Re-surveying the insectivorous bats of northern Kruger National Park, South Africa

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Dedication

This thesis is dedicated to my family: John, Robin, and Taylor Brinkley. Thank you for all the love, support, and encouragement that made this opportunity possible.

"Therefore encourage one another and build each other up, just as in fact you are doing."

1 Thessalonians 5:11

Abstract

With over 1,300 known species, the order Chiroptera makes up about 20 percent of all mammalian species. Due to its sub-tropical climate, the northern part of Kruger National Park (KNP) in South Africa is believed to have the highest bat species richness in the country. However, the last comprehensive assessment of bat diversity in the region was conducted more than 30 years ago. In 2017 and 2018, I undertook the first detailed re-assessment of the bat communities of the northern KNP since the early 1980's. I used both live-capture (harp traps and mist-netting) and acoustic technology (SM2 and SM4 Songmeters, Wildlife Acoustics. MA, USA) to sample bats at 24 sites across the northern region of KNP. Through live-capture (336 trapping hours), 155 bats representing 13 species from five families were recorded including *Cloetis pervicali*, which has never been recorded within the borders of the KNP before. The echolocation calls of all captured bats were recorded to develop a site-specific call reference library that was used (in combination with existing reference calls) as a guide for the identification of bat calls recorded using the acoustic detectors set across 24 sites (278 sampling nights). The acoustic monitoring identified 22 species from six families and two unknown sets of calls. Compared to the historical data of 40 documented species (collected over a 30-year period), the current survey (27 species) resulted in a lower species richness. However, this is likely due to the lower overall sampling effort during my survey. By re-surveying the bats of northern KNP, I have contributed towards an overall bat species inventory for this region. In addition, I have generated an important baseline dataset for the future monitoring of bat diversity across the KNP. Due to bats being important biological indicators, increased research on the various species and their behaviours is essential for improving our understanding of climate change effects as well as the overall health of the environment, especially in protected areas.

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TABLE OF CONTENTS

Chapter 1: General introduction

With fossils dating back over 50 million years, bats have evolved into one of the most specious mammalian orders (Simmons et al. 2008). With over 1,300 known species, the order Chiroptera makes up about 20 percent of all mammals (Flaquer et al. 2007, Russo et al. 2018). Bats are one of the few mammalian orders with the ability to echolocate and the only order that can fly (Schnitzler & Kalko 2001, Estrada & Coates-estrada 2002, Thomas et al. 2004). Through the power of flight, and the ability to echolocate, bats dominate the night skies (Simmons et al. 2008). This has led to increased lifespans, averaging about 15 years (Monadjem et al. 2010). The combination of flying and echolocation has also resulted in bats being able to adapt to a large diversity of vegetation communities ranging from woodlands, caves, floodplains, and even urban areas (Jones & Teeling 2006, Russo & Ancillotto 2015). Bats have been documented globally except for Antarctica, the Artic, and a few oceanic islands. Species richness is the highest in neotropical areas due to increased resources of food, water sources and warmer climates (Mickleburgh et al. 2002, McCain 2007).

1.1 Biology and behaviour of bats

Depending on the type of vegetation structure and foraging strategy, wing characteristics and flight patterns in bats vary among families and even species (Norberg & Rayner 1987). Open-air foragers usually hunt above the tree canopy and must be able to fly quickly to catch prey in mid-air (Fullard et al. 1991). Most open-air foragers (Molossidae and Emballonuridae) have long, narrow wings that are capable of fast, agile flying (Norberg & Rayner 1987, Monadjem et al. 2010). The families Hipposideridae, Nycteridae and Rhinolophidae are known as clutter foragers that hunt for prey within dense vegetation (Aldridge & Rautenbach 1987,

Monadjem et al. 2009). They are adapted to quickly avoid collisions within clutter habitats and have short, broad wings (Monadjem et al. 2009, 2010). Having wings with low aspect ratio allows for slow flight and the ability to manoeuvre around obstacles (Fullard et al. 1991). Clutter-edge foragers are bats that fly in-between dense vegetation habitats and open-air spaces (Lee & McCracken 2004). The families Vespertilionidae and Miniopteridae are commonly known to be clutter-edge foragers and must be able to fly in both clutter and open spaces (Lee & McCracken 2004). There is a great diversity in wing morphology between clutter-edge species but most bats have wings with round tips that are intermediate in size (Norberg & Rayner 1987). With intermediate sized wings, bats are able to fly fairly quickly and manoeuvre around obstacles (Monadjem et al. 2010).

As a group, bats feed on a diverse range of food including fruit, small amphibians, fish, blood, insects, and even other bats (Kunz et al. 2011). Fruit bats (from the family Pteropodidae) feed on a variety of plant-based foods including fruits, seeds, nectar, and leaves (Fujita 1991). They are pollinators for many plant species belonging to the families Myrtaceae (59 species), Leguminosae (12 species), Bignoneaceae (nine species), and Proteaceae (six species); these species are almost completely reliant on fruit bats for pollination (Fujita 1991, Mickleburgh et al. 1992). By comparison, insectivorous bats make up approximately 70 percent of bat species worldwide and hunt for prey through the use of echolocation (Monadjem et al. 2010). Insectivorous bats feed on a large selection of insects ranging from small insects like mosquitoes (family Culicidae) to large beetles (Order Coleoptera) and moths (Order Lepidoptera) (Freeman 1979, Champ & Highley 1985, Wilkerson et al. 2015, Wray et al. 2018). Some bats are selective while others are generalist predators and feed on a wide diversity of insects (Kasso & Balakrishnan 2013). The type of prey that a bat hunts is dependent on their jaw shape and

strength (Freeman 1979). Stronger jaws with longer teeth are capable of eating insects with hard shells, while weaker jaws with shorter teeth are only able to eat insects with soft bodies such as mosquitoes (Freeman 1979).

Echolocation is the transmitting of sonar signals into the environment and the analysing of the returning echoes to navigate around objects and to find food (Schnitzler & Kalko 2001, Thomas et al. 2004). Echolocation calls are generated in the larynx and emitted through either the mouth (the families Vespertilionidae, Emballonuridae, and Molossidae) or nose (the families Rhinolophidae, Hipposideridae, and Nycteridae) of the bat (Monadjem et al. 2010, Elemans et al. 2011). Echolocating bats use a wide range of species-specific frequencies and durations (Schnitzler & Kalko 2001). There are two types of echolocation behaviours; low duty-cycles which are used by the majority of echolocating bats, and high duty-cycles which are only used by a few families, such as Rhinolophidae and Hipposideridae (Jones & Teeling 2006, Monadjem et al. 2010). Low duty-cycles are short to intermediate duration calls that are followed by longer breaks between pulses (Jones & Teeling 2006, Elemans et al. 2011). During the break, the bat is able to receive the returning echo, process the call, and characterize the surrounding environment (Elemans et al. 2011).

Depending on species, low duty-cycle calls can either have broad or narrow bands (Jones & Teeling 2006). When a bat emits a low duty-cycle call, the frequency of the call sweeps downward, changing the frequency in the process (Hiryu et al. 2010). Broadbands reach as many frequencies as possible during a short duration (2 to 5 ms) and are commonly used by the families Vespertilionidae and Miniopteridae (Monadjem et al. 2010). Calls are usually intermediate to high frequencies (30 to 75 kHz), used by clutter-edge bats that forage for prey in both dense vegetation and open spaces (Russo et al. 2018). Species within these families are able

to shift their echolocation calls over a range of frequencies to best suit their needs when hunting (Parsons & Jones 2000). Narrow bandwidth calls cover fewer frequencies compared to broadband calls and typically have short or long durations (2 to 11 ms) (Russo et al. 2018). Narrow band calls are commonly used by the families Molossidae and Emballonuridae (Monadjem et al. 2010). Calls are usually low in frequency (8 to 30 kHz) and are generally used by open-air foragers (Russo et al. 2018). Low frequency calls are best for open-air foragers because the calls do not attenuate quickly when traveling through space and are capable of reaching distances of up to 30 meters (Monadjem et al. 2017).

High duty-cycle calls have intermediate to long duration (10 to >100 ms) and, unlike low duty-cycle calls, do not sweep downward and thus have a constant frequency through the pulse (Jones & Teeling 2006). When approaching prey, some bats also employ Doppler-shift, where calls are not separated by breaks between pulses but rather by subtle changes in frequency, where one pulse is a slightly different frequency than the returning echo (Fenton 2003, Monadjem et al. 2010). High duty-cycle calls are common in families (Hipposideridae and Rhinolophidae) that forage within dense vegetation (Parsons & Jones 2000). With little breaks in-between pulses and at intermediate to high frequencies (40 to >200 kHz), bats foraging in dense vegetation are able to quickly distinguish between prey and obstacles (Parsons & Jones 2000).

Being nocturnal, bats must find protective roosts to sleep in during the day (Kasso & Balakrishnan 2013). Bats are known to roost within many different vegetation types and microclimates (Monadjem et al. 2010). The use of a roost can also provide social structure for many communities and can include hundreds of individual bats (Bouchard 2001). Moreover, females will often form maternity roosts to keep their young protected while they search for food during the night (Lausen & Barclay 2006). Foliage-roosting bats use many parts of trees for

roosting including hanging from branches while using clumps of leaves to hide in, clinging inside a hollow part of the tree trunk or under loose bark, inside abandoned bird nests, and hanging from exposed roots (Bernard & Fenton 2003, Willis & Brigham 2004, Carter & Feldhamer 2005). Hollow-roosting bats are known to roost in hollow places such as caves or man-made structures like bridges and buildings (Bernard & Fenton 2003, Lausen & Barclay 2006). Bats roosting in buildings are found in attics, basements, gaps under floors, and hollows within the wall (DePaepe et al. 1996, Lausen & Barclay 2006). Caves are known to be occupied by a large variety of bat species as well as large abundances of individual bats numbering in the thousands and in few situations, even millions (Iskali & Zhang 2015). Caves provide many forms of microclimates varying in temperature, humidity, and the amount of light (Monadjem et al. 2010, Klüg-Baerwald et al. 2017). While certain species are very specific in their roost selection, such as *Rhinolophus swinnyi* which roosts in caves, other species are capable of roosting in multiple habitats, such as *Taphozous mauritianus* which roosts in both trees and buildings (Fenton 1992, Monadjem et al. 2009).

1.2 Importance of bats

Bats are often viewed as unwanted pests, which can lead to many human-bat conflicts, especially when bats use man-made structures as roosts (Kunz et al. 2011, Cooper-Bohannon et al. 2016). Bats are usually feared because of their ability to transmit diseases such as rabies, but only ~1 percent of bats contract rabies and are unlikely to pass diseases on to humans (DePaepe et al. 1996, Kunz et al. 2011). Contrary to popular myths, bats are shy and non-aggressive animals that prefer to stay away from humans (DePaepe et al. 1996). Even though bats are commonly viewed as unwanted, they provide many important ecological and economic benefits for both humans and the environment (Iskali & Zhang 2015).

No matter what type of prey, bats are capable of consuming on average about 1.5 times their body weight within one night (Estrada et al. 1993). While lactating, females are known to increase their consumption for the extra energy needed to feed their young (Kasso & Balakrishnan 2013). Due to the amount of prey consumed within one night, bats have proven to be vital in controlling insect pests (Iskali & Zhang 2015). When bat colonies are stationed near agricultural farms, crops have less damage by insect pests compared to farms without bat colonies present (Jones et al. 2009). The control of unwanted insect pests can thus provide important economic benefits for farmers (Iskali & Zhang 2015).

Bats are sensitive to changes in climate, temperature, resource abundance, and habitat change which makes them valuable indicators for monitoring environmental health (Russo & Ancillotto 2015). Increases or decreases in bat populations can indicate loss of vegetation communities, pollution, diseases, changes in the availability of water sources, and climate change (Stahlschmidt & Brühl 2012). Due to their roosting behaviours and foraging habits, many bat species are dependent on certain vegetation communities (Mickleburgh et al. 2002). *Rhinolophus fumigatus*, for example, is restricted to riparian forests (Monadjem & Reside 2008). Declines in this species within a particular area could therefore indicate a loss of trees that are used as roosts or declines in available prey (Monadjem & Reside 2008). The abundance of bats around riparian habitats can also indicate the quality in water sources (McCain 2007). Bats rely on water sources to drink and to hunt for prey above the surface (Downs & Racey 2006, Sherwin et al. 2013). Declines in water quality can result in lower abundances of prey and cause decreases in bat activity (Jones et al. 2009). Streams contaminated from pollution can therefore be identified when bat activity declines (Jones et al. 2009).

The monitoring of bat's hibernation periods (also known as torpor), reproductive cycles, as well as changes in distribution can also be indicators for climate change (Jones et al. 2009, Sherwin et al. 2013). Torpor is the state of lowering the body metabolism to spend less energy both on a daily or seasonal bases (Audet & Fenton 1988). Seasonal periods of torpor and reproduction are closely linked in many bat species (Johnson et al. 2017). Mating usually occurs before entering torpor and females are able to delay implantation or halt embryonic development until conditions become favourable (van der Merwe & Rautenbach 1990). As temperatures rise due to climate change, seasonal torpor periods can decrease causing bats to be active longer during the year and the birth of pups to occur sooner (Sherwin et al. 2013). The monitoring of torpor periods and the timing of births could give insight into the effects of climate change on various species of bats (Sherwin et al. 2013). Changes in species distributions could also indicate changes in temperature (Lundy et al. 2010). Rising temperatures could cause a northward shift in bat distributions as temperate regions become better suited habitats for many tropical bat species (Lundy et al. 2010). Increased shifts in species distributions could be caused by increases in insect abundance, more water being available, and warmer temperatures that require less torpor periods (Lundy et al. 2010). On the other hand, regions that experience both increases in temperature and decreases in rainfall could result in a decline in bat species richness due to habitats becoming unsuitable (Sherwin et al. 2013).

1.3 Global concern and sampling of bats

Globally, about 66 percent of bat species are listed as least concern by the International Union for Conservation of Nature (IUCN) red list (Monadjem et al. 2010). About 17 percent of species are listed as vulnerable, four percent listed as endangered, and three percent listed as critically endangered (Monadjem et al. 2010). Even though a small percentage of bats species are

listed as endangered or critically endangered, bat species are facing declines in populations due to major threats (Mickleburgh et al. 2002). One major threat is habitat loss due to logging activities in forests where many bat species are dependent on the trees to use as roosts and forage for food (Braun de Torrez et al. 2018). Seventy percent of bats species feed on insects and the use of pesticides can greatly reduce the amount of available food (Kunz et al. 2011). Illegal hunting for bushmeat of larger bat species has been shown to have negative impacts on bat populations as thousands of bats, mostly species from the genus *Pteropus* and *Acerodon*, are captured and sold as food (Mickleburgh et al. 2002, Kunz et al. 2011). Conservation efforts to help preserve bat species has been difficult due to a lack of information (Mickleburgh et al. 2002). Knowledge of roost selection, echolocation calls, distribution ranges, and statuses is limited due to lack of research (Gelderblom et al. 1995, Mickleburgh et al. 2002, Russo et al. 2004). Even though many regions worldwide still lack distributional data, a few areas (Europe and North America) have been successful in mapping bat species distribution and statuses (Fukui et al. 2001). Through increasing knowledge of bat species, conservation effects can continue to expand (Mickleburgh et al. 2002).

In order to increase the knowledge of bats, surveys must be conducted to gain insight into bat communities, foraging behaviours, and roost locations (Mickleburgh et al. 2002). Live-capture and acoustic technology are the two main methods used to study bats (O'Farrell & Gannon 1999, Adams et al. 2012). Traps used in live-capture (mist nets and harp traps) offer many benefits when conducting bat surveys (Francis 1989). Traps provide an opportunity to handle bats for the collection of body measurements (i.e. mass, forearm length, and head size), faeces for dietary studies, overall descriptions, and echolocation calls that is used for acoustic surveys (Francis 1989, Fukui et al. 2001, Berry et al. 2004). Studies have shown that harp traps

are more effective in capturing small (<15g) to medium (15-25g) sized bats compared to mist nets that are more likely to capture medium to large (>25g) bats (Francis 1989). Mist nets are useful for trapping in slightly more open areas to cover more surface area but they must be monitored during the time of use (Taylor et al. 2013). Due to needing less space, harp traps are useful when trapping in dense vegetation and do not require constant monitoring overnight (Fukui et al. 2001, Taylor et al. 2013).

The use of acoustic bat detectors to record echolocation calls can improve our understanding of bat behaviours, such as hunting and activity levels, distribution patterns, and species richness within a particular study area (Adams et al. 2012). Acoustic methods have become a common and powerful technique in surveying bat communities due to their non-invasiveness that can collect large quantities of data over short periods of time (Adams et al. 2012, Skalak et al. 2012). Bat detectors are also beneficial in the detection of species that are difficult to live-capture due to the ability of some species to fly out of reach or detect the trap and quickly avoid capture (Berry et al. 2004). Consequently, the use of acoustic detectors usually results in higher species richness estimates compared to live-capture methods (O'Farrell & Gannon 1999).

1.4 Motivation and broad aims

The Kruger National Park (KNP) is located in the north-eastern corner of South Africa and borders Mozambique and Zimbabwe (Eckhardt et al. 2000). Due to its sub-tropical climate, the northern KNP is predicted to have the highest bat species richness in South Africa (Rautenbach et al. 1985, Venter & Gertenbach 1986). In northern KNP, research was conducted in 1979, 1982, 1983, 1984, and 1985 to determine the number of species occurring in the region (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987). In 1985, one QMC S-200 bat

detector (Ultrasound Advice, London, United Kingdom) was used to record release calls from 16 bat species of five different families but no comprehensive acoustic methods were used in the assessment of KNP bat communities historically (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987). Additionally, between 1960 and 1990 some bat specimens were collected from KNP but no complete assessment was conducted (Monadjem et al. 2010). Since the last comprehensive assessment in KNP, bat diversity in the region has been under studied (Monadjem & Reside 2008).

Constant monitoring of bat communities provides important insight into the environmental health (Fukui et al. 2001, Mickleburgh et al. 2002). Unfortunately, due to their nocturnal behaviour, use of multiple (difficult to access) roosts, and their variation in flight patterns, bats can be difficult to survey but are often overlooked in diversity studies (Adams et al. 2012, Berry et al. 2004, Gelderblom et al. 1995, Monadjem & Reside 2008). In 2017 and 2018, through the use of live-capture and acoustic technology, I was able to re-survey the insectivorous bats of northern KNP. Through this study I aimed to (i) contribute towards the inventory of bat species found within northern KNP, (ii) compare changes in bat diversity with historical data, (iii) develop a site-specific echolocation release call library to be used for current and future acoustic surveys and (iv) generate baseline data to be used for long-term monitoring of bats within KNP.

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Chapter 2: Study area

2.1 Kruger National Park

The Kruger National Park (KNP) is located in the north-eastern corner of South Africa and covers both the Mpumalanga and Limpopo provinces (Eckhardt et al. 2000). The park borders Mozambique and Zimbabwe (Du Toit et al. 2003). KNP was originally proclaimed as the Sabie Game Reserve between the Sabie and Crocodile rivers in 1902 (Du Toit et al. 2003). In 1903, an additional game reserve, the Shingwedzi Game Reserve, was proclaimed between the Letaba and Limpopo rivers (SANParks 2018). In 1916, the two game reserves were consolidated into one park (Pienaar 1969). The National Parks Act of 1926 proclaimed the consolidated land as South Africa's first national park and it was named after South Africa's first president, Paul Kruger (SANParks 2018). KNP is currently 1,918,140 ha, 350 km long north to south and 60 km wide from west to east (Figure 2.3) (Roux et al. 2008).

KNP protects some of Africa's most endangered plant and animal species (Gelderblom et al. 1995). KNP is also well-known for its diversity in species and is considered an important biodiversity hotspot (Gelderblom et al. 1995). Significantly for my study, about twenty percent of the mammalian diversity in the KNP comprises insectivorous bats with 40 species historically documented in the northern regions of the park (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987, Mickleburgh et al. 2002, Monadjem et al. 2010, Adams et al. 2015).

Biological Regions

KNP is located within the Savanna Biome (Du Toit et al. 2003). The Savanna Biome is the largest and most widespread biome in Africa and is located mostly in the north-eastern parts of South Africa (Mucina & Rutherford 2006). The Savanna Biome is located primarily in the southern countries of Africa and is generally subtropical to tropical in climate (Staver et al.

2011). Due to the influences of the Atlantic and Indian Oceans, the biome has many microclimatic regions with seasonal precipitation periods (Mucina & Rutherford 2006). The biome occurs at low level altitudes, mostly below 1,500 m (Mucina & Rutherford 2006). It has been separated into six biological regions, Central Bushveld, Mopane, Lowveld, Sub-Escarpment Savanna, Eastern Kalahari Bushveld, and Kalahari Duneveld (Mucina & Rutherford 2006). All of KNP is covered by the Savanna Biome (Du Toit et al. 2003). There is a distinct difference in the vegetation of the northern region (north of the Olifants River) of the park compared with the southern region (Codron et al. 2007). The northern region is dominated by broad-leaved trees while the southern region is dominated by fine-leaved trees (Codron et al. 2007). Broad-leaved trees are found in nutrient poor soils that originated from granite or sandstone, mostly found in the arid regions in northern KNP (Du Toit et al. 2003). The southern region has moister and more nutrient-rich clay soil, causing fine-leaved vegetation growth (Du Toit et al. 2003). The research described below was conducted within the northern region of KNP. Northern KNP is made up of two biological regions; Lowveld and Mopane (Mucina & Rutherford 2006).

The Mopane biological region is the smallest of the Savanna Biome and is found at low altitudes (Mucina & Rutherford 2006). It is positioned in the Limpopo Province, north of the Soutpansberg Mountains, in a semi-arid to arid area (Mashabane et al. 2001). The Mopane region has an average rainfall of about 527 mm with the rainy season being between October and April (Rutherford et al. 2012). The bioregion is dominated by mixture of Bushveld, Mopaneveld, and Shrubland (Du Toit et al. 2003, Mucina & Rutherford 2006). Bushveld is made up of mostly *Colophospermum mopane* and *Combretum apiculatum* growing within a mixture of clay and sandy soils (Mucina & Rutherford 2006). Mopaneveld is a medium to high shrub savanna dominated mostly of *C. mopane* located on a mixture of clay, sand, and gravel soil (Mucina & Rutherford 2006). Shrubland is the smallest area of the bioregion with mostly grassy plains and

scattered medium to low *C. mopane* shrubs positioned in high clay soils (Mucina & Rutherford 2006). The Mopane bioregion is well conserved within protected reserves but outside these areas, it is under threat due to clearing for agriculture and the use of the wood (Mashabane et al. 2001, Rutherford et al. 2012).

The Lowveld biological region is located throughout most of KNP (Kleynhans et al. 2005). This ecoregion is a hot and dry region with a moderate annual precipitation (Kleynhans et al. 2005). The Lowveld is one of the two Savanna bioregions with the highest mean annual precipitation of 580 to 800 mm (Wessels et al. 2011). Mean annual temperature is 16^{o}C to 22^{o}C and has an altitude of 0 to 700 meters (Kleynhans et al. 2005, Wessels et al. 2011). This ecoregion's terrain consists of mostly plains with patches of hills and mountains (Kleynhans et al. 2005). Dominant vegetation types are Bushveld and Lowveld (Mucina & Rutherford 2006). Bushveld consists of mostly tall shrubs to tree savannas of *Terminalia sericea* and *C. apiculatum* growing in deep sandy soils (Mucina & Rutherford 2006). Lowveld is dominated by tall shrubs of *Acacia nigrescens* and *Dichrostachys cinerea* located on sandy soils (Mucina & Rutherford 2006). Portions of the Lowveld biological region are conserved through the protection of KNP, but outside the park it is vulnerable to ranching, rural agriculture, and urban expansion (Wessels et al. 2011).

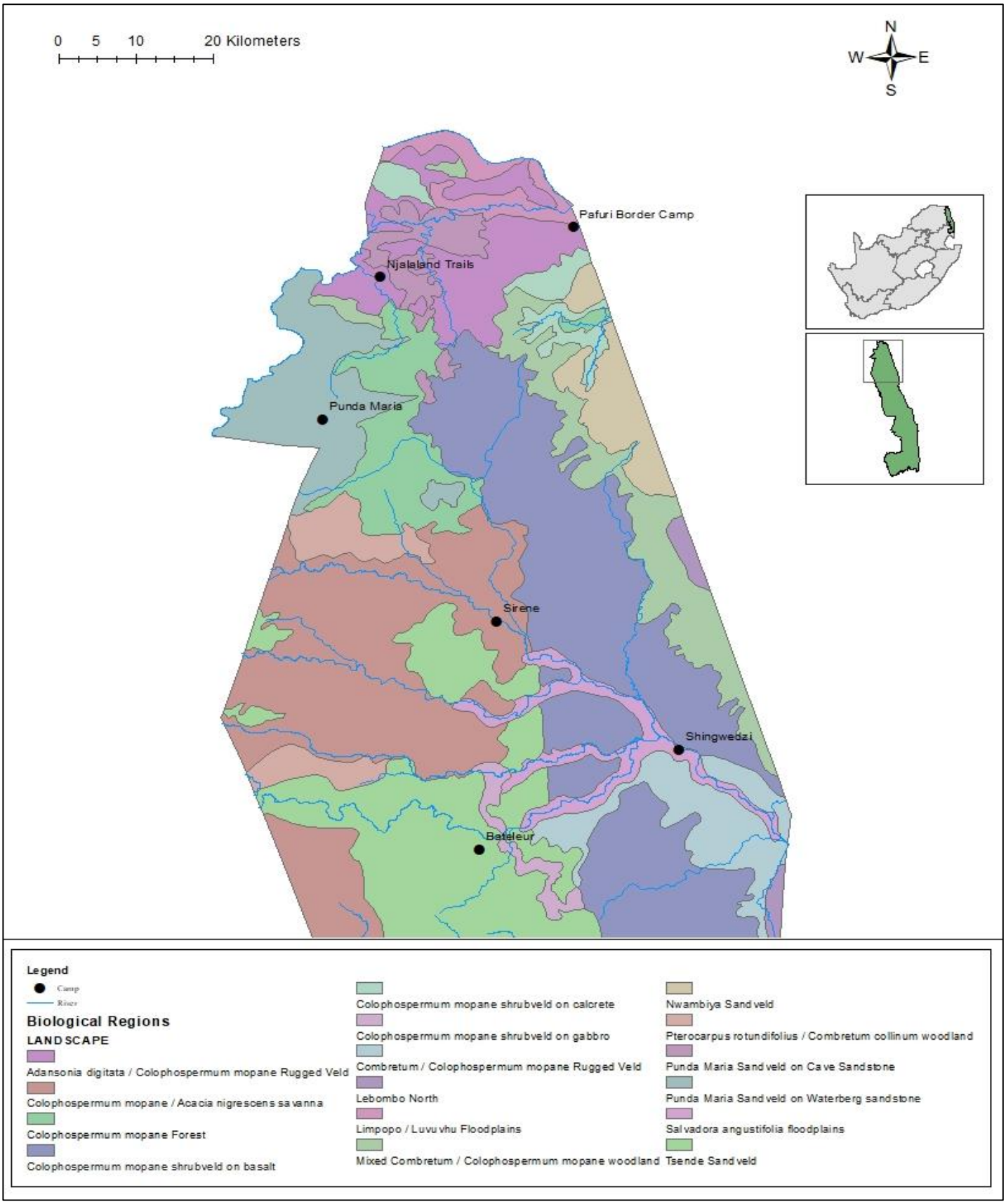

Figure 2.1: Landscape map of northern Kruger National Park displaying vegetation units present within the study area. Each polygon represents a dominated vegetation type. The black dots represent camps located in northern Kruger National Park.

