

**Assessment of the life history characteristics of *Rhinolophus  
blasii* (Mammalia, Chiroptera) in South Africa**

by

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## **DECLARATION**

I, Mengjing Wei, declare that this thesis, which I hereby submit for the degree, MSc Wildlife Management, at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

**DISCLAIMER:** This thesis was structured with two data chapters (Chapter 2 and 3) written up as independent research manuscripts and therefore some repetition might occur, or reference made to either data chapter.

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## Summary

This study investigated variation in activity, relative abundance, and body condition of Blasius's horseshoe bat (*Rhinolophus blasii*) in relation to temperature, season, rainfall, and wind speed across seven years at the Meletse area, Limpopo, South Africa. The study was divided into two chapters: passive (Chapter 2) and active monitor (Chapter 3). An SM2+ bat detector recorded echolocation calls emitted by individual bats, which were used as a measure of the species activity and relative abundance. I also included active monitoring data, which involved the capture of bats (mist-nets and harp traps), where captured individuals were measured to obtain body condition information. Rainfall and season were the main factors that best explained the variation in the response variables relative abundance and activity for *R. blasii*. Relative activity peaked during winter to spring and reduced from summer to autumn. The largest proportion of *R. blasii* calls were recorded in the 2016 and 2017 sample periods, lowest in 2019. *Rhinolophus blasii* shows female-biased sexual dimorphism in body mass. Female body mass increased in winter and spring decreased in summer and autumn and had the lowest body mass in spring. This is the first known study on a southern African population of *R. blasii*, where southern African populations have different life-history characteristics compared to the European populations.

This study would suggest that for southern African populations of *R. blasii*, monitoring can focus on females and males during spring to predict population trends. Further work is required for both females and males on how the two sexes increase body weight under the lowest food abundance during winter months. This focused monitoring can provide quantitative data that may be used for reporting; on the

conservation risk (IUCN Red List) that southern African populations of *R. blasii* may be experiencing.

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## Chapter 1 Introduction

### 1.1 Genus *Rhinolophus*

The family Rhinolophidae is composed of a single genus *Rhinolophus* (horseshoe bats), the second-largest bat's genus (Presetnik 2000). Tsubamoto et al. (2004) report that Rhinolophidae likely has originated in Asia's tropical rainforests during the Eocene in south-eastern Asia. From the early to the middle Eocene, this family was recorded in Africa and Asia (Janis 1993). In late Eocene (ca.37-33 MYA), Rhinolophidae family experienced a stage of increase in faunal endemism happened in South-eastern Asia. Over the last 16 years, the number of its recognised species has grown from 77 to 106 (Taylor et al. 2019), which occur throughout the Afrotropical, Australian, Indomalayan, Oceanian and Palaeartic regions (Csorba et al. 2003). Horseshoe bats are characterised by the “intricate folds of skin in the nasal region like a horseshoe”. Their nose-leaves are usually composed of three sections which may have variations in arrangement, length and shape depending on the species. The popular name “horseshoe bats” comes from this characteristic.

Many horseshoe bats (genus *Rhinolophus*) use echolocation calls characterised by high frequencies (typically above 60 kHz). Echolocation is the process of using the larynx and vocal cords to broadcast sound pulses to locate things based on their echoes (Griffin 1960). Bats that employ echolocation can be classified into two groups based on the length of time they spend "calling": low duty-cycle (LDC) and high duty-cycle (HDC) (Fenton and Bell 1981); Bats with a high duty cycle (HDC) use calls that are longer than the inter-call interval (Fenton and Bell 1981). The "auditory fovea" in Rhinolophid bats is like the fovea in certain animals' visual systems; within the basilar membrane of HDC bats, there is an area of

thickening and lengthening that correlates to the specific frequency element of the echolocation call. (Schuller and Pollak 1979).

Horseshoe bat populations have been implicated as host reservoirs for the Severe Acute Respiratory Syndrome (SARS) outbreak of 2002 in China (Lau et al. 2010, Hu et al. 2017). While the COVID-19 outbreak in China, Wuhan city in 2019-2020, has been proposed to be linked to horseshoe bats (*Rhinolophus*) that host viruses similar to the COV-SARS-2 (Markotter et al. 2020). It is essential to survey related viruses in this genus of bats elsewhere, including South Africa (Markotter et al. 2020). The unique characteristic of horseshoe bats, including life history, distribution, and ecology, makes them unique among most mammals and contributes to their potential as viral reservoirs (Luis et al. 2013). Stoffberg (2010) recognised two monophyletic *Rhinolophus* clades; the first clade comprised *Rhinolophus* species principally from the African biogeographical area, the second comprised species from the Oriental area. *Rhinolophus blasii* is within the African clade.

### **1.2 *Rhinolophus blasii***

The Blasius's Horseshoe bat (*Rhinolophus blasii*) has an extensive distribution in the Palearctic and the Afrotropics. Currently, *R. blasii* is widely distributed through Europe, Asia, and Africa (Csorba et al. 2003). In Asia, *Rhinolophus blasii* has a patchy distribution from west Turkey to the east of Pakistan east (Csorba et al. 2003). In Europe, *Rhinolophus blasii* is the rarest horseshoe bat (Kryštufek 1999). In Africa, *R. blasii* is mainly restricted to southern Africa, with the reported distribution extending into Malawi and Zambia, occurring marginally beyond the eastern Democratic Republic of Congo (DRC) (Csorba et al. 2003) and Tanzania. Within South Africa, *R. blasii* is reported to occur in Limpopo, Mpumalanga, and KwaZulu-Natal provinces of South Africa (Taylor 2016).

According to the IUCN Global Red List assessment, *R. blasii* is classified as Least Concern (LC). However, threats to the species are listed as the continued loss of habitats such as Mediterranean woodlands, habitat disturbance, and cave disturbance. Even so, *R. blasii*'s population was reduced regionally. In Slovenia, *R. blasii* not only vanished from Postojnska Jama but also has been extirpated from its marginal distribution in the northern Dinaric karst (Krystufek 2008). A regional IUCN assessment for South Africa in 2016, *R. blasii*, is classified as Near Threatened (D1) (Jacobs et al. 2016).

Most of the research on *R. blasii* is based on the European populations about their current distribution (Popov and Ivanova 2002, Siemers and Ivanova 2004, Siemers et al. 2005, Krystufek 2008, Javid et al. 2015, Kearney et al. 2016, Taylor 2016, Jéré et al. 2017) and few studies report on *R. blasii* hunting or feeding behaviour and echolocation call frequency study (Siemers and Ivanova 2004, Shahabi et al. 2019). Hutterer (2005) reported that *R. blasii* hibernates in underground sites during the wintertime in Europe, seasonal migration up to 100 km. In Bulgaria, *R. blasii*'s echolocation constant characteristic frequency (CF) is  $95.14 \pm 0.74$  kHz, range from 92.21 kHz to 96.02 kHz. In Iran, *R. blasii* use CF frequency of echolocation call is around 92.85kHz. In Eswatini (Swaziland) and in the southern African range, their CF component of echolocation call is a bit lower than above two places, around 86 kHz (Monadjem 2006). From the CF difference of *R. blasii* in certain regions, their life history may also be slightly different. There are no known published studies that have investigated life-history characterises in South Africa on *R. blasii*. The *R. blasii* population from South Africa could also be considered a population with likely differences in molecular phylogenetic data (Demos et al. 2019).

The drivers behind the phenology of bats are less understood (Menzel 2002, Ciechanowski and Jarzembowski 2010, Rodrigues and Palmeirim 2008), and there are no known studies that have investigated phenology in *R. blasii*. Therefore, this study (thesis) will provide valuable insight into this species life history and ecology.

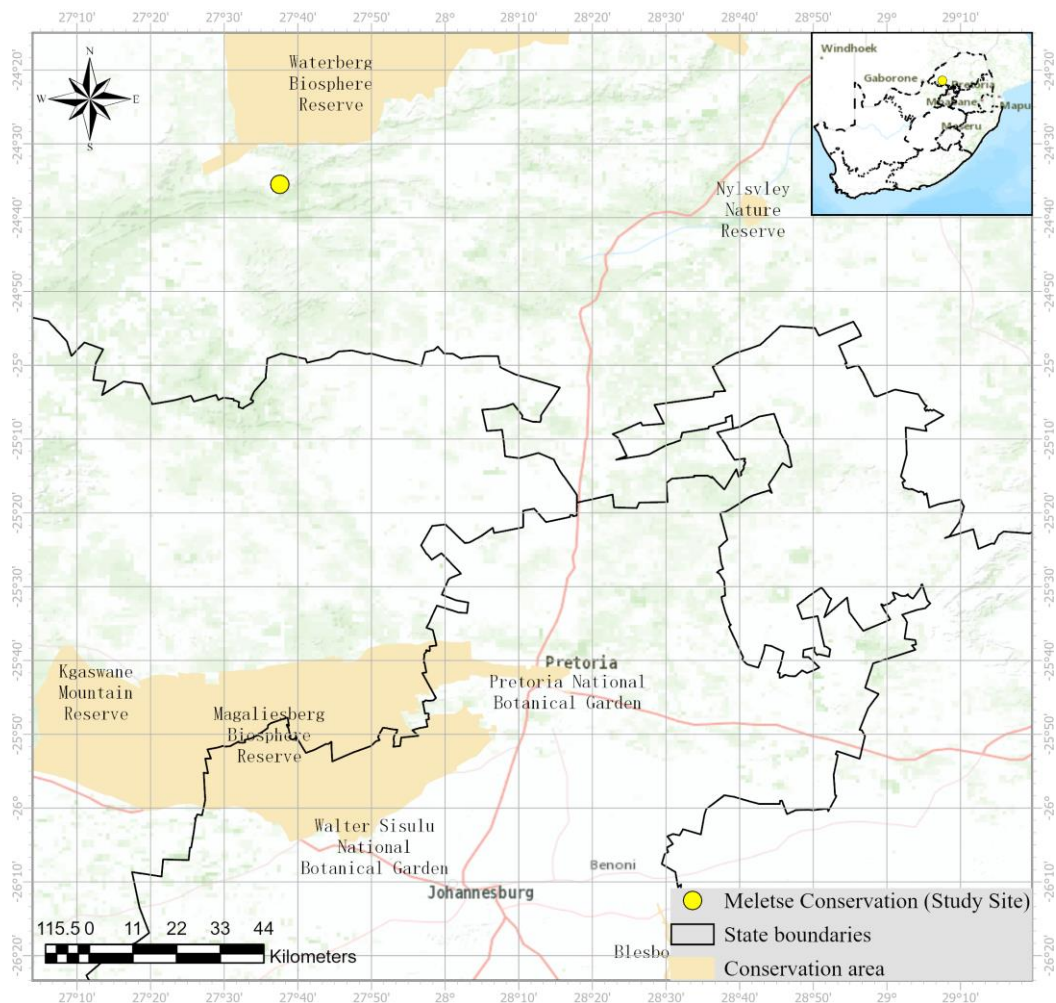


Figure 1-1 Map of study area. The location of the karst landscape systems of Meletse in Limpopo Province, South Africa (include insert map).

