperature (˚C), relative humidity (%), atmospheric pressure (kPa), wind speed (m/s) and direction were recorded at two-minute intervals. This allowed sound pressure levels of the echolocation pulses recorded by the microphones to be adjusted for errors caused by absorption in the atmosphere and spreading losses by taking the bats' distance from the microphone into account.

3.2.5 Flight path reconstruction

A first step involved selecting sequences of echolocation pulses that offered good signal quality (good signal-to-noise ratio) and favourable flight paths towards the microphone array. By using the time of arrival differences (TOADs) of the incoming pulses at each microphone (Avisoft Bioacoustics, Glienicke, Germany) in the array, the flight paths of the bats were plotted in three dimensions with respect to every array (Holderied & von Helversen, 2003; Gillette & Silverman, 2008). From TOADs of pulses measured at each microphone, the exact location of the sound source (the bat) and the two central microphones, each in the middle of the array (microphones 1 and 5) was determined. The time of arrival of a pulse at each microphone was accurately computed by cross correlating the same echolocation pulses (Holderied & von Helversen, 2003) using a customized script in MATLAB 8.1 (The Math Works Inc., Natick, MA, USA) developed by Marc Holderied. The time of arrival was entirely based on the FM components for LDC and HDC bats in the assemblage. In high duty cycle bats, the CF pulse does not allow precise measurement of time delays between channels because the cross-correlation function cannot be precisely determined. Thus, this component was erased from each pulse in the recorded sequence, leaving only the FM components. By combining the different positions of each pulse, we were able to reconstruct the 3D flight path for each recorded sequence. Only flight paths for bats flying towards the microphone array were

considered for analyses except for those when bats approached the arrays from behind. Bats are assumed to direct their sonar beam probably in the direction they fly, particularly on straight parts of the flight route without sharp turns pre or post. Individual points in the selected flight trajectories supported each other in speed and space. Any erroneous points along the flight path were removed. The construction of bat's 3D flight trajectory made it easy to determine if the bat was flying in front of or behind the microphone. Hence, the 3D flight paths with the recorded echolocation pulses allowed the determination of how bats change their pulse parameters, including source levels, and therefore their detection distances at any point along the bats' flight paths (Fig. 3.2 a & b).

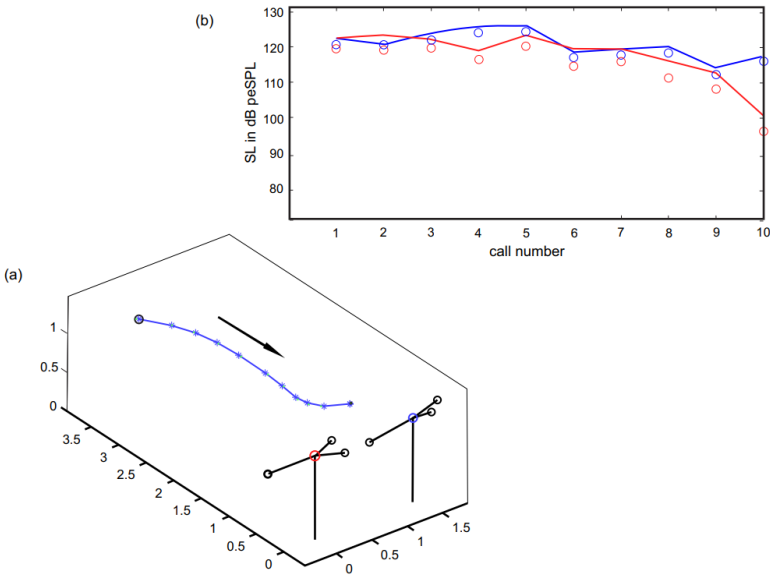

Figure 3.2. Illustration of a representative 3D spatial flight trajectory as the bat approached the microphones (a). The echolocation pulses (1-10) with their source levels in dB peSPL at microphone 1 (in red) and microphone 5 (in blue) (b).

3.2.6 Acoustic analyses of echolocation pulses

Acoustic parameters were measured in Avisoft-SASLAB Pro (Version 5.2.09, Avisoft Bioacoustics, Berlin, Germany), supported by USB key (dongle) device driver (MAX HL MN720427/OZRWQ/31060647-1, Berlin, Germany). Prior to the measurements, good quality echolocation recordings were subjected to appropriate filters as a prerequisite for analysis. Both low-pass filter (removes signal components above a specified cut-off frequency) and high-pass

filter (removes signal components below the specified cut-off frequency) were applied to the selected recordings using the Time Domain FIR Filter function, Hamming window type of Avisoft. FIR (Finite Impulse Response) filters allow the removal of any background noise from recordings without phase distortion of signals. In cases where the background noise overlapped with the echolocation pulse signal, it was possible to completely remove that noise manually by using the command Tools/Cursors/'Standard eraser cursor' (See details in Chapter 2, Table 2.1). The acoustic measurements for HDC bats was made from the second harmonic of the pulses because most energy is placed in this harmonic (Pye & Roberts, 1970; Jacobs & Bastian, 2016). Conversely, low duty cycle (LDC) bats place most of the pulse energy in the first (fundamental) harmonic (reviewed in Jacobs & Bastian, 2016). Therefore, these harmonics have the best signal-to-noise ratio. The time and frequency parameters were measured in considerable detail using the Automatic Parameter Measurement tool of the spectrogram window (Avisoft-SASLAB Pro). All the spectral and temporal measurements were calculated to a threshold that was normally set to -20 dB, which was adjusted depending on the structure of the echolocation pulses and the background noise level. The threshold was automatically referenced to the maximum magnitude of values ranging from -9 dB to -15 dB, which changes depending on the quality of the echolocation pulses and the level of the background noise. In recordings with a high level of background noise, this threshold was not set too low. The (+) and (–) buttons were used to interactively find the best settings. In this way, the option was used to safely detect elements exhibiting varying amplitudes and was recognized more precisely. Spectrograms for analysis of the bat species were generated using the FlatTop window, Fast Fourier Transform length (FFT length) 256 points, frame size 100% and overlap 50%. Temporal and spectral parameters measured were pulse duration (the time from the beginning to the end of the pulse; ms), start time (time at the beginning of the pulse; ms), end time (time at the end of the pulse; ms), distomax (time between the start of the pulse to the

maximum amplitude of the pulse; ms), peak-to-peak amplitude (the change between highest amplitude value and the lowest amplitude value of the pulse; Volts), peak frequency (frequency at the peak (maximum amplitude) in the spectrum of the entire pulse; kHz) as well as peak amplitude (the maximum amplitude (magnitude) of the instantaneous spectrum at the peak frequency of the pulse: dB). Measurements of these parameters from the first and fifth channels of the spectrogram display were saved as an excel file and imported into custom-made script, on MATLAB to calculate source levels (dB peSPL).

3.2.7 Calculating echolocation pulse source levels

The source levels were first determined at the microphones. Taking this into account, the source levels as Sound Pressure Level (SPL) are ultimately converted in decibels (dB) at a distance of 10 cm in front of the bat's position, since this is the standard method of expressing source levels. The distance to the microphone based on the TOAD's was used to calculate source levels. The calculation of source levels considers transmission loss due to geometric spreading, atmospheric absorption and, microphone directionality as the sound traverses the distance from the bat to the microphones (Holderied & von Helversen, 2003). Only the echolocation pulse with the maximum source level value in a series of all species' reconstructed three-dimensional flight trajectories was used for additional analyses. The highest value in a sequence is presumed to be the maximum source level emitted by the bat. This was considered to minimize any directionality although this approach does not always and completely resolve the problem. The problem arises because incoming pulses to the microphone array from the bat may point away from the microphone array. This imply that the source levels in the present research are unlikely to match the actual source levels. The validation process of source levels for all species was implemented by visually checking the source levels derived from each array to ensure they are comparable. Source levels were also validated to guarantee that they were not affected by background noise derived from wind. Using linear regression analysis, the investigation was

carried out to determine whether there were any relationships between the source levels and wind speed.

3.2.8 Calculating detection distance

Distances at which bats detected a range of insect prey sizes (large, medium, small) were calculated for each species in each assemblage using the sonar equation (Møhl, 1988): DT = (SL + 2TLA + TLS + TS) - noise term (dB), where DT is the detection threshold; SL is source level; TLA is the transmission loss due to atmospheric absorption; TLS is the transmission loss resulting from spherical spreading and TS is the target strength. TLA and TLS are distance-based functions. The SL was based on the averages of the loudest echolocation pulse found from each flight path for each species and used to calculate detection distances. The TLA was computed using a formula via a link (resource.npl.co.uk /acoustics/ tech guides/ absorption/) as the attenuation of echolocation signal due to atmospheric absorption at a distance from the microphone dB per meter (dB/m) (UK National Physics Laboratory). The dB/m is a function of echolocation frequency (Hz), local atmospheric conditions [temperature (^{o}C), relative humidity (%) and the atmospheric pressure (kPa)] recorded at the same time while recording echolocation pulses in the field. The formula also has values for reflection loss (c1; -40, -50 & -65), spreading loss (c2; -40, -50 & -65) given by Stilz & Schnitzler (2012). The above values correspond to the acoustic power known as target strength (TS) of the echo reflected off three different prey size categories: Large (TS = -40 dB), medium (TS = -50 dB) and small (TS = -65 dB). Target strength of an echo off a target varies depending on how large the target is in comparison to its wavelength. For instance, the target strength of a solid sphere with a diameter of 4 cm is -40 dB at 1 m which corresponds to -20 dB at 10 cm. Insect prey and targets vary in size, therefore bats often detect echoes from them at a maximum distance that is influenced by their sizes. Usually, an insect/target's echo is weaker when it is smaller, and stronger when it is larger. Moreover, the maximum distance a bat can detect is determined by the frequency of

their echolocation pulses. The point-reflector function and the sonar equation were used to generate estimates of the maximum detection distances of different insect sizes. Given the diverse bat species in biomes that either fly in open space or at edges of clutter and/or within cluttered habitat, it is likely that large, medium and small preys are hunted. Since there was no information on hearing thresholds for the species in the assemblages in this study, I relied on prior studies elsewhere that provided approximate values of bats hearing threshold ranging from 0-20 dB SPL (e.g., Neuweiler et al., 1984). The DT for bat species in the present study was therefore assumed to be 20 dB SPL in line with several studies (Griffin et al., 1960; Holderied & von Helversen, 2003; Stiltz & Schnitzler, 2012; Lewanzik & Goerlitz, 2018; Goerlitz et al., 2020). The 20-dB SPL detection threshold represents the actual sensitivity bats exhibit under natural conditions (when noise is present). The detection distance was calculated computing the signal pulse with the maximum source level, in a series of calculated source levels along with its peak echolocation pulse frequency, as described in Holderied & von Helversen (2003).

3.2.9 Statistical analyses

Statistical analyses were performed in R software (R-version-3.6.1; R core team, 2017) and PAST 3.14 (Hammer et al., 2001). Linear regression was used to test the influence of nightly wind speed (m/s) on source levels and whenever there was any effect detected, source levels were further regressed with peak frequency (kHz) of the echolocation pulses. Descriptive statistics for the average maximum source levels (dB peSPL), frequency (kHz), duration (ms) and detection distances (m) among species (described in Chapter 2) was performed. The normality of data for detection distances for large, medium and small insect sizes was tested using Shapiro-Wilks test (Shapiro & Wilk, 1965). Shapiro-Wilks tests are robust even with small sample sizes. Then Q-Q scatter plots for detection distances were generated by plotting sample quantiles against theoretical quantiles and all these were performed in R statistical

software. Where data were not normal, data were log transformed and reassessed. In cases where normality was not achieved after transformation, a non-parametric Kruskal-Wallis test was applied on a general linear model (GLMs) (Bolker et al., 2009) in PAST 3.14 to test whether detection distances among species within an assemblage were similar according to prediction; (i) different among assemblages from one biome to the next according to prediction, (ii) of the AAH and prediction, (ii) of the alternative hypothesis. Where significant differences were indicated, post hoc multiple pair wise comparisons of the ranked data were done using Dunn's test, showing differences between and within assemblages. Since data for detection distances for *Miniopterus natalensis* were normal, a parametric test one-way ANOVA was performed on GLM, with subsequent post hoc multiple pair wise comparisons of the ranked data that were done using Tukey's test, to test prediction (ii) of the alternative hypothesis. I had limited sample size for *Hipposideros caffer* and therefore excluded it in the statistical analysis.

I also tested the combined effect of echolocation pulse traits (source levels, frequency and duration) on detection distances (Brinkløv et al., 2010). I did so by performing multiple linear regression analyses in GLM to evaluate the effect of the three predictor variables (source levels, frequency and duration) on detection distances for bats within species and among assemblages. A *priori* correlation analysis was performed to examine the robustness of the relationships between model variables, that is, source levels versus duration as well as source levels versus frequency. Correlation analysis was important in this case because changes in frequency and duration of bats could increase detection distances.

3.3 Results

3.3.1 Source levels

There was a low negative correlation between source levels of bat assemblage and wind speed in desert biome ($R^2 = 0.051$, $F_{1,119} = 7.4$, $p < 0.01$; Fig. S3.1), indicating that source levels were

not affected by prevailing wind speeds when pulses were recorded. Thus, it was necessary to perform a test to see whether differences in source levels could be explained by the echolocation peak frequency of the species in that assemblage, and the results revealed a moderate negative relationship (R^2 = 0.224, $F_{1,119}$ = 35.8, p < 0.001). Higher source levels in the desert assemblage were associated with species in the family's Molossidae (e.g., *Sauromys petrophilus*) and Cistugidae (e.g., *Cistugo seabrae*) and their source levels were within the range of similar species as reported by Surlykke & Kalko (2008). Source levels associated with *Rhinolophus capensis* and *Rhinolophus damarensis* were also high although 5-10 dB lower than those achieved by *S. petrophilus* and *C. seabrae*. However, there was no correlation between the source levels of bat assemblages and wind speed in the Fynbos (R^2 = 0.023, $F_{1,133}$ = 1.3, p = 0.2), Savanna (R^2 = 0.018, $F_{1,107}$ = 2.9, p = 0.08), Albany thicket, (R^2 = 0.037, $F_{1,46}$ = 2.8, p = 0.1), Coastal Belt (R^2 = 0.013, $F_{1,63}$ = 0.1, p = 0.8) or Nama Karoo (R^2 = 0.015, $F_{1,55}$ = 0.9, p = 0.3) biomes (Fig. S3.1). On that account, source levels obtained for all bats in the assemblages were considered for subsequent analyses. Source levels across bat assemblages were negatively correlated with frequency (R^2 = 0.076, $F_{1,533}$ = 44.06, p < 0.001; Fig. S3.2) and duration (R^2 = 0.018, $F_{1,533}$ = 9.78, p < 0.01; Fig. S3.3). Considering within species, *R. capensis* revealed a weak negative correlation with source levels and peak frequency (R^2 = 0.054, $F_{1,138}$ > 7.95, *p's* = 0.0055) but not with duration (R^2 = 0.0029, $F_{1,138}$ = 0.4, p = 0.53). Conversely, other species in the assemblages showed no correlation between their source levels and peak frequency (all R^2 < 0.13, *p's* > 0.06) or between the source levels and duration of the pulses (all R^2 < 0.17, *p's* > 0.21).

The regression analysis indicated that detection distances across all prey sizes are explained by the large significant differences in frequency (kHz; *p's* < 0.001), source levels (dB peSPL; *p's* < 0.001) and duration of pulses (ms; *p's* < 0.001) across bat assemblages, with the model explaining the large proportion of the variance > 79%. A similar trend of larger variations in

frequency (kHz; *p's* < 0.001), source levels (dB peSPL; *p's* < 0.001) and duration, (ms; *p's* < 0.01) occurred within five bat assemblages except for duration in the Savanna biome (*p*'*s* > 0.15). The proportion of variance within bat assemblages was also high ranging from 85% to 97%).

Considering species-specific effect of source levels (dB peSPL), frequency (kHz) and duration (ms) of pulses on detection distances, there were considerable and significant variations of parameters even though there were none in some instances. Large and statistically significant differences were found in all parameters according to mean ± SD and regression analyses, for species such as *Neoromicia capensis* (source levels: 136.8 ± 5.8, $F_{1,52}$ >357.1, *p'*s < 0.001, frequency: 36.5 ± 5.4, $F_{1,52}$ > 368.7, *p'*s < 0.001 and duration: 4.8 ± 2.04, $F_{1,52}$ > 0.2, *p'*s < 0.05: all R^2 > 0.93 in all prey sizes), *Rhinolophus clivosus* (source levels: 126.2 ± 6.6, frequency: 90.7 ± 1.04 and duration: 37.1 ± 4.9: $F_{1,39}$ > 27.7, *p's* < 0.001: R^2 > 0.96 in all prey sizes) and *Rhinolophus simulator* (source levels: 127.2 ± 9.8, frequency: 81.9 ± 1.3, $F_{1,12}$ > 118.2, *p's* < 0.001 and duration: 40.7 ± 7.7, $F_{1,12}$ > 8.5, *p*'s < 0.01: R^2 > 0.98 in all prey sizes). Species with considerable variability in source levels and duration but not in frequency include: *Miniopterus fraterculus* (source levels: 128.9 ± 4.9, $F_{1,23}$ > 87.3, *p's* < 0.001, duration: 2.5 ± 0.7, $F_{1,23}$ > 8.3, *p*'*s* < 0.01 and frequency: 62.7 ± 1.8, $F_{1,23}$ > 0.95, *p*'*s* > 0.18: R^2 > 0.80 in all prey sizes), *R. damarensis* (source levels: 125.04 ± 7.4, duration: 42.1 ± 9.9, $F_{1,28}$ > 11.4, *p*'*s* < 0.001 and frequency: 83.5 ± 1.3, $F_{1,28}$ > 0.003, *p's* > 0.51: R^2 > 0.91 in all prey sizes) and *Rhinolophus swinnyi* (source levels: 127.5 ± 8.6, duration: 42.7 ± 7.4, $F_{1,11}$ > 41.5, *p*'*s* < 0.001 and frequency: 99.8 ± 2.5, $F_{1,11}$ *p's* > 0.01: R^2 > 0.98 in all prey sizes). Whilst other species showed large significant variation in both source level and frequencies, the duration of pulses had no effect e.g., *M. natalensis* (source levels: $F_{1,95}$ > 662.2, *p's* < 0.001: frequency: $F_{1,95}$ > 121.5, *p's* < 0.001 and duration: $F_{1,95}$ > 0.20, *p's* > 0.55: R^2 > 0.89 in all prey sizes), *C. seabrae* (source levels:138.01 ± 5.1, $F_{1,13}$ > 25.7, *p's* < 0.001: frequency: 35.01 ± 3.3, $F_{1,13}$ > 2.3, *p's* <

0.05 and duration: 6.1 ± 1.3, $F_{1,13} > 0.6$, *p's* > 0.29: $R^2 > 0.73$ in all prey sizes), *Myotis tricolor* (source levels: 127.6 ± 8.6, frequency:47.3 ± 3.2, $F_{1,32} > 165.5$, *p's* < 0.001 and shortest duration: 2.4 ± 0.5, $F_{1,32} > 0.2$, *p's* > 0.16: $R^2 > 0.94$ in all prey sizes) and *S. petrophilus* (source levels: 129.9 ± 9.6, $F_{1,4} > 122.6$, *p's* < 0.001: frequency: 31.6 ± 2.3, $F_{1,4} > 8.3$, *p's* < 0.05 and duration: 6.01 ± 0.8, $F_{1,4} > 0.18$, *p's* > 0.60: $R^2 > 0.97$ in all prey sizes). *R. capensis*, like the latter three species showed significant differences in source levels and frequency: $F_{1,136} > 934.0$, *p's* < 0.001, although not in duration: $F_{1,136} > 0.1$, *p's* > 0.08: $R^2 > 0.96$ in all prey sizes. The populations' *R. capensis* over its whole range of biomes exhibit a notable frequency range of 75-86 kHz (Table 3.1). Therefore, environmental selection shapes the diversity of echolocation pulse structure of *R. capensis* populations that live apart geographically. Lastly, *Tadarida aegyptiaca* had large differences only in source levels: 134.6 ± 12.7, $F_{1,8} > 18.2$, *p's* < 0.001 but not in duration: 14.9 ± 1.6 or frequency: 21.09 ± 0.9, $F_{1,8} > 0.03$, *p's* > 0.24: $R^2 >$ 0.70 in all prey sizes, whereas none of the parameters for *Nycteris thebaica* were statistically significant (source levels: 113.4 ± 3.5: frequencies: 85.6 ± 2.8 and duration: 1.7 ± 0.5, $F_{1,4} >$ 0.10, *p's* > 0.16) whereby $R^2 < 0.47$. Despite some instances of parameter overlap among species, major differences within and between assemblages can be attributed to species phylogenetic relatedness and also to differences in foraging modes for the diverse species in assemblages (Table 3.1).

Table 3.1. The mean ± SD for pulse frequency (kHz) duration (ms), average of maximum source levels (dB peSPL) of bat species and their detection distances (m) of three prey sizes across six sites in biomes of South Africa

Biome	Species	FG	FP (n)	Frequency (kHz)	Duration (ms)	Source level (dB peSPL)	Detection distance (m) TS -40	TS -50	TS -65
Fynbos	*Rhinolophus capensis*	C	28	81.5 ± 2.4 (77.07-85.0)	39.05 ± 7.2 (26.4-53.3)	122.3 ± 4.5 (117.7-133.01)	6.1 ± 0.5 (5.4-7.3)	4.9 ± 0.5 (4.3-6.1)	3.4 ± 0.4 (3.04-4.4)
	Miniopterus natalensis	CE	21	52.9 ± 1.3 (52.3-55.7)	4.5 ± 1.3 (2.4-7.03)	135.6 ± 8.8 (117.6-146.3)	10.2 ± 1.7 (12.3-7.01)	8.4 ± 1.5 (5.5-10.3)	6.01 ± 1.3 (4.1-7.7)
	Myotis tricolor	CE/T	12	47.3 ± 3.2 (39.9-52.7)	2.4 ± 0.5 (1.6-3.3)	127.6 ± 8.6 (114.8-146.7)	9.7 ± 1.9 (7.2-13.5)	7.7 ± 1.7 (5.5-11.4)	5.2 ± 1.4 (3.4-8.4)
	Neoromicia capensis	CE	37	40.03 ± 2.3 (34.6-44.5)	5.8 ± 1.9 (2.9-9.7)	129.9 ± 7.6 (118.5-147.8)	11.5 ± 2.04 (7.4-15.7)	9.1 ± 1.8 (5.5-13.1)	6.09 ± 1.5 (3.3-8.6)
	Tadarida aegyptiaca	OA	12	21.09 ± 0.9 (19.7-22.7)	14.9 ± 1.6 (12.2-17.4)	134.6 ± 12.7 (117.6-148.7)	21.9 ± 6.9 (13.1-29.9)	17.04 ± 6.1 (9.2-23.9)	9.8 ± 4.3 (5.04-15.9)
Desert	*Rhinolophus capensis*	C	40	73.6 ± 1.4 (71.02-76.2)	43.1 ± 10.5 (21.2-63.8)	128.8 ± 4.2 (117.5-134.5)	7.1 ± 0.6 (5.8-7.9)	5.9 ± 0.5 (4.7-6.6)	4.20± 0.4 (3.1-4.8)
	Rhinolophus damarensis	C	32	83.5 ± 1.3 (80.02-87.2)	42.1 ± 9.9 (21.8-59.7)	125.04 ± 7.4 (116.7-141.4)	6.2 ± 0.8 (5.3-8.2)	5.1 ± 0.8 (4.1-6.9)	3.6 ± 0.7 (2.7-5.3)
	Miniopterus natalensis	CE	24	50.3 ± 2.4 (45.8-56.3)	3.6 ± 0.5 (2.7-4.6)	132.1 ± 4.7 (126.5-144.8)	9.7 ± 1.3 (7.9-12.9)	7.90 ± 1.1 (6.4-10.8)	5.5 ± 0.8 (4.4-7.9)
	Sauromys petrophilus	OA	8	31.6 ± 2.3 (28.2-35.3)	6.01 ± 0.8 (4.7-7.4)	129.9 ± 9.6 (118.3-146.6)	11.7 ± 3.5 (7.3-16.9)	9.2 ± 3.1 (5.4-13.9)	6.04 ± 2.5 (3.1-9.7)
	Cistugo seabrae	CE	17	35.01 ± 3.3 (29.3-41.1)	6.1 ± 1.3 (4.1-8.7)	138.01 ± 5.1 (123.7-146.6)	14.5 ± 1.9 (11,3-18.9)	11.8 ± 1.7 (8.7-15.5)	8.1 ± 1.3 (5.4-10.9)

Albany Thicket	*Rhinolophus capensis*	C		83.3 ± 0.6	36.8 ± 7.6	125.4 ± 5.7	6.3± 0.6	5.2 ± 0.6	3.7 ± 0.5
				(81.9-84.3)	(21.2-48.5)	(118.6-136.8)	(5.2-7.3)	(3.9-6.1)	(2.6-4,54)
	Miniopterus natalensis	CE		55.6 ± 1.6	4.5 ± 0.9	126.9 ± 9.6	8.4 ± 1.7	6.8 ± 1.6	4.7 ± 1.3
				(53.3-58.8)	(3.3-6.03)	(117.3-142.4)	(6.5-11.2)	(5.2-9.1)	(3.4-6.9)
	Neoromicia capensis	CE	7	42.3 ± 1.1	4.1 ± 0.4	136.8 ± 5.8	12.4 ± 1.4	10.1 ± 1.3	7.1 ± 1.1
				(40.5-44.4)	(3.5-4.8)	(128.1-142.7)	(10.2-13.9)	(8.1-11.5)	(5.4-8.2)
	Nycteris thebaica	G	8	85.6 ± 2.8	1.7 ± 0.5	113.4 ± 3.5	5.01 ± 0.5	4.0 ± 0.5	2.7 ± 0.5
				(81.4-89.8)	(1.3-2.9)	(110.7-119.4)	(4.7-5.9)	(3.5-4.9)	(2.3-3.7)
Savanna	*Rhinolophus clivosus*	C	43	90.7 ± 1.04	37.1 ± 4.9	126.2 ± 6.6	6.6 ± 0.8	5.9 ± 0.7	3.9 ± 0.6
				(88.5-92.8)	(27.8-49.4)	(110.3-140.4)	(4.8-8.1)	(3.8-6.9)	(2.5-5.2)
	Rhinolophus swinnyi	C	14	99.8 ± 2.5	42.7 ± 7.4	127.5 ± 8.6	6.4 ± 1.0	5.3 ± 0.9	3.8 ± 0.8
				(89.9-101.9)	(21.1-53.1)	(109.9-139.3)	(5.2-7.4)	(3.6-6.3)	(2.3-4.8)
	Miniopterus fraterculus	CE	11	62.7 ± 1.8	2.5 ± 0.7	128.9 ± 4.9	8.7 ± 0.9	7.1 ± 0.8	4.9 ± 0.6
				(58.1-65.04)	(1.3-4.02)	(118.1-134.1)	(7.1-10.1)	(5.6-8.2)	(3.6-5.7)
	Miniopterus natalensis	CE	16	57.2 ± 4.04	3.9 ± 1.2	127.1 ± 7.2	8.7 ± 0.9	6.9 ± 0.9	4.8 ± 0.8
				(46.3-63.1)	(2.3-6.1)	(110.03-137.2)	(6.4-9.1)	(4.8-7.6)	(2.9-5.5)
	Myotis tricolor	CE/T	24	48.8 ± 3.7	2.5 ± 0.8	125.4 ± 6.1	9.1 ± 1.2	7.2 ± 1.1	4.8 ± 0.9
				(40.8-56.7)	(1.2-4.3)	(114.5-135.9)	(7.1-11.6)	(5.4-8.9)	(3.4-6.2)
Coastal Belt	*Rhinolophus simulator*	C	16	81.9 ± 1.3	40.7 ± 7.7	127.2 ± 9.8	5.9 ± 1.1	4.9 ± 0.9	3.6 ± 0.8
				(78.4-83.3)	(20.7-49.8)	(104.6-144.5)	(3.8-7.8)	(2.9-6.7)	(1.9-5.2)
	Miniopterus fraterculus	CE	16	61.4 ± 2.3	3.04 ± 0.8	125.9 ± 4.7	7.3 ± 0.7	5.9 ± 0.7	4.1 ± 06
				(57.8-66.3)	(1.9-5.2)	(119.2-135.4)	(6.2-9.1)	(5.3-7.6)	(3.4-4.5)
	Miniopterus natalensis	CE	13	55.2 ± 2.8	3.6 ± 1.2	129.5 ± 4.9	8.6 ± 1.1	7.04 ± 1.1	4.9 ± 0.8
				(47.6-57.6)	(2.1-6.8)	(122.1-136.6)	(7.1-10.4)	(5.7-8.5)	(3.8-6.1)
	Neoromicia capensis	CE	20	37.5 ± 5.5	4.8 ± 2.1	131.1 ± 6.3	13.5 ± 3.1	10.6 ± 2.6	7.1 ± 1.8