Climate

Northern KNP is located in a subtropical climate (SANParks 2018). The region experiences hot, humid summers and mild, frost-free winters (Wessels et al. 2011, Tshiala et al. 2011). The average temperature in the summer has a mean of 27°C with an average high of 32°C and an average minimum of 19°C (Figure 2.2) (Venter & Gertenbach 1986, Tshiala et al. 2011). The winter season is mild with a mean of 20°C, an average maximum of 26°C and an average minimum of 12°C (Figure 2.2) (Venter & Gertenbach 1986, Tshiala et al. 2011).

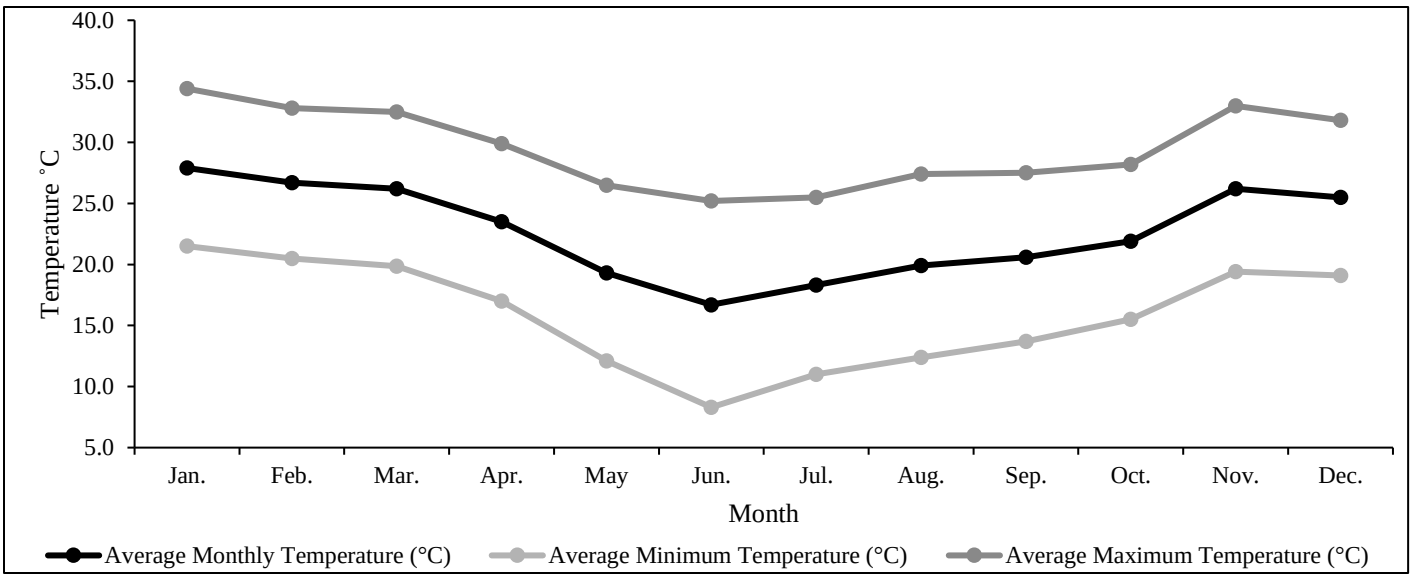

Figure 2.2:Average temperature (°C) of northern Kruger National Park with average minimum and maximum temperature (°C) (Venter & Gertenbach 1986). Dark grey represents the average maximum temperatures, light grey represents the average minimum temperatures, and black represents the average monthly temperatures.

The rainy season is during the summer from October to April with an average annual precipitation of approximately 500 mm (Tshiala et al. 2011). May to September is the winter and dry season with an average of about 40 mm of precipitation (Venter & Gertenbach 1986). The average annual rainfall in the north varies between 300 to 500 mm compared to the region south of the Olifants River with an annual precipitation of 500 to 700 mm (Figure 2.3) (MacFadyen et al. 2018).

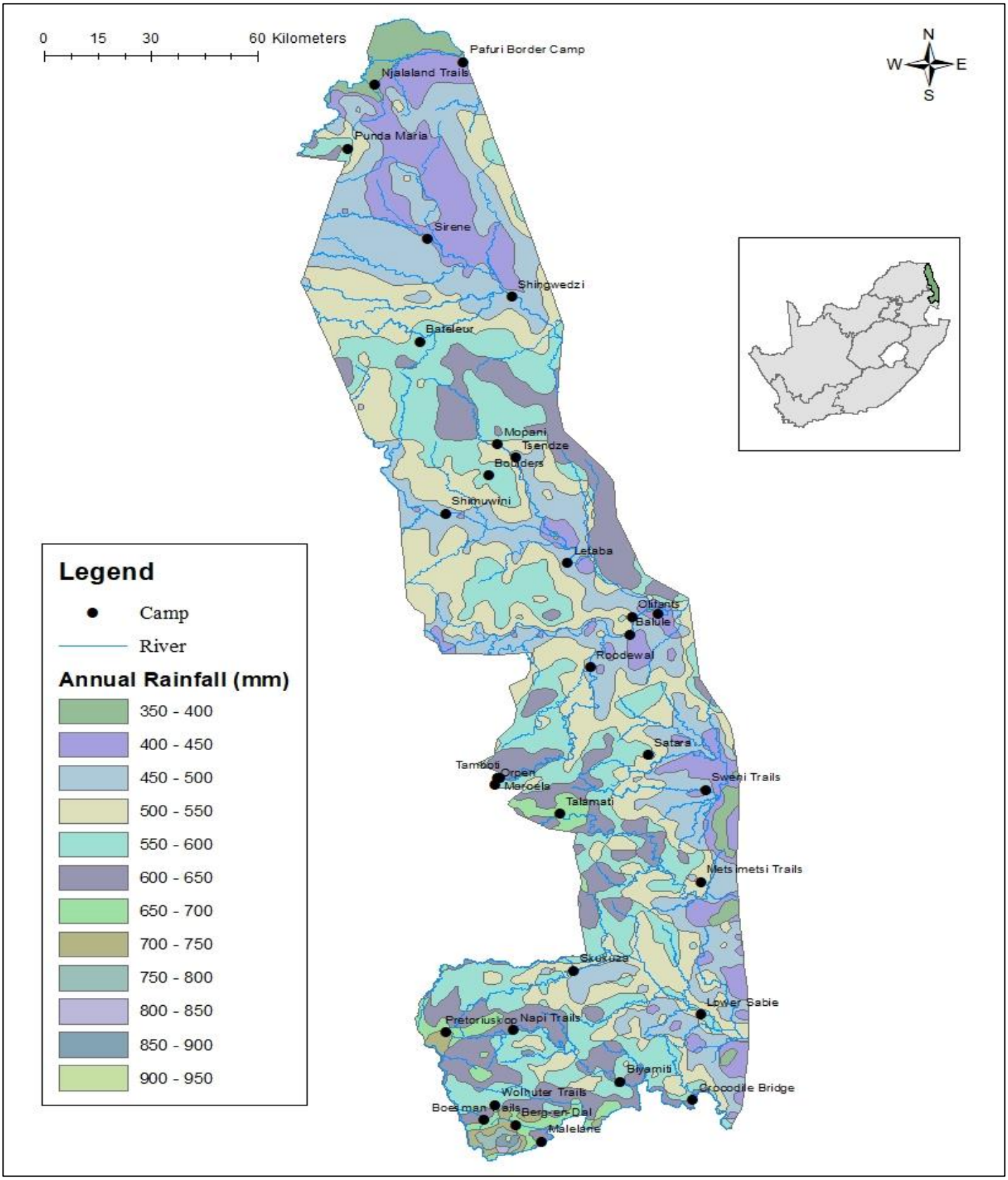

Figure 2.3: Average annual rainfall (mm) within Kruger National Park. Each polygon represents the amount of rainfall (mm) within each area. Black dots represent camps.

Rivers

Northern KNP has two major perennial rivers, the Limpopo River and the Luvuvhu River, and one major seasonal river, the Shingwedzi River (Roux et al. 2008). These rivers run through the park and supply water for the region as well as provide nutrients for the soil (Roux et al. 2008). These rivers were formed from mountainous terrain outside the park and all flow in an eastward direction (Roux et al. 2008).

The Limpopo River flows along the northern border of KNP and separates South Africa from Mozambique, Botswana, and Zimbabwe (Ashton et al. 2001). The river has numerous tributaries, including the Crocodile, Luvuvhu, and Sand rivers (Jacobsen & Kleynhans 1993). The sediment is mostly sand and mud, providing nutrients to the many trees, shrubs, and grasses that grow along the banks of the river (Jacobsen & Kleynhans 1993). Due to increasing agriculture and mining outside of KNP, there has been an increase in sediment into the river causing major impacts on aquatic diversity (Ashton et al. 2001).

The Luvuvhu River is located in the north-eastern part of the Limpopo Province (Onyari & Ilunga 2013). Although only about 34 percent of the river length is located within KNP, 61 percent of the river's catchment can be found within the park (Pollard et al. 2011). The river flows through South Africa then joins the Limpopo River at an area known as Crook's Corner located at the three-way border between South Africa, Zimbabwe, and Mozambique (Onyari & Ilunga 2013). The Luvuvhu River supports many wildlife and vegetation species by providing resources to the Makuleke Wetland (SANParks 2018). The Makuleke Wetland is made up of riparian floodplain forest, floodplain grassland, and riverine forest (SANParks 2018).

The Shingwedzi River flows through northern South Africa into Mozambique (Swanepoel et al. 2000). Three major tributaries, the Phugwane River, the Mphongolo River, and

the Shisha River, flow into the Shingwedzi River within KNP (Ashton et al. 2001). It is a seasonal river that regularly flows during the rainy season but during the dry season there is often no surface flow (Ashton et al. 2001). The majority of the river's surface is loose sandy soil with sandy-loam soil in western portions of the river (Ashton et al. 2001). The Shingwedzi River is mostly used for wildlife conservation within parks, such as KNP, and agriculture outside of nature reserves (Ashton et al. 2001).

2.2 Overall sampling approach

Data were collected in the form of morphometric measurements from live-captured bats and bioacoustics recordings using bat detectors (Taylor et al. 2013). Data were collected during four fieldtrips per year over two years (2017-2018). In total, 26 sampling sites were sampled over the two years. Eighteen of these sites were used as acoustic-only sampling sites, two sites were used for the live-capture of bats only, and six of the sites were used as both acoustic and live-capture sites (Figure 2.4). Site selection was based on covering the broad range of historical sampling sites in northern KNP (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987). In 2017, 12 sites (eight acoustic-only, one live-capture only, and three both) were sampled in the Pafuri region (Figure 2.4) while 14 sites (eleven acoustic-only, one live-capture only and two both) were sampled in the Punda Maria/ Shingwedzi region in 2018 (Figure 2.4).

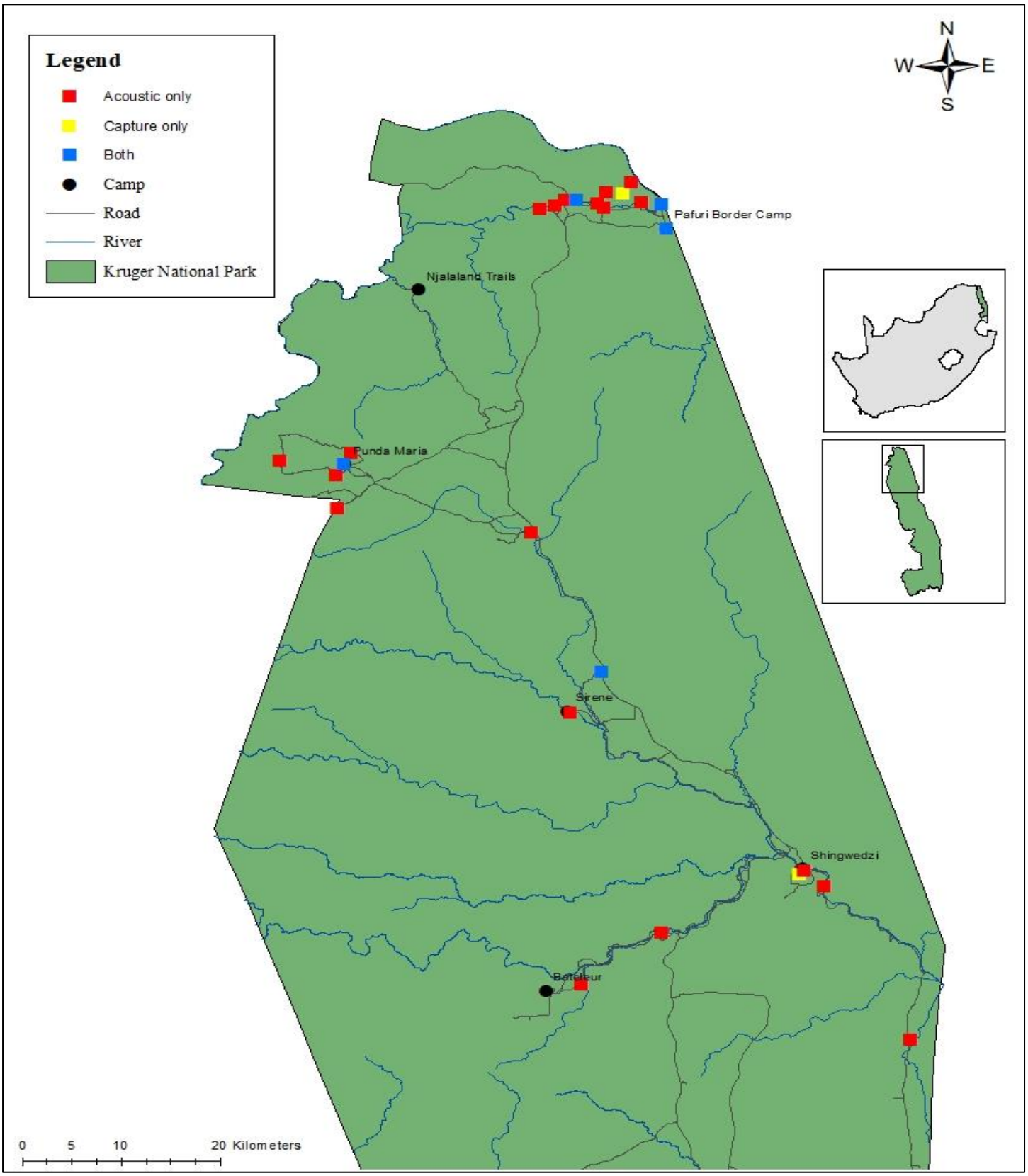

Figure 2.4: Study area indicating bat population sampling sites within northern Kruger National Park (green polygon). The black dots represent camps located within northern Kruger National Park. The red squares represent sites sampled through acoustic monitoring only. The yellow squares represent sites sampled through live-captures only. The blue squares represent sites sampled through both methods of acoustic monitoring and live-captures.

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Chapter 3: Bat communities of the northern Kruger National Park, South Africa as determined by live-capture

3.1 Introduction

Found on every continent except for Antarctica, bats are the second most diverse of all mammalian orders with over 1,300 known species recorded (Mickleburgh et al. 2002, Russo et al. 2018). Bats are the only mammalian order with the capability to fly, contributing to their widespread distribution (Jones et al. 2009). The diversity of bats is also due to their ability to adapt to a variety of habitats around the world (McCain 2007). Tropical and subtropical regions have the highest species richness of bats compared to any other region due to the increased abundance of food, water, and warm climates (McCain 2007).

Bats are important indictors for environmental health (Kasso & Balakrishnan 2013). Thus, by monitoring bats, changes to the environment can be detected (Jones et al. 2009). The decline in abundance of bats can indicate changes in environments through, for example, the loss of habitats, decreases in water sources, increased pollution from pesticides, and increases in diseases (Mickleburgh et al. 2002). For example, *Rhinolophus darlingi* is almost entirely restricted to riparian habitats and a decline in their abundance could indicate loss of such habitat (Monadjem & Reside 2008, Jones et al. 2009). In addition, changes in climate can be detected by monitoring bat species (Jones et al. 2009). The reproductive cycles of bats are closely linked to temperature, abundance of food sources, and hibernation (Jones et al. 2009). Climate change can therefore cause shifts in species distributions caused by warmer temperature (Jones et al. 2009).

Even though bats make up over 1,300 species around the world, our understanding of bats is still fairly limited (Cooper-Bohannon et al. 2016, Russo et al. 2018). Bats can be difficult

to study due to their nocturnal behaviour, their use of multiple (difficult to access) roosts, and their ability to go undetected by traps and/or bat detectors (Berry et al. 2004, Monadjem & Reside 2008, Adams et al. 2012). In addition, variation in flight patterns can cause difficulties in surveying bat populations. The family Molossidae, for example, are capable of swift flying above the tree canopy line and out of reach of humans (Cotterill & Fergusson 1993, Fenton et al. 2004), while the family Rhinolophidae are capable of quick manoeuvres when flying that can aid in avoiding traps (Berry et al. 2004). The lack of data and understanding of the distribution of many bat species has resulted in poor conservation status and little to no protection in many countries (Cooper-Bohannon et al. 2016).

The Kruger National Park (KNP) has a high diversity of bats due to its subtropical climate and is estimated to have a higher bat diversity than any other region in South Africa (Rautenbach et al. 1985, Venter & Gertenbach 1986). In northern KNP, research was conducted in 1979, 1982, 1983, 1984, and 1985 to determine the number of species occurring in the region (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987). Additionally, between 1960 and 1990 specimens were collected from KNP but no complete assessment was conducted. Since the last comprehensive assessment in KNP, bat diversity has been under studied and unmonitored (Monadjem & Reside 2008).

The goal of this chapter was to provide an up to date study on the bat diversity of northern KNP. By comparing the current survey with historical data (the 1980's surveys and the collected museum specimens), I would be able to determine if there have been any changes in bat diversity. Specifically, I aimed to (i) contribute towards the inventory of bat species found within northern KNP through live-capture methods, (ii) estimate the current diversity of bat species to compare with historical data and (iii) develop a comprehensive bat echolocation call

reference library generated from captured bats. The echolocation call reference library would be used as a guide for the acoustic identification portion of my project (see Chapter 4).

3.2 Materials and methods

Live-captures

The live-capture of bats was done using mist-netting and a two-bank harp trap (Aldridge & Rautenbach 1987). Mist-nets (either 6, 9, or 12 m in length; Ecotone, Gdynia, Poland) were utilized within fenced restcamps and a harp trap (Faunatech, Victoria, Australia) was used within restcamps and at designated picnic sites. The mist nets and the harp trap were set up no later than 30 minutes before sunset near either a water source (swimming pool, pan, or stream) or a man-made structure that displayed evidence of bat activity. Evidence of activity included observations of flying bats in the evening, faeces on the ground, or brown stains under a hole on the surface of a building caused by urine (DePaepe et al. 1996).

Mist-nets were operated for two to four hours and were monitored throughout. Since harp traps can be left unattended overnight, the harp trap was left out for between 12 and 13 hours each night it was in use and was checked at sunrise (Taylor et al. 2013a, 2013b). Sampling took place during eight, five-night fieldtrips spread over two years (2017 and 2018). In 2017, sampling was done in March/April, May, August, and October. In 2018, sampling was conducted in March, May, September, and October. The selection of capture sites was completed on the first day of each trip. This was done by confirming the presence of bat activity at a site. For example, employees of KNP aided in the understanding of where bats were known to roost or expected to be active.

There were a total of eight separate sites that were sampled over a total of 33 nights for the two years (Figure 3.1 and 3.2). In 2017, there were four sites (n = 13 sampling nights) within the Pafuri region (Table 3.1). In 2018, there were four sites (n = 20 sampling nights) within the Punda Maria/ Shingwedzi region (Table 3.2). When captured, bats were carefully removed from either the mist net or the harp trap and placed in individual cloth bags for processing and released the following day (Taylor et al. 2013a, 2013b).

Table 3.1: Live-capture locations within the Pafuri region of northern Kruger National Park with the number of nights and hours that each site was sampled within a given year. Method of capture was either mist-net, harp-trap, or alternated between both forms.

2017 Pafuri				
Site	**Nights Sampled**	**Total Capture Hours**	**Method**	**Month of Trapping**
Rietbok Vlei	2	6	Mist Net	April, August
Pafuri Border Camp	1	25	Mist Net, Harp Trap	May, October
Pafuri camp	8	35	Mist Net, Harp Trap	March, May, August
Crooks Corner	2	26	Harp Trap	October
Total	13	92		

Table 3.2: Live-capture locations within the Punda Maria/ Shingwedzi region of northern Kruger National Park with the number of nights and hours that each site was sampled within a given year. Method of capture was either mist-net, harp-trap, or alternated between both forms.

2018 Punda Maria/ Shingwedzi				
Site	**Nights Sampled**	**Total Capture Hours**	**Method**	**Month of Trapping**
Shingwedzi Research camp	8	104	Harp Trap, Mist Net	May, October
Punda Maria restcamp	6	64	Mist Net, Harp Trap	March, September
Babalala Picnic site	4	52	Harp Trap	March, May
Shingwedzi restcamp	2	24	Harp Trap	October
Total	20	244		

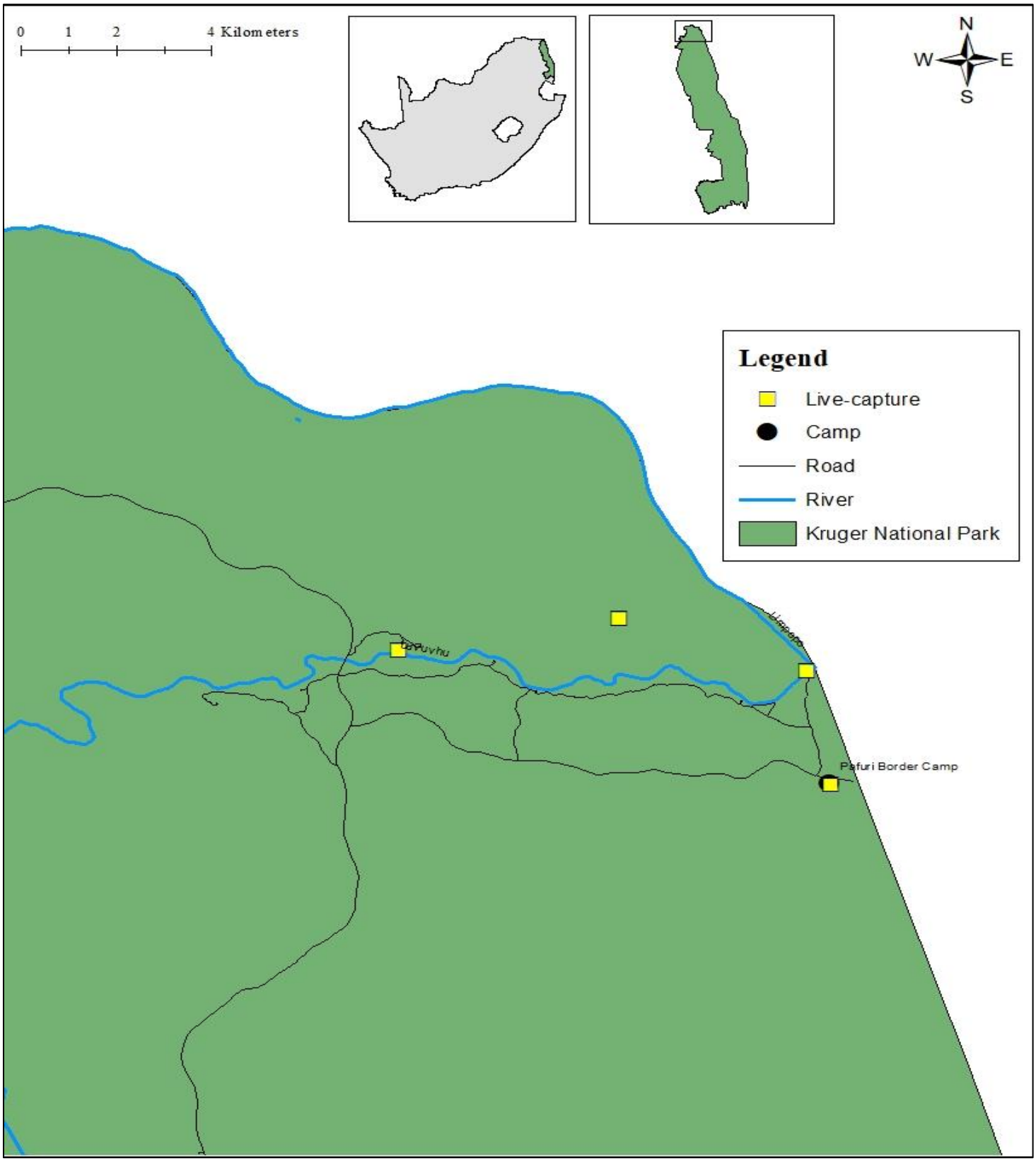

Figure 3.1: Study area indicating sampling sites in the Pafuri region of northern Kruger National Park (green polygon). The black dots represent camps. The yellow squares represent sites sampled.

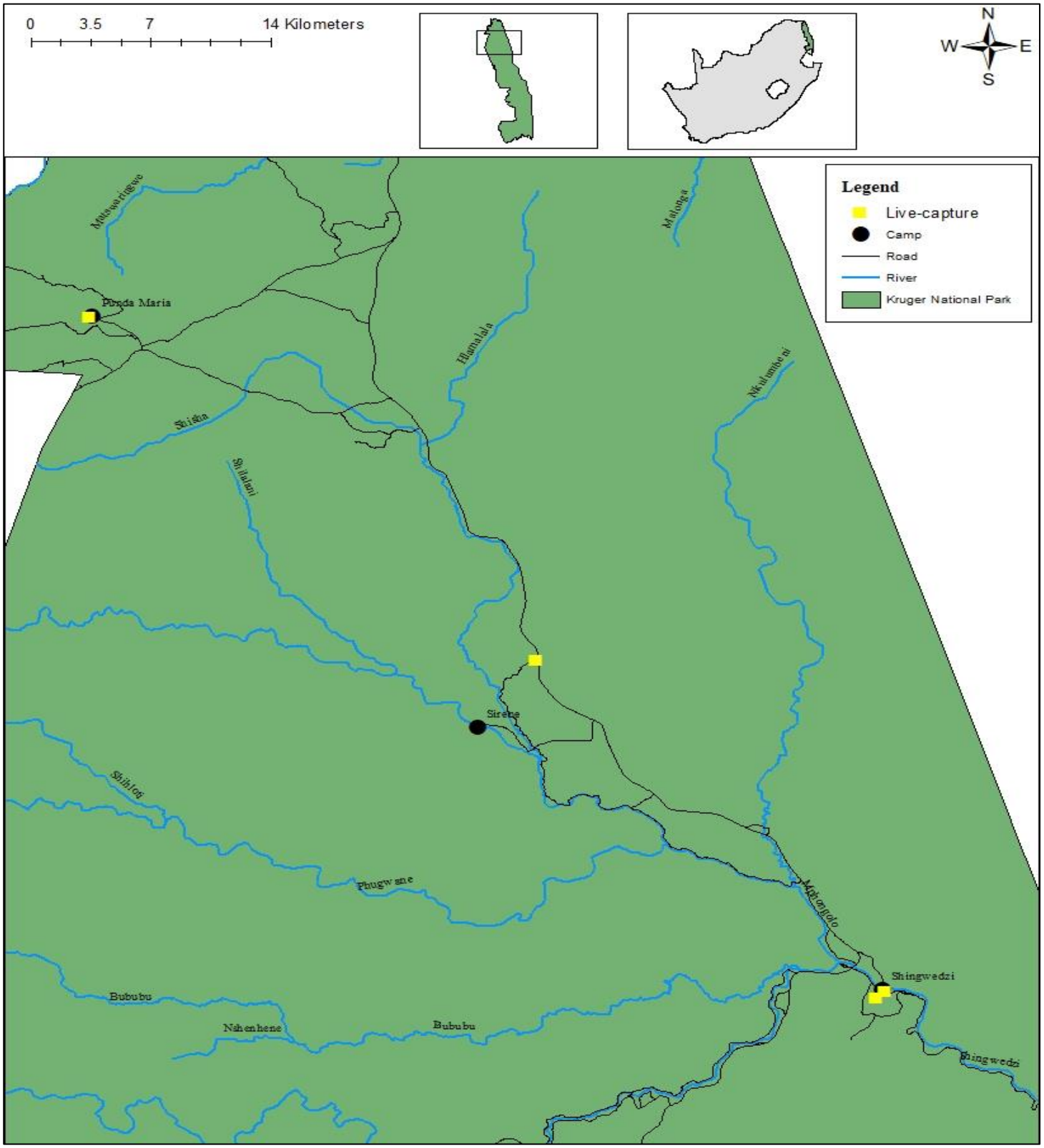

Figure 3.2: Study area indicating sampling sites in the Punda Maria/Shingwedzi region of northern Kruger National Park (green polygon). The black dots represent camps. The yellow squares represent sites sampled.