### 1.3 Bat Phenology and Ecology

Phenology, derived from the Greek word *phaino*, is defined as annually recurring life cycle events (Schmeller et al. 2018). Critical life-cycle events such as reproduction, migration, hibernation must be keyed to the appropriate seasonal cycles of temperate zones (Schwarz

2003). Ciechanowski and Jarzembowski reported (2010) that the signal of seasonal climatic more pronounced, the stronger the need for appropriate change maintained through phenology. These may be regulated along with changes in biotic and abiotic factors and the interaction among interspecific species and intraspecific species (Lieth 1974). Abiotic changes can be the first indicators of critical ecological changes in terrestrial, freshwater, and marine environments (Schmeller et al. 2018). Changes in phenology can result in a decoupling of temporally matched biotic interaction. Hence, identification of changes in phenology within a population may be used as an early indicator of significant alteration in ecological relationships in terrestrial, freshwater, and marine environment (Schmeller et al. 2018).

Bats use *k*-selected life-history strategies with small body sizes (Becker et al. 2003), which means they have a long generation time and low reproductive rate. Bats are likely to be the most significantly affected by changes that result in resources and breeding phenology (Canale and Henry 2010). Wickramasinghe et al. (2003) and Kalcounis-Rueppell et al. (2007) demonstrate that bats can be bioindicators of habitat change, as bats are sensitive to anthropogenic change. Because a considerable percentage of metabolic energy is spent keeping a stable body temperature. As small endothermic mammals, insectivorous bats such as *R. blasii* are vulnerable to shifting climatic conditions (Fristoe et al. 2015). Therefore, this species as well as other bats will reflect ecological changes before organisms at lower trophic levels (Alleva et al. 2006).

Many aspects of bat ecology are not clearly understood and limits conservation efforts (Popa-Lisseanu and Voigt 2009). Frick et al. (2019) reviewed that globally almost 15% of bats species are threatened and 19.18% data deficient. Significant threats to bats include logging,

harvesting, and mining. Even though forests are the most important habitat type for many mammals, cave roost habitats play a key role in supporting species diversity at a global scale to bats (Voigt and Kingston 2016). Human activities and disturbance are also significant, especially for cave-dwelling bats that are particularly threatened by their large form and concentrated aggregations (Voigt and Kingston 2016). Agricultural production is one of the major land-use transitions across the planet, which has been designated as a threat in IUCN Red List assessments for over half of bat species. Climate change leads to changes in species richness and distribution patterns at an unprecedented rate (Pecl et al. 2017). It may shift, disrupt, or change migratory behaviour, survival, and reproduction, foraging behaviour and, disrupt food availability (Thuiller et al. 2005, Both et al. 2006, Lundy et al. 2010, Frick et al. 2012, Adams 2018). Bats are vulnerable to variations in temperature and rainfall due to the fluctuation in food availability and thermoregulation constraints (Johnson et al. 2017). Thus, developing frameworks to identify species responses to shifts and changes are needed (Rebelo et al. 2010). This study's result may be used to understand any change that occurs within populations in the sub-region and at species level. We can then also evaluate if that change can be used as an early warning indicator of possible threats to the species/environment.

Even though some bat monitoring systems are in place, bat diversity is drastically declining (Voigt and Kingston 2016). Multiple studies investigate bat ecology and conservation plans are known to include body condition and relative abundance as monitoring techniques (Wiederholt et al. 2013, Rughetti and Toffoli 2014, Amorim et al. 2015).



## 1.4 Study Objective

This study investigated some of life history characteristics of *Rhinolophus blasii* population in Meletse area in Limpopo Province, South Africa. This study also included environmental factors such as temperature, rainfall, wind speed and seasons, investigated how these factors are correlated to the variations in the bats. The study period across years from 2014 to 2019, as a large time-scales study, which gives insight as to how to management this species in the vary over time and seasons.

## 1.5 Research Questions

This study aimed at investigating the temporal variation of the *Rhinolophus blasii* in Meletse area.

Q1- Determine if *R. blasii* in South Africa show any annual or seasonal phenology and if so, is this driven by environmental factors? there are significant difference among above five categories in body mass. Hypothesis two: there are significant difference body mass seasonal and yearly change in *R. blasii*.

Q2- Determine if *R. blasii*'s body mass changes seasonally?

## 1.6 Outline of thesis

The study investigated the *R. blasii* life-history in the Meletse region in Limpopo Province, South Africa. This study included environmental factors such as temperature, wind speed, and rainfall and investigated how these factors are related to *R. blasii* activity and body condition variation. This was undertaken by using historical data collected by AfricanBats NPC (Not for Profit Company). Data included variation in species-specific relative

abundance, activity using echolocation data, and body mass of the *R. blasii* population in the Meletse area.

The thesis was written in the format of two independent – free-standing manuscripts, and the following outlines for each of the two chapters are

Chapter 2. Annual and seasonal phenology of *Rhinolophus blasii* (Mammalia: Chiroptera) in relation to climatic factors.

This first research chapter investigated the measure of the activity of *R. blasii* to annual and seasonal changes in relation to temperature, wind speed and rainfall variation. I utilised passive monitoring (echolocation recordings) to derive a measure of the activity index from 2014 to 2019.

Chapter 3 Seasonal body mass change of *Rhinolophus blasii* (Mammalia: Chiroptera)

This chapter investigated the temporal variation in body condition of the *R. blasii* seasonally. I also investigated the body condition variation within the population based on each captured animal's sex, age, and reproductive classes for the period.

The thesis is ended by Chapter 4 Conclusions

This chapter combines findings of Chapter 2 and 3, linked the *R. blasii* activity changes to the body mass change cross the season and the year. The discussions therefore and the meaning of the findings, which may also provide insights for future studies.

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## **Chapter 2 Relative activity of *Rhinolophus blasii* (Mammalia: Chiroptera) in relation to climatic factors: insight into *R. blasii* phenology**

### **Abstract**

Bats are sensitive to anthropogenic change, especially for the insectivorous bats, which are susceptible to climate change. The activity of *Rhinolophus blasii* is still understudied, the cue to start their activity are not well known. In this study, I determined if *R. blasii* in South Africa show any annual or seasonal phenology and if so, is this driven by environmental factors? I investigated the measure of the activity of *R. blasii* to annual and seasonal changes in relation to temperature, wind speed, rainfall. I utilised passive monitoring (echolocation recordings) to derive a measure of the activity index from 2014 to 2019. The largest proportion of *R. blasii* calls were recorded in the 2016 and 2017 sample period, lowest in 2019. As the result, rainfall and season were the main factors that correlated with variations in the relative abundance and activity for *R. blasii*. Relative activity peaked from winter to spring and reduced from summer to autumn. The Meletse area population of *R. blasii* did not show hibernation or long periods of torpid characteristics during winter compared to the European population. Variation within species provides an understanding into the factors that influence populations such as survival, reproductive timing, and success. This study provides a baseline for future monitoring programs which can aid national and international reporting organisations by monitoring *R. blasii* to detect early signs of population depressions.

## 2.1 Introduction

Phenology can show considerable variation across the annual year, with certain events taking place assuredly at a definite time and some presenting high fluctuation between years (Menzel 2002). Phenological cycles on the southern side of the equator are less distinct than those on the northern side, which are clarified by extreme temperature changes (Chambers et al. 2013). By tracking the seasonal activities of certain species, researchers can infer the influence of climate change and land transformation (Menzel 2002).

Bats (Chiroptera) are likely to be especially affected by changes in resources relied on for breeding (Canale and Henry 2010). Wickramasinghe et al. (2003) and Kalcounis-Rueppell et al. (2007) demonstrate that bats are sensitive to anthropogenic change and can be used as indicators of habitat change. Insectivorous bats are susceptible to climate change, as they spend a large amount of metabolic energy on maintaining constant body temperature (Pretorius 2020, Fristoe et al. 2015).

Within the Pacific Northwest USA, temperature and rainfall have been proposed as phenological indicators of bat activity, in which low temperatures and rainfall suppresses bat activity (Erickson and West 2002). Ambient temperature has been identified as an environmental factor linked to bat activity, especially for hibernating species (Berková and Zúkal 2010). Parsons et al. (2003) recorded a decrease in the activity of cave bat species in England when temperatures decreased below 13°C. Berková and Zúkal (2010) recorded that bat increase their activity when temperatures rise above 6.2°C in winter in temperate zones (Europe). Therefore, bat activity fluctuates with ambient temperature changes that differ between species and geographically (Racey and Swift 1985, Maier 1992, Kusch and Idelberger 2005, Biłas et al. 2007). At the same time, Morellato et al. (2020) linked rainfall,

more than the temperature, to phenology in tropical and subtropical regions, where the dry-wet cycles are pronounced.

Little is known about temporal and seasonal activity of *R. blasii* and the effect of bioclimatic factors, and which cues start their activity are not well known. Factors that may influence this species activity patterns may include temperature, rainfall, barometric pressure, and wind speed. Investigating the relationship between these factors and activity is essential in understanding the bats' basic ecology, which can better inform conservation strategies. Many studies use active methods (for example, using mist nets/ harp traps to catch bats) to allow calculating the number of bats caught during the year (Neuweiler et al. 1987, Coburn and Geiser 1998, Weller et al. 2000, Estrada 2002, Fraser et al. 2010, Benda et al. 2016, Culina et al. 2017). Nevertheless, these methods have limitations, such as causing extra stress for animals which may reduce their survival rate. Passive methods, such as acoustic monitoring, have been effectively used to observe sensitive animals (Dawson and Efford 2009). Echolocation calls can be decidedly reliable taxonomic features, especially for constant frequency bats (Fenton and Thomas 1980, Brunet-Rossinni and Wilkinson 2009, Hayes et al. 2009). The power of acoustic monitoring is through the detection of species that can easily be 'heard' but are not easily seen. Although there is no direct proportionality relationship between call counts and bat population numbers, echolocation calls can provide a relative indication of bat activity and abundance in the landscape (Ford et al. 2011).

Miller (2001) presented an acoustic activity index (AI) that represents the relative activity of free-flying bats based on echolocation pulses per time frame. This method does not require the definition, identification, and counting of bat passes and provides an easy way to quantify activity (Miller 2001). Many bats' studies have used the Miller index to analyse bats' activity

(Fiedler 2004, Avila-Flores and Fenton 2005, Ford et al. 2005, Gorresen et al. 2008, Herkt et al. 2017).

As already stated, many studies of *Rhinolophus blasii* focus on the European populations (Krystufek 2008, Javid et al. 2015, Jéré et al. 2017), while few studies have investigated the African population (Jacobs et al. 2016, Demos et al. 2019). These studies have investigated the taxonomic relationships, and few have explored the phenological relationship to climate and environmental variables and the potential shifts associated with change.

In this chapter, I investigated the variation in a southern African population of *Rhinolophus blasii* activity in relation to the temporal variation of environmental factors, specifically temperature, rainfall, and wind speed.

## **2.2 Study area**

The study area is located at Madimatle Cave (also known as Gatkop Cave or Sandspruit Cave Number one); (24° 36'59 "S, 27° 39'10 "E) in the Limpopo province, South Africa. Madimatle Cave is an important cave roost for species such as *Miniopterus natalensis*, *Rhinolophus blasii*, *Rhinolophus simulator*, *Hipposideros caffer* and *Myotis tricolor* (Kearney et al. 2016). The cave is located on a farm (Randstephan455 KQ) approximately 25 km east of Thabazimbi (van der Merwe 1975). The vegetation in this area may be divided into two regional vegetation types: The Waterberg Mountain Bushveld and Western Sandy Bushveld (Mucina and Rutherford. 2006). Summer rains characterizes the area, with a hot, humid temperature during the summer months (Mucina and Rutherford. 2006). The regional climate of the study area is described as semi-arid savannah with daily temperatures vary from a maximum average of 20°C to 32°C but can go up to 45°C in summer periods (Van Staden 2009). The minimum average temperatures vary between 5°C and 19°C with an 18.4°C Mean

Annual Temperature (MAT) in winter (Mucina and Rutherford 2006, Schroder et al. 2006, Van Staden 2009). The annual rainfall ranges between 500 - 700mm, Mean Annual Precipitation (MAP) during summer rainfall periods spanning from November to April (Mucina and Rutherford. 2006, Van Staden 2009).

### **2.3 Materials and methods**

#### *Annual or seasonal phenology and possible abiotic drivers*

I used activity data that had been collected from a bat detector station for six years (January 1<sup>st</sup>, 2014, to December 30<sup>th</sup> 2019) by AfricanBats NPC—counting each year for 365/366 days. An SM2+ detector (Wildlife Acoustics Song Meter – bat SM2+) was deployed 600 meters from the Madimatle cave at the Meletse Bat Research and Conservation Training Centre. The bat detector was connected to an external 45aH battery and charged by a 50w solar panel. The recordings of echolocation calls started 30 minutes before sunset and 30 minutes after sunrise in zero-crossings format.

Recorded echolocation calls were analysed in AnalookW 4.5d software (Corben 2001), where a species-specific filter for *R. blasii* was used (AfricanBats NPC unpublished) to identify calls, which is uniquely distinguishable from sympatric bat species for this species, call range is 85kHz to 90kHz.

Daily climatic data were gained from weather stations (Davis Vantage Vue R 6250 weather station, Davis Instruments Corporation) started at the site in 2014. Minimum temperature, rainfall rate, dew point, wind averaged speed over the period of activity monitoring will be the main abiotic factors to consider.

The South African Weather Service provided the seasonal calendar dates used to categorise climatic data (Weather S.A., 2013-2019) and seasons were defined accordingly: Autumn: March 1<sup>st</sup> – May 31<sup>st</sup>, Winter: June 1<sup>st</sup> – August 31<sup>st</sup>, Spring: Sept 1<sup>st</sup>–November 30<sup>th</sup>, and Summer: Dec 1<sup>st</sup> – February 28<sup>th</sup>. For a broader categorization, I grouped the calls from May to September to be occurring during the dry season, and from November to April as the wet season in South Africa. By counting the number of one-minute time blocks, an Activity Index for *R. blasii* was calculated, where one or more call sequences was detected (n) as being present (P):  $AI = \sum_1^n P$  (Miller 2001). As a result, for each sample night, I can provide an estimate of bat activity at that location.

Due to the weather station and bat detector failing, there were some data missing. The bat detector failed in 2014, May 10<sup>th</sup> - May 30<sup>th</sup>; in 2017, May 16<sup>th</sup>, May 21<sup>st</sup> - May 31<sup>st</sup>, June 1<sup>st</sup> - 12<sup>th</sup>. The Weather station failed in 2014, January 3<sup>rd</sup> - 26<sup>th</sup>; in 2015, March 26<sup>th</sup>, 27<sup>th</sup>, 28<sup>th</sup>; April 11<sup>th</sup>; December 13<sup>th</sup> to 19<sup>th</sup>. In 2018, December 28<sup>th</sup> – 31<sup>st</sup>; in 2019, January 1<sup>st</sup> - 21<sup>st</sup>, and 2019 from November 1<sup>st</sup> to December 31<sup>st</sup>.

All statistical analyses were conducted using R-Studio (version 1.3.1073), with selected packages (Car, Pscl, Mass). I used the Shapiro test to assess the nature of the data to see if my data distributed normally. I used Tukey's honestly significant difference (HSD) post hoc test following Kruskal Wallis ANOVA to see if there a significant of *R. blasii* activity differed between the sample years and among the four seasons across the different sample years.

The impacts of climatic variables on bat activity were tested by using general linear models (package Car). Variance inflation factors (VIF) (package Car) were used as an indication of the degree of collinearity between independent variables in regression models. VIF's were

calculated for each covariate. All VIF were less than 5, indicating relatively low collinearity between covariates. Based on the data I collected (activity index, which had a high number of zeros), I used the zero-inflated Poisson regression (ZIP) model to transform data for further analysis

## 2.4 Result

*Rhinolophus blasii* calls were recorded from January 1<sup>st</sup> 2014, until December 30<sup>th</sup> 2019. The largest proportion of *R. blasii* calls were recorded in the 2016 and 2017 sample period (23.3% and 23%, respectively), while in 2014, 2015 and 2018, *R. blasii* calls had the lower proportion (16%, 14%, 17%, respectively) and in the 2019 year had the lowest (4%).

Although there was no significant difference in *R. blasii* activity in comparison to any subsequent years (Tukey HSD test;  $p > 0.05$  among each year), there was a significant seasonal difference of *R. blasii* activity across sample years. Between spring and autumn (Tukey HSD test; diff =4.95,  $p < 0.001$ ), winter and summer (Tukey HSD test; diff =5.004,  $p < 0.001$ ), winter and autumn (Tukey HSD test; diff =3.59,  $p < 0.001$ ), summer and spring (Tukey HSD test; diff = -6.36,  $p < 0.001$ ). There were no significant differences between summer and autumn, winter, and spring (Tukey HSD test; diff = 0.72,  $p > 0.05$ ). In spring throughout all years, *R. blasii* activity was highest (AI index =  $1.2 \pm 1.17$ ), then it was in winter (AI index =  $1.15 \pm 0.69$ ), and autumn (AI index =  $0.98 \pm 0.3$ ), with summer being the lowest (AI index =  $0.16 \pm 0.16$ ) (Fig. 2-2).

There was no significant difference between the wet season (September to March) and the dry season (April to August) (Tukey HSD test; diff =3.304,  $p > 0.05$ ). A pulse of activity (Fig. 2-2) was generally seen across most of the years for *R. blasii* activity around late winter to

early spring. In 2014, 2016, 2017 and 2018, this pulse all appears on nights 240- 260. In 2015, *R. blasii* range of activity didn't show a spike. In 2019, I detected a low *R. blasii* activity for most of the months, with AI <50. Alternatively, activity increases in spring and winter and decreases in summer (Table 2-1).

Total cumulative rainfall was the highest in 2017 (674mm), followed by 2016 (567.2mm) and 2018 (565.4mm) (Table 2-4). The lowest cumulative rainfall was in 2015 (322mm). Furthermore, the pooled mean temperature across all seasons was at its highest in 2015 (22.5°C) followed by 2016 (22.2°C), 2018 (21.57°C), 2019 (21.38°C), 2017 (21.04°C) and 2014 (20.6°C) (Table 2-4).

The VIF test results indicate that the factors which may influence *R. blasii* activity are rainfall (GVIF=1.06), seasons (GVIF=2.86), temperature (GVIF=2.55), and wind speed (GVIF=1.25). All VIF's were less than 5, indicating low covariates collinearity. Following that, all covariates were maintained for the global model. Three models were constructed to identify the best model fit of *R. blasii* activity (Table 2-3).

Rainfall and season were the predictor variables, with bat activity increasing significantly as rainfall decreased (Fig 2-3). Temperature and wind speed showed very little effect on activity. The relationship between per year activity of *R. blasii* and cumulative rainfall per year is positive, with weather data in 2019 being excluded (Table. 2-4).

## 2.5 Discussion

The Meletse area *R. blasii* population, which can be considered a representative of southern Africa savannah population presented activity strongly associated with the winter and spring seasons. Hutterer et al. (2005) reported *R. blasii* European population hibernates underground during the winter period. Based on the data collected, the Meletse area population of *R. blasii*



did not show hibernation or long period torpid characteristics during winter. From the weather station data, the average temperature in winter time at the study area is  $14 \pm 1.3^{\circ}\text{C}$ , the lowest temperature can reach  $-1^{\circ}\text{C}$ . Webb (2005) reported some of the *Rhinolophus* species only hibernate at ambient temperatures below  $2^{\circ}\text{C}$ , not like other bats where a frequency distribution temperature range for hibernating was  $11^{\circ}\text{C}$ . But Dulic (1959) and Gaisler (1970) recorded torpid in hibernacula at  $10.5^{\circ}\text{C}$  to  $16.2^{\circ}\text{C}$  for some populations of *R. blasii* in Europe. Thus, the temperature may not be the main driver shaping different overwintering strategies between the European population and southern African population. Other factors such as interspecies relationship, prey abundance etc. (Fiedler 2004, Maier 1992, Ciechanowski 2007) need to be further explored.

As I predicted, the data suggest that the *R. blasii* activity shows seasonal changes, an increase in winter and spring activity, and a decrease in summer and autumn, showing the same pattern with their body mass seasonal change (see Chapter 3).

*Rhinolophus blasii* activity was strongly related with rainfall and not temperature and wind speed. In Limpopo province, rainfall is primarily in the summer with thunderstorms during late afternoons (Newbould 2003). At study area the rainfall occurs from October to May. In the seven years of data collection, each year rainfall time range did not have significant changes. There was a significantly negative relationship between *R. blasii* activity and rainfall within the years. When rainfall within a day becomes high ( $> 20$  mm per day), bat activity decreases, *R. blasii* activity approached zero (Fig. 2-3). This phenomenon has also been found in another insectivorous bat by Erickson and West (2002). In England, cave bat activity decreased on days where rainfall exceeded 15mm rain per day (Parsons et al. 2003). Additionally, rainfall causes thermoregulatory constraints due to wet fur, which increases the

energetic cost and increases the smaller bat's body mass (Voigt et al. 2011). Rain additionally interferes with atmospheric propagation of echolocation, and heavy rain can impact bats' thermoregulation, thereby hindering foraging activity and influencing flight capacity (Appel et al. 2019).

In reference to the ZIP model, it shows season has more influence on *R. blasii* activity than climate factors such as rainfall (Table 2-3). The study revealed that *R. blasii* activity had increased in winter and Spring (June to September) and decreased in summer and showed a seasonal difference.