				(30.2-47.3)	(2.9-9.7)	(118.8-143.4)	(10.1-19.04)	(8.1-15.4)	(4.8-10.6)
Nama Karoo	*Rhinolophus clivosus*	C	18	90.2 ± 0.5	47.1 ± 5.8	134.1 ± 7.04	6.6 ± 0.8	5.6 ± 0.7	4.3 ± 0.7
				(90.6-92.6)	(30.02-59.8)	(120.5-144.5)	(5.2-7.8)	(4.3-6.6)	(3.1-5.1)
	Rhinolophus capensis	C	14	84.8 ± 1.04	37.4 ± 7.6	132.1 ± 3.4	6.9 ± 0.5	5.9 ± 0.4	4.3 ± 0.4
				(83.2-86.7)	(21.2-47.9)	(125.5-136.4)	(6.3-7.5)	(5.2 -6.4)	(3.7-4.8)
	Miniopterus natalensis	CE	15	52.7 ± 3.7	4.1 ± 0.8	130.3 ± 4.9	9.4 ± 0.9	7.6 ± 0.8	5.3 ± 0.7
				(42.2-58.1)	(2.9-5.7)	(122.6-141.4)	(8.3-11.5)	(6.5-9.04)	(4.3-6.6)
	Neoromicia capensis	CE	13	36.5 ± 5.4	4.8 ± 2.04	133.1 ± 5.8	12.04 ± 2.5	9.2 ± 2.3	6.2 ± 1.2
				(31.3-46.3)	(2.9-8.9)	(121.9-143.3)	(9.3-13.6)	(7.2 -10.6)	(5.5-7.3)

OA = open air; C = cluttered; G = gleaner; CE = clutter-edge (Schoeman & Jacobs, 2008); CE/T = clutter-edge/trawler (Moyo & Jacobs, 2020) TS = Target strength (dB) where TS -40 = large prey; TS -50 = medium prey; TS -65 = small prey; FG = foraging group; FP = flight paths; () = range

3.3.2 Comparing detection distances within sites (testing predictions (i), (ii) & (iii))

Shapiro-Wilk normality test (Shapiro & Wilk, 1965) indicated that data for detection distances for large (L), medium (M), and small (S) prey sizes within all assemblages were not normally distributed (W = 0.4356; $p < 0.05$; $F_{5, 529} = 6.125$) even after data were log transformed (Fig. S3.4).

Detection distances were significantly different within each assemblage for all three sizes classes of prey (Kruskal-Wallis > 34.27; df = 4 or 3; *p's* < 0.001) (Table 3.1). In each assemblage the LDC aerial hawkers generally had longer detection distances than the clutter specialist HDC rhinolopids (Dunn's post hoc *p's* < 0.001). The open-air aerial hawkers such as *T. aegyptiaca* (L: 21.9 ± 6.9 m, M: 17.04 ± 6.1 m and S: 9.8 ± 4.3 m) and *S. petrophilus* (L: 11.7 ± 3.5 m, M: 9.2 ± 3.1 m and S: 6.04 ± 2.5 m) had the longest detection distances of all species (*p's* < 0.001) with the exception of the clutter-edge forager *C. seabrae* which had similar detection distances for large (14.5 ± 1.9 m) and medium (11.8 ± 1.7 m) prey size to those of *S. petrophilus* (*p's* > 0.11) but longer detection distances for the small prey size class (8.1 ± 1.3 m) than that of *S. petrophilus* bats ($p < 0.05$). This is probably the result of its higher source levels combined with a frequency that is not much higher than that of *S. petrophilus* (Table 3.1). These differences in detection distances within assemblages do not support prediction (i) of the AAH but instead supports prediction (i) of the alternative hypothesis.

Hipposideros caffer was excluded from the final analysis due to small sample size. However, the selected flight trajectory revealed the shortest detection distances across prey sizes (L: 2.53 m, M: 1.98 m and S:1.29 m) even shorter than that of the gleaner *N. thebaica* (L: 5.01 ± 0.5 m, M: 4.0 ± 0.5 m and S: 2.7 ± 0.5 m).

3.3.3 Detection distances across sites in biomes

Detection distances across assemblages were significantly different for large (Kruskal-Wallis = 35.51; df = 5; $p < 0.001$), medium (Kruskal-Wallis = 32.29; df = 5; $p < 0.001$) and small (Kruskal-Wallis = 27.55; df = 5; $p < 0.001$) prey sizes (Figs. 3.3, 3.4 & 3.5). Dunn's post hoc test indicates detection distances for assemblage in the Savannah site (L: 7.6 ± 1.5 m, M: 6.2 ± 1.2 m and S: 4.3 ± 0.9 m) were shorter than those in Fynbos (L: 9.8 ± 4.7 m, M: 7.9 ± 3.7 m and S: 5.3 ± 2.3 m) ($p < 0.001$), Coastal Belt (L: 9.1 ± 3.5 m, M: 7.4 ± 2.7 m and S: 5.02 ± 1.8 m) ($p < 0.05$) and Albany thicket (L: 7.2 ± 2.4 m, M: 5.9 ± 2.1 m and S: 4.1 ± 1.4 m) ($p < 0.05$) sites across all prey sizes but with site in the Desert biome ($p < 0.05$) for small prey size only (4.9 ± 1.8 m). Detection distance for a site in the Fynbos was nearly 4 m longer than in both Nama Karoo (L: 7.6 ± 1.4 m, M: 6.3 ± 1.1 m and S: 4.5 ± 0.7 m) ($p < 0.01$) and Albany thicket ($p < 0.001$), with the latter site having the shortest detection distance of all sites ($p < 0.01$).

However, similarities in detection distances of prey across some assemblages also occurred. For instance, detection distances of large (8.7 ± 3.1 m) and medium size (7.1 ± 2.5 m) prey for a site in the Desert biome was predominantly not significantly different from Savanna (L: 7.6 ± 1.5 m and M: 6.2 ± 1.2 m) ($p = 0.08$ and $p = 0.07$), Fynbos (L: 9.8 ± 4.7 m and M: 7.9 ± 3.7 m) ($p = 0.08$ and $p = 0.16$), Nama Karoo (L: 7.6 ± 1.4 m and M: 6.3 ± 1.1 m) ($p = 0.15$ and $p = 0.33$) and Coastal Belt (L: 9.1 ± 3.5 m and M: 7.4 ± 2.7 m) ($p = 0.48$ and $p = 0.53$) sites. Detection distances in Coastal Belt site was not statistically different from both Fynbos ($p = 0.47$ and $p = 0.59$) and Nama Karoo ($p = 0.07$ and $p = 0.17$) sites, although detection distance in the latter site was not significantly different from Savannah ($p = 0.89$ and $p = 0.66$) for large and medium prey. Detection distance for small insect prey in Nama Karoo site (4.5 ± 0.7 m) was not significantly different from Fynbos (5.3 ± 2.3 m) ($p = 0.47$. Thus, differences in detection distances among assemblages only partially supports prediction (ii) of the AAH.

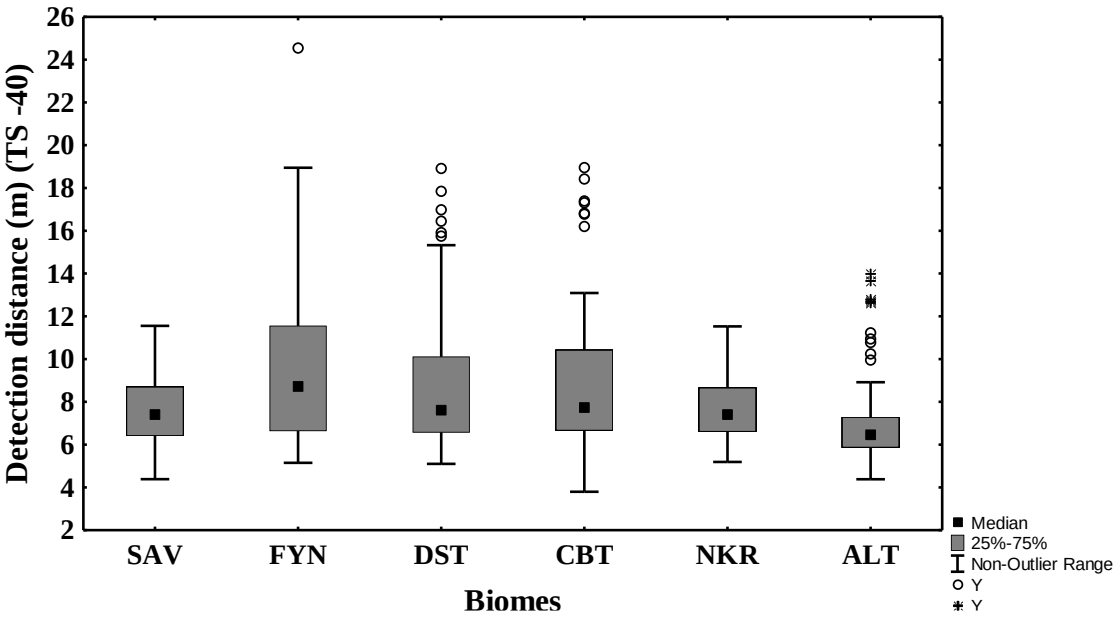

Figure 3.3. Box and whisker plots of median detection distance (m) (TS = -40 dB) for bat assemblages across six biomes (SAV-Savanna; FYN-Fynbos; DST-Desert; CBT-Coastal Belt; NKR-Nama Karoo; ALT-Albany thicket; Y-outlier).

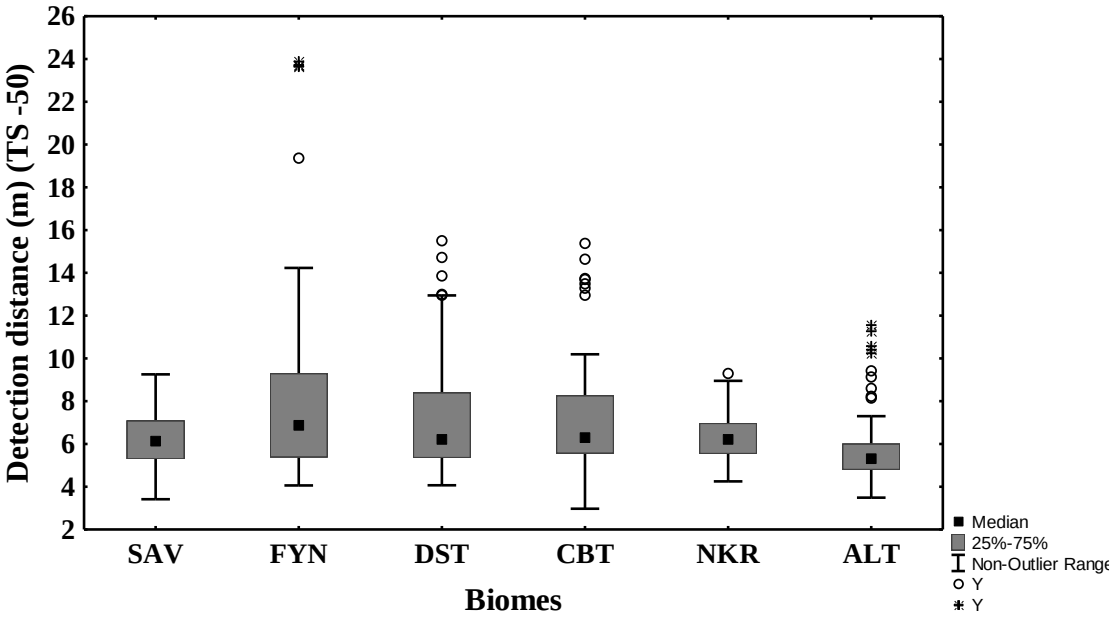

Figure 3.4. Box and whisker plots of median detection distance (m) (TS = -50 dB) for bat assemblages across six biomes (SAV-Savanna; FYN-Fynbos; DST-Desert; CBT-Coastal Belt; NKR-Nama Karoo; ALT-Albany thicket; Y-outlier).

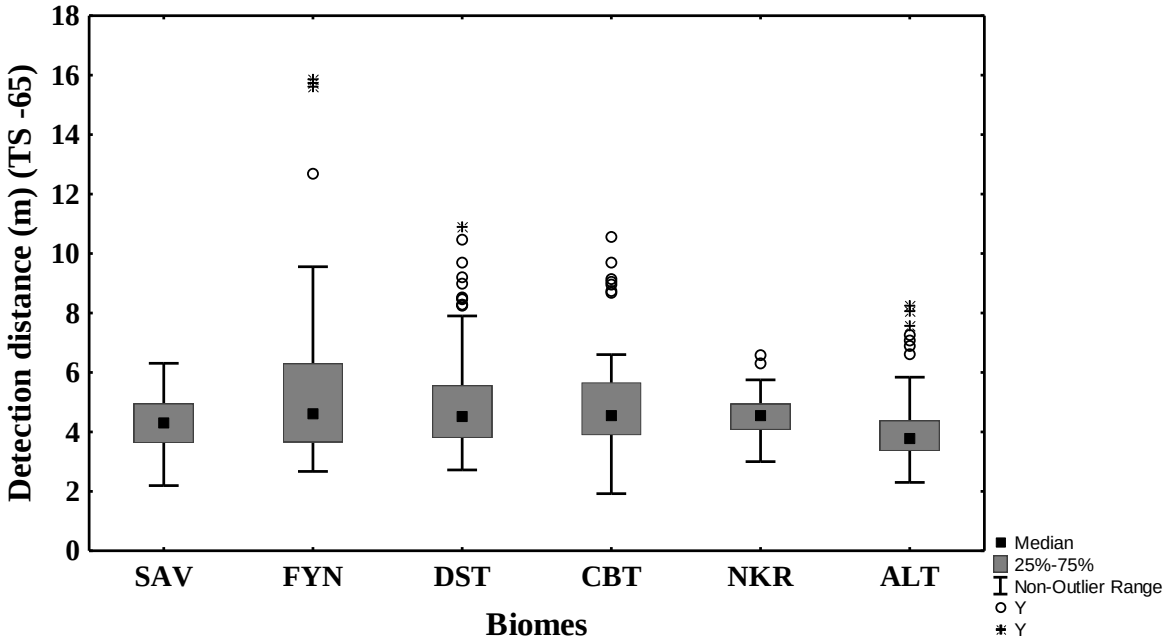

Figure 3.5. Box and whisker plots of median detection distance (m) (TS = -65 dB) for bat assemblages across six biomes (SAV-Savanna; FYN-Fynbos; DST-Desert; CBT-Coastal Belt; NKR-Nama Karoo; ALT-Albany thicket; Y-outlier).

3.3.4 Detection distances for Miniopterus natalensis between sites (testing prediction (ii) of the alternative hypothesis).

Shapiro-Wilk normality test (Shapiro & Wilk, 1965) showed that data for detection distances for the three prey sizes across all sites were normally distributed (W = 0.987; $p = 0.526$; $F_{6, 99} = 5.284$; Fig. S3.5). Variances of detection distances between sites were equal (all *p's* > 0.05), so the ANOVA was used to compare detection distances. Detection distances for large (GLM ANOVA = 17.35; df = 6; $p < 0.01$), medium (ANOVA = 18.30; df = 6; $p < 0.01$) and small (Anova = 18.24; df = 6; $p < 0.01$) prey across sites was statistically significant (Fig. S3.6, S3.7 & S3.8). Tukey post hoc test indicate that detection distances across all prey sizes for *Miniopterus natalensis* in a site in the Fynbos biome (L: 10.2 ± 1.7 m, M: 8.4 ± 1.5 m and S: 6.02 ± 1.3 m) were 1-2 m longer than those in the Savannah (L: 8.7 ± 1.05 m, M: 7.1 ± 0.9 m and S: 4.8 ± 0.8 m) ($p < 0.05$), Grassland (L: 8.5 ± 1.3 m, M: 6.7 ± 1.1 m and S: 4.5 ± 0.9 m) ($p < 0.05$), Coastal Belt (L: 8.6 ± 1.1 m, M: 7.1 ± 1.03 m and S: 4.9 ± 0.8 m) ($p < 0.01$) and

Nama Karoo (L: 9.4 ± 0.9 m, M: 7.6 ± 0.8 m and S: 5.3 ± 0.7 m) ($p < 0.01$) biome sites. The latter two sites had similar detection distances; however, they were on average 1m shorter than those in the Desert (L: 9.7 ± 1.3 m, M: 7.9 ± 1.1 m and S: 5.5 ± 0.8 m) ($p < 0.05$). Detection distances between most sites were not significantly different (p's > 0.05) (Table 3.1). Similar detection distance within species between sites contradict the AAH.

Table 3.2. *Miniopterus natalensis*; average maximum source levels (dB peSPL; Mean ± SD) and detection distances (m; Mean ± SD) of three insect sizes across seven biomes in South Africa.

Biome	***n***	**Frequency (kHz)**	**Source Levels (dB peSPL)**	**Detection Distances (m) (TS = -40 dB)**	**(TS = -50 dB)**	**(TS = -65 dB)**
Fynbos	21	53	135.6 ± 8.8	10.2 ±1.7	8.4 ± 1.5	6.0 ± 1.3
Desert	24	54	132.1 ± 4.7	9.7 ± 1.3	7.9 ± 1.1	5.5 ± 0.8
Albany Thicket	10	56	127.0 ± 9.6	8.4 ± 1.7	6.8 ± 1.6	4.7 ± 1.3
Savanna	16	57	127.1 ± 7.2	8.7 ± 1.0	7.0 ± 0.9	4.8 ± 0.8
Coastal Belt	13	55	129.5 ± 4.9	8.6 ± 1.1	7.0 ± 1.0	4.9 ± 0.8
Nama Karoo	15	53	130.3 ± 5.0	9.4 ± 0.9	7.6 ± 0.8	5.3 ± 0.7
Grassland	7	57	123.8 ± 8.0	8.5 ± 1.3	6.7 ± 1.1	4.5 ± 0.9

3.4 Discussion

The study showed that prediction (i) of the AAH was not confirmed, prediction (ii) only partially confirmed, and prediction (iii) was not confirmed. This implies that the AAH was not supported because detection distances appear to be species-specific rather than habitat-specific for bat assemblages. Bats in the same assemblage in the same habitat had varying body sizes, foraging strategies and using different source levels, which were associated with differences in detection distances, confirming prediction (i) of the alternative hypothesis; but prediction (ii)

was only partially supported as *M. natalensis* did not fully support the latter prediction of the alternative hypothesis.

This study shows that bats within assemblages of different frequencies emitted high source levels regardless of body sizes and had variable detection distances; this demonstrates the relationship between echolocation pulse parameters with detection distances for bats in assemblages. A similar pattern is shown by Holderied & von Helversen (2003) who found that bat species in their study emitted intense echolocation pulses. Larger species such as *Nyctalus lasioperus* emitted echolocation pulses with maximum source levels of 133 dB peSPL, which is 5 dB greater than those emitted by *Pipistrellus pygmaeus* (small). Large bat species like *Rhinolophus ferrumequinum* using high frequency and *Eptesicus bottae* using low frequency, emit intense pulses of 130 dB peSPL and 133 dB peSPL, respectively (Holderied et al., 2005; Goelitz et al., 2020). This indicates that the species in the aforementioned studies had varied detection distances. However, Surlykke & Kalko (2008) reported that smaller bats using higher frequencies had similar detection distances to larger bats using lower frequencies because the smaller bats emitted their pulses at higher source levels. Therefore, differences and similarities in detection distances are an indicator of a selective process involving an intricate relationship between echolocation pulse parameters, flight morphology and foraging sites in habitats.

Bat assemblages consist of species of different body sizes and wing designs that are optimally influenced by many selection processes, to meet physiological (e.g., thermoregulation) and foraging demands (Norberg & Rayner, 1987; Blackburn et al., 1999). The balance between these selection processes may differ with environment, foraging habitat, and prey availability, and may as well vary in morphology. Observations have shown that wing morphology is highly correlated with a bat's foraging habitat (Saunders & Barclay, 1992; Jacobs, 1999a), and

echolocation pulse characteristics (Jacobs, 1999a), and in combination these constitute an adaptive complex (Aldridge & Rautenbach, 1987). There is a close relationship between echolocation frequency and body size in bats (Jacobs et al., 2007; Stoffberg et al., 2011). Large bats emit echolocation pulses with long duration at low frequency than small bats (Zhang et al., 2000; Jacobs et al., 2007). The negative allometric relationship between pulse frequency and body size is clearly demonstrated in bat families, e.g., Vespertilionidae, Emballonuridae, Molossidae, Hipposideridae and Rhinolophidae (Jones, 1996; Jacobs et al., 2007; Stoffberg et al., 2011). Changes in body size and echolocation pulse frequency may further be reflected in wing morphology and in the end impact on foraging behaviour of bats. If so, bats may employ different foraging strategies, resulting to differences prey detection distances within an assemblage, which may vary from one assemblage to another.

This study revealed how bats modify their echolocation pulse features (source levels, frequency and duration) to achieve the optimal detection distances. Bats flying in open space (*T. aegyptiaca* and *S. petrophilus*) not only produced long and the loudest pulses, but they also attained the longest estimated detection distances across the prey sizes. Indeed, detection distances were within the general range of open-space foraging bats in other studies (Holderied et al., 2005; Surlykke & Kalko, 2008). The latter two species are of large and medium body sizes, respectively, with long and narrow wings, and wing aspect ratios and loads that are above average compared to bats that fly in closed habitats (Norberg & Rayner, 1987). They are thought to be energetically adapted to swift flight between resource fields and for that reason, they produce loud pulses at low frequency to probe signals further in relatively open space to detect prey without reacting to clutter (Neuweiler, 1990). Both *M. natalensis* and *M. fraterculus* are phylogenetically related, have similar medium body sizes (Miller-Butterworth et al., 2005) and have similar high echolocation pulse source levels, relatively higher frequencies, shorter

duration and detection distances. However, the former has high wing loading and aspect ratio suited to forage on aerial prey in relatively open or less cluttered habitat (Jacobs, 1999b; Schoeman & Jacobs, 2003). The latter has low wing loading suited to maneuver in open space and relatively closed habitats (Jacobs, 1999) just like *M. tricolor* of similar body size. *N. capensis* has an intermediate wing loading while *C. seabrae* with low wing-loading (Schoeman & Jacobs, 2008) although both are of similar small body sizes and they emit echolocation pulses of optimal high source levels at low frequencies and long duration that contribute to longer detection distances as they search for prey in respective sites. Low frequency pulses and the increased duration reduce the effect of atmospheric attenuation. Atmospheric attenuation increases significantly with frequency (Lawrence & Simmons, 1982) which supposedly poses a limitation such bats have to compromise between detection distance on one hand, and simultaneously resolve and localize on the other (Kalko & Schnitzler, 1993). Detection distances plays an important role in relation to flying speed and maneuverability when clutter is detected although it might be inconsequential for *Rhinolophus* and *Hipposideros* species due to their ability to use acoustic glints within vegetation (Kalko & Schnitzler, 1993). Among Rhinolophidae and Hipposideridae, echolocation pulse frequency scales negatively with body size (Heller & Helversen, 1989; Jones, 1996; Stoffberg et al., 2011) although *Rhinolophus* species e.g., *R. clivosus* deviate from the trend (Jacobs et al., 2007). However, *Rhinolophus* species have characteristically similar wing proportions and a low wing load (Jacobs, 2000). Low wing loading enables slow flight and shorter wings allow easier negotiation in narrow space making it possible to detect fluttering prey against background clutter at short distances. This study confirmed that some species in the family Rhinolophidae, occurring in different biomes showed variations in echolocation pulse source levels, duration and frequency (*R. clivosus* and *R. simulator*), others with source levels and duration but not frequency (*R. damarensis R. swinnyi*), and *R. capensis* with source levels and frequency but not duration.