During processing, all captured bats were identified to species using Monadjem et al. (2010). Morphometric measurements were also recorded for each bat and these included body mass (g) and forearm length (mm) (Appendix 3.1). Body mass was recorded with the use of a Pesola balance and measured to the nearest 0.5 gram. The forearm length was measured by a 150 mm digital calliper (Insize, Germany) and measured to the nearest 0.01 mm. Assessment of overall size, shape, and colouration aided in the identification of each individual bat (Monadjem et al. 2010). The sex and age (adult, sub-adult and juvenile) of each bat were also recorded (Appendix 3.1). Age was determined by the extent of ossification between the metacarpal bones and proximal phalanx of the fourth digit (Kunz & Anthony 1981). In juvenile bats, the region between these bones is made up entirely of cartilage and is seen as a white band (gap) when the bat's wing is held up to the light (Kunz & Anthony 1981). As the bat ages, the cartilage begins to ossify (Kunz & Anthony 1981). Sub-adults have a narrower white band compared to juveniles, but it is not completely ossified like adults (Kunz & Anthony 1981).

After processing, bats were normally released about half an hour before sunset. Upon release, echolocation calls from each bat were recorded using either an Anabat SD2 (Titley Electronics, Australia) or Echo Meter Touch 2Pro (Wildlife Acoustics, USA) or both. The recordings of the calls were used to confirm the identification of the bats and to generate a call reference library for the acoustic component of the project (See Chapter 4). Although almost all of the captured bats were released, some could not be identified in the field (2017 = 9; 2018 = 3) and were therefore taken as museum specimens to confirm identification using cranial and dental characteristics (Taylor et al. 2013b). All specimens were sent to the Durban Natural Science Museum to be cleaned, analysed and accessioned. Permission to conduct the research within KNP was granted by SANParks (Permit #: PARD1401). All live-captures were approved by the

SANParks Animal Use and Care Committee (Approval #: 025/16) and fell under the provincial capture and handling permit issued to Professor PJ Taylor (Permit code: 0089-MKT001-00004). Ethical clearance for the study was granted by the University of Venda (Permit No. SMNS/17/SARC H1/01/2006).

Historical captures

Historical data were drawn from the 1984, 1985, and 1987 papers describing three bat surveys conducted in northern KNP (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987). Additional data were drawn from Monadjem et al. (2010) that describes the majority of bat specimens collected within the study area between 1960 and 1990, predominantly from the Ditsong National Museum of Natural History (formerly Transvaal Museum). All references provided detailed accounts of each of the species captured including the number of individuals, location of capture, sex, and morphometric descriptions (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987, Monadjem et al. 2010). The recorded species mentioned in these sources were combined into a Microsoft Excel spreadsheet. This spreadsheet listed each individual bat captured along with family name, species name, location of capture, year, and paper reference in which the bat was recorded (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987, Monadjem et al. 2010). Several species names have been changed over the past thirty years due to increased genetic research. Thus, the most current and accepted species names were used and were based on Monadjem et al. (2010) and subsequent papers (Goodman et al. 2017, Taylor et al. 2018).

Species richness and heterogeneity measurements

Bat species richness and diversity were determined in both 2017 and 2018, and for all existing historical data (Menhinick 1964). Species richness is the number of different species residing together in the same area (Derry et al. 1998). Diversity is defined as a function of species richness and evenness and is commonly calculated by Shannon-Wiener diversity index which estimates diversity (Peet 1974). Evenness is the equability of species within a community (Derry et al. 1998). Both the Shannon-Wiener diversity index and the Shannon evenness were calculated for each year to determine any changes in bat communities over time. The Shannon-Wiener index was calculated using the following equation:

$$H' = -\sum_{i=1}^{s} p_i ln p_i$$

and Shannon evenness using the equation:

$$J' = H'/H_{max} (H_{max} = log_2 s)$$

where p_i is the relative abundance of species, i, ln is the natural log of p_i, and s is the total number of species found (Pearson & Rosenberg 1977, Magurran 2011). The Shannon-Wiener index was calculated using R studio (R Core Team 2017). The package 'vegan' was used to calculate diversity through the Shannon-Wiener equation with the code written as diversity (file name $ Captured, index = "shannon") (Oksanen et al. 2018). The package 'vegan' is used for descriptive community ecology for analysis of diversity, community ordination, and dissimilarity evaluation (Oksanen et al. 2018). Shannon evenness was calculated manually once diversity had been determined.

Statistical analyses

All statistical analyses were conducted using R studio (R Core Team 2017). To test whether there were any differences between the current surveys (2017 vs 2018) t-tests were performed (Lu et al. 2005). In addition, any differences between the combined current surveys (2017 and 2018) and the combined historical data were also tested using t-tests (Lu et al. 2005). A .csv file was created for both comparisons. Each file contained a column labelled 'year', 'diversity', 'richness', 'abundance', and 'evenness'. The variables of interest in these analyses were bat diversity, richness, abundance, and evenness for each current fieldtrip and the historical data. After each variable was tested for normality, the package 'tidyverse' was used to conduct the t-tests.

Species accumulation curve

A species accumulation curve was constructed to provide a meaningful interpretation of the species richness found during the current survey for northern KNP (Flaquer et al. 2007). A species accumulation curve is a linear model that calculates an estimated number of species within an area compared to the number of sample sites surveyed (Skalak et al. 2012). The model presumes that as the number of sample sites increases, the number of observed new species within the study area will decrease (Skalak et al. 2012). EstimateS 9.10 software (Colwell & Elsensohn 2014) was used to generate the individual-based accumulation curve (Chao et al. 2000). EstimateS is a programme that calculates and compares the various diversities and species richness within a sample site (Colwell & Elsensohn 2014). A .txt file was created to be placed into EstimateS that contained the number of captures from each species recorded from each site (Table 3.1 and 3.2). Using the .txt file, EstimateS computes a mean and variance for species richness by randomly selecting a sample size from the total data sample to generate a S(est), Incidence Coverage-based Estimator (ICE), and Chao2 line (Flaquer et al. 2007, Parker &

Bernard 2018). S(est) is the calculated species richness from the observed data (Parker & Bernard 2018). Both ICE and Chao2 calculate the sampling efficiency to generate an estimated species richness within the study area (Parker & Bernard 2018).

3.3 Results

Historical data

Three surveys were conducted in northern KNP in the1980's. Combined, the three published records describe 175 individual bat captures representing 31 species in six families (Table 3.3). The six families were Vespertilionidae, Molossidae, Emballonuridae, Nycteridae, Rhinolophidae, and Hipposideridae (Table 3.3). *Neoromicia stanleyi* (20 individuals captured) was the dominant species followed by *Pipistrellus rueppelli* (19 individuals captured), both from the family Vespertilionidae (Table 3.3).

Additional data were drawn from Monadjem et al. (2010) that describes all bat specimens collected within the study area between 1960 and 1990, including some of the specimens from the 1980's surveys mentioned previously. A total of 142 individuals were added from this dataset, representing 35 species from eight families (Table 3.3). The eight families were Vespertilionidae, Molossidae, Miniopteridae, Nycteridae, Rhinolophidae, Emballonuridae, Pteropodidae, and Hipposideridae (Monadjem et al. 2010). Thus, historically, the region, as a whole, had a total of 317 individual bats captured from 40 species (Table 3.3) resulting in high species diversity (3.1; standard deviation of 1.9) and a moderate species evenness (0.59; standard deviation of 0.06).

Table 3.3: Bat species captured within the northern parts of Kruger National Park between 1960 and 1990. 'Years' represents year of specimen collection. 'Date Unknown' represents an individual bat in which the date of collection was unknown. 'Individuals' represents the total number of each species recorded during that particular year (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987, Monadjem et al. 2010).

Historical Live-captures			
Family	**Species**	**Individuals**	**Years**
Emballonuridae	*Taphozous mauritianus*	4	1983, 1984, 1985
Hipposideridae	*Hipposideros caffer*	18	1979, 1982, 1984, 1985, 1988
	Hipposideros vittatus	6	1985, 1987, 1988, 1990
Miniopteridae	*Miniopterus natalensis*	3	1979, 1984
Molossidae	*Chaerephon ansorgei*	11	1985, 1986, 1988
	Chaerephon pumilus	7	1960, 1961, 1979, 1983, 1984, 1985
	Mops condylurus	7	1984, 1985
	Mops midas	7	1979, 1983, 1984, 1985
	Tadariada aegyptiaca	3	1982, 1984
	Tadariada fulminans	3	1984, 1985
Nycteridae	*Nycteris thebaica*	3	1960, 1985
	Nycteris woodi	3	1979
Pteropodidae	*Epomophorus wahlbergi*	3	1975, 1979
	Rousettus aegyptiacus	9	1979, 1983, 1983
Rhinolophidae	*Rhinolophus clivosus*	1	1990
	Rhinolophus darlingi	3	1984, 1985, 1990
	Rhinolophus fumigatus	10	1975, 1983, 1985, 1989, 1990
	Rhinolophus landeri	7	1985
	Rhinolophus simulator	5	1983, 1985, 1986
	Rhinolophus smithersi	14	1979, 1982, 1983, 1984, 1985, 1989
	Rhinolophus swinnyi	2	1985
Vespertilionidae	*Eptesicus hottentotus*	10	1982, 1984, 1985
	Glauconycteris variegata	8	1979, 1982, 1982, 1983, 1984, 1985, 1986
	Kerivoula argentata	1	1985
	Kerivoula lanosa	1	1984
	Laephotis botswanae	8	1985
	Myotis bocagei	12	1979, 1982, 1985
	Myotis tricolor	4	1979, 1984, 1985
	Myotis welwitschii	1	Data Unknown
	Neoromicia capensis	16	1979, 1985, Date Unknown
	Neoromicia nana	11	1985, Date Unknown
	Neoromicia stanleyi	26	1985, Date Unknown
	Neoromicia zuluensis	10	1985, Date Unknown
	Nycticeinops schlieffeni	23	1979, 1982, 1985, 1988, 1990
	Pipistrellus hesperidus	1	1990
	Pipistrellus rueppelli	10	1983, 1984, 1985
	Pipistrellus rusticus	3	1985, Date Unknown
	Scotophilus dinganii	26	1979, 1983, 1984, 1985
	Scotophilus leucogaster	12	1979, 1982, 1984, 1988
	Scotophilus viridis	5	1985
Total:	40	317	

Current data

In 2017, a total of 106 individual bats were captured within the Pafuri area. Seven species, representing four families (Molossidae, Vespertilionidae, Rhinolophidae and Pteropodidae) were captured (Table 3.4). *Mops condylurus* (Molossidae) was the dominant species with a total of 79 captured. Of the 79 *Mops condylurus* bats, 71 were captured within one night at the Pafuri Border Camp. *Chaerephon pumilus* (Molossidae) and *Scotophilus dinganii* (Vespertilionidae) were both the least represented species in 2017 with only two individuals of each species captured (Table 3.4). Our target was insectivorous bats but we also captured four *Roussetus aegyptiacus* bats from the fruit bat (Pteropodidae) family during the August 2017 field visit. The most bats were captured during May 2017 (75 individuals) and the fewest bats (2) were captured in October 2017 (Table 3.4).

In 2018, a total of 49 individuals, representing nine species from four families, were captured (Table 3.4). The four families represented in 2018 were Molossidae, Vespertilionidae, Hipposideridae, and Pteropodidae. *Mops condylurus* (Molossidae) was again the dominant species with a total of 18 captured. *Cloeotis percivali* (Hipposideridae) and *Neoromicia capensis* (Vespertilionidae) had the lowest captures (1 individual each) during the year (Table 3.4). We also captured two *Epomophorus wahlergi* bats from the fruit bat (Pteropodidae) family during the May 2018 field visit. The most bats were captured during May 2018 (22 individuals) and the fewest bats (0) were captured in October 2018 (Table 3.4).

Table 3.4: Bat species captured within the northern parts of Kruger National Park between 2017 and 2018. 'Years' represents year of species captured. 'Individuals' represents the total number of each species recorded during that particular year.

2017 and 2018 Live-capture			
Family	**Species**	**Individuals**	**Years**
Hipposideridae	*Cleotis percivali*	1	2018
	Hipposideros caffer	4	2018
Molossidae	*Chaerephon pumilus*	4	2017, 2018
	Mops condylurus	97	2017, 2018
Pteropodidae	*Epomophorus wahlbergi*	2	2018
	Rousettus aegyptiacus	4	2017
Rhinolophidae	*Rhinolophus fumigatus*	6	2017
Vespertilionidae	*Neoromicia capensis*	1	2018
	Neoromicia nana	12	2017, 2018
	Neoromicia zuluensis	8	2018
	Nycticeinops schlieffeni	7	2017
	Pipistrellus rusticus	2	2018
	Scotophilus dinganii	7	2017, 2018
Total:	13	155	

The 2017 survey had a higher abundance of captures compared to the 2018 survey, but there was no statistically significant difference ($t = 0.60$, $df = 3.39$, $P > 0.05$). Even though a greater number of individual bat captures were made in 2017 (Figure 3.3), species diversity was higher in 2018. In 2018, the species diversity was 1.91 (standard deviation of 0.94), slightly higher than the species diversity of 2017 of 1.00 (standard deviation of 0.83). However, the difference between the two surveys was not statistically significant ($t = -1.26$, $df = 4.56$, $P > 0.05$). Species richness was greater in the 2018 survey (ten species) compared to the 2017 survey (seven species), however, the difference was not statistically significant ($t = -1.47$, $df = 4.23$, $P > 0.05$). The 2018 survey had a higher Shannon evenness measurement (0.57; standard deviation of 0.14) compared with 2017 (0.51; standard deviation of 0.34) but the difference between the two evenness values was not statistically significant ($t = -0.32$, $df = 4.50$, $P > 0.05$). Combined, a

total of 155 individual bats from 13 species were captured over the two years of study (Figure 3.3). In addition, the study area, as a whole, had a diversity of 1.51 and an evenness of 0.41.

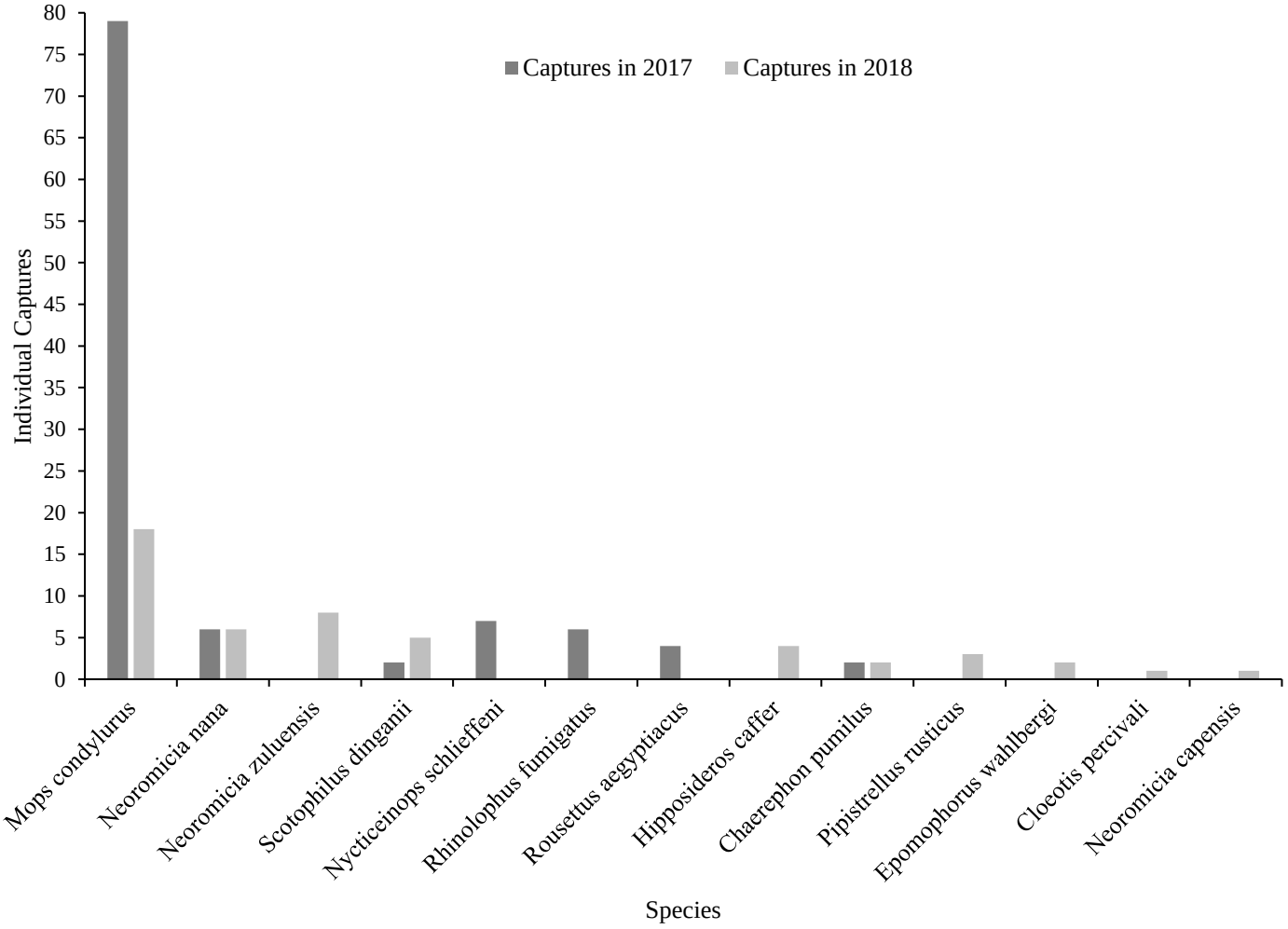

Figure 3.3: A comparison of the number of individual bat species captured during 2017 and 2018 in northern Kruger National Park. Dark grey represents bats captured in the 2017 survey. Light grey represents the bats captured in the 2018 survey.

Comparing species richness and heterogeneity measurements

Even though the historical surveys, combined, resulted in a higher diversity of bat species compared to the current survey, the difference was not statically significant ($t = -2.19$, $df = 3.67$, $P > 0.05$). Nevertheless, the number of bat species recorded from the historical surveys was 40, almost four times more than the number recorded in the current survey (13). Even though species richness and abundance were higher in the historical data (Table 3.3, 3.4), the difference was not statistically significant for either richness ($t = -1.87$, $df = 3.05$, $P > 0.05$) or abundance ($t = -1.76$, $df = 3.54$, $P > 0.05$). The distribution between species in the combined historical surveys was fairly even with the dominant species, *Scotophilus dinganii* and *Neoromicia stanleyi*, each making up 8.3% of the captured individuals resulting in a Shannon evenness of 0.59. The distribution between species in the current assessment had *Mops condylurus* making up for 62.6% of the captured bats (Figure 3.3) resulting in a lower Shannon evenness of 0.41. However, the difference between the historical surveys and current surveys evenness was not statistically significant ($t = -0.81$, $df = 7.55$, $P > 0.05$).

Species accumulation curve

A total of eight sites were sampled using live-capture within the study area of northern KNP over a two-year period (Table 3.1; Table 3.2). From the eight sample sites, 13 species (S(est)) were detected. The S(est) and Chao 2 (11.99) estimator lines demonstrates that species richness continued to increase throughout the sample sites (Figure 3.5). Both the S(est) and Chao 2 curves suggests that there was an incomplete sampling and there should be more than eight sampling locations within the study area to observe most of the detectable bat species. In

addition, the ICE (19.36) estimator indicated that the survey missed approximately six species over the eight sampling sites (Figure 3.4).

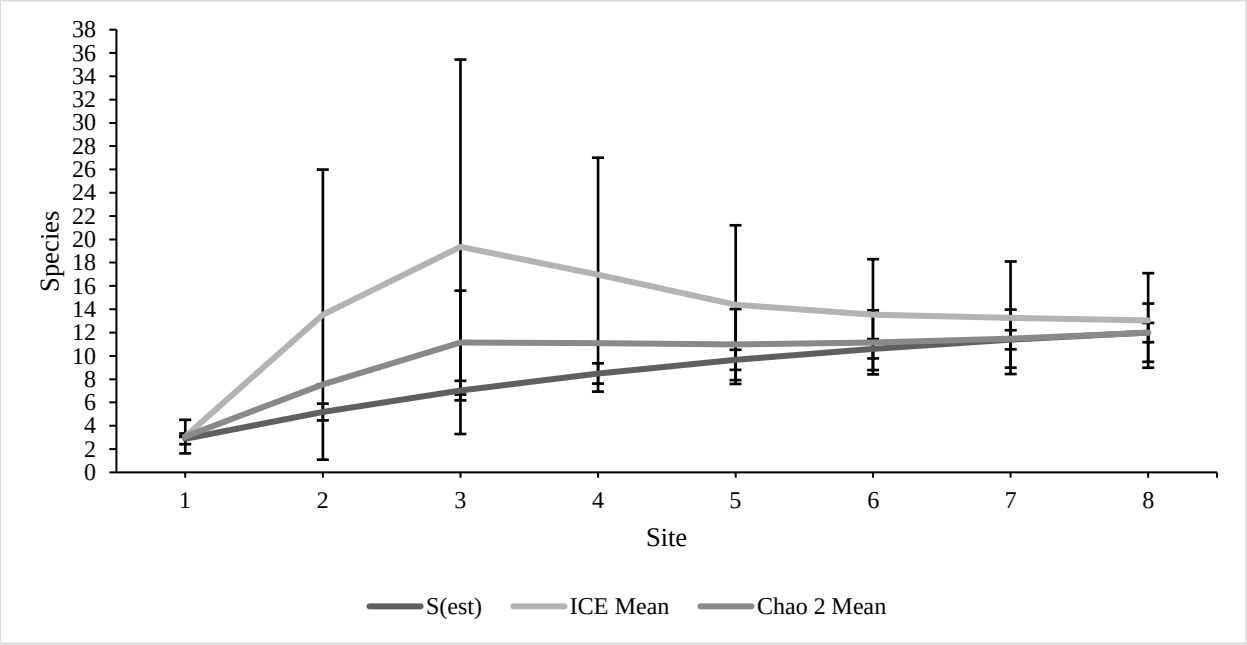

Figure 3.4: Species accumulation curve for all live-capture data recorded from current survey within northern Kruger National Park. S(est) is the observed data and ICE mean and Chao 2 mean are the estimated species richness. Error bars represent standard deviation.

3.4 Discussion

Comparing historical data with the current survey

Northern KNP has historically had a high diversity of bats with 40 species documented (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987, Monadjem et al. 2010). Over the two-years of my study, 13 species from five families were recorded. The historical data shows a much higher diversity of bat species and greater number of individuals captured within the study area however the data was collected over a 30-year time span causing for a greater sampling effect compared to the current survey. The longer sampling period from the historical data increased the likelihood of capturing a greater number of rare, non-common species (Larsen et

al. 2007). However, environmental effects, biological aspects, and overall study limitations also likely contributed to the observed differences (McCain 2007).

The majority of the historical individual bat captures were documented during the 1980's (Table 3.3). During this time, KNP experienced two years (1980, and 1984) of slightly higher than average rainfall while 1982, 1986, and 1988 experienced slightly lower average rainfall (MacFadyen et al. 2018). The two years of increased rainfall could have caused an increase in insect prey, resulting in an increase in bat activity (Pinheiro et al. 2002, Monadjem et al. 2010, MacFadyen et al. 2018). Areas with increased water sources might also experience higher abundances of bats due to the utilizing of the water resource to drink and hunt for insects (McCain 2007). By comparison, in 2015 and 2016, KNP experienced a severe drought caused by the third strongest El Niño event recorded since climate recordings (Urban et al. 2018). The decrease in rainfall leading up to the beginning of the current survey might have caused a decrease in insect abundance (Pinheiro et al. 2002). The decrease in water and insect abundance could cause bat activity to decline, resulting in few individuals captured during the current survey (McCain 2007).

In addition, a variety of bat species are dependent on large trees and shrubs as roosts and use the surrounding habitat as a food source while hunting (Monadjem et al. 2010). Foliage-roosting bat species, such as *Taphozous mauritianus* and *Neoromicia nana*, use the leaves, roots, trunks, and branches of trees and shrubs as roosts; these bat species are dependent on the protection of large trees and shrubs during the day (Fenton 1992, Willis & Brigham 2004). KNP monitors tree and shrub density through the use of aerial photography and data has shown a decline in the prevalence of large trees since the 1960's (Trollope et al. 1998, Wigley et al. 2014). Both over-browsing by herbivores and fire restricts the growth processes of trees smaller

than 3 m which causes the recruitment of young trees to decline (Trollope et al. 1998, Wigley et al. 2014). Elephants (*Loxodonta africana*) are known to damage large trees by over browsing, bark-stripping, snapping off branches, and even killing trees by knocking them over (Midgley et al. 2005). Areas that have experienced increases in elephant populations and fires show significant declines in large trees (Trollope et al. 1998). In addition, many large riparian trees were severely destroyed by flood events around the Shingwedzi River in 2000 (Bonaccorso et al. 2014). The loss of several large trees has caused a decline in recruitment of young tress around effected areas (Bonaccorso et al. 2014). The historical trap locations were all within riparian habitats where large trees are known to grow and many bat species hunt (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987, Monadjem & Reside 2008). Many of the species recorded from the historical data are reliant on habitats with dense, woody vegetation (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987, Monadjem et al. 2010). Even though the current survey sites were located within and around the historical sites, there were fewer tree dependent bat species captured. This may explain why there was an increase number of open-air and cluster-edge bat species captured in the current study. The decrease in bat species that depend on habitats with dense, woody vegetation could have been caused by the continuous decline in large trees (McCleery et al. 2018).

Both current and historical surveys showed a greater number of individuals from the families Molossidae and Vespertilionidae compared to any other family. The Vespertilionidae has about 19 species known to inhabit KNP and is one of the largest families of bats (Hoofer & Bussche 2003, Monadjem et al. 2010). Species in the Vespertilionidae family are known to be clutter-edge foragers that fly between open air and dense vegetation habitats to hunt for insects (Norberg & Rayner 1987, Lee & McCracken 2004). They are known to roost in both foliage and

man-made structures (Lee & McCracken 2004). Due to their behaviour as clutter-edge foragers, Vespertilionids can be captured when the trap is placed both in open air areas (i.e. pool or pan) or when placed within dense vegetation areas (Monadjem et al. 2010). They are also known to be the most active in the evening, during the same time period the mist nets were setup in the current survey (Lee & McCracken 2004). The behaviour of flying and time of activity could have resulted in the capturing of many species belonging to the family in both the historical data and current survey.

Even though the Molossidae is not the largest family, it is the most common bat family in southern Africa and the family inhabits every continent except Antarctica (Mickleburgh et al. 2002, Monadjem et al. 2010). There are at least six species known to inhabit KNP and many of these species are widespread (Monadjem et al. 2010). *Tadarida aegyptiaca*, for example, is widespread and abundant throughout all of South Africa up to southern Zambia (Monadjem et al. 2010). Members of the Molossidae are known to roost both within man-made structures and large trees (Bouchard 2001, Monadjem et al. 2010). Roosts can number from just a few individuals to hundreds of individuals making them very common around buildings and bridges (Vivier & van der Merwe 2007). Even though many Molossid species give birth to one pup at a time, many are capable of multiple birth seasons a year (van der Merwe et al. 1986). For example, *Mops condylurus* and *Chaerephon pumilus* are both capable of two to three births per year (Bouchard 2001). Both historical and current surveys captured many individuals from the family Molossidae, most likely due to their multiple roosting habitats and abundance in numbers. However, the current survey was biased towards *Mops condylurus* due to traps being set near known roosting sites. For example, 71 individual *Mops condylurus* were captured within one

night at the Pafuri Border Camp. The harp trap was placed near a building that was a roosting site for *Mops condylurus*, resulting in the high abundance of individuals.

Compared to historical data, the current survey had a low number of individuals from the family Hipposideridae and Rhinolophidae. Both families have low number of species found within KNP with three species recorded for Hipposideridae and seven species for Rhinolophidae (Monadjem et al. 2010). In addition, both families are known to hunt within dense vegetation and are capable of quick manoeuvres due to their broad wings to avoid objects within the vegetation (Aldridge & Rautenbach 1987). The roosting behaviour of the family Hipposideridae are not completely known although most species are associated to dense vegetation and caves/ mines (Bernard & Fenton 2003, Zhang et al. 2009, Iskali & Zhang 2015). The males of the species *Hipposideros caffer* are known to have solitary roosts, while females roost in numbers varying between a few individuals to hundreds (Monadjem et al. 2010). Species of the Rhinolophidae family have similar roosting behaviour to the Hipposideridae with roosts found mainly within caves/mines or hollow trees (Bernard & Fenton 2003, Zhang et al. 2009). Roosts are normally low in numbers, ranging between one to less than two hundred individuals (Zhang et al. 2009). Reproduction in both families is lower compared to the Molossidae in which only one pup is born every year (Monadjem et al. 2010). Due to their ability to manoeuvre between obstacles (vegetation and traps, for example), a lack of knowledge of roosting sites, fewer individuals within roosts, and a likely low abundance of each species might have caused for the reduced number of captures for both families within the historical data and current survey.

The families Emballonuridae, Nycteridae, and Miniopteridae were recorded for the historical data but not for the current survey. Each family has very few species known to occur in KNP, with the Emballonuridae having one species (*T. mauritianus*), Nycteridae having two

(*Nycteris thebaica* and *Nycteris woodi*), and Miniopteridae with one (*Miniopterus natalensis*) (Monadjem et al. 2010). Even though I observed two *Taphozous mauritianus* individuals at the Bababala Picnic site during the day on the March 2018 field trip, the species was not captured and thus was not included in the data set. *Taphozous mauritianus* is known to roost in small numbers varying normally between one to five individuals located within rocks, tree trunks, and man-made walls (Fenton 1992). The species is capable of hunting in open-air, similar to bats in the family Molossidae (Fenton 1992) and females are known to give birth twice a year (Monadjem et al. 2010). *Nycteris thebaica* and *N. woodi*, much like the Rhinolophidae, roost in caves/ mines, large trees, and rarely within man-made structures (Monadjem 2001, Monadjem et al. 2009). Both species give birth to one pup a year (Monadjem et al. 2010). The Nycteridae, as a family, are known as whispering bats due to their soft, high frequency echolocation calls adapted for hunting in dense vegetation thus providing the capability to easily detect and avoid traps (Monadjem et al. 2009, 2010). *Miniopterus natalensis* roost in large numbers but the species is cave-dependent (Goodman et al. 2009) and there may not have been any suitable roost sites near my sampling locations. *Miniopterus natalensis* gives birth to one pup a year and is known to be clutter-edge hunter (Monadjem et al. 2010). Since my sampling sites were not positioned within all possible combinations of habitat and/ or roosting locations, this is likely a major factor in why I did not capture all possible species that are present in northern KNP.

Capture site locations can cause for a variation in captured species, species richness, as well as the abundance of individuals (Monadjem & Reside 2008). Historical data were drawn from three papers describing bat surveys conducted in northern KNP in 1979, 1982, 1983, 1984, and 1985 (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987). Additional data were drawn from Monadjem et al. (2010) that describes all bat specimens collected within the study

area between 1960 and 1990. All data were collected from various locations within the study area (over 15 individual sites) and could have caused for the greater diversity in species compared to the current survey. Due to safety concerns during the current survey, capture sites were located mostly within rest camps and designated picnic areas. The increase in man-made structures and decrease in dense vegetation around some of the trap sites could have caused for the increase in the open-air and clutter-edge flying species and the increase in species known to roost within buildings (DePaepe et al. 1996).

Even though the historical data shows a high diversity in species, the data were collected over a thirty-year time span compared with the current survey. In addition, both mist nets and harp traps can result in bias data towards common species and rare species can be missed (Larsen et al. 2007). Many species are capable of detecting traps and are able to avoid capture by flying around or over the trap (Larsen et al. 2007). By expanding efforts through increasing the numbers of capture sites and sampling hours, the possibility of recording a non-common bat species could increase (Larsen et al. 2007). During the two-year survey, sampling efforts totalled 33 nights with 336 hours of trapping. This resulted in about 32.5% of the historical species recorded being in the current survey. However, *Cloeotis pervicali*, from the family Hipposideridae, was captured and recorded in the Punda Maria rest camp on the September 2018 trip. *Cloeotis percivali* has never been documented for KNP before but has been recorded within areas surrounding KNP (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987, Monadjem et al. 2010). By expanding the survey into multiple years, with several sites throughout the year, the possibility of recording more of the less common species increases (Larsen et al. 2007).

Current data

A higher abundance of species was recorded within the 2017 survey located in the Pafuri region compared to the 2018 survey located in the Punda Maria/ Shingwedzi region. Ecosystems, biological aspects, and sampling limitations could have all played a role in these observed differences (McCain 2007). Even though the Pafuri region receives less rainfall than Punda Maria/ Shingwedzi, many baobab trees (*Adansonia digitata*) are present within the region and the trees are known to be used by many bat species (Gebauer et al. 2002, Baum et al. 1998). In addition, the Limpopo and Luvuvhu rivers, located within the Pafuri region, provide many riparian forests in which bats use as roosts and areas to hunt for insects (Monadjem & Reside 2008). Certain species, such as *Rhinolophus fumigatus*, are restricted to riparian forests and are rarely found beyond this habitat (Monadjem & Reside 2008). By comparison, the Punda Maria/ Shingwedzi region is dominated by mopani trees (*Colophospermum mopane*) which do not grow as large as many riparian trees and thus cannot provide adequate roosts for many species of bats (Mucina & Rutherford 2006, Monadjem & Reside 2008). The Pafuri region as a whole has a slightly warmer climate which in return provides a larger insect activity (Pinheiro et al. 2002). The presence of baobab trees, large riparian trees, more rivers, and warmer climate could have all resulted in a higher abundance of bats in the Pafuri region.

Even though the 2017 survey had a higher abundance in live-captures, the 2018 survey had slightly higher diversity and evenness scores. Evenness is based on how close the total number of each species are to each other at the time of sampling; the larger the Shannon evenness product, the more even the species are to each other (Derry et al. 1998). In 2018, the abundance of captured individuals from each species were closer in total number compared to the 2017 survey; this caused the Shannon evenness product to be greater. In 2017, a trap was placed in front of a daytime roost and 71 *Mops condylurus* were caught. These captures could

have caused the Shannon evenness in 2017 to be lower. The Shannon-Wiener diversity index equation factors evenness within the equation causing for higher or lower results (Peet 1974). The 2018 survey had a higher species richness and a greater Shannon evenness score compared to 2017; both of which could have caused the diversity to be greater in 2018 compared to 2017.

The location of capture sites and the types of traps used could have also affected the observed differences in the diversity of bat species recorded in 2017 and 2018. Studies have shown that harp traps are biased in terms of capturing small (<15g) to medium (15-25g) size bats compared to mist nets that are more likely to capture medium to large (>25g) sized bats (Francis 1989). The size of the traps can also influence captures. Mist nets can be extended up to 12 meters across and two to three meters in height covering more surface area (Taylor et al. 2013b). By contrast, harp traps take up less space with approximately two meters across and about two meters in height (Fukui et al. 2001). Mist nets are useful for trapping in slightly more open areas to cover more surface area but they must be monitored during the time of use (Taylor et al. 2013b). Due to needing less space, harp traps are useful when trapping in dense vegetation and do not require consent monitoring overnight (Fukui et al. 2001, Taylor et al. 2013b). These factors, combined, can influence which trap to use within a particular location, thus influencing the type of bats most likely to be captured. During 2017, trapping took place in more open areas around pools and pans, creating the need to use mist nets to cover the sides of the water sources. Compared to mist nets, the harp trap was used less within suitable dense vegetation sites. In 2018, both traps were used more equitably. Mist nets were used to trap around a pool or in front of a roost while the harp trap was left overnight at a picnic spot or on a trail within Punda Maria restcamp. The slight difference in bat species captured between each year could have been caused by the type of trap used within each capture site.

My study aimed to provide a current assessment of bat species found within northern KNP while also comparing bat species richness to historical data. Through live-capture methods, 13 bat species were recorded, including one species (*Cloetis pervicali*) which has not been documented in KNP before this study. Comparably, the historical data recorded 40 species but many factors could have caused for a lower species count in the current assessment. Decline in both presence of large trees and annual rainfall were observed during the current survey which could have influenced species richness and abundance (Wigley et al. 2014, MacFadyen et al. 2018). In the current survey, the low numbers of non-common species could have been caused by biological behaviours (hunting, flying, echolocation, and roost selections) resulting in trap avoidance (Francis 1989). Sampling effort would have also played a major role. The time span of the historical data was 30 years compared to the current assessment of only two years. Expanding capture locations and sampling hours could increase the possibility of capturing more non-common species (Larsen et al. 2007). Due to study limitations, it is difficult to determine if decline in bat diversity has occurred since the last assessments. Non-capture methods, such as acoustic technology, could greatly increase sampling efforts and reduce bias results which may result in higher species richness.

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Chapter 4: The use of acoustic technology to survey bat communities in northern Kruger National Park, South Africa

4.1 Introduction

Bats are one of the most successful mammalian orders in the world with over 1,300 species found on every continent expect for Antarctica (Mickleburgh et al. 2002, Russo et al. 2018). Tropical and subtropical regions have the highest species richness of bats compared to other regions due to the increased abundance of food, plentiful water, and warm climates (McCain 2007). Bats are the only mammalian order with the capability to fly, contributing to their widespread distribution (Jones et al. 2009). Bats are also one of the few mammalian orders with the ability to echolocate which allows them to adapt to a large variety of environments (Schnitzler & Kalko 2001). The power of flight and echolocation combined are responsible for the high species richness of bats that occupy a diverse range of habitats globally (Jones & Teeling 2006).

Echolocation is the transmitting of sonar signals into the environment and the analysing of the returning echoes to navigate around objects while also locating resources such as food (Thomas et al. 2004, Russo et al. 2018). Over 70 percent of insectivorous bats use echolocation calls (with frequencies ranging between 8 and > 200 kHz) to navigate and forage depending on the species and the surrounding environments (Russo et al. 2018). Echolocation calls are generated in the larynx and emitted through either the mouth or nose of the bat (Monadjem et al. 2010, Elemans et al. 2011). Low to intermediate frequency calls (8 to 75 kHz) are generally produced through the mouth in the families Vespertilionidae, Miniopteridae Emballonuridae, and Molossidae (Monadjem et al. 2010). Lower frequency calls (8 to 30 kHz) do not attenuate

quickly when traveling through space and are capable of reaching distances up to 30 meters (Monadjem et al. 2017). Due to the ability to travel greater distances, lower frequency calls are generally used by species known as open-air foragers to hunt for prey above the vegetation canopy (Russo et al. 2018). Intermediate frequency calls (30 to 75 kHz) are used mainly by clutter-edge foraging species (the Vespertilionidae and Miniopteridae) to hunt for prey in both dense vegetation and in open spaces (Monadjem et al. 2010). Intermediate frequency calls can travel a distance of about 10 to 15 m (Monadjem et al. 2017). Species within the families Vespertilionidae and Miniopteridae are also able to shift their echolocation calls over a range of frequencies to best suit their needs when hunting, depending on whether they are foraging in either open air or dense vegetation (Russo et al. 2018). *Neoromicia capensis*, for example, has a frequency range of between 36 and 54 kHz (Appendix 4.1). Intermediate to high frequency (40 to > 200 kHz) "high duty cycle" calls are often dominated by constant frequency components (Fenton 2003). The calls are generally produced through the nose of clutter foraging bats in the families Rhinolophidae, Hipposideridae, and Nycteridae (Parsons & Jones 2000, Monadjem et al. 2010). Each species within these families have a complex organ called a noseleaf that is located on the nose that releases the echolocation calls (Feng et al. 2012). Higher frequency calls are used to find prey and avoid collisions within cluttered spaces (Schnitzler & Kalko 2001). Compared to low frequencies, high frequency calls used by the Hipposideridae and some Rhinolophidae species cannot travel further than a few meters before attenuating (Monadjem et al. 2017).

The use of acoustic bat detectors to record echolocation calls can improve our understanding of bat behaviour, distribution patterns, and species richness within a particular study area (Fenton 2003, Adams et al. 2012). In addition, the use of bat detectors is a non-

invasive method that can collect large quantities of data over a short period of time (Adams et al. 2012). In addition, bat detectors can be placed almost anywhere (Skalak et al. 2012). Bat detectors are beneficial in the detection of species that are difficult to capture with a live-trap due to the ability of some species to fly out of reach or detect the trap and quickly avoid capture (Berry et al. 2004). Consequently, the use of acoustic detectors usually results in higher species richness estimates compared to live-capture methods (O'Farrell & Gannon 1999) and the approach has become common in the monitoring of bat communities (Adams et al. 2012).

However, there are several limitations to the use of bat detectors, including the overall sensitivity of microphones and the variability of echolocation calls between species and individual bats (Adams et al. 2012). Lower call frequencies can travel greater distances and can be recorded further away from a microphone compared to higher call frequencies which can only be recorded from a short distance (up to a few meters) from the microphone (Monadjem et al. 2017). Highly sensitive microphones are able to detect bats from a greater distance but bats with lower frequency calls (e.g. those in the Molossidae) can appear to dominate a particular area of study (Adams et al. 2012). Less sensitive microphones have a shorter detection range and can only record bats that fly close to the microphone (Adams et al. 2012). Less sensitive microphones are therefore better for recording high frequency calls but the probability of actually detecting a bat decreases (Adams et al. 2012). No matter which type of microphone is used, maximum detection range for echolocation calls is dependent on the placement of the microphone (Agranat 2014). Microphones should be placed as close as possible to where bats are known to fly, such as a flyway between vegetation, over a water sources, or next to an exit point of a roost (Agranat 2014).

Once recorded, calls are normally analysed using computer software programmes that can automatically analyse and identify the calls to species (Rydell et al. 2017). However, different species can often use similar calls, making identification difficult (Rydell et al. 2017). If not properly reviewed, the process of using automated identification of bat calls can cause false-positive results, effecting overall species richness (Clement et al. 2014, Rydell et al. 2017). Nevertheless, the use of bat detectors and automatic identification computer software tools can be a powerful approach in the assessment of bat communities (Adams et al. 2012).

The Kruger National Park (KNP) has a high diversity of bats and is estimated to have the highest bat diversity in South Africa (Rautenbach et al. 1985, Venter & Gertenbach 1986). Assessments of bat species richness were conducted in northern KNP through the use of live-capture methods in 1979, 1982, 1983, 1984, and 1985 (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987). Additionally, between 1960 and 1990 specimens were collected on an *ad hoc* basis by various researchers (Monadjem et al. 2010). In 1987, one QMC S-200 bat detector (Ultrasound Advice, London, United Kingdom) was used to record release calls from 16 bat species of five different families, but no comprehensive acoustic methods were used in the assessment of KNP bat communities historically (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987).

The goal of this chapter was to provide an up to date study on bat richness in northern KNP by adding a new technique for detecting bat species. Through this study, a site-specific identification classifier tool was developed to automatically identify bat echolocation calls. Bat activity was also monitored to compare two regions of northern KNP, Pafuri and Punda Maria/Shingwedzi. I aimed to (i) contribute towards the overall inventory of bat species found

within northern KNP, (ii) develop a classifier tool for identification to be used for future assessments and (iii) compare bat activity between the two regions of northern KNP.

4.2 Materials and methods

Acoustics protocol

SM4 and SM2 Songmeter detectors (Wildlife Acoustics, Concord, MA, USA) were used to record the echolocation calls of insectivorous bats in northern KNP (Adams et al. 2012). The songmeters were powered internally by four D-cell batteries throughout each field trip. All data were stored internally on an SD card which was transferred onto a hard drive after every trip. Each songmeter was equipped with a waterproof case and an SMM-U1or SMX-US ultrasonic microphone. When a detector was positioned at a site, the microphone was placed at least 1.5 meters above the ground by mounting the microphone to a pole or a tree (Clement et al. 2014). By placing the microphone above the ground, non-bat recordings caused by wind and rain were avoided (Parker & Bernard 2018). Each microphone was placed at a 45-degree angle to keep it moisture free and positioned to face the direction of a water source (Clement et al. 2014). Each songmeter was programmed to start recording at sunset and to stop recording at sunrise.

Sampling took place over two years (2017 and 2018). In 2017, sampling was done in March/April, May, August, and October. In 2018, sampling was conducted in March, May, September, and October. Site selection was determined before the first trip of each year and was based on covering the broad range of historical sampling sites in northern KNP (see Chapters 2 & 3) (Rautenbach et al. 1984, 1985, Aldridge & Rautenbach 1987). There was a total of 24 sites used for acoustic sampling over the two years. Eleven of these sites were located in the Pafuri region (surveyed in 2017) and 13 were located in the Punda Maria/ Shingwedzi region (surveyed

in 2018) (Figure 4.1 and Figure 4.2). Each detector was active for between three and five nights during each field trip. In 2017, there was a total of 148 nights of sampling and there were 130 nights of recording in 2018 (Table 4.1 and Table 4.2). A total of 278 nights of recordings was completed during the two-year survey. Each site was located near a water source such as a pan, stream, or river to enhance the detection of insectivorous bats (Monadjem et al. 2010).

Table 4.1: Acoustic sampling locations within northern Kruger National Park in 2017 showing the number of nights each site was sampled.

2017	
Site Name	**Total Acoustic Nights**
Site 1- Mapimbi Pan	14
Site 2- Crooks Corner	18
Site 3- Pafuri Picnic Site	13
Site 4- Nwambi Pan	16
Site 5- Luvhuvu River Bridge	19
Site 6- Opposite old Bobameng Campsite	14
Site 7- Luvuvhu River near Thulamela	3
Site 8- Between Pafuri picnic site and Crooks Corner	5
Site 9- Pafuri Border Camp	17
Site 10- Nyala Pan	16
Site 11- Pafuri Camp	13
Total	148

Table 4.2: Acoustic sampling locations within northern Kruger National Park in 2018 showing the number of nights each site was sampled.

2018	
Site Name	**Total Acoustic Nights**
Site 1- Babalala Picnic site	16
Site 2- Kaniedood bird hide	13
Site 3- Tshanga lookout	8
Site 4- Shingwedzi rest camp	17
Site 5- Nyawatsi bird hide	8
Site 6- Red Rocks lookout	8
Site 7- Sirheni restcamp	8
Site 8- Punda Maria restcamp	18
Site 9- Maritube Pan	10
Site 10- Matukwala Pan	7
Site 11- Coetzer Pan	7
Site 12- Dzundzwini Road	6
Site 13- Punda Maria Gate	4
Total	130

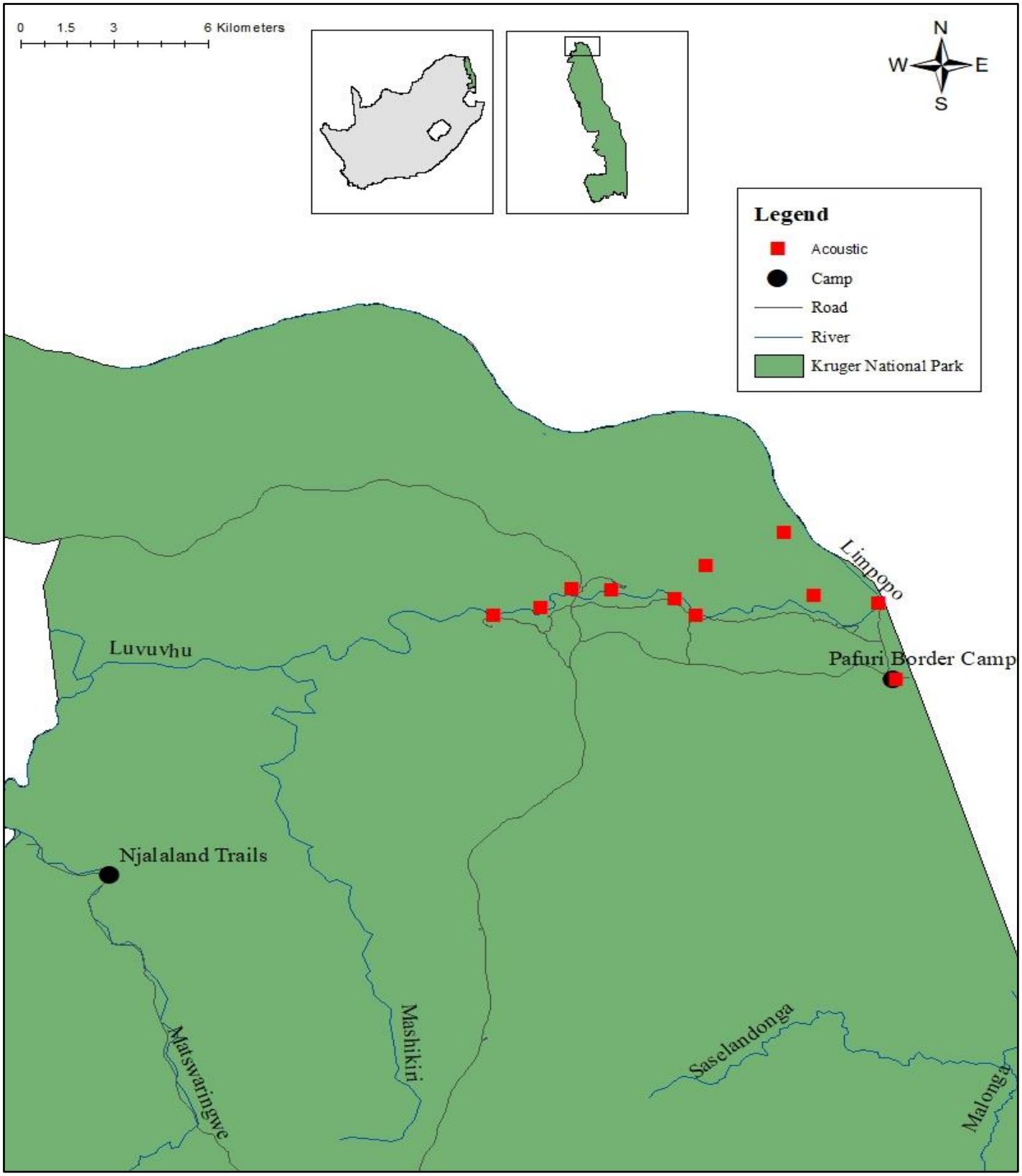

Figure 4.1: Study area indicating sampling sites in the Pafuri region of northern Kruger National Park (green polygon). The black dots represent camps. The red squares represent sites sampled.

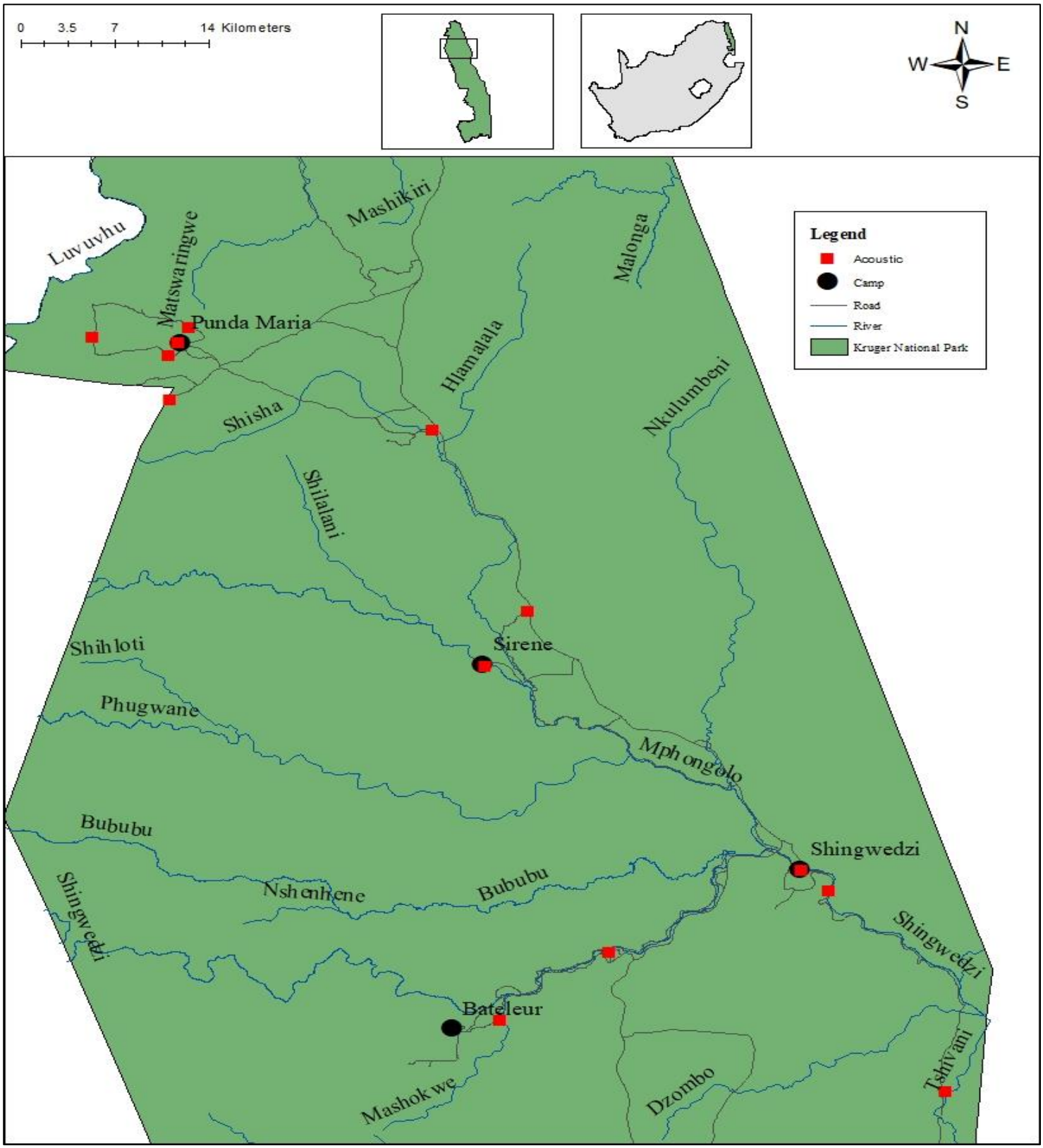

Figure 4.2: Study area indicating sampling sites in the Punda Maria/ Shingwedzi region of northern Kruger National Park (green polygon). The black dots represent camps. The red squares represent sites sampled.

Kaleidoscope Pro software (Wildlife Acoustics, Concord, MA, USA) was used to analyse and identify each bat call series recorded. Kaleidoscope is a computer software programme made to identify bats (Rydell et al. 2017). Kaleidoscope is able to automatically identify recordings based on the characteristics of each call series through the use of built-in classifier tools for different regions of the world (Rydell et al. 2017). To enhance the accuracy of this automated identification function, I developed a KNP-specific bat species classifier and uploaded it into the programme (Linden et al. 2014). A comprehensive bat echolocation call reference library was created using the echolocation calls of bats that had been released during the live-capture component of my project (see Chapter 3). In addition, unpublished echolocation calls of known bat species held by Professor P. Taylor and Professor D. Parker were also included to supplement the call reference library. The call reference library was used as an identification guide for the development of the KNP-specific classifier (Taylor et al. 2013b). The call reference library consisted of call sequences from 516 individual bats of 29 species from seven families (Appendix 4.1).

To develop the KNP-specific classifier, a subset of 8,118 individual bat call sequences were used. The classifier is a function used as a filter to speed up sorting and subsequent final identification of each call. The 8,118 recordings were collected during the first night of sampling in 2017 from nine sites across the Pafuri area. Using the built-in “cluster tool” of Kaleidoscope, the 8,118 recordings were clustered/classified into 43 initial clusters based on their call characteristics. Call characteristics included total duration of the call (Dur), characteristic frequency (Fc), minimum call frequency (Fmin), maximum call frequency (Fmax), and frequency at the slope of the call (Fk) (Monadjem et al. 2010). Each of the individual bat call sequences within each of the newly recognized clusters was then manually identified to species

level using the call reference library. Recorded call sequences were only positively identified/labelled to species if there was a sequence of three or more bat calls. If a call sequence could not be positively identified, it was left blank in the results spreadsheet of Kaleidoscope. Call sequences were also left blank when there was clearly more than one bat species that had been recorded in a call sequence. This conservative approach was adopted to avoid committing a Type I statistical error (Clement et al. 2014, Parker & Bernard 2018) and to improve the overall accuracy of the classifier (see below). All manual species assignments were then verified by two additional observers (Professor D. Parker and Professor P. Taylor). Using these new species identifications, the "cluster tool" in Kaleidoscope was then re-run on the same subset of call sequences to produce a final .kml file (i.e. the KNP-specific classifier) that could be used to cluster all echolocation call recordings from each site (over both years) into putative species categories based on their call characteristics.

All acoustic data collected from each site (during each fieldtrip) was then processed through Kaleidoscope and putatively identified to species or as "noise". Kaleidoscope, through the characteristic of the KNP-specific classifier, considered noise to be any recordings that were blank, had low-quality call sequences (less than three individual bat calls), or files that did not have any call sequences recorded (i.e. background noise). Even though Kaleidoscope automatically identified each recording, the software is not completely accurate (Rydell et al. 2017). Therefore, I manually verified each putative species identification made by Kaleidoscope using the call reference library and the conservative criteria described above. Using this procedure, passing bats could be identified to species and the accuracy of the KNP-specific classifier (i.e. % of correct identifications) could be assessed. To keep the identification

consistent throughout the study and to fully test the accuracy of the classifier, new species and unidentifiable echolocation calls were not added to the classifier tool.

Assignment of species

Over the two-year survey of northern KNP, 22 species and two unknown set of calls were documented through the use of acoustic detectors (see results below). Of the 22 species, I am most confident in the identification of 15 species (*Eptesicus hottentotus, Hipposideros caffer*, *Miniopterus natalensis, Mops midas*, *Myotois tricolor*, *Neoromicia nana, Otomops martiensseni*, *Pipistrellus hesperidus, Pipistrellus rusticus, Rhinolophus darlingi*, *Rhinolophus fumigatus, Rhinolophus hildebrandtii*, *Rhinolophus simulator*, *Scotophilus dinganii* and *Taphozous mauritianus*). These species have echolocation calls that are distinctive in terms of the characteristics within each call (e.g. shape, frequency, and or duration) which makes it unlikely that they were incorrectly identified (Appendix 4.1, 4.2). *Taphozous mauritianus,* for example, calls at a frequency similar to *Chaerephon pumulis* but it is unlikely to be incorrectly identified due to its unique shape and a very short call duration (Appendix 4.2) (Monadjem et al. 2010). The remaining seven species (*Chaerephon ansorgei*, *Chaerephon pumulis, Mops condylurus, Tadarida aegyptiaca*, *Neoromicia capensis, Neoromicia zuluensis,* and *Nycticeinops schlieffeni*) were challenging to assign to species level due to substantial overlap in call parameters (Appendix 4.1, Appendix 4.2). Both *Chaerephon ansorgei* and *Tadariada aegyptiaca* echolocation calls are similar in frequency, causing difficulty in distinguishing between the two species. Similarly, *Chaerephon pumulis* and *Mops condylurus* proved to be a challenge for the same reason. In addition, *Neoromicia capensis* has a broad range of frequencies, resulting in an overlap in call parameters between both *Neoromicia zuluensis* and *Nycticeinops schlieffeni*. The call reference library (Appendix 4.1) included variables of many parts of a call including

duration (length of call), Fmin (lowest frequency), Fmax (highest frequency), Fk (change in slope), and Fc (flattest portion); all which were used in the identifying of calls from species that generally overlap each other in frequencies. As a result, I tried to reduce the error involved in the assignment of species identifications for the bats that overlapped significantly in terms of their call characteristics.

Species richness and heterogeneity measurements

Bat species richness and diversity were determined in 2017, 2018, and over the two years combined. Species richness is the number of different species residing together in the same area (Derry et al. 1998). Species richness is calculated by counting the number of species found within a region (Magurran 2011). Diversity is defined as a function of species richness and evenness and is commonly calculated by Shannon-Wiener diversity index which estimates diversity (Peet 1974). Evenness is the equability of species within a community (Derry et al. 1998). Both the Shannon-Wiener diversity index and the Shannon evenness were calculated for each year to determine any changes in bat communities over time. The Shannon-Wiener index was calculated using the following equation:

$$H' = -\sum_{i=1}^{s} p_i ln p_i$$

and Shannon evenness using the equation:

$$J' = H'/H_{max} (H_{max} = log_2 s)$$

where p_i is the relative abundance of species, i, ln is the natural log of p_i, and s is the total number of species found (Pearson & Rosenberg 1977, Magurran 2011). The Shannon-Wiener index was calculated using R studio (R Core Team 2017). The package 'vegan' was used to

calculate diversity through the Shannon-Wiener equation with the code written as diversity(file name $ Captured, index = “shannon”) (Oksanen et al. 2018). The package ‘vegan’ is used for descriptive community ecology for analysis of diversity, community ordination, and dissimilarity evaluation (Oksanen et al. 2018). Shannon evenness was calculated manually once diversity had been determined.

Species accumulation curve

A species accumulation curve was constructed to provide a meaningful interpretation of the species richness for northern KNP (Flaquer et al. 2007). A species accumulation curve is a linear model that calculates an estimated number of species within an area compared to the number of sample sites surveyed (Skalak et al. 2012). The model presumes that as the number of sample sites increases, the number of observed new species within the study area will decrease (Skalak et al. 2012). EstimateS 9.10 software (Colwell & Elsensohn 2014) was used to generate the individual-based accumulation curve (Chao et al. 2000). EstimateS is a programme that calculates and compares the various diversities and species richness within a sample site (Colwell & Elsensohn 2014). A .txt file was created to be placed into EstimateS that contained the number of passes from each species recorded from each site. Using the .txt file, EstimateS computes a mean and variance for species richness by randomly selecting a sample size from the total data sample to generate a S(est), Incidence Coverage-based Estimator (ICE), and Chao2 line (Flaquer et al. 2007, Parker & Bernard 2018). S(est) is the calculated species richness from the observed data (Parker & Bernard 2018). Both ICE and Chao2 calculate the sampling efficiency to generate an estimated species richness within the study area (Parker & Bernard 2018).

Bray-Curtis cluster

A Bray-Curtis cluster analysis was used to determine the difference between sampling sites based on the presence/ absence of species recorded. Bray-Curtis is a form of multivariate analysis that is calculated based on the similarities of a variable between two defined groups (Clarke et al. 2006). The software programme PAST (Paleontological Statistics) (Hammer et al. 2001) was used to calculate the Bray-Curtis cluster. PAST is a programme used to generate statistical and graphical algorithms (Hammer et al. 2001). Community composition was compared between sample sites in Pafuri (labelled PA) and Punda Maria/ Shingwedzi (labelled PS) to determine the similarity of species presence between the two regions.

Activity index

Bat activity was measured based on the presence or absence of calls sequences per hour after sunset for each bat species (Parker & Bernard 2018). Activity of bats presence over an individual time block can be calculated using the Activity Index (AI) formula:

$$AI = \sum_{\bar{e}}^{n} P^*$$

were P* is the presence of a species within the sum of each time block (n) and divided by the time period of collected data ($\bar{e}$) (Skalak et al. 2012). AI was used to compare the activity of each insectivorous bat family. Due to not being completely confident in the identification of seven species (*Chaerephon ansorgei*, *Chaerephon pumulis*, *Mops condylurus*, *Tadarida aegyptiaca*, *Neoromicia capensis*, *Neoromicia zuluensis,* and *Nycticeinops schlieffeni*; see above), the AI was simplified into families. Calculating AI was also used as a way to compare total bat activity between both years within the study area. AI is not calculated based on the

frequency of calls but rather the presence or absence of a species (1 or 0) in each hour after sunset for each site (Parker & Bernard 2018). Time blocks were labelled based on the number of hours after sunset (i.e. 0 – 13). The number of individual presence records (i.e. the number of "1s") per time block were summed and divided by the total number of hours (i.e. time blocks) sampled per night (Parker & Bernard 2018). For example, if the Rhinolophidae were recorded as being present in three of the time blocks in a night, the family would have received an AI score of 0.23 (=3/13) for that night.

4.3 Results

Accuracy of Kaleidoscope classifier tool

The classifier tool in Kaleidoscope software had a relatively high average accuracy in terms of identifying echolocation calls to species. The classifier tool had an average percent accuracy of 79.3% in 2017. The 2018 survey had a slightly higher percentage accuracy (80.0%). Overall, the classifier tool demonstrated a 79.7% accuracy in correctly identifying the echolocation calls to species (Figure 4.3).

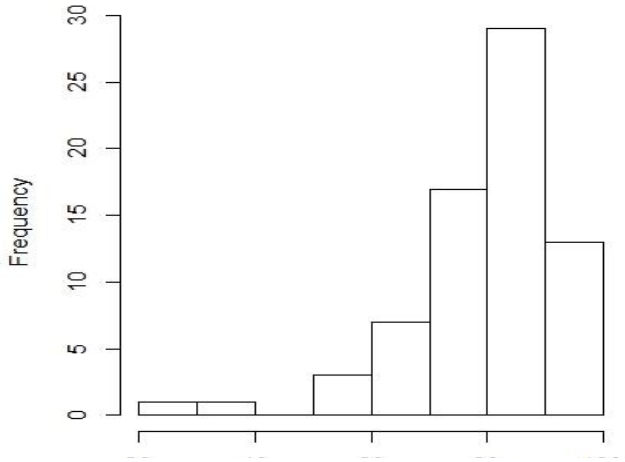

Figure 4.3: Frequency histogram of 'Accurate' identifications for both 2017 and 2018 combined. The x-axis represents the eight percentage accuracy categories. Using each site's acoustic data (i.e. all acoustic recordings from one site during one fieldtrip), the y-axis represents the number of times each percentage accuracy category was achieved.

The cluster tool demonstrated to have a higher identification accuracy for the families Hipposideridae (99.5%) and Miniopteridae (98.8%). The families Molossidae (83.7%) and Vespertilionidae (83.4%) both had the fairly high identification accuracy. The identification of Rhinolophidae (60.2%) and Emballonuridae (51.8%) had the lowest accuracy percentage.

Species richness and heterogeneity measurements

In 2017, a total of 71,512 echolocation call sequences were recorded within the Pafuri area. Twenty-two species, representing six families (Emballonuridae, Hipposideridae, Miniopteridae, Molossidae, Rhinolophidae, and Vespertilionidae), and one unknown call, labelled Unknown 75, were recorded (Table 4.3). The family Molossidae was the dominant family with 57,896 calls recorded from six species (*Chaerephon ansorgei*, *Chaerephon pumilus*, *Mops condylurus*, *Mops midas*, *Otomops martiensseni* and *Tadarida aegyptiaca*). The most echolocation calls recorded were in October 2017 (21 species) with 25,856 recordings (Table 4.3). The fewest bat echolocation calls were recorded during August 2017 (22 species) with 7,640 recordings (Table 4.3).

In 2018, a total of 48,982 echolocation call sequences were recorded within the Punda Maria/ Shingwedzi area. Twenty-two species, representing six families (Emballonuridae, Hipposideridae, Miniopteridae, Molossidae, Rhinolophidae and Vespertilionidae), and two unknown calls, labelled Unknown 75 and Unknown 34, were recorded (Table 4.3). The family Molossidae was again the dominant family with 32,785 call sequences recorded from six species (*Chaerephon ansorgei, Chaerephon pumilus, Mops condylurus, Mops midas, Otomops martiensseni* and *Tadarida aegyptiaca*). The most echolocation calls were recorded during March 2018 (22 species) with 18,556 recordings (Table 4.3). The fewest bat echolocation calls were recorded during October 2018 (21 species) with 6,300 recordings (Table 4.3).

Table 4.3: Bat species recorded within the northern parts of the Kruger National Park in 2017. The numbers for each month are the total number of calls recorded for that particular trip. 'Total Detection' represents the total number of calls for each species.

Family	Species	March/April	May	August	October	2017 Total Detection
Emballonuridae	*Taphozous mauritianus*	404	256	23	212	895
Hipposideridae	*Hipposideros caffer*	375	193	284	231	1083
Miniopteridae	*Miniopterus natalensis*	88	143	154	226	611
Molossidae	*Chaerephon ansorgei*	1659	1293	1205	5492	9649
	Chaerephon pumilus	3212	1741	750	2333	8036
	Mops condylurus	5838	3511	624	5133	15106
	Mops midas	534	1847	1251	2049	5681
	Otomops martiensseni		36	1	2	39
	Tadarida aegyptiaca	3039	6436	1792	7329	18596
Rhinolophidae	*Rhinolophus darlingi*	1				1
	Rhinolophus fumigatus	98	74	74	61	307
	Rhinolophus simulator	12	7	26	11	56
	Rhinolophus smithersi	197	856	51	90	1194
Vespertilionidae	*Eptesicus hottentotus*	549	282	45	318	1194
	Myotois tricolor	25	3	71	5	104
	Neoromicia capensis	132	96	400	322	950
	Neoromicia nana	2165	636	483	1279	4563
	Neoromicia zuluensis	27	29	164	149	369
	Nycticeinops schlieffeni	87	34	50	86	257
	Pipistrellus hesperidus	27	4	4		35
	Pipistrellus rusticus	5	2	26	21	54
	Scotophilus dinganii	1052	176	162	505	1895
Unknown	Unknown 34					
	Unknown 75	31	2	3	2	38
	Grand Total	19557	17657	7643	25856	70675
	No. Species	22	22	22	21	23

Table 4.4 continue: Bat species recorded within the northern parts of Kruger National Park in 2018. The numbers for each month are the total number of calls recorded for that particular trip. 'Total Detection' represents the total number of calls for each species.

Family	Species	March	May	September	October	2018 Total Detection	Total Detection
Emballonuridae	*Taphozous mauritianus*	516	36	41	10	624	1519
Hipposideridae	*Hipposideros caffer*	24	35	6	197	262	1345
Miniopteridae	*Miniopterus natalensis*	338	461	615	259	1673	2284
Molossidae	*Chaerephon ansorgei*	691	899	1006	1064	3660	13309
	Chaerephon pumilus	3379	332	2291	343	6345	14381
	Mops condylurus	5146	338	2749	151	8384	23490
	Mops midas	272	386	392	321	1371	7052
	Otomops martiensseni	2		1	30	33	72
	Tadarida aegyptiaca	3926	2196	4817	2053	12992	31588
Rhinolophidae	*Rhinolophus darlingi*	1	2		30	33	34
	Rhinolophus fumigatus	25	4	15	5	49	356
	Rhinolophus simulator	24	26	104		154	210
	Rhinolophus smithersi	433	30	69	9	541	1735
Vespertilionidae	*Eptesicus hottentotus*	537	310	354	69	1270	2464
	Myotois tricolor	70	1	17	10	98	202
	Neoromicia capensis	1435	233	2165	554	4387	5337
	Neoromicia nana	244	405	887	222	1758	6321
	Neoromicia zuluensis	512	187	1156	468	2323	2692
	Nycticeinops schlieffeni	412	22	400	385	1219	1476
	Pipistrellus hesperidus			14		14	49
	Pipistrellus rusticus	165	313	105	12	593	649
	Scotophilus dinganii	1083	304	170	102	1659	3554
Unknown	Unknown 34	12	3	67	6	88	88
	Unknown 75		1	2		3	41
	Grand Total	18719	6520	17443	6300	48982	119657
	No. Species	22	22	23	21	24	24

The 2017 survey had a higher abundance of echolocation calls compared to the 2018 survey, but there was no statistically significant difference between the two years ($t = 1.08$, $df = 5.92$, $P > 0.05$). Even though a greater number of calls were recorded in 2017 (Table 4.3), species diversity was higher in 2018. In 2018, the species diversity was 2.34 (standard deviation of 0.14), slightly higher than the species diversity in 2017 (2.14; standard deviation of 0.17). However, the difference between the two surveys was not statistically different ($t = -1.73$, $df = 3.16$, $P > 0.05$). The 2018 survey had a slightly higher Shannon evenness measurement (0.51; standard deviation of 0.02) compared with 2017 (0.47; standard deviation of 0.05) but the value differences were not statistically significant ($t = -1.60$, $df = 3.62$, $P > 0.05$). Combined, a total of 119,657 echolocation calls were recorded from 22 species and two unknown species over the two years of study (Table 4.3). In addition, the study area, as a whole, had a diversity of 2.28 and evenness of 0.50.

Species accumulation curve

In 2017, there were 11 sample sites within the Pafuri region. From the 11 sites, 23 species (S(est)) were detected. The S(est)) line demonstrates that species richness no longer increased passed the 9th sample site after 23 species were detected (Figure 4.4). The Chao 2 (22.63) and ICE (23.03) estimators indicated a completeness of sampling after the inclusion of the 10th sampling site (Figure 4.4). Both the Chao 2 and ICE estimators suggest that sampling 10 sites should observe most of the detectable species within the Pafuri region.

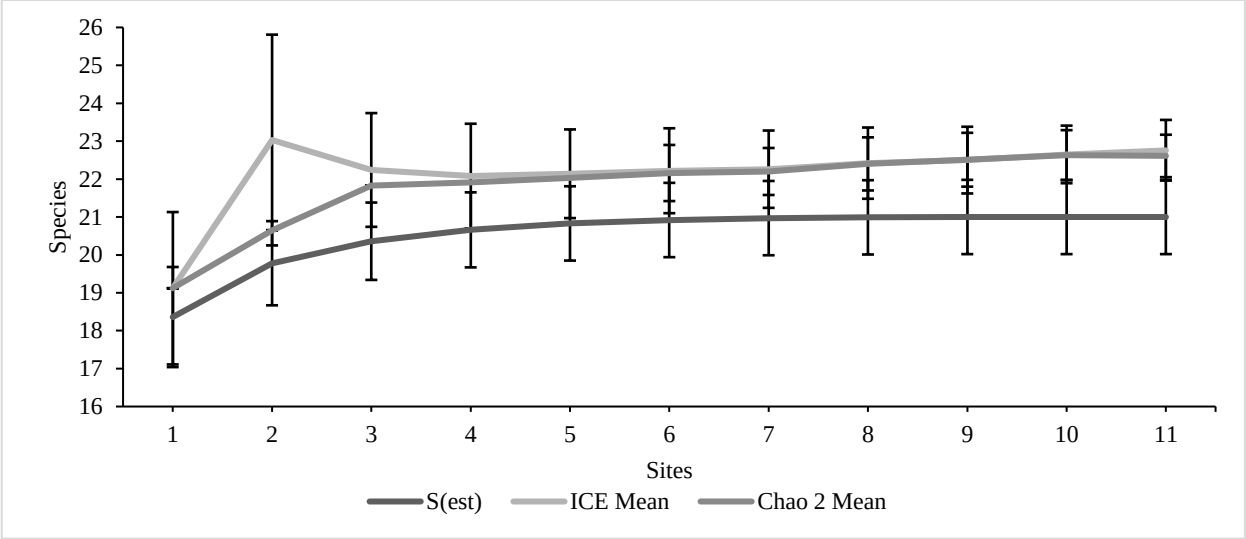

Figure 4.4: Species accumulation curve for acoustic data recorded within the Pafuri region. S(est) is the observed data and ICE mean and Chao 2 mean are the estimated species richness. Error bars represent standard deviation.

In 2018, there were 13 sites sampled within the Punda Maria/ Shingwedzi region. From the 13 sites, 24 species (S(est)) were detected. The S(est)) line demonstrates that species richness no longer increased passed the 12th sample site after 24 species were detected (Figure 4.5). The Chao 2 (23.87) estimator indicated a completeness of sampling after the inclusion of the 13th sampling site. However, the ICE (26.55) estimator indicated that the survey missed approximately two species over the 13 sampling sites (Figure 4.5).

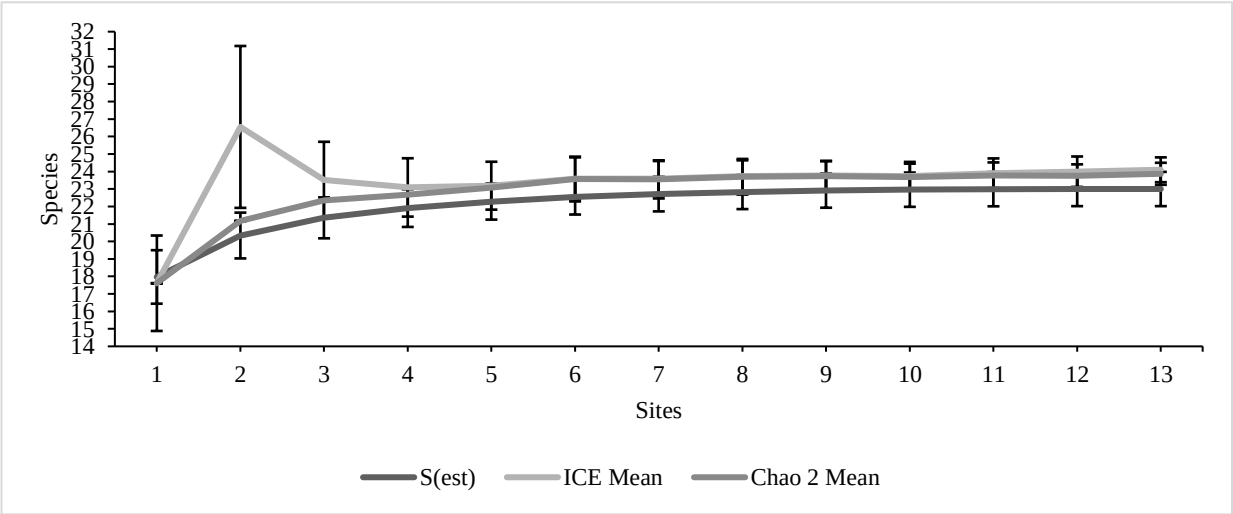

Figure 4.5: Species accumulation curve for acoustic data recorded within the Punda Maria/ Shingwedzi region. S(est) is the observed data and ICE mean and Chao 2 mean are the estimated species richness. Error bars represent standard deviation.

A total of 24 sites were sampled using acoustic detectors within northern KNP over a two-year period (Table 4.1; Table 4.2). From the 24 sample sites, 24 species (S(est)) were detected. The S(est) line demonstrates that species richness no longer increased passed the 20th sample site after 24 species were detected (Figure 4.6). The S(est) curve suggests that sampling 20 locations should observe most of the detectable bat species within the study area. Chao 2 (24.06) estimator indicated completeness of sampling after the inclusion of the 11th sampling site (Figure 4.6). However, the ICE (24.98) estimator indicated that the survey missed approximately one species over the 24 sampling sites (Figure 4.6).

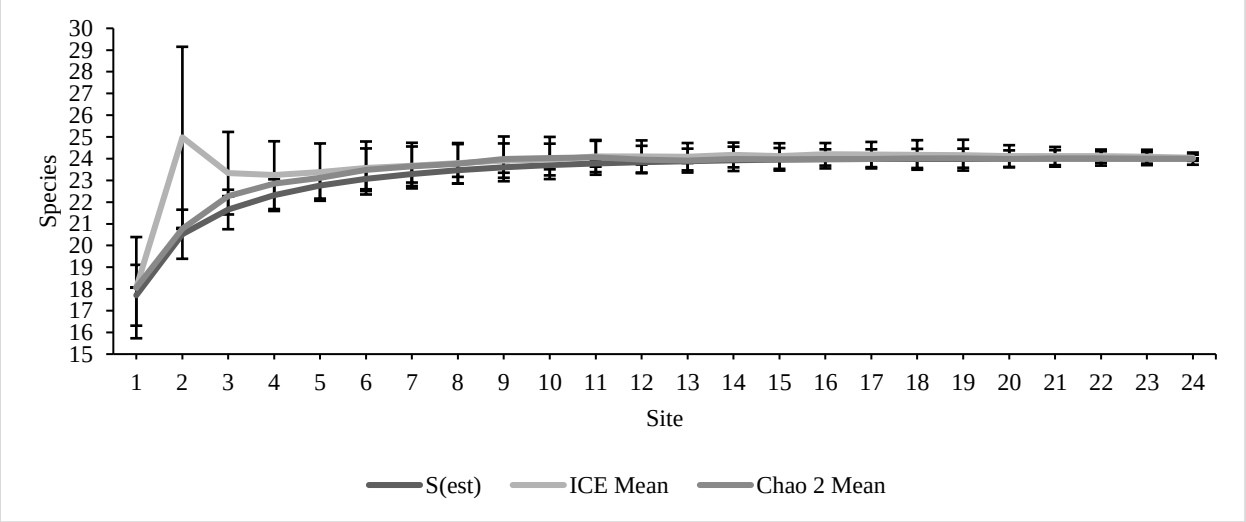

Figure 4.6: Species accumulation curve for all acoustic data recorded within northern Kruger National Park. S(est) is the observed data and ICE mean and Chao 2 mean are the estimated species richness. Error bars represent standard deviation.

Bray-Curtis cluster

A Bray-Curtis cluster analysis was used to determine the difference between sampling sites based on presence-absence of species recorded. The Pafuri sites (labelled PA) were about 90 percent similar in recorded species while the Punda Maria/ Shingwedzi (labelled PS) had about 85 percent similarity of recorded species (Figure 4.7). There was about an eight percent

difference in species presence between the regions Pafuri and Punda Maria/ Shingwedzi during the time of the study. The study area as a whole had about 87% similarity in species presence between all sample sites (Figure 4.7).

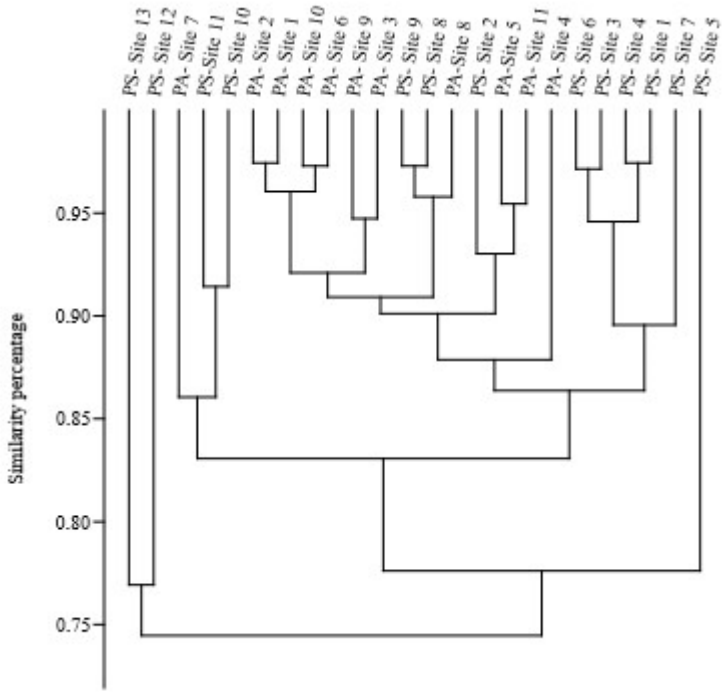

Figure 4.7: Cluster analysis of Bray-Cutis distance based on presence-absence of species. PA represent sites sampled in the Pafuri region. PS represent sites sampled in the Punda Maria/Shingwedzi region.

Activity index

In 2017, the family Molossidae was the most detected group with the highest activity levels and these remained fairly consistent throughout each sample night (Figure 4.8). The Vespertilionidae had the second highest activity levels with a spike at the first hour after sunset then gradually decreasing until a second peak in activity at the eleventh hour. The unknown species labelled as Unknown 75 had the lowest activity with a small peak at the second hour after sunset. All other families had similar activity levels with peaks ranging between the first

and second hour and remaining relatively consistent throughout the nights sampled (Figure 4.9). Both the Rhinolophidae and Miniopteridae had a small secondary peak ranging between the tenth and eleventh hour after sunset.

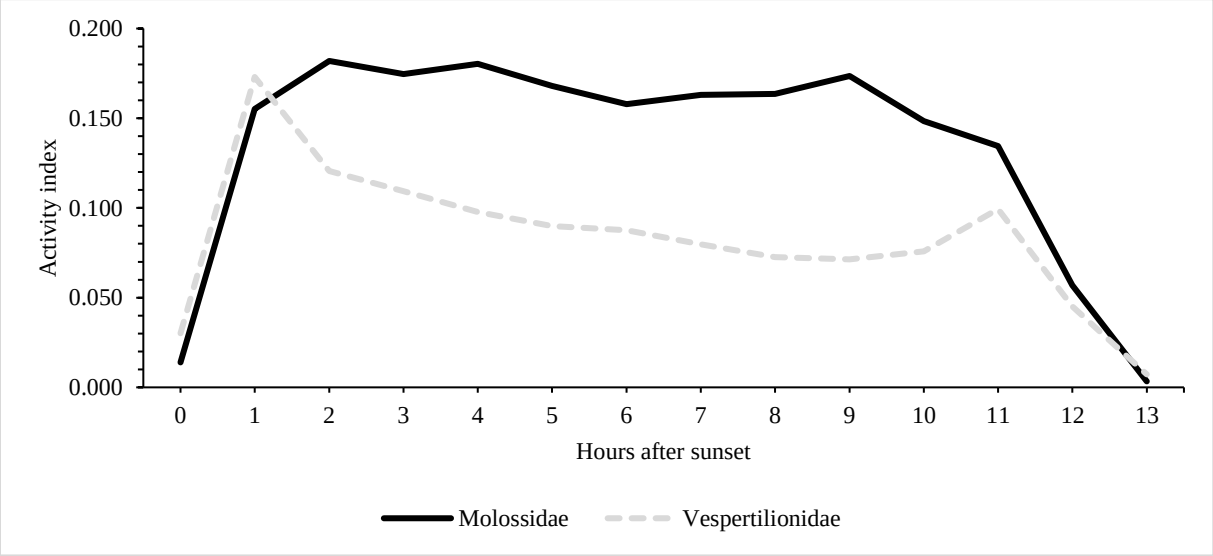

Figure 4.8: Changes in bat activity for the families Molossidae and Vespertilionidae from 11 sites (148 sampling nights) in 2017 within the northern Kruger National Park.

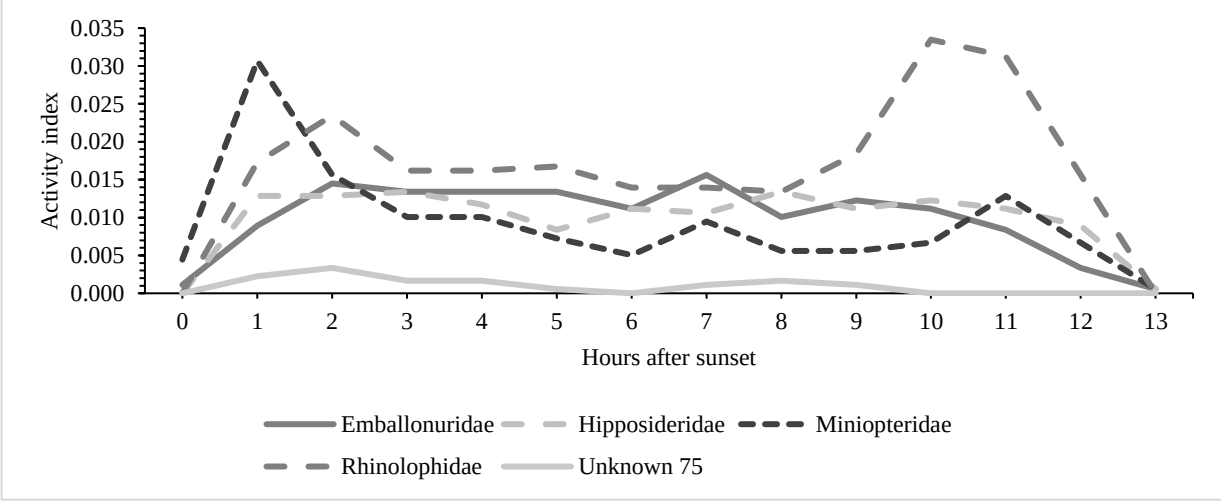

Figure 4.9: Changes in bat activity for the families Emballonuridae, Hipposideridae, Miniopteridae, Rhinolophidae, and Unknown 75 from 11 sites (148 sampling nights) in 2017 within northern Kruger National Park.

In 2018, the family Vespertilionidae had the second highest activity level after the family Molossidae which showed to have the greatest activity levels with a peak at the first hour after sunset and remained consistent throughout the night (Figure 4.10). The Vespertilionidae had the highest peak in activity at the first hour after sunset and then decreased throughout the night until a secondary peak in activity at the twelfth hour (Figure 4.10). The Miniopteridae showed the third highest activity levels that remained consistent through the first and fourth hours and then gradually decreased throughout the rest of the nights sampled (Figure 4.11). The unknown species labelled as Unknown 75 had the lowest activity level with a small peak at the first and fifth hours after sunset. All other families had similar activity levels with peaks ranging between the first and third hours and remaining consistent throughout the nights sampled (Figure 4.11). The Rhinolophidae, Emballonuridae, and Unknown 34 had a small secondary peak between the eleventh and twelfth hour after sunset.

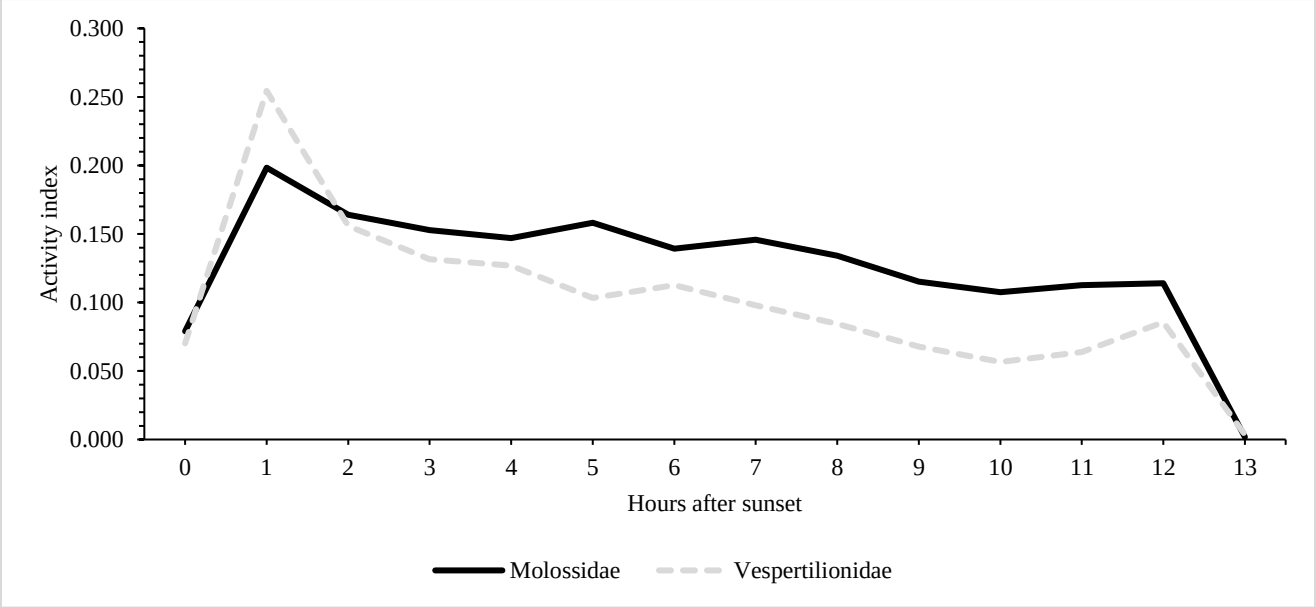

Figure 4.10: Changes in bat activity for the Molossidae and Vespertilionidae from 13 sites (130 sampling nights) in 2018 within the northern Kruger National Park.

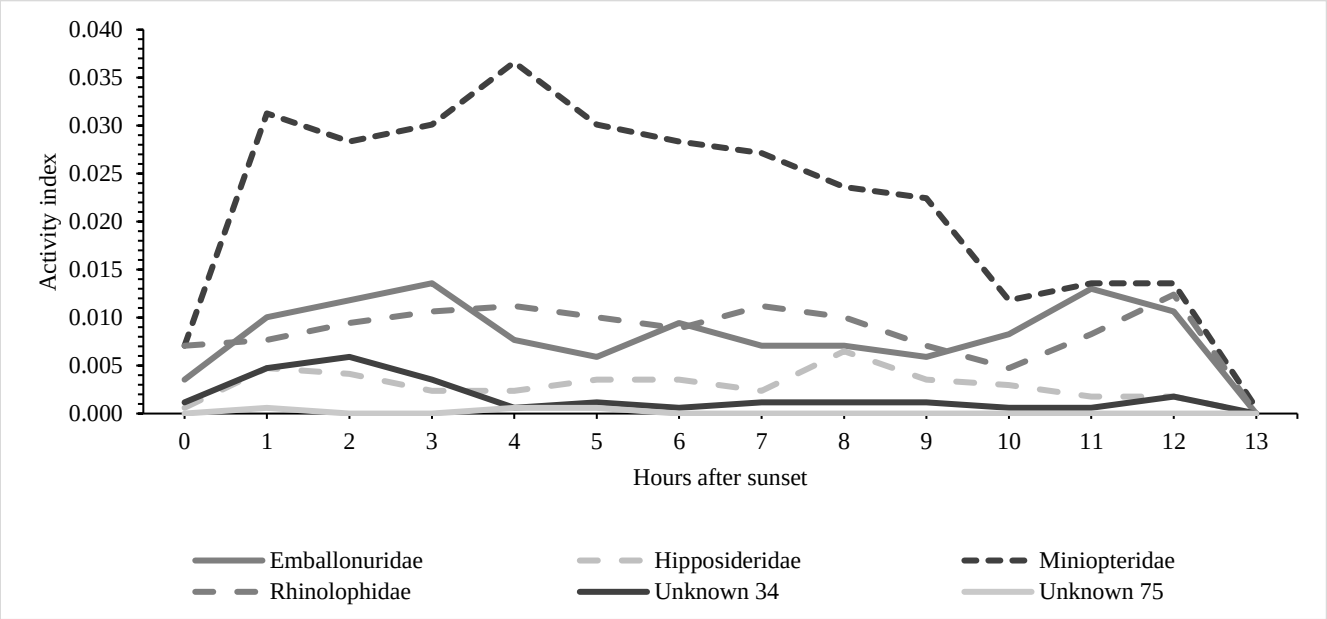

Figure 4.11: Changes in bat activity for families of Emballonuridae, Hipposideridae, Miniopteridae, Rhinolophidae, Unknown 34, and Unknown 75 from 12 sites (130 sampling nights) in 2018 within northern Kruger National Park.

Overall, bat activity levels showed similar trends across the two years of the survey with the highest activity peak at the first hour and then gradually declining as the night progressed with a steep decline in activity after the eleventh hour (Figure 4.12). Compared to 2017, 2018 had the highest activity peak at the second hour after sunset. In addition, 2018 had a secondary activity peak between the eleventh and twelfth hours after sunset.

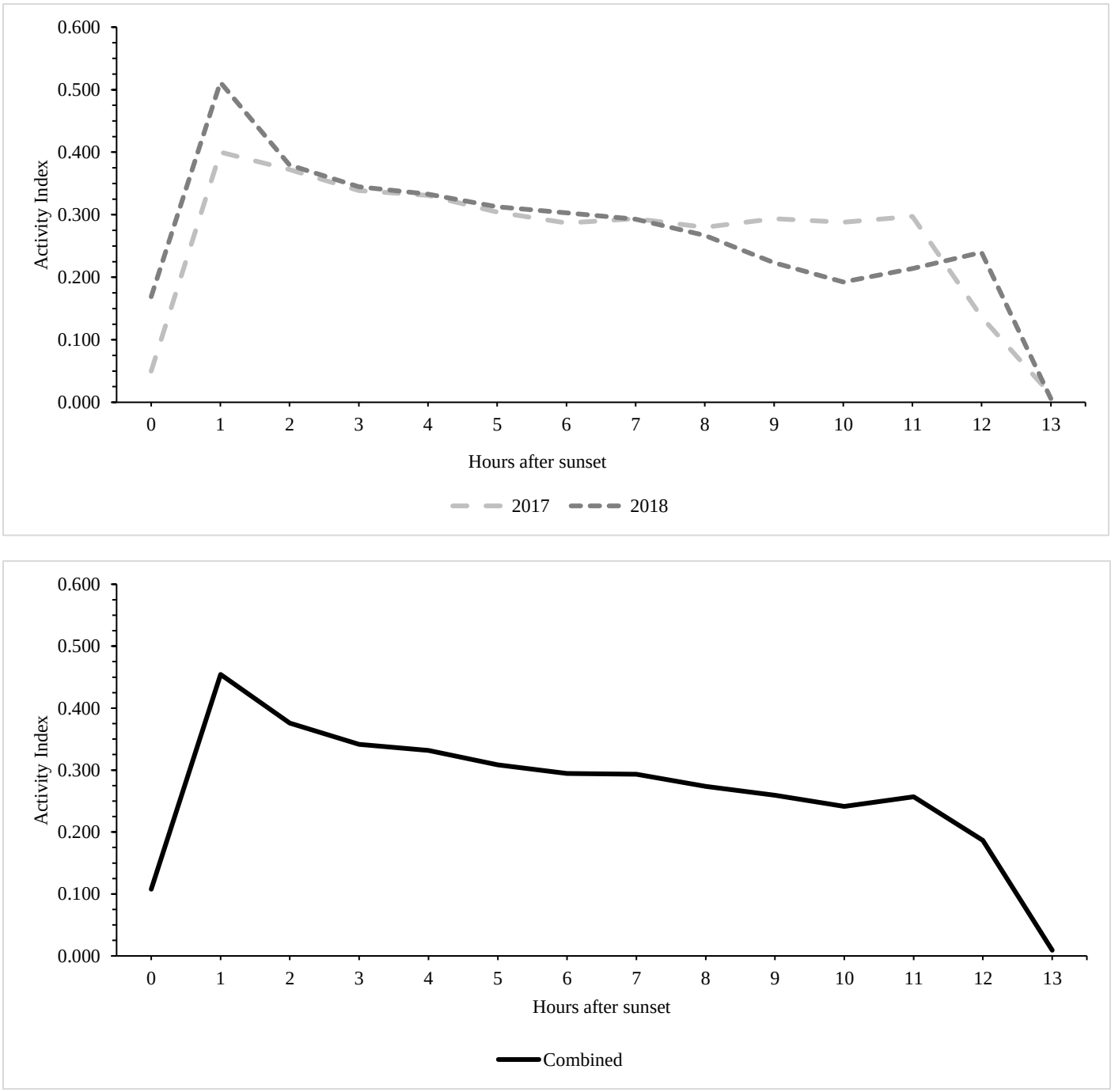

Figure 4.12: Changes in overall nightly bat activity in 2017 and 2018 with the bat activity of both years combined within northern Kruger National Park. The light grey line represents 2017 and the dark grey line represents 2018. The black solid line represents both years combined.

4.4 Discussion

Accuracy of Kaleidoscope classifier tool

Even though the Kaleidoscope classifier tool had a relatively high percentage accuracy (~ 79%), manual identification was still required to avoid false-positives (Clement et al. 2014). The programme was successful in clustering all processed calls into species groups based on the classifier tool (Rydell et al. 2017). Kaleidoscope was effective in identifying calls from common bat species such as *Mops condylurus* and *Neoromicia nana*, as well as the identification of calls that are distinctive in terms of their characteristics (e.g. frequency and/or duration) which makes them unlikely to be incorrectly identified (e.g. *Hipposideros caffer*). However, Kaleidoscope was not as successful in distinguishing between common and non-common species that shared similar call characteristics of frequency and duration (Rydell et al. 2017). *Myotois tricolor*, for example, is a non-common species and although it was placed into the classifier tool as its own group, many calls were misidentified as *Neoromicia capensis*, a common species, due to the similarities in the two species' call parameters (Monadjem et al. 2010). It is possible to minimize misidentification of non-common species when using the automatic identifier by placing more calls of each non-common species into the cluster tool (Rydell et al. 2017). This will enable the classifier tool to better distinguish between the differences in each species, thus minimizing misidentification (Rydell et al. 2017). In addition, the classifier tool misidentified species, such as *Taphozous mauritianus* and *Rhinolophus darlingi*, that overlapped frequencies with other species but has a unique call shape. When using the cluster tool, Kaleidoscope identifies all calls based only on the species used within the classifier tool (Rydell et al. 2017). This reliance can cause false-positives of species not included into the tool but were detected during the study (Rydell et al. 2017). For example, *Otomops martiensseni* was not recorded in

the 8,118 calls used to construct the cluster tool and was therefore always initially identified as *Mops midas* when processed. *Otomops martiensseni* has only been recorded for KNP once before my survey (Adams et al. 2015) and the current calls were detected through manual identification. Thus, after files are automatically identified, manual identification is important to minimize misidentification of non-common species and for the detection of species not included in the cluster tool (Rydell et al. 2017). Calls manually identified as non-common species can be collected through a study and placed into the classifier tool to keep the tool updated while aiding in improving the accuracy. Despite these limitations, the Kaleidoscope cluster tool was extremely valuable in streamlining the identification and processing of large volumes of bat calls from multiple sites (Rydell et al. 2017).

Species richness and heterogeneity measurements

The abundance of recorded echolocation calls in the 2017 survey (located in the Pafuri region) was higher than the abundance of calls recorded in the 2018 survey (located in the Punda Maria/ Shingwedzi region). Ecosystems, biological aspects, and sampling limitations could have all played a role in these observed differences (McCain 2007). Compared to the Punda Maria/ Shingwedzi region, many riparian forests are located within the Pafuri region along the Limpopo and Luvuvhu rivers (Monadjem & Reside 2008). Riparian forests likely provided ample hunting opportunities for bats because of increased insect activity over the water. In addition, large trees growing within the riparian forests are used as roosting sites for many bat species, such as *Nycticeinops schlieffeni* (Monadjem et al. 2010). Certain species of bats, such as *Pipistrellus rusticus*, are also believed to be restricted to riparian forests and are rarely found beyond this habitat (Monadjem & Reside 2008). In comparison, the decrease in abundance of recorded calls in 2018 could have been caused by the domination of mopani trees

(*Colophospermum mopane*) with in the Punda Maria/ Shingwedzi region (Mucina & Rutherford 2006). Mopani trees do not grow as large as many riparian trees and are thus unlikely to provide adequate roosts and/or prey for as many bat species (Monadjem & Reside 2008).

The presence of large water sources, such as rivers, are known to influence the abundance of bats (Downs & Racey 2006). Due to their large size, rivers have increased insect activity compared to smaller pans and streams which can influence the abundance of insectivorous bats (Downs & Racey 2006). Vegetation in riparian habitats, such as trees, grow larger around rivers and provide an increase in prey, roosting sites, and cover for protection for many bat species (Downs & Racey 2006). The Pafuri region has two major perennial rivers, the Limpopo and the Luvuvhu rivers, while the Punda Maria/ Shingwedzi region has only one major seasonal river, the Shingwedzi River (Roux et al. 2008). Bat detectors were placed around the major rivers within the Pafuri region in 2017 while detectors were placed around the Shingwedzi River, streams, and pans within the Punda Maria/ Shingwedzi region. Due to having smaller water source, vegetation grows smaller in riparian habitats around pans and streams thus correlating a decline in prey activity and roosting sites along with a decrease in bat abundance within the Punda Maria/ Shingwedzi region (Downs & Racey 2006).

The placement of bat detectors could also have influenced the abundance of calls recorded. In 2017, sampling sites were all located along both the Limpopo and the Luvuvhu rivers. One detector was placed on the Luvuvhu River bridge that could have been a roosting site for many bat species and resulted in an increase in call abundance in 2017. The Luvuvhu bridge site had 28,872 calls recorded and made up 40.4% of the total call abundance in 2017. In 2018, sample sites were located around various water sources such as pans, streams, and the Shingwedzi River. The decrease in bat calls in 2018 could have therefore been caused by the

sampling around smaller water sources and the decline in water in the Shingwedzi river during the dry season (Ashton et al. 2001, McCain 2007).

Even though the 2017 survey had a higher abundance in recorded calls, the 2018 survey showed a slightly higher diversity and evenness. Evenness is based on how close the total number of each species are to each other at the time of sampling; the larger the Shannon evenness product, the more even the species are to each other (Derry et al. 1998). In 2018, the abundance of calls recorded from each species were closer in total number compared to the 2017 survey. The 2018 survey had a slightly higher species richness due to the recording of an unknown echolocation call labelled Unknown 34 that was not recorded in the 2017 survey. The Shannon-Wiener diversity index equation factors both evenness and species richness within the equation to determine higher or lower results (Peet 1974). The 2018 survey had a higher species richness recorded as well as a greater Shannon evenness; both of which could have caused the diversity to be greater in 2018 than in 2017.

Biological behaviours of bat species, such as roost selections and frequency of echolocation calls, could have further affected the observed difference in the number of recorded calls between families in the combined survey. The Molossidae was the dominating family with 89,892 recorded calls from both years combined. Molossidae have some of the most common species in southern African and at least six species are known to inhabit KNP (Monadjem et al. 2010). Roosts can number from a few to hundreds of individuals making them very common around buildings and bridges (Vivier & van der Merwe 2007). The Molossidae are open-air flyers (i.e. they fly above tree canopy) and use low frequency calls ranging between 9 and 27 kHz to search for food (Norberg & Rayner 1987, Monadjem et al. 2010). Low frequency calls do not attenuate as quickly as higher frequencies and thus are capable of traveling further distances

(Monadjem et al. 2017). Due to their ability to call at relatively low frequencies, species from the family Molossidae can easily be detected through ultrasonic microphones up to a maximum distance of 30 m (Monadjem et al. 2017). The family Molossidae was most likely the dominant family due the large number of individuals within roosts and their ability to call at low frequencies, inflating their prevalence amongst the bat calls recorded (Monadjem et al. 2010, 2017).

The family Vespertilionidae was the second most dominant family with 22,744 calls recorded. The Vespertilionidae is one of the largest families of insectivorous bats and has about 19 species known to inhabit KNP (Hoofer & Bussche 2003, Monadjem et al. 2010). Species of the Vespertilionidae family are clutter-edge foragers that fly between open air and dense vegetation with echolocation calls at intermediate frequencies (Lee & McCracken 2004, Russo et al. 2018). Within the family, frequencies range between 27 and 73 kHz with short durations ranging between 2 to 5 milliseconds (Appendix 4.1). Compared to the family Molossidae, the Vespertilionidae have higher frequency calls with a maximum detection distance of 10 to 15 m (Monadjem et al. 2017). Due to their foraging behaviours and the ability to be detected from a reasonable distance, the family Vespertilionidae are also commonly recorded using bat detectors.

The family Hipposideridae was the family with the fewest calls (1,345 calls recorded). The Hipposideridae is a small family with three species (*Cloeotis percivali*, *Hipposideros caffer*, *Hipposideros vittatus*) found within KNP (Monadjem et al. 2010). The family hunts for prey within dense vegetation and uses high frequency echolocation calls ranging between 66 and 208 kHz (Monadjem et al. 2010). *Hipposideros caffer* was the only species from this family to be recorded using the acoustic detectors. With the highest call frequencies recorded

during the survey, *Hipposideros caffer* has the shortest detection range of about 0.3 m and was most likely only recorded when an individual flew close to the microphones (Monadjem et al. 2017, Parker & Bernard 2018). The echolocation calls of *Cloetis percivali* and *Hipposideros vittatus* were not recorded during the acoustic survey. *Hipposideros vittatus* mostly occurs in countries north of South Africa with Pafuri being the southern border of their distribution and are dependent on caves to roost (Monadjem et al. 2010). The combination of distribution and bat detectors not being placed near caves most likely caused for the species not to be recorded within the survey. *Cloetis percivali* is not abundant and is sparsely distributed in the north-eastern portions of South Africa (Monadjem et al. 2010). This species has recently been documented for KNP (see Chapter 3) and the expansion of sampling sites into woodlands and near caves will most likely increase the chances of recording *Cloetis percivali* with bat detectors in the future.

Throughout the survey, two sets of calls were recorded that did not match any known species from the call reference library and current literature (Unknown 34 and Unknown 75) (Appendix 4.2; Monadjem et al. 2010). Unknown 34 appears to be a higher frequency Molossid call that ranges between 33 and 35 kHz. The call was recorded during each trip in the 2018 survey throughout the Punda Maria/ Shingwedzi region. The call has been recorded in past acoustic surveys within the Soutpansberg and Blouberg Mountains (Taylor et al. 2013a, 2013b). Unknown 75 appears to be a higher frequency Vespertilionid call that ranges between 73 and 76 kHz. The call was recorded during both 2017 and 2018 surveys. The call has also been recorded in past acoustic surveys at Mapungubwe National Park (MNP), South Africa (Parker & Bernard 2018). Both unknown sets of calls cannot be identified through acoustic methods but a concerted effort should be made to capture and describe these potentially new species (Parker & Bernard 2018, Taylor et al. 2018).

Sampling effort

Over the two-year acoustic survey, 24 bat species were recorded from 24 sampling sites. The species accumulation estimators indicated that at least one species was likely missed during the study. Bat species that call at very high frequencies (80 to >200 kHz), such as *Kerivoula lanosa*, are able to go undetected by acoustic surveys (Monadjem et al. 2017). High frequency calls attenuate very quickly and are not capable of traveling distances further than a few meters thus making the possibility of recording an individual very low (Monadjem et al. 2017). In addition, the family Nycteridae are known as whispering bats due to their soft, low-intensity echolocation calls (Monadjem et al. 2010). It is very difficult to record the family through acoustic technology even from a distance of 0.1 m (Monadjem et al. 2017). As such, this group is regularly under-represented in bat acoustic surveys (Monadjem et al. 2017, Parker & Bernard 2018). Expanding the number of sample sites to include additional roost locations of bats that have high frequencies and/or soft echolocation calls could provide the potential for recording more species for the study area (Flaquer et al. 2007).

Bray-Curtis Cluster

There was about an eight percent difference in species presence between the Pafuri and Punda Maria/ Shingwedzi regions. The difference between the regions was most likely caused by the difference in habitat. The Pafuri region has many riparian forest caused by the Limpopo and the Luvuvhu rivers (Roux et al. 2008, Monadjem & Reside 2008). In comparison, the Punda Maria/ Shingwedzi region is dominated by mopani trees and has only one major seasonal river, the Shingwedzi River, located within the area (Mucina & Rutherford 2006, Roux et al. 2008). The sites within the Punda Maria/ Shingwedzi were sampled throughout a variety of habitats such as mopani woodlands, savannas, and riparian habitats compared the sites within the Pafuri

region that were sampled mostly within riparian habitats. The types of habitats could have caused the slight differences in species presence between the regions.

Activity index

Bat activity was higher in 2018 compared to 2017 within the first five hours after sunset. The higher activity was most likely due to the increase in Vespertilionidae activity during the first three hours after sunset. The family Vespertilionidae is known to be the most active during the evening times (Monadjem et al. 2010). The increase in activity from the family could have been caused by the various habitats (mopani woodlands, savanna, and riparian habitats) surveyed in the Punda Maria/ Shingwedzi region compared to Pafuri where sampling sites were located mostly within riparian habitats. Vespertilionidae species are clutter-edge hunters capable of flying within both open air and dense vegetation habitats, thus the ability to move easily between multiple habitats in search of prey (Lee & McCracken 2004).

The family Molossidae was the most active family in both the 2017 and 2018 (AI consistently > 1.8) surveys. The higher and consistent activity levels were likely caused by the high abundance of the family found during the survey. Molossid species are also easier to detect through ultrasonic microphones due to their lower frequency calls that can travel up to a maximum distance of 30 m (Monadjem et al. 2017). The 2017 survey showed a slightly higher Molossidae activity compared to the 2018 survey. The majority of the Molossidae were recorded on the Luvuvhu River Bridge in 2017, which could have been a roosting site, contributing to the higher activity levels.

Overall, bat activity had the highest peak at the first hour of the time period and then gradually declined as the night progressed with a steep decline in activity after the eleventh

hour. The peak in activity within the first couple of hours after sunset was likely caused by an increase in abundance of nocturnal insects that prefer warmer temperatures (O'Donnell 2000). Insect activity is linked to nightly temperatures which is warmer right after sunset and steadily cools as the night progresses (O'Donnell 2000). As the temperature steadily declines throughout the night, insect abundance will decline too (O'Donnell 2000). This could have been the cause of the gradual decrease in bat activity (O'Donnell 2000).

Conclusion

My study was successful in determining the species richness of bats through the use of acoustic technology for the first time in northern KNP. The use of a site-specific classifier within Kaleidoscope was also successful in grouping putative bat species but it must be manually checked to minimize mistakes and detect any species that were not included in the initial clustering exercise. Bats are indicators of environment health and can be monitored through their activity (Mickleburgh et al. 2002). If there is a decline in activity of bats, it could indicate loss of habitat, a decrease in the number of water sources, or increases in disease prevalence (Mickleburgh et al. 2002). Using acoustic detectors, bat activity can be determined throughout the night and compared across years to monitor environmental health (Jones et al. 2009). Lastly, even though the species accumulation curves showed that the survey may have missed at least one species, acoustic detectors were able to detect the majority of the present species as well as uncommon species such as *Otomops martiensseni*, which is a near-threatened species that has only been documented in KNP once before (Adams et al. 2015). Expanding the number of sample sites within a variety of habitats could increase the possibility of recording more non-common species (Larsen et al. 2007). In the future, the use of acoustic technology can continue

to provide important data (i.e. species richness, bat activity, and distribution) for the surveying of bat communities within KNP.

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Chapter 5: Summary and concluding remarks

The primary goal of my study was to contribute towards the bat species list for northern Kruger National Park (KNP) by using live-capture methods and acoustic technology. The secondary goal of my study was to generate baseline data to be used for long-term monitoring of bats within KNP by creating a bat echolocation call reference library and an automatic classification tool used within Kaleidoscope software.

Combined, the historical data for northern KNP indicated a total of 40 species recorded over a 30-year period. About 63% of species recorded historically were also documented during my two-year survey. However, 15 species previously documented for northern KNP were not recorded using either live-capture or acoustic detectors during my survey. Nevertheless, I did detect two species that were not recorded historically. *Otomops martiensseni* echolocation calls were recorded through the acoustic detectors and this species has only been documented in KNP once before in 2015 (Adams et al. 2015). In addition, *Cloetis pervicali* was captured in September 2018 and this species has never been documented to occur within the borders of the KNP before my study. Although I did not record all of the species previously known from this part of KNP (probably because my study only lasted two years), I do believe that I have contributed meaningfully to the bat species inventory of northern KNP.

Limpopo is the north-eastern province of South Africa and has been shown to have the highest bat species richness compared to the rest of the country (Herkt et al. 2016). In addition, Rautenbach et al. (1985) predicted that the northern parts of KNP, specially the Pafuri region, had the highest concentration of bat species compared to other parts of South Africa. Regions within the Limpopo province were surveyed in recent years and thus species richness can be

compared to determine the hotspots of bat species richness. Within the Soutpansberg mountain range in Limpopo, South Africa (west of KNP), a survey used both acoustic technology and live-capture methods and recorded 18 bat species (Weier et al. 2017). Another survey using both acoustic detectors and live-capture methods was conducted in Soutpansberg and Blouberg Mountains that documented a bat species richness of 25 within the area (Taylor et al. 2013). Using acoustic detectors only, an assessment of bat species was conducted within human settlements bordering the northern parts of the KNP and recorded 22 bat species (Foord et al. 2018). Within northern boarders of the KNP, the current survey recorded 27 species overall and when compared to surrounding regions, the study area, as a whole, continues to have one of the highest species richness.

Through live capture and bat detectors combined, my survey recorded a total of 27 species within northern KNP. Of the total species richness count, 48% of the species were detected through the use of live-capture but most (89%) were recorded by the bat detectors. Both acoustic detectors and live-capture methods can have bias results towards certain species and when using only one method, non-common species are most likely to be overlooked (Francis 1989, Flaquer et al. 2007, Monadjem et al. 2017). Combining techniques can result in a greater species richness estimate and a better understanding of bat diversity (Flaquer et al. 2007, Taylor et al. 2013). Bat detectors are able to detect a greater species richness over a shorter period of time compared to live-capture (O'Farrell & Gannon 1999). However, when identifying recorded echolocation calls, it is important to know and understand the call parameters from species within the study area (Adams et al. 2012, Taylor et al. 2013). Through live-capture, echolocation calls can be collected from each bat species captured and placed into a call reference library (Taylor et al. 2013). Having a strong reference library decreases the chances of mis-identifying

calls recorded from acoustic detectors (Parker & Bernard 2018). My study was therefore successful in showing that through the combination of live-capture and bat detector methods, most (~68%) of the detectable bat species were recorded within the study area.

In addition to a strong call reference library, an accurate classification tool is beneficial for the identification process of recorded echolocation calls. A reliable classifier tool is able to automatically sort through large data-sets to select and identify quality bat calls, creating a quicker screening process (Rydell et al. 2017). With an accuracy of about 79%, the site-specific classifier tool developed for KNP was successful in the identification of common species but it was generally prone to mis-identifying non-common species. My research supports recent work that has demonstrated that manual identification of bat calls is essential to detect any false-positives of non-common species as well as species not included in the classifier tool (Rydell et al. 2017).

Continuous monitoring of bat species can provide insight into effects of climate change and overall health of the environment as well as to conserve bats species that face many global threats (Mickleburgh et al. 2002, Russo & Ancillotto 2015). By re-surveying the insectivorous bats of northern KNP, I have been able to generate an important baseline dataset for the future monitoring of bat diversity and this is recommended. Through increased research on bats and their behaviours, conservation efforts can continue to expand within KNP as well as throughout South Africa.

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Appendices

Appendix 3.1: Comprehensive summary of each species documented through live-capture from each sampling site in 2017 and 2018. Measurements of mass and forearm length were averaged for each species at each site. The total number of males and females from each species at each site was documented as well as age classes (Adult, Sub-adult, and Juvenile). S.D. represents standard deviation. Age class for the species *Mops condylurus* and *Nycticeinops Schlieffeni* captured at the Pafuri Border Camp site were not recorded.

Live Capture										
Location	**Species**	**Average Mass (g)**	**Mass S.D. (g)**	**Average Forearm length (mm)**	**Forearm length S.D. (mm)**	**Male**	**Female**	**Adult**	**Sub-adult**	**Juvenile**
Pafuri Border Camp	*Chaerephon pumilus*	22.5	2.8	46.7	1.2		1	1		
	Mops condylurus	12.0		39.5		32	39			
	Nycticeinops schlieffeni	5.0	1	30.2	1.1	2	1			
Crooks Corner	*Rhinolophus fumigatus*	20		51.9			1	1		
Pafuri Camp	*Nycticeinops schlieffeni*	4.9	0.3	29.8	1.2	2	2	3	1	
	Rhinolophus fumigatus	12.6	1.6	51.94	0.3		5	1		
	Rosettus aegyptiacus	106.5	9.3	89.9	4.6	1	3	4		
Rietbok Vlei	*Chaerephon pumilus*	10.5		37.6			1	1		
	Mops condylurus	20.9	3.6	46.5	1.5	2	6	8		
	Neoromicia nana	3.3	0.7	28.5	1.0	5	1	6		
	Scotophilus dinganii	28.5	10.6	53.9	2.9	1	1	2		
Babalala	*Chaerephon pumilus*	11.8	1.8	38.9	0.5	1	1	2		
	Epomophorus wahlbergi	64.5	29.0	70.2	7.2		2	1		1
	Mops condylurus	21.0	4.8	44.9	2.3	2	1	3		
	Pipistrellus rusticus	4.8	0.4	28.0	0.4	1	1	2		
Punda Maria restcamp	*Cloeotis percivali*	4.5		33		1		1		
	Hipposideros caffer	7.4	1.4	44.3	1.7	4		4		
	Neoromicia nana	3.7	0.4	27.6	0.9	3	2	5		
	Neoromicia zuluensis	4.1	0.6	27.6	0.8	3	4	7		
	Scotophilus dinganii	26.0	6.4	52.68	2.1	1	4	5		
Shingwedzi Research Camp	*Mops condylurus*	25.7	4.5	46.0	1.3	13	2	12	3	
	Neoromicia nana	4.5		29.6			1		1	
	Neoromicia zuluensis	5.0		28.9			1	1		
	Pipistrellus rusticus	4.0		29.4			1		1	
Total						74	81	63	6	1

Appendix 4.1: A summary of the echolocation call characteristics of released bats during the 2017/2018 survey as well as unpublished release calls held by Professor P. Taylor and Professor D. Parker; n represents the number of individual calls recorded from each species. Fmin is the lowest frequency of a call. Duration is the length of a call. Fmax is the highest frequency of a call. Fk is the change in slope of a call. Fc is the flattest portion of a call. S.D. represents the standard deviation of the mean.

Release Call Parameters							
Family	**Species**		**Fmin**	**Duration**	**Fmax**	**Fk**	**Fc**
Emballonuridae	*Taphozous mauritiana* (n= 24)	Mean	25.7	3.0	33.2	30.1	27.9
		S.D.	2.2	0.5	8.0	3.7	2.5
		Min	23.7	2.1	26.9	24.6	24.3
		Max	34.7	3.8	40.2	42.5	35.9
Hipposideridae	*Hipposideros caffer* (n= 14)	Mean	124.6	4.6	142.8	141.1	139.2
		S.D.	15.9	1.3	3.1	3.4	3.6
		Min	99.1	3.2	134.7	132.1	130.6
		Max	140.4	7.5	145.1	143.9	142.7
	Hipposideros vittatus (n= 7)	Mean	58.3	8.8	64.4	63.6	63.3
		S.D.	1.5	2.2	1.6	1.4	1.4
		Min	56.4	5.9	61.5	61.1	60.9
		Max	60.4	11.7	66.3	65.2	64.9
Miniopteridae	*Miniopterus natalensis* (n= 23)	Mean	50.5	3.2	65.8	53.8	51.9
		S.D.	2.6	0.7	8.3	3.6	2.9
		Min	46.6	2.4	51.5	48.5	47.8
		Max	54.2	4.6	85.9	60.3	57.0
Molossidae	*Chaerephon ansorgei* (n= 15)	Mean	18.8	6.7	22.4	19.8	19.4
		S.D.	1.0	2.3	1.8	1.1	1.0
		Min	17.6	3.8	19.5	18.5	18.2
		Max	20.4	10.9	26.3	21.6	21.0
	Chaerephon pumulis (n= 14)	Mean	22.0	7.6	26.2	23.7	22.9
		S.D.	1.4	2.0	4.8	3.2	2.2
		Min	20.1	3.2	21.9	21.3	20.9
		Max	25.4	10.9	36.9	33.8	29.4

Family	Species		Fmin	Duration	Fmax	Fk	Fc
Molossidae	*Mop condylurus* (n= 26)	Mean	25.4	4.6	38.4	32.3	29.5
		S.D.	1.4	1.2	4.9	4.2	3.2
		Min	22.5	2.9	28.6	25.5	25.4
		Max	27.2	8.6	45.4	42.1	37.9
	Mops midas (n= 7)	Mean	13.7	9.6	15.8	14.4	14.2
		S.D.	1.8	2.2	2.5	2.0	1.9
		Min	11.7	6.0	12.8	12.1	12.1
		Max	17.3	13.2	18.9	17.9	17.6
	Otomops Martiensseni (n= 3)	Mean	10.5	10.6	10.9	10.6	10.6
		S.D.	1.1	2.0	1.2	1.1	1.1
		Min	9.7	8.4	10.0	9.8	9.8
		Max	11.8	12.4	12.3	11.8	11.8
	Tadarida aegyptiaca (n= 16)	Mean	21.3	7.9	26.6	23.5	22.6
		S.D.	2.2	3.0	3.9	1.9	1.3
		Min	16.3	3.5	22.3	21.3	21.0
		Max	25.2	11.2	35.2	27.0	25.3
Rhinolophidae	*Rhinolophus capensis* (n= 4)	Mean	76.2	25.9	84.5	83.7	83.9
		S.D.	2.1	15.8	1.0	1.0	0.7
		Min	73.2	9.6	83.2	82.2	82.8
		Max	77.9	42.1	85.5	84.3	84.4
	Rhinolophus clivosus (n= 47)	Mean	75.0	18.7	92.6	108.1	90.4
		S.D.	3.2	7.0	0.6	119.7	0.6
		Min	67.6	7.7	91.4	89.4	89.1
		Max	81.9	32.0	93.5	91.1	91.4
	Rhinolophus darlingi (n= 4)	Mean	70.4	27.3	82.0	80.7	80.8
		S.D.	4.3	1.0	3.7	3.6	3.5
		Min	67.6	26.5	80.1	78.8	79.0
		Max	76.8	28.7	87.5	86.1	86.1

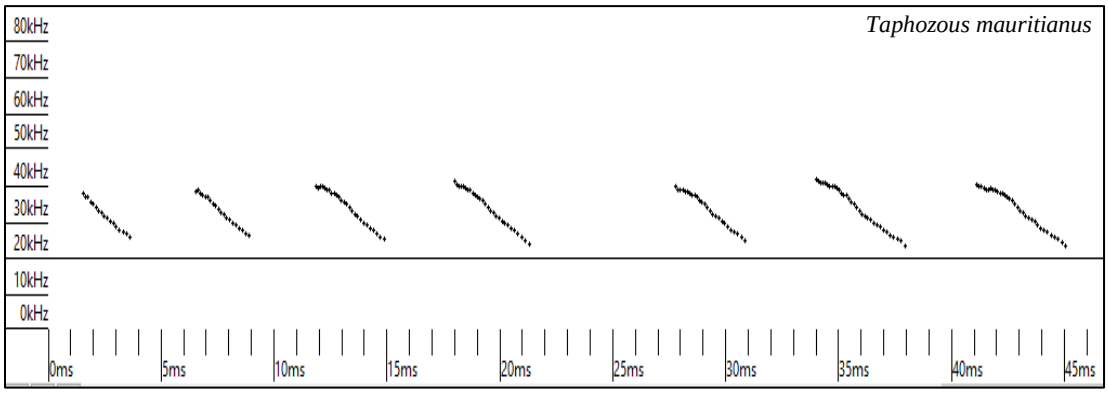
Family	Species		Fmin	Duration	Fmax	Fk	Fc
Rhinolophidae	*Rhinolophus fumigatus* (n= 83)	Mean	50.2	25.4	55.8	54.7	54.7
		S.D.	2.6	8.6	0.9	0.7	0.6
		Min	43.4	6.3	53.2	52.5	52.7
		Max	54.2	41.5	57.7	56.2	55.9
	Rhinolophus rhodesiae (n= 2)	Mean	93.3	15.5	101.0	99.1	99.6
		S.D.	2.5	1.6	0.1	0.1	0.2
		Min	91.5	14.4	100.9	99.0	99.4
		Max	95.0	16.6	101.0	99.2	99.7
	Rhinolophus simulator (n= 41)	Mean	68.4	15.7	80.7	79.3	79.2
		S.D.	3.4	4.6	60.4	3.5	3.5
		Min	56.7	5.4	3.6	59.7	59.5
		Max	76.6	24.2	83.1	82.2	82.0
	Rhinolophus smithersi (n= 23)	Mean	45.5	21.0	47.8	47.2	47.1
		S.D.	1.1	9.1	0.5	0.6	0.5
		Min	43.2	9.4	46.2	45.6	45.7
		Max	47.4	49.7	48.8	48.3	48.1
Vespertilionidae	*Eptesicus hottentotus* (n= 13)	Mean	30.4	3.3	55.9	36.9	33.5
		S.D.	1.8	0.7	9.0	2.2	2.5
		Min	26.9	2.2	40.1	32.3	31.5
		Max	33.8	4.5	70.2	41.1	40.1
	Laephotus botswanae (n= 4)	Mean	32.9	2.1	66.3	35.7	33.3
		S.D.	1.1	0.1	1.6	1.1	0.7
		Min	31.7	2.0	64.5	34.5	32.5
		Max	34.2	2.2	68.4	37.2	34.2
	Myotis tricolor (n= 3)	Mean	40.3	2.5	60.8	44.9	42.7
		S.D.	7.5	0.4	24.1	7.0	4.9
		Min	31.7	2.2	38.7	36.8	37.1
		Max	45.0	3.8	86.5	49.4	46.3

Family	Species		Fmin	Duration	Fmax	Fk	Fc
Vespertilionidae	*Myotis welwitchii* (n= 4)	Mean	30.3	2.8	69.1	60.7	49.2
		S.D.	3.7	0.7	3.8	11.5	7.0
		Min	25.3	2.2	66.2	44.4	39.6
		Max	34.2	3.8	74.7	71.5	56.0
	Neoromicia capensis (n= 12)	Mean	39.4	4.1	53.1	40.2	38.7
		S.D.	4.9	1.1	8.0	2.0	1.5
		Min	36.2	2.3	40.1	36.7	36.2
		Max	54.2	5.7	65.1	43.5	41.2
	Neoromicia nana (n= 21)	Mean	63.9	3.1	89.0	72.8	67.7
		S.D.	2.0	0.8	16.9	7.5	3.4
		Min	60.3	2.2	70.0	66.1	63.5
		Max	67.2	4.8	118.5	93.3	78.5
	Neoromicia zuluensis (n= 27)	Mean	46.0	3.7	58.2	48.1	46.7
		S.D.	1.2	0.7	7.9	1.4	1.3
		Min	43.9	2.6	32.7	45.3	44.4
		Max	48.7	4.9	69.7	51.3	49.3
	Nycticeinops schlieffeni (n= 1)	Mean	40.1	3.9	42.8	42.2	42.1
		S.D.					
		Min					
		Max					
	Nycteris thebaica (n= 1)	Mean	62.4	1.7	83.4	63.1	42.1
		S.D.					
		Min					
		Max					
	Pipistrellus hesperidus (n= 42)	Mean	44.6	3.3	63.0	48.5	45.3
		S.D.	1.6	1.0	11.1	2.8	1.6
		Min	39.6	2.0	48.7	44.3	42.1
		Max	47.8	5.9	86.6	54.9	49.2

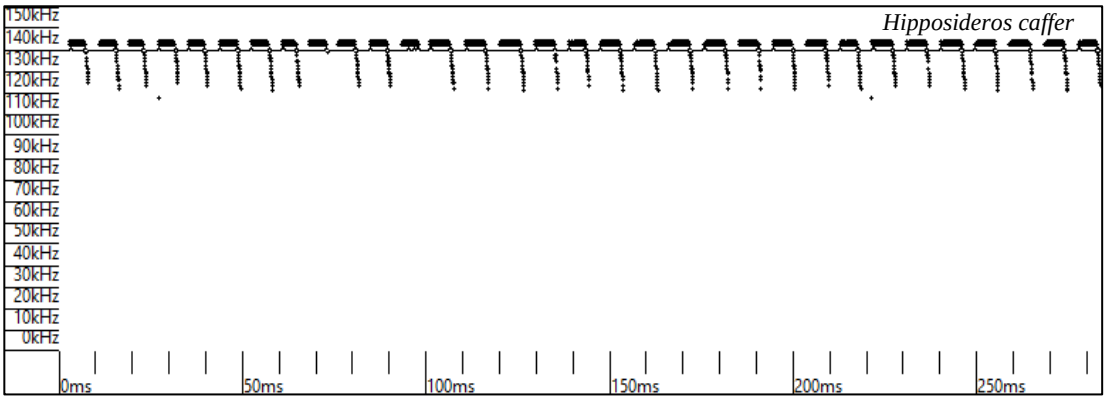
Family	Species		Fmin	Duration	Fmax	Fk	Fc
Vespertilionidae	*Pipistrellus rusticus* (n= 9)	Mean	50.8	2.4	70.3	48.2	51.9
		S.D.	2.9	0.4	7.0	16.0	2.4
		Min	46.8	2.1	62.7	5.9	48.5
		Max	55.2	3.1	82.0	57.9	55.5
	Scotophilus dinganii (n= 26)	Mean	32.3	3.7	53.6	35.0	33.0
		S.D.	1.1	0.9	8.1	2.3	1.2
		Min	30.1	2.2	40.2	31.6	30.4
		Max	34.7	5.8	64.8	42.1	35.9