Furthermore, some African insectivorous bats' reproductive cycle is correlated with rainfall, where various species give birth a month before the peak rainfall season (Cumming et al. 1997). If *R. blasii* females were to be pregnant in Spring, heavy rainfall might be exceedingly costly to reproductive females already under severe energy load due to lactation (Koehler and Barclay 2000), leading bats to stay in the cave rather than hunt. A visible activity peak occurred in several years from 2014 to 2018 except 2015, after 20 to 40 days of yearly rainfall peak. This phenomenon could prove to be an interesting question to address in the future study. That activity peak may be a result of reproductive behaviour. Madimatle cave hosts multiple bat families *Rhinolophidae*, *Miniopteridae* and *Vespertilionidae* (Kearney et al. 2016). Within the Meletse bat assemblage other than *R. blasii*, temperature rather than rainfall is more strongly correlated with increased bat relative activity, and they are more active in summer (Shanahan 2020). Pretorius et al. (2019) reported that an influx of *M. natalensis* females occur from October. In terms of increasing interspecies competition, this may explain why *R. blasii* activity is lower in summer. The lack of interspecies competition during winter may also be another reason for *R. blasii* has high activity in winter (June to

August) because most of the Meletse bat assemblage had the lowest recorded relative activity during midwinter to summer (Shanahan 2020).

From 2014 to 2016, *R. blasii* activity increased every year and reached the highest activity in 2017. In the years 2018 and 2019, *R. blasii* activity decreased dramatically. In 2019, the lowest level of *R. blasii* activity was recorded. El Niño-Southern Oscillation weather systems has strong impact to South Africa (Chambers et al. 2013). The El Niño episode from 2014 to 2016 was the fiercest ever recorded (Zhai et al. 2016, Benkenstein 2017, Yin et al. 2018). The weather station that had been set up was 500m away from the cave, shows that Meletse has its unique rainfall system. In 2016, 2017 and 2018, the precipitation was higher than other years, where 2017 had the highest total rainfall (674mm). Though rainfall affects the food supply of African insectivorous bats, rainfall especially constrains reproduction timing (Cumming et al. 1997). In 2017 January and February, the total rainfall was higher than any of the other years. Naturally, food abundance was higher; the survival rate of sub-adult *R. blasii* increased with enough food. *Rhinolophus blasii* had the lowest activity in 2019. Declines in relative bat activity could help highlight the decline of specific species that are more sensitive to climate variation changes, habitat destruction and environmental change (Jones et al. 2009).

Regardless of the focal species of an acoustic investigation, technological advancements have a significant impact on the feasibility of using acoustic techniques. Nowadays, technology still can't determine whether sounds are coming from one or multiple individuals moving past a microphone, so acoustic records can help most effectively offer a relative index of activity levels (Hayes 2000). Additionally, the dominant frequencies in many bat species' calls (20-60 kHz) indicate a balance call range due to attenuation and detection resolution

(Fenton et al. 1998). For *R. blasii*, which will have a lower effective range of echolocation due to attenuation due to higher-frequency components in their calls (Fenton et al. 1998). As a result, detectors may have a great difficulty detecting it. The decrease in activity frequency of *R. blasii* year by year can also be caused by the loss of sensitivity of the equipment we used (Wildlife Acoustics Song Meter – bat SM2+). For further study, I would suggest attempt variate equipment, there are differences between brands and even individual detectors of the same model (Hayes 2000, Larson and Hayes 2000, Miller 2002), differences between time expansion and zero-crossing detectors (Fenton et al. 2001).

Understanding the environmental cue that *R. blasii* bats utilize to perform their activities can aid in their conservation, especially during some critical life history phase like spring breeding season (Ciechanowski et al. 2010). Additionally, the seasonal, temporal variation of bats in the Meletse area underscores the importance of adaptive management systems and conservation actions for the persistence and survival of these animals' groups.

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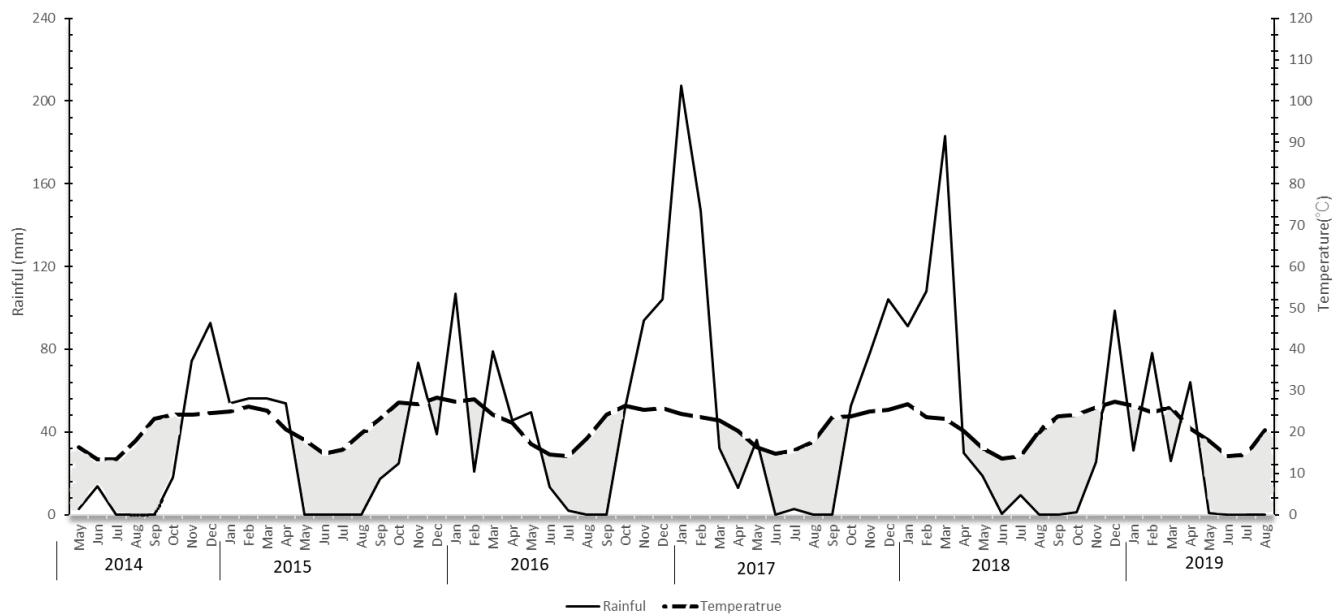


Figure 2-1 Ombrothermic diagram in Meletse area from 2014 to 2019 with rainfall and temperature measured for the research area

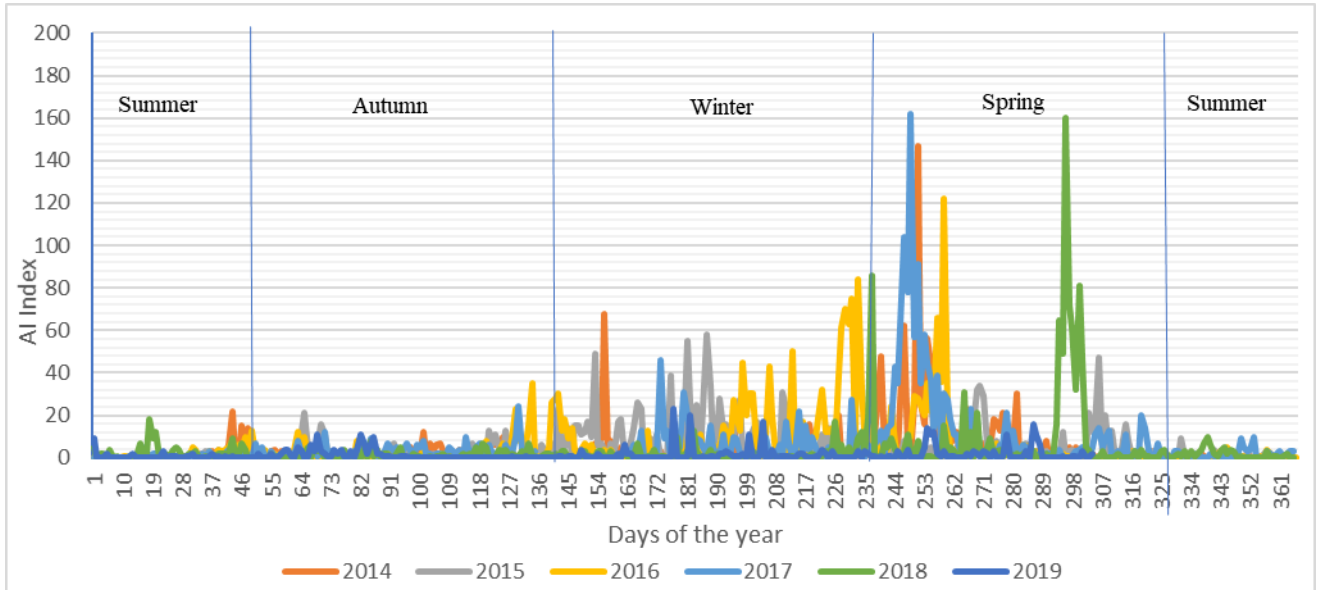


Figure 2-2: The variation in the relative activity of the *R. blasii* at Meletse area site with SM2-BAT detector bat detector from January 2014 to October 2019. January 1st to December 31<sup>th</sup> is day 1 to day 365.

Table 2-1: Akaike Information Criterion (AIC) general linear regression models of climatic factor effects on the nightly activity of *R. blasii*. Climatic factors are mean day temperature (Mean Temp), day wind speed (Mean Windspeed), and day cumulative rainfall (Rainfall). "X" in columns means that factor included in the models, "\" means not.

Model. No	Mean Temp(°C)	Season	Mean Wind speed(m/s)	Rainfall(mm)	AIC
1	X	X	X	X	9884.06
2	\	X	X	X	9882.08
3	\	X	\	X	9880.64

Table 2-2: Parameter estimates, standard error, t-value, and P-value for the variable in the best candidate model with several climatic variables for explaining *R. blasii* bats' activity at the Bat conservation and study centre Limpopo, South Africa.

	Estimate	Std. Error	t value	Pr(> t )
Intercept	2.92	0.50	5.77	0.0000000008
Rainfall	-0.099	0.046	-2.14	0.0323
Season spring	4.89	0.70	6.93	0.00000000000056
Season summer	-1.25	0.72	-1.72	0.0846
Season winter	3.43	0.70	4.89	0.0000001

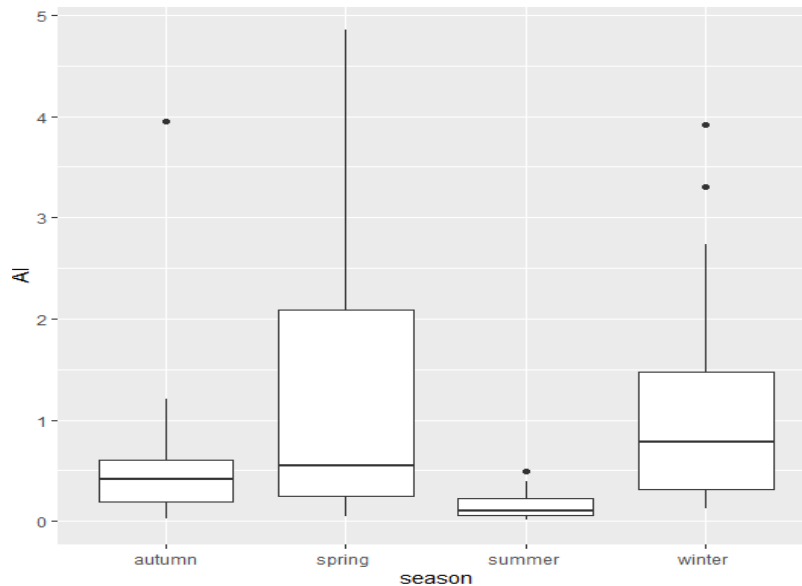


Figure 2-3: Boxplot showing medians at 95% CI of *R. blasii* Activity Index per season across six years (from 2014 to 2019) periods collected at Madimatle Cave, Limpopo, South Africa.

Table 2-3: Parameter estimates, standard error, Z-value, and P-value for the variable in the ZIP model with several climatic variables for explaining *R. blasii* bats' activity at the Madimatle Cave Limpopo, South Africa

	Estimate	Std. Error	Z vale	Pr(> z )
(Intercept)	1.47	0.027	53.34	< 2e-16 ***
Rain	-0.09	0.0074	19.48	< 2e-16 ***
Season spring	1.01	0.031	31.88	< 2e-16 ***
Season summer	-0.40	0.052	-7.73	<1.04e-14 ***
Season winter	0.63	0.032	19.48	< 2e-16 ***

Table 2-4: *Rhinolophus blasii* bat activity data across seven sample years at Madimatle Cave, Limpopo, South African

Month	2014	2015	2016	2017	2018	2019
Jan	11	2	12	24	86	24
Feb	106	26	89	48	39	17
Mar	45	103	56	93	51	101
Apr	95	45	26	103	40	4
May	51	192	293	74	47	12
Jun	160	346	55	172	46	38
Jul	99	433	425	156	43	75
Aug	203	98	659	188	204	31
Sep	580	183	490	1065	147	71
Oct	161	110	49	107	615	61
Nov	11	151	18	129	34	19
Dec	9	15	20	61	59	6
Total	1531	1704	2192	2220	1411	459



Table 2-5: Summary the mean climatic variables (temperature (°C) and wind speed (m/s)) and cumulative rainfall (mm) across six years by using weather station at the Madimatle Cave, Meletse, Limpopo, South Africa.

Month	2014			2015			2016			2017			2018			2019		
	Temp	rain	WS	Temp	rain	WS	Temp	rain	WS	Temp	rain	WS	Temp	rain	WS	Temp	rain	WS
Jan	25.1	31	0.46	24.97	54	0.51	27.27	107	0.75	24.41	207.4	0.53	26.7	91	0.81	26.3	31	1.85
Feb	25.05	39.8	0.46	26.04	56	0.52	27.84	21	0.73	23.6	147	0.41	23.6	108	0.41	24.7	78	1.28
Mar	21.97	199.4	0.27	25.12	56	0.64	24.08	79	0.37	22.73	32.2	0.27	23.1	183	0.32	26	26	1.88
Apr	18.51	11.2	0.229	20.57	53.8	0.33	22.25	45.6	0.36	20.3	13	0.24	20.2	29.8	0.86	20.8	64	1.07
May	16.32	2.8	0.24	18.14	0	0.28	17.13	49.6	0.36	16.27	36.2	0.26	16.2	18.8	0.92	17.8	0.6	0.77
Jun	13.43	13.6	0.26	14.67	0	0.58	14.56	13.2	0.23	14.75	0	0.26	13.5	0.4	1	14.2	0	0.65
Jul	13.45	0	0.34	15.78	0	0.51	14.07	1.8	0.48	15.5	2.8	0.32	14.2	9.6	1.4	14.5	0	0.91
Aug	17.97	0	0.74	19.57	0	0.55	18.4	0	0.58	17.62	0	0.57	20.3	0	3.37	20.4	0	1.85
Sep	23.13	0	0.86	23.1	17.4	0.91	24.13	0	1.03	23.52	0	0.91	23.7	0	3.89	22.1	0	2.48
Oct	24.17	18	1.03	27.13	24.6	1.06	26.23	51.8	1.12	23.68	52.8	0.99	24.2	1.2	3.18	27.2	0.4	3.71
Nov	24.18	74.4	0.79	26.69	73.6	1.11	25.31	94	0.94	24.87	77.8	0.91	25.9	25.5	4.16	NA	NA	NA
Dec	24.5	92.6	0.56	28.27	38.8	0.775	25.72	104	0.71	25.3	104.2	0.72	27.4	98.6	3.28	NA	NA	NA
<b>Total rainfall</b>		<b>482.8</b>			<b>374</b>			<b>567</b>			<b>674</b>			<b>566</b>			<b>200</b>	

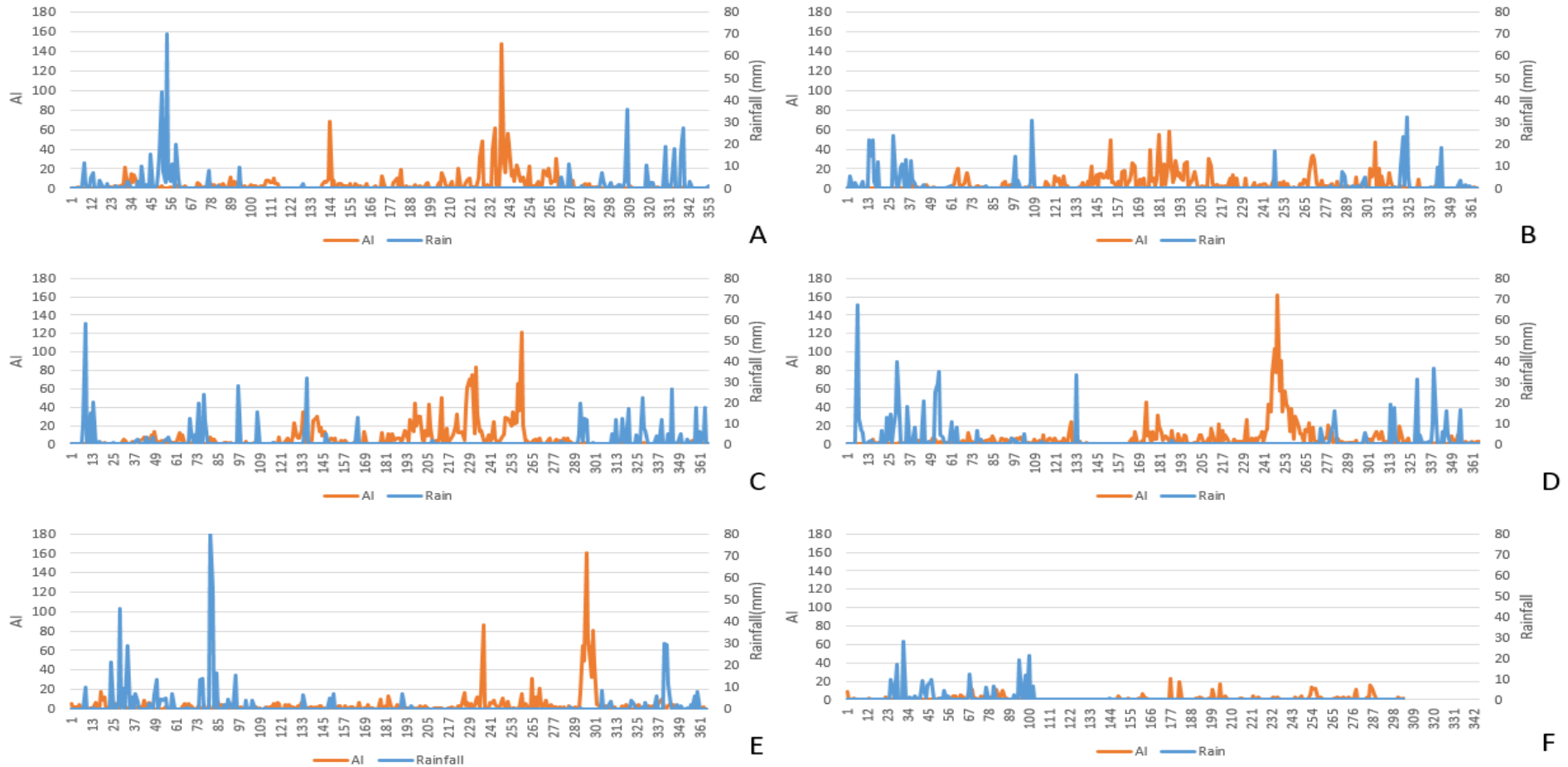


Figure 2-4: The temporal variation in cumulative rainfall (mm) of each day related to *R. blasii* activity (AI) recorded in the Meletse area. From 2014 to 2019 (A to F)

## Chapter 3 Seasonal body mass change of *Rhinolophus blasii* (Mammalia: Chiroptera)

### Abstract

Body size is known to affect an animals' morphology, physiology, and life history. Measurement of mass is a non-destructive method used in condition indices (CIS). For small mammal's seasonal adjustment in body mass is one survival mechanism and there is no known data on the physiology of Blasius's Horseshoe bat (*Rhinolophus blasii*). In this study, the aim was to determine if the body mass of *R. blasii* changed seasonally. Mist net and harp traps were used to capture the bat in Meletse area from 2011 to 2019. Sex, reproduction, age, and mass were recorded for each individual caught. Five categories were created based on age and reproductive condition, where body mass was assessed for seasonal differences. A significant difference in *R. blasii*'s body mass between age, sex and season was found. A female biased sexual dimorphism in body mass. *R. blasii* body mass changed seasonal that increasing in winter and spring decrease in summer and autumn and having the lowest body mass in spring.

### 3.1 Introduction

Bats are essential in sustaining ecosystem services by suppressing insect populations and contribute through pollination, seed dispersal and nutrient cycling (Kunz et al. 2011, Culina et al. 2017). As insectivorous bats, the ecosystem roles of *R. blasii* have a positive influence on humans, which is the pest control, mainly nocturnal moths (Csorba 2003). And they are also known for their excrement has exceedingly phosphorus and nitrogen that is useful to soil (Dietz 2006). The IUCN Red List conservation status of the *Rhinolophus blasii* global population is listed as Least Concern (Taylor 2016), while the regional assessment for South Africa is listed as Near Threatened (Jacobs et al. 2016). Both Taylor (2016) and Jacobs et al.

(2016) suggest that monitoring and protection of caves are required, which are a key resource for *Rhinolophus blasii*. While Jacobs et al. (2016) state that in South Africa, no specific interventions are present, and populations are suspected to be stable. The global populations have been highlighted to be under threat and population numbers are reported to be decreasing (Taylor 2016) due to habitat loss. Very little is known on the life history and population-specific threats of *R. blasii*, especially the southern African populations. It is currently not even known if this may be a different species (Jacobs et al. 2016, Taylor 2016) as to the populations in the northern parts of Africa and in Europe. Both Jacobs et al. (2016) and Taylor (2016) highlight the need for monitoring of this species across its distributional range.