Despite obvious variations in traits amongst *Rhinolophus* species, the species exhibited equivalent and shortest detection distances across prey sizes. A previous study (Mutumi et al., 2016) also determined detection distances of large prey size for two sibling species whereby *R. swinnyi* and *R. simulator* had 6 m and 8 m, respectively. In support, detection distance of the former species was similar with the current findings, unlike the latter species whose detection distance was 2 m longer than reported here. *Rhinolophus* species normally emit high frequency pulses (75-100 kHz; Table 3.1) that are also highly directional, increases resolution and localization (Kalko & Schnitzler, 1993). At the same time, they emit intense pulses (> 120 dB peSPL; Table 3.1), possibly to compensate for frequency-dependant atmospheric attenuation, which confer *Rhinolophus* species superior ability to detect small prey sizes. Both *Hipposideros caffer* and *N. thebaica* have morphological similarities in wing design of low wing loading and low aspect ratio (Bowie et al., 1999). Similarity is also reflected in their flight pattern as both species ably hover (Bell & Fenton, 1984) and use a combination of aerial pursuit and gleaning to capture prey (Norberg & Rayner, 1987; Aldridge & Rautenbach, 1987). Thus, their feeding strategies may suggest why they emit low source levels (86.2 versus 113.4 dB peSPL mean values, respectively) combined with high frequencies (133-144 versus 85.6 kHz mean values, respectively) and reduced duration (14 versus 1.7 ms mean values, respectively) of their pulses. Higher frequency pulses are heavily attenuated (Lawrence & Simmons, 1982) though enhances directionality and resolution of targeted objects, hence suitable for detection at short distances (Neuweiler, 1984). Hence, within an assemblage of bats, differences in body sizes and foraging strategies may result in differences in echolocation pulse frequency and detection distances. For these reasons, prediction (i) of the AAH was not confirmed and prediction (ii) only partially confirmed. It has been shown that bat wing morphology determines their ability to disperse into new geographic locations (Norberg & Rayner, 1987). This is because bats with higher wing loading, and higher aspect ratio fly at higher speed and longer distances. Accordingly, *M.*

natalensis is one of the species that exhibit intraspecific variation in wing morphology within biomes and from one biome to another (Jacobs, 1999a; Miller-Butterworth et al., 2003). Some individuals forage in the open, others in cluttered habitats, and both have low wing loading although the latter group has wings with low aspect ratio (Jacobs, 1999). Since the group has relatively low wing loading plus its low aspect ratio, confers the species high maneuverability for foraging in open habitats as well as dense habitats. At the same time, the species adjusts its echolocation pulse frequency to the varying degree of clutter such that it emits relatively low frequency pulses of longer duration in the open than cluttered habitat. Echolocation pulses emitted for longer duration at low frequency travels further increasing the probability of prey detection, which is crucial for *M. natalensis* during fast flight. Therefore, *M. natalensis* exhibit flexibility of wing morphology as it occupies a niche and may change from one niche to another suggesting that the flying ability of this species is adapting to either habitat structure or migration or both. This could possibly contribute to species using similar detection distances in some sites but also vary detection distances in other sites. This partially supports prediction (ii) of the alternative hypothesis. Equally, some bird species shows no relationship of their signals with acoustic properties of habitat type (Naguib & Wiley, 2001; Ey & Fischer, 2009), other studies show only fairly minor differences in song signals of habitat types (Boncoraglio & Saino, 2007; Catchpole & Slater, 2008). Lack of evidence for a habitat effect may not absolutely discredit the Acoustic Adaptation Hypothesis, but rather demonstrates the possibility of other crucial determinants affecting structures of signal production, e.g., phylogeny. Phylogeny may constrain acoustic signals and is limited to species body size (Ryan & Brenowitz, 1985). However, in this study, lack of evidence of AAH was because the numbers of replicates within each biome were not sufficient to account for variability within biomes.

These findings have significant implications for the role of echolocation signals for resource partitioning as evidenced by detection probabilities. Some bat species achieved almost thrice or twice longer prey detection distances than others (Table 3.1). A similar pattern was reflected in their foraging modes, with open-space foragers achieving significantly longer detection distances than clutter-edge foragers, gleaner and clutter foragers; the latter two having much shorter detection distances. It simply indicates how niche space is structured between distinct foraging modes, as it is widely accepted. Since species within foraging modes exhibited differences in detection distances across prey sizes, probably indicates that differences in echolocation traits contribute to fine-grained niche differentiation. All of this relates sensory abilities of a group of potentially competing bat species to a direct indicator of their corresponding foraging effectiveness (based on prey detection ability), implying that sensory ecology plays an essential part in bat community structuring.

Basically, the echo strength of insects is virtually independent of the emitted frequency within the 20-100 kHz frequency range (Waters & Jones, 1995). It accordingly implies that bats within this range of the said frequencies would still be able to detect even acoustically small size insects whether at a short or long distances. Jones (1994) showed that aerial hawking bats that used high source levels echolocation pulses and low frequencies between 20 and 30 kHz had diet that constituted not only large but even small insect sizes. This suggests that low atmospheric attenuation at low frequencies permits the detection of even small targets at greater distances. However, a minimum operating distance could be imposed by pulse-echo-overlap (Schnitzler & Kalko, 2001; Thomas et al., 2004) and therefore long signal duration used by LDC bats may introduce another limit on the detectability of small insects. To overcome this problem, aerial feeding bats use long signal duration that increases at lower frequencies, so the effect of low frequency and long duration is combined in many bat species (Jones, 1999). HDC

bats using high frequency signal is suited to detect wider range of insect sizes although it is limited at short distances due to severe atmospheric attenuation. Ultimately, when a bat's signal performs well in detecting small targets, the minimum prey sizes may be limited not by detection constraint, but by the size distribution of insects in the habitat or by maneuverability of the bats. However, smaller insects may be less profitable to consume than larger insects, and this may also influence the choice of minimum prey size by a bat.

In summary, whether there were variations in source level, duration and frequency among bat assemblages or not, the prey detection distances varied. A two- to seven-fold variation in detection distances was observed across all target strengths. Flight speed, distance from ground surface, background clutter and other species-specific differences are also likely to influence echolocation pulse characteristics associated with phylogenetic relatedness. Based on the present results, it is evident that detection distance seems to be a critical evolutionary factor in adapting pulse source levels to attain detection distances for prey. Closely related species in assemblages that are essentially associated with a particular foraging strategy would have same detection distance. Dissimilarity in habitat structure and environmental conditions across sites may suggest that bat species access the variety of available insect prey. Finally, the present study emphasizes the importance of intensity measurement in the field as source level plays a crucial and to a great extent, the underestimated role in bat echolocation. It is quite evident that bats may respond differently to varying degree of vegetation clutter (Brinkløv et al., 2010). Further understanding the influence of environmental variables on detection distance of bat assemblages would be invaluable. These involves testing whether proxies of climate (e.g., temperature and relative humidity) correlate with detection distance across several sites in different biomes (Chapter 4) and, would represent the final test of the Acoustic Adaptation Hypothesis.

CHAPTER 4

Climatic correlates of detection distances for bat assemblages and *Miniopterus natalensis* across sites in biomes of South Africa:

‘‘A test of the Acoustic Adaptation Hypothesis’’

Abstract

Climatic conditions present varying environmental challenges for echolocation systems. As such, the quality and content of information derived from echolocation pulses reflects these environmental challenges. Temperature, relative humidity and atmospheric pressure impact the transmission of acoustic signals due to atmospheric attenuation which alters prey detection distances. Thus, echolocation pulses within or among species may differ from one habitat to the next due to climate-induced variable selection pressure, resulting in local adaptation. The Acoustic Adaptation Hypothesis (AAH) proposes that acoustic properties of the environment influence sound propagation and ultimately the evolution of echolocation pulses. The AAH predicts that bat detection distances should be: (i) similar in the same assemblage exposed to the same climatic conditions, (ii) different across bat assemblages, and (iii) different for species populations, occupying localities that differ in climatic conditions. As an alternative, body size, life history strategies, and phylogeny may affect detection distances more strongly. If so, detection distances should: (i) differ within and across bat assemblages occupying different habitats and exposed to different climatic conditions, and (ii) be species-specific and remain constant across localities despite differences in climatic conditions. Here, I used multiple microphone arrays to measure the source levels of echolocation pulses of bat assemblages (comprising 14 bat species) across six sites and the ubiquitous species, *Miniopterus natalensis* across the same six sites and a seventh site in South African biomes. Results shows that within and among bat assemblages detection distances were different because assemblages comprised

species with a wide range of body sizes, life histories or phylogenies, which may influence detection distances more strongly. These considerations contradict prediction (i) of the AAH, instead they provided support for prediction (i) of the alternative hypothesis. The detection distances were different between some bat assemblages but not others, which partially supports the AAH prediction (ii). The detection distances of *M. natalensis* populations were similar, supporting prediction (ii) of the alternative hypothesis but not predictions (iii) of the AAH. Predictive modelling results revealed that the differences in detection distances for bat assemblages were correlated with temperature and longitude. The detection distances of *M. natalensis* were associated with longitude. The detection distances for bat assemblages and *M. natalensis* were, however, not correlated to relative humidity, atmospheric pressure, and latitude. Paradoxically, because of the composite effect of individual PCs, all climatic variables appeared to be stronger predictors of the differences in detection distances for bat assemblages and similar detection distance for *M. natalensis*. Because temperature and relative humidity may change at different longitudes and latitudes owing to diverse geographical features that affect atmospheric circulation, it suggests that temperature and relative humidity are the most important climatic variables that impacts echolocation. The data suggest that any human induced climate changes will ultimately cause changes in temperature and relative humidity, which will consequently impact the survival of bats.

Key words: atmospheric attenuation, climatic conditions, evolution, local adaptation, microphone arrays, sound propagation

4.1 Introduction

It is widely acknowledged that evolution generates and sustains biodiversity through adaptive changes in ecologically relevant traits (Schluter, 2001; Losos et al., 2013). In animals, for instance, traits may diverge and as a consequence prompt ecological speciation which is the

primary cause of evolutionary diversification (Schluter, 2001; Rundle & Nosil, 2005; Wilkins et al., 2013). Several events and mechanisms exist that may interact with one another in various ways; the mechanisms also vary across space and time during speciation (Nosil, 2012; Wilkins et al., 2013). The adaptive processes like natural selection (on characters associated with survival) and sexual selection (on characters associated with mating), and non-adaptive processes such as genetic drift may lead to divergence (Dieckmann et al., 2004; Irwin et al., 2008; Funk et al., 2009; Wilkins et al., 2013). Among adaptive processes, climatic and environmental selection pressures have been shown to contribute to divergence of essential attributes like acoustic signals (Jones & Teeling, 2006; Wilkins et al., 2013; Maluleke et al., 2017). These acoustic signals are highly specialized and responsible for mediating mate choice, defend resources and recognize species in a wide variety of taxa, including bats (Finger et al., 2017; Pettitt et al., 2020; Warren et al., 2020).

The relative significance of ecological processes that contribute to acoustic divergence has received greater attention in the broader sensory drive framework coined by Endler (1992), as shown in several studies (Jones & Teeling, 2006; Mutumi et al., 2016; Jacobs et al., 2017). The framework includes the Acoustic Adaptation Hypothesis (Hansen, 1979), which states that the acoustic signals are selected based on habitat type so that sound propagation in different environments is optimized. For example, the songs of Rufous-and-white wren (*Thryophilus rufalbus*) tend to be more optimized for transmission in densely vegetated habitats than in open areas (Mennill et al., 2009). Moreover, individuals may adjust their acoustic signals in distinctly different environments in response to prevailing environmental conditions, to maximize signal transmission. Indeed Wiley and Richards (1978), found that vegetation density, temperature and humidity had an effect on the sound propagation properties of bird songs. Whereas the Acoustic Adaptation Hypothesis (hereafter AAH) was originally formulated in the context of

long-distance transmission of bird song, this hypothesis should be applicable to bat echolocation even though bats echolocation operates over shorter distances than bird song because bat echolocation frequencies are generally higher than those in bird song.

Several researchers have investigated environmentally driven variation in echolocation frequency mostly taking a keen interest in the influence of humidity on echolocation pulses (Guillén et al., 2000; Xu et al., 2008; Sun et al., 2013; Jiang et al., 2013). According to the concept of sensory drive, which encompasses the acoustic adaptation hypothesis (Sun et al., 2013; Wilkins et al., 2013), the frequency of bat echolocation pulses are adapted to climatic conditions. Climatic conditions (e.g., humidity) can directly impact sound transmission through the air (Snell-Rood, 2012; Jiang et al., 2015; Goerlitz, 2018) as a result of atmospheric attenuation. Atmospheric attenuation increases exponentially as frequency increases, and attenuation rapidly increases above 90 kHz (Lawrence & Simmons, 1982). Such frequency-dependent atmospheric attenuation may therefore be a major constraint on echolocation pulse frequencies and prey detection distances. However, other studies have nonetheless contested the effect of humidity on echolocation pulse frequency attenuation, showing that changes in humidity of up to 10% do not change acoustic transmission on a fine scale (Armstrong & Kerry, 2011). The habitat preferences of bats and their insect prey may also be influenced by humidity (Guillén et al., 2000). In this way, the bats adapt their pulse frequencies in response to changing acoustic environment, for example changing local humidity, habitat, and preferred insect prey. Temperature gradients may also influence acoustic sound transmission although shifts between 15°C to 30°C may have insignificant effects of sound attenuation in the atmosphere (Lawrence & Simmons, 1982; Luo et al., 2014; Goerlitz, 2018). Atmospheric attenuation, caused by the scattering and absorption of sound as it travels through the atmosphere, is the result of a complex interaction between sound frequency, humidity, temperature, and atmospheric pressure (Luo et al., 2014; Mutumi et al., 2016). More recently authors have emphasized the

interactive effect of climatic conditions on echolocation pulses (Jacobs et al., 2017; Maluleke et al., 2017; Goerlitz, 2018). For instance, relative humidity interacts non-linearly with temperature; as such factoring in the effects of these climatic variables is fundamental to understanding their effect on the acoustic signal divergence. According to aforementioned research in addition to humidity, climatic variables like temperature, latitude and altitude may also affect echolocation pulse frequency variation in bats. Specifically, spatial predictors (longitude and latitude) have indeed been reported to be useful in predicting acoustic divergence (e.g., Jacobs et al., 2017); primarily because they carry climatic effects of temperature and humidity that directly influence acoustic signals. Therefore, variations in relative humidity and temperature throughout a species' natural distribution may, therefore, lead to the selection of different echolocation pulse frequencies and source levels to compensate for atmospheric attenuation and optimize detection distances. Numerous field research has established that gradients in temperature and humidity influence echolocation frequencies of bat species (Guillén et al., 2000; Jiang et al., 2010; Mutumi et al., 2016; Maluleke et al., 2017; Jacobs et al., 2017). Even though the latter researchers provided an empirical foundation for climate-based selection on bat echolocation, given biosonar's significance in the evolutionary history of bats, this subject remains poorly understood. An interesting question is the interplay between direct and indirect effects of long-term climate, in a particular location, on acoustic divergence of signals. Tackling such a question would require the integration of several climatic variables to predict the patterns that drive acoustic variations. It further necessitates an integrative approach that combines analyses such as predictive modelling (to test the effect of several climatic variables while holding one parameter constant) and the composite climatic variables to test their effects on acoustic parameters. To date, no work has been done on how climatic conditions affect prey detection distances in bats. The present study sought to address this gap, by comparing the effect of climatic variables (temperature, humidity, atmospheric

pressure, longitude, and latitude) on prey detection distances of bat species over an evolutionary time. Detection distances were measured from echolocation pulses of free-flying bats in the field.

The focal system chosen comprises bat assemblages that are widely distributed (Monadjem et al., 2010) across climatically and geographically distinct biomes of Southern Africa (Mucina & Rutherford, 2006). If, as proposed by the AAH, bat echolocation parameters are influenced by climatic conditions so that signal transduction is optimized in the habitat they occupy, then under the same prevailing climatic conditions, bats should have the same detection distances, despite being of different sizes, and having different life history strategies and phylogenetic histories. Bats in an assemblage, particularly when the assemblage is composed of cave roosting bats using the same cave roost and provided bats do not commute great distances from their foraging grounds; therefore, should experience the same climatic conditions. Because bats in an assemblage experience similar climatic condition, they presumably undergo signal evolution that minimizes atmospheric attenuation and optimizes detections distances. Under these circumstances bats in the same assemblage should have similar detection distances. This might explain why bats in an assemblage in Panamá had similar detection distances despite differing in their echolocation parameters, body sizes, foraging microhabitats and phylogenetic history (Surlykke & Kalko, 2008). The species in the assemblage in Panamá used different echolocation frequencies but compensated for frequency dependent attenuation by adjusting the source levels at which they emitted their echolocation pulses. It is worth noting that, the aforementioned generalization applied only to the species in the assemblage in a single habitat that was studied (Surlykke & Kalko, 2008). As such, it is imperative to document if such generalization from a single habitat may apply to heterogeneous habitats with varying climatic conditions.

If this is a general response of bats to atmospheric attenuation caused by local climatic conditions then within bat assemblages detection distances should be similar but across bat assemblages, occupying habitats that differ in climatic conditions, detection distances should be different. The latter should also be apparent in the case of a single species occupying localities that differ in climatic conditions. An investigation of detection distances in a single species occupying a range of climatic conditions allows for the control of interspecific variations in life-history strategies and phylogenetic history.

There are many other factors that influence bat echolocation and therefore detection distances. For examples, pulse frequency and source level are correlated with body size; larger bats tend to fly faster and emit their pulses at higher source levels. This is because a big bat flying faster, would need to detect targets at further detection distances than a smaller bat, to be able to maneuver in time to intercept a target or avoid an obstacle. Furthermore, the echolocation parameters of bats are adapted to their foraging strategies and the structural components of their habitats. This raises questions around why detection distances should be the same even in the same habitat, as reported by Surlykke and Kalko (2008). It is possible, probably likely, that optimal detection distances will vary among species of an assemblage. Secondly, climatic factors are known to impact acoustic parameters and so does habitat structure. Detection distances are dependent on these parameters so it is reasonable to expect that even if there are optimal detection distances influenced by habitat and climate, that detection distances should differ within and across bat assemblages occupying different habitats.

The aim of this chapter is to test whether differences in detection distances among bat assemblages and the ubiquitous species *M. natalensis* are the result of climate-mediated selection in accordance with Acoustic Adaptation Hypothesis. The following predictions of the

AAH were tested: (i) bats in the same assemblage exposed to the same climatic conditions, should have similar detection distances, (ii) detection distances should be different across bat assemblages occupying habitats that differ in climatic conditions and should be correlated with climatic conditions, and (iii) detection distances should be different for a single species, *M. natalensis* occupying localities that differ in climatic conditions. Alternatively, if body size, life-history strategies and phylogenetic history have a greater influence on detection distances then: (i) detection distances should differ among species within and across bat assemblages occupying different habitats and exposed to different foraging conditions and, (ii) that detection distances should be species-specific and remain constant across localities despite differences in climatic conditions. This research contributes to understanding the influence of climate on sensory traits and shows the possible impacts of future climatic change on acoustic signals.

4.2 Material and methods

4.2.1 Study animals

The echolocation pulses of free-flying insectivorous bats were recorded from a wide range of sites in biomes of South Africa (Mucina & Rutherford, 2006). Bat assemblages comprised of species from different families varying in body sizes and foraging strategies. Some species were restricted to one or two sites only, while others were common to more than two sites. *M. natalensis* was distributed at all seven sites. Detailed information for both bat assemblages and *M. natalensis* can be found in Chapters 2 and 3.

4.2.2 Study sites

Free flying bats were recorded at seven sites, each associated with a different biome. The coordinates of study sites in different biomes were recorded using a hand-held Global Positioning System (GPS) unit (model Colorado 300, Garmin International Inc, Kansas). Detailed descriptions of the sites are provided in Chapters 2 and 3 based on (Mucina & Rutherford, 2006).

4.2.3 Recording of echolocation pulses and weather variables

Echolocation pulses were recorded in the field using a real-time 8-channel recorder, Avisoft-UltraSoundGate 816H (Avisoft Bioacoustics, Berlin, Germany). Climatic conditions were recorded using a portable weather station (Model WMR89/WMR89A, Oregon Scientific Inc., Tualatin, Oregon, USA). A detailed description of how to record echolocation pulses and climatic conditions is provided in Chapter 3.

4.2.4 Flight path reconstruction

The time of arrival differences (TOADs) of pulses at each microphone (Avisoft Bioacoustics, Glienicke, Germany) in the array was used to plot the bats' flight paths in three dimensions in relation to each array (Holderied & von Helversen, 2003; Gillette & Silverman, 2008). Detailed descriptions are provided in Chapter 3.

4.2.5 Acoustic parameters of echolocation pulses

Acoustic parameters were measured in Avisoft-SASLAB Pro (Version 5.2.09, Avisoft Bioacoustics, Berlin, Germany), supported by USB key (dongle) device driver (MAX HL MN720427/OZRWQ/31060647-1, Berlin, Germany). Detailed descriptions of these measurements are provided in Chapters 2 and 3.

4.2.6 Echolocation pulse source levels

Echolocation pulse source levels were calculated as described in Chapter 3.

4.2.7 Calculating detection distance

Distances at which bats detected a range of insect prey sizes (large, medium and small) were calculated for each species in each assemblage using the sonar equation (Møhl, 1988): DT = (SL + 2TLA + TLS + TS) - noise term (dB), where DT is the detection threshold; SL is source level; TLA is the transmission loss due to atmospheric absorption; TLS is the transmission loss resulting from spherical spreading and TS is the target strength. TLA and TLS are distance-

based functions. The SL was based on the averages of the most intense echolocation pulse found from each flight path for each species and used to calculate detection distances. The TLA was calculated using online formular (resource.npl.co.uk /acoustics/ tech guides /absorption/) as the attenuation of echolocation signal due to atmospheric absorption at a distance from the microphone dB per meter (dB/m) (UK National Physics Laboratory). The dB/m is a function of echolocation frequency (Hz), atmospheric conditions that include temperature (^{o}C), relative humidity (%) and the atmospheric pressure (kPa). The averages of 40 years (1979-2019) of monthly and annual climatic data were used to determine atmospheric conditions. The formula also has values for reflection loss (c1; -40, -50 & -65), spreading loss (c2; -40, -50 & -65) given by Stilz and Schnitzler (2012). The above values correspond with the acoustic power known as target strength (TS) of the echo reflected off three different prey size categories: Large (TS = -40 dB), medium (TS = -50 dB) and small (TS = -65 dB). Target strength of an echo off a target varies depending on how large the target is in comparison to its wavelength. For instance, the target strength of a solid sphere with a diameter of 4 cm is -40 dB at 1 m which corresponds to -20 dB at 10 cm. Insect prey and targets vary in size, therefore bats often detect echoes from them at a maximum distance that is influenced by their sizes. Small and large insect/targets equate to weaker and greater echo, respectively. The overall maximum detection distance also depends on the frequency of the emitted pulse by the bat. The point-reflector fitted in the sonar calculator customised the values of the reflection loss so that estimates of the detection distances for different insect sizes are generated. Given the diverse bat species in biomes that either fly in open space or at edges of clutter and/or within cluttered habitat, it is likely that large, medium, and small preys are hunted. There was no information on hearing thresholds for the species in the assemblages in this study. I, therefore, relied on prior studies elsewhere that provided approximate values of bats hearing threshold ranging from 0-20 dB SPL (Neuweiler, 1984). The DT for bats in the present study was assumed to be 20 dB SPL as described by

several previous studies (Griffin et al., 1960; Holderied & von Helversen, 2003; Stilz & Schnitzler, 2012; Lewanzik & Goerlitz, 2018; Goerlitz et al., 2020). The 20-dB SPL detection threshold represents the actual sensitivity bats exhibit under natural conditions (when noise is present). Signal pulse with the maximum source level in a series plus its frequency was used to calculate the detection distance according to the methods used by, e.g., Holderied et al. (2005) and Holderied & von Helversen, (2003). A statistical comparison of detection distances for bat assemblages and populations of *M. natalensis* was performed to test various predictions.

4.2.8 Climatic variables

The KNMI (The Royal Netherlands Meteorological Institute) Climate Explorer web-based high-resolution research tool was used to download climate data spanning 40 years from 1979-2019 (https://climexp.knmi.nl/selectfield). The tool has an ERA5 (the fifth generation of atmospheric reanalyses of the global climate) segment that provided estimates for the following atmospheric variables: mean annual temperature (^{o}C), relative humidity (%) and atmospheric pressure (kPa). Variables were taken on land points as specific areas within a grid box based on the coordinates of each site. Therefore, mean annual temperature on land points at 2 m/10 m above the surface were considered while relative humidity measured using data obtained at 10 m above the Earth's surface. All climatic variables considered were based on monthly and yearly averages of a time series between 1979-2019. Such averages would include extreme climatic conditions that occur throughout the year. The timing of climatic conditions may vary annually (e.g., hot summer and cold winter, rainy summers and dry winters or dry summers and rainy winters), causing seasonal or annual fluctuations that put strong evolutionary pressures on bats. Another reason for using climate variables as averages was to reduce the numerous instances of selecting climate data based on the timing of extreme climatic conditions including some unusual events such as El Niño, that lasts for an appreciably longer time.

4.2.9 Statistical analyses

4.2.9.1 Testing the AAH's prediction (i), (ii) and (iii) as well as prediction (i) and (ii) of the alternative hypothesis

Statistical analyses on detection distances between assemblages and, between populations for *M. natalensis* followed methods in Chapter 3 and were preceded by tests for homogeneity of variance and normality (Figs. S4.1, S4.2, S4.3 & S4.4).

4.2.9.2 Analysis of detection distances and climatic conditions to predict detection distances

To establish whether the detection distances were associated with environmental factors, the statistical analysis approach followed the methods used in Mutumi et al. (2016) and Maluleke et al. (2017). Environmental variables considered were relative humidity, mean annual temperature, atmospheric pressure, longitude and latitude. Atmospheric attenuation (dB/m) that limits the propagation of sound, and can therefore impact the detection distance of targets by echolocation, is dependent on the interactive effect of relative humidity (%), mean annual temperature (^{o}C) and atmospheric pressure (kPa) (Lawrence & Simmons, 1982; Luo et al., 2014; Goerlitz, 2018). Principal component analysis (PCA) was used to create an uncorrelated principal component; PCs, set of environmental variables which are meant to adjust for possible multicollinearity among variables in the environment (Dormann et al., 2013). Separate PCAs were performed on environmental variables: the first was for the six sites where echolocation pulses for bat assemblages were recorded, and the second was for the seven sites where *M. natalensis* echolocation pulses were recorded. As part of the PCA, atmospheric pressure was integrated, and latitude and longitude were kept as separate predictors since atmospheric pressure could have a strong climate effect but has a lesser spatial dependence on climate. The PCs were later used to test their influence on detection distances and since they constituted the influence of climate (relative humidity, mean annual temperature and atmospheric pressure), the presumption was that they reflect a possible latent effect of environmental variability on detection distances. Latitude and longitude were also added as environmental variables for

spatial prediction, to evaluate for a possible spatial structure at a wider scale of sampling sites based on distance. Detection distances, included as a response variable, was based on estimated maximum detection distances for each reconstructed flight path for each species in each assemblage, and maximum detection distance for each reconstructed flight path for *M. natalensis* across the seven sites. Only the detection distances (DD) for medium prey (TS = -50 dB) were considered as a test case to evaluate how they could be affected by environmental variables across sites.

Linear mixed effects models (LMEs) were utilised to investigate whether there was a relationship between detection distances and climatic variables (relative humidity, temperature and atmospheric pressure) contained in PC1 and PC2 and, spatial (latitude and longitude) as explanatory variables. The model incorporates two types of effects: (i) fixed effects to evaluate, to a large extent the impacts across all sites, and (ii) random effects to evaluate the impact of fixed effect variables between sites (Zuur et al., 2009; Bell et al., 2019).