Appendix 4.2: Sonographs of recorded bat species

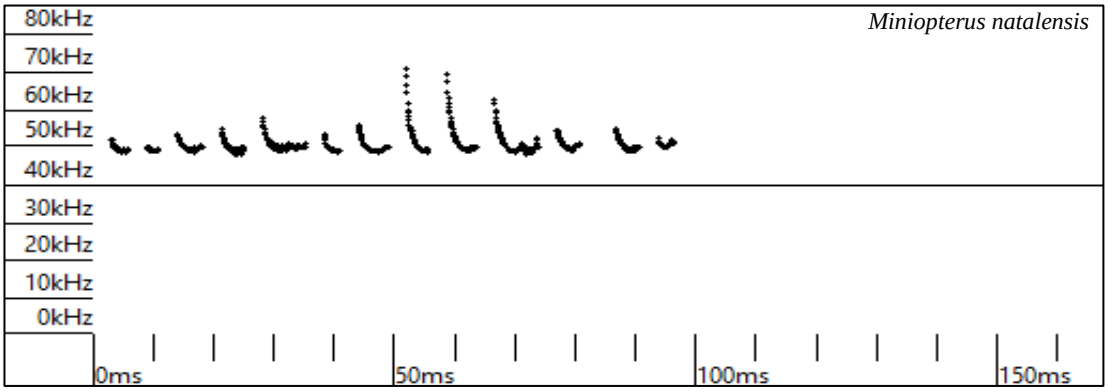
Emballonuridae



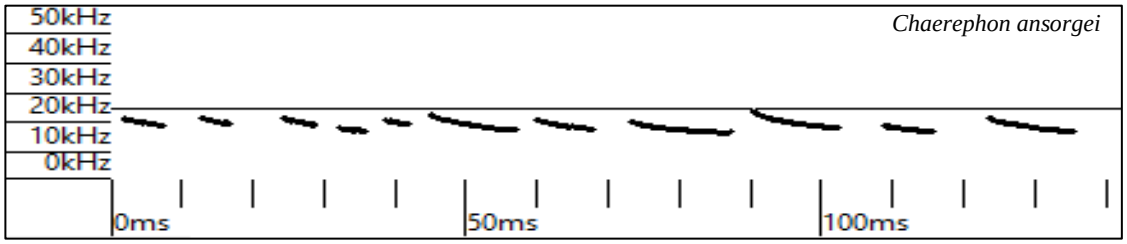
Hipposideridae

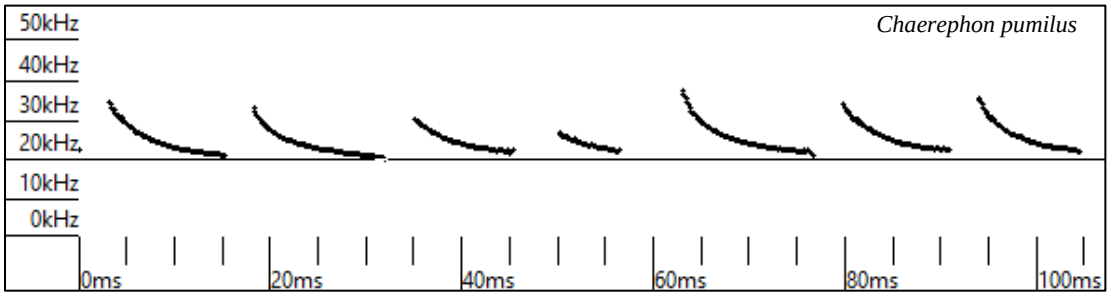


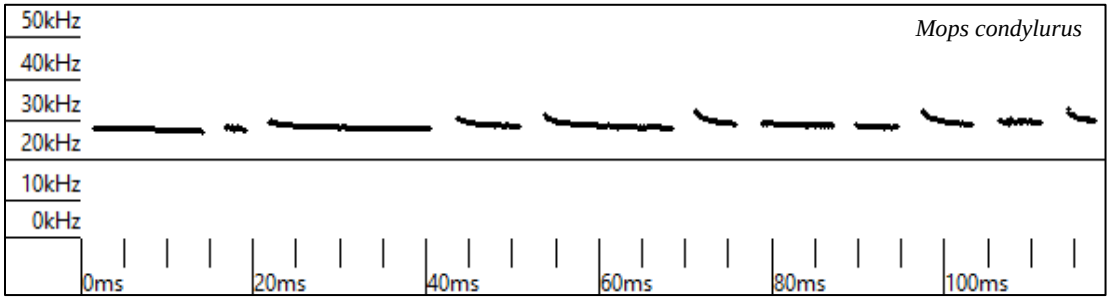
Miniopteridae

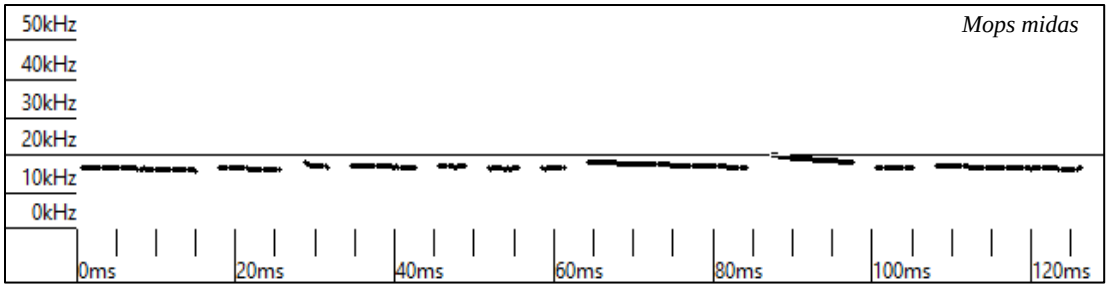


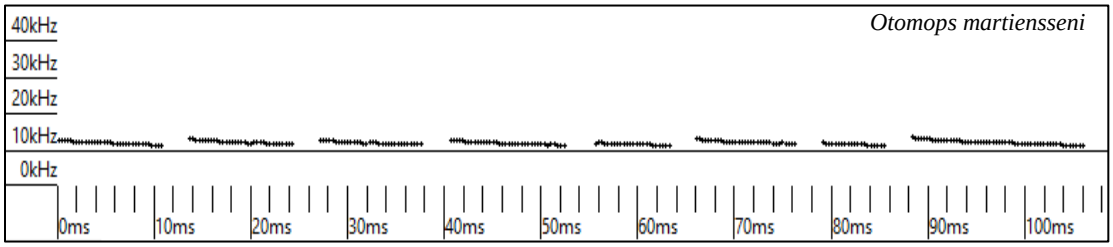
Molossidae

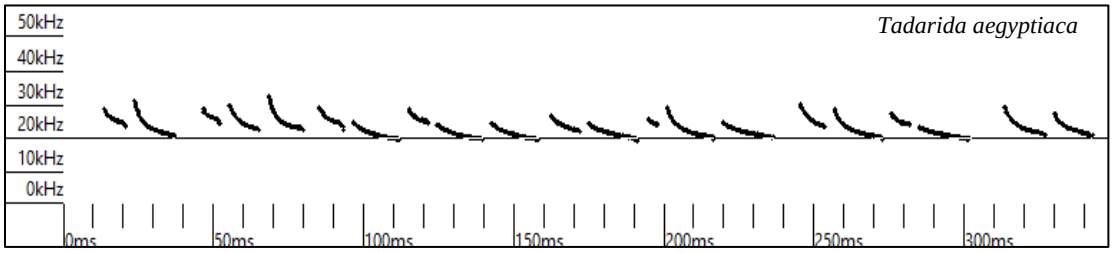




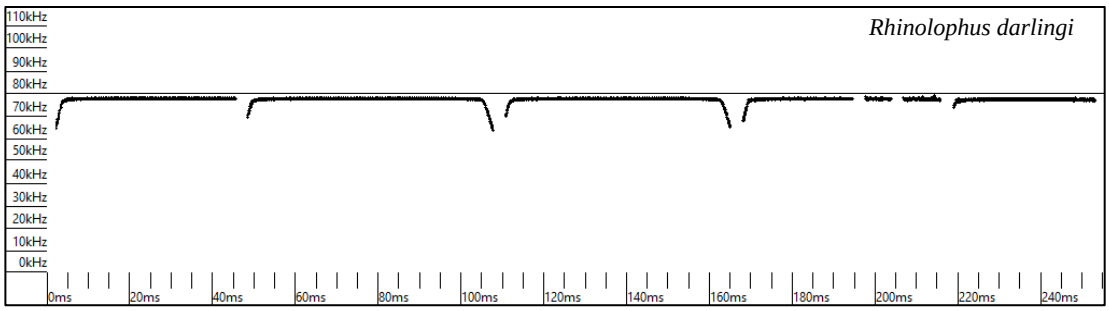


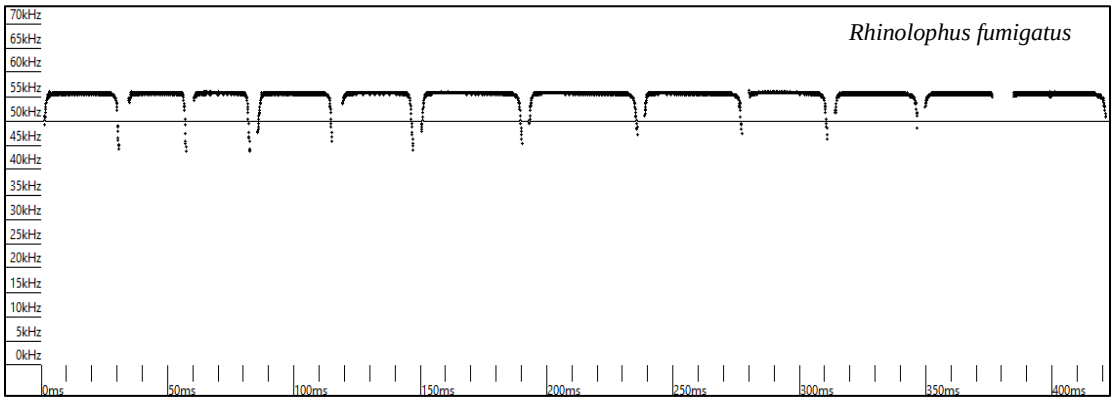


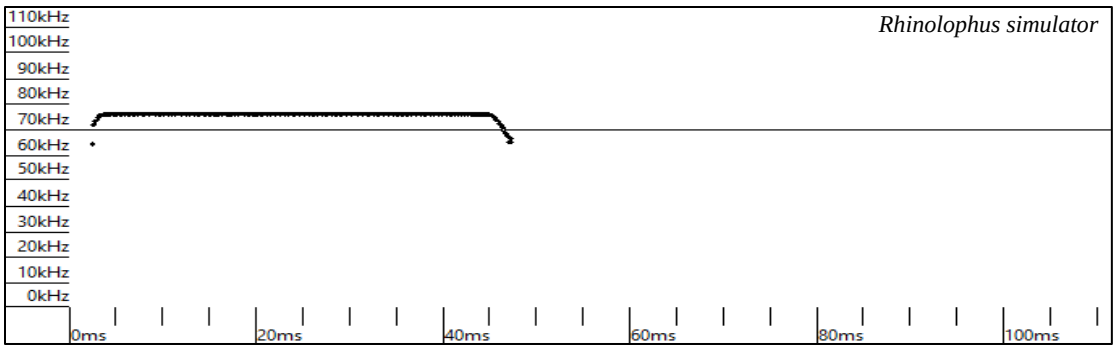


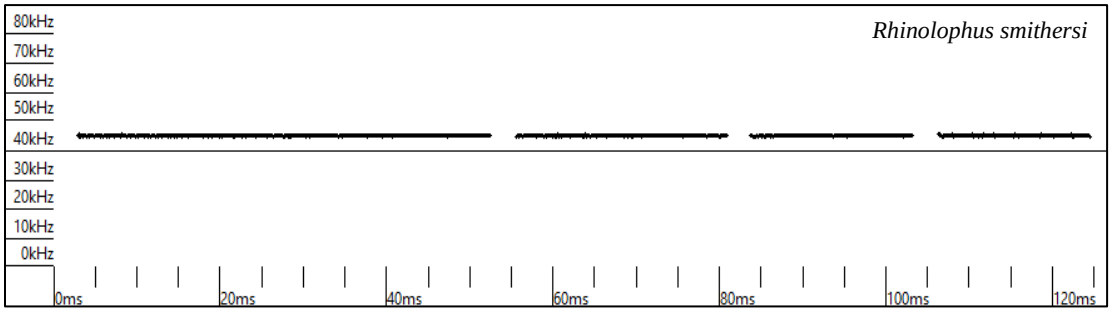


Rhinolophidae

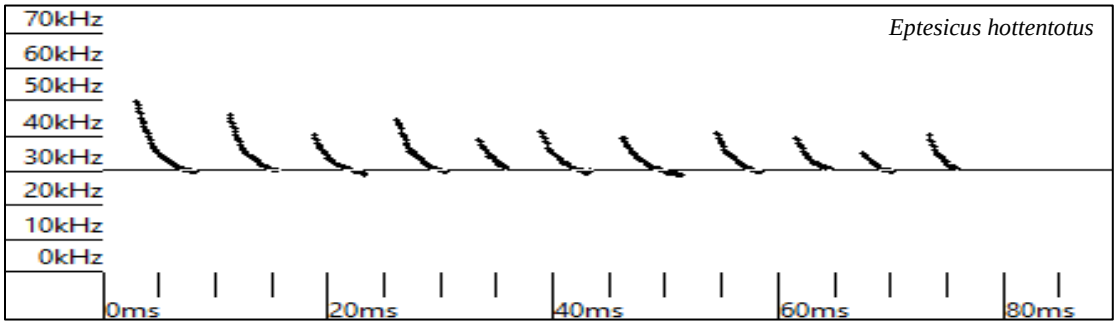


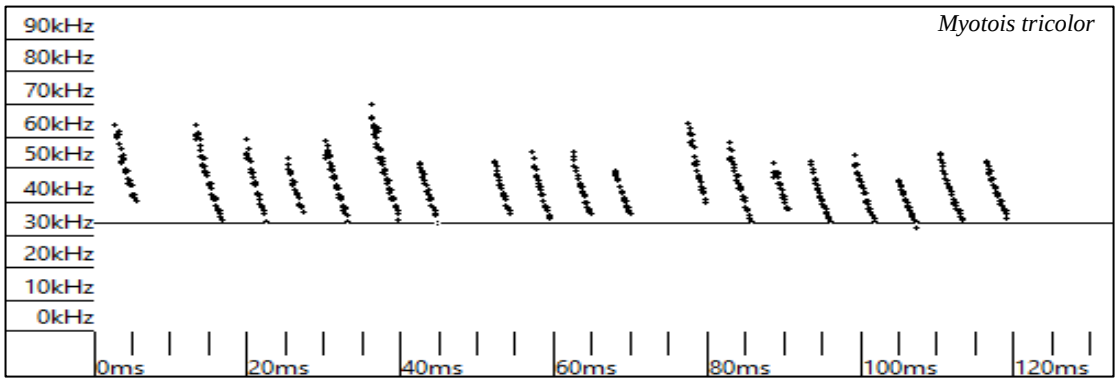


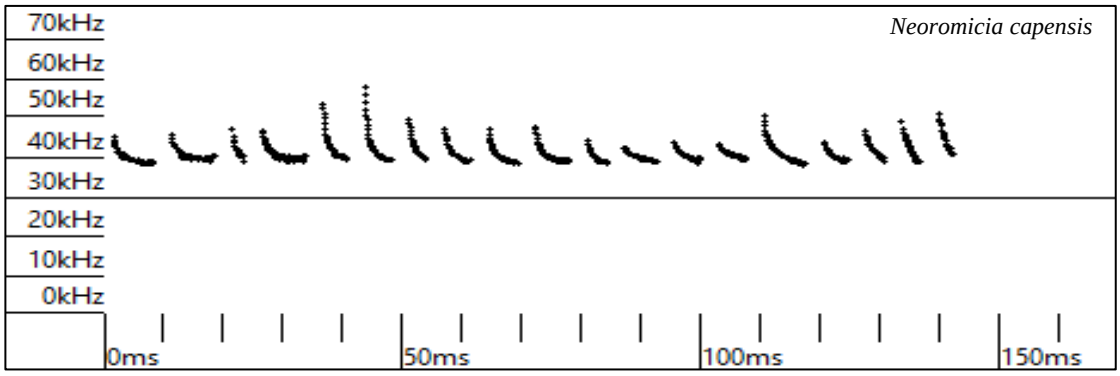


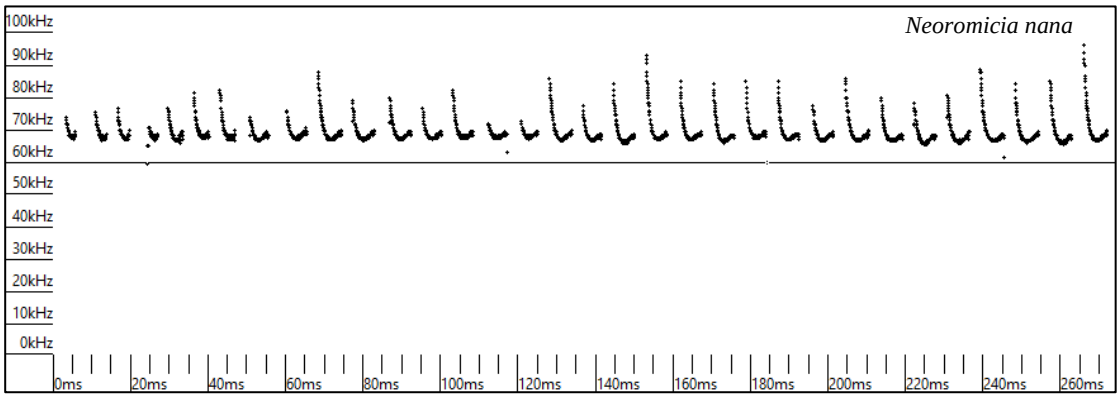


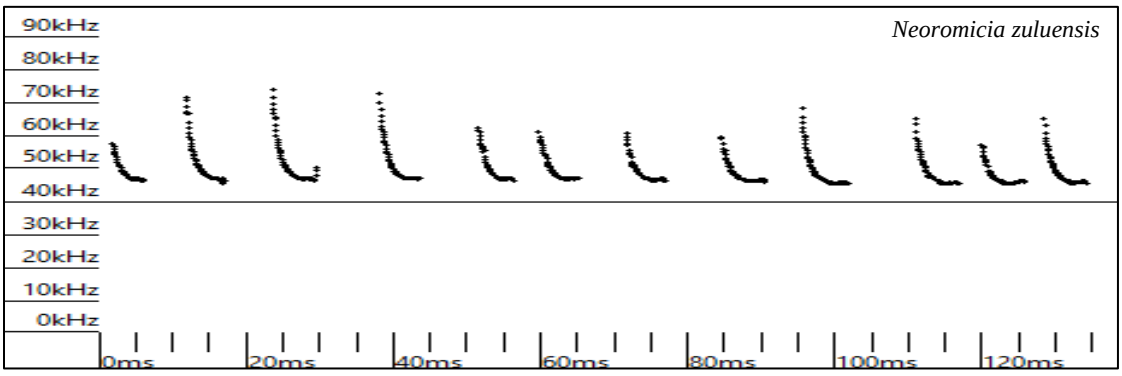
Vespertilionidae

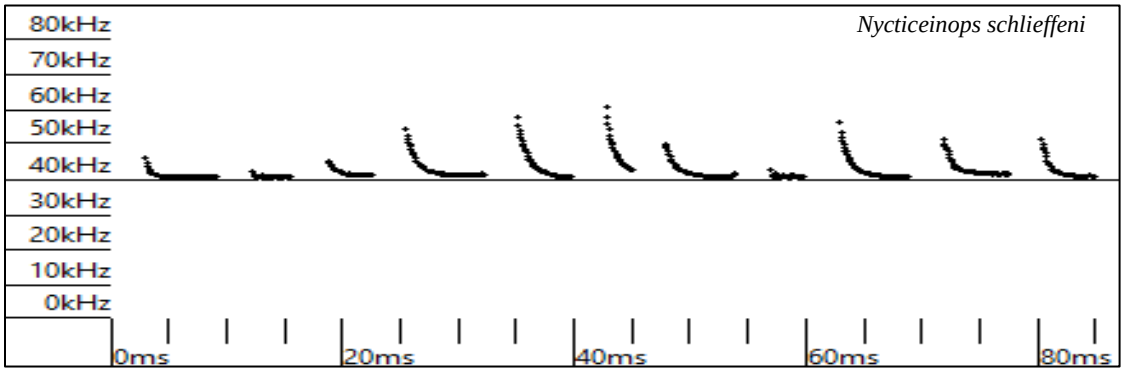


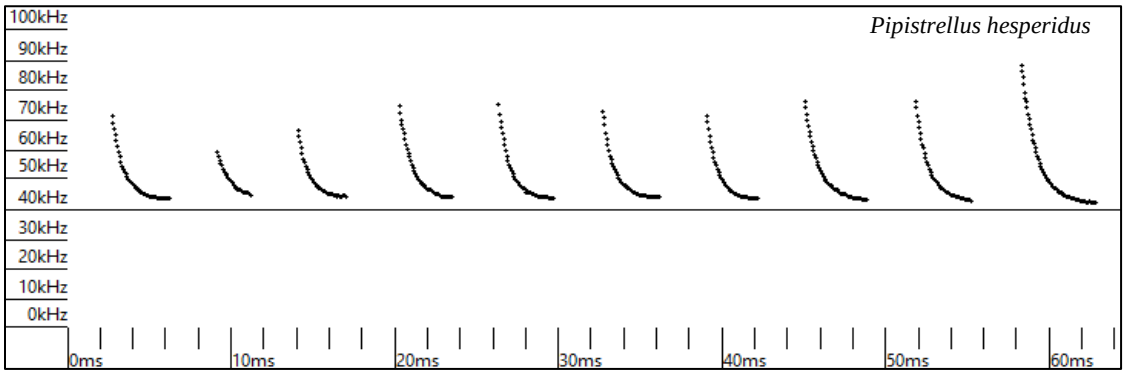

Myotois tricolor
90kHz
80kHz
70kHz
60kHz
50kHz
40kHz
30kHz
20kHz
10kHz
0kHz
0ms
20ms
40ms
60ms
80ms
100ms
120ms

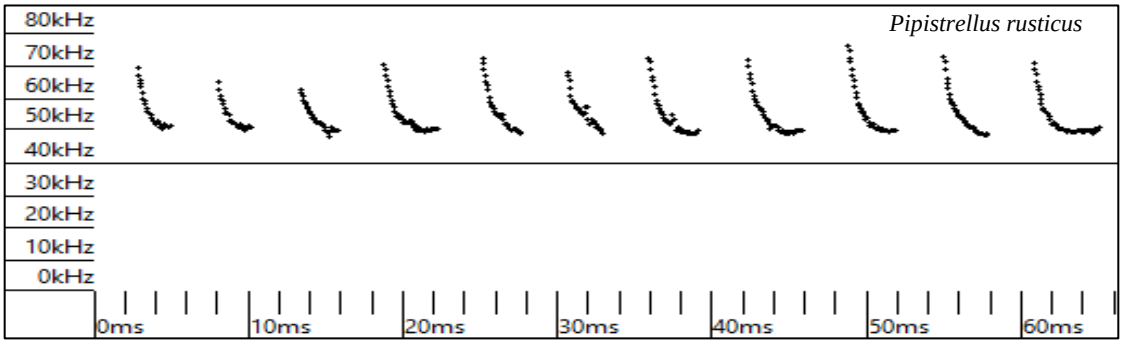

Neoromicia capensis
70kHz
60kHz
50kHz
40kHz
30kHz
20kHz
10kHz
0kHz
0ms
50ms
100ms
150ms

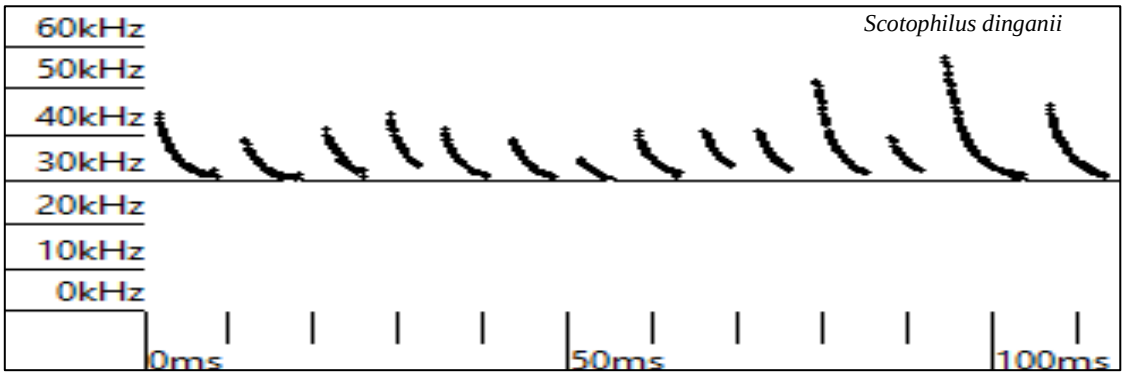

Neoromicia nana
100kHz
90kHz
80kHz
70kHz
60kHz
50kHz
40kHz
30kHz
20kHz
10kHz
0kHz
0ms
20ms
40ms
60ms
80ms
100ms
120ms
140ms
160ms
180ms
200ms
220ms
240ms
260ms


Neoromicia zuluensis
90kHz
80kHz
70kHz
60kHz
50kHz
40kHz
30kHz
20kHz
10kHz
0kHz
0ms
20ms
40ms
60ms
80ms
100ms
120ms

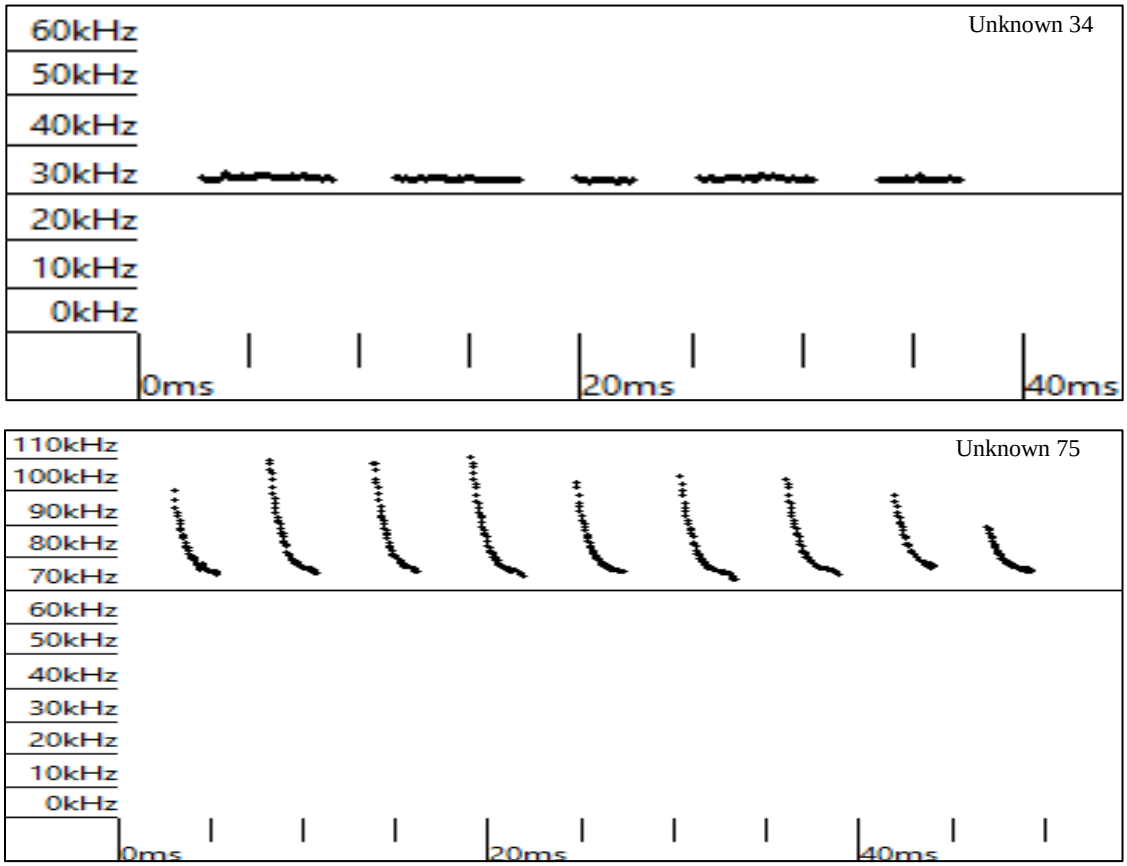

Nycticeinops schlieffeni
80kHz
70kHz
60kHz
50kHz
40kHz
30kHz
20kHz
10kHz
0kHz
0ms
20ms
40ms
60ms
80ms


Pipistrellus hesperidus
100kHz
90kHz
80kHz
70kHz
60kHz
50kHz
40kHz
30kHz
20kHz
10kHz
0kHz
0ms
10ms
20ms
30ms
40ms
50ms
60ms


Pipistrellus rusticus
80kHz
70kHz
60kHz
50kHz
40kHz
30kHz
20kHz
10kHz
0kHz
0ms
10ms
20ms
30ms
40ms
50ms
60ms


Scotophilus dinganii
60kHz
50kHz
40kHz
30kHz
20kHz
10kHz
0kHz
0ms
50ms
100ms

Unknown calls



Appendix 4.2: Sonograms of each species recorded during the acoustic portion of the 2017/2018 survey. The x axis represents time (ms) to display the duration of a call. The y axis represents frequency (kHz).