Body size is known to affect an animal's morphology, physiology, and life history (Peters 1983, Schmidt-Nielsen 1984). Measurement of mass is a non-destructive method used in condition indices (CIS) (Green 2001). The body condition refers to an animal energetic state, the indices of body condition attempt to determine the mass of the individual associated with energy reserves after correcting structural body size (Schulte-Hostedde et al. 2005). Body condition indices have reflected the resource availability, sustainability of habitat, environmental pressures, and individual animal health status (Sztatecsny and Schabetsberger 2005). Low body condition indicates a reduced immune function that decreases individuals' survivability (Schmeller et al. 2018). Body condition is linked to resource availability which varies seasonally between species and within sex, age, and reproductive groups (Schmeller et al. 2018).

Various studies have examined the seasonal influence on the expression of ectotherm life-history traits, especially for body size (Ballinger 1977, Dunham 1977, Jones and Ballinger 1987, Adolph, and Porter 1996). Body mass is a balance between expenditure and energy

intake (Merritt et al. 2001). In the temperate zone, Li and Wang (2005) found that seasonal adjustment in body mass is essential for the survival of small mammals (Li and Wang 2005). Most small mammals, such as prairie voles (*Mesocricetus ochrogaster*), South American field mice (*Abrothrix andinus*), Djungarian hamsters (*Phodopus sungorus*) and meadow voles (*M. pennsylvanicus*) decrease their body mass in the cold season. However, Syrian hamsters (*Mesocricetus auratus*), and Pampas mice (*Akodon azarze*) increase their body mass by accumulating energy reserves for the coming winter (Bartness and Wade 1984, Nagy et al. 1995, Del Valle and Busch 2003). Rughetti and Toffoli (2014) found that the Daubenton's Bat (*Myotis daubentonii*) and Savi's Pipistrelle Bat (*Hypsugo savii*) body conditions vary seasonally and between different sexes. Amorim et al. (2015) recorded the European Free-tailed Bat (*Tadarida teniotis*) to decrease body mass in the reproductive season.

Bats in temperate zones are dependent on seasonal food availability (Siemers et al. 2005). When considering energetic constraints within a population structure, the demands are higher for breeding females than in non-breeding females and males (Federico et al. 2008). Corburn and Geiser (1998) found that subtropical bats from warm climates, followed the same thermal pattern as those observed from temperate regions, but less. Zuercher et al. (1999) found that change in body mass reflected adjustment or acclimatization to different selective environmental conditions for summer as opposed to winter. Therefore, Dason and Oli (2008) recommended that the study of body mass changes within a population over time may be used to predict mortality rates.

Sex Size Dimorphism (SSD) in mammals is normally biased towards males and infrequently towards females (Myers 1978). However, with bats, Myers (1978) found that only some families follow the SSD model, while the families Rhinolophidae (included Rhinolophidae, Hipposiderodae, Rhynonyctidae) and Vesperionidae (included Vespertilionidae,

Cistugonidae and Miniopteridae) females were usually larger than males (Williams and Findley 1979). It may be because the female bats' energetic costs are associated with gestation and lactation (Clutton-Brock and Parker 1992, Rughetti and Toffoli 2014, Gittleman and Thompson 1988), which have higher demands in maintaining body condition during the breeding season (Rughetti and Toffoli 2014).

Comparing to males, females not only have to maintain their bodies for existence but also nourish their growing offspring. As female body mass decreases, the energy prerequisites of milk production grow exponentially and the period of lactation increase this burden on female small mammals (Gittleman and Thompson 1988, Pretorius et al. 2020). Thus, the population may have fewer offspring in a year in with fewer resources. While population change being observed the following year. In bats, body mass played an important role for survival of in first year young especially before the onset of winter (Koehler and Robert 2000).

In mammals, body mass variations depend on seasonal basis as food availability fluctuates (Rughetti and Toffoli 2014). Bats are expected to have increased fat reserves before entering torpor or enable them to survive the winter. Body mass is a phenotypic feature under severe selection in bats, as they are the only mammals capable of powered flight. Jacobs et al (2016) show *R. blasii* has a short-wing length that is unable to disperse large distances. As cave roosting bats, *R. blasii* might have higher energy expenditures compared to other species that migrate or go into hibernation or torpor, such as Natal Long-fingered Bat (*Miniopterus natalensis*), Temminck's Mouse-eared Bat (*Myotis tricolor*) due to increased competition for resources because of movement being centred around the cave roosts in the area (McDonald et al. 1990). As it is a short-winged species that are unable to disperse considerable distance, it is unlikely to be experiencing immigration from neighbouring countries. They are also

exposed to parasites and diseases due to large aggregations of multiple species occupying the same roost space (McDonald et al. 1990). In terms of seasonal change in specific areas, these factors might reflect more pronounced in non-hibernation bats species body condition such as *R. blasii* than migratory species or species that enter hibernation (Arita 1996).

This study investigated the temporal variation in *Rhinolophus blasii* body mass from the Madimatle Cave, in Limpopo Province, South Africa. Also, this investigation will provide insights into seasonal and annual changes in the body mass of *R. blasii*, which can be used in the development of an early warning system for possible threats to the population.

### **3.2 Study area**

The study was conducted on the farm Randstephan455 KQ, located approximately 25 km east of Thabazimbi (van der Merwe 1975). Sampling was undertaken at the Meletse Bat Research and Conservation Training Centre (MBRCTC) and Madimatle Cave (24° 36'59 "S, 27° 39'10 "E) in the Limpopo province, South Africa. The MBRCT is located 600m from the entrance to the Madimatle Cave. The vegetation of the area varies from tall open to low woodlands, broad-leaved as well as microphallus tree species are prominent, and it is defined as the Western Sandy Bushveld vegetation and Sweet Lowveld and Mixed Lowveld Bushveld vegetation types (Mucina and Rutherford 2006). The area is characterized by summer rainfall, with a hot, humid climate throughout the summer months (Mucina and Rutherford 2006).

### **3.3 Methods**

Data for this study were obtained by AfricanBats NPC (for bats caught from 2011 to 2019. unpublished data).

Bats were captured at the Meletse Bat Research and Conservation Training Centre (MBRCTC) and the Madimatle cave entrance, using harp traps and mist nets. Harp traps were set from sunset to sunrise, and mist nets were opened 30 minutes before sunset kept open for one hour. Individual bats were weighted using Pesola balances ( $\pm 0.1\text{g}$ ), age was determined by examining whether epiphyses were fully fused or not, fused represented as an adult (Brunet-Rossinni and Wilkinson 2009). Sex was determined by inspecting external genitalia. Females were characterized as pregnant (pulpation of abdomen) or not pregnant; males were characterized as scrotal or not scrotal (Kunz et al. 2011). Fieldwork took place four times a year during each season; each trip collected data 4 to 5 nights.

The South African Weather Service provided the seasonal calendar dates used to categorise climatic data (Weather S.A., 2013-2019), Autumn: 1 March – 31 May, Winter: 1 June – 31 August, Spring: 1 September – 30 November, and Summer: 1 December – 28 February. May to September is the dry season, from November to April is the wet season in South Africa.

To assess the difference in body mass of *R. blasii* bats in different age and reproductive categories, I classified five categories: adult non-reproductive female (ANF), adult male (AM), pregnant female (PF), sub-adult female (SF) and sub-adult male (SM).

The sub-adult female, sub-adult male and pregnant female bats, were excluded for seasonal body change analysis because sub-adult bats were only recorded in a single seasonal (summer), and pregnant females only recorded in spring, making further seasonal comparison analyses impossible

The software RStudio (version 1.2.1073), with selected packages: Car, Ggplot2 and FSA were used for analyses (Fox 2007, Wickham 2016, Ogle 2021).



Hypothesis one: there are significant difference among above five categories in body mass.  
Hypothesis two: there are significant difference body mass seasonal and yearly change in *R. blasii*. To tested hypothesis one, a Kruskal-Wallis ANOVA test was applied to determine if there were differences among reproductive categories and seasons and a Tukey-HSD multiple comparisons of the means test was used to test whether there was a significant difference between each category, with  $p < 0.05$ . Similarly, to tested hypothesis two: the differences between seasons and years the mass of *R. blasii* in different categories using the Turkey-SD test with  $p = 0.05$ .

### 3.4 Results

A total of 310 *R. blasii* bats were caught from 2011 to 2019, representing five categories: adult-male (n=102), Sub-adult male (n=29), adult non-pregnant female (n=123), sub-adult female (n=30) and pregnant female (n=26) (Table 3-1). Adult non-pregnant female bats were recorded each year of the study (2011 to 2019). In 2016, adult non-pregnant female *R. blasii* had the largest body mass ( $\bar{x} = 10.9 \pm 1.18$  grams) among seven years (Fig. 3-1). The most numerous captures were recorded in 2018 (Table 3-1).

Pregnant females were only caught in spring (September to November), and sub-adult bats only appeared from the beginning of December to the middle of February (Table 3-2) confirming that this species is a seasonal breeder.

All reproductive categories were significantly different from each other (see Table 3-2). Adult male body mass ranges were 7.10g-10g ( $\bar{x} = 8.85 \pm 0.89$ g), the sub-adult male is 6.50-9.50g ( $\bar{x} = 8.21 \pm 0.72$ g). The body mass of adult females tended to be larger than that of males (6%). Adult *R. blasii* body mass in both sexes is heavier than sub-adult (male 12.2%; female

10.2%). Comparing between the sexes, sub-adult males were 7.7% lighter in body mass relative to sub-adult females (Table 3-2).

Comparisons of other seasons, there is a higher number of captured bats in summer. Body mass of adult non-pregnant *R. blasii* increased in winter and spring, decreasing in summer and autumn, and having the most solemn body mass in spring, lightest in autumn (Fig 3-2). Significate differences ( $p < 0.05$ ) in body mass was found between spring and autumn (Table 3-7).

Table 3-1. A total number of *R. blasii* bats caught in mist nets and harp traps within the Meletse area (MBRCTC), including records from Madematle cave from 2011 to 2019.

Year	Adult male	Adult female	Sub-adult male	Sub-adult female	Pregnant-female	Total number per year
2011	1	1	1	2	0	5
2012	0	0	0	0	0	0
2013	11	8	5	4	11	39
2014	0	1	0	1	0	2
2015	24	18	0	0	12	54
2016	13	14	4	6	1	38
2017	9	17	8	6	12	52
2018	26	37	11	10	0	84
2019	18	28	0	0	0	46
<b>Total</b>	<b>102</b>	<b>123</b>	<b>29</b>	<b>30</b>	<b>26</b>	<b>310</b>

Table 3-2. Comparison of *Rhinolophus blasii* body mass in different reproductive conditions for sample years 2011 to 2019 within the Meletse area. Mean body mass in grams (g) in mean  $\pm$  SE and sample size in brackets

Category	Body mass
Adult male	9.21 $\pm$ 0.72 (102)
Sub-adult male	8.21 $\pm$ 0.73 (29)
Adult-non-pregnant female	9.78 $\pm$ 0.99 (123)
Pregnant female	11.2 $\pm$ 1.28 (26)
Sub-adult female	8.85 $\pm$ 0.81 (30)

Table 3-3. Analysis of variance in a different category of *R. blasii* by using Tukey-HSD test.

Category	Analysis of Variance	
	F-statistic ( <i>d.f</i> )	P ( <i>adj</i> )
Sub-adult female vs Sub-adult male	56	0.012
Adult non-pregnant female vs Adult male	16.87	0.0023
Non-pregnant female vs Pregnant female	148	0.00057
Sub-adult male vs Adult male	135	0.00000025
Sub-adult female vs Adult female	151	0.0045
Sub-adult male vs Adult Female	135	0.0000000000000022

Table 3-4. Total number of *R. blasii* bats caught in mist nets and harp traps within the Meletse area, including records from Madematle cave in different seasons.

Number of bats					
Season	Adult-male	Adult-female	Sub adult-male	Sub adult-female	Pregnant-female
summer	37	61	30	29	0
autumn	24	29	0	0	0
winter	21	22	0	0	0
spring	24	16	0	0	26

Table 3-5. *Rhinolophus blasii* mean body mass standard deviation and sample size in brackets across different conditions from 2011 to 2019 at Meletse area,

Year	Body mass(g)			
	Adult male	Adult female	Sub-adult male	Sub-adult female
2011	8.3(1)	9.9(1)	7.35±0.45 (2)	7.1 (1)
2012	NA	NA	NA	NA
2013	9.53±0.47(11)	9.83±0.61(8)	NA	NA
2014	NA	9.8(1)	8.18±0.41 (4)	8.76±0.58 (5)
2015	9.34±0.71 (24)	9.94±1.04 (18)	6.5 (1)	NA
2016	9.69±1.03(13)	10.59±1.18 (14)	NA	NA
2017	9.14±0.55(9)	9.51±1.09 (17)	8.73±0.67 (6)	9.55±0.34 (4)
2018	8.97±0.52(26)	9.91±1.86 (37)	8.74±0.42 (6)	9.56±0.22 (8)
2019	8.91±0.71(18)	9.58±1.01 (28)	7.94±0.52 (10)	8.27±0.58 (11)

Table 3-6: Body mass of adult *R. blasii* from 2011 to 2019 in four seasons. Body mass is given in mean (unit is grams) ± SE.

season	Body mass	
	Adult-female	Adult-male
Summer	9.74±1.00 (53)	9.26±0.73 (35)
Autumn	9.11±1.38 (28)	8.96±0.69 (23)
Winter	9.62±1.44 (15)	8.99±0.72 (19)
Spring	10.63±1.36 (27)	9.53±0.72 (25)

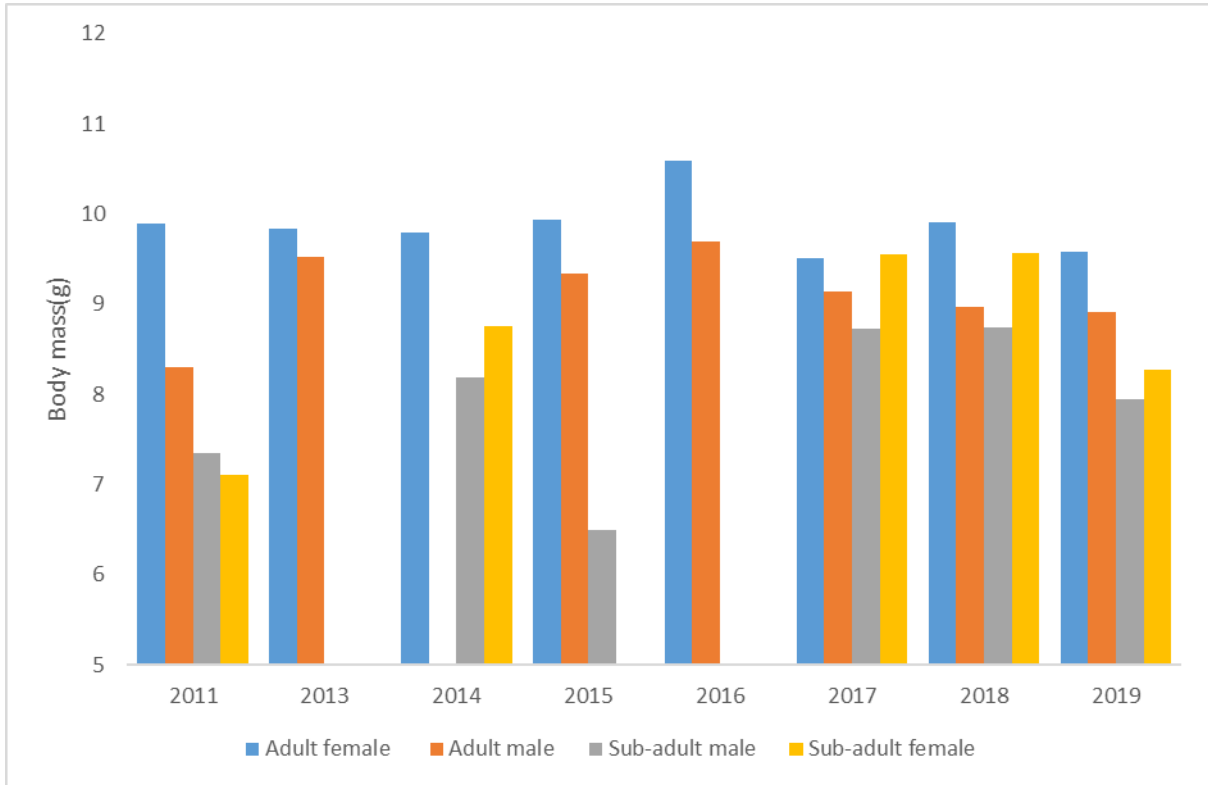


Figure 3-1. Temporal yearly variation in mean body mass of adult non-pregnant female, adult male, sub-adult male and sub-adult female *R. blasii* in the Meletse Area recorded from 2011 to 2019. In 2013 and 2016, we only recorded adult non-pregnant females and adult males, in 2014, we recorded adult male, adult female and sub-adult male. In 2015, sub-adult females were not recorded.

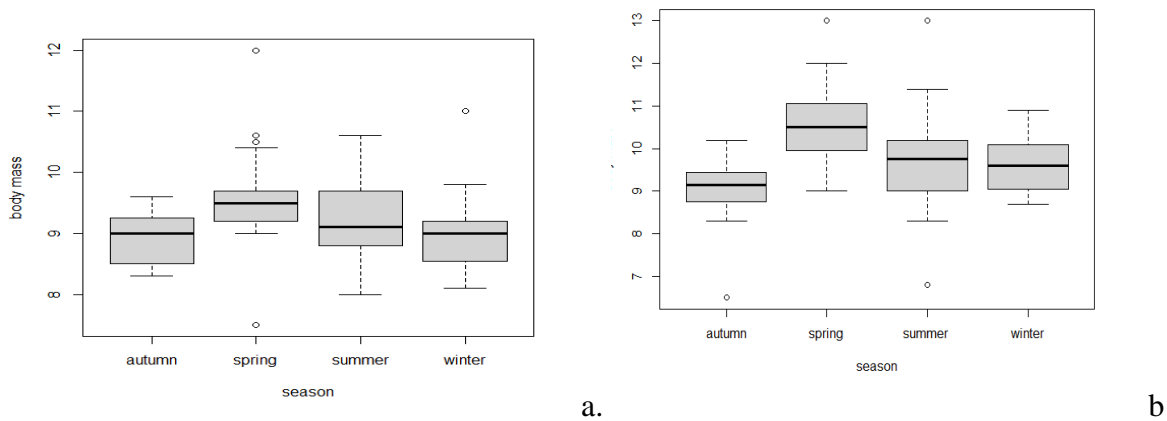


Figure 3-2: Temporal seasonal variation in mean body mass of *R. blasii* in the Meletse Area recorded from 2013 to 2019. a. Adult male b. Adult non-pregnant female

Table 3-7. Analysis of variance in a different season of adult- female *R. blasii* by using Tukey-HSD test. Significantly different values in bold

Season	Analysis of Variance			
	Adult-male		Adult-female	
	<i>diff</i>	<i>p adj</i>	<i>diff</i>	<i>p adj</i>
spring-autumn	0.57	0.02	1.52	0.0000957
summer -autumn	0.30	0.36	0.80	0.03
winter-autumn	0.03	0.99	0.80	0.59
summer -spring	-0.22	0.45	0.50	0.07
winter-spring	-0.54	0.05	0.50	0.06
winter-summer	-0.27	0.52	-0.30	0.84

### 3.5 Discussion

*Rhinolophus blasii* showed signs of major body mass change over the sampling period; they increased their body mass in winter and spring and decreased in summer and autumn. The results from this study indicates that *R. blasii* had a weight difference between males and females at the Meletse study site.

#### *Sexual size dimorphism*

Bats mainly display female-biased sexual size dimorphism, such as many species of Pteropodidae and Vespertilionidae (Myers 1978, Lisón et al. 2012). Hui (2014) reports that *Rhinolophus ferrumequinum* (Greater horseshoe bat) in China also presented female-biased sexual dimorphism. There are two theories of why the bats females are bigger than males 1) females with larger body size can ensure a constant temperature in the case of a lower ambient temperature, then the foetus can develop faster (Stevens et al. 2013); 2) some fruit bat females have a larger body size in order to have a relatively large airfoil to enhance its flight performance so that they can carry cubs during flying (Myers 1978); For further research, forearm length (FA) could be considered as a factor to test sexual dimorphism.

Forearm length may intuitively display that females have proportionately larger wings than males to offset the additional weight caused by foetus and pup carrying; also, body mass can be influenced by effects of different amounts of food in the intestinal system, seasonal differences and reproduction condition within individuals (Ransome 1968).

### *Seasonal change of body mass*

*Rhinolophus blasii* in the Meletse region Limpopo Province presents itself as a single-season breeder, female bats pregnant and give birth in late spring and have young who grow up in one season (summer). Monadjem (2005) collected a lactating female and had a small offspring in Eswatini in late October also support this discovery. For the seasonal variations in body mass, I only analysed adult male and non-pregnant adult females of *R. blasii*. From the lowest body weight values were recorded in autumn, bats regained weight in winter to reach a maximum weight in late spring for females and adult males. The increase can be related to breeding season, In Malawi, *R. blasii* parturition occurs early in wet season, from November to January followed by a one-month lactation period (Happold and Happold 1990); females increase their body mass faster than males. The summer body mass decreases are in both sexes and especially in females, which reaches its lowest in autumn. This decrease also was recorded in Saudi Arabian *Pipistrellus kuhlii*, Alagaili (2008), who proposed that is the impact of moulting.

Changes in the body mass of females reflect energetic demands inflicted by reproduction and lactation for some bats (Koehler and Robert 2000). Summer decrease in female *R. blasii* body mass was mainly associated with the proportion of lactating in the study population. Lactating females have increased energy demands, which may be associated with reducing body mass (Welbergen 2011, Gittleman and Thompson 1988). In many mammals, the



breeding season for males is correlated with an increase in energy spend and a decrease in foraging time; due to sexual activities, which cause male body mass to decrease in summer.

Most of the research examine seasonal cycles in the setting of fall fat accumulation prior to hibernation (Kunz et al. 1998, Speakman and Rowland 1999) or food availability (McNab 1976, Racey 1982). The energy requirements for body temperature maintenance increase during low temperatures, periods of high precipitation, and when prey abundance is low (Avery 1985, Racey and Speakman 1987). However, weight increase for *R. blasii* was starting to increase by the onset of southern hemisphere winter; around that time, food availability and the temperatures were the lowest (Welbergen 2006), and from the results presented in Chapter 2, *R. blasii* do not hibernate (Chapter 2). In South Africa, insectivorous bats' food abundance is closely linked to rainfall (Davies et al. 2015). Lisón et al. (2015) found that the meridional serotine bats (*Eptesicus isabellinus*) s body mass increase could be related to prey captures. Thus, *R blasii* may need to increase prey capture during periods of low food availability (winter).

Seasonality may produce two consequences to body mass evolution: reduced density-dependent competition or increased fasting endurance for individuals of considerable size (Zuercher et al. 1999). Another possible reason that female bats increase their body mass during winter might indirectly or directly affect other bats species (low competition). In the study area, Madimatle cave servers as a maternity roost for several bat species, which may share a similar ecological niche with *R. blasii* such as Sundevall's Roundleaf Bat (*Hipposideros caffer*), Bushveld Horseshoe Bat (*Rhinolophus simulator*), Smithers's Horseshoe Bat (*Rhinolophus smithersi*), *M. natalensis* and Temminck's Mouse-eared Bat. These large aggregations of *M. natalensis* are likely highly competitive in terms of resource and space utilization (Morris 1983, van der Merwe 1987). Although in the wintertime, most

of the *M. natalensis* would leave the study area, resident species such as Sundsvall's Roundleaf Bat (*Hipposideros caffer*) enter hibernation in winter (Pretorius 2020). Thus, *R. blasii*, as a non-hibernation resident species, might face low competition in low prey abundance winter.

The few reports about *R. blasii* are based in Europe on distribution and echolocation (Ransome 1968, Hoven 1996, Stoffberg et al. 2010, Javid et al. 2015). Little published information about *R. blasii*'s life history is known. Body mass may be impacted, from daily variations due to a strong foraging pattern to annual fluctuations. Unfortunately, the data were collected during the long term (performed one sampling for each season every year for seven years) but not continuous, which likely masks variations within the season. Although not investigated as part of this study and inferred, it opens a new avenue for further research.

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## Chapter 4 Conclusion

One major challenge in Africa for bat conservation is the lack of data on species ecology and population status (Frick et al. 2020). Effective monitoring programmes focusing on these basics' aspects, across a longer period and, seasons, can help increase our knowledge of species variations and responses to changes in climatic variables. Wildlife management plans, conservation assessments and strategies require monitoring to track temporal changes in populations and adjust managing objectives based on these findings (Gibbs et al. 1999).

This current study investigated the temporal variation in relative activity and body mass change of the Peak-saddled Horseshoe bat, also known as Blasius's horseshoe bat (*Rhinolophus blasii*) in southern Africa associated with temperature, rainfall, and wind speed across seasons. The aim was to examine if *R. blasii* populations in southern Africa show any annual or seasonal phenology in activity and if so, is driven by environmental factors. The second aim was to determine if the body mass of *R. blasii* changed seasonally. To test this, I used *R. blasii* echolocation activity data and body condition data across the seven-year period in the Meletse area, Limpopo Province collected by AfricanBats NPC.

Hutterer (2005) reported European populations of *R. blasii* hibernates in underground sites in the winter. Based on the *R. blasii* activity data analysed in the current study they have a relatively high activity during the winter and spring (June to November). The southern African population of *R. blasii* did not show hibernation characteristics during winter (Chapter 2). There is no known reports or data currently available to indicate if individuals are migratory or resident. In this study, we detected *R. blasii* activity calls in four seasons, but the relative activity of *R. blasii* was low in summer and early autumn and increased in winter

and spring (Chapter 2). From the capture data (Chapter 3), female *R. blasii* were found to be pregnant in spring. Male *R. blasii* were found to be scrotal throughout the year, but most males were reported to be scrotal in spring. No information was found on mating system of *R. blasii* (Csorba et al. 2003; Schober and Grimmberger 1987; Csorba et al. 2003; Nowak 1999; Schober and Grimmberger 1987), the findings from this study supports that *R. blasii* mating time may be during the winter (June to August).

Rainfall also played a role in the relative activity of *R. blasii* in the Meletse area. The main rainy season in the Meletse area is from October to May; the peak rainfall time (>20mm per day) occurs in December, January, and February (weather station data). *Rhinolophus blasii* activity decreased with increased rainfall (Chapter 2). More specifically, heavy rainfall days (over 20mm per day) were the main factor that was positively correlated with the temporal variation in relative activity for *R. blasii*. There is a positive relationship with this species activity across years' cumulative rainfall. In the cumulative higher rainfall year, *R. blasii* body mass also was higher. Cumulative rainfall with associated increased resource (insects) availability (Geisser and Reyer. 2005, Descamps et al. 2008), may explain why in 2017, *R. blasii* had the highest activity during the study period. Rainfall seems to have direct and indirect effects on *R. blasii*, heavy rainfall directly limited the activity of *R. blasii* as rain droplets interfere with echolocation, and heavy rain can influence bats' thermoregulation (chapter 2); Rainfall also affects the prey availability, which indirectly influence the activity and body mass of *R. blasii*. Thus, the monitoring of *R. blasii* can be used as environmental bio-indicators, for early warnings systems for changes in population based on changes in the environment. These warnings can help managers and landowners respond to changes before major shifts in the environment occur.

Although *R. blasii* did correlate to rainfall and seasons to some extent, it does not mean that these correlations are the ultimate cause. Various other factors could also influence them, such as water source availability, moonlight, habitat structure, sunlight duration, interspecies competition (Ciechanowski et al. 2006, Biłás et al. 2007). Including those factors in future studies would build on the data that this study provides.

Sexual dimorphism in body mass was documented in *R. blasii* at Meletse area, where on average, adult and sub-adult's females have greater body mass than males. Significant seasonal variation in the body mass of *R. blasii* was documented. Female body mass increased during winter and spring, decreasing in summer and autumn. Male body mass increased in spring while decreasing in summer and autumn. *Rhinolophus blasii* body mass increased at about the onset of the southern hemisphere winter (June to August) when food availability is lowest. The result suggests that food availability is not likely the primary driver of body mass in *R. blasii* during this period. Therefore, changes in the seasonal body mass of *R. blasii* may be associated with the maximum reproductive effort of both sexes. Insect abundance and availability within the Meletse area were highest during periods of increased temperature and rainfall (Nkoana 2020); this supports the theory that breeding seasons overlap with high productivity in terms of available prey and water resources, allowing breeding females and sub-adult to increase the survival rate (Bernard and Cumming 1997, Rughetti and Toffoli 2014). Sub-adults were only recorded in summer, while pregnant females in spring. During the winter, the peaks in body mass correlate with bat activity, which need to increase their fat stores for mating, rearing offspring, and survival (Rughetti and Toffoli 2014). At Meletse, *R. blasii* give birth during the late Spring to early summer (chapter 3). These breeding patterns were also documented in other African bat populations located across South Africa, Eswatini, Lesotho, Southern Mozambique, Namibia, Zimbabwe,

and Botswana (Cumming and Bernard 1997). The Madimatle Cave is a maternity roost for several bat species (Kearney et al. 2016), which could also further explain the change in *R. blasii* body mass, which may be related to interspecific competition. The influx of the migrant, *Miniopterus natalensis* also add further interspecific competition during Nov-December (Pretorius 2020, Van der Merwe 1975). Thus, it is important to not only focus on single species monitoring, but to also understand possible interspecific competitive species that may affect the interpretation of results from other sites where there may not be high interspecific competition.

Interestingly, as *R. blasii* activity decreases, the body mass also decreases over the years. Except for 2016-2017, where both activity and body mass were the highest recorded. At the same time, the number of captured sub-adults was highest in 2018 and lowest in 2019. Although the sample size was small, I assume the *R. blasii* population had increased between 2016 to 2018 and decrease in 2019. The activity data also support this hypothesis; in 2019, *R. blasii* activity rate dropped dramatically 2019, has a higher activity rate from 2016 to 2018. Land-use change has been reported in South African, the mineral-rich geology of Meletse has been prospected for mining (Almond 2012). Kearney and Seamark (2012) also suggested that with further development of roads, pits and processing areas resulting from the mining activities could cause the loss of foraging areas for bats species in Meletse. Kearney and Seamark (2014) reported loss of forage habitat and prey base also can have an impact on *R. blasii* Cleared natural vegetation for the development of the infrastructure required such as game farm with camps, which reduced access to natural foraging area and food resources negatively influence the fitness and survival of the *R. blasii*.

As a specialist bat, *R. blasii* has a lower tolerance to vegetation change (affect prey abundance and foraging habitats). Thus, any habitat change activity which results in changes

to forage habitat may have a broader impact on the region's *Rhinolophus* species which use high-frequency echolocation and are clutter foragers (Findley and Black, 1983; Schoeman and Jacobs, 2003).

Nkoana (2020) report on insect activity in the Meletse area, where Lepidoptera abundance was lower in the wintertime, which is the main published prey of *R. blasii*. This may be the reason *R. blasii* is more active in winter due to foraging time increased. During the initial research in the area undertaken by AfricanBats NPC, sample collection was not focused on *R. blasii*. For more accurate population research in the future, I would suggest picking the wintertime for a long-term single-season continually study to investigate why and how *R. blasii* increase their body mass, especially females.

My study serves as a baseline that could aid some organisations such as the International Union for Conservation of Nature (IUCN) Red List in reporting on this species' status within the Meletse area. The *R. blasii* population in Meletse provide insight into differences in southern African population, where this population have a different life-history characteristic compared to the European population (Csorba et al. 2003). The key finding from this study can support management programs to isolate target objectives such as lifespan. The life history studies such as this would include long-term monitoring programmes that will allow for the early warning of population depressions, also allow for insight derived from short-term temporal monitoring programmes to accurately assess the specific variation in *R. blasii* within the same season, such as to monitor females or sub-adults in spring and summer to predict the risk of population depressions, non-breeding, and breeding seasons to monitor population variations. We need to continue to monitor this population to allow for an adaptive management approach, for active adaptive management responses for best applied conservation efforts. Thus, the management of bats during the non-breeding season would

have different objectives than management in breeding seasons. This study adds to the growing knowledge of *Rhinolophus blasii* life-history characterise and ecology. It also can aid the development of wildlife management programs for the conservation of this species in the Meletse area

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