The standardized residuals were obtained from LMEs which indicated that their distribution and quantile-quantile plots for the distribution of detection distances was normal as earlier presented in Chapter 3. In addition, evaluation of residuals in comparison with fitted values as well as correlograms, clearly demonstrates that residuals were spatially structured, which is inconsistent with the assumption that data are spatially independent (BjØrnstad & Falck, 2001; Fletcher & Fortin, 2018). Testing various models with and without random effects was performed to identify the optimal error structure based on the covariates, in conjunction with various spatial autocorrelation functions (where distances were specified by latitude) which includes corrGaussian, corrExponential, corrSpherical, corrRatio, corrARI. The random effect

for site was factored into the equation in nested sampling procedure since more than one individual was sampled from a single site (Zuur & Ieno, 2016; Harrison et al., 2018).

LME model fit by REML (restricted maximum likelihood) with a random effect for site and with the absence of spatial autocorrelation was the most parsimonious model structure (a model with the smallest Akaike's Information Criterion, AICc). The AICc has a correction for small sample sizes to address the potential fitting problems in a model (Burnham & Anderson, 2004; Mazerolle, 2006). Check of spline correlograms proved the random factor adequately eliminated the spatially lagged correlations between residuals (Fig. 4.1) and tests of residual values against predicted values were clear of any violations of homogeneity of variance and normality assumptions (Figs. S4.1, S4.2, S4.3 and S4.4).

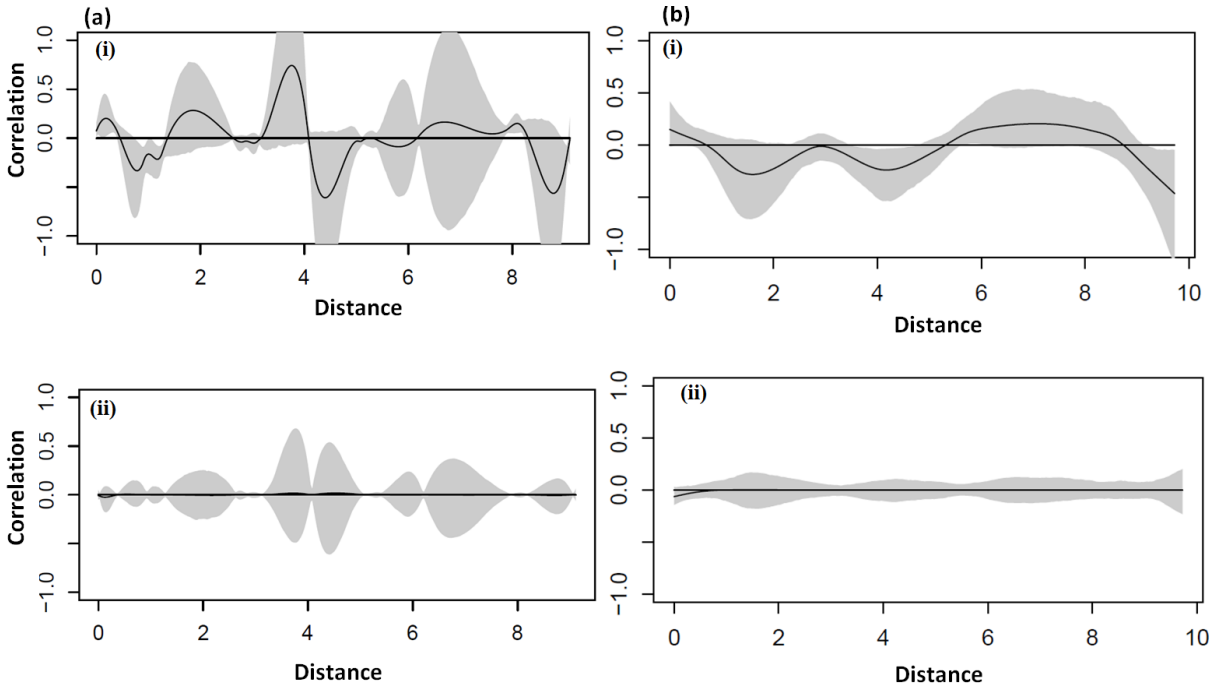

Figure 4.1. Spatial autocorrelation in observed data due to spatial dependence, i.e., temperature, relative humidity, atmospheric pressure, latitude and longitude (i) and A graph with no spatial autocorrelation after spatial dependencies are taken into account by the simple random-effects model (ii). The climatic variables used in (a) were from six sites where bat assemblages were recorded and (b) from seven sites where *M. natalensis* was recorded.

A forward-backward-stepwise variable selection procedure was used to determine a simplified combination of covariates with PC1 and PC2 (PCs containing relative humidity, temperature and atmospheric pressure), latitude and longitude as predictors based on AICc. This was performed using the step-AICc function in R package MASS which has a correction factor to account for small sample sizes (Burnham et al., 2011; Harrison et al., 2018). In step-AICc, the selection procedure is automatically performed by selecting the variables sequentially and ending with the best model. Final models selected were DD ~ PC1 * PC2 + latitude + longitude; AIC = 2536.98 and DD ~ PC1 * PC2 + latitude + longitude; AIC = 325.18, for bat assemblages and *M. natalensis*, respectively. A simplified combination of covariates considered as the best subset model was summarized statistically with an analysis of variance to evaluate how each variables accounted for the variation in the detection distances (Crawley, 2012). The final model was only fed with variables accounting for a large percentage of the variation in detection distances. This tested the predictions (i) and (ii) of the Acoustic Adaptation Hypothesis as to whether or not environmental/climatic factors (relative humidity, mean annual temperature and atmospheric pressure, spatial structuring) explained the variation in detection distances for bat assemblages across six sites. The same approach was used to evaluate the impact of environmental factors on the detection distances of *M. natalensis* across the seven sites to test prediction (iii) of the Acoustic Adaptation Hypothesis.

The covariates were considered separately to determine the nature of their relationship with detection distances for bat assemblages and *M. natalensis* across sites. To achieve this, individual-specific effects were predicted by adjusting each covariate individually, but not the effect of concern. It involved a two-step process by separating out the individual environmental effects, relative humidity, mean annual temperature and atmospheric pressure. In the first place, "standardized" PCs for each variable i.e., relative humidity, mean annual temperature and

atmospheric pressure were created by fixing two variables to their mean values while permitting others to vary. For instance, the designation PC (atmospheric pressure) referred to mean annual temperature and relative humidity that were fixed while atmospheric pressure was subject to variation. Then, the 'standardized' PCs were matched with the other covariates to forecast the influence of the environment for a variable of interest through the 'best' LME. Data were analyzed in statistical software R version 4.0.1 (RCore, 2016), loaded with R package MASS. The MASS package has a stepAICc function, which allows for stepwise model selections based on corrected Akaike information criterion (AICc) (Venables & Ripley, 2002; Ripley et al., 2013), the 'nlme' function was used to suit the LME model by integrating other effects so that the mean of the response variable could be computed (Bates et al., 2014), the 'effects' for showing the effect sizes of linear, generalized linear and other customized models (Kuznetsova et al., 2017), Randtests' performs Bartels rank test of randomness, which tests if a sample is randomly sampled from an underlying distribution of the population (Bartels, 1982; Mateus & Caeiro, 2014), 'car' a tool used in conjunction with applied regression to perform regression tests along with applied regression (Fox, 2002), the 'mgcv' functions for generalized additive modelling and generalized additive mixed modelling as domains for automatic smoothness selection (Wood, 2011) of more than one variable and the 'ncf' a spatial tool for making correlograms and to check if splines are polynomial fit with continuous function that interpolates the data and is constructed from linear function (Fletcher, 2017). The 'ncf' also useful checking spatial autocorrelation (BjØrnstad & Falck, 2001). Moreover, the above-stated analyses were supported by evaluating the relationships between environmental variables and echolocation pulse parameters; the Pearson correlation test was conducted to evaluate statistical evidence for a linear relationship among variables.

4.3 Results

4.3.1 Comparing detection distances within assemblages (testing prediction (i) of the AAH)

Detection distances for large (L), medium (M) and small (S) prey sizes within all assemblages were not normally distributed (Shapiro-Wilk normality test; W's > 0.81; *p's* < 0.0001; $F_{5, 529}$ > 6.12). My data showed that bat assemblages do not have equal variances in detection distances (Leven's test; *p's* < 0.001). Non-parametric tests were therefore used to compare detection distances. Within each assemblage, the detection distances were significantly different for the three size classes of prey (Kruskal-Wallis > 34.27; df = 4 or 3; *p's* < 0.001; Table S4.1). Compared to other assemblages, the Fynbos assemblage displayed a greater variance in detection distances, as reflected on the mean squares (MS) of three prey sizes (MS = L: 6.7 m M: 5.2 m, S: 3.1 m). However, there were a few exceptions, where the detection distances were significantly different only for large and medium-sized or medium and small size classes of prey (Kruskal-Wallis > 34.27; df = 4 or 3; *p's* < 0.001; Table S4.1). In each assemblage, the LDC aerial hawkers generally had longer detection distances than the clutter specialist HDC rhinolopids (Dunn's post hoc tests *p's* < 0.001). The open-air aerial hawkers such as *T. aegyptiaca* (L: 20.5 ± 7.7 m, M: 15.8 ± 6.6 m and S: 9.6 ± 5.0 m) and *S. petrophilus* (L: 12.9 ± 4.1 m, M: 10.6 ± 3.6 m and S: 6.5 ± 2.8 m) had the longest detection distances of all species (*p's* < 0.001), except for the clutter-edge forager *C. seabrae* which had similar detection distances to those of *S. petrophilus* bats for large (15.4 ± 1.5 m) and medium (12.4 ± 1.4 m) prey size (*p's* > 0.24) but 1.6 m longer for the small prey size class (8.4 ± 1.1 m) (*p* < 0.05). In comparison to other clutter edge species, detection distances for *C. seabrae* were 3-4 m and 4-5 longer than *N. capensis* (L: 12.2 ± 1.3 m, M: 9.9 ± 1.2 m and S: 6.8 ± 0.9 m) and *M. tricolor* (L: 9.1 ± 1.2 m, M: 7.1 ± 1.1 m and S: 4.8 ± 0.9 m, respectively, across their distribution ranges. As for the latter two species, however, detection distances between them were approximately 1.5 m, even though detection distances for *N. capensis* were nearly 2 m longer than *M.*

fraterculus (L: 8.7 ± 0.9 m, M: 7.1 ± 0.8 m and S: 4.9 ± 0.6 m), which were similar to those of *M. tricolor (p* > 0.05) across prey sizes. Also, these species were characterized by low to high frequencies (21-62 kHz) coupled with high source levels, > 125 dB peSPL (Table S4.1). The rhinolophids, for example, *R. capensis* (L: 6.1 ± 0.5 m, M: 5.3 ± 0.6 m and S: 3.4 ± 0.4 m), *R. swinnyi* (L: 6.4 ± 1.0 m, M: 5.3 ± 0.9 m and S: 3.8 ± 0.8 m) and *R. clivosus* (L: 6.6 ± 0.8 m, M: 5.9 ± 0.7m and S: 3.9 ± 0.6 m) had shorter detection distances, which were, however, 1 m longer than those of gleaner, *N. thebaica* (L: 5.01 ± 0.5 m, M: 4.0 ± 0.5 m and S: 2.7 ± 0.5 m). *N. thebaica* thus had the shortest detection distance. Since the assemblages comprised both LDC and HDC bats, differences in detection distances observed within assemblages under similar climatic conditions are inconsistent with prediction (i) of the AAH. Instead, they supported prediction (i) of the alternative hypothesis.

4.3.2 Comparing detection distances between assemblages (testing prediction (ii) of the AAH)

All bat assemblages had unequal variances in detection distances (Leven's test; *p's* < 0.001), so non-parametric tests were used. Detection distances between assemblages were significantly different for large (Kruskal-Wallis = 63.58; df = 5; $p < 0.001$), medium (Kruskal-Wallis = 77.0; df = 5; $p < 0.001$) and small (Kruskal-Wallis = 85.16; df = 5; $p < 0.001$) prey sizes. Dunn's post hoc test indicates the detection distances for the bat assemblage in the Savanna biome site(L: 6.9 ± 1.6 m, M: 5.7 ± 1.3 m and S: 4.0 ± 0.9 m) were shorter than those in the Fynbos (L: 9.6 ± 4.5 m, M: 7.7 ± 3.6 m and S: 5.2 ± 2.4 m) ($p < 0.001$), Coastal Belt (L: 9.0 ± 3.1 m, M: 7.3 ± 2.4 m and S: 5.02 ± 1.6 m) ($p < 0.05$), Albany thicket (L: 8.2 ± 2.1 m, M: 6.8 ± 1.7 m and S: 4.9 ± 1.2 m) ($p < 0.05$) and Desert biome (L: 10.1 ± 3.1 m, M: 8.1 ± 2.5 m and 5.6 ± 1.7 m; $p < 0.05$) sites across all prey sizes ($p < 0.05$). Detection distances in the Fynbos biome site

was nearly 4 m longer on average than sites in the Nama Karoo (L: 7.9 ± 2.4 m, M: 6.3 ± 2.0 m and S: 4.4 ± 1.5 m) ($p < 0.01$) and Albany thicket ($p < 0.001$).

Nevertheless, some assemblages showed similarities in detection distances across prey sizes as well. For instance, detection distances of large (L: 9.6 ± 4.5 m) medium size (M: 7.7 ± 3.6 m) and small (S: 5.2 ± 2.4 m) prey in Fynbos biome site were not significantly different from Coastal Belt (L: 9.0 ± 3.1 m, M: 7.3 ± 2.4 m and S: 5.0 ± 1.6 m) ($p = 1$, $p = 0.06$ and $p = 0.07$) and Albany thicket sites (L: 8.2 ± 2.1 m, M: 6.8 ± 1.7 m and S: 4.9 ± 1.2 m) ($p = 1$, $p = 1$ and $p = 1$). Detection distances in the Coastal Belt site (L: 9.0 ± 3.1 m, M: 7.3 ± 2.4 m and S: 5.0 ± 1.6 m) did not differ significantly from sites in the Desert (L: 10.1 ± 3.1 m, M: 8.1 ± 2.5 m and S: 5.6 ± 1.7 m) ($p = 0.5$, $p = 0.3$ and $p = 0.2$) and Albany thicket (L: 8.2 ± 2.1 m, M: 6.8 ± 1.7 m and S: 4.9 ± 1.2 m) ($p = 0.5$, $p = 0.53$ and $p = 0.8$) biomes. However, the latter two sites were not significantly different either ($p = 0.7$, $p = 0.9$ and $p = 1$) for large, medium and small prey sizes, respectively. Additionally, detection distances in the Savanna (L: 6.9 ± 1.6 m, M: 5.7 ± 1.3 m and S: 4.01 ± 0.9 m) did not differ significantly from a site in the Nama Karoo (L: 7.9 ± 2.4 m, M: 6.3 ± 2.0 m and S: 4.4 ± 1.5 m) ($p = 1$, $p = 0.6$ and $p = 0.3$) for all prey sizes and Albany thicket biomes for only large prey (8.2 ± 2.1 m; $p= 0.7$). Thus, differences in detection distances among some assemblages under different climatic conditions only partially support prediction (ii) of the AAH.

4.3.3 Comparing detection distances for Miniopterus natalensis between sites in different biomes (testing prediction (iii) of AAH and prediction (ii) of the alternative hypothesis).

The Shapiro-Wilk normality test (Shapiro & Wilk, 1965) indicated that the data for detection distances of *M. natalensis* for the three prey sizes across all sites were normally distributed (W's > 0.96; *p's* > 0.21; $F_{6, 97} > 7.8$; Fig. S4.4, S4.5 & S4.6). Detection distances between sites

also had equal variances (Leven's test; *p's* > 0.05), so the ANOVA was used to compare detection distances. Detection distances for large (GLM ANOVA = 7.02; df = 6; $p < 0.001$), medium (ANOVA = 10.2; df = 6; $p < 0.001$) and small (ANOVA = 13.51; df = 6; $p < 0.001$) prey across sites were statistically significant. Dunn's post hoc analysis indicates that detection distances across all prey sizes for *M. natalensis* at the site in the Desert biome (L: 11.4 ± 1.1 m, M: 9.2 ± 1.0 m and S: 6.2 ± 0.9 m) were on average 0.8-3.5 m longer than those in the Savanna (L: 8.1 ± 0.9 m, M: 6.7 ± 0.8 m and S: 4.7 ± 0.7 m) ($p < 0.05$), Grassland (L: 7.9 ± 1.1 m, M: 6.3 ± 1.1 m and S: 4.2 ± 0.9 m) ($p < 0.05$), Coastal Belt (L: 8.8 ± 1.8 m, M: 7.2 ± 1.5 m and S: 5.0 ± 1.2 m) ($p < 0.01$), Nama Karoo (L: 9.1 ± 1.9 m, M: 7.3 ± 1.7 m and S: 5.0 ± 1.4 m) ($p < 0.01$) and Albany thicket (L: 9.2 ± 0.9 m, M: 7.5 ± 0.8 m and S: 5.2 ± 0.7 m) ($p < 0.01$) biomes. The latter two sites in the biomes had similar detection distances. However, the two sites were on average 1 m shorter than those in the Fynbos (L: 10.2 ± 1.7 m, M: 8.4 ± 1.6 m and S: 6.0 ± 1.3 m) site ($p < 0.05$). Detection distances across prey sizes at the site in the Fynbos biome were not significantly different from a site in the Desert (*p's* > 0.96), Coastal Belt (*p's* > 0.32), Albany thicket (*p's* > 0.36) and Nama Karoo (*p's* > 0.52) biomes. In contrast, detection distances between sites in the Coastal Belt biome and the latter two biomes were not significantly different (*p's* > 0.056) as well as sites in the Grassland (*p's* > 0.06) and Savanna (*p's* > 0.77) biomes. Furthermore, detection distances for sites in the Albany thicket biome did not differ significantly from those in the Grassland (*p's* > 0.36), Nama Karoo (*p's* > 0.97), and Savanna (*p's* > 0.40) biomes, whereas those latter two biomes were neither significantly different from each other (*p's* > 0.86) nor from Grassland biome (*p's* > 0.51). In essence, most sites had no statistically significant differences, in detection distances across prey sizes (*p's* > 0.05; Table S4.1). Such similarities in detection distances between populations of a single species between sites that differ in climatic conditions contradict prediction (iii) of the AAH, but support prediction (ii) of the alternative hypothesis.

4.3.4 Separation of study sites along principal components (PC's)

PCA results suggested that sites were distinct in terms of relative humidity, mean annual temperature and atmospheric pressure. PC1 accounted for 59.21% and PC2 = 36.67% of the variance and cumulatively the two PCs accounted for 95.88% of the variation in the six study sites where bat assemblages occurred (Fig. 4.2). The variables with the highest loading on PC1 were RH, Temp and Atm in order of the magnitude of their effect and on PC2 were Temp and Atm (Fig. 4.2). While in the analyses for *M. natalensis*, PC1 accounted for 56.26% and PC2 = 36.14% of the variance and cumulatively accounting for 92.40% of the variation in climatic variables among the seven sites in which *M. natalensis* occurred (Fig. 4.3). The variables with the highest loading on PC1 were RH and Atm in order of the magnitude of their effect and on PC2 was Temp, in seven study sites, with RH showing a weak effect along PC2 (Fig. 4.3).

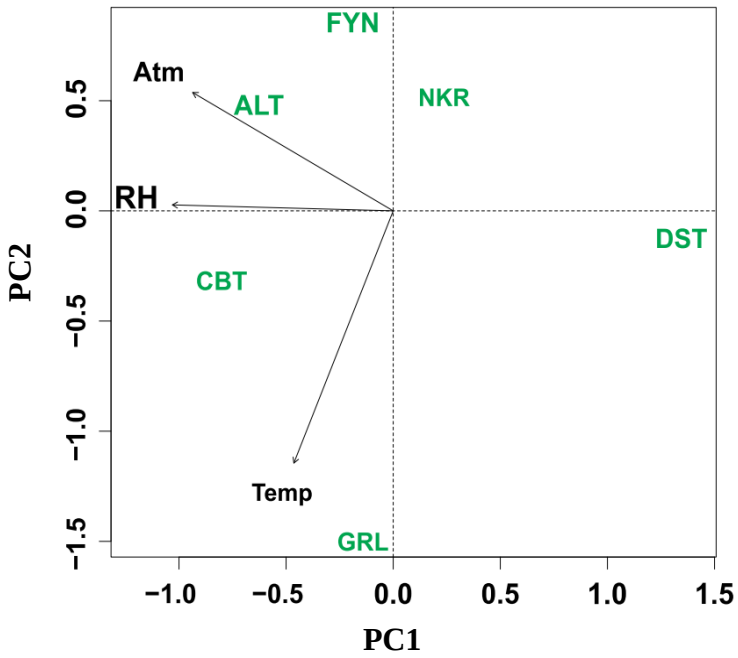

Figure 4.2. Variation in climatic variables (relative humidity; RH, mean annual temperature; Temp, atmospheric pressure; Atm) across biomes from which the echolocation pulses were recorded for bat assemblages (where PC1 and PC2 accounted for 95.88% of variation). Grassland; GRL, Coastal Belt; CBT, Albany Thicket; ALT, Fynbos; FYB, Nama Karoo; NKR, Desert; DST.

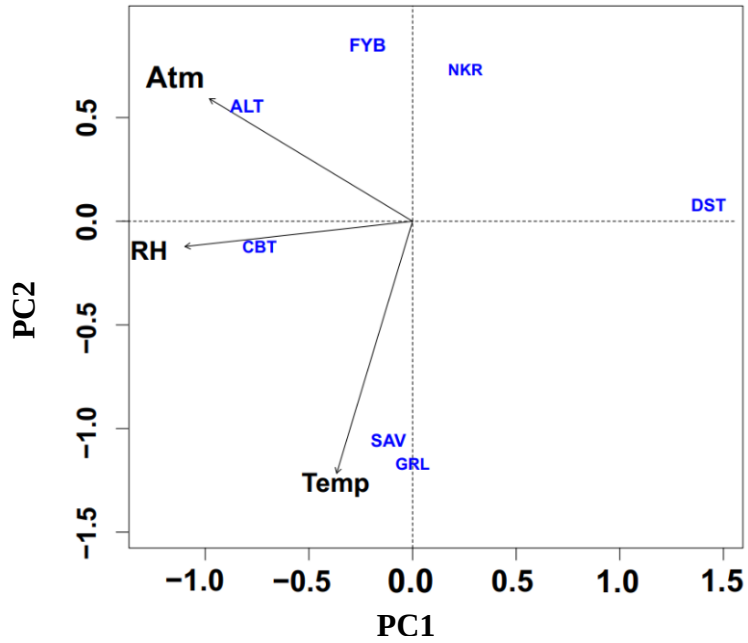

Figure 4.3. Variation in climatic variables (relative humidity; RH, mean annual temperature; Temp, atmospheric pressure; Atm) across biomes from which the echolocation pulses were recorded for *M. natalensis* (where PC1 and PC2 accounted for 92.40% of variation). Grassland; GRL, Savanna; SAV, Coastal Belt; CBT, Albany Thicket; ALT, Fynbos; FYB, Nama Karoo; NKR, Desert; DST.

4.3.5 Impact of climatic variables on detection distances

Although the best model explaining differences in detection distances across assemblages included PCI, PC2, latitude, longitude and the interaction between PC1 & PC2, only PC2 and longitude were significant (Table 4.1).

Table 4.1. 'Best' model for the global model of environmental variables versus detection distances for bat assemblages selected by a forward-backward stepwise process. Statistics are shown only for variables included in the best model.

	numDF	denDF	ANOVA-F-value	*p* -value
(Intercept)	1	501	433.622	<.0001
PC1	1	2	5.138	0.067
PC2	1	2	18.628	0.041
Latitude	1	501	2.061	0.151
Longitude	1	501	4.871	0.029
PC1:PC2	1	2	0.197	0.701
Number of Observations: 509				
Number of Groups: 6				

Abbrev: PC1 and PC2 = Principle component factor 1 and 2 derived from relative humidity, mean annual temperature and atmospheric pressure; numDF= numerator degrees of freedom ; denDF = denominator degrees of freedom.

PC1, PC2 and longitude were significant predictors of the detection distances of *M. natalensis* (Table 4.2).

Table 4.2. 'Best' model for the global model of environmental variables versus detection distances for *Miniopterus natalensis*. Statistics are shown only for variables included in the best model.

	numDF	denDF	ANOVA-F-value	*p* -value
(Intercept)	1	90	4337.769	<.0001
PC1	1	3	30.272	0.0118
PC2	1	3	21.606	0.0188
Latitude	1	90	2.859	0.0944
Longitude	1	90	5.309	0.0236
PC1:PC2	1	3	3.216	0.1708
Number of Observations: 99				
Number of Groups: 7				

Abbrev: PC1 and PC2 = Principle component factor 1 and 2 derived from relative humidity, mean annual temperature and atmospheric pressure; numDF= numerator degrees of freedom ; denDF = denominator degrees of freedom.

4.3.6 Predictive modelling to test the separate effects of environmental variables on detection distances for bat assemblages (testing prediction (i) and (ii) of the AAH, and prediction (i) of the alternative hypothesis).

The effects of each of the environmental variables (relative humidity, mean annual temperature, atmospheric pressure, longitude and latitude) were isolated by holding (controlling) the other variables constant at the across-site mean. Temperature had an inverse relationship with detection distances and the effect was moderate (Fig. 4.4; Table 4.1). There was no relationship between detection distances and relative humidity, atmospheric pressure or latitude for bat assemblages across sites (Fig. 4.4). Longitude was found to have a positive linear relationship with detection distances for bat assemblages across sites, with a strong effect (Fig. 4.4; Table 4.1)

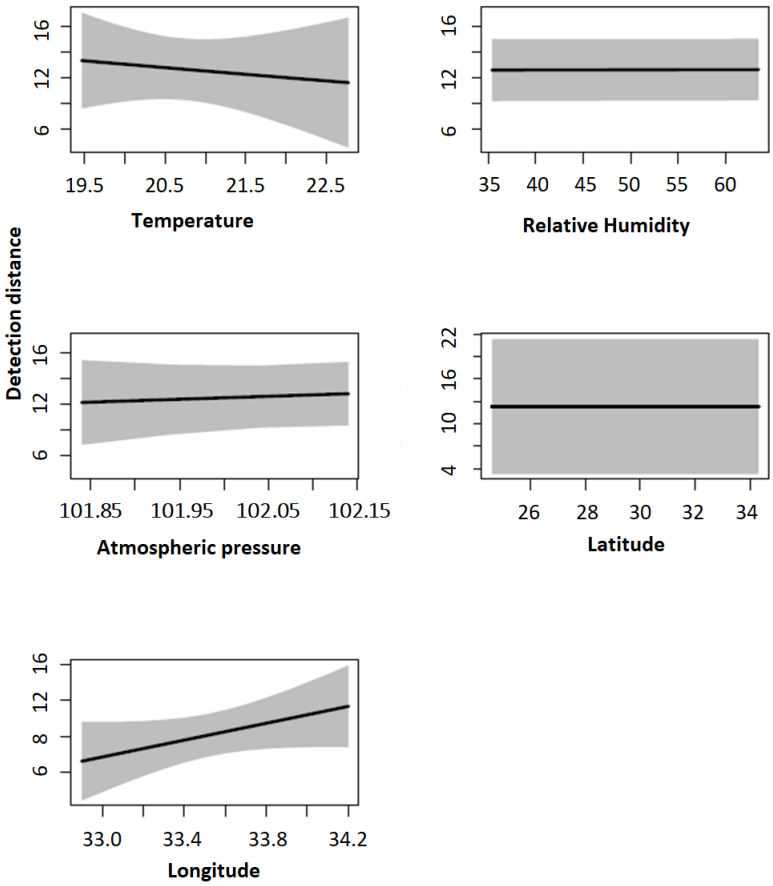

Figure 4.4. The effect of environmental variation: annual temperature, relative humidity, atmospheric pressure, longitude and latitude on detection distances of bat assemblages. Predictions and 95% confidence intervals are depicted as black lines and grey areas, respectively.

4.3.7 Predictive modelling to test the separate effects of environmental variables on distances for bat assemblages (testing prediction (i) and (ii) of the AAH, and prediction (i) of the alternative hypothesis).

The effects of each variable, that is, relative humidity, mean annual temperature, atmospheric pressure, latitude and longitude on detection distances of *M. natalensis* populations were isolated by holding the others constant at the across-site mean. Longitude revealed a strong positive linear relationship with detection distances across sites. (Fig. 4.5; Table 4.2). However, relative humidity, atmospheric pressure and latitude did not exhibit any relationship with detection distances for *M. natalensis* across sites (Fig. 4.5; Table 4.2).

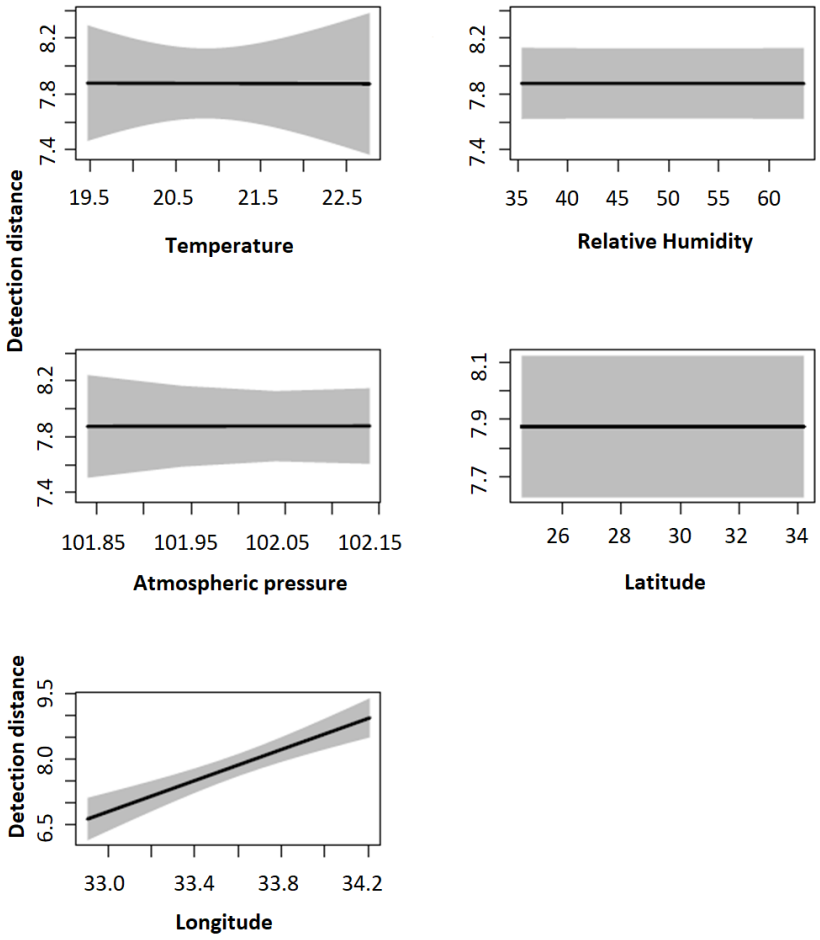

Figure 4.5. The effect of environmental variation: annual temperature, relative humidity, atmospheric pressure, longitude and latitude on detection distances of *M. natalensis*. Predictions and 95% confidence intervals are depicted as black lines and grey areas, respectively.

4.3.8 Test of the separate effects of PCs on detection distances for bat assemblages (testing prediction (i) and (ii) of the AAH, and prediction (i) of the alternative hypothesis).

Analyses of the individual effects of PCs on detection distances for bat assemblages showed that PC1 (relative humidity), was responsible for 59.21% of the changes in the climate data (Table 4.1) had a stronger effect on detection distance (Fig. 4.6). PC2 (temperature), on the other hand, explained the minimal variation in the data set (36.67%, Table 4.1), positively influenced detection distance (Fig. 4.6). Spatial predictors (longitude and latitude) had effects on the detection distance and were incorporated in the climatic effect (Fig. 4.6). The trends in spatial effects indicated strong inverse linear associations.

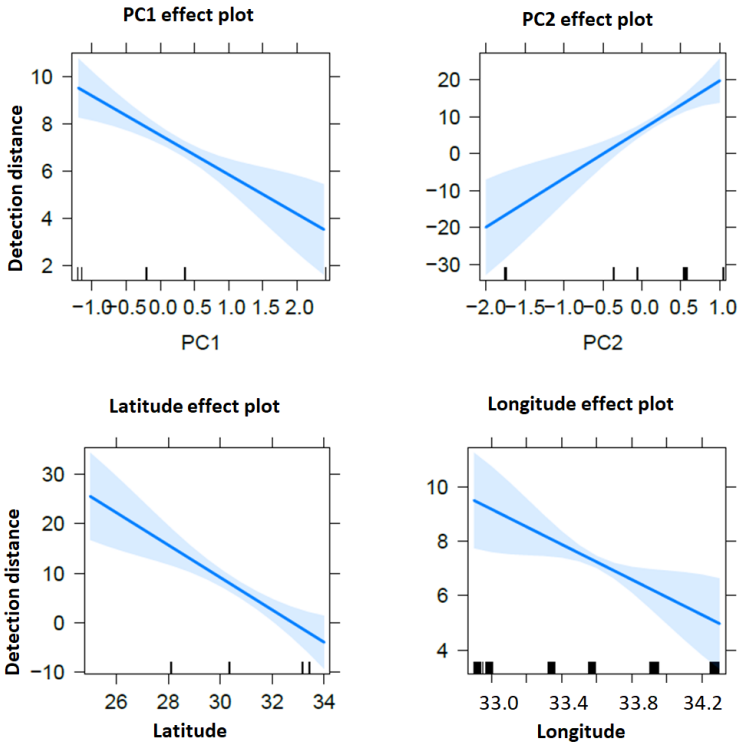

Figure 4.6. The relationship between detection distance and PC1, PC2, latitude and longitude for bat assemblages across study sites.

The interacting effects of PC1 (relative humidity) and PC2 (temperature) revealed the existence of a complex nonlinear relationship between detection distance for bat assemblages across sites and climatic conditions (Fig. 4.7). The effect plots illustrate that temperature was not mediated by relative humidity levels and neither of these two climatic factors (relative humidity and temperature) influenced the detection distance (Fig. 4.7).

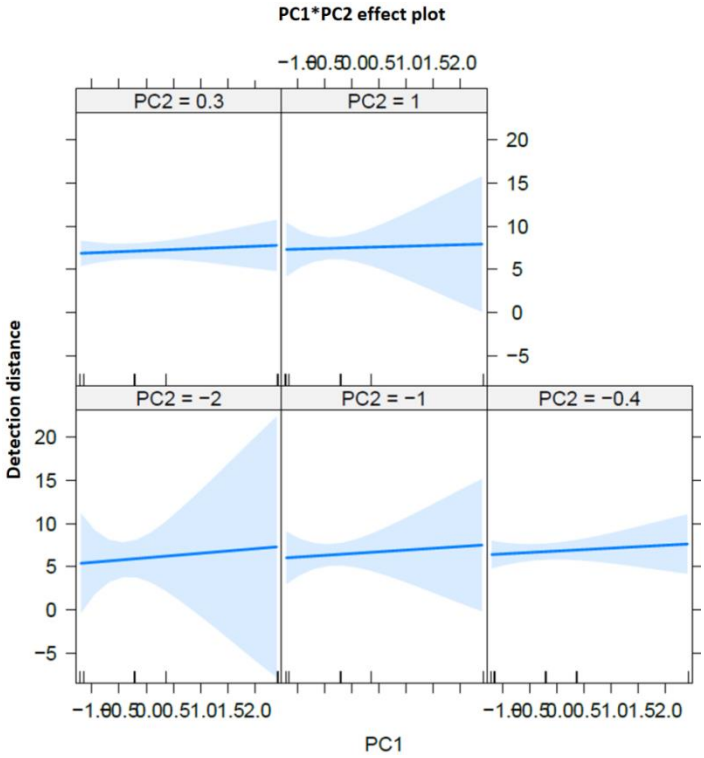

Figure 4.7. Interactive effects of PC1 and PC2 on detection distances bat assemblages across study sites.

4.3.9 Test of the separate effects of PCs on detection distances for Miniopterus natalensis (testing prediction (iii) of the AAH and prediction (ii) of the alternative hypothesis).

Analyses of the individual effects of PCs on detection distances for *M. natalensis* showed that PC1 (RH and Atm), which accounted for 56.26% of the variation in the climatic data (Table 4.2) had a stronger negative effect on detection distance (Fig. 4.8). PC2 (Temp) which also accounted for slightly less variation in the climatic data (36.14%, Table 4.2), had a strong positive influence on detection distance (Fig. 4.8). The detection distance was affected by spatial effects in both longitude and latitude, which were integrated into the climatic effect (Fig. 4.8). Longitude and latitude indicated inverse and positive linear relationships, respectively (Fig. 4.8).

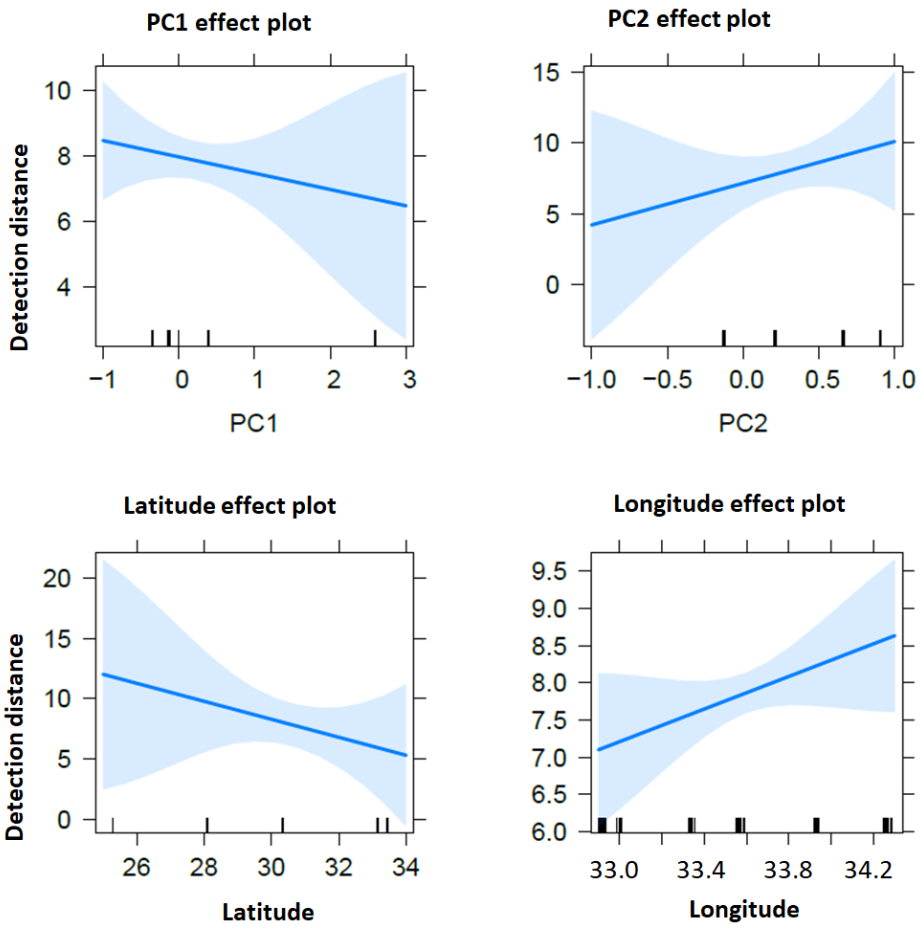

Figure 4.8. The relationship between detection distance and PC1, PC2, latitude and longitude for *M. natalensis* across study sites.

The interactive effects of PC1 (relative humidity) and PC2 (temperature) indicated the existence of a complex interaction between detection distance for *M. natalensis* and climatic conditions (Fig. 4.9). The effect plots illustrate the effect of temperature on detection distance was mediated by relative humidity. When relative humidity is high, there is a surprisingly modest increase in temperature, which causes the detection distance to increase (Fig. 4.9). Temperature lowers and stabilizes (levels off) over time as relative humidity rises, negatively affecting the detection distances (Fig. 4.9).

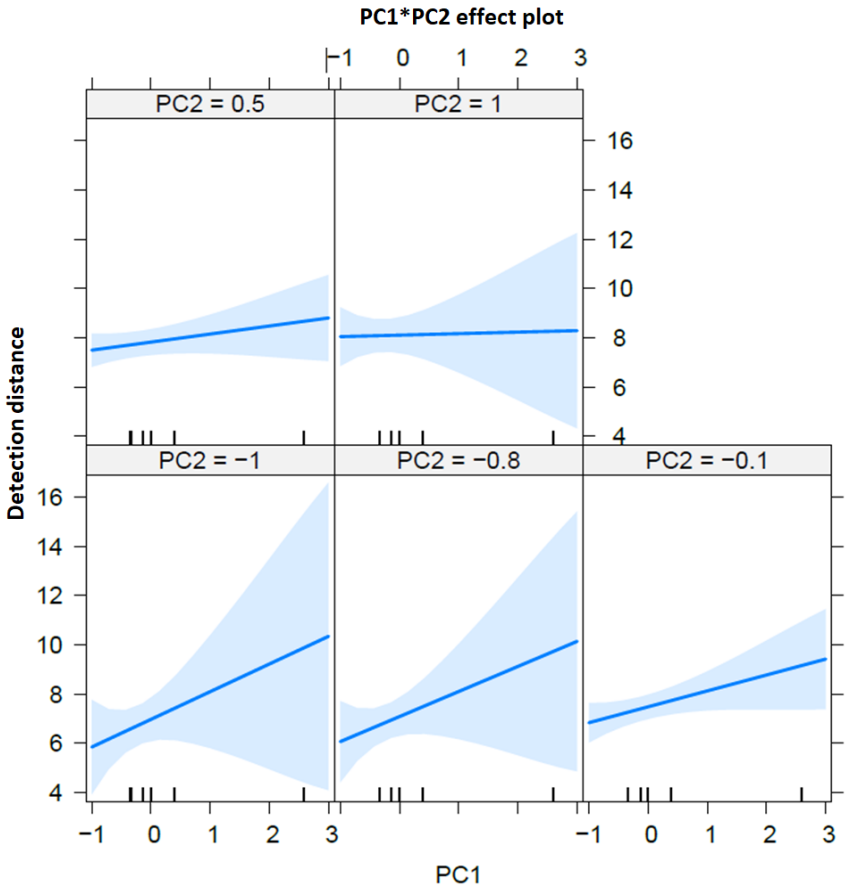

Figure 4.9. Interactive effects of PC1-relative humidity (RH; %) and PC2-mean annual temperature on detection distances bat assemblages across study sites

There is a difference between the results of predictive analysis and pure PCs analysis. In summary, these results show that climatic variables have less influence on detection distance individually than they do in combination with other variables.

4.3.10 Correlation analyses between echolocation parameters bat assemblages and climatic variables across sites

Pearson correlations indicated that there was a significant positive association between source levels and detection distance, [r (535) = 0.667, $p < 0.001$; Fig. S4.6]. Relative humidity was not correlated with the echolocation pulse frequency [r (535) = -0.017, $p = 0.698$; Fig. S4.5] and with source levels [r (535) = 0.029, $p = 0.499$].

4.3.11 Correlation analyses between echolocation parameters Miniopterus natalensis and climatic variables across sites

Pearson correlations showed that there was a moderate and significant negative association between echolocation pulse frequency and source levels [r (99) = 0.325, p = 0.001; Fig. S4.7]. Relative humidity was not correlated with source levels [r (99) = -0.089, p = 0.380] and detection distances [r (99) = -0.164, p = 0.105]. There was a strong and significant positive association between temperature and longitude [r (99) = 0.744, p = 0.000; Fig. S4.8], and moderate association between longitude and relative humidity [r (99) = 0.309, p = 0.002; Fig. S4.9].

4.4 Discussion

Bats in the same assemblage exposed to the same climatic conditions had different detection distances, and as a result, prediction (i) of the AAH was not supported. Detection distances were different across some but not all bat assemblages occupying foraging habitats with varying climatic conditions; this indicates that prediction (ii) of the AAH was only partially supported. Body size, life-history strategies or phylogenetic history, and the selection pressures that shape them within and across species appear to exert a greater influence on detection distances among bat assemblages. Thus, the variations seen within and across bat assemblages occupying habitats with varying climatic conditions, supported prediction (i) of the alternative hypothesis. Moreover, detection distances were similar for a single species, *M. natalensis* despite it occupying localities that differed in climatic conditions. This and dissimilar detection distances within an assemblage in the same foraging habitat and climates did not therefore support prediction (iii) of the AAH. Instead, these results support prediction (ii) of the alternative hypothesis. Other factors like body size and evolutionary history appear to have exerted a greater influence on detection distances than climate. Nevertheless, both of these factors are influenced by climate hence the partial support of prediction (ii) of the AAH.

In the present study, bats had different echolocation parameters, body sizes, foraging microhabitats, and phylogenetic histories, all of which impact bat echolocation, thus, detection distances. The different phylogenetic histories of LDC and HDC bats, for example, result in different detection distances even within the same climatic zone. LDC bats such as *N. capensis* that forage close to dense vegetation have on average higher detection distances than HDC bats because LDC bats have to avoid echoes being masked when they overlap with echoes from the background vegetation. In contrast, HDC bats use Doppler-shift compensation to circumvent such masking effects, allowing them to use shorter detection distances closer to vegetation. The LDC specialist gleaners, like *N. thebaica* use high frequency, multi-harmonic pulses of short duration, often at low source levels, while listening for faint rustling sounds of their prey on the ground surface to circumvent masking. This allows them to have the shortest detection distances despite being exposed to the same climate. The weak influence of climate on detection distances is influenced by the ubiquitous *M. natalensis* which occupies heterogeneous habitats with diverse climates. Despite its wide geographic distribution, populations of *M. natalensis* achieved similar detection distances regardless of foraging climate, suggesting that climate does not exert an overriding influence on detection distances. Instead, life history appears to be more important. The similar detection distances by *M. natalensis* is probably achieved through compensation for frequency-dependent atmospheric attenuation in each locality by adjusting echolocation pulse frequencies (49-58 kHz) and source levels (128-140 dB peSPL) so as to optimize detection distances given its phylogenetic constraint.

Similarly, body size and foraging mode differences can impact on detection distances independently of climate. LDC bats such as *T. aegyptiaca* and *S. petrophilus*, have large body sizes and emit low frequency (21 and 26 kHz) pulses at high source level > 125 dB peSPL to increase their detection distances in accordance with the fast flight demanded by their open

space mode of foraging. They need to detect prey at further distances than a smaller bat, to be able to maneuver in time to intercept prey. In contrast, LDC bats like *M. tricolor*, an aerial hawker, is small and forages at the edge of vegetation, use echolocation pulses of high-frequency (45-55 kHz) and high source levels > 128 dB peSPL during foraging. Here, the primary concern is to improve resolution to detect prey against complex clutter while enhancing detection distances. Higher frequency signals are attenuated by a large magnitude than lower frequency signal (Lawrence & Simmons, 1982), thus there is more likelihood of noticing variations in the frequency among bat species that use pulses of high frequency because of attenuation. Bat species like *R. capensis, R. clivosus, R. damarensis, M. fraterculus* and *M. tricolor* emitted high source levels to counteract this effect of atmospheric absorption and optimize detection distances in their respective foraging conditions. In contrast, bats in the assemblages in the present study also comprised species that emitted pulses of high frequency and low source levels, e.g., *N. thebaica, R. simulator*. Some species like *C. seabrae, S. petrophilus, T. aegyptiaca* in the assemblages however emitted pulses of low frequency and high source levels as a means to minimize the effect of atmospheric attenuation and enhance detection distances.

With their diverse wing morphologies and echolocation pulse frequencies, bats of different body sizes adapt to a diverse range of foraging habitats and climates (Norberg & Rayner, 1987; Schnitzler et al., 2003; Varzinczak, 2020). Relatively large-bodied bats (e.g., *T. aegyptiaca, S. petrophilus*) are characterized by different flight modes related to their distinct wings, as well as long duration and low-frequency pulses. Such wing modification, boosts the bat's ability to fly fast and catch flying insects in open spaces, and thus the low-frequency pulses emitted are meant to maximize bat's detection distances (Norberg & Rayner, 1987). Clutter-adapted bats (e.g., *R. capensis, R. simulator, R. damarensis, R. clivosus*) that are in a range of small to large

body sizes can distinguish prey from surrounding vegetation using high frequency, wide bandwidth pulses, and can maneuver well in small spaces because their wings are short, broad, with low wing loadings and aspect ratios (Norberg & Rayner, 1987; Blakey et al., 2019). Such wing designs are compatible with slow, maneuverable flight in cluttered habitats as the species emit pulses of high frequency to detect prey closer to vegetation (Jacobs & Bastian 2016). A detailed discussion of these relationships is provided in Chapter 3.

The present study showed that among bat species in different assemblages' echolocation pulse frequencies, their source levels, and the resultant product, namely, the detection distances varied. Conversely, Surlykke and Kalko (2008) report of similar detection distances among bat species in the assemblage, whose echolocation pulse frequencies and source levels varied. Although the bat species they recorded were of different body sizes and foraging strategies, the similar detection distances found may be attributed to a set of circumstances under which the echolocation pulses were recorded. Almost all recordings were taken at a forest habitat edge environment, only a couple near water bodies, all under the same atmospheric conditions. Though the bat species in their study differed in body sizes, phylogenetic history (11 species from 5 families) and pulse frequencies, they had similar hunting habits. This might have limited their flight speed and maneuverability in similar ways. Their detection distances were similar, and independent of phylogeny and body size, because of each species adjusting their source levels to compensate for frequency-dependent atmospheric attenuation (Surlykke & Kalko, 2008). In contrast, the assemblages in the present study consisted of species of diverse phylogenetic history and, more importantly, foraging habits including, gleaning, clutter specialists and aerial hawking close to vegetation or in the open.

Several trends were observed regarding detection distances with respect to climatic conditions in different habitats, notwithstanding differences in the relationships. The association between

detection distances and climatic variables were only partially supported despite the fact that acoustic signals are known to be influenced by atmospheric conditions. However, this is probably because bats are able to adjust source levels and frequency of their echolocation to mitigate the impact of changing atmospheric conditions on their detection distances. For example, although some LDC bats emit pulses at low frequencies, the source levels output is high, e.g., *S. petrophilus* (28 kHz; > 130 dB peSPL), *T. aegyptiaca* (21 kHz; > 135 dB peSPL), *C. seabrae* (38 kHz; > 128 dB peSPL). Other species emit pulses at high frequencies and high source levels e.g., *M. fraterculus* (63 kHz; > 130 dB peSPL) *M. natalensis* (55 kHz; > 127 dB peSPL) *M. tricolor* (48 kHz; > 128 dB peSPL). The echolocation pulses for HDC bats with much higher frequencies within a range of 75-100 kHz, also had high source levels > 120 dB peSPL, they were typically 5-10 dB lower than those for most LDC bats.

Nevertheless, in some instances, detection distances are influenced by climatic variables. For bat assemblages, detection distances correlated with temperature and longitude, whereas for *M. natalensis*, a correlation was found between detection distances and longitude. The only exception was *M. natalensis*; it maintained similar detection distances. Since temperature and longitude influenced the detection distances for bat assemblages, in part supported prediction (ii) of the AAH. All these correlations were based on predictive analyses that tested the separate effects of environmental variables. In contrast, relative humidity, temperature and spatial predictors (longitude and latitude) were correlated with detection distances only when the individual PCs were analyzed separately: PC1 (relative humidity) and PC2 (temperature). Nonetheless, the interaction between PC1 and PC2 have no influence on detection distances for bat assemblages and have only a modest effect on the detection distances of *M. natalensis*. In spite of the differences in climatic conditions between habitats, detection distances for populations of *M. natalensis* are similar. A two-pronged approach (predictive analysis and

individual PCs), therefore, suggests that the influence of environmental variables on detection distances is the result of complex interactions between multiple climatic variables, for example, with the natural and phylogenetic history of a lineage.

An apparent lack of correlation between detection distances for bat assemblages and relative humidity could possibly be attributed to two possible explanations. Firstly, it is due to the complex relationship between frequency, temperature and relative humidity. Secondly, species differences in pulse frequency exist within bat assemblages, and the majority have relatively high source levels to compensate for atmospheric attenuation. Nevertheless, despite differences in relative humidity across sites, relative humidity neither correlated with echolocation pulse frequency ($r = -0.017$; Fig. S4.5) nor source levels ($r = 0.029$) for bat assemblages. Congruent to my findings, studies by Odendaal et al. (2014) and Maluleke et al. (2017) found no relationship between relative humidity and resting frequency in *Rhinolophus* species occurring in different sites although the humidity profiles varied less. A strong positive correlation between source levels and detection distances exists ($r = 0.67$; Fig. S4.6) indicating the vital role of high source level output, which enhances sound propagation over greater distances (Holderied et al., 2005; Jakobsen et al., 2013). Thus, depending on species differences in emitted pulse source levels within assemblages, response to local environmental conditions may vary between assemblages occupying different sites.

Only longitude showed a considerable influence on detection distances for *M. natalensis* across sites. *M. natalensis* occupies different habitats, for example, Jacobs (1999) reports that the morphology and echolocation pulse of *M. natalensis* is adapted to the different microhabitats they occupy. Furthermore, Miller-Butterworth et al. (2003) report genetic substructure across the range of *M. natalensis* and this substructure is correlated with morphology and ecology.

Therefore, the species' phenotypic traits (wing shape) as well as genotypic character traits reflect its adaptation to its local environment, including its prey detection system. *M. natalensis* also adjusted echolocation pulse source levels at an average range of 127-136 dB peSPL (Table S4.1) across sites to match both the habitat type and climatic conditions, while optimizing prey detection distances. This was supported by a moderate and negative correlation between source levels and temperature (r = -0.33; Fig. S4.7); however, there was no link between relative humidity and source levels (r = -0.089) or detection distances (r = -0.164). Still, less variation in echolocation pulse frequency ranging between 53-56 kHz for *M. natalensis* across its distributional range casts doubt on the extent to which frequency-dependent atmospheric attenuation was responsible for the correlation between longitude and detection distances.

Nevertheless, climatic conditions may change according to relationship with longitude and latitude (Shao et al., 2012; Matthews & Mazer, 2016). Basically, both temperature and humidity variability along longitude and latitude direction result from the regular change of elevation. This is evidenced by a strong positive correlation between longitude and temperature (r = 0.74; Fig. S4.8) and longitude that is also moderately correlated with relative humidity (r = 0.31; Fig. S4.9). Other geographical features such as mountains and seas at different longitudes and latitudes greatly affect atmospheric circulation, rainfall pattern, vegetation, and thus regional climate (Shao et al., 2012). In South Africa, there is a precipitation gradient with increased aridity from east to west, i.e., along a longitudinal gradient (Wessels et al., 2007). High precipitation increases the relative humidity, suggesting that the two climatic conditions follow a similar gradient from east to west. However, the latter relationship may not always be true at broader scales because water vapour in the atmosphere condenses as part of the rainfall effect. Furthermore, it implies that over larger areas, rain can reduce humidity, which varies in different climates due to differences of saturation vapour pressure in the atmosphere. Since

saturation is a function of atmospheric pressure, and especially of temperature, it may possibly vary along longitudes (Lawrence, 2005). Variations are also dependent on where longitude intersects with latitude (Matthews & Mazer, 2016). At higher longitudes (Fig. S4.9), there is a possibility of a saturation deficit; this deficit increased with temperature leading to a drop in relative humidity for localities in the east. Detection distances have followed a trend to this gradient, that is, as you move further east, detection distances increase, suggesting that a decrease in relative humidity reduces atmospheric attenuation, and thus has lesser effect on detection distances. The different models used here provided complex results and the implications of using regional climate to predict detection distances. They provide an indication of how spatial patterns in climates combine to affect the local climate response (a nonlinear interaction), and how those patterns may impact detection distances. For example, the inverse linear relationship between longitude and latitude with detection distances for bat assemblages; Fig. 4.6 and *M. natalensis*; Fig. 4.8) was probably due to increased relative humidity along longitudinal gradient. Therefore, populations of the species have phenotypic traits (wings shape) adapted to forage in their respective habitats that differ in humidity. Predictive modelling analyses revealed that neither atmospheric pressure nor latitude influenced detection distances for bat assemblages and *M. natalensis* across sites (Fig. 4.4 & 4.5). The main reason latitude was not an important factor was that sites are less separated along latitude than they are along longitude (see map in Chapter 2 and Table S4.2). In general, however, latitude comprises several environmental and complex effects (Meiri & Dayan, 2003), such as altitude and aridity, which may substantially affect temperatures differently, influencing the acoustic properties of the environment as revealed by analyses of individual PCs (Fig. 4.6 & 4.8). Congruent to these findings, Mutumi et al. (2016) found a latitudinal cline in resting frequency for populations of *Rhinolophus simulator*. Temperature can affect atmospheric pressure at different altitudes between locations due to disparity in air density (Nauenberg, 2017; Lu & Tu,

2021). A complex non-linear interaction between temperature and relative humidity exist, differs between sites in different biomes, and these variables are correlated with detection distances, offering partial support to prediction (ii) of the AAH. Generally, the relationship between the latter climatic variables changes over spatial scales and over time (Wiley & Richards, 1978). Due to these changes in atmospheric conditions, bat species in assemblages and populations of *M. natalensis* in different sites may have adapted their echolocation pulses to the local atmospheric conditions over evolutionary time.

To conclude, my study shows that bats have a diverse suit of echolocation pulse parameters, body sizes and phylogenetic history, which exert a greater influence on prey detection distances than climate gradients. However, climatic changes have profound effects on sensory ecology because the efficacy of the signal in a given environment is largely dependent on the atmospheric conditions (Ferrari et al., 2011; Luo et al., 2014). Bats echolocating at low and high frequencies may simultaneously use moderately high source levels to minimize the severity of atmospheric attenuation to increase detection distances. Climatic gradients are associated with detection distances and the association is further linked to the natural and evolutionary history of bats. Therefore, the combination of echolocation pulses, body sizes, wing morphology, and climatic conditions results in a complex adaptive relationship that may promote selective pressure on the prey detection system of bats.

CHAPTER 5

Synthesis and Conclusions

The focus of the whole study was to unravel the hitherto unexplored source level and detection distances for bat fauna across their geographical distribution in South Africa. Advanced technology that is, the multiple microphone array system, allows the reconstruction of bat flight trajectories so that the source levels at which bats emit their echolocation pulses can be measured from the recorded echolocation pulses of free flying bats in the wild. Data on source levels and weather parameters were used to calculate prey detection distances for bat species comprising bat assemblages and populations of a single species *Miniopterus natalensis*. Then, detection distances were used to test the Acoustic Adaptation Hypothesis; AAH (Morton, 1975; Hansen, 1979) (Chapters 3 and 4) which proposes that the acoustic properties of the environment, as a result of habitat structure or climate, influence sound propagation and ultimately the evolution of echolocation pulses. This research therefore explored whether differences in detection distances for free-flying bats, identified from their echolocation pulses (Chapter 2) could be explained by local adaptation to habitat structure (Chapters 3) or to climate regimes (Chapter 4).

5.1 Identification of species based on acoustic signals

The use of acoustic signals to identify species is common in many species that communicate acoustically, including cetaceans, bats, lacewings, orthopterans, birds, and anurans (Russo & Jones, 2002; Fox et al., 2008; Vidaña-Vila et al., 2020); for example, the calls for *Leptodactylus* of the *marmoratus* group of the Amazon Basin amphibians, and their associated environments and the songs of male crickets in Australia (Kok et al., 2007; Angulo & Icochea, 2010). Most of these populations exhibit distinct advertisement calls that demonstrate their species-specificity, but that can sometimes show intraspecific variability

because age, gender, and size can influence different call parameters such as frequency and amplitude (Russo et al., 2018; Pereira et al., 2020). Acoustic signals are also deemed useful in the identification of morphologically cryptic species, and their use has been explored notably among groups that are taxonomically complex (Koehler et al., 2017; Garg et al., 2021). In the present study (as revealed in Chapter 2), bat species were reliably identified from their echolocation pulses, using several parameters including peak frequency, maximum frequency, duration, bandwidth and peak amplitude. Species identification was facilitated by the low levels of phylogenetic relatedness in the bat assemblages, which typically consisted of several families with one to three species per family. Differences in foraging modes such as clutter-edge foragers, open-air high-flying foragers, clutter foragers, narrow space gleaning foragers and gleaning/trawling (Moyo & Jacobs, 2020) also contributed to the differences in echolocation pulses that facilitated species classification. However, the acoustic identification of some species was somewhat confounded due to overlapping echolocation parameters with other species e.g., *M. natalensis* and *Myotis tricolor*, but because pulses of the latter species are more steeply frequency modulated without a quasi-constant frequency component, it was possible to tell them apart. Identification of rhinolophids proceeded with caution, as the frequency of the constant component of some rhinolophids overlapped; thus, the use of the frequency of the constant frequency component alone could not always be reliable. This hurdle was resolved using available species distribution records (Schoeman & Jacobs, 2003, 2008; Monadjem et al., 2010) and confirmed by previous genetic identification of all species included in this study, and their echolocation pulses, in the laboratory of David Jacobs, University of Cape Town. Consistent with this findings, other taxa, such as birds, insects and anurans have diverse and complex songs and, can be challenging to identify (Sasahara et al., 2012; Greenfield, 2016; Chen et al., 2020; Santos et al., 2021). Nevertheless, vocalizations in certain

groups of birds as a complement to genetic and morphological information is commonly used to determine species identity (Slabbekoorn & Smith, 2002; Renner et al., 2018).

5.2 Acoustic signal adaptation to the acoustic environment

Across diverse taxa, animals produce acoustic signals for communication (Chaverri et al., 2018; Penar et al., 2020). A signal can give valuable information about an individual, such as their identity, sexual orientation, size, or aggression, which makes them extremely important to social animals. (Bradbury & Vehrencamp, 2011b). Bat echolocation, on the other hand has evolved predominantly for orientation and to aid food acquisition and with some evidence of communication (Heller & von Helversen, 1989; Schnitzler et al., 2013). Several studies have shown that bat echolocation is adapted to bats' habitat and foraging strategy, which in turn impacts their detection system. Hence, echolocation is an ideal way to study how adaptation affects the variation of phenotypic traits important for survival and reproduction. It's important to note, however, that acoustic signals degrade when transmitted through water and air, and they degrade faster than expected (Wiley & Richards, 1978; Forrest, 1994). If signal quality is reduced, information may be lost, while communication failure may adversely affect resource defense, attraction of mate, or other critical survivorship and reproduction behaviours (Reby & McComb, 2003; Bradbury & Vehrencamp, 2011a). For example, it may impede bats' prey detection system, reducing their foraging effectiveness (Goerlitz, 2018). Thus, signal transmission and the conditions leading to degradation of the signal and the possible means to counteract it have drawn much attention, particularly as it applies to mammals, insects, birds, and anurans.

Habitats with varying habitat structure and prevailing climatic conditions provide different acoustic environments, which determine how signals are degraded. Attenuation of sound in the environment is well documented and occurs when sound is absorbed by atmospheric pressure,

attenuated by ground, scattered by beams, or reflected by vegetation (Huisman & Attenborough, 1991; Wahlberg & Larsen, 2017). According to the sensory drive hypothesis, environmental differences that affect sensory perception may influence a species' choice and signalling attributes. (Endler, 1992; Schaefer & Ruxton, 2015). In connection with this, the Acoustic Adaptation Hypothesis (AAH) proposes that animals should therefore use acoustic signals that are well adapted to propagate in their environment (Hansen, 1979). Since its founding, wide - ranging comprehensive research on acoustic signals has been performed to ascertain the AAH's predictions. In most studies, acoustic features are examined in relation to the habitats in which they occur and whether the features of the acoustics are as predicted by the AAH. Most of them are in favour of some of the AAH's predictions, but not all of them. (Boncoraglio & Saino, 2007). Acoustic signals are generally reported to vary in the way predicted by the AAH across seemingly heterogeneous habitats, as exemplified by studies of birds, mammals, and anuran species (Peters & Peters, 2010; Tobias et al., 2010; Velásquez et al., 2018;). Moreover, some taxa may exhibit acoustic signal divergence in direct correlation with body size and foraging strategies (Tobias et al., 2010).

Despite a number of studies showing relationships between acoustic signal structure with environmental conditions and providing a proper evaluation of the AAH signal structure predictions, they do not test signal transmission directly. The foundational prediction of the AAH expressly indicate that sound signals that reduces degradation in a particular environment should be preferred (Morton, 1975). Thus, investigations that directly tests signals transmission, therefore, provides a robust a test of the AAH. One classic example is the echolocating bats' prey detection system, where the predictive power of AAH is tested. As such, I report results that determined detection distances within and between bat assemblages in different habitats to test the AAH. Within and between assemblages, bats used varying

frequencies (kHz) and source levels (dB peSPL) to minimize atmospheric attenuation (dB/m) to enhance signal transmission in specific habitats and under prevalent climatic conditions. In Chapter 3, I report that detection distances within bat assemblages were different and similar across some but not all assemblages. Similarly, detection distances for *M. natalensis* were significantly different across some sites but similar across other sites. The AAH was thus only partially supported. This concurs with other investigations in groups of frogs, birds and mammals (Saunders & Slotow, 2004; Vargas-Salinas & Amézquita, 2014). The partial support is not surprising due to the intricacies of acoustic signals and limitations, the numerous factors that can influence signal evolution, and the interconnectedness of these factors. The results in Chapters 3 and 4 are contrary to those of Surlykke and Kalko (2008) in that detection distances were not similar within assemblages. In general, the acoustic properties of habitat could not fully explain detection distances of bat assemblages. Thus, body size and foraging strategy probably influences detection distances to a greater extent than the acoustic properties of the habitat. This explains why the AAH was only partially supported in so many different studies as mentioned above, including in the present study. Differences in acoustic properties of the habitat are probably dealt with through behavioural changes in source levels or frequency in LDC and only in source levels in HDC bats because the acoustic fovea does not allow flexible echolocation frequency in HDC bats. Differences in detection distances are attributable to local adaptation because populations of *M. natalensis* show variations in phenotypic (wing shape) and genotypic traits (Miller-Butterworth et al., 2003, 2005). The species also adjusted echolocation pulse source levels across sites in biomes, possibly to optimise prey detection distance. The bat assemblages studied here consisted of species of different body size and wing shapes that are probably the result of many selection processes, allowing bats to meet physiological and foraging demands (Norberg & Rayner, 1987; Blackburn et al., 1999). Given that echolocation pulse frequency is associated with body size in bats (Jacobs et al., 2007;

Stoffberg et al., 2011) it may be further reflected in wing morphology and, ultimately, in foraging behaviour of bats. For example, bats may employ different foraging strategies, resulting in differences in prey detection distances within an assemblage, which may vary from one site to another. Open-space foragers such as *Tadarida aegyptiaca* and *Sauromys petrophilus* of relatively large and medium body sizes, respectively, have long and narrow wings, and an above average aspect ratio and wing loading compared to bats that fly in closed habitats (Norberg & Rayner, 1987). They produce loud pulses at low frequency allowing them to probe the space in front of them at greater distances. Similarly, both *M. natalensis* and *Miniopterus fraterculus* are phylogenetically related, have similar body sizes (Miller-Butterworth et al., 2005) and have similarly high echolocation pulse source levels, relatively higher frequencies although their detection distances vary. They have high and low wing loading and aspect ratio, respectively (Jacobs, 1999a; Schoeman & Jacobs, 2003, 2008), are suited to forage on aerial prey in relatively open or less cluttered habitat, and should alter their source levels appropriately to suit their flight speeds and maneuverability. The wing morphology of *M. natalensis* appears to be locally adapted to the different habitats it occupies (Miller-Butterworth et al., 2003). Such adaptation may be influenced either by habitat structure or migration or both. This could possibly contribute to this species using similar detection distances in some sites but different detection distances in other sites.

In Chapter 4, it was established that the AAH prediction (i) was not supported as bats in the same assemblage had different detection distances. Prediction (ii) of the AAH was only partially supported because detection distances were different across some but not all bat assemblages under varying climatic conditions. A diverse array of factors, including species body size, life-history strategies and phylogenetic history (as described in Chapter 3), and selection pressures, such as climatic conditions can result in differences in acoustic signals in

different taxa, including bats. Moreover, a similarity in detection distances for *M. natalensis* occupying climatically different sites failed to support prediction (iii) of the AAH but supported prediction (ii) of the alternative hypothesis. It implied that climate does not have an overriding influence on detection distances. Similar detection distances were likely achieved via compensation for frequency-dependent atmospheric attenuation at each of the sites by adjusting echolocation pulse frequencies and source levels to optimize detection distances within phylogenetic constraints. I report results in Chapter 4 based on predictive model showing correlations between detection distance and environmental factors like temperature and longitude for bat assemblages. Similar detection distances for *M. natalensis* were associated with longitude. As an interesting paradox, all climatic variables appeared to be better predictors of detection distances for bat assemblages and *M. natalensis*. It was because of the composite effect of individual principal components which constitute climatic variables. Since temperature and relative humidity may change at different longitudes and latitudes owing to diverse geographical features that affect atmospheric circulation, it suggests that temperature and relative humidity are the most important climatic variables that impacts echolocation. Variation in local temperatures occurred along the environmental gradient and the species' populations have adapted to these changes. Bats, for example (as illustrated in Chapter 3) adjust echolocation pulse source levels across localities to counter the effects of sound attenuation so that prey detection distance is optimized. Apart from bats, studies have revealed that temporal patterns of calling activity among birds and anurans also experience attenuation as the acoustic signals are transmitted (Snell-Rood, 2012; Goutte et al., 2018). Such sound attenuation is a nonlinear function of the sound frequency, relative humidity, temperature and atmospheric pressure (Luo et al., 2014). A few studies have authenticated that relative humidity influences pulse frequency of bats in a negative way (Guillén et al., 2000; Mutumi et al., 2016). Relative humidity, however, exerts its influence through a complex and nonlinear interaction with

temperature (Stilz & Schnitzler, 2012; Luo et al., 2014). It is, therefore, not surprising that relative humidity across sites did not correlate with echolocation pulse frequency for bat assemblages. In concordance with this, two previous studies found no link between relative humidity and resting frequency in *Rhinolophus* species (Odendaal et al., 2014; Maluleke et al., 2017). Similarly, the chorus of the songs of bulbuls (*Pycnonotus leucotis*) and sparrows (*Passer domesticus*), are unaffected by changes in relative humidity (Hasan, 2017).

In general, there is compelling proof that acoustic signals linked to sensory systems are tuned to the environment in a variety of animals (Haven & Richards, 1982). Climatic conditions may exert an influence on acoustic signals for species in terrestrial environment (e.g., bats, birds, insects, and anurans) and aquatic environment (e.g., dolphins) (Hauselberger & Alford, 2005; Snell-Rood, 2012; Veca et al., 2020). For example, relative humidity and temperature influence the resting frequency of *Rhinolophus clivosus* in different localities (Jacobs et al., 2017). Birds, reptiles, and fish, for example, show visual signals corresponding to their environments (Tobias et al., 2010; Caves et al., 2018; Gunderson et al., 2018). This is demonstrated by the evidence that light intensity, background color or turbidity can affect signal progression as a consequence of sensory drive (Tobias et al., 2010).

5.3 Hearing sensitivity of animals

The sensory systems demonstrate unifying, phylogenetically determined principles, but each species has distinct adaptations. This highlights the existence of the multiple evolutionary pressures at play in sensory perception. This further illustrates how evolutionary pressures affected sensory perception (Yost et al., 2007; Lattenkamp et al., 2021). Even though most species have similar hearing ranges, there is appreciable variation in frequency (high and low) sensitivity as well (Mooney et al., 2020; Lattenkamp et al., 2021). Grounded on the results from only a few species, generalizations regarding the sensitivity of mammals, birds, and

invertebrates can be made (Southall et al., 2007; Goerlitz et al., 2010; Larsen et al., 2020; Mooney et al., 2020). This has brought about very limited knowledge of species auditory variability both within and between species. For example, the bottlenose dolphin (*Tursiops truncatus*), regardless of frequency, the species display variations in auditory thresholds > 80 dB (Finneran & Houser, 2006) compared to belugas (*Delphinapterus leucas*) which exhibit less variation (Castellote et al., 2014). Invertebrates have thresholds that differ from fish, such as goldfish (*Carassius auratus*), in similar situations (Sisneros & Rogers, 2016). Some of this variation may result from factors such as age or prior noise exposure histories, as well as the acoustics of the environment or the method of establishing threshold levels (Houser & Finneran, 2006; Finneran & Schlundt, 2007; Martin et al., 2020).

Real life hearing thresholds are often affected by environmental conditions rather than sensitivity (Griffin, 1971; Cunningham et al., 2014; Römer & Holderied, 2020). Therefore, hearing thresholds determined in conjunction with ambient noise exposure should be considered. Since sounds in the wild are much more complex and masking effects such as spatial or temporal can reduce hearing thresholds, these factors can contribute to reductions in hearing thresholds (Schlundt et al., 2008; Erbe et al., 2016). Among bats, hearing sensitivity is species-specific and dependent on the type of acoustic signal and the circumstances under which it is produced in audible range, owing to the need to detect objects and prey (Neuweiler, 1984). Early studies estimated the bats' hearing threshold to be between 0-20 dB SPL (Neuweiler et al., 1984). Hearing thresholds measured in recent years for bat species in Europe have been determined to be 20 dB SPL (Lewanzik & Goerlitz, 2018; Goerlitz et al., 2020). Accordingly, the detection threshold for different species of bats in the present study was 20 dB SPL based on the latter studies, since no information concerning the hearing thresholds for bats in Africa was available. Thus, the detection distances for all prey sizes were calculated at

the chosen threshold. Other taxa, such as birds, have hearing thresholds ranges between 0 -120 dB SPL below the mean ambient noise level (Dooling et al., 2000; Zeyl et al., 2020). Birds typically do not hear much above 10 kHz (Dooling et al., 2000; Strawn & Hill, 2020). For instance, the Japanese quail's (*Coturnix japonica*) with a high frequency, upper limit to its hearing range occurred at 16 Hz to 8 kHz (Strawn & Hill, 2020). The species' frequency of maximal sensitivity occurred at 2 kHz, with all frequencies from 1 to 5.6 kHz having thresholds under 20 dB SPL (Dooling et al., 2000; Dooling & Prior, 2017).

Despite efforts made to determine the threshold for species in different taxa, the hearing threshold of many mammal species, including bats, are not known. Consequently, there is still a great deal of uncertainty about how they detect and respond to sound. The lack of data regarding hearing thresholds results in uncertainty regarding hearing thresholds, which is a significant limitation. The possibility of testing all species is impossible, however there is an open question over whether the results from one species can be extrapolated to a different species within the same taxonomic group. The degree of uncertainty may be higher for a species with limited data and whose sensitivity will vary with age (Strawn & Hill, 2020).

5.4 Conclusions

Bat assemblages comprise species that vary in frequencies and source levels and that, in turn, determine detection distances across habitats. Prey detection distances varied within and across bat assemblages and were influenced by several environmental and life-history factors. The interspecific variation was probably influenced mostly by differences in body size, and foraging strategies resulting from disparate phylogenetic histories (Demery et al., 2021). The assemblages were largely composed of species from different families with a diverse range of foraging strategies. However, climate partially influenced detection distances which appeared to be locally adapted to local climates, thus providing an empirical test for the role that selection

might influence the variations in pulse signals among echolocating bats. The combination of analytical models allows a thorough analysis of how adaptive processes contribute to pulse variations. Through such a holistic strategy, we can examine how climate interactions, and in this case temperature and relative humidity, can affect patterns of detection distances among assemblages and a single species. The paradoxical results stemming from this integrated approach of analysis about the relationship between detection distances and climatic factors reflects the complexity of natural selection.

5.5 Directions for future research

Future studies should investigate detection distances among assemblages and populations of a species in conjunction with the vegetation density (NDVI Index or above-ground biomass) and possibly consider precipitation, for example, as a proxy for relative humidity. Studies should also include multiple sites from different biomes as this would boost the sample sizes of the analyses and could provide more information about effect sizes on detection distances. Finally, future studies should consider controlling phylogenetic effects, as attempted in this study by focusing on a single species, *M. natalensis,* when determining the relative influence of phylogeny and natural selection on detection distances. This would help disentangle the processes influencing patterns of detection distances across species. The hearing thresholds of more species would also greatly facilitate such studies and our understanding of how animals use sound.

6.0 References

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7.0 Appendices

Chapter 2

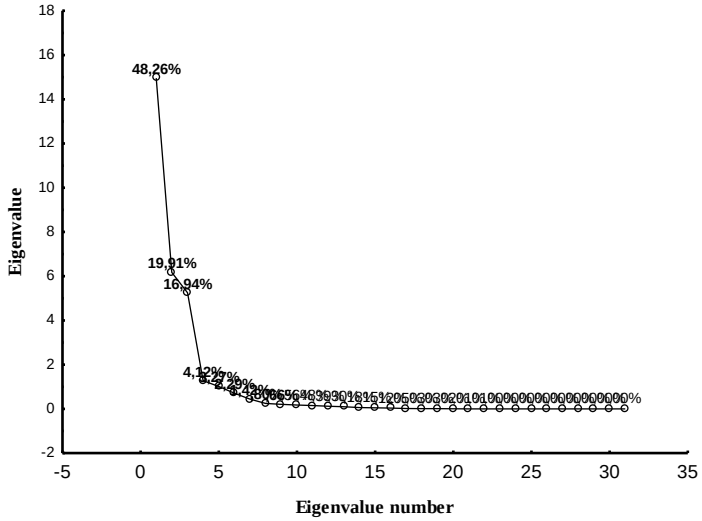

Figure S2.1. Eigen values of correlation matrix for variables of bats

Table S2.1. Classification matrix of species where rows show the observed classifications and columns showing predicted classifications.

Group	% correct	RCA p=.14	RDA .04	RCL .08	RSW .04	RSM .03	HCA .04	MN .02	MF .07	NC .10	MT .08	TA .05	CS .04	SP .04	NT .03
RCA	82.69	43	6	2	0	1	0	0	0	0	0	0	0	0	0
RDA	81.25	2	13	1	0	0	0	0	0	0	0	0	0	0	0
RCL	75.86	6	0	22	1	0	0	0	0	0	0	0	0	0	0
RSW	92.86	0	0	1	13	0	0	0	0	0	0	0	0	0	0
RSM	53.85	6	0	0	0	7	0	0	0	0	0	0	0	0	0
HCA	85.71	2	0	0	0	0	12	0	0	0	0	0	0	0	0
MN	87.50	0	0	0	0	0	0	70	7	2	0	0	1	0	0
MF	71.43	0	0	0	0	0	0	8	20	0	0	0	0	0	0
NC	82.93	0	0	0	0	0	0	5	1	34	0	0	0	1	0
MT	100.0	0	0	0	0	0	0	0	0	0	32	0	0	0	0
TA	83.33	0	0	0	0	0	0	0	0	2	0	15	1	0	0
CSB	76.47	0	0	0	0	0	0	0	0	1	0	1	13	2	0
SP	85.71	0	0	0	0	0	0	0	0	1	0	0	1	12	0
NT	83.33	0	0	0	0	0	0	0	2	0	0	0	0	0	10
Total	**83.16**	**59**	**19**	**26**	**14**	**8**	**12**	**83**	**30**	**40**	**32**	**16**	**16**	**15**	**10**

Table S2.2. Factor-variable correlations as factor loadings that are based on correlations.

Variables	Factor 1	Factor 2	Factor 3	Factor 4
Duration	0.4173	0.7687	-0.0326	-0.1932
Interval	-0.6447	0.5299	-0.2023	-0.2507
Disttomax	0.4278	0.7234	-0.0616	-0.2039
Start time	-0.6542	0.4614	-0.1372	-0.2674
End time	-0.6347	0.4935	-0.1383	-0.2751
Peak-to-peak ampl	0.0767	0.0904	0.8954	-0.0333
Peak freq(start)	0.7879	-0.5206	0.1121	0.1460
Min freq(start)	0.8401	-0.4167	0.1472	0.1106
Peak ampl(start)	-0.0489	0.1746	0.9116	-0.0878
Max freq(start)	0.7605	-0.5631	0.0849	0.1525
Bandw(start)	-0.0559	-0.6802	-0.1909	0.1930
Peak freq(end)	0.9536	0.1916	-0.1544	0.0251
Peak ampl(end)	0.0479	0.0299	0.8928	-0.0590
Min freq(end)	0.9479	0.2101	-0.1539	0.0614
Max freq(end)	0.9588	0.1613	-0.1608	-0.0319
Bandw(end)	0.2536	-0.3556	-0.0846	-0.7431
Peak freq(centre)	0.9850	0.0823	-0.0391	-0.0951
Peakampl(centre)	0.0789	0.1619	0.9526	0.0443
Min freq(centre)	0.9837	0.1146	-0.0511	-0.0752
Max freq(centre)	0.9867	0.0368	-0.0376	-0.1088
Bandw(centre)	-0.0081	-0.8544	0.1494	-0.3637
Peak freq(max)	0.9821	0.1395	-0.0668	-0.0410
Peak ampl(max)	0.1139	0.1855	0.9520	0.0162
Min freq(max)	0.9780	0.1682	-0.0778	-0.0242
max freq(max)	0.9859	0.0984	-0.0660	-0.0505
Bandw(max)	0.0333	-0.8663	0.1497	-0.3235
Peak freq(mean)	0.9762	0.1597	-0.0821	-0.0659
Peak ampl(mean)	0.2137	0.5589	0.7618	0.0513
Min freq(mean)	0.9588	0.2449	-0.1130	0.0023
Max freq(mean)	0.9402	-0.2601	0.0665	-0.1092
Bandw(mean)	-0.1453	-0.8586	0.3083	-0.1835

Table S2.3. Mahalanobis distance of species for HDC bats (RCA-*Rhinolophus capensis*, RDA-*Rhinolophus damarensis*, RCL-*Rhinolophus clivosus*, RSW-*Rhinolophus swinnyi*, RSM-*Rhinolophus simulator* and HCA-*Hipposideros caffer*).

Species comparison	F-value; df =4,336	Squared Mahalanobis Distances	*p*-values
RCA & RDA	7.91	10.44	0.000
RCA & RCL	10.54	9.14	0.000
RCA & RSW	16.48	24.13	0.000
RCA & RSM	5.42	8.41	0.000
RDA & RCL	8.59	13.45	0.000
RDA & RSW	10,89	23.55	0.000
RDA & RSM	9.50	21.40	0.000
RCL & RSW	5.51	9.42	0.000
RCL & RSM	8.57	15.41	0.000
RSW & RSM	10.54	25.24	0.000
HCA & Rhinolophids	(31-47)	(68-85)	0.000

Table S2.4. Mahalanobis distance of species for LDC bats (MN-*Miniopterus natalensis*, MF-*Miniopterus fraterculus*, NC-*Neoromicia capensis*, MT-*Myotis tricolor*, TA-*Tadarida aegyptica*, SP-*Sauromys petrophilus*, NT- *N. thebaica* and CS-*Cistugo seabrae.*

Species Comparison	F-value; df = 4.336	Squared Mahalanobis Distances	*p*-values
MN & MF	6.32	6.92	0.004
MN & NC	16.32	9.73	0.000
MN & MT	150.83	106.57	0.000
MN & TA	69.43	76.31	0.000
MN & CS	24.88	28.65	0.000
MN & SP	26.47	35.88	0.000
MN & NT	87.51	135.44	0.000
MF & NC	16.37	15.89	0.000
MF & MT	88.20	95.39	0.000
MF & TA	52.97	78.08	0.000
MF & CS	20.06	30.63	0.000
MF & SP	24.77	42.86	0.000
MF & NT	59.61	114.60	0.000
NC & MT	113.13	101.65	0.000
NC & TA	37.94	48.99	0.000
NC & CS	10.92	14.67	0.000
NC & SP	9.28	14.36	0.000
NC & NT	84.82	147.55	0.000
MT & TA	99.61	139.64	0.000
MT & CS	68.91	100.23	0.000
MT & SP	58.50	97.00	0.000
MT & NT	83.05	153.68	0.000
TA & CS	26.57	49.08	0.000
TA & SP	25.73	52.77	0.000
TA & NT	88.64	198.81	0.000
CS & SP	8.62	18.12	0.000
CS & NT	61.91	142.13	0.000

Table S2.5. Initial classification of species based on their duty cycle, echolocation pulse structure, peak frequency (kHz).

Family	Species	Duty Cycle	Pulse Structure	Peak Freq. (kHz)
Rhinolophidae	*Rhinolophus capensis*	HDC	FM-CF-FM	80.2±4.5
	Rhinolophus damarensis	HDC	FM-CF-FM	83.4±0.5
	Rhinolophus clivosus	HDC	FM-CF-FM	90.7±0.8
	Rhinolophus swinnyi	HDC	FM-CF-FM	99.9±0.6
	Rhinolophus simulator	HDC	FM-CF-FM	82.1±1.0
Hipposideridae	*Hipposideros caffer*	HDC	CF-FM	131.6±5.2
Miniopteridae	*Miniopterus natalensis*	LDC	FM-QCF	55.5±5.3
	Miniopterus fraterculus	LDC	FM-QCF	63.1±3.0
Verspertilionidae	*Myotis tricolor*	LDC	FM	55.8±9.6
	Neoromicia capensis	LDC	FM-QCF	39.8±4.8
Cistugidae	*Cistugo seabrae*	LDC	FM-QCF	36.0±3.3
Molossidae	*Sauromys petrophilus*	LDC	FM-QCF	30.4±1.1
	Tadarida aegyptiaca	LDC	FM-QCF	21.3±1.2
Nycteridae	*Nycteris thebaica*	LDC	FM	82.4±3.4

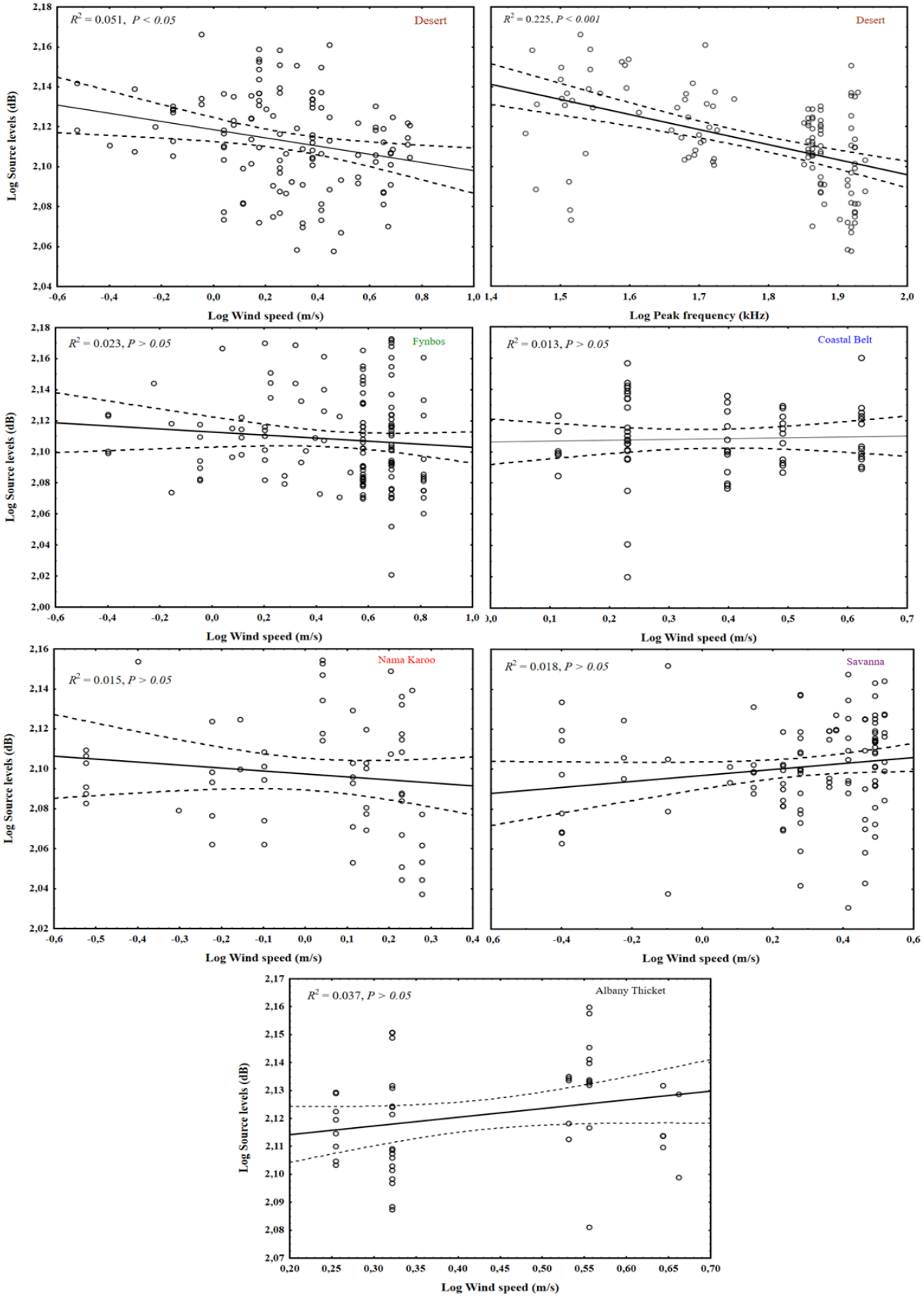

Figure S3.1. Regression of the log source levels (dB) against the log of wind speed (m/s) in six biomes, and with peak frequency (kHz) in one biome.

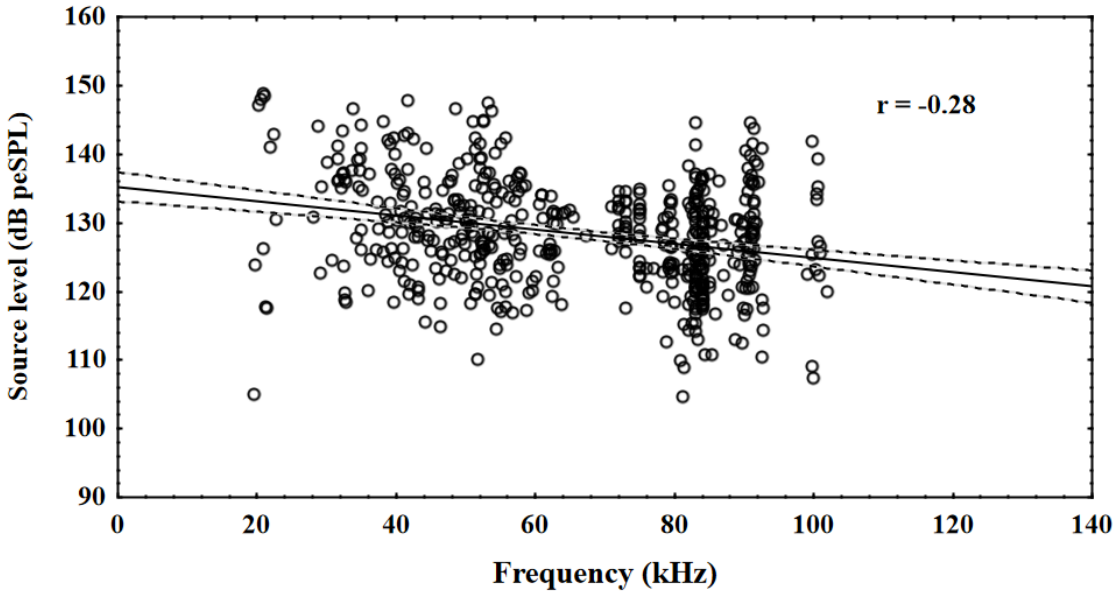

Figure S3.2. Correlation between source levels and frequency of echolocation pulses for bat assemblages across sampling sites.

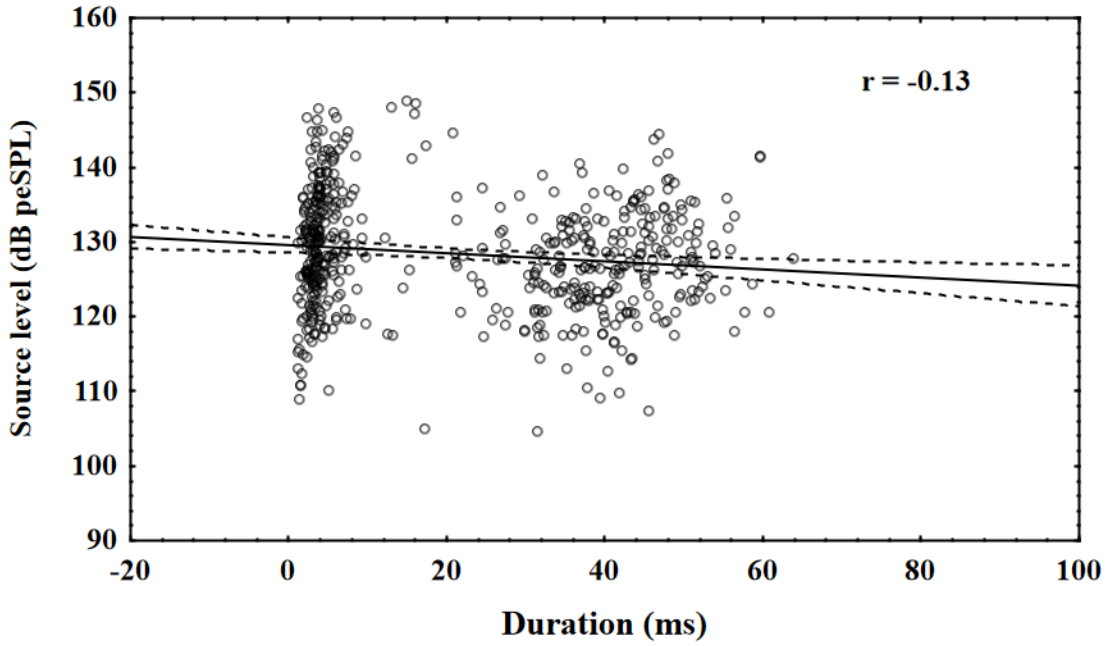

Figure S3.3. Correlation between source levels and duration of echolocation pulses for bat assemblages across sampling sites.

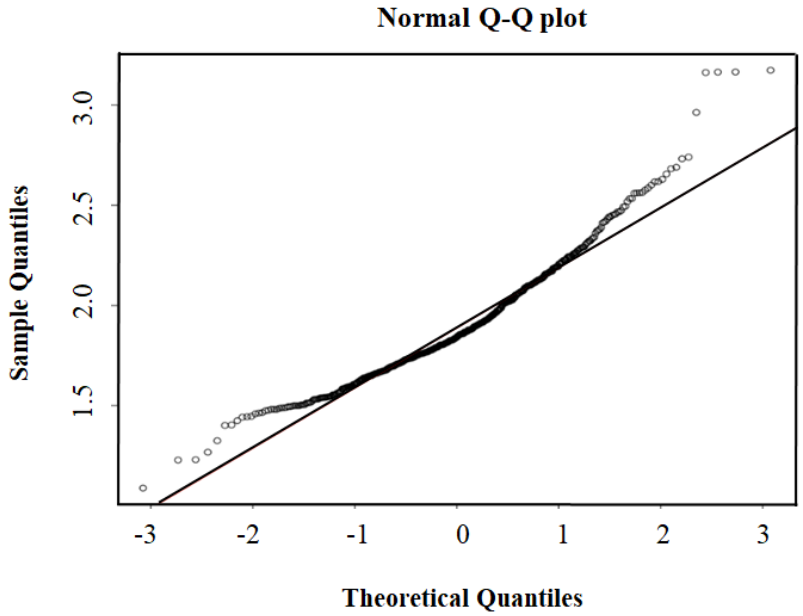

Figure S3.4. Plots to test for normality for detection distances for bat assemblages.

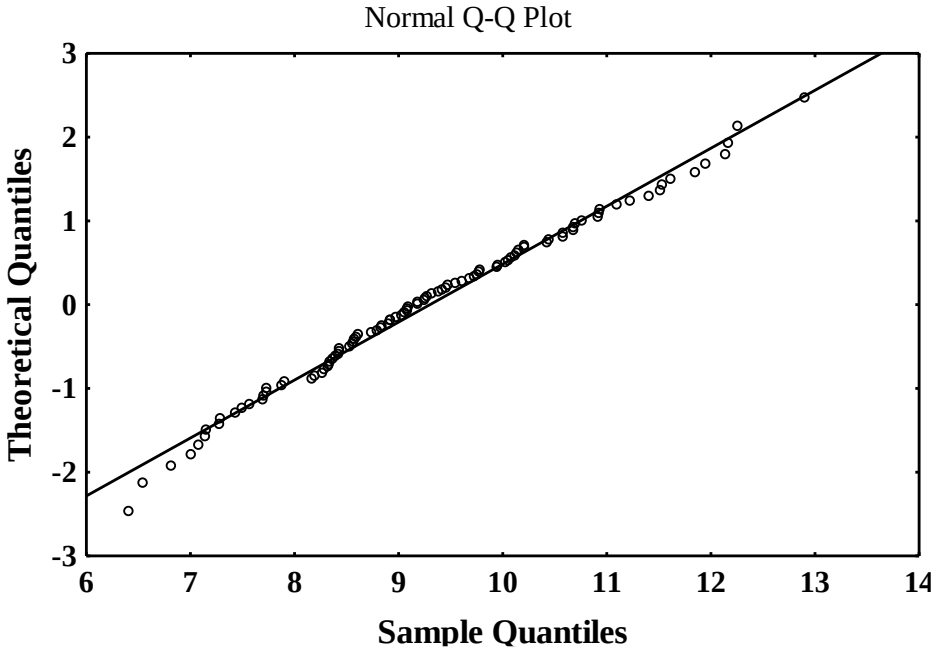

Figure S3.5. Plots to test for normality for detection distances for *Miniopterus natalensis*).

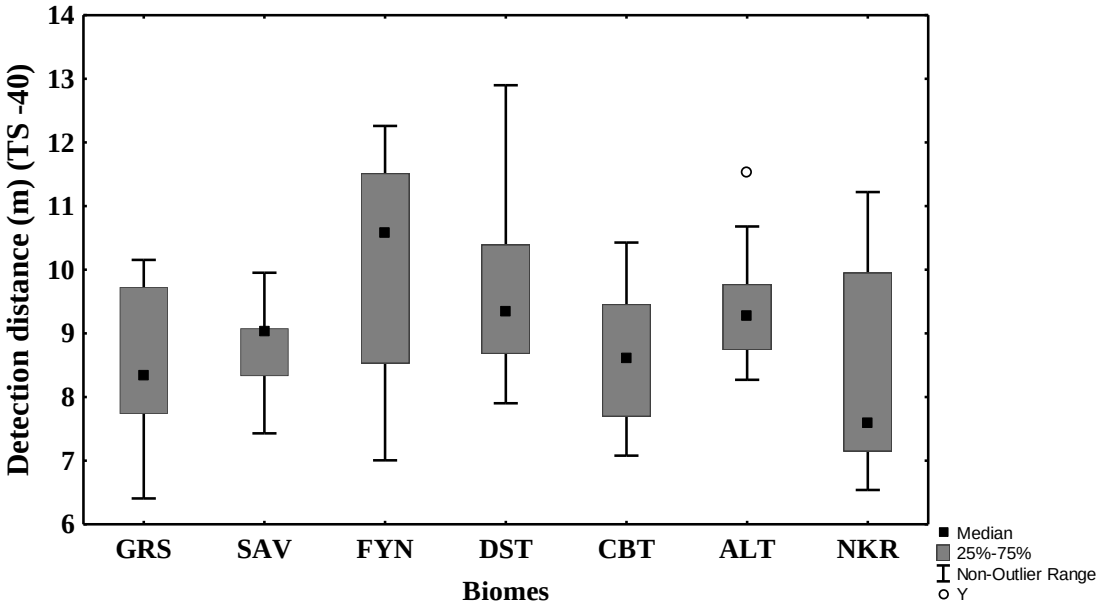

Figure S3.6. Box and whisker plots of detection distance (m) (TS -40 dB) for *Miniopterus natalensis* across seven sites (GRS-Grassland; SAV-Savanna; FYN-Fynbos; DST-Desert; CBT-Coastal Belt; NKR-Nama Karoo; ALT-Albany thicket; ○Y- Outlier).

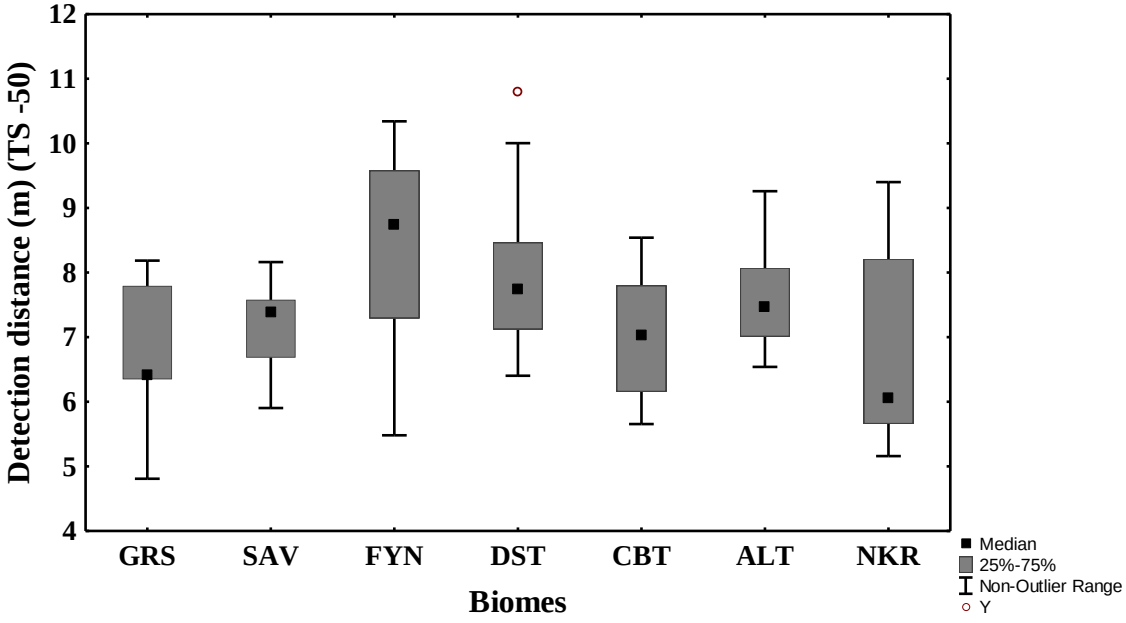

Figure S3.7. Box and whisker plots of detection distance (m) (TS -50 dB) for *Miniopterus natalensis* across seven sites (GRS-Grassland; SAV-Savanna; FYN-Fynbos; DST-Desert; CBT-Coastal Belt; NKR-Nama Karoo; ALT-Albany thicket; ○Y- Outlier).

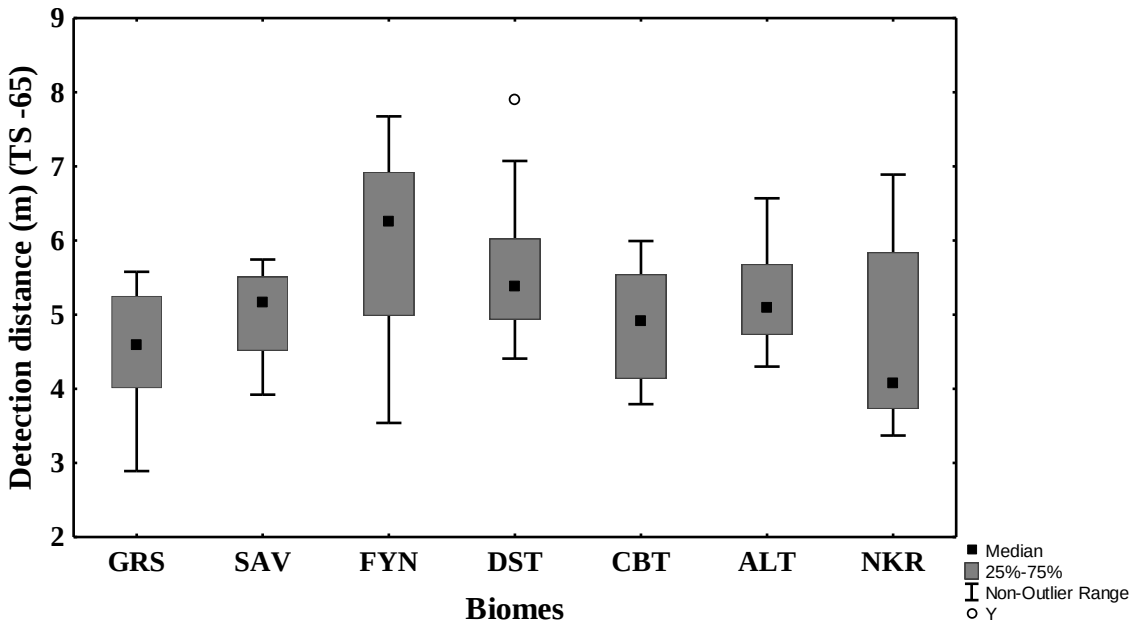

Figure S3.8. Box and whisker plots of detection distance (m) (TS -65 dB) for *Miniopterus natalensis* across seven sites (GRS-Grassland; SAV-Savanna; FYN-Fynbos; DST-Desert; CBT-Coastal Belt; NKR-Nama Karoo; ALT-Albany thicket; ○Y- Outlier).

Table S3.1. Bat assemblages with their echolocation types, recorded in biomes of South Africa.

Species >>>>	***R. ca***	***R. da***	***R. cl***	***R. sm***	***R. sw***	***H. ca***	***M. na***	***M. fr***	***M. tr***	***N. ca***	***T. ae***	***N. th***	***S. pe***	***C. se***
Echolocation type >	FM/CF/FM	FM/CF/FM	FM/CF/FM	FM/CF/FM	FM/CF/FM	FM/CF/FM	FM/QCF	FM/QCF	FM	FM/QCF	FM/QCF	FM	FM/QCF	FM/QCF
Fynbos	✓	X	X	X	X	X	✓	X	✓	✓	✓	X	X	X
Desert	✓	✓	X	X	X	✓	✓	X	X	X	X	X	✓	✓
Albany Thicket	✓	X	X	X	X	X	✓	X	X	✓	X	✓	X	X
Savanna	X	X	✓	X	✓	X	✓	✓	✓	X	X	X	X	X
Coastal Belt	X	X	X	✓	X	X	✓	✓	X	✓	X	X	X	X
Nama Karoo	✓	X	✓	X	X	X	✓	X	X	✓	X	X	X	X

✓ presence of species in a biome; X absence of species in a biome (Mucina & Rutherford 2006)

R. ca =Rhinolophus capensis; R. da = Rhinolophus damarensis; R. cl = Rhinolophus clivosus;R. sm=Rhinolophus simulator; R. sw = Rhinolophus swinny; H. ca = Hipposideros caffer; M. na = Miniopterus natalensis; M. fr = Miniopterus fraterculus; M. tr = Myotis tricolor; T. ae= Tardarida aegyptiaca; N. ca = Neoromicia capensis; N. th = Nycteris thebaica; S. pe = Sauromys petrophilus; C. se = Cistugo seabrae

CHAPTER 4

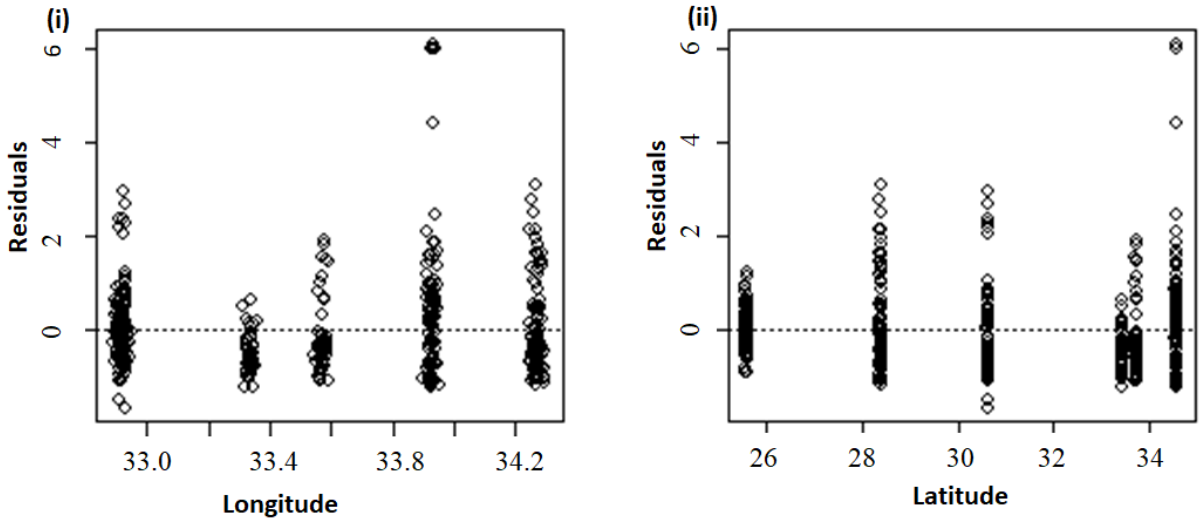

Figure S4.1. Validation graphs demonstrating residuals that estimate a normal distribution without breaching presumption of homogeneity of variance of the detection distances for bat assemblages. The graphs are (i) and (ii) predicted values against residuals explicitly distributed.

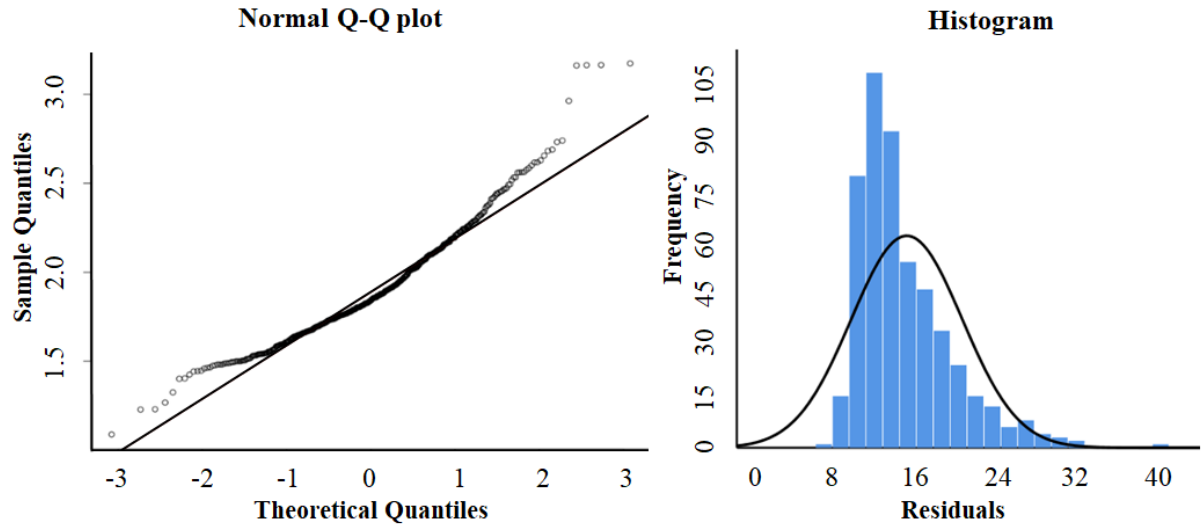

Figure S4.2. Plots to test for normality for detection distance (m) (TS = -50) for bat assemblages.

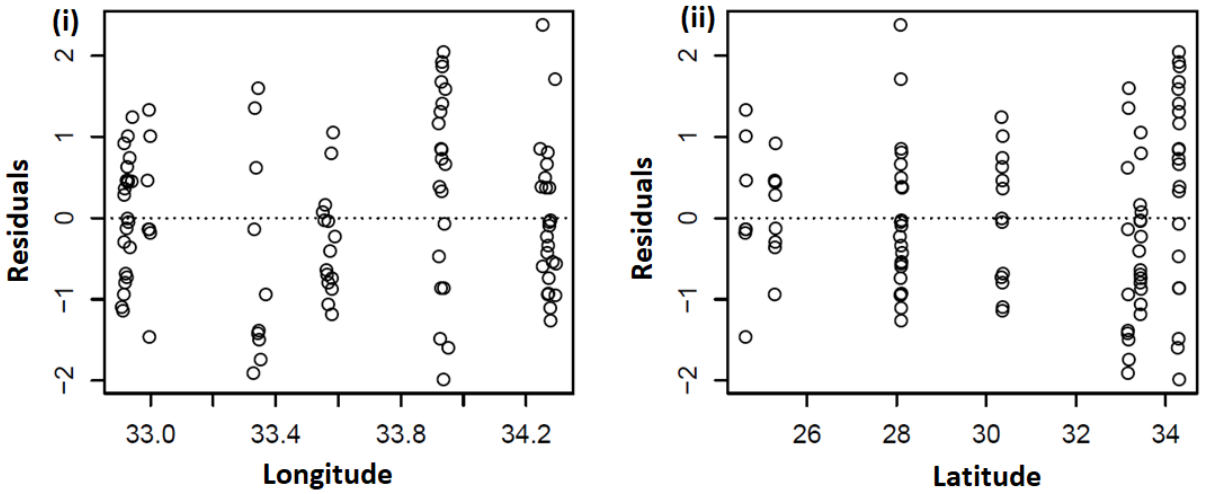

Figure S4.3. Graphs of model validation with residuals close to a normal distribution without violating the assumption of homogeneity of variance of the detection distances for *Miniopterus natalensis*. Graphs (i) and (ii) show predicted values versus residual values that are clearly spread out.

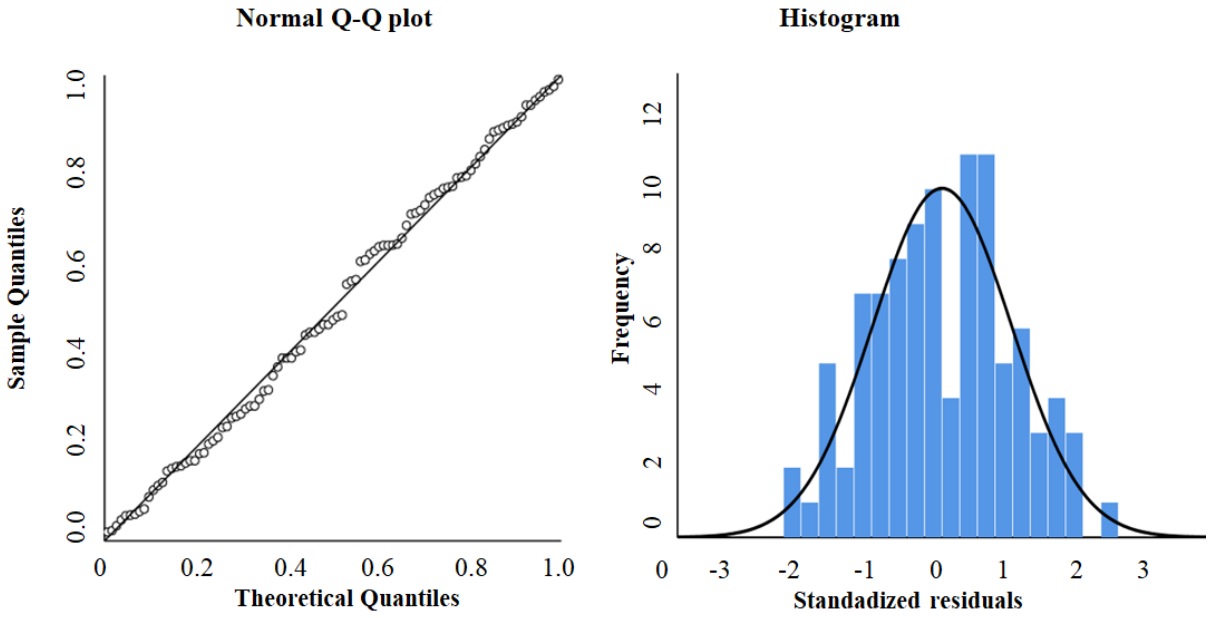

Figure S4.4. Plots to test for normality for detection distance (m) (TS -50) for *Miniopterus natalensis.*

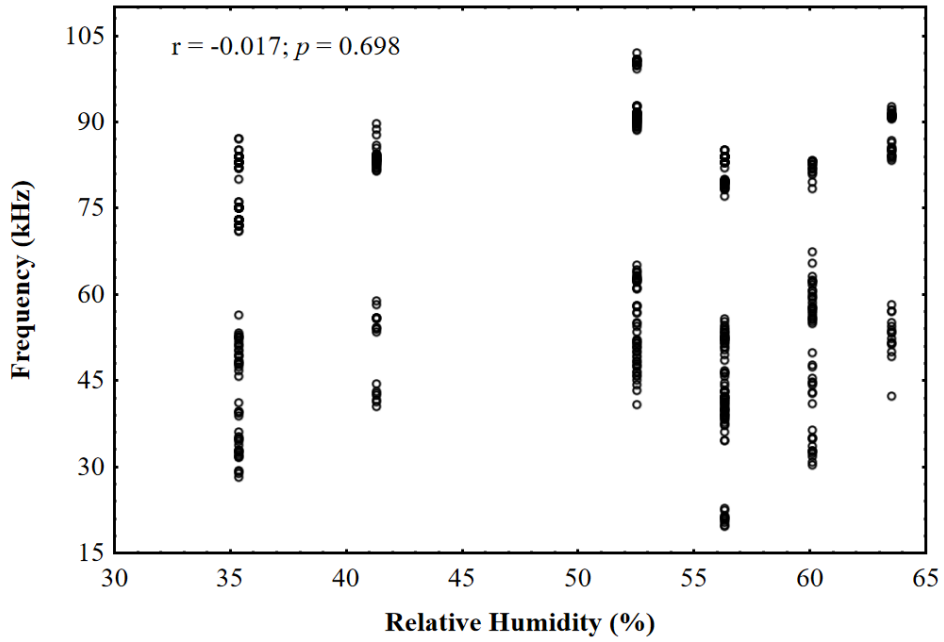

Figure S4.5. Scatter plot to show the association between echolocation pulse frequency for bat assemblages and relative humidity.

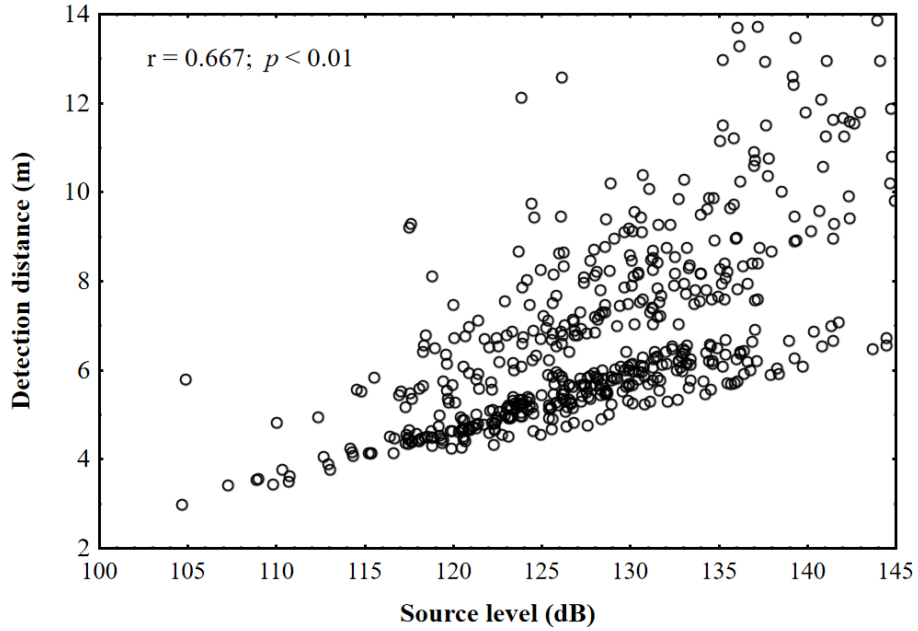

Figure S4.6. Scatter plot to show the association between echolocation pulse source levels and detection distances for bat assemblages across sites.

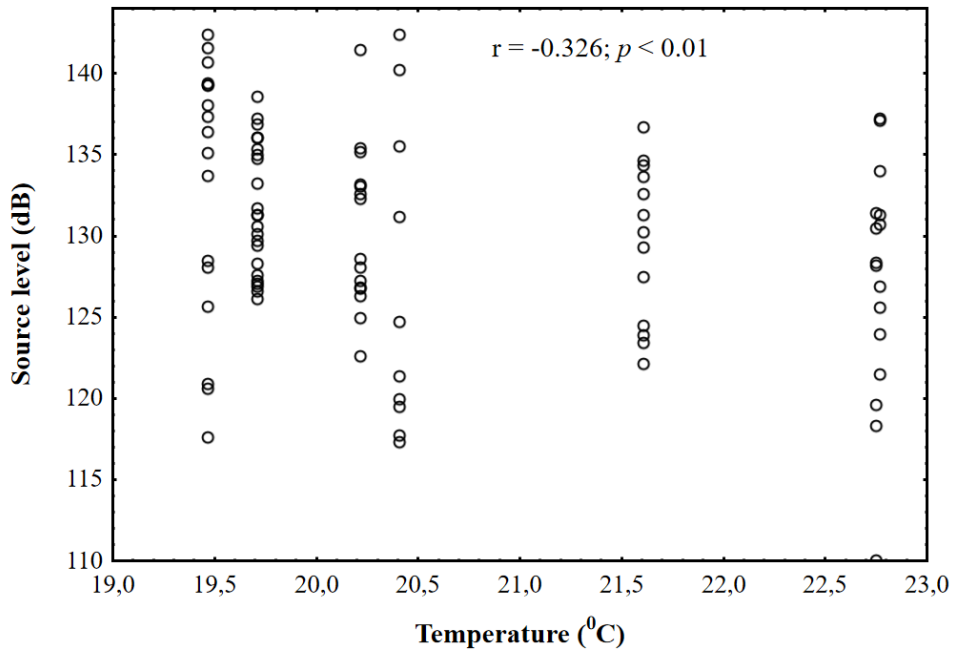

Figure S4.7. Scatter plot to show the association between echolocation pulse frequency for *Miniopterus natalensis* and relative humidity.

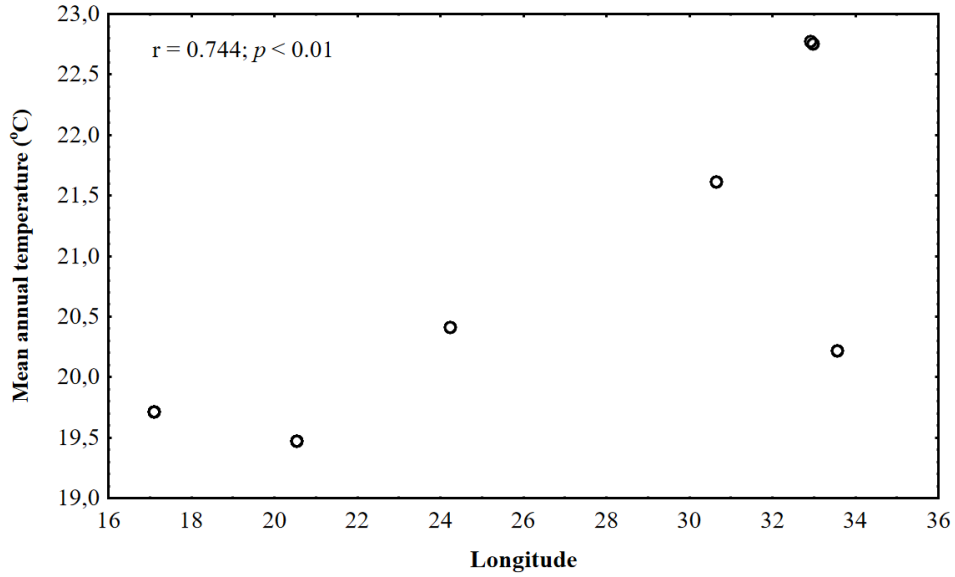

Figure S4.8. Scatter plot to show the association between mean annual temperature and longitude in sites where *Miniopterus natalensis* was recorded.

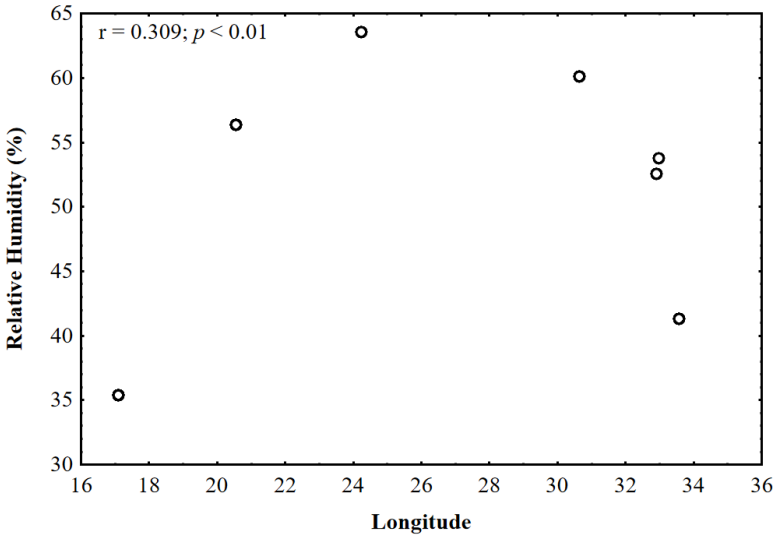

Figure S4.9. Scatter plot to show the association between relative humidity and longitude in sites where *Miniopterus natalensis* was recorded.

Table S4.1. The mean ± SD for pulse frequency (kHz), duration (ms), average of maximum source levels (dB peSPL) of bat species and their detection distances (m) of three prey sizes across six biomes of South Africa.

Biome	Species	FG	FP (n)	Frequency (kHz)	Duration (ms)	Source level (dB peSPL)	Detection distance (m) TS -50
Fynbos	*R. capensis*	C	54	81.5 ± 2.4	39.05 ± 7.2	122.3 ± 4.5	5.3± 0.6
				(77.07-85.0)	(26.4-53.3)	(117.7-133.01)	(4.3-6.1)
	M. natalensis	CE	21	52.9 ± 1.3	4.5 ± 1.3	135.6 ± 8.8	8.4 ± 1.5
				(52.3-55.7)	(2.4-7.03)	(117.6-146.3)	(5.5-10.3)
	M. tricolor	CE/T	11	47.3 ± 3.2	2.4 ± 0.5	127.6 ± 8.6	7.6 ± 1.6
				(39.9-52.7)	(1.6-3.3)	(114.8-146.7)	(5.2-11.2)
	N. capensis	CE	40	40.03 ± 2.3	5.8 ± 1.9	129.9 ± 7.6	8.5 ± 2.0
				(34.6-44.5)	(2.9-9.7)	(118.5-147.8)	(5.1-13.2)
	T. aegyptiaca	OA	11	21.09 ± 0.9	14.9 ± 1.6	134.6 ± 12.7	15.8 ± 6.6
				(19.7-22.7)	(12.2-17.4)	(117.6-148.7)	(5.7-23.4)
Desert	*R. capensis*	C	40	73.6 ± 1.4	43.1 ± 10.5	128.8 ± 4.2	7.0± 0.7
				(71.02-76.2)	(21.2-63.8)	(117.5-134.5)	(5.4-8.0)
	R. damaresis	C	32	83.5 ± 1.3	42.1 ± 9.9	125.04 ± 7.4	6.1 ± 1.0
				(80.02-87.2)	(21.8-59.7)	(116.7-141.4)	(4.1-6.9)
	M. natalensis	CE	23	50.3 ± 2.4	3.6 ± 0.5	132.1 ± 4.7	9.2 ± 1.0
				(45.8-56.3)	(2.7-4.6)	(126.5-144.8)	(7.7-11.9)
	S. petrophilus	OA	8	31.6 ± 2.3	6.01 ± 0.8	129.9 ± 9.6	10.6 ± 3.6
				(28.2-35.3)	(4.7-7.4)	(118.3-146.6)	(5.8-15.7)

	C. seabrae	CE	17	35.01 ± 3.3	6.1 ± 1.3	138.01 ± 5.1	12.4 ± 1.4
				(29.3-41.1)	(4.1-8.7)	(123.7-146.6)	(8.8-15.1)
Albany Thicket	*R. capensis*	C		83.3 ± 0.6	36.8 ± 7.6	125.4 ± 5.7	5.7± 0.4
				(81.9-84.3)	(21.2-48.5)	(118.6-136.8)	(5.0-6.0)
	M. natalensis	CE	15	55.6 ± 1.6	4.5 ± 0.9	126.9 ± 9.6	7.5 ± 0.8
				(53.3-58.8)	(3.3-6.03)	(117.3-142.4)	(6.3-9.1)
	N. capensis	CE	7	42.3 ± 1.1	4.1 ± 0.4	136.8 ± 5.8	9.9 ± 1.2
				(40.5-44.4)	(3.5-4.8)	(128.1-142.7)	(8.1-11.5)
	N. thebaica	G	8	85.6 ± 2.8	1.7 ± 0.5	113.4 ± 3.5	4.0 ± 0.5
				(81.4-89.8)	(1.3-2.9)	(110.7-119.4)	(3.5-4.9)
Savanna	*R. clivosus*	C	43	90.7 ± 1.04	37.1 ± 4.9	126.2 ± 6.6	5.9 ± 0.7
				(88.5-92.8)	(27.8-49.4)	(110.3-140.4)	(3.8-6.9)
	R. swinnyi	C	14	99.8 ± 2.5	42.7 ± 7.4	127.5 ± 8.6	5.3 ± 0.9
				(89.9-101.9)	(21.1-53.1)	(109.9-139.3)	(3.6-6.3)
	M. fraterculus	CE	11	62.7 ± 1.8	2.5 ± 0.7	128.9 ± 4.9	7.1 ± 0.8
				(58.1-65.04)	(1.3-4.02)	(118.1-134.1)	(5.6-8.2)
	M. natalensis	CE	16	57.2 ± 4.04	3.9 ± 1.2	127.1 ± 7.2	6.9 ± 0.9
				(46.3-63.1)	(2.3-6.1)	(110.03-137.2)	(4.8-7.6)
	M. tricolor	CE/T	24	48.8 ± 3.7	2.5 ± 0.8	125.4 ± 6.1	7.2 ± 1.1
				(40.8-56.7)	(1.2-4.3)	(114.5-135.9)	(5.4-8.9)
Coastal Belt	*R. simulator*	C	16	81.9 ± 1.3	40.7 ± 7.7	127.2 ± 9.8	5.3 ± 1.0
				(78.4-83.3)	(20.7-49.8)	(104.6-144.5)	(3.1-7.1)
	M. fraterculus	CE	16	61.4 ± 2.3	3.04 ± 0.8	125.9 ± 4.7	6.1± 0.6
				(57.8-66.3)	(1.9-5.2)	(119.2-135.4)	(5.1-7.6)
	M. natalensis	CE	12	55.2 ± 2.8	3.6 ± 1.2	129.5 ± 4.9	7.2± 0.8
				(47.6-57.6)	(2.1-6.8)	(122.1-136.6)	(5.8-8.1)
	N. capensis	CE	20	37.5 ± 5.5	4.8 ± 2.1	131.1 ± 6.3	9.9 ± 2.3
				(30.2-47.3)	(2.9-9.7)	(118.8-143.4)	(8.1-15.4)
Nama Karoo	*R. clivosus*	C	18	90.2 ± 0.5	47.1 ± 5.8	134.1 ± 7.04	5.6 ± 0.7
				(90.6-92.6)	(30.02-59.8)	(120.5-144.5)	(4.3-6.6)
	R. capensis	C	14	84.8 ± 1.04	37.4 ± 7.6	132.1 ± 3.4	5.8 ± 0.9
				(83.2-86.7)	(21.2-47.9)	(125.5-136.4)	(8.4-11.9)
	M. natalensis	CE	10	52.7 ± 3.7	4.1 ± 0.8	130.3 ± 4.9	7.6 ± 0.8
				(42.2-58.1)	(2.9-5.7)	(122.6-141.4)	(6.5-9.04)
	N. capensis	CE	7	36.5 ± 5.4	4.8 ± 2.04	133.1 ± 5.8	9.2 ± 2.3
				(31.3-46.3)	(2.9-8.9)	(121.9-143.3)	(7.2 -10.6)

OA = open air; C = cluttered; G = gleaner; CE = clutter-edge (Schoeman & Jacobs, 2008); CE/T = clutter-edge/trawler (Moyo & Jacobs, 2020); TS -50 = medium prey; FG = foraging group; FP = flight paths; () = range

Table S4.2. Shows yearly averages of climatic variables for 40 years (1979-2019) from different localities within biomes of South Africa.

Biome	Locality	Lat	Long	Am-Temp	RH	Atm-Pr
Fynbos	DeHoop Nature Reserve	34.29	20.55	19.47	56.34	102.12
Grassland	Sudwala Caves	24.63	32.99	22.75	53.75	102.01
Savanna	Kalkoenkrans Cave	25.29	32.92	22.77	52.53	101.99
Desert	Lekkersing	28.10	17.10	19.71	35.39	101.84
Coastal Belt	Bazley Beach Tunnel	30.43	30.65	21.61	60.12	102.17
Albany Thicket	Table Farm	33.44	26.43	20.22	41.30	102.18
Nama Karoo	Baaviansklоof	33.63	24.24	20.41	63.53	102.16

Abbriviation:Lat, Latitude; Long, Longitude; Am-Temp,Annual mean temperature; Atm-Pr,Atmospheric pressure; RH, Relative Humidity