

Seasonal small mammal diversity, endocrine correlates, and diet behaviour in
rodents across transformed and pristine ecosystems in the Magaliesberg

by

Mmatsawela Ramahlo

10097687

Supervisor: Prof. Andre Ganswindt

Co-supervisor: Prof. Michael J. Somers

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DECLARATION

I, Mmatsawela Ramahlo, declare that this thesis, which I hereby submit for the degree Doctor of Philosophy (Zoology) at the University of Pretoria, is my own work and has not been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

I, Mmatsawela Ramahlo, declare that the research described in this work has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Research and the Policy and Procedures for Responsible Research. The work was approved by the University of Pretoria Animal Ethics Committee.

DISCLAIMER

All data chapters were structured and formatted according to the scientific journal to be submitted for publication. Therefore, there is an inevitable overlap and repetition in the introduction and methods sections between chapters.

Signature:



Mmatsawela Ramahlo

Date: 07/02/2025

SUMMARY

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Mmatsawela Ramahlo

Supervisors: Prof. A. Ganswindt

Prof. M. J. Somers

Department: Department of Zoology and Entomology

University: University of Pretoria

Degree: PhD in Zoology

Keywords: Abundance, anthropogenic activity, biological indicators, diet, diversity, ecosystem health, enzyme immunoassays, faecal glucocorticoid metabolites, keratin, land transformation, rodents, southern Africa, species richness, stress-related hormones

Landscape transformations due to a growing human population lead to increased resource use, declines in natural habitat quality and increased human-wildlife conflicts across ecosystems. As a result, animal population dynamics, stress-related behaviour, and diet may be affected in unknown ways. Rodents can be ideal model species for monitoring ecosystem health. Their relatively small size allows them to use various niches that are inaccessible to larger animals. By investigating the effects of anthropogenic activities and changes in global climate on rodent species, we can assess the impacts these variables will have on wildlife over time. We can also determine the effects of these variables on species abundance, diversity, stress-related endocrine correlates, and diet preference at inter- and intra-specific levels.

This study investigated the effects of anthropogenic activity and human-mediated land transformation on capture/recapture frequencies, species richness, and diversity of native small mammal community assemblages in the Magaliesberg Biosphere, North West province, South

Africa. Additionally, the study aimed to establish minimally invasive methods for quantifying faecal glucocorticoid metabolites (fGCMs) in six southern African rodent species (*Aethomys ineptus*, *Lemniscomys rosalia*, *Mastomys coucha*, *Micaelamys namaquensis*, *Otomys irroratus*, and *Steatomys pratensis*) by evaluating five enzyme immunoassays (EIAs). Following this, the adrenocortical responses of dominant rodent indicator species to human-mediated practices and seasonal variation were monitored to determine their efficacy in informing on ecosystem health. Methods of keratinaceous (hair and nails) tissue sample collection and preparation methods for rodent diet stable isotope analysis (SIA) were also investigated. The least minimally invasive method was used to assess the diet preferences of two dominant rodent species across differently disturbed landscapes.

Five anthropogenically transformed land-use types were investigated: animal rehabilitation and ecotourism centre, agricultural farmstead, residential farmstead, mine-adjacent agricultural farmstead, and nature conservancy. Over three consecutive years, live traps were used to sample rodents during the dry and wet seasons. For validation of fGCM quantification methods, faecal samples were collected daily from enclosures of captive females and males and frozen immediately at -20°C. Biological and physiological stimuli were used, and fGCM concentrations were determined from samples collected before, during, and after each event. Following this, a suitable assay was found to assess the adrenocortical hormone responses of two dominant southern African murids, *Lemniscomys rosalia* and *Mastomys coucha*. Faecal samples were collected from traps of captured individuals and measured for fGCM concentrations using the previously validated assay. For diet analysis, hair and nail samples were prepared for SIA mass spectrometry, and carbon and nitrogen isotope ratios were compared.

My study found that capture/recapture frequencies differed significantly between sites and seasons, with the highest capture frequencies recorded at the agricultural and residential farmsteads. Species richness and diversity were highest at the residential and industrial-agricultural farmsteads, both of which experienced intermediate levels of anthropogenic disturbance throughout the sampling period. For quantifying fGCMs in rodents, my study identified the 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA (detecting fGCMs with a 5 α -3 β , 11 β -diol structure) as most suited in all six species assessed. Using this EIA, it was found that fGCM concentrations of *M. coucha* were significantly higher in the wet season compared to the dry. Land-use variation had no significant impact on either species, which supports reported

synanthropic behaviour (to various extents) in both species. The reduced adrenocortical responses observed in these dominant species in human-transformed areas suggest that when monitoring fGCM concentrations, dominant species such as *M. coucha* and *L. rosalia* are not always an ideal indicator of anthropogenic disturbance.

Diet-related sampling methods were compared, and hair sample collection was found to be faster and more animal-friendly, resulting in a calmer demeanour and reduced anxious behaviour in animals compared to the effects of nail sample collection. Correlation analyses of hair and nails showed high similarity in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios within four of the six species analysed. Hair clipping may be classified as the less invasive sampling method and can be used exclusively on rodents, as isotopic variation between hair and toenail samples is negligible.

Overall, the findings of the study lend support to the use of minimally invasive methods of data collection and a combination of both dominant and low-population rodent species as biological indicators of ecosystem health to ensure repeatable, long-term data collection and capturing of ecological responses from both generalist and specialist species to elucidate the impacts of anthropogenic activities on wildlife populations and their natural environments.

OUTPUTS FROM THIS THESIS

Journal Article

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Conference Presentations

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Poster presentation

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Ramahlo, M.; Somers, M.J.; Ganswindt, A. Southern African murids (*Lemniscomys rosalia* and *Mastomys coucha*) respond to seasonal variation and land transformation.

LIST OF ABBREVIATIONS

ABR	Animal Breeding and Rehabilitation
ACTH	Adrenocorticotrophic Hormone
CMR	Capture-Mark-Recapture
DW	Dry Weight
EIA	Enzyme Immunoassay
fGCMs	Faecal Glucocorticoid Metabolites
GCs	Glucocorticoids
HPA	Hypothalamic-Pituitary-Adrenal
IAR	Industrial Agricultural Residential
NCR	Nature Conservancy Residential
RFS	Residential Farmstead
SD	Standard Deviation
SIA	Stable Isotope Analysis

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CHAPTER 1: GENERAL INTRODUCTION

1.1 BACKGROUND AND SCOPE

Global anthropogenic landscape transformations are a result of a growing human population and the associated expansion and development of agriculture, urbanisation, and industrial practices such as mining (Irwin et al. 2010). The subsequent loss of natural habitats, urban encroachment, increasing human-wildlife conflicts, and local species extinctions across various ecosystems emphasise the importance of monitoring ecosystem health and wildlife responses to anthropogenic activities (Waters et al. 2016). Investigating ecosystem health requires the assessment and monitoring of several variables and indices. These can inform on the level of disturbance resulting from anthropogenic activities and provide management strategies on how to rehabilitate disturbed landscapes and restore displaced species. Good study designs that include monitoring of multiple variables, although costly and time-consuming, can provide far more insight than single-variable studies. By carrying out robust studies on ecosystem health using biological indicator species, we can work to improve the quality of ecological monitoring research and better inform policy makers and management strategies (Cadotte et al. 2016, Fanson et al. 2017). Many studies show both small- and large-scale temperature and rainfall shifts annually. With a yearly increase in temperature and decrease in rainfall, continued monitoring of previously identified species of interest is important to track their progress. The monitoring of “Least Concern” species is also crucial as their responses to environmental changes are often the most telling due to their resilient and adaptive nature.

Rodents are valuable biological indicators of anthropogenic disturbance and, subsequently, ecosystem health. Monitoring their population dynamics, behaviour, and physiology, specifically measuring their adrenocortical activity, can provide insights into the impact of anthropogenic disturbance and seasonal variation in transformed landscapes. Using minimally invasive sample collection methods on sentient animals means minimal pain, relatively quick recovery of individuals from potentially induced stress, and release of wild-caught species. By highlighting the efficacy and convenience of these data collection methods, we can reduce animal casualties in biological studies conducted on both captive and free-ranging species. Additionally, minimally invasive techniques can be beneficial for assessing the impact of anthropogenic disturbance on natural environments and their associated fauna.

The importance of monitoring ecosystems and their associated species stems from human encroachment and the irreversible changes that humans have made to the natural environment (Umetsu & Pardini 2007, Irwin et al. 2010, Waters et al. 2016). Altering landscapes ultimately leads to habitat degradation or destruction, and depletion of natural resources. These changes to natural habitats of wildlife species lead to niche variation and leave three courses of action: adapt, move, or die. As such, wildlife species population fluctuations are linked with human population growth and the presence or absence of human-mediated activities such as agriculture, urbanisation, and mining, among others (Marris 2008, World Wildlife Fund 2016, Christie et al. 2019). By monitoring a wide variety of land use types, we can assess the level of anthropogenic impact on the natural environment, free-ranging species, and their responses to these changes. Additionally, animal responses to changing global climatic conditions such as rainfall and temperature patterns should be investigated. This makes the incorporation of seasonal variation into landscape monitoring an important aspect, as data collected on the presence, abundance and behaviour of some animals may be season dependent. Investigating associated impacts of both land transformation and seasonal variation on wildlife species may highlight which factors are most detrimental and inform on suitable management strategies.

Seasonal differences in rainfall and temperature can influence populations and shift community structure both directly and indirectly (Sánchez-Cordero 2001, Fox 2011, Massawe et al. 2011). Rainfall and temperature can affect the germination and growth of plants that provide food resources and vegetation cover for many species, and so, aseasonal fluctuations in these variables can significantly impact the population numbers and community structure within an ecosystem. Rainfall plays a key role in the success of populations as many mammal species depend on rainfall as an ecological cue, such as for breeding, as the wet season results in an abundance of resources such as food and plant cover that offers protection from predators (Taylor & Green 1976, Meheretu et al. 2015). As such, extreme rainfall conditions (i.e., drought or flood) can have adverse effects on wildlife, with larger-sized animals being able to migrate to avoid harsh seasons and return when conditions are more favourable (Hurst et al. 2014, Lininger & Wohl 2019). Many smaller-sized animals, however, cannot cover vast distances and thus rely on the use of microhabitats and refugia, such as burrows and fallen trees, that assist with moderating environmental conditions (Jorgensen 2004, Swan et al. 2016). Small mammals and their limited ranges can, therefore, offer insight into long-term population and behavioural responses to land transformation and seasonal variation. As they are restricted in their movements, their diversity and behaviour can be monitored and correlated to their

immediate environmental conditions. This makes small mammals, such as rodents, ideal biological indicators of environmental changes and ecosystem health.

The use of minimally invasive techniques for monitoring species of interest has become more popular for researchers because we can minimise animal discomfort and improve the accuracy of collected data through replicable study designs as the animal can be sampled from as needed (Cadotte et al. 2016, Fanson et al. 2017). Many invasive techniques may not be effective for long-term monitoring, and so minimally invasive data collection allows for long-term patterns and trends within the study species and at the population and community levels. As such, my study used the capture-mark-recapture technique for population studies, faecal sample collection for monitoring adrenocortical activity, and keratinised structures such as hair and nails for analysing diet selection in rodents. These sampling methods and sample types allowed for repeated measurements and reduced impacts of the overall study on the free-ranging animals being studied.

1.2 SMALL MAMMAL DIVERSITY IN RESPONSE TO LAND TRANSFORMATION AND SEASONAL VARIATION

Human threats to biodiversity and subsequent animal responses should be closely monitored to inform on ecosystem health and the overall impact of anthropogenic activities on the natural environment (Marris 2008, World Wildlife Fund 2016, Christie et al. 2019). Ecosystem health is often monitored using variables such as species richness, distribution, and community assemblage in small mammals across transformed and untransformed landscapes to assess ecosystem health (Umetsu & Pardini 2007, Christie et al. 2019).

Landscape diversity has also been used as a measure of ecosystem health and refers to the range and abundance of species in an area (Monadjem & Perrin 2003, Cadotte et al. 2016, Simelane et al. 2018). Monitoring species abundance and diversity of a region over time can highlight the impact of anthropogenic activities on natural environments (Umetsu & Pardini 2007, Christie et al. 2019). Declines in landscape diversity can result in homogenisation of landscapes and local species extinction (Monadjem & Perrin 2003, Magurran 2004).

Monitoring adaptive taxonomic groups that are not categorically at risk of extinction, however, can inform on a wide range of species in the environment as anthropogenic activities that can

impact resilient species may cause irreparable damage to species that are less able to adapt (McKee et al. 2004, Watson et al. 2004, Colles et al. 2009). Therefore, rodents are reliable short-lived model species that can be studied over several generations (Galetti et al. 2009, Merritt 2010). The use of these adaptive and resilient species in research allows us to investigate the likelihood of wildlife populations and ecosystems recovering from human-induced disturbances. It can also help to improve the management strategies of threatened species in disturbed landscapes.

When assessed using the IUCN Red Data List of Threatened Species, rodents are capable adapters to many environmental changes (Joyce et al. 1997, Skinner & Chimimba 2005, IUCN 2021). Although rodents can use microhabitats, they are not restricted by their macrohabitat and can thus persist in a wide range of landscapes (Skinner & Chimimba 2005). Successful establishment of rodents in a landscape does not require interspecific interactions with other taxonomic groups. They, themselves, are ecosystem drivers who cycle nutrients through the soil, disperse seeds to aid in plant growth, and provide food for predators (Joyce et al. 1997, Monadjem et al. 2015). With the increased human expansion and subsequently transformed landscapes, favourable conditions prove more challenging to maintain (Rymer et al. 2013, Spear et al. 2013). In addition to an increase in land transformations due to more significant anthropogenic needs, climate change may exaggerate disruptions to ecosystem health. Thus, diversity-based research should include seasonal variation (Ellis et al. 2013, Cadotte et al. 2016). Due to the resilient characteristics displayed by rodents, it is essential to monitor them in both natural and transformed landscapes to gauge the critical limit of their tolerance for environmental changes brought about by climate change and anthropogenic activities.

1.3 STRESS-RELATED FGCM CONCENTRATIONS AS A PROXY FOR ECOSYSTEM HEALTH

1.3.1 Biological and physiological validation of minimally invasive methods for quantifying fGCM concentrations in rodents

Adrenocortical activity from stress-related physiological responses of wildlife and associated behavioural alterations can be used as indicators of animal welfare and add to our understanding of their ecology (Reeder & Kramer 2005, Angelier & Wingfield 2012). Quantifying physiological responses to anthropogenic activity enables us to investigate the effects of human-mediated disturbance on the environment and its fauna and flora. Stress is a

general syndrome, either acute or chronic, that is brought on when a perceived stressor threatens the homeostasis of an individual (Palme et al. 2005, Hulsman et al. 2011). When a stressor is perceived, activation of the hypothalamic-pituitary-adrenal (HPA) axis stimulates the secretion of glucocorticoids (GCs), which are then metabolised and circulated through matrices such as blood, urine, and faeces (Reeder & Kramer 2005, Touma & Palme 2005). Short-term elevations of GCs can benefit the animal, for example, as a fight or flight response can allow it to evade predators. Long-term elevations, however, can lead to health issues such as immune response suppression, reduced reproductive success, and impaired HPA regulation (Dobson & Smith 2000, Hing et al. 2014). Therefore, monitoring stress-related biomarkers like concentrations of GCs or their metabolites can be an important aspect of wildlife management and conservation.

Physiological and behavioural monitoring of captive animals can be invasive and negatively affect the welfare of study animals. This can be due to life-long captivity requirements or euthanasia at the end of a study (Hodges et al. 2010, Mason 2010). Minimally invasive quantification of faecal glucocorticoid metabolites (fGCMs) is a viable method for monitoring stress-linked hormone activity (Palme 2005). Faecal sample collection does not require the animal to be restrained or immobilised, and in many cases, faeces can be collected discretely from study animals (Palme et al. 2005). Monitoring rodent stress-related responses to environmental stressors can improve our understanding of anthropogenic impacts on their surrounding ecosystems. Most stress-related rodent research has been conducted on laboratory reared animals, and the majority of established enzyme immunoassays (EIAs) have been validated using captive animals in the northern hemisphere (Touma et al. 2004, Touma & Palme 2005, Sheriff et al. 2011). It is, therefore, vital to validate assays for free-ranging southern African species. My study used biological and physiological methods to validate the suitability of five different EIAs for quantifying fGCMs in several southern African rodent indicator species.

1.3.2 Rodent stress-related fGCM responses to land transformation and seasonal variation

Once a reliable EIA has been identified, monitoring the physiological responses of free-ranging indicator species to anthropogenic disturbance and seasonal variation can inform on ecosystem health and identify potential environmental stressors (Reeder & Kramer 2005, Angelier & Wingfield 2012). Stressors such as extreme environmental changes and invasive research methods can have detrimental effects on animal health and welfare, and can lead to conditions

such as repetitive pacing, self-harm, weakened immunity and increased disease susceptibility, increased blood pressures and inflammatory responses, among others (Aplin et al. 2003, Angelier & Wingfield 2012, Wingfield 2013). Some of these conditions can become epigenetic and spread to future generations, further weakening the population (Braastad 1998, Mason et al. 2013). Using physiological stress-related hormone responses of wildlife to human-mediated disturbance may provide insight into animal welfare and ecology, and potentially improve our understanding of the anthropogenic activities of greatest concern to wildlife populations (Nováková et al. 2008, Harper & Austad 2012). Stress hormone monitoring is thus a vital part of wildlife management and conservation as the impact of environmental stressors on natural populations can be measured. Minimally invasive and sustainable means of measuring stress-related hormone responses in rodent indicator species can thus add to our knowledge of animal ecology and the effects of human encroachment on the natural world. Therefore, this study aimed to assess the effects of land use and seasonal variation in two dominant rodent species using stress-related faecal endocrine correlates as a physiological measure of disturbance across untransformed and transformed landscapes in my study area.

1.4 STABLE ISOTOPE ANALYSIS OF RODENT SPECIES DIET ACROSS LANDSCAPES AND SEASONS

Globally, we see an exponential increase in human populations and anthropogenic activities, resulting in declining natural landscapes due to habitat destruction or fragmentation (Turner et al. 2001, Sasidhran et al. 2016). Small mammals such as rodents can be used as biological indicators of ecosystem health and landscape disturbance, revealing the effects of human-mediated land transformation on the natural environment and biodiversity in transformed areas (Avenant et al. 2008, Wilson et al. 2018). With the rapid transformation of natural landscapes, research on the behavioural responses of wild populations to these changing environments is crucial. Land transformation and the resultant displacement and loss of species have led to a need for non-invasive methods of sample collection. These methods can further our understanding of the impacts of anthropogenic activities on natural habitats and their fauna without sacrificing animals for research. This can ensure repeatable, sustainable, and environmentally friendly research. In ecosystem monitoring efforts, it is crucial to obtain up-to-date data on the diet and foraging preferences of indicator species to determine how they are persisting in transformed landscapes under changing seasonal conditions. By monitoring diet selection and trophic ecology, we can assess the effects of human practices on ecosystem health

and wildlife behaviour such as foraging, as significant differences in diet selection and foraging behaviour may be indicative of food resource shortages and can possibly lead to declining animal welfare and local species extinctions (Dickman 1999, McCarty 2001).

The diet of an animal can reveal many details about its ecology and welfare, and as such, an increasing number of studies are investigating the diets of various animal species and the factors that may affect their foraging behaviour (Kerley et al. 1990, Robb et al. 2012). Stable isotope analysis allows for the majority of digested food resources to be detected as their isotope ratios are accumulated in animal tissue such as blood (recent diet), muscle (average indication of diet), bone (long-term diet), and other keratinized structures, such as hair, feathers, and nails (Tieszen et al. 1983, Lourenço et al. 2015). The use of stable isotopes in ecology involves the determination of the abundance of two commonly used elements in food webs and diet studies: 1) carbon (C) and 2) nitrogen (N) (Tieszen et al. 1983, Layman et al. 2012). By measuring the stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$), diet patterns and the relative trophic positions of individuals can be assessed (Barquete et al. 2013, Van Den Heuvel & Midgley 2014). Stable carbon isotope values ($\delta^{13}\text{C}$) are used to determine the source of nutrients as $\delta^{13}\text{C}$ is well-conserved in food chains, while stable nitrogen isotope values ($\delta^{15}\text{N}$) indicate the trophic level of individual animals (Layman et al. 2012, Van Den Heuvel & Midgley 2014). Carbon isotopes can provide distinctions between browsers and grazers, or forest floor and canopy feeders, while nitrogen isotopes in a consumer's tissues increase proportionally with its position in the food web, and can help us to differentiate between herbivores, carnivores, and omnivores (Barquete et al. 2013, Van Den Heuvel & Midgley 2014).

Natural carbon and nitrogen isotopes occur in varying compositions in animal tissue and can be used to analyse diet as these compositions reflect the stable isotopes found in the animal's diet (Miller et al. 2008). Carbon isotope ratios provide data on consumed food types and show little change in the trophic positions of the species under study, while stable nitrogen isotope ratios change to reflect the trophic position of the animal (Miller et al. 2008, Caut et al. 2009). Typically, the carbon ratio is used to identify carbon fixed by marine or terrestrial C_3 plants and C_4 plants as this ratio moves through food webs relatively stably (changing approximately 1‰), while the nitrogen ratios found in the bodies of most animals are more positive (by approximately 3-4‰) than those found in their diets (Deniro & Epstein 1981, Peterson & Fry 1987). Additionally, the $\delta^{15}\text{N}$ ratios obtained from animals are dependent on individual diet,

and the type of tissue analysed as $\delta^{15}\text{N}$ can vary in its level of preservation in different tissues (Deniro & Epstein 1981, Caut et al. 2009). As such, the nitrogen isotope ratio is used to elucidate the trophic level ecology of animals.

Tissue sample collection for diet studies often involves blood, muscle, and bone, along with liver, kidney and stomach content, depending on the research questions being asked (Hobson & Quirk 2014, Rosenblatt et al. 2015). The type of tissue selected for analysis determines the invasiveness of the sample collection. The level of invasiveness can range from drawing blood, where the animal experiences some pain and discomfort, to using muscle, liver, or stomach content, where the animal is euthanised. Tieszen et al. (1983) proposed keratinized structures such as hair or nails as a viable sample. They showed that, while sample turnover rate was slow, hair was approximately 1% more enriched in ^{13}C relative to the diet and was also more enriched than tissues such as fat, brain, muscle, and liver. Conversely, an approximate 3-4% increase in enrichment in ^{15}N relative to diet was recorded (Caut et al. 2009). An important aspect of using sample tissue as a proxy for diet content in animals is having an appropriate diet to tissue discrimination factor, which can vary according to tissue type, species studied, and even sex or age of individuals within a species (DeMots et al. 2010, McLaren et al. 2015). A limiting factor to the understanding of southern African rodent ecology is the minimal research available on diet behaviour of free-ranging indigenous species, and as such, information regarding their assimilation of dietary sources into different tissue types is not well studied. Diet-tissue discrimination studies have been limited to captive rodent species in the northern hemisphere. These studies require captive animals with a controlled diet, which does not often account for free-ranging dietary behaviour of similar species (DeMots et al. 2010). By investigating the diet of free-ranging small mammals, we can improve our knowledge of wild species and identify variables that influence their behaviour in the natural environment.

Rodents have a diverse dietary range, including seeds and grains, leaves and roots, and insects, with most observed inter-specific differences in diet being related to body size or habitat preferences (Canova & Fasola 1993). Studies conducted on the diet of small mammals have shown that food resource quality and abundance are directly linked to litter size, weaning success, and nesting survival (Gilbert & Krebs 1991, Monadjem & Perrin 2003). This means that species richness and abundance are correlated to food resource quality and quantity in natural landscapes, and with increased human activity, both food quality and quantity are affected (Massawe et al. 2011, Ademola et al. 2021). Diet studies on rodent species, therefore,

allow for long-term monitoring of the extent of anthropogenic disturbance on the natural environment.

Currently, researchers are relying on more ethical and sustainable research methods, and so sampling methods that result in reduced invasion and stress to animals have become increasingly popular. In dietary studies, sample collection regularly involves the collection of keratin structures such as hair, nails, fin clips, and feathers, as they are relatively easy to obtain and, more importantly, cause nominal discomfort to the animal (Roth & Hobson 2000, Barquete et al. 2013, Van Den Heuvel & Midgley 2014). Minimally invasive sample collection means the animal can be released after sampling and, if adequately marked, recaptured, and resampled to determine potential variation in the diet of species of interest over time. My study aimed to compare sample collection protocols, preparation, and stable isotope analysis of two keratin sample materials, namely hair and nail clippings, from nine southern African rodent species. I aimed to determine whether the sample types have similar carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), and which sample type is the more animal- and researcher-friendly option for long-term diet studies. Using the identified keratin sample type, my study further aimed to assess the effects of landscape transformation and seasonal variation on the diet of rodent species. Two stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were compared to determine whether any inter- or intra-specific variation was observed between southern African rodent species in the region. Carbon and nitrogen isotope ratios of each species were compared across seasons (dry and wet) and landscapes (minimally disturbed to very disturbed) to investigate how the abovementioned variables impact diet selection of rodents in the region.

1.5 MAP OF THE STUDY AREA AND SUMMARY OF STUDY SITES

Study animals were captured at several sites located around the Magaliesberg mountain range (25°41'00.1"S 27°57'51.8"E), which extends through the Gauteng and North West provinces of South Africa (Figure 1.1). Study sites are located in the De Wildt (25°40'05.5"S 27°55'23.1"E) and Zilkaatsnek (25°39'25.8"S 27°56'34.9"E) areas of the region, and are characterised by hot, wet summers (November to March) and cold, dry winters (April to October) (Boonzaaier & Hough 2011) (Table 1.1). The start of the wet season and fluctuations in inter-annual rainfall are variable. The Magaliesberg Biosphere falls within the Savannah Biome and is characterised by two vegetation types: the Gold Reef Mountain Bushveld vegetation type at higher altitudes, and the Marikana Thornveld and Moot Plains Bushveld in lower lying areas (Rutherford & Mucina 2006). The region is dominated by thorny *Vachellia*

species and semi-open thickets, combined with a predominantly grassy and herbaceous layer. The underlying geology is comprised of igneous rocks from the Rustenberg Layered Suite and the Rooiberg Groups (Rutherford & Mucina 2006).



Figure 1.1. Orientation map of the North West province showing the locations of the five study sites where rodents were captured between 2018 and 2021 in South Africa.

Disturbed or transformed land can be classified as a landscape that has been physically altered by human activity or invaded by non-native species and has not retained its natural land cover or land use (Foley et al. 2005, Bongaarts 2009). Land transformation is achieved by altering land use entirely, or through the modification or intensification of pre-existing land use (Parody et al. 2001). Intensive land use can include dairy farming, and cultivating of crops such as fruits and vegetables, which are regarded as high-value crops due to their importance in human and animal dietary requirements (Ehrlich & Ehrlich 1991, Eccard et al. 2000). Intensive land use can lead to environmental degradation such as soil erosion, increased water use, and overall loss of biodiversity (Scott et al. 2006, Ribeiro et al. 2009). Despite these drawbacks, many people still rely on intensive land use as it increases productivity in various ways: higher crop and livestock yields in agriculture, greater mining yields in the industrial sector, increased

housing opportunities in urbanisation, increased tourist numbers and human activity, among others (Evenson 2003, Pei et al. 2015).

The five study sites used in this study experience varying levels of intensive land use (Table 1.1). To approximate the relative levels of disturbance for my five study sites, I used the several variables: primary land use, primary land cover, proportion of the study site that has been transformed (altered through human activity), proportion of landscape that is adjacent to a main road and increased traffic, and average weekly human population density (the average number of landowners, staff members, and tourists who visit each site per week). Residential land use was not considered as a variable because all sites have at least two permanent occupants, but rather, the mean number of weekly visitors was used.

Table 1.1. Summary of study sites including abbreviations, primary land use, primary vegetation cover, total area covered, proportion and percentage of disturbed area, total boundary distance, proportion and percentage of boundary that is adjacent to a main or dirt road analysed in the Magaliesberg Biosphere, North West province, South Africa. ARF – Agricultural Residential Farmstead; ABR – Animal Breeding and Rehabilitation; IAR – Industrial Agricultural Residential; RFS – Residential Farmstead; NCR – Nature Conservancy Residential. Anthropogenic activities that are considered intensive land use practices have been highlighted in red.

	ABR	ARF	IAR	RFS	NCR
Primary land use	Animal breeding and rehabilitation Eco-tourism Permanently cleared land for animal exhibitions	Agriculture - crop (vegetables, fruit) - livestock (cattle, chicken, donkey, rabbit)	Agriculture - crop (vegetables) - livestock (cattle, horse)	Residential Remote office	Nature conservation
Secondary land use	Residential (permanent staff)	Residential (landowners and permanent staff)	Platinum mine-adjacent Residential (permanent staff)	Donkey enclosure	Residential (landowners)
Average weekly human density	>50	<10	<10	<5	<6
Primary vegetation cover	Natural	Natural (grasses) Planted crops Fruit trees	Natural Planted crops	Natural	Natural
GPS Location	25°40'25.2" S 27°55'17.4" E	25°39'31.3" S 27°55'08.0" E	25°38'43.3" S 27°55'48.8" E	25°39'20.3" S 27°55'15.7" E	25°40'39.6" S 27°57'48.9" E
Total area	1.13 km ²	0.22 km ²	0.33 km ²	0.20 km ²	1.63 km ²
Proportion area transformed	42.70 %	54.40 %	34.70 %	7.00 %	7.90 %

Total perimeter	5.55 km	1.91 km	2.42 km	1.82 km	5.30 km
Proportion perimeter adjacent to main road	13.24 %	51.64 %	22.13 %	28.69 %	17.47 %
Proportion perimeter adjacent to dirt road / footpath	86.76 %	48.36 %	46.30 %	48.55 %	17.80 %
Number of relatively intense land use variables	4	4	2	0	0

Using the variables listed previously, an approximation of land transformation and land use intensity was made, in order of most to least transformed and intensive: ABR and ARF; IAR, RFS; and NCR.

1.6 STUDY SPECIES

The study investigated nine southern African rodent species from the Muridae and Nesomyidae families to assess the effects of land transformation and seasonal variation on the population dynamics and community structure, stress-related endocrine correlates, and diet selection of small mammals of the Magaliesberg region, North West province, South Africa (Figure 1.2, Table 1.2).

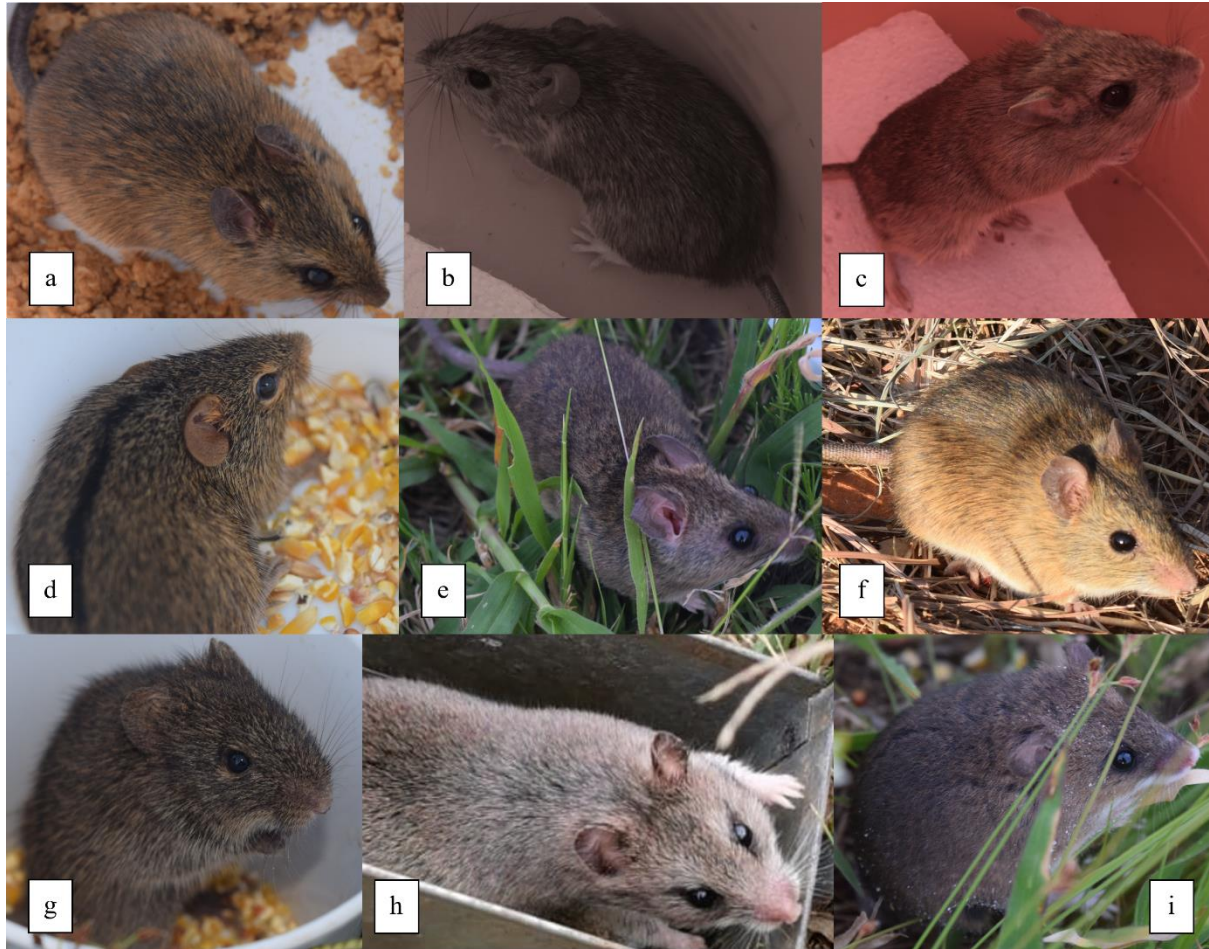


Figure 1.2. Rodent species investigated in the present study. Pictured from left to right: a) *Aethomys ineptus*, b) *Gerbilliscus brantsii*, c) *Gerbilliscus leucogaster*, d) *Lemniscomys rosalia*, e) *Mastomys coucha*, f) *Micaelamys namaquensis*, g) *Otomys irroratus*, h) *Saccostomus campestris*, and i) *Steatomys pratensis*.

Table 1.2. Summary of species and common names, habitat, and ecology of study animals captured and analysed in the Magaliesberg Biosphere, North West province, South Africa (Davis 1973, Skinner & Chimimba 2005, Chimimba & Linzey 2008, Stuart & Stuart 2015, Monadjem et al. 2015).

Species	Common name	Average adult mass	Habitat	Ecology	Reproduction	Feeding patterns and average % diet composition
<i>Aethomys ineptus</i> (Thomas & Wroughton 1908)	Tete veld rat	♀ - ♂ -	Savannah-woodlands with abundant ground cover	Can be synanthropic Nocturnal Communal living	Poly-oestrous 1-3 young Nipple-cling	Omnivorous (32% herbage, 47% seeds, 21% insects)
<i>Gerbilliscus brantsii</i> (Smith 1836)	Highveld gerbil	♀ - 81 g ♂ - 79 g	Open savannahs and wooded grasslands	Can be synanthropic Nocturnal Communal living	Poly-oestrous 1-3 young Do not nipple-cling	Omnivorous (20% herbage, 75% seeds, 5% insects)

<i>Gerbilliscus leucogaster</i> (Peters 1852)	Bushveld gerbil	♀ - 68 g ♂ - 71 g	Bushveld and grasslands	Can be synanthropic Nocturnal Communal living	Poly-oestrous 1-4 young Do not nipple-cling	Omnivorous (32% herbage, 26% seeds, 41% insects)
<i>Lemniscomys rosalia</i> (Thomas 1904)	Single-striped grass mouse	♀ - 52 g ♂ - 56 g	Savannahs, grasslands, and cultivated lands	Can be synanthropic Crepuscular, with diurnal habits Communal living	Poly-oestrous 4-5 young Do not nipple-cling	Omnivorous (48% herbage, 50% seeds, 2% insects)
<i>Mastomys coucha</i> (Smith 1834)	Southern multimammate mouse	♀ - 39 g ♂ - 48 g	Savannahs, grasslands, cultivated lands Disturbed landscapes	Synanthropic Nocturnal Communal living	Polyoestrous 1-27 young Do not nipple-cling	Omnivorous (3% herbage, 79% seeds, 18% insects)
<i>Micaelamys namaquensis</i> (Smith 1834)	Namaqua rock mouse	♀ - 47 g ♂ - 48 g	Highveld rocky grasslands and outcrops	Synanthropic Nocturnal Communal living	Polyoestrous 3-5 young Nipple-cling	Omnivorous (90% herbage, 2% seeds, 8% insects)
<i>Otomys irroratus</i> (Brants 1827)	Southern African vlei rat	♀ - 114 g ♂ - 122 g	Mesic grasslands and marshes	Free-living Diurnal Live singly or paired	Polyoestrous 2-5 young Nipple-cling	Herbivorous (100% herbage, 0% seeds, 0% insects)
<i>Saccostomus campestris</i> (Peters 1846)	South African pouched mouse	♀ - 47 g ♂ - 50 g	Savannah woodlands	Free-living Crepuscular, with nocturnal habits Solitary living	Polyoestrous 3-13 young Do not nipple-cling	Omnivorous (12% herbage, 79% seeds, 9% insects)
<i>Steatomys pratensis</i> (Peters 1846)	Fat mouse	♀ - 25 g ♂ - 21 g	Open grasslands and savannahs, cultivated lands	Can be synanthropic Nocturnal Live singly or paired	Poly-oestrous 3-6 young Do not nipple-cling	Omnivorous (4% herbage, 83% seeds, 13% insects)

1.7 PROJECT RATIONALE

By investigating the effects of anthropogenic activities and changes in global climate on rodent species, we can assess the impacts these variables will have on wildlife over time and generate models to infer approximate distributions and timespans for many species. We can also determine the effects of these variables on species population density, diversity, endocrine-related correlates, diet, behaviour, and differences in sympatric intra-specific variables. Although complex study designs are often more expensive and challenging, they are still more effective and should be the first choice in experimental designs. Low-complexity designs may threaten the accuracy of the data collected and the findings of a study, which can translate to incorrect management implications on how best to conserve ecosystems and the biodiversity within them (Christie et al. 2019). Good study designs, although costly and time-consuming, are heavily encouraged. By carrying out robust studies with variable indicators of ecosystem health, we can work to improve the quality of ecological monitoring research and better inform policy-makers and management strategies.

Many studies show both small- and large-scale temperature and rainfall shifts annually (Norton 1996, Rahel & Olden 2008, Mulder & Spellman 2019). With this yearly increase in temperature and decrease in rainfall, continued monitoring of previously identified species of interest is important to track their progress. Monitoring “Least Concern” species is also crucial as their responses to environmental changes are often the most telling due to their resilient and adaptive nature (Ramahlo et al. 2019, Majelantle et al. 2020). Additionally, several of these species may become prominent across disturbed landscapes, leading to increased interactions with humans and the designation of pest status to some species.

The first part of this study (Chapter 2) investigated the effects of season and land use on the biodiversity of small mammal species and overall community structure. The following population characteristics were assessed: species abundance, richness, diversity, composition, evenness, and community dissimilarity. The second part of the study (Chapters 3 and 4) assessed the effects of season and land use on the physiology of small mammals and focused on monitoring and comparing stress-related endocrine correlates in species across seasonal and land use differences. The third part of the study (Chapter 5) investigated the effects of season and land use on the diet selection of small mammals. The final chapter (Chapter 6) provides a general discussion and conclusions on the findings of this study and highlights its limitations with recommendations for future studies.

1.8 PROJECT AIM, OBJECTIVES, AND PREDICTIONS

The present study aimed to investigate the effects of land transformation and seasonal variation on the population dynamics and community structure, stress-related physiology, and diet selection of small mammal species of the Magaliesberg region, North West province, South Africa.

1.8.1 Research objectives

1. Diversity and community structure

- To assess the effects of season and land transformation on species abundance.
- To assess the effects of season and land transformation on species richness.
- To assess the effects of season and land transformation on small mammal diversity.

- To assess the effects of season and land transformation on species composition within the community.

2. Stress-related fGCM responses

- To biologically and physiologically validate a suitable enzyme immunoassay (EIA) for southern African rodent species using five EIAs that were previously validated for northern hemisphere rodent species.
- To determine baseline and peak fGCM hormone concentrations of captured southern African rodent species.
- To assess the effects of season and land transformation on fGCM concentrations of captured small mammal species.

3. Diet-related responses

- To investigate minimally invasive methods of monitoring diet in rodent species.
- To assess the effects of season and land transformation on the diet preference of rodent species.

1.8.2 Predicted outcomes

1. Diversity and community structure

- Species abundance, richness and diversity would be highest during the wet season and inversely proportional to the level of transformation in a landscape.
- Community composition and evenness would be most balanced during the wet season and most disrupted during the dry season with an increase in disturbance within a landscape.

2. Stress-related fGCM responses

- Captured murid species would have a common EIA that shows reliable sensitivity across an interspecific range.
- Stress-related hormone responses would be directly correlated with the level of disturbance and human presence in a landscape.
- fGCM concentrations of captured rodent species would be higher during the dry season.

3. Diet-related responses

- Comparison of minimally invasive data collection methods would reveal a method that can be used for multiple southern African rodent species.
- Carbon and nitrogen isotope values would differ seasonally across rodent species occupying differently transformed landscapes.

CHAPTER 2: SMALL MAMMAL DIVERSITY IN RESPONSE TO LAND TRANSFORMATION AND SEASONAL VARIATION IN SOUTH AFRICA

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2.1 ABSTRACT

Anthropogenic land transformation is a consequence of human population growth and the associated agricultural, residential, and industrial needs. This study aimed to investigate the effects of anthropogenic activity and human-mediated land transformation on capture/recapture frequencies, species richness, and diversity of native small mammal community assemblages in the Magaliesberg Biosphere, North West province, South Africa. Five anthropogenically transformed land-use types were investigated: an animal rehabilitation and ecotourism centre, an agricultural farmstead, a residential farmstead, a mine-adjacent agricultural farmstead, and a protected nature conservancy. I used live traps to sample small mammals during the dry and wet seasons over three consecutive years and compared population numbers and species composition across study sites and seasons. Capture/recapture frequencies differed significantly between sites and seasons, with the highest capture frequencies recorded at the agricultural and residential farmsteads. Species richness and diversity were highest at the residential and mine-adjacent farmsteads, both of which experienced intermediate levels of anthropogenic disturbance throughout the sampling period. The study shows that while natural and protected landscapes with low levels of disturbance are preferred, transformed landscapes can also be managed effectively to benefit native small mammal populations by regulating the frequency and intensity of human-mediated activities.

2.1.1 Keywords: abundance; agriculture; anthropogenic activity; community assemblage; industrial activities; intermediate disturbance; rodents; species richness

2.2 INTRODUCTION

Due to the rapidly increasing human population and the far-reaching impacts of anthropogenic activities such as agriculture, industrialisation and urbanisation, fewer ecosystems remain untransformed and undisturbed (Umetsu & Pardini 2007, Irwin et al. 2010, Waters et al. 2016)). Monitoring threats to biodiversity that stem from anthropogenic activities is vital in mitigating and managing their effects (Marris 2008, World Wildlife Fund 2016, Christie et al. 2019). Ecosystem health is often monitored using several environmental variables, and any one of these variables can be a proxy for health. In the past, similarities in terms of species richness, distribution, and community assemblage in small mammals were investigated across transformed and untransformed landscapes to assess ecosystem health (Umetsu & Pardini 2007, Christie et al. 2019). Whereas some studies focus on the impact of one variable at a time, an integrated investigation of several variables can give a more insightful view of the state of both biotic and abiotic components within the system (Cadotte et al. 2016, Wilson et al. 2018). The investigation of the presence of land-use-based species can inform on the impact of various alterations to landscapes and which human activities are most impactful to the ecosystem and resident species (Heroldová et al. 2007, Foord et al. 2018).

Diversity refers to the range and abundance of species in an area and is also a reliable indicator of ecosystem health (Monadjem & Perrin 2003, Cadotte et al. 2016, Simelane et al. 2018). Along with the presence and number of species, it is essential to record the diversity of a region over time, as this further demonstrates how impactful anthropogenic activities are on natural environments (Umetsu & Pardini 2007, Christie et al. 2019). Reduced community evenness can indicate species and diversity declines, resulting in the homogenisation of formerly diverse landscapes and the extinction of local species (Monadjem & Perrin 2003, Magurran 2004). The presence of invasive species in a landscape can also indicate land disturbance, displacement of indigenous species, and most likely, declines in biodiversity (Wilson et al. 2018, Ramahlo et al. 2019).

It is essential to research species prone to population fluctuations in response to landscape changes, as these species are the most vulnerable to environmental and anthropogenic threats (Cincotta et al. 2000, Scott et al. 2006). To improve the identification of vulnerable species, the IUCN (World Wildlife Fund 2016) has identified several relevant biological traits that make species more at risk. These include the use of specialised habitats or microhabitats, a

narrow environmental tolerance, a dependence on environmental cues, a dependence on relationships and interactions with other species, and a poor ability to disperse from low-quality habitats and establish in landscapes with more favourable conditions. Similarly, it is also important to investigate those taxonomic groups that occur on the other end of the spectrum and do not generally operate within the ecological constraints of at-risk species (Colles et al. 2009). However, it can be assumed that environmental changes that affect these tolerant taxonomic groups may have devastating consequences for at-risk species that are dependent on specific ecological and climatic cues (McKee et al. 2004, Watson et al. 2004, Colles et al. 2009). Small mammals, such as rodents, are a reliable model for shorter-lived species that can be easily studied, as they have relatively short generation times that can be observed over a human lifetime (Galetti et al. 2009, Merritt 2010). The use of these adaptive and resilient species in research allows us to investigate the likelihood of wildlife populations and ecosystems recovering from human-induced disturbances. It can also help to improve the management strategies of threatened species in disturbed landscapes.

Most rodents are not on the IUCN Red Data List of Threatened Species and are classified as ‘Least Concern’, with only a handful being assessed as threatened or endangered (IUCN 2021). When assessed using the IUCN-identified biological traits, they are shown to be capable of either adapting to most environmental changes or finding more favourable conditions (Joyce et al. 1997, Skinner & Chimimba 2005). Although rodents can use microhabitats, they are not restricted by their macrohabitat and can thus persist in a wide range of landscapes (Skinner & Chimimba 2005). Successful establishment of rodents in a landscape does not require interspecific interactions with other taxonomic groups. They, themselves, are ecosystem drivers who cycle nutrients through the soil, disperse seeds to aid in plant growth, and provide food for predators (Joyce et al. 1997, Monadjem et al. 2015). With increased human expansion and subsequently transformed landscapes, favourable conditions prove more challenging to maintain (Rymer et al. 2013, Spear et al. 2013). In addition to an increase in land transformations due to more significant anthropogenic needs, climate change may exaggerate disruptions to ecosystem health, and thus, seasonal variation should be included in diversity-based research (Ellis et al. 2013, Cadotte et al. 2016). Due to the resilient characteristics displayed by rodents, it is essential to monitor them in both natural and transformed landscapes to gauge the critical limit of their tolerance for environmental changes brought about by climate change and anthropogenic activities.

Additionally, due to the heavy reliance of small mammals on resources and shelter in their environments, it is important to monitor any external factors that may influence these environments. Seasonal variation is one such variable, as it can have immediate and delayed effects on natural landscapes. In terms of immediate effects, wet season climatic conditions may lead to physical alterations in the landscapes, such as softer soil substrates, whereas dry season climates can harden said substrates (Rymer et al. 2013). Many habitats found in southern Africa can be adversely affected by climatic conditions but can also be renewed by wet season rains and produce vegetation that small mammals use for cover and food (Spear et al. 2013). Conversely, the dry season may mean that food and cover are limited, often leading to inter- and intraspecific competitive interactions for these resources (McKee et al. 2004, Watson et al. 2004). This study aimed to investigate the effects of land transformation and seasonal variation on small mammal biodiversity in the Magaliesberg Biosphere of South Africa by examining the impacts of season and land transformation on (a) capture frequency, (b) species richness, and (c) community diversity, evenness, and similarity. I predicted that an increase in landscape disturbance would be inversely proportional to population health and community diversity. Furthermore, I anticipated that seasonal variation would reduce community diversity during the dry season and increase population abundance in the wet season.

2.3 MATERIALS AND METHODS

2.3.1 Study area

The Magaliesberg mountain range (25°41'00.1" S 27°57'51.8" E) expands through the Gauteng and North West provinces, South Africa. The mountain range spans a length of approximately 140 km, with a maximum elevation of 1852 m.a.s.l. The Magaliesberg is primarily characterised by the Savannah Biome, which covers the Gold Reef Mountain Bushveld vegetation type at higher altitudes and Marikana Thornveld and Moot Plains Bushveld in lower-lying areas (Rutherford & Mucina 2006). The vegetation in these bioregions is woody and consists of a predominantly grassy herbaceous layer. Thorny *Vachellia* species and semi-open thickets dominate the region. The region comprises igneous rocks from the Rustenberg Layered Suite and the Rooiberg Group (Rutherford & Mucina 2006).

Trapping localities were in the De Wildt (25°40'05.5" S 27°55'23.1" E) and Zilkaatsnek (25°39'25.8" S 27°56'34.9" E) areas, with land-use types such as agriculture, residential,

tourism, conservation, and mining (Figure 2.1). I named the sites as follows: the animal breeding, rehabilitation, and ecotourism centre (ABR); the agricultural and residential farmstead (ARF); the residential farmstead (RFS); the industrial and agricultural farmstead (IAR); and the nature conservancy and residential site (NCR). The climate is characterised by hot, wet summers and cold, dry winters, with April until October referred to as the dry season and November to March typically referred to as the wet season (Louw & Louw 2020). However, the start and patterns of annual rainfall are unpredictable each year. I used five closely located study sites (the maximum distance between sites is 15 km) to discount climatic variability and determine whether the size of study sites and their proximity to each other allows for species turnover (Table 2.1). All five sites shared similar natural vegetation structures (Rutherford & Mucina 2006), apart from additional cultivation of commercial vegetation in some areas (Table 2.1). The savannah vegetation composition differs across land-use types, with the level of clearing increasing with an increase in anthropogenic activity. This means that land-use types in the same region with similar vegetation differ in plant species assemblages due to human-mediated land development, clearing, and burning, among other factors. Study sites with infrequent human activity therefore experience less vegetation clearing, resulting in high richness of plant species, with a few examples of cleared areas being roads and footpaths for occasional tourists. Despite the differences in composition, however, the effects of seasonal variation influence the different study sites in similar ways. In the dry season, the area experiences low rainfall conditions, which leaves habitats dry with little green vegetation for animals to use. The soil is dry and compacted, making burrowing more arduous for some species (Rutherford & Mucina 2006). Due to the dry climate and grass, locals around the area practice controlled burning and slashing techniques to ensure the areas do not experience uncontrollable and dangerous veld fires. This alters the landscape and may influence natural vegetation and wildlife in various ways. In the wet season, rainfall experienced in the region results in green vegetation and softer soil, which makes burrowing easier for small mammals (Newbery 1999, Louw & Louw 2020). Additionally, the increase in vegetation can result in increased grazing activity by livestock in the surrounding areas (Newbery 1999).



Figure 2.1. Orientation map of the North West province showing the locations of the five study sites where rodents were captured between 2018 and 2021 in South Africa.

Table 2.1. Study-area information on the locations and land-use types from which small mammals were sampled in the Magaliesberg Biosphere, North West province, South Africa. The five land-use types were designated as follows: animal breeding and rehabilitation centre (ABR); agricultural and residential farmstead (ARF); residential farmstead (RFS); industrial, agricultural, and residential (IAR); and nature conservancy and residential (NCR).

Study Site	GPS Location	Land Use	Primary Vegetation	Topography
ABR	25°40'25.2" S 27°55'17.4" E	Animal breeding, rehabilitation Ecotourism	Thorny, semi-open thickets	Rocky, slight incline near the base of the mountain
ARF	25°39'31.3" S 27°55'08.0" E	Agricultural Residential	Grassy herbaceous layer	Flat at the base of the mountain
RFS	25°39'20.3" S 27°55'15.7" E	Residential	Herbaceous and semi-open thickets	Flat at the base of the mountain
IAR	25°38'43.3" S 27°55'48.8" E	Industrial Agricultural Residential	Herbaceous and semi-open thickets	Flat at the base of the mountain
NCR	25°40'39.6" S 27°57'48.9" E	Conservation Residential	Herbaceous and semi-open thickets	Rocky, steep incline along the mountain

2.3.2 Data collection

For this study, I sampled five study sites during four consecutive seasons (two dry and two wet seasons) for 16,000 trap nights between 2018 and 2021. For each season, all five sites were monitored concurrently for five consecutive nights (Sunday to Thursday), after which, traps were closed on Friday afternoons and reopened and baited on Sunday afternoons. This was repeated four times for a total of 20 nights of trapping per site, per season. At each site, I

deployed 40 Enviro-Care live traps (imported from Cangzhou Jinglong Technology Co., Ltd., Hebei Province, China), for a total of 3200 trap nights per site. The traps were made of galvanized sheet metal and had the following dimensions (length $\times$ height $\times$ width): 255 $\times$ 78 $\times$ 80 mm. I baited each trap with a mixture of oats and peanut butter and placed them 10 m apart in a 5 $\times$ 8 trapping grid, following standard procedures (Aplin et al. 2003). All traps remained open during the day and night to ensure diurnal and nocturnal species trapping. I checked all traps in the morning from 05:30 until 10:00 and again from 15:00 until 17:00 every day. All captured animals were identified and sexed where possible (Joyce et al. 1997, Skinner & Chimimba 2005, Fleckman et al. 2013). I weighed individuals using a spring scale (PESOLA Präzisionswaagen AG, Schindellegi, Switzerland) and marked them using numbered clipping of toenails to identify recaptured individuals (Aplin et al. 2003). As many small mammals contain blood vessels in their toenails, which may become damaged if clipped, I treated toenail-clipped individuals with antiseptic Mercurochrome (Barrs Pharmaceutical Industries, Cape Town, South Africa) to prevent infection and ensure that the nails would heal with minimal contamination (Meheretu et al. 2015). Toenail clipping had a dual function of marking individuals and providing keratinised materials to be analysed in a separate diet-related study. Additionally, I collected <1 mm ear clips by clipping the upper right ear using sterilized dissecting scissors. These ear clips were used as genetic material for resolving individuals from cryptic genera, namely *Aethomys* and *Mastomys*, into their respective species (Aplin et al. 2003). After processing, I released individuals at their respective capture sites (Newbery 1999, Anderson et al. 2011). I emptied the traps of all contents, rebaited, and returned them to their original positions in the trapping grid (Aplin et al. 2003). This study was performed with the approval of the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (Ethics clearance number EC044-18).

2.3.3 Statistical analysis

All statistical analyses were done with the software program R 4.1.0 (R Core Team 2021) using the RStudio interface (Version 1.4.1103). For the current study, I only included the first capture of an individual. However, a figure with a summary of capture and recapture numbers is provided in the Results section. Sequence analyses, details on DNA extraction and nucleotide sequencing of the genetic material are included in Appendix A (Bastos et al. 2011). Sequence chromatographs were visualised and edited in the Chromas program embedded in MEGA 7 and used to generate contiguous sequences (contigs) (Kumar et al. 2016). The final aligned database for the cryptic species was used to infer a maximum-likelihood (ML) phylogenetic

tree in MEGA 7 (Kumar et al. 2016). Details on the generation of the database are included in Appendix A. The best-of-fit model of sequence evolution was determined under the Bayesian information criteria (BIC), and the Akaike information criteria (AIC) in MEGA 7 were used for the ML analysis, with the nodal support being assessed through 5000 bootstrap replications (Kumar et al. 2016).

Cryptic *Aethomys* species were identified as *A. ineptus*, whereas *Mastomys* species were identified as *M. coucha*. These resolved species were specified in the dataset and included in further analysis. Furthermore, all sequences were deposited in GenBank (*A. ineptus*: OM055762, OM055763, OM055764, OM055772, OM055773, OM055774, OM055775, OM055776, OM055777, OM055778; and *M. coucha*: OM055765, OM055766, OM055767, OM055768, OM055769, OM055770, OM055771). The accession numbers represent unique haplotypes identified in the study and include geographical information. The maximum-likelihood tree is included in Appendix A. I investigated nestedness and trap dependency in R using the *beta.multi* function in the *betapart* package, which computes multiple-site dissimilarities and accounts for the nestedness components of beta diversity (Baselga 2008, 2009). The function uses beta diversity to measure site dissimilarity resulting from nestedness patterns in the community (Baselga 2009). In the event of high nestedness between sites, a linear model was employed to account for inter- and intraspecific trap dependency at spatial and temporal scales:

$$\text{Abundance} \sim \text{Season} + \text{Land Use} + \text{Species} + \text{Trapline}$$

where Abundance is the total number of newly captured individuals within a land-use type, Season refers to the dry and wet sampling periods, Land Use denotes the five sites sampled, and Trapline refers to the numbered trap in the trapping grid where each individual was captured. A *p*-value of ≤ 0.05 was defined as significant.

To compare species richness (alpha diversity), I used descriptive statistics, namely the total number of species captured at each site. To compare diversity across sites, I used the *vegan* package to calculate Shannon and Simpson's diversity indices, as well as Pielou's evenness index (Oksanen et al. 2019). Additionally, I used the *betadisper* analytical function in the package to test for homogeneity of groups (or similarity) by calculating Bray–Curtis distances between land-use types (Saetnan & Skarpe 2006, Oksanen et al. 2019).

2.4 RESULTS

Both season (dry season, $n = 693$; wet season, $n = 824$; $p < 0.001$; $df = 2$) and land-use type ($p < 0.0001$; $df = 4$) resulted in significantly distinct abundance across study sites (Figure 2.2). Seasonal abundance fluctuated most at the agricultural farmstead (ARF) and the animal breeding and rehabilitation centre (ABR) and least at the residential farmstead (RFS) and industrial and agricultural farmstead (IAR). Small mammal captures were low during the dry season at the transformed sites (ABR and ARF), increasing with a decrease in human-intensive land disturbance. Wet season captures showed an inverse trend, except for RFS, which showed consistent capture numbers overall. During the dry season, 66.7% of all encountered individuals were recaptured animals, while the recapture rate during the wet season was 42.6%.

Trap dependency, however, did not have a significant effect on capture and recapture numbers ($n = 1516$; $df = 41$; $p = 0.201$). Species nestedness accounted for a large proportion of the beta diversity in the region ($\beta_{\text{NES}} = 0.93$).

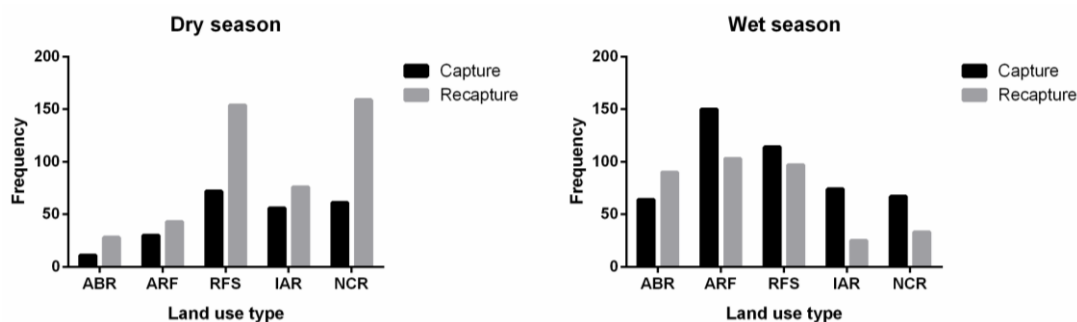


Figure 2.2. Seasonal capture and recapture numbers for small mammals for a three-year sampling period (2018, 2019, and 2020) across five land-use types: animal breeding and rehabilitation centre (ABR); agricultural and residential farmstead (ARF); residential farmstead (RFS); industrial, agricultural, and residential (IAR); and nature conservancy and residential (NCR).

All captured species were indigenous to southern Africa. Species richness did not differ significantly across seasons ($p = 0.137$; $df = 1$) or sites ($p = 0.799$; $df = 4$) (Table 2.2). Shannon's and Simpson's diversity indices, as well as Pielou's evenness index, were not significantly different between seasons or sites. (Table 2.2). Beta diversity, represented by Bray–Curtis dissimilarity, varied seasonally and across sites (Table 2.3). Mean dissimilarity was calculated as 0.37 during the dry season and 0.36 during the wet season.

Table 2.2. Dry and wet season counts of small mammal species and community structure indices across five land-use types in the Magaliesberg Biosphere, North West province, South Africa. Land-use types were designated as follows: animal breeding and rehabilitation centre (ABR); agricultural and residential farmstead (ARF); residential farmstead (RFS); industrial, agricultural, and residential (IAR); and nature conservancy and residential (NCR). * Denotes statistically significant differences between sites.

Species Name	ABR		ARF		RFS		IAR		NCR	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Macroscelidea										
<i>Elephantulus brachyrhynchus/myurus</i> (Elephant shrew)	0	0	0	0	0	0	0	0	1	0
Rodentia										
<i>Aethomys ineptus</i> (Tete veld rat)	3	21	0	0	0	0	0	3	10	17
<i>Dendromus mystacalis</i> (Climbing mouse)	0	0	0	0	0	0	0	0	1	0
<i>Gerbilliscus brantsii/leucogaster</i> (Bushveld/Highveld gerbil)	0	0	0	0	0	0	20	2	0	2
<i>Lemniscomys rosalia</i> (Single-striped mouse)	1	8	3	7	15	14	11	10	9	0
<i>Mastomys coucha</i> (Multimammate mouse)	4	19	20	120	45	70	12	29	14	2
<i>Micaelamys namaquensis</i> (Namaqua rock mouse)	2	16	0	18	0	12	0	21	23	46
<i>Mus minutoides</i> (Pygmy mouse)	2	0	6	1	10	0	8	1	2	0
<i>Otomys irroratus</i> (Vlei rat)	0	0	0	0	2	3	0	2	3	0
<i>Rhabdomys pumilio</i> (Three-striped mouse)	0	0	0	0	0	0	2	0	0	0
<i>Saccostomus campestris</i> (Pouched mouse)	0	0	0	3	0	1	2	6	0	0
<i>Steatomys pratensis</i> (Fat mouse)	0	0	0	1	0	15	2	2	0	0
Captures (N) *	12	64	29	150	72	115	57	76	63	67
Species richness	5	4	3	6	4	6	7	9	8	4

Shannon diversity index (<i>H</i>) *	1.52	1.33	0.82	0.72	0.99	1.20	1.64	1.66	1.66	0.82
Simpson diversity index (<i>D</i>)	0.78	0.73	0.47	0.34	0.55	0.59	0.82	0.75	0.76	0.46
Pielou's evenness index (<i>J</i>)	0.94	0.96	0.74	0.40	0.71	0.67	0.84	0.76	0.80	0.59

Table 2.3. Bray–Curtis dissimilarity indices between study sites during the dry and wet seasons across five land-use types in the Magaliesberg Biosphere, North West province, South Africa. Land-use types were designated as follows: animal breeding and rehabilitation centre (ABR); agricultural and residential farmstead (ARF); residential farmstead (RFS); industrial, agricultural, and residential (IAR); and nature conservancy and residential (NCR).

	ABR		ARF		RFS		IAR	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
ARF	0.25	0.40	-	-	-	-	-	-
RFS	0.33	0.40	0.14	0.17	-	-	-	-
IAR	0.50	0.38	0.40	0.20	0.45	0.20	-	-
NCR	0.23	0.25	0.45	0.60	0.33	0.60	0.60	0.38

2.5 DISCUSSION

This study demonstrates that seasonal and land-use-specific variation can influence the abundance of small mammal species to different extents in the Magaliesberg Biosphere, South Africa. Minimally transformed landscapes, such as the residential farmstead and the nature conservancy, had the highest abundance during the dry season. Conversely, study sites that experienced moderate to high levels of anthropogenic activity, i.e., the animal breeding and rehabilitation centre and the agricultural and residential farmstead, had fewer captures during the dry season. This could be due to the high fire risks associated with the region, specifically during the dry season, and the resulting management strategies to prevent or reduce fire damage (Louw & Louw 2020). Fire risk-management efforts appear to increase with an increase in land-use complexity and associated practices. Activities such as controlled burning, livestock grazing, and mechanical slashing directly impact small mammal populations (Saetnan & Skarpe 2006, Blaum et al. 2007). Additionally, in many areas with a high human presence, humans actively try to reduce the number of small mammals and their pest-related activities in and around their households through live and snap trapping, and poisoning, as well as biological means, such as pets (Mason & Littin 2003, Welegerima et al. 2020). This may further reduce population numbers in transformed areas with a constant human presence. During the wet season, the abovementioned minimally transformed sites had lower capture numbers, as

small mammals were no longer reliant on temporary food sources due to improved plant growth after the rains.

Conversely, the more transformed sites had more frequent small mammal captures, possibly due to the resurgence and re-establishment of population numbers following the dry season. Additionally, the breeding season of many observed species coincides with the wet season, which could result in an overall increase in capture numbers (Skinner & Chimimba 2005, Van Den Heuvel & Midgley 2014, Meheretu et al. 2015). Species ecology and behaviour can also impact seasonal population numbers, as many southern African small mammals are seasonal breeders. For example, *M. namaquensis* reproductive behaviour is mainly confined to rainy summer months, which correlates with an abundance of nutrient-rich food resources (Muteka et al. 2006). Similarly, *M. coucha* has been reported to breed primarily during the wet season, and this behaviour has been linked to the growth of new grass shoots (Leirs et al. 1994). Grass shoot growth can also be seen after fire events, and this could explain the upsurge in *M. coucha* during the dry season months after vegetation-control methods are implemented.

In this study, overall species richness was not significantly different across study sites, although a downward trend was associated with increasing and frequent land-use intensification. The ARF site, with several families permanently residing on the plot, and farm staff and machinery operating consistently year-round, and ABR site, with permanent staff residences scattered around the property and variable daily tourist densities, saw the lowest species numbers (five and six, respectively). These findings align with Flynn et al. (2009) and Horváth et al. (2001), who showed that intensification of agriculture resulted in reduced species richness. Further, species richness did not differ across seasons and sites, but the highest species richness values were recorded at the intermediately disturbed residential farmstead and mine-adjacent farmstead sites. This may be due to the management strategies employed, which involve alternating between rest and intensive tilling and planting activity (Schmidt et al. 2005, Chia et al. 2016). Intermediately disturbed landscapes have been linked with increased species richness, as some species can persist and even thrive in these environments (Ferreira & Van Aarde 2000). At IAR, species from the genus *Gerbilliscus* were captured almost exclusively, suggesting that this particular landscape and level of disturbance are optimal for their survival (Wright 2006). Conversely, *L. rosalia* was mainly found across agricultural landscapes, which correlates with several studies that have reported that the species is commonly found along the fringes of agricultural landscapes (Monadjem & Perrin 1997). Ecological niche preferences

often drive habitat selection, and this is seen with *A. ineptus*, a species that prefers rocky outcrops (Skinner & Chimimba 2005). These outcrops are found primarily at two study sites (ABR and NCR) which are characterised by a rocky incline near the base of the Magaliesberg. Animal behaviour can also drive habitat selection and population numbers, as seen in *E. myurus* and *O. irroratus*. The territorial behaviour exhibited by these species may explain the low capture numbers observed in my study (Skinner & Chimimba 2005). Dietary preference may also explain the disappearance of some species from landscapes, as some small mammal species show seasonal shifts in diet (Field 1975). These shifts may result in a change in food selection or a shift in distributional range in search of favourable food resources (Leirs et al. 1994). Another potential behavioural driver of species capture numbers can be adjustments in activity patterns, as some small mammals, such as *S. pratensis*, can reportedly go into torpor (Richardson & Perrin 1992). These and other drivers are often linked with seasonal variation. However, the overall lack of significant variation in species richness across seasons and land-use types is consistent with the findings of Sánchez-Cordero (Richardson & Perrin 1992, Sánchez-Cordero 2001). Contrary to my findings, however, Umetsu and Pardini (2007) found that species richness across land-use types varied, leading to strong variation in small mammal community assemblages.

As far as beta-diversity-related nestedness, the five land-use types sampled are similar, and a large portion of this similarity is due to nestedness. The disappearance of some species across land-use types and seasons may, therefore, be a cause for concern, as this may support the theory that specialist species are being excluded from increasingly transformed landscapes and warmer climates (Heroldová et al. 2007, Blaum et al. 2007, Colles et al. 2009). In my study, the results obtained for community assemblage diversity, evenness, and similarity highlight the importance of holistic approaches to biodiversity research (Christie et al. 2019). At first, the results suggest that the animal rehabilitation and ecotourism site has the most diverse and ecologically ordered community assemblage. However, when incorporating the biodiversity variables mentioned above, it appears to be a skewed view. Due to its low sample size, the findings from this heavily transformed site cannot be resolved into an accurate ecological representation. Community diversity and evenness refer to sample size, and a smaller dataset can lead to a distorted output that is heavily biased (Magurran 2004). By including different ecosystem health and biodiversity measures, such as diversity, richness, evenness, and composition, the actual state of community assemblages could be more accurately interpreted across all sites (Christie et al. 2019).

The nature conservancy site showed high diversity and evenness during the dry season, which declined during the wet season. This is contrary to the findings of Sánchez-Cordero (2001), who demonstrated that small mammal diversity was higher during the wet season. The residential, as well as the industrial and agricultural farmsteads also showed high diversity. These sites were exposed to intermediate levels of activity throughout the year, as the intensity and frequency of farming and grazing at these sites were well managed. My findings are consistent with those of Horváth et al. (2001) and Sánchez-Cordero (2001), who reported that small mammal diversity is associated with habitat complexity. They further show that intensively farmed agricultural landscapes reduce habitat heterogeneity and subsequent declines in diversity and balance in community assemblages, whereas heterogeneously managed sites show high diversity.

2.6 CONCLUSION

My study found that seasonal and land-use variation influences small mammal community assemblages, resulting in fluctuations in population numbers, as well as temporal and spatial differences in species richness. As evidenced by the results, particularly those of the natural and intermediately disturbed landscapes, continuously healthy and diverse wildlife populations in a changing world may rely on an environment that experiences some level of periodic disturbance. Although relatively undisturbed landscapes are preferable, it is possible to manage transformed landscapes to safeguard the species richness and community diversity of the region (Magurran & Henderson 2018). Therefore, it is important to carefully manage and monitor the frequency and intensity of anthropogenic disturbance to ensure that native populations can re-establish and stabilise after a disturbance event and continue to do so sustainably for extended periods. Considering the ecology, behaviour, and physiology of different species, we can implement careful land-use management strategies to reduce the homogenisation of natural landscapes and potentially slow the loss of species in southern African environments that are undergoing anthropogenic transformation.

CHAPTER 3: VALIDATION OF ENZYME IMMUNOASSAYS FOR QUANTIFYING FAECAL GLUCOCORTICOID METABOLITES IN SIX SOUTHERN AFRICAN RODENT SPECIES USING BIOLOGICAL AND PHYSIOLOGICAL STIMULI

3.1 ABSTRACT

Global landscape transformations result from growing human populations and increased resource use, leading to declines in ecosystem health and natural habitat quality. Small mammals can be used as biological indicators of ecosystem health and anthropogenic disturbance by monitoring their behaviour and physiology, specifically measuring their stress-related hormones. My study aimed to establish minimally invasive methods for quantifying faecal glucocorticoid metabolites (fGCM) in six southern African rodent species (*Aethomys ineptus*, *Lemniscomys rosalia*, *Mastomys coucha*, *Micaelamys namaquensis*, *Otomys irroratus*, and *Steatomys pratensis*) by evaluating five enzyme immunoassays (EIAs). Rodents were live trapped in the Magaliesberg Biosphere, North West province, South Africa. Where possible, one male and one female from each species were captured and individually housed on-site for assay validation experiments. Biological (handling and temporary removal from enclosure) and physiological (Synacthen-induced Adrenocorticotrophic Hormone challenge test) stimuli were administered, faecal samples were collected daily, and fGCM concentrations were determined from samples collected before, during, and after each event. The reliability of an assay for a species was denoted by its ability to detect a two-fold (100%) or more increase in fGCM concentrations (ug/g DW) after either biological or physiological validation, or both. Additionally, the assay should be able to detect mean absolute fGCM concentrations greater than 1. My study identified a 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA (detecting fGCMs with a 5α - 3β , 11β -diol structure) as the most suitable assay as it provided a reliable signal (two-fold increase in fGCMs) and detected fGCMs in greater amounts than the other EIAs for the six species assessed. Further, two additional EIAs appear suitable for some of the species tested: Oxoetiocholanolone II, measuring fGCMs with a 5β - 3α -ol- 11 -one structure (*A. ineptus*, *O. irroratus*, and *S. pratensis*); and a Corticosterone EIA (*M. coucha*, *O. irroratus*, and *S. pratensis*). Establishing minimally invasive techniques for monitoring stress-related animal responses can be beneficial for assessing the impact of anthropogenic disturbances on natural environments and their associated fauna.

3.1.1 Keywords: Adrenocorticotrophic Hormone challenge test, biological validation, faecal glucocorticoid metabolites, enzyme immunoassays, physiological validation, rodents, southern Africa, stress-related bio-indicator

3.2 INTRODUCTION

Evaluating stress-related physiological responses of wildlife and associated behavioural alterations has become popular in recent research, and can be used to indicate animal welfare and add to our understanding of faunal ecology (Reeder & Kramer 2005, Angelier & Wingfield 2012). Historically, animal physiology and behaviour have been used as measures of animal welfare and, more recently, environmental health (Łopucki et al. 2019, Crossey et al. 2020, Majelantle et al. 2020). By quantifying physiological responses to anthropogenic activity, we can assess the impact of human-mediated disturbance on various habitats and the fauna and flora that inhabit them (Cadotte et al. 2016, Jepsen et al. 2021). Stress is a general syndrome, either acute or chronic, that occurs when a perceived stressor threatens the homeostasis of an individual (Palme et al. 2005, Hulsman et al. 2011). When a stressor is perceived, activation of the hypothalamic-pituitary-adrenal (HPA) axis stimulates the secretion of glucocorticoids (GCs), which are then metabolised and circulated through various matrices including blood, urine, and faeces (Reeder & Kramer 2005, Touma & Palme 2005). Short-term elevations of GCs can benefit the animal, for example, as a fight or flight response can allow it to evade predators. Long-term elevations, however, can lead to health issues such as suppression of the animal's immune response, reduced reproductive success, and impaired HPA regulation (Dobson & Smith 2000, Hing et al. 2014). Therefore, monitoring stress-related biomarkers like concentrations of GCs or their metabolites can be an important aspect of wildlife management and conservation.

Physiological and behavioural monitoring of fauna conducted on captive animals can be invasive and negatively affect the welfare of study animals. This can be due to prolonged (possibly life-long) captivity requirements and, in many cases, euthanasia after the completion of experimental protocols (Hodges et al. 2010, Mason 2010). Monitoring stress-related bio-indicators through faeces is becoming more common because quantifying faecal glucocorticoid metabolites (fGCMs) employs minimally invasive methods compared with other matrices (Palme 2005). The collection of faecal samples does not require the animal to be restrained or

immobilised, and in many cases, faeces can be collected discretely from study animals (Palme et al. 2005). Minimally invasive methods have become more important in recent years as sustainable research methodology and long-term monitoring of wildlife for more robust datasets become more commonplace (Ganswindt et al. 2003, Laver et al. 2012). Validated methods that quantify fGCMs can provide researchers with ethically sound experimental protocols and opportunities for long-term monitoring of populations.

Small mammals such as rodents are reliable biological indicator species in various environments globally (Horváth et al. 2001, Avenant et al. 2008, Cadotte et al. 2016). Finding reliable methods for monitoring their adrenocortical activity in response to environmental stressors can improve our understanding of anthropogenic impacts on their habitat. Most stress-related rodent research has been conducted on laboratory-reared animals, and most established enzyme immunoassays have been validated using captive animals in the northern hemisphere (Touma et al. 2004, Touma & Palme 2005, Sheriff et al. 2011).

There are several enzyme immunoassays (EIAs) that are commonly used to quantify adrenocortical activity in rodent species found in the northern hemisphere, but few have been validated for southern African species. This study examined the suitability of five different EIAs for quantifying fGCMs in six southern African rodent species (*Aethomys ineptus*, *Lemniscomys rosalia*, *Mastomys coucha*, *Micaelamys namaquensis*, *Otomys irroratus*, and *Steatomys pratensis*) by a) inducing a potential biological stress event through direct animal handling, temporary removal from enclosure, and altering of the immediate environment, and b) conducting an ACTH challenge involving pharmacological stimulation of the HPA axis. I aimed to investigate whether EIAs used to quantify fGCMs in northern hemisphere rodent species could be validated for southern African species. An additional objective was to determine whether a general EIA could be identified for several species, thus potentially simplifying future research in adrenocortical monitoring of southern African rodent species.

3.3 MATERIALS AND METHODS

3.3.1 Study area

The study animals were captured around the Magaliesberg mountain range (25°41'00.1"S 27°57'51.8"E), which extends through the Gauteng and North West provinces of South Africa. The Magaliesberg Biosphere is characterised by the Savannah Biome, which covers the Gold

Reef Mountain Bushveld vegetation type at higher altitudes and the Marikana Thornveld and Moot Plains Bushveld in lower lying areas (Rutherford & Mucina 2006). The study sites are located in the De Wildt (25°40'05.5"S 27°55'23.1"E) and Zilkaatsnek (25°39'25.8"S 27°56'34.9"E) regions of Magaliesberg, and are characterised by hot, wet summers (November to March) and cold, dry winters (April to October) (Boonzaaier & Hough 2011). However, the start of the wet season and interannual rainfall fluctuations are unpredictable.

3.3.2 Sampling protocol

The sites were sampled using 40 Enviro-Care live traps (Cangzhou Jinglong Technology Co. Ltd., Hebei Province, China) over a four-week period between December 2020 and January 2021. The traps (length x height x width: 255 x 78 x 80 mm) were constructed out of galvanised sheet metal and were placed 10 m apart in a 5 x 8 trapping grid following standard procedures (Aplin et al. 2003, 2011). Each trap was baited with a mixture of oats and peanut butter. The traps remained open throughout the day and night to ensure diurnal and nocturnal species trapping and were checked in the morning between 05:30 and 09:00 and again between 15:00 and 17:00 daily. Captured animals were sexed and identified up to species level (Joyce et al. 1997, Newbery 1999, Skinner & Chimimba 2005). Individuals were weighed using a spring scale (PESOLA Präzisionswaagen AG, Schindellegi, Switzerland) and transported to an on-site animal housing research facility for fGCM validation experiments. Traps were rebaited and returned to their original positions in the trapping grid daily (Aplin et al. 2003).

This study was approved by the Natural and Agricultural Sciences Faculty Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (Ethics clearance number EC044-18) and complied with Section 20 of the Animal Diseases Act of South Africa, 1984 (SDAH-Epi-18111309000).

3.3.3 Study species

The six study species were selected based on their capture numbers during initial sampling to investigate community structure and population dynamics. The species highlighted in this study were the most commonly caught species and were termed as dominant due to their relatively large sample sizes and the ease with which they were captured. The study aimed to highlight minimally invasive research methodology and demonstrate the effective application of low-impact sampling protocols. Due to the invasive nature of the validation experiments (prolonged captivity, handling, and medically induced physiological responses), only two

individuals from each species were used in the study, resulting in 12 focal animals. Additionally, because I planned to release all study animals after the experiment, numbers were kept low in case of long-lasting negative effects post-release. Where possible, one female and one male from each species were assessed for biological and physiological validation experiments. Two species, *M. namaquensis* (two females) and *M. coucha* (two males), deviated from the standard protocol because individuals from the necessary sexes were not captured. To supplement the low sample size, a second type of validation (biological) was included in the study, along with the physiological validation. A summary of species characteristics, their preferred habitat, ecology, and reproductive characteristics can be found in Table 3.1.

Table 3.1. Summary of species and common names, average body mass of adults, ecology, and reproduction of study animals captured and analysed in the Magaliesberg Biosphere, North West province, South Africa (Davis 1973, Skinner & Chimimba 2005, Stuart & Stuart 2015, Monadjem et al. 2015).

Species	Common name	Habitat	Ecology	Reproduction
<i>Aethomys ineptus</i> (Thomas & Wroughton 1908)	Tete veld rat	Savannah-woodlands with abundant ground cover	Can be synanthropic Nocturnal Communal living Omnivorous	Poly-oestrous 1-3 young Nipple-cling
<i>Lemniscomys rosalia</i> (Thomas 1904)	Single-striped grass mouse	Savannah, grasslands, and cultivated land	Can be synanthropic Crepuscular, recent reports of increasing diurnal activity Communal living Omnivorous	Poly-oestrous 4-5 young Do not nipple-cling
<i>Mastomys coucha</i> (Smith 1834)	Southern multimammate mouse	Savannahs, grasslands, agricultural landscapes Disturbed landscapes	Synanthropic Nocturnal Communal living Omnivorous	Polyoestrous 1-27 young Do not nipple-cling
<i>Micaelamys namaquensis</i> (Smith 1834)	Namaqua rock mouse	Highveld rocky grasslands and outcrops	Synanthropic Nocturnal Communal living Omnivorous	Polyoestrous 3-5 young Nipple-cling
<i>Otomys irroratus</i> (Brants 1827)	Southern African vlei rat	Mesic grasslands and marshes	Free-living Diurnal Live singly or paired Herbivorous	Polyoestrous 2-5 young Nipple-cling
<i>Steatomys pratensis</i> (Peters 1846)	Fat mouse	Open grasslands and savannahs, cultivated lands	Can be synanthropic Nocturnal Live singly or paired Omnivorous	Poly-oestrous 3-6 young Do not nipple-cling

3.3.4 Animal housing

Animals were temporarily housed on site to facilitate release and re-introduction into their natural environment at the end of the study. Individuals were kept in a separate room (approximately 8 m x 15 m) at the field research site. The experimental room was equipped with an air conditioning unit that was used daily at intermittent times to allow air circulation and prevent rodents from overheating during summer. The room was kept closed to minimise the risk of infiltration by pets of on-site staff and snakes from the surrounding fields. Animals were housed individually in enclosures of 380 mm length x 110 mm height x 284 mm width. Fresh grass was collected daily and placed in the enclosures for feeding or nesting. For species between 6 g and 60 g, tins (73 mm length x 62 mm height x 62 mm width) were used for nesting, whereas larger species (> 60 g) were given buckets (120 mm length x 130 mm height x 130 mm width). In addition, depending on the individual's body size, enrichment objects (cardboard rolls and different-sized boxes) were placed in enclosures. Animals were additionally fed once a day on mixed feed grains, a vegetable (carrot, cucumber, bell pepper), and a fruit (apple, pear) option, and water was provided ad libitum.

3.3.5 Experimental design

Animals were captured and housed for up to 40 days. Faecal samples were collected from all captive individuals at 0-8 hours post-defecation three times a day (morning, afternoon, and evening). To investigate the effects of handling and temporary removal of individuals from their enclosures for approximately 30 minutes so they could be cleaned (biological validation), faecal samples collected within two days before and after the biological event were considered. During the biological validation, animals were removed from their enclosures using a non-transparent handling bag, weighed using a scale and body mass recorded, then placed in a clear 110-litre storage container (length x width x height: 800 x 400 x 430 mm) for observation while enclosures were cleaned. Following cleaning, animals were returned to their enclosures which had different olfactory and visual cues from the cleaning agent used (70 % ethanol and water) and the newly added enrichment objects. The combination of these activities would likely result in a quantifiable physiological response. Two days after the biological validation, the same animals underwent a physiological validation via an ACTH challenge, and faecal samples were collected for seven days post-injection.

For biological validation, faecal samples collected from one female and one male from each species were analysed, except for *M. coucha* (two males) and *M. namaquensis* (two females), where the relevant sexes could not be captured. Samples were collected for two days before

the animals were removed from their enclosures and weighed. While the animals were being weighed, their enclosures were cleaned with 70% ethanol, and enrichment objects were replaced. Animal handling, temporary removal from the enclosure and return to an altered environment were identified as potential stressors for individuals, and it was assumed that these actions might elicit a stress response in affected animals (Ramahlo et al. 2019). For 48 h post re-introduction, faecal samples were collected three times a day to monitor for any elevations in fGCM concentrations. Approximately 48 hours after that, faecal samples were collected (between 0-8 hours post-defecation three times a day) for analysis to determine whether individual fGCM concentrations had returned to pre-stressor levels and, if so, how long this took in each species.

Two days after the biological validation experiment was concluded, the same individuals (Appendix B) were subjected to a physiological validation experiment (ACTH stimulation test). Each animal received a single dose (2.5 IU) of a synthetically prepared Synacthen-saline solution (Synacthens/Synacthen Depots; Novartis, Basel, Switzerland) administered intramuscularly, after which they were returned to their enclosures for careful monitoring over 24 h. All injections were administered by a veterinary nurse practitioner. Animals were monitored by a veterinary nurse, the primary researcher, and a research technician. During this 24-h period, enclosures were checked hourly for faecal samples which were collected and frozen immediately. Following the 24-h period, the sampling protocol reverted to faecal collection three times a day for the following seven days. At the end of the physiological validation experiment, all animals were released back into the environment at their capture sites. All frozen faecal material was transported to the Endocrine Research Laboratory of the University of Pretoria, South Africa, for further processing.

3.3.6 Steroid extraction

Frozen faecal material was freeze-dried and pulverised following Fraňková et al. (2012). Subsequently, dried faecal powder was extracted with 80 % ethanol in water (0.016 – 0.024 g of faecal powder in 0.5 ml solvent, 0.025 – 0.037 g in 1 ml solvent, and 0.038 – 0.055 g in 1.5 ml solvent) (Touma et al. 2003). The suspensions were vortexed for 15 min and then centrifuged at 1500g for 10 min. Supernatants were decanted into 1.5 ml Eppendorf tubes and stored at -20 °C until analysis.

3.3.7 Hormone analysis

Steroid extracts were quantified for immunoreactive fGCMs using five different enzyme immunoassays (EIAs): (i) a 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA (detecting fGCMs with a 5α - 3β , 11β -diol structure); (ii) an 11-oxoetiocholanolone (detecting 11,17-dioxoandrostanes; hereon referred to as Oxoetiocholanolone I); (iii) an 11-oxoetiocholanolone (detecting fGCMs with a 5β - 3α -ol- 11 -one structure; hereafter referred to as Oxoetiocholanolone II); (iv) a Corticosterone EIA; (v) and a Cortisol EIA. Selection of these five EIAs was based on their successful application in hormone quantification in laboratory-reared rodents, and availability of their characteristics in literature. Detailed assay characteristics including the full descriptions of assay components and cross-reactivities, have been provided by Touma et al. (2003) for the 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA, by Palme and Möstl (1997) for the 11-oxoetiocholanolone I, Corticosterone, and Cortisol EIAs, and by Möstl et al. (2002) for the 11-oxoetiocholanolone II EIA. The assay sensitivity and respective intra- and inter-assay coefficients of variance are provided in Appendix B. Parallelism tests were conducted on all species for which an assay proved reliable, and respective serial dilutions of the faecal extracts gave displacement curves parallel to the standard curves. The relative variation in the slopes of the respective trendlines for each assay and its associated species are listed in Appendix B. All values obtained are reported as $\mu\text{g/g}$ DW unless otherwise stated.

3.3.8 Data analysis

Presumed non-stressor baseline fGCM concentrations of samples obtained 24-72 hours after the Synacthen injection were used to calculate individual median baseline fGCM values. Peak fGCM values were determined as the highest fGCM concentration obtained from all experimental individuals up to 24 hours post-injection. The reliability of an assay was determined by a 2-fold increase (100% increase) in fGCM concentrations ($\mu\text{g/g}$ DW) resulting from the biological validation, the physiological validation, or both. An assay that detected at least a 2-fold increase in fGCM concentrations in a species following animal handling and re-introduction to an enclosure or ACTH injection was denoted as a suitable assay for that particular species. Additionally, assays that were unable to detect mean absolute fGCM concentrations greater than $1 \mu\text{g/g}$ DW (trace amounts) were regarded as unsuitable assays due to the potential for conflated percentage increases between baseline and peak values that are extremely small.

The fold or percentage increases in fGCMs following the biological and physiological validation experiments were calculated using the highest (peak) fGCM concentration and median (baseline) values obtained for each species post-experiment. The median values were calculated using fGCM concentrations acquired between 24 and 72 h after injection and are representative of baseline fGCM concentrations for each species. Each individual had a mean baseline value that was used for both biological and physiological peak fGCM concentration calculations. For each species, the highest (biological and physiological peak) fGCM concentration was divided by the mean baseline concentration to determine the fold or percentage increase in fGCM concentrations:

$$\text{Percentage biological increase} = (\text{fGCM peak post-biological stimuli} \div \text{baseline fGCM}) \times 100$$

and

$$\text{Percentage physiological increase} = (\text{fGCM peak post-physiological stimuli} \div \text{baseline fGCM}) \times 100$$

3.4 RESULTS

All individuals responded to biological validation or ACTH injection with an increase in fGCM levels, but the assays with the highest percentage increase responses varied between species. Assays detected fGCMs with their associated structures in varying absolute amounts, with the 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA detecting mean fGCM concentrations greater than 1 $\mu\text{g/g}$ DW for the six species (Figure 3.1). Based on absolute values, two additional assays detected mean fGCM concentrations greater than 1 $\mu\text{g/g}$ DW in several species: Oxoetiocholanolone II EIA measuring fGCMs with 5 β -3 α -ol-11-one structure (*A. ineptus*, *O. irroratus*, and *S. pratensis*) and Corticosterone (*A. ineptus*, *M. coucha*, *M. namaquensis*, and *O. irroratus*). The Oxoetiocholanolone I EIA measuring 11,17 dioxoandrostanes and Cortisol detected associated fGCM structures in trace amounts for all six species, with the highest absolute concentrations detected being 0.3 for both assays.

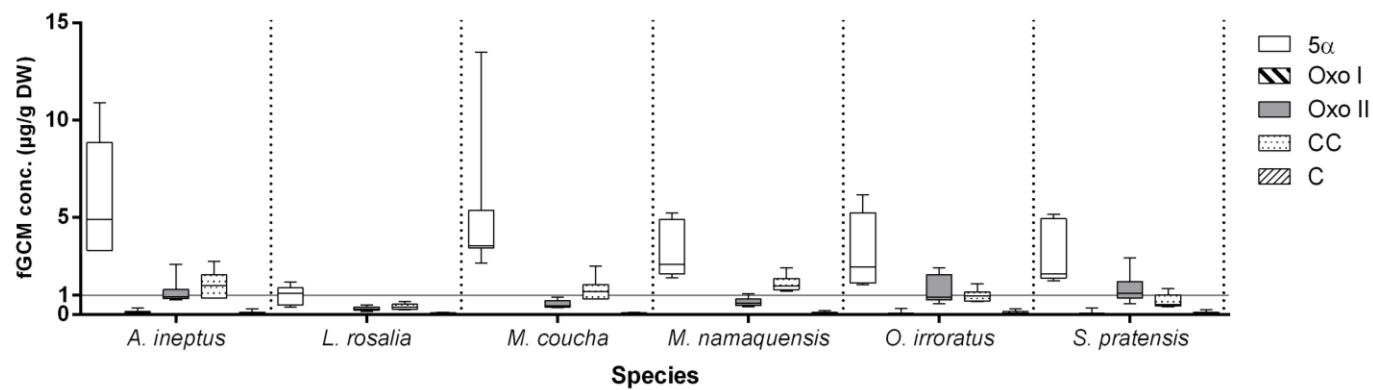


Figure 3.1. Summary of mean absolute immunoreactive faecal glucocorticoid metabolite (fGCM) concentrations determined using five enzyme immunoassays: 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA, Oxoetiocholanolone I EIA (measuring 11,17 dioxoandrostanes), Oxoetiocholanolone II EIA (measuring fGCMs with 5β - 3α -ol-11-one structure), Corticosterone EIA, and Cortisol EIA. Assays were primarily tested on one adult female and one adult male of the species after a perceived potential stressor (handling, weighing, and enclosure cleaning) and adrenocorticotrophic hormone (ACTH) administration. Vertical dotted lines denote a separation between six species: *A. ineptus*, *L. rosalia*, *O. irroratus*, *S. pratensis*, *M. coucha*, and *M. namaquensis*. The solid, horizontal line ($y = 1$) represents the minimum selected absolute fGCM concentration ($\mu\text{g/g DW}$). Assays with mean values below the line are classified as detecting faecal GCs in trace amounts.

I observed variation in the species-specific assay suitability across genetically related individuals. The five species in the Muridae family (*A. ineptus*, *L. rosalia*, *M. coucha*, *M. namaquensis*, and *O. irroratus*) showed differences in assay suitability, with the 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA being the only common assay between the species (Appendix B). The assays reliably detected a greater than 2-fold percentage increase in the six species with varying levels of success (Table 3.2). The Cortisol EIA detected fGCMs in trace concentration amounts across all six species, despite showing 2-fold or more increases between baseline and peak values and was thus denoted as an unreliable assay for quantifying stress-related fGCMs in the individuals tested in the present study. Conversely, the 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA proved to be a reliable assay for the tested species and, potentially for other murid species. The assay performed well both inter- and intra-specifically, reliably detecting 100% increases in fGCM concentrations of 10 of the 12 individuals tested across all six species, with the exception of the female *L. rosalia* and one male *M. coucha*. It was noted that the *L. rosalia* fGCM concentrations obtained were relatively lower than those of the other species, and a

100% increase was recorded only for the male individual. The Oxoetiocholanolone I and Oxoetiocholanolone II EIAs performed relatively similarly, both reliably detecting 100% increases in three of the same species: *A. ineptus*, *O. irroratus*, and *S. pratensis*. However, differences were observed in the ability of each assay to reliably detect fGCMs in *L. rosalia* (reliably detected by Oxoetiocholanolone I), and *M. namaquensis* (reliably detected by Oxoetiocholanolone II). Similar to Cortisol, the Corticosterone EIA did not perform as well as the other assays and detected 2-fold increases in three species (*M. coucha*, *O. irroratus*, and *S. pratensis*).

Table 3.2. Summary of fold increases per enzyme immunoassay denoting suitability for six Rodentia species (*A. ineptus*, *L. rosalia*, *M. coucha*, *M. namaquensis*, *O. irroratus*, and *S. pratensis*) captured in the Magaliesberg Biosphere, North West province, South Africa after experiencing a potential biological stressor (handling and temporary removal from enclosure), and a physiological stressor (ACTH administration) for each of the five enzyme immunoassays tested: 5 α -pregnane-3 β ,11 β ,21-triol-20-one (5 α); Oxoetiocholanolone I (Oxo I); Oxoetiocholanolone II (Oxo II); Corticosterone (CC); Cortisol (C). Highlighted cells indicate a 2-fold or more increase between fGCM concentrations pre- and post- stressor for biological and physiological validation. The associated relative variations of slopes of the respective parallelism trendlines (%) are presented along with baseline and peak fGCM concentrations, percentage increase, faecal sample number, and hours post-injection for all individuals in Appendix B.

Species EIA	Validation	<i>Aethomys ineptus</i>		<i>Lemniscomys rosalia</i>		<i>Mastomys coucha</i>		<i>Micaelamys namaquensis</i>		<i>Otomys irroratus</i>		<i>Steatomys pratensis</i>	
		♀	♂	♀	♂	♂	♂	♀	♀	♀	♂	♀	♂
5 α	Biological	2.13	1.58	1.50	2.74	3.93	1.44	2.64	2.15	2.15	3.98	2.39	2.97
	Physiological	2.36	2.58	1.35	1.05	1.79	1.40	2.53	2.39	2.12	2.18	2.42	2.24
Oxo I	Biological	3.02	1.33	4.50	2.13	1.88	1.44	1.64	1.22	6.33	2.47	0.99	7.16
	Physiological	1.99	2.68	4.12	1.74	1.51	1.10	1.98	1.19	1.51	0.93	1.03	2.39
Oxo II	Biological	1.40	1.58	1.68	1.59	2.05	1.80	1.71	2.73	3.17	2.72	2.11	1.42
	Physiological	1.65	2.93	1.69	1.13	1.66	1.85	1.22	2.00	2.49	2.18	2.97	1.86
CC	Biological	1.83	1.15	1.78	1.77	3.16	1.30	1.51	1.76	2.03	1.63	1.42	3.06
	Physiological	1.54	1.81	1.71	1.48	1.87	1.44	1.59	1.12	1.49	1.12	2.16	2.24
C	Biological	2.58	1.29	2.86	1.28	2.43	1.17	1.54	2.94	3.62	4.09	1.36	2.21
	Physiological	0.98	1.63	2.13	8.27	1.63	1.36	1.84	2.31	1.62	1.96	2.06	4.43

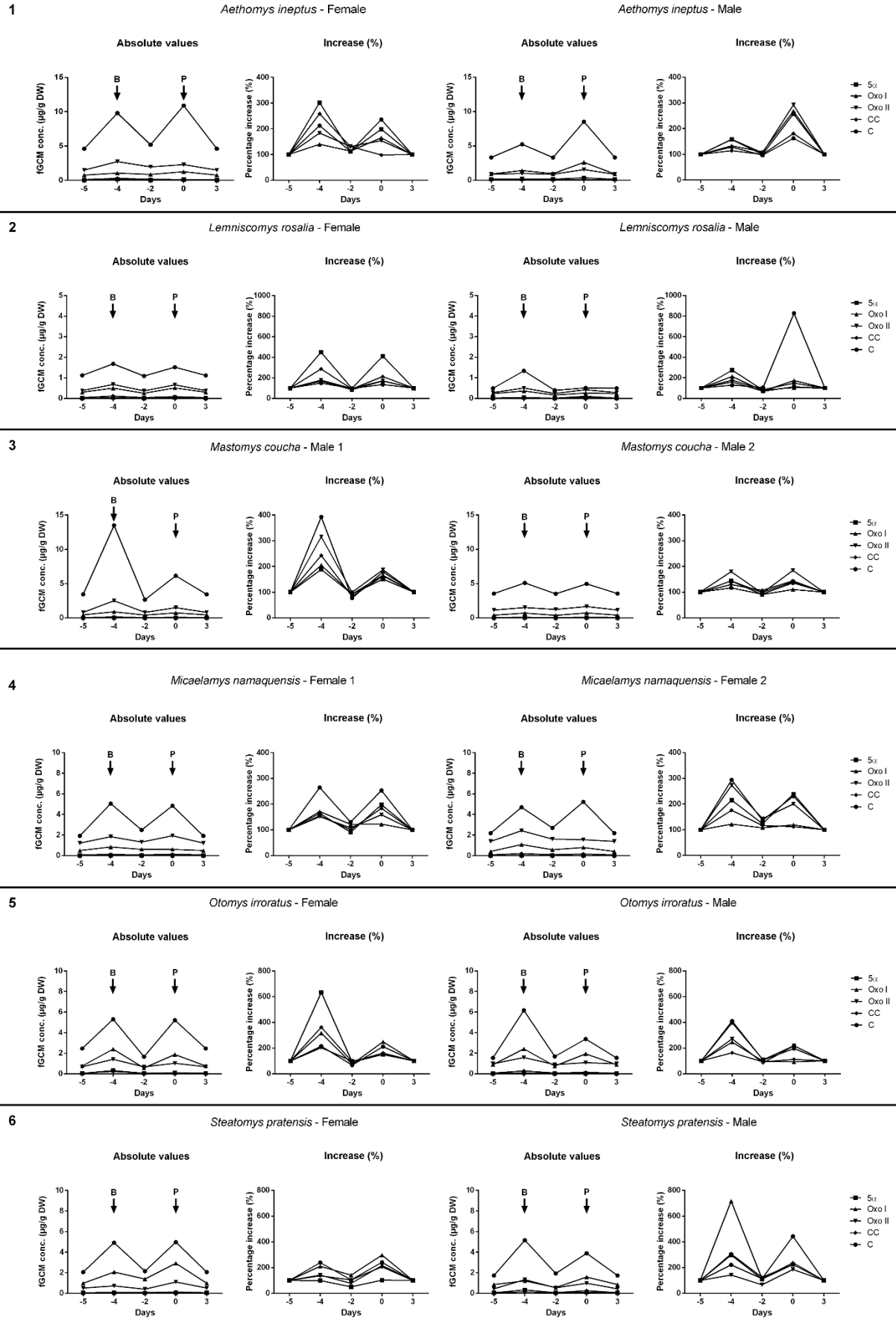


Figure 3.2. Longitudinal profiles of immunoreactive faecal glucocorticoid metabolite (fGCM) concentrations and associated percentage increase differences between calculated mean baseline and peak (highest) fGCM concentrations determined using five enzyme immunoassays: 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA, Oxoetiocholanolone I EIA (measuring $11,17$ dioxoandrostanes), Oxoetiocholanolone II EIA (measuring fGCMs with $5\beta-3\alpha$ -ol- 11 -one structure), Corticosterone EIA, and Cortisol EIA. Assays were primarily tested on one adult female and one adult male of the species after a perceived potential stressor denoted by **B** (handling, weighing, and enclosure cleaning) and adrenocorticotrophic hormone (ACTH) administration denoted by **P**. Vertical black arrows indicate individual peak fGCM concentrations associated with biological and physiological validation experiments for the six species: 1) *A. ineptus*, 2) *L. rosalia*, 5) *O. irroratus*, 6) *S. pratensis*, except for 3) two males for *M. coucha*, and 4) two females for *M. namaquensis*. Days plotted on the x-axis represent experimental protocol days: -5 (24 hours before biological validation), -4 (day of biological validation), -2 (two days after biological validation), 0 (day of physiological validation in the form of an ACTH injection), and 3 (three days after physiological validation). Concentrations plotted pre- and post-experiments denote baseline fGCM concentrations calculated using values obtained 48-120 hours pre- and 24-72 hours post-experiment as per the iterative approach outlined by Brown *et al.* (1994). For the peaks, the single highest absolute values after biological validation and during the 24-hour observation period post-injection were used. All individuals were captured in the Magaliesberg Biosphere, North West province, South Africa.

Fold-increase and absolute value detection were thus combined to determine that three of the five assays are suitable for the species under study: the 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA (all six species); the Oxoetiocholanolone II EIA (*A. ineptus*, *O. irroratus*, and *S. pratensis*), and the Corticosterone EIA (*M. coucha*, *O. irroratus*, and *S. pratensis*). The 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA was thus identified as a potential common EIA for reliable detection of fGCMs across various southern African species.

3.5 DISCUSSION

My study compared the ability of five EIAs to reliably detect variation in fGCM concentrations of six southern African rodent species in response to adrenocortical stimulation through biological and physiological means. The 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one enzyme

immunoassay was identified as a potential common EIA for southern African rodents due to its reliable detection of fGCM concentrations in calculable absolute amounts in all six rodent species. Two assays were also able to reliably detect fGCMs in non-trace amounts: the Oxoetiocholanolone II EIA (*A. ineptus*, *O. irroratus*, and *S. pratensis*); and the Corticosterone EIA (*M. coucha*, *O. irroratus*, and *S. pratensis*). Conclusions drawn from the results are based on small sample sizes and should be interpreted as such. Future research should include fGCM quantification of larger sample sizes preferably using the 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA to allow intra-specific responses to perceived stressors to be compared across studies.

The study identified assays with varying detection rates of fGCM concentrations related to varying degrees of assay suitability within five rodent species from the Muridae family (*A. ineptus*, *L. rosalia*, *M. coucha*, *M. namaquensis*, *O. irroratus*) and one from the Nesomyidae family (*S. pratensis*) (Skinner and Chimimba, 2005). This species-specific variation in assay suitability within seemingly closely related rodent species highlights the importance of enzyme immunoassay validation when using such assays for the initial quantification of hormones in a species (Ganswindt et al. 2003, Millspaugh & Washburn 2004).

In terms of intraspecific sex-related variation in assay suitability, baseline and peak biological and physiological absolute fGCM values differed across species. Baseline and both peak values were higher in females of *A. ineptus* and *L. rosalia* compared to their male counterparts, while only the physiological peak was higher in females of *O. irroratus* and *S. pratensis* relative to their male counterparts. Hill et al. (2015) reported that rodents have alternative reproductive tactics (ARTs) that are influenced by their varying living strategies (communal, returning, and solitary, among others). These tactics can affect endocrine control and steroid hormones in adult individuals. The relatively high fGCM levels seen in the females of the communal-living species (*A. ineptus* and *L. rosalia*) compared to males may be explained by the stress associated with group-living social dynamics (Hill et al. 2015). Van Kesteren and Schradin (2012) showed that communal living leads to physiological stress in reproductive individuals, which is supported by the findings of my study. Females of solitary-living species have lower corticosterone levels as living singly does not require as much cooperative behaviour and its associated stress responses (Hill et al. 2015). This variability within and between species further emphasises the physiological and behavioural hormone-linked differences between related taxonomic groups. As such, comparisons should not be made between the absolute fGCM concentrations of individuals from different species as there are many factors that can

influence how their fGCMs are metabolised and, subsequently, the values quantified by enzyme immunoassays.

This research on rodent species indicates that there is interspecific variation in fGCM concentrations and responses to experimental protocols such as handling and injection during ACTH tests, which further emphasises the need for assay validation before fGCM quantification. To understand this variation and potentially improve our ability to extrapolate assay reliability in large taxonomic groups, future research should also investigate glucocorticoid composition and metabolism as a function of phylogenetic relatedness (Goncalves et al. 2016). Such research should include the characterisation of glucocorticoid metabolite compositions associated with the study species and the determination of gut passage time in said species. Such information may improve the accuracy of a priori hormone assay selection because some assays can be eliminated from validation experiments if their associated hormone structures are not found in a species. Determining the gut passage time of different species may also improve our understanding of glucocorticoid metabolism and faecal excretion. A longer gut passage time may indicate prolonged digestion and, potentially, increased metabolic activity of some hormones and not others. This may explain the trace amounts of hormones detected by some EIAs in this study, whereas others were present in greater concentrations. Additionally, temporal niches should be considered when conducting future research on adrenocortical activity in diurnal versus nocturnal species because circadian rhythms and activity patterns may affect hormone activity in different species (MacDougall-Shackleton et al. 2019).

Of the five assays, the Oxoetiocholanolone I and Cortisol EIAs were determined as unreliable due to their inability to reliably detect absolute fGCM concentrations greater than 1 ug/DW and a 2-fold or more increase. With hormones detected in trace amounts, there is potential to exaggerate any differences observed in baseline and peak values, thus making any fold-increases unreliable. The remaining three were able to detect fGCMs at non-trace amounts for several species: the 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA (all six species); the Oxoetiocholanolone II EIA (*A. ineptus*, *O. irroratus*, and *S. pratensis*); and the Corticosterone EIA (*M. coucha*, *O. irroratus*, and *S. pratensis*).

The 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA appears to be a reliable assay for detecting fGCM concentrations in rodent species from the Muridae and Nesomyidae families and could

potentially be used as a common EIA in future studies. However, the low fGCM detection rates observed in *L. rosalia* highlight the importance of assay validation across a wide range of species, as interspecific variation can arise in distinct species of the same taxonomic order. In addition, Oxoetiocholanolone I and Cortisol appear to be poor matches for measuring fGCM concentrations in southern African murids, as the hormones are not excreted in clearly measurable amounts in these species (Goncalves et al. 2016). Future studies that aim to investigate adrenocortical activity in southern African rodent species should, ideally, avoid using Oxoetiocholanolone I and Cortisol assays because of their presence in trace amounts in rodent species of interest.

3.6 CONCLUSION

The 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA can be used to monitor stress-related fGCM concentrations in selected southern African rodent species, with the potential for use in other untested species. A common EIA can standardise future research so that intraspecific comparisons can be made across different studies of the same species. This has various implications for small mammal research because researchers conducting captive studies can reliably quantify the impact of their experiments on their study animals and, where necessary, adjust their protocols to improve their ethical compliance. In addition, southern African murids can be used as biological indicators of ecosystem health by monitoring their stress-related physiological responses to various anthropogenic disturbances and land transformations in a minimally invasive manner.

CHAPTER 4: FAECAL GLUCOCORTICOID METABOLITE CONCENTRATIONS OF TWO DOMINANT SOUTHERN AFRICAN MURIDS (*LEMNISCOMYS ROSALIA* AND *MASTOMYS COUCHA*) ACROSS VARIOUSLY TRANSFORMED LANDSCAPES

4.1 ABSTRACT

Global landscape transformations are a result of the growing human population and the subsequent loss of natural habitats. Local species extinctions across various ecosystems emphasise the importance of monitoring wildlife responses to anthropogenic activities. Small mammals such as rodents are valuable biological indicators of anthropogenic transformation and, subsequently, ecosystem health. The study aimed to investigate the effects of seasonal and land use conditions on adrenocortical activity in quantified faecal glucocorticoid metabolites (fGCMs) as a potential physiological biomarker of ecosystem health in two dominant southern African murids. *Lemniscomys rosalia* (single-striped grass mouse) and *Mastomys coucha* (southern multimammate mouse) were live-trapped across dry and wet seasons within minimally and moderately transformed landscapes over a three-year sampling period in the Magaliesberg Biosphere, South Africa. Faecal samples were collected from captured individuals and measured for fGCM concentrations using a previously validated 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one enzyme immunoassay detecting fGCMs with a 5α - 3β , 11β -diol structure. *Mastomys coucha* showed significantly higher fGCM concentrations during the wet season, unlike *L. rosalia*, which may reflect the species-specific dynamics associated with activity patterns. Land-use variation had no significant impact on either species, which supports reported synanthropic behaviour (to various extents) in both species. When investigating animal welfare, measuring stress-related responses to anthropogenic transformation in dominant species such as *M. coucha* and *L. rosalia* may not be an ideal approach as their phenotypic plasticity and subsequent behavioural adaptability enable them to acclimate to new and altered environments, thus minimising the quantifiable impact of anthropogenic transformation on their adrenocortical hormone responses.

4.1.1 Keywords: anthropogenic activities, faecal glucocorticoid metabolites, land transformation, single-striped grass mouse, southern multimammate mouse

4.2 INTRODUCTION

The natural world is constantly changing, with almost all recent land transformations being human-mediated (Holdren & Ehrlich 1947, Ellis et al. 2013). Two such large-scale changes are human population growth and global climatic variation (Foley et al. 2005, Bowman et al. 2011, Palomo et al. 2013). The expansion of human populations into previously natural landscapes has led to habitat degradation and subsequent adverse and, often, irreversible effects on animal and plant population numbers (Ehrlich & Ehrlich 1991, Weibull et al. 2003, Brinkmann et al. 2014). Anthropogenic land transformations such as deforestation, land development for agriculture and urbanisation, as well as industrial activities like mining often lead to altered wildlife community structures across various ecosystems, with some species adapting to the new environments while others disappear, leading to local extinctions (Blaum et al. 2007, Ribeiro et al. 2009, Van Der Merwe & Hellgren 2016).

Monitoring threats to biodiversity that stem directly and indirectly from anthropogenic activities is a vital first step in mitigating and managing their effects (Christie et al. 2019). To assess ecosystem health, it is necessary to determine which variables directly impact biodiversity and how to quantify this impact. With the increasing rate of global change, there is a rise in the amount of research conducted on wildlife and natural environments in response to these changes (Turner et al. 2001, Arturo Sánchez-Azofeifa et al. 2002). As a result, the measure of ecosystem health has evolved over the years to incorporate not only the abiotic factors affected within the ecosystem but also the responses of biotic components such as biological indicator species to anthropogenic activities (Cadotte et al. 2016, Turak et al. 2017). Monitoring the physiological responses of free-ranging indicator species to anthropogenic transformation can inform on ecosystem health and identify potential stressors impacting local wildlife (Reeder & Kramer 2005, Angelier & Wingfield 2012). Quantifying physiological adrenocortical responses of wildlife to human-mediated transformation may provide insight into animal welfare and ecology, and potentially improve our understanding of the anthropogenic activities of greatest concern to wildlife populations (Nováková et al. 2008, Harper & Austad 2012).

Glucocorticoids (GCs) are stress-related hormones that are metabolised and secreted throughout the body in matrices such as urine and faeces (Touma & Palme 2005). GCs can increase in response to various stimuli, including stress, circadian rhythms, heightened

emotional responses, and illness, among others (Palme 2005, Nováková et al. 2008, MacDougall-Shackleton et al. 2019). In recent literature, GCs have been used primarily to measure stress-related responses to stimuli, but they can also be indicative of other physiological conditions (Romero & Beattie 2021). To improve the precision of quantifying GC metabolite concentrations induced by a specific condition or stimulus, other GC-linked stimuli should be accounted for as much as possible. Research studies should aim to standardise data collection protocols and assess individuals' overall conditions to minimise the effects of other GC-linked stimuli. In terms of stress-related GC monitoring, body condition, age, and reproduction should also be assessed, as they can influence GC concentrations.

Stress can be defined as a general syndrome, acute or chronic, which occurs in response to a stimulus or perceived stressor with the potential to impact the homeostasis of an animal (Hulsman et al. 2011). When an animal perceives a potential stressor, the hypothalamic-pituitary-adrenal (HPA) axis is activated and stimulates the secretion of stress hormones known as glucocorticoids (GCs) (Reeder & Kramer 2005). The short-term elevation of GCs relative to their baseline concentrations in an animal can be beneficial, allowing the animal to evade predators, among other behaviours, while long-term elevations from baseline can cause health issues such as impaired HPA regulation, reduced reproductive success, and immune suppression (Dobson & Smith 2000, Hing et al. 2014). Monitoring HPA activity is thus a vital part of wildlife management and conservation, as the impact of environmental stressors on natural populations can be measured. Quantifying faecal glucocorticoid metabolites (fGCMs) has become popular as data collection is minimally invasive, and faeces can often be collected discretely (Palme et al. 2005, Sheriff et al. 2011). Measuring stress-linked biomarkers in indicator species can thus add to our knowledge of animal ecology and the effects of human encroachment on the natural world.

Small mammals such as rodents are ideal indicator species as they serve various ecological roles, including seed dispersal, nutrient cycling, and pollination (Dickman 1988, Skinner & Chimimba 2005). Additionally, rodents are pioneer species whose presence or absence in an ecosystem can assist with categorising levels of transformation and the rehabilitation potential of affected landscapes (Ferreira & Van Aarde 1999, 2000, Hagenah et al. 2009). By investigating the stress-linked responses of rodent species in natural and human-populated areas, we can improve our understanding of the effects of anthropogenic land transformation and seasonal variation on natural populations in a minimally invasive and sustainable way.

This may highlight the scope of influence (adverse or otherwise) that human development has on natural ecosystems.

Therefore, this study aimed to assess the effects of land use and seasonal variation in two rodent species, the single-striped grass mouse (*Lemniscomys rosalia*) and the southern multimammate mouse (*Mastomys coucha*), using adrenocortical activity as a physiological measure of disturbance across untransformed and transformed landscapes in the Magaliesberg Biosphere, South Africa. I predicted that, for both species, fGCM concentrations would be positively correlated with the level of disturbance and human presence in a landscape. Additionally, I predicted that fGCM concentrations would be higher in the dry season compared to the wet season, because of reduced vegetation cover and food resource availability associated with low rainfall and dry conditions in the dry season.

4.3 MATERIALS AND METHODS

4.3.1 Study area

The study was conducted in the Magaliesberg mountain range (25°41'00.1"S, 27°57'51.8"E), which spans the Gauteng and North West provinces of South Africa. This area is part of the Magaliesberg Biosphere, located within the Savannah Biome. The region features two primary vegetation types: the Gold Reef Mountain Bushveld at higher elevations and the Marikana Thornveld and Moot Plains Bushveld in the lower areas (Rutherford & Mucina 2006). The vegetation consists mainly of woody plants with a grass-dominated herbaceous layer. The landscape is characterized by thorny *Vachellia* species, semi-open thickets, and igneous rocks from the Rustenberg Layered Suite and Rooiberg Groups (Rutherford & Mucina 2006). The study sites are situated in the De Wildt (25°40'05.5"S, 27°55'23.1"E) and Zilkaatsnek (25°39'25.8"S, 27°56'34.9"E) areas, which experience hot, wet summers (November to March) and cold, dry winters (April to October) (Boonzaaier & Hough 2011). The onset of the wet season and annual rainfall patterns show considerable variability. Study sub-sites were pooled to increase sample size, resulting in samples being collected at two sites or land use types for comparison: 1) a minimally transformed landscape which is made up of a nature conservancy and a small residential homestead, and 2) a moderately transformed landscape which comprises a crop and livestock farm, residential farmsteads, an eco-tourism centre, and a platinum mine (Table 4.1).

Table 4.1. Summary of study sites including landscape properties that aided with categorising land use types as either minimally or moderately disturbed: primary land use, primary vegetation cover, total area covered, proportion and percentage of disturbed area, total boundary distance, proportion and percentage of boundary that is adjacent to a main or dirt road analysed in the Magaliesberg Biosphere, North West province, South Africa.

Landscape properties	Land use type	
	Minimally transformed	Moderately transformed
Primary land use	Nature conservation Residential	Agriculture (crop and livestock) Animal breeding and rehabilitation Eco-tourism Residential
Primary vegetation cover	Natural	Planted crops Planted fruit trees Natural
Total area (km ²)	1939793.7 km ²	1699366.6 km ²
Proportion transformed area (km ²)	150907.0 km ² (7.8%)	722536.2 km ² (42.5%)
Total study site perimeter (km)	7050.0	8730.0
Study site perimeter flanked by road (km)	3308.6	7970.0
Study site perimeter flanked by road (%)	46.9	91.3

4.3.2 Study species

My study investigated stress-linked faecal GC metabolite responses of two southern African species from the Muridae family. *Lemniscomys rosalia* is a terrestrial rodent that can tolerate various savannah habitats, grasslands, and the fringes of cultivated landscapes (Monadjem & Perrin 1997, 2003). This species is communal living and omnivorous, with a polyoestrous reproductive cycle where approximately 4-5 young are born (Skinner & Chimimba 2005). The species is primarily diurnal, although some crepuscular activity has been reported (Monadjem & Perrin 2003, Skinner & Chimimba 2005, Stuart & Stuart 2015). *Mastomys coucha* is also a terrestrial, synanthropic rodent found across savannahs, grasslands, and agricultural landscapes (Skinner & Chimimba 2005). The rodent is communal living and omnivorous, with a polyoestrous reproductive cycle where approximately 1-27 young are born (Monadjem & Perrin 2003, Skinner & Chimimba 2005, Stuart & Stuart 2015). The species is primarily nocturnal and is often found in disturbed landscapes, with its dominance in small mammal communities being regarded as an indicator of poor ecosystem health (Avenant et al. 2008, Avenant 2011). Both species were captured across untransformed and transformed study sites in relatively high numbers and were classified as dominant species in the area and are thus the focus of this study. With minimal endocrine research conducted on southern hemisphere rodent

species, pre-existing data on the physiological responses of *L. rosalia* and *M. coucha* are not available for comparison. As such, it is even more essential to investigate these dominant species as their responses to anthropogenic activities can provide meaningful data towards effective and sustainable land use management.

4.3.3 Sampling protocol

The study sites were sampled using 40 Enviro-Care live traps (Cangzhou Jinglong Technology Co. Ltd., Hebei Province, China) between October 2018 and January 2021. The traps (length x height x width: 255 x 78 x 80 mm) were made of galvanised sheet metal and were placed 10 m apart in a 5 x 8 trapping grid following standard procedures (Aplin et al. 2003, 2011). Each trap was baited with a mixture of oats and peanut butter. Each season, sites were monitored simultaneously over five consecutive nights (Sunday to Thursday). Traps were closed on Friday afternoons and reopened with fresh bait on Sunday afternoons. This cycle was repeated four times, resulting in a total of 20 nights of trapping per site per season. The traps remained open throughout the day and night to ensure diurnal and nocturnal species trapping and were checked in the morning between 05:30 and 09:00 and again between 15:00 and 17:00 daily. Captured animals were sexed and identified up to species level (Joyce et al. 1997, Newbery 1999, Skinner & Chimimba 2005). Individuals were weighed using a spring scale (PESOLA Präzisionswaagen AG, Schindellegi, Switzerland) and marked using numbered toenail clipping to ensure that recaptured individuals could be identified (Aplin et al. 2003). Many small mammals contain blood vessels in their toenails that may become damaged if clipped. Thus, toenail-clipped individuals were treated with antiseptic Mercurochrome (Barrs Pharmaceutical Industries, Cape Town, South Africa) to prevent infection and ensure the nails re-grow with minimal contamination (Fleckman et al. 2013). Animals were then released at their respective capture sites. Faecal samples were collected from the previously occupied traps and stored in labelled cryotubes at -20°C. Traps were then re-baited and returned to their original positions in the trapping grid (Aplin et al. 2003). All faecal samples were kept frozen until transported to the Endocrine Research Laboratory of the University of Pretoria, South Africa, for further processing. This study was conducted with the approval of the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (Ethics clearance number EC044-18) and complied with Section 20 of the Animal Diseases Act of South Africa, 1984 (SDAH-Epi-18111309000).

4.3.4 Steroid extraction

Frozen faecal materials were freeze-dried and pulverised (Fraňková et al. 2012). Subsequently, dried faecal powder was extracted with 80 % ethanol in water (0.016 – 0.024 g: 0.5 ml solvent; 0.025 – 0.037 g: 1 ml solvent; 0.038 – 0.055 g: 1.5 ml solvent) (Touma et al. 2003). The suspensions were vortexed for 15 min and then centrifuged at 1500g for 10 min. Supernatants were decanted in 1.5ml Eppendorf containers and stored at -20°C until analysis.

4.3.5 Hormone analysis

Assay validation conducted in Chapter 3 for both species showed that steroid extracts can be quantified for immunoreactive fGCMs using a 5 α -pregnane-3 β ,11 β ,21-triol-20-one enzyme immunoassay (EIA) detecting fGCMs with a 5 α -3 β , 11 β -diol structure. Since this assay was identified as a reliable detector of fGCM activity in six southern African rodent species, it was selected for its potential application as a common EIA for many southern African rodents. Additionally, the use of a common or standardised EIA may allow for more effective comparison of a species across research studies. Detailed assay characteristics, including the full descriptions of assay components and antibody cross-reactivities, have been provided by Touma et al. (2003). The intra-assay coefficients of variance (CVs) were 3.15% and 3.61%, and the inter-assay CVs were 8.87% and 10.44%. The sensitivity of the assay was 2.4 ng/g faecal dry weight. The slope given in the linear equation for the linear range of the assay standard curve and a respective dilution series of a sample were compared and defined as parallel if the difference in slope is <5%. Parallelism tests with serial dilutions of faecal extracts gave displacement curves parallel to the standard curve (relative variation of the slopes of respective trend lines were <3% for *L. rosalia* and <5% for *M. coucha*).

4.3.6 Data analysis

All statistical analyses were conducted in R using the RStudio interface (R Core Team 2020). The data were tested for normality using a Shapiro-Wilk test (Royston 1992). In the event of normality, a t-test was used to assess sex-related differences between species, while for a non-normal distribution, a Kruskal-Wallis test was used to assess sex-related differences between species (Vargha & Delaney 1998, Ruxton & Beauchamp 2008). In the event of statistically significant sex-related variation, fGCM concentrations of female and male individuals within each species were analysed separately. Conversely, if the sex-related variation was not statistically significant, subsequent analyses were conducted on a pooled dataset of female and male fGCM concentrations for each species.

Of the 131 samples analysed, 55 were from individuals captured at the untransformed site (dry season = 36; wet season = 19) and 76 from individuals captured at the transformed site (dry season = 29; wet season = 47). The data were computed as non-normal for both *L. rosalia* ($W = 0.78$; p -value < 0.001) and *M. coucha* ($W = 0.81$; p -value < 0.001). Sex-related differences did not result in statistically significant variation in fGCM concentrations for either species: *L. rosalia* ($H = 1.13$; p -value = 0.29; $n = 59$) and *M. coucha* ($H = 1.42$; p -value = 0.23; $n = 72$), and thus data from females and males were pooled for subsequent analyses. In Chapter 3, baseline fGCM concentrations for both species were calculated using captive animals during validation of assay experiments for *L. rosalia* (0.68 ug/g DW) and *M. coucha* (2.65 ug/g DW). These values were plotted against those of individuals captured across the untransformed and transformed sites for a comparison between baseline values of each species and the absolute fGCM values obtained for my captive validation individuals.

To assess fGCM responses of each species, a linear regression model of best fit used two variables (season and land use):

$$\text{fGCM concentration} \sim \text{Season} + \text{Land Use}$$

where Season refers to the time of capture (dry or wet season), and Land Use refers to the study site where animals were captured (minimally or moderately disturbed). For both variables, a p -value greater than 0.05 and an F -value lower than 2.5 meant that the variable was unlikely to explain any variability observed in the fGCM concentrations of *L. rosalia* and *M. coucha*, and vice versa (Frieden 2001).

4.4 RESULTS

Seasonal variation resulted in significantly elevated fGCM concentrations in *M. coucha* (F -value = 5.49; p -value = 0.02; $df = 1$) during the wet season (summer), but not in *L. rosalia* (F -value = 1.10; p -value = 0.30; $df = 1$). Percentage elevation in fGCMs from the dry to wet seasons for *L. rosalia* at the minimally (-46.6 %) and moderately (-21.8 %) transformed sites shows a trend towards declining fGCM concentrations associated with the wet season. For *M. coucha*, minimally (59.1 %) and moderately (66.8 %) transformed site percentage elevations from the dry to wet seasons indicate an inverse response to seasonal shifts. Landscape-specific

fGCM concentrations did not differ significantly in either *L. rosalia* (F -value = 0.88; p -value = 0.56; df = 1) or *M. coucha* (F -value = 0.06; p -value = 0.42; df = 1) (Figure 4.1).

Comparisons between the calculated baseline values for each species and the absolute fGCM values of individuals captured across minimally and moderately transformed landscapes showed varying results. Baseline values obtained from my temporarily captive *L. rosalia* (0.68 ug/g DW) were much lower than those of my capture-mark-recapture (CMR) individuals (Figure 4.1). In terms of *M. coucha*, baseline values (2.65 ug/g DW) were comparable to those of CMR individuals during the dry season, but higher during the wet season.

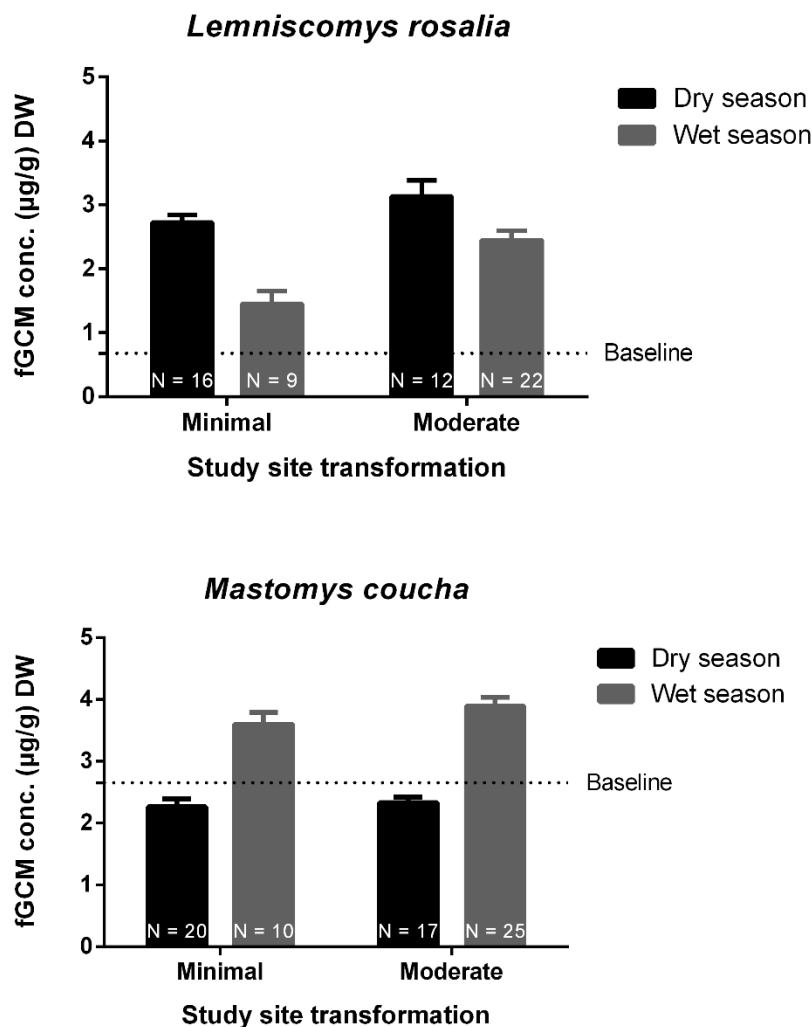


Figure 4.1. Seasonal (dry and wet) and site (untransformed and transformed) comparisons of immunoreactive faecal glucocorticoid metabolite (fGCM) concentrations of *Lemniscomys rosalia* (single-striped grass mouse) and *Mastomys coucha* (southern multimammate mouse)

sampled in the Magaliesberg Biosphere, North West province, South Africa. Baseline fGCM concentrations (indicated by dotted lines) were calculated using captive animals during validation of assays for *L. rosalia* (0.68 ug/g DW) and *M. coucha* (2.65 ug/g DW) in Chapter 3.

4.5 DISCUSSION

The study investigated the physiological stress-linked endocrine responses of two southern African rodent species (*L. rosalia* and *M. coucha*) to seasonal variation and land transformation. I found that seasonal variation significantly impacted fGCM concentrations of the nocturnal, *M. coucha*, and, to a lesser extent, the diurnal *L. rosalia*, while land transformation did not significantly affect either species.

Considering the calculated baseline fGCM values obtained from captive individuals in Chapter 3 compared to values obtained from free-ranging individuals, captive *L. rosalia* has relatively low baseline values compared to its free-ranging counterparts. With daily food, water and a temperature-controlled room, the main issues associated with free-living species are accounted for, leaving only researchers entering the room three times a day to feed animals and collect faecal samples. The species' diurnal and sometimes crepuscular activity patterns often see it interact, directly or indirectly, with humans, and may have led to the habituation seen in the baseline fGCM values of these captive *L. rosalia* (Monadjem & Perrin 1997). The nocturnal *M. coucha*, however, which is habituated to living concurrently with humans by occupying a different temporal niche may have found difficulty in encountering researchers directly during daily feedings and sample collections, thus resulting in the relatively high baseline values observed in Chapter 3 (Skinner & Chimimba 2005). Alternatively, the consistently high fGCM values seen in *M. coucha* may be indicative of a species with perpetually high acute GC concentrations, which can sometimes reflect an animal that is coping in its environment and is ready to handle potential stressors (Romero & Beattie 2021).

4.5.1 Seasonal variation

Seasonal variation had a minimal impact on fGCM levels of *L. rosalia*, with the wet season seeing lower fGCM concentrations in both minimally and moderately transformed landscapes. The dynamically crepuscular-diurnal *L. rosalia*'s exposure to varying seasonal temperatures throughout the day may explain the relatively low impact of seasonal changes on fGCM levels

(Skinner & Chimimba 2005, Monadjem et al. 2015). In warmer conditions, individuals may prefer to adopt more crepuscular activity patterns as an avoidance mechanism, while colder conditions may see the species being more active later in the day, thus minimising its temperature-related stress responses. Although seasonal variation did not have a significant impact, I observed higher overall fGCM concentrations for *L. rosalia* during the dry season, specifically in minimally transformed landscapes. Natural landscapes do not have the benefit of year-round food availability associated with human-altered areas, which may explain the sharp decline in fGCM levels during the food-rich wet season. With the species showing adaptive activity patterns, it is less likely that warmer temperatures are the reason for the elevated fGCM concentrations observed in the dry season. However, the dry season is associated with lower rainfall, and the dry season months in the Magaliesberg region are becoming increasingly drier as rainfall patterns shift due to global climate change (Waters et al. 2016, Power et al. 2019, Louw & Louw 2020). This decline in water availability influences food availability, vegetation cover, and reproductive behaviour in many rodent species, and may explain the increased fGCM concentrations seen in my study relative to the wet season months (Taylor & Green 1976, Monadjem & Perrin 1997, Meheretu et al. 2015). A diurnal species showing elevated fGCM levels in hot, dry conditions may be cause for concern as the species is adapted to withstand daily temperatures, but low water availability may prove an unavoidable consequence of global climate change.

Conversely, fGCM concentrations of the nocturnal southern multimammate mouse were significantly influenced by seasonal variation. The activity patterns of *M. coucha* mean that the species is rarely exposed to as wide a range of temperatures as its diurnal counterpart, with most overnight temperatures in southern Africa being significantly lower than daytime temperatures (Van der Walt & Fitchett 2020). My study found that adrenocortical responses in *M. coucha* were more elevated in the wet season compared to the dry season. In southern Africa, the dry season is often associated with winter months and colder climates, while the wet season is associated with summer months, which are becoming increasingly warmer as global climatic patterns continue to shift (Engelbrecht & Engelbrecht 2016). *Mastomys coucha* is adapted to nocturnal conditions and the associated cooler temperatures, and the increasingly warmer summer temperatures may be perceived as an environmental stressor by these murids, as seen in other mammalian and non-mammalian species (Morgan & Tromborg 2007, Young et al. 2014, Ngcamphalala et al. 2021).

4.5.2 Land use variation

Unlike seasonal variation, land transformation had no significant influence on the fGCM levels of either species in the present study. These species are reported to exhibit synanthropic behaviour to varying extents (Skinner & Chimimba 2005), which may explain the low impact of land transformation in my study. *Lemniscomys rosalia* occurs on the fringes of agricultural landscapes, which may have influenced its habituation in anthropogenically transformed areas (Monadjem & Perrin 1997, 2003). This may explain the lack of significant variation in stress-responses of this species across untransformed and transformed landscapes. Despite this possible habituation, the species still showed higher fGCM concentrations at the transformed site, which suggests some sensitisation to anthropogenic transformation in *L. rosalia*.

Mastomys coucha is widely considered a synanthropic species (Skinner & Chimimba 2005), and this is supported by the present study. Stress-related hormone concentrations were similarly lower at both sites during the dry season, and marginally higher during the wet season, with the main differences seen across seasons and not sites. The high similarity in fGCM concentrations of *M. coucha* across sites supports studies showing the species' habituation to human areas, with the associated access to year-round food resources and reduced reliance on natural vegetation for cover around human infrastructure (Yarnell et al. 2007, Avenant et al. 2008, Angelier & Wingfield 2012).

4.5.3 Comparisons to assay validation baselines

Pre-stressor fGCM concentrations of captive individuals obtained in Chapter 3 were compared to those of free-ranging individuals in this study. The stress-related responses of free-ranging *L. rosalia* across all seasons and sites were higher than those of their captive counterparts. Since the individuals used in Chapter 3 were captured in the same localities as the free-ranging individuals in this study and given an acclimation time of approximately three weeks, this showcases the highly adaptive behaviour of the species. In the wild, small mammals such as rodents often become prey items for larger predators, which may explain the 300% (dry season) and 110% (wet season) elevations in fGCM concentrations seen in the individuals captured at the untransformed site relative to the captive *L. rosalia*. Individuals captured in the transformed landscapes showed a 360% (dry season) and 260% (wet season) increase in fGCM concentrations compared to captive individuals. This may be explained by environmental stressors perceived in the transformed environment and an increased human presence relative to the untransformed environment.

Conversely, the captive *M. coucha* individuals appear to have difficulty adapting to captivity, as suggested by the high baseline values recorded in captive individuals. Baseline values obtained in captivity were greater than those of individuals at the untransformed (-15%) and transformed (-12%) sites during the dry season, while wet season fGCM values at both sites were greater than those of captive individuals (36% increase at the untransformed site and 47% increase at the transformed site). Environmental stressors during the wet season appear to impact the species more prominently, possibly due to warmer temperatures and population explosions brought on by the rainy season and the increased intra- and inter-specific competition for resources associated with the breeding season (Massawe et al. 2011, Meheretu et al. 2015).

4.6 CONCLUSION

Our findings inform on the resilience of both species in a world that is continuously changing by anthropogenic means. With increasing land transformation and human encroachment into natural landscapes, continued exposure to anthropogenic transformation and dense human populations may lead to increased habituation by these and other indigenous species. Prolonged exposure to a perceived stressor may lead to acclimation and reduced sensitivity to the said stressor (Bashaw et al. 2016). Increasing human encroachment and the subsequent behavioural and physiological responses of some species showing reduced sensitivity to anthropogenic transformation and dense human populations may be a cause for concern as a growing number of species may become more synanthropic. Small mammals and other species may be forced to adopt an increasingly synanthropic lifestyle, leading to more occurrences of human-wildlife conflicts and potential classifications of indigenous species as pests. This could result in increased use of pest-control methods that are not environmentally friendly and have adverse effects on small mammal populations, community structures, and their associated food webs across various ecosystems. Future studies should investigate whether behavioural habituation to transformed landscapes can be seen in other indicator species, and whether we can quantify the extent of habituation in these species and use it as a minimally invasive indicator of ecosystem health in a fast-changing world.

CHAPTER 5: THE EFFECTS OF LAND TRANSFORMATION AND SEASONAL VARIATION ON DIETARY SELECTION OF SOUTHERN AFRICAN RODENT SPECIES IN THE MAGALIESBERG BIOSPHERE, SOUTH AFRICA

5.1 ABSTRACT

Investigating diet-related resource partitioning of biological indicator species can improve our understanding of the behavioural mechanisms employed by animals to survive in a rapidly changing world. Assessing the diet preferences of sympatric species across various seasonal and landscape conditions is vital for managing natural areas and rehabilitating transformed areas. Changing landscapes can influence vegetation and species in the environment, and subsequently, animal diet selection. Long-term stable isotope dietary responses can be investigated as a tool for measuring ecosystem health. The study compared carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios obtained from hair samples of four dominant southern African small mammal species from the Magaliesberg, North West province, South Africa: *Aethomys ineptus* (Thomas and Wroughton, 1908), *Lemniscomys rosalia* (Thomas, 1904), *Mastomys coucha* (Smith, 1834), and *Micaelamys namaquensis* (Smith, 1834). Species were live-trapped, and hair clippings were collected over a three-year sampling period during dry and wet seasons. Samples were collected across untransformed and transformed landscapes. Hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios were compared to determine whether there was any significant diet variation due to land transformation and seasonality. Land transformation resulted in significant isotopic variation in *A. ineptus* (p -value < 0.01 ; $R^2 = 0.40$) and *M. coucha* (p -value < 0.001 ; $R^2 = 0.16$), whereas *M. namaquensis* was significantly affected by both land transformation (p -value = 0.001 ; $R^2 = 0.44$) and seasonality (p -value < 0.001 ; $R^2 = 0.44$). *Lemniscomys rosalia* was minimally affected by either variable. Generalist and omnivorous species are more likely to persist in cases of altered landscapes and increased human encroachment, whereas specialist species may struggle to adapt and face local extinction.

5.1.1 Keywords: *Aethomys ineptus*, carbon isotope ratio, diet, hair, indicator species, keratin, *Lemniscomys rosalia*, *Mastomys coucha*, *Micaelamys namaquensis*, nitrogen isotope ratio, rodents, southern Africa, stable isotope analysis

5.2 INTRODUCTION

Human-mediated land transformation is a global practice aimed at meeting the varied needs of a growing human population through the alteration of natural landscapes for agriculture, urbanisation, industrialisation, and recreation (Ehrlich & Ehrlich 1991, Turner *et al.* 2001). These landscape changes may negatively influence native fauna and flora in the immediate environment. These negative effects may be further exacerbated by seasonal shifts in rainfall and temperature. In the present study, seasonal variation refers to temperature and rainfall differences between the dry and wet seasons in the southern hemisphere, which can affect food resource availability throughout the year (Kerley 1992, Massawe *et al.* 2011, Young *et al.* 2014). Dry seasons, typically characterised by low rainfall (between April and September), lead to reduced vegetation for food and cover, whereas wet seasons, typically characterised by high rainfall (between October and March), lead to abundant vegetation for food and cover. Historically, ecosystem threats were natural disasters and uncharacteristically extreme climatic events (wildfires, floods, heat waves, frost, or droughts), which occurred intermittently, allowing native flora to regrow and wildlife to establish (Townsend *et al.* 1997, Aplin *et al.* 2011). In recent times, anthropogenic threats (land transformation for agriculture, natural habitat degradation due to deforestation and mining of natural resources) to ecosystem health have increased in frequency and intensity, leading to reduced numbers of endemic species. Anthropogenic activities have transformed natural ecosystems and disrupted habitats and preferred niches for many species by reducing their physical suitability and food resource availability (Cadotte *et al.* 2016). Many species may be forced to adapt their diet or disperse to regions with favourable conditions, shifting the trophic ecology of an ecosystem.

Biological indicators of ecosystem health allow us to monitor resilient taxonomic groups and assess the level and intensity of anthropogenic transformation and seasonal variation (Townsend *et al.* 1997, Aplin *et al.* 2011). In ecosystem monitoring efforts, it is crucial to obtain up-to-date data on the dietary and foraging preferences of indicator species to determine whether they persist in these transformed landscapes under changing seasonal conditions. By monitoring diet selection and trophic ecology, we can assess the effects of human practices on ecosystem health and wildlife behaviour, such as significant variation in diet selection and shifts in foraging behaviour. Changes may be indicative of food resource shortages and may lead to declining animal welfare and local species extinctions (Dickman 1999, McCarty 2001).

The diet of an animal can reveal many details about its ecology and welfare. An increasing number of studies have investigated the diets of various animal species and the factors affecting their foraging behaviour (Kerley *et al.* 1990, Robb *et al.* 2012). Historically, diet studies were mainly observational and required many hours of observational research to acquire data (Harcourt 1986, Berteaux 1998). The implementation of faecal data collection methods has reduced observation time and allowed researchers to collect samples at intermittent rates to obtain longer-term data depending on the gut-passage time of a particular species (Jones *et al.* 1979). Faecal sample collection can be challenging if the source cannot be positively identified. An important factor to consider is that certain food resources are easily broken down and digested before excretion, leaving little to no trace in the animal's faecal material (Begg and Dunlop, 1985; Churchfield and Brown, 1987). As such, some easily digestible dietary items may be underrepresented or omitted from analyses leading, to inaccuracies in the reported diet preferences of some species.

Stable isotope analysis (SIA) allows for the detection of most digested food resources as their isotope ratios are assimilated into animal tissues such as blood (recent diet), muscle (average indication of diet), bone (long-term diet), and keratinised structures, such as hair, feathers, and nails (Tieszen *et al.* 1983, Lourenço *et al.* 2015). The use of stable isotopes in dietary ecology involves the measurement of the ratio of two common elements: 1) carbon and 2) nitrogen (Tieszen *et al.* 1983, Layman *et al.* 2012). The carbon and nitrogen used by an animal to build tissue and obtain energy are derived directly from their diet. Stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) allow the assessment of dietary patterns and the relative trophic positions of individuals (Barquete *et al.* 2013, Van Den Heuvel & Midgley 2014). Carbon isotope ratios ($\delta^{13}\text{C}$) are used to determine the basal source of nutrients as $\delta^{13}\text{C}$ ratios are well-conserved in food chains, whereas nitrogen isotope ratios ($\delta^{15}\text{N}$) indicate the trophic level of individual animals (Layman *et al.* 2012, Van Den Heuvel & Midgley 2014).

Carbon isotope ratios provide data on consumed food types and show little change in the trophic positions of the species under study, whereas stable nitrogen isotope ratios change to reflect the trophic position of the animal (Miller *et al.* 2008, Caut *et al.* 2009). The carbon isotope ratio is used to identify carbon fixed by marine or terrestrial C_3 plants, and C_4 plants remaining relatively unchanged (approximately 1‰) in food webs. The nitrogen ratios of the animal become more positive (approximately 3-4‰) than those of their diets (Deniro & Epstein 1981, Peterson & Fry 1987). Consumer $\delta^{15}\text{N}$ ratios are dependent on individual diet and the

type of tissue analysed as $\delta^{15}\text{N}$ ratios can vary in different tissues (Deniro & Epstein 1981, Caut *et al.* 2009). As such, nitrogen isotope ratios are used to elucidate the trophic ecology of animals.

Tissue collection for diet studies involves the collection of blood, muscle, and bone, along with liver or kidney samples, and/or stomach contents, depending on the research questions being asked (Hobson & Quirk 2014, Rosenblatt *et al.* 2015). The tissue selected for collection and subsequent analysis determines the level of invasiveness of collection methods. This can range from drawing blood or muscle biopsies, where the animal experiences some pain and discomfort, to using organ samples such as liver tissue, or stomach contents, where the animal is euthanised. Tieszen *et al.* (1983) proposed keratinised structures such as hair or nails as a viable sample and showed that, while sample turnover rate was slow, hair was approximately 1% more enriched in ^{13}C relative to the diet and was also more enriched than tissues such as fat, brain, muscle, and liver. An approximate 3-4% increase in enrichment in ^{15}N relative to diet was recorded (Caut *et al.* 2009). An important aspect of using sample tissue as a proxy for diet content in animals is having an appropriate diet to tissue discrimination factor. These can vary according to tissue type, species, and even sex or age of individuals within a species (DeMots *et al.* 2010, McLaren *et al.* 2015). The discrimination factor denotes the amount of change in diet-related stable isotope values once they are assimilated into various tissues within the consumer's body (DeMots *et al.* 2010, McLaren *et al.* 2015).

A currently limiting factor in southern African rodent ecology is minimal research on diet behaviour of free-ranging indigenous species, and details regarding their assimilation of dietary sources into tissue types. The majority of research into small mammal diet, discrimination factors, and foraging behaviour has been conducted on northern hemisphere captive or laboratory reared species such as *Peromyscus*, *Clethrionomys*, *Apodimus*, *Myodes*, among others (Canova 1993, DeMots *et al.* 2010, Khanam *et al.* 2016). These studies require captive animals with a controlled diet, and observed feeding behaviour is primarily influenced by the chosen diet and captive environment (DeMots *et al.* 2010, Eccard & Herde 2013). This limits their applicability as comparative resources in free-ranging diet studies. By investigating the diets of free-ranging small mammals, specifically rodents, we can improve our knowledge of wild species and identify variables that influence their behaviour in the natural environment.

Because of their relatively short gestation periods and rapid generation times, small mammals such as rodents are ideal study species for monitoring environmental (Joyce *et al.* 1997, Skinner & Chimimba 2005). Rodents have a diverse dietary range, including seeds and grains, leaves and roots, and insects, with most observed interspecific differences in diet being related to body size or habitat preference (Canova & Fasola 1993). Dietary studies on small mammals have shown that food resource quality and abundance are directly linked to litter size, weaning success, and nesting survival (Gilbert & Krebs 1991, Monadjem & Perrin 2003).

Species richness and abundance correlated with food resource quality and quantity in natural landscapes. With increased human activity, both food quality and quantity can be affected in detrimental (potential loss of natural food sources) and beneficial (abundance of anthropogenic food items) ways (Massawe *et al.* 2011, Ademola *et al.* 2021). Dietary studies on rodent species allow for long-term monitoring of the extent of anthropogenic transformation on the natural environment. Continuous minimally invasive monitoring of biological indicator species such as rodents can inform on their adaptive ability, and whether they can persist in heavily transformed areas due to continued human encroachment into natural habitats. This data may serve as a proxy indicator for ecosystem health.

Researchers are relying on more ethical and sustainable research methods; therefore, sampling techniques that result in reduced invasion and stress to animals have become increasingly popular. In dietary studies, sampling now regularly involves the collection of keratinous tissues such as hair, nails, fin clips, and feathers, which are relatively easy to obtain, and cause nominal discomfort when taken (Roth & Hobson 2000, Barquete *et al.* 2013, Van Den Heuvel & Midgley 2014). Using minimally invasive sampling, the animal can be released after initial sampling and, if adequately marked, can be recaptured and resampled to determine potential dietary variation over time.

The study aimed to assess the effects of landscape transformation and seasonal variation on the diet of rodent species across the Magaliesberg Biosphere, South Africa. Stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were compared to determine any interspecific or intraspecific variation between four dominant southern African rodent species from the study area. The carbon and nitrogen isotope ratios of each species were compared across seasons (dry and wet) and landscapes (minimally disturbed to very disturbed) to investigate how the abovementioned variables impact the diet selection of rodents in the region. Plant samples were collected and

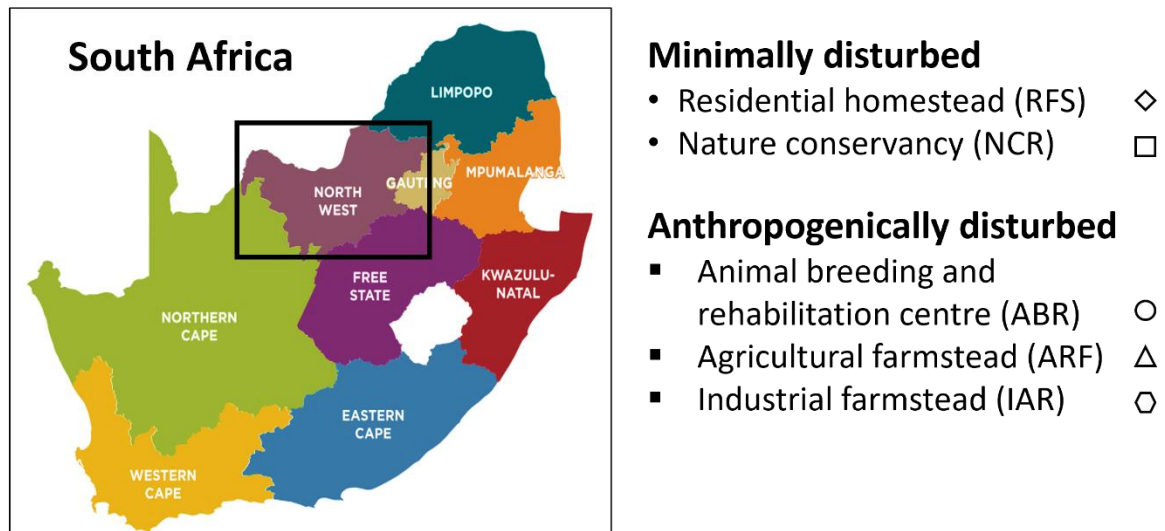
measured to determine isotopic baseline values for comparison between producers and primary consumers. This baseline plant isotope data was compared with the stable isotope ratios of the study species to estimate their carbon sources, i.e., C₃, C₄ or a mixed diet.

5.3 MATERIALS AND METHODS

5.3.1 Study area

Animals were captured at multiple locations across the Magaliesberg mountain range (25°41'00.1"S, 27°57'51.8"E), which spans the Gauteng and North West provinces of South Africa (Figure 5.1). The specific study sites were situated in the De Wildt (25°40'05.5"S, 27°55'23.1"E) and Zilkaatsnek (25°39'25.8"S, 27°56'34.9"E) areas. These sites experience hot, wet summers (from November to March) and cold, dry winters (from April to October), with the onset of the wet season and interannual rainfall varying annually (Boonzaaier & Hough 2011). The Magaliesberg Biosphere, which falls within the Savannah Biome, consists of two main vegetation types: the Gold Reef Mountain Bushveld at higher elevations and the Marikana Thornveld and Moot Plains Bushveld in lower-lying areas (Rutherford & Mucina 2006). The area is dominated by thorny *Vachellia* species and semi-open thickets, with a predominantly grassy and herbaceous understory. The region's geology consists of igneous rocks from the Rustenberg Layered Suite and the Rooiberg Groups (Rutherford & Mucina 2006).

For efficient statistical analysis, data from the five study sites were pooled into two broad categories: minimally disturbed and anthropogenically disturbed. The minimally disturbed landscape consists of a residential homestead and a nature conservancy with little to no human activities on the surrounding land. The anthropogenically disturbed landscape comprises an agricultural farmstead, an animal breeding and rehabilitation centre, and an industrial farmstead. These three sub-sites experience moderate to high levels of human practices and high human density.



North West Province – Bojanala District



Figure 5.1. Orientation map of the North West province (location denoted by a black rectangle on the map of South Africa) showing the location of the study area in the Bojanala District (denoted by a black circle) where rodents were captured between 2018 and 2021.

5.3.2 Study species

My study investigated the diet preferences of four southern African rodent species from the Muridae and Nesomyidae families (Table 5.1 and Figure 5.2).

Table 5.1. Summary of selected species, including common names, habitat, and ecology from the Magaliesberg Biosphere, North West province, South Africa (Davis 1973, Skinner & Chimimba 2005, Chimimba & Linzey 2008, Stuart & Stuart 2015, Monadjem et al. 2015).

Species	Common name	Habitat	Ecology
<i>Aethomys ineptus</i> (Thomas & Wroughton 1908)	Tete veld rat	Savannah-woodlands with abundant ground cover	Can be synanthropic Nocturnal Communal living
<i>Lemniscomys rosalia</i> (Thomas 1904)	Single-striped grass mouse	Savannahs, grasslands, and cultivated lands	Can be synanthropic Crepuscular, diurnal habits Communal living
<i>Mastomys coucha</i> (Smith 1834)	Southern multimammate mouse	Savannahs, grasslands, cultivated lands Disturbed landscapes	Synanthropic Nocturnal Communal living
<i>Micaelamys namaquensis</i> (Smith 1834)	Namaqua rock mouse	Highveld rocky grasslands and outcrops	Synanthropic Nocturnal Communal living

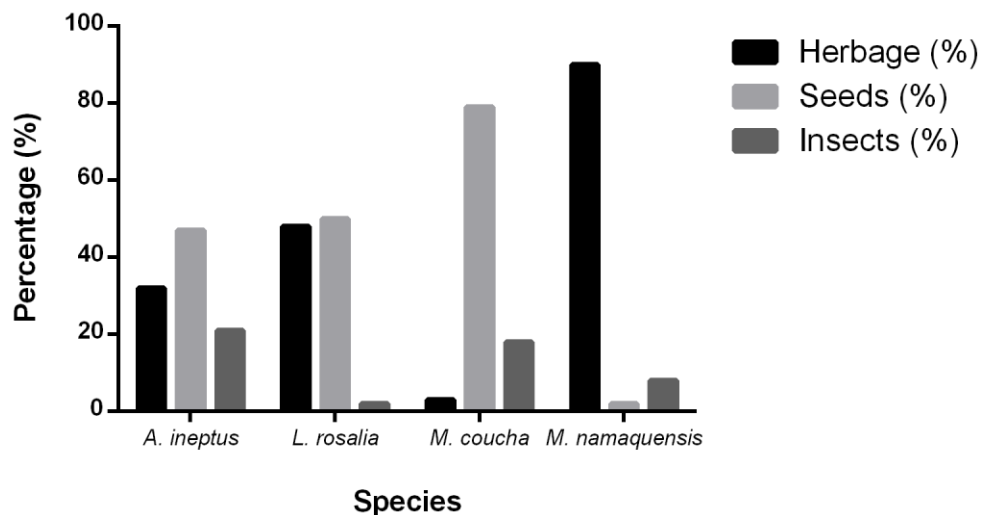


Figure 5.2. Literature-reported percentage contributions of broad food groups to the diet of four dominant indigenous rodent species (*A. ineptus*, *L. rosalia*, *M. coucha*, and *M. namaquensis*) sampled in the Magaliesberg Biosphere, North West province, South Africa (Davis 1973, Skinner & Chimimba 2005, Chimimba & Linzey 2008, Stuart & Stuart 2015, Monadjem et al. 2015).

5.3.3 Sampling protocol

Rodents were captured using 200 Enviro-Care live traps (Cangzhou Jinglong Technology Co. Ltd., Hebei Province, China) from October 2018 to January 2021. Galvanized sheet metal traps (dimensions: 255 x 78 x 80 mm) were set in 5 x 8 trapping grids with a 10-meter spacing between traps, following established protocols (Aplin *et al.* 2003, 2011). Each trap was baited with a combination of oats and peanut butter. The traps remained open continuously, both day and night, to capture diurnal and nocturnal species. Traps were checked twice daily, once in the morning between 05:30 and 09:00, and again in the afternoon between 15:00 and 17:00. The captured animals were sexed and identified to the species level (Joyce *et al.* 1997, Newbery 1999, Skinner & Chimimba 2005). Where possible, animals were aged by comparing their body mass with literature-reported masses, identifying whether females had perforated vaginas and pronounced mammary glands, and noting whether males were scrotal or not (Aplin *et al.* 2003, Skinner & Chimimba 2005, Muteka *et al.* 2006, Odhiambo *et al.* 2008). Cryptic species (*Aethomys* spp. and *Mastomys* spp.) were resolved using DNA extraction and molecular identification (Appendix A).

For stable isotope analysis, hair samples were clipped from captured individuals using scissors and collected using forceps. A 70% ethanol solution was used to clean the equipment, preventing cross-contamination. Hair samples were collected from the same location on all individuals (right side rump) and trimmed as close to the base of the follicle as possible. The animal was held upright, steady, and facing away from the trimming procedure. The animals were then released at their respective capture sites. Traps were rebaited and returned to their original positions in the trapping grid (Aplin *et al.* 2003).

Samples of the most common plants in the region were collected and analysed to provide a baseline of potential dietary items. Transect lines that coincided with traplines were walked, and, where possible, entire plant samples in and around the trapping grid were uprooted, cleaned, and labelled. Plant samples were identified to species level where possible through consultation with a field botanist (Appendix C). Collected plants were categorised into three groups: 1) Cultivated / anthropogenic species, 2) Indigenous wild species, 3) Non-native wild species. Plant isotope ratios were compared with isotope ratios obtained from rodent individuals to identify a potential baseline trophic level separation and specific diet selection in rodents across different landscapes in the dry and wet seasons.

Hair samples were stored in labelled 1.5 ml Eppendorf tubes, and plant samples were labelled, stored in clear bags and frozen at -20 °C. All samples were taken to the UP Stable Isotope Laboratory (UPSIL), Mammal Research Institute of the University of Pretoria, South Africa, for further processing. This study was approved by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (Ethics clearance number EC044-18) and complied with Section 20 of the Animal Diseases Act of South Africa, 1984 (SDAH-Epi-18111309000).

5.3.4 Sample preparation and stable isotope analysis

Following Bearhop *et al.* (2003), hair samples were placed in clean glass test tubes (5 ml), treated with a 2:1 Methanol/Chloroform (1 ml) solution, and agitated using an ultrasonic cleaner (Eins Sci Model: E-UC3-HD-D) for 10 min at room temperature. Samples were removed from the ultrasonic cleaner, and the used solvent was decanted. For particularly dirty samples, this process was repeated until the solvent remained clear. Samples were dried in an oven at 70°C for 24 h. Aliquots of hair weighing between 0.3 and 0.6 mg were placed in tin capsules that were pre-cleaned in toluene and compacted.

Plant samples were dried in an oven at 70°C for 24 h and homogenised using a pestle and mortar. For harder samples, a mechanical homogeniser (IKA MF 10 basic Wiley mill) was used. Aliquots of plant samples weighing between 0.6 and 0.9 mg were placed in tin capsules pre-cleaned in toluene and compacted.

All samples were combusted at 1020°C using an elemental analyser (Flash EA 1112 Series) coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany), housed at the UP Stable Isotope Laboratory, Mammal Research Institute, University of Pretoria.

Two laboratory running standards (Merck Gel: $\delta^{13}\text{C}$ = -20.26‰, $\delta^{15}\text{N}$ = 7.89‰, C% = 41.28, N% = 15.29) and (DL-Valine: $\delta^{13}\text{C}$ = -10.57‰, $\delta^{15}\text{N}$ = -6.15‰, C% = 55.50, N% = 11.86) and a blank sample were run after every 11 unknown samples.

The carbon and nitrogen ratios for the laboratory running Merck and DL-Valine standards were calibrated using the following primary standards: IAEA-CH-3 (Cellulose), IAEA-CH-6 (Sucrose), IAEA-CH-7 (Polyethylene foil), IAEA N-1 & IAEA N-2 (Ammonium sulphate), and IAEA NO-3 (Potassium nitrate).

All results are referenced to Vienna Pee-Dee Belemnite for carbon isotope values and to air for nitrogen isotope values. Results are expressed in delta (δ) notation using a per mille (‰) scale using the standard equation:

$$\delta X(\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} - 1]$$

where X = ^{15}N or ^{13}C and R represents $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$, respectively (Coplen 2011).

5.3.5 Statistical analyses

All statistical analyses were conducted in R using the 2020 R Studio interface (Version 2.1) (RStudio Team 2022). Statistical significance was inferred at 5% and alpha set at 0.05.

A total of 388 samples from four dominant species were analysed, with 180 collected from the untransformed landscape (106 in the dry season and 74 in the wet season) and 208 from the transformed landscape (53 in the dry season and 155 in the wet season). To determine whether sex-related differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios were statistically significant, an analysis of variance (ANOVA) was used (Ruxton & Beauchamp 2008). In the case of non-significance, female and male datasets for each species were pooled for analysis because any differences observed between female and male samples would be considered negligible (Crossey *et al.* 2020, 2021).

To determine intraspecific variation between carbon and nitrogen isotope ratios, linear regression models of best fit identified four variables (season, land use, sex, and age group). These were used to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of individuals across dry and wet seasons and, untransformed and transformed landscapes. Carbon and nitrogen isotope ratios were plotted by species (Parnell 2021).

5.4 RESULTS

An initial comparison of keratin sample collection techniques showed that hair collection is faster and minimally invasive to the animals under study. For a more detailed description of

methodologies used and stable isotope data, refer to Appendix C. A total of 22 plant samples were analysed and compared with small mammal samples (Appendix C).

Stable isotope data obtained support literature reports on the generalist feeding behaviour of the four omnivorous species under study (Figure 5.3) (Davis 1973, Skinner & Chimimba 2005, Chimimba & Linzey 2008, Stuart & Stuart 2015, Monadjem *et al.* 2015). The reported percentage food item contribution for species (summarised in Figure 5.2) shows high herbage consumption and high seed consumption in three of the four species (*A. ineptus*, *L. rosalia*, and *M. namaquensis*). All four species primarily consume C_4 plants, with *A. ineptus*, showing a proclivity for C_3 plants (Joyce *et al.* 1997, Skinner & Chimimba 2005). *Aethomys ineptus* is reported to have the most even percentage food item preference of herbage, seeds, and insects, which may explain this deviation from the other omnivores.

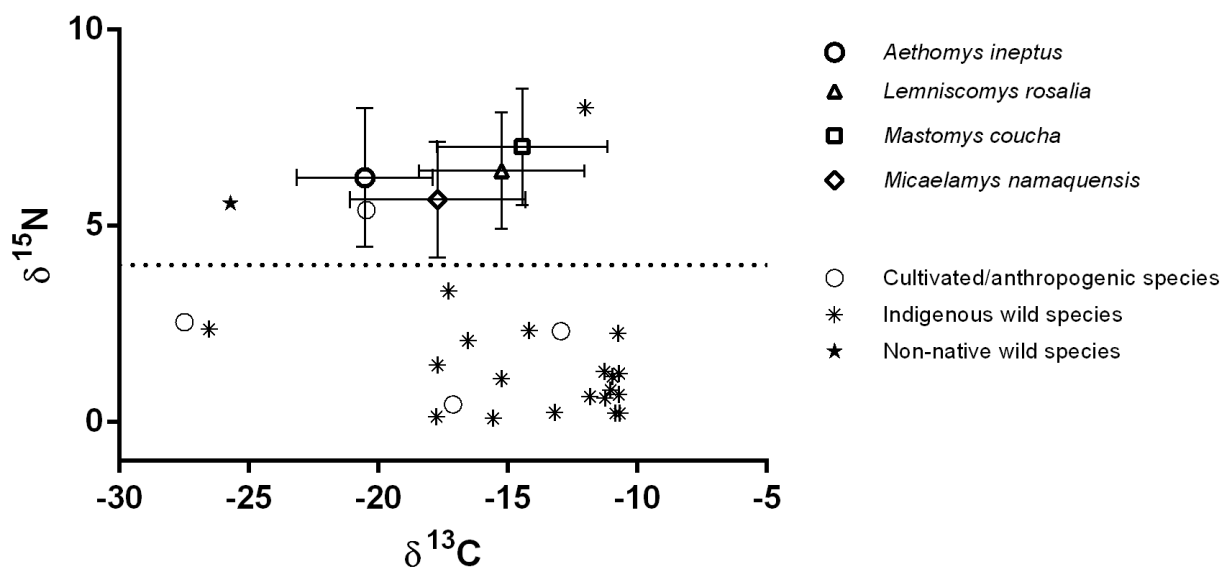


Figure 5.3. Comparison of mean ($\pm$ SD) carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of four dominant rodent species (*A. ineptus*, *L. rosalia*, *M. coucha*, and *M. namaquensis*) and plant species from the study area. The dotted line, based on nitrogen isotope values, represents a potential trophic level baseline that separates producers (plants) from primary consumers. This baseline can be dynamic because, prior to senescence, nitrogen isotope ratios in plants change throughout the growing season as plants redirect resources in response to the production of leaves, flowers, fruit and/or seeds. Additional information on the plant reference categories is provided in Appendix C.

A linear model of best fit was identified and used to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of species across dry and wet seasons, and minimally disturbed and anthropogenically disturbed landscapes:

$$\delta^{13}\text{C} / \delta^{15}\text{N} \sim \text{Season} + \text{Land use} + \text{Sex} + \text{Age group}$$

For the carbon isotope ratio: F -statistic = 20.94, $df = 9$ and 377, $p < 0.001$; Adjusted AICc = 1982.60; Adjusted R-squared = 0.32. For the nitrogen isotope ratio: F -statistic = 12.25, $df = 9$ and 377, $p < 0.001$; Adjusted AICc = 1382.45; Adjusted R-squared = 0.21.

The model above was used to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of four species across dry and wet seasons, and minimally disturbed and anthropogenically disturbed landscapes. Seasonal variation appears not to have a significant impact on the diet selection of three of the four species (*A. ineptus*, *L. rosalia*, and *M. coucha*), while landscape variation had a significant effect on *A. ineptus*, *M. coucha*, and *M. namaquensis* (Table 5.2, Figure 5.4).

Table 5.2. Summary of the best fit model for comparison of carbon and nitrogen isotope ratios across minimally disturbed and anthropogenically disturbed landscapes in the dry and wet seasons ($\delta^{13}\text{C} / \delta^{15}\text{N} \sim \text{Season} + \text{Site} + \text{Sex} + \text{Age group}$) for *A. ineptus*, *L. rosalia*, *M. coucha*, and *M. namaquensis*. Statistically significant values are in bold and indicated by *.

Variable	Df	Species (N)							
		<i>A. ineptus</i> (29)		<i>L. rosalia</i> (65)		<i>M. coucha</i> (218)		<i>M. namaquensis</i> (76)	
		P-value							
		δ ¹³ C	δ ¹⁵ N	δ ¹³ C	δ ¹⁵ N	δ ¹³ C	δ ¹⁵ N	δ ¹³ C	δ ¹⁵ N
Season	1	0.95	0.98	0.34	0.27	0.11	0.32	0.04*	<0.001*
Land use	1	0.11	0.01*	0.95	0.05	0.001*	0.12	0.05	0.001*
Sex	1	0.92	0.36	0.26	0.36	0.37	0.32	0.76	0.06
Age group	2	0.79	0.15	0.28	0.02*	<0.001*	0.001*	0.09	0.27
AICc		152.43	112.99	343.80	233.03	1101.95	786.17	405.65	240.25
Adjusted R ² value		-0.06	0.40	0.02	0.18	0.16	0.05	0.07	0.44

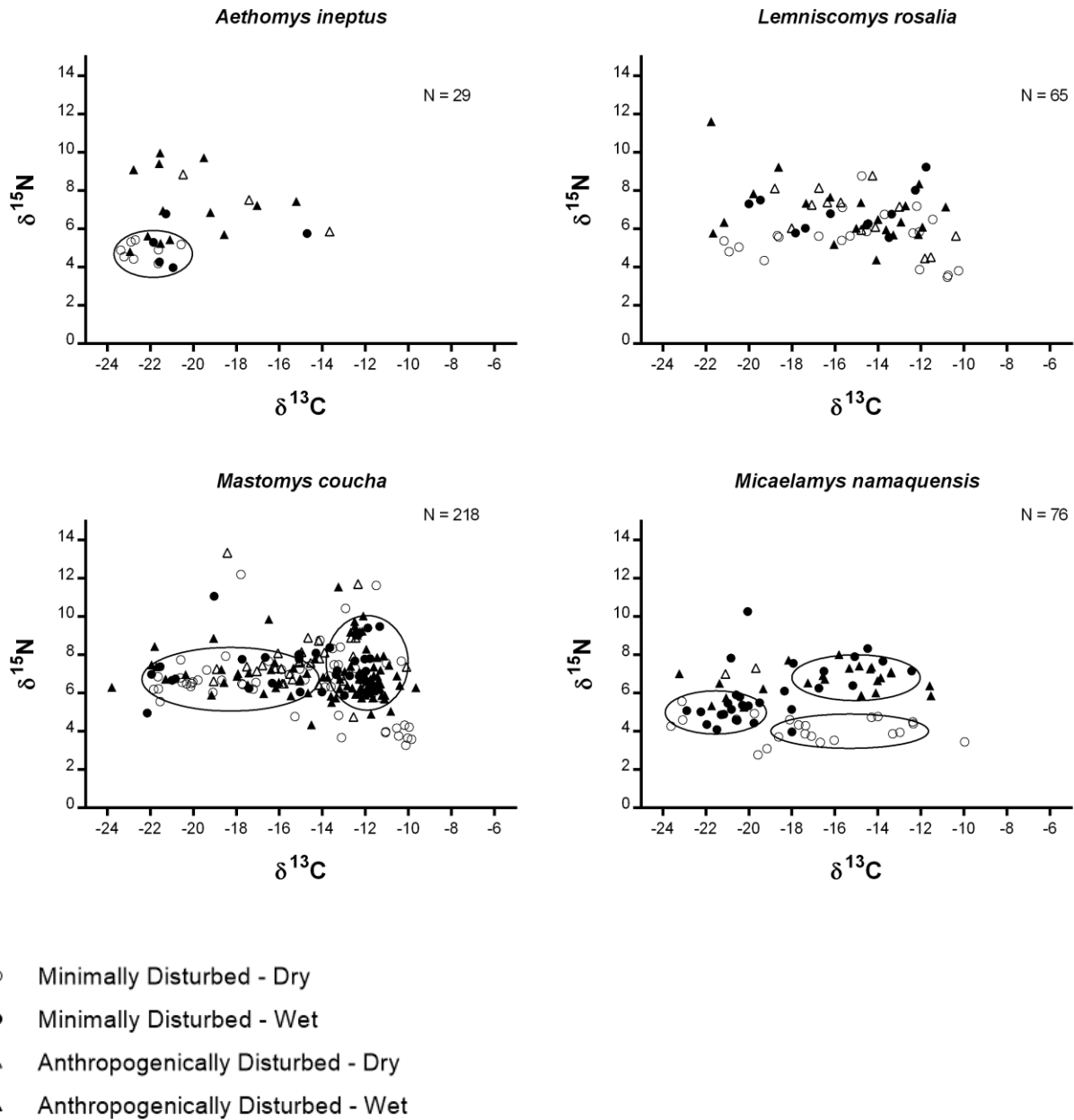


Figure 5.4. Comparisons of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios across minimally and anthropogenically disturbed landscapes in the dry and wet seasons of four dominant rodent species (*A. ineptus*, *L. rosalia*, *M. coucha*, and *M. namaquensis*) with N values representing sample sizes. Data points in ellipses were plotted in *GraphPad Prism* (Version 9.5.0) (GraphPad Software 2023) using best fit models calculated in the R Studio interface (RStudio Team 2022). These points indicate statistically significant variation between individuals captured across seasons (*M. namaquensis*) and landscapes (*A. ineptus*, *M. coucha*, and *M. namaquensis*).

5.5 DISCUSSION

My study aimed to assess the effects of landscape transformation and seasonal variation on the diet of four dominant southern African rodent species across the Magaliesberg Biosphere by comparing the hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios across dry and wet seasons and minimally disturbed and anthropogenically disturbed landscapes.

All four species are classified as omnivorous or generalist feeders with varying proportions of seeds, leaves and insects making up their diet; however, *M. coucha* is primarily a granivore, and *M. namaquensis* feeds primarily on herbage (Joyce *et al.* 1997, Skinner & Chimimba 2005). The stable isotope data from this study support the reported generalist feeding behaviour of the four species under study. Despite having varying percentages of food item contributions to their diet, all four species primarily consume C_4 plants, whereas *A. ineptus* appears to prefer C_3 plants. Literature reports (Chimimba 2001, Chimimba & Linzey 2008) show that *A. ineptus* has the most even composition of herbage, seeds, and insects relative to the other species, which may explain the varied mix of C_3 and C_4 plants in the diet (Chimimba 2001, Chimimba & Linzey 2008). The species may rely on wild indigenous grasses (C_4) and surrounding agricultural landscapes with planted crops such as maize (C_4) for seed intake.

Seasonal rainfall variation appeared to significantly affect the carbon and nitrogen isotope ratios of *M. namaquensis*. The change in isotope ratios may have resulted from changes in the nitrogen isotope composition of some plants as they transfer resources to different parts (roots to leaves to fruit and seeds) throughout the growing season. When the plant's leaves begin to senesce, it can reabsorb nitrogen into its roots (Karasov 1982, Nilsen & Strand 2008). However, this is less likely to explain the nitrogen increase observed in *M. namaquensis* because the remaining three species showed no significant variation in nitrogen isotope ratios between the dry and wet seasons. Rather, the species relies on habitats with abundant ground cover, which varies between the dry and wet seasons (Kerley *et al.* 1990, Abu Baker & Brown 2012). With this variation in vegetation cover across seasons, food selection may have been altered to ensure survival. During the wet season, individuals within the species recorded higher carbon and nitrogen isotope ratios than their dry season counterparts. Because the diet of *M. namaquensis* is primarily comprised of leaves, a shift in diet (and potentially trophic niche) between the dry and wet seasons may be more pronounced as individuals are forced to

temporarily consume more grains or insects, which can be foraged more easily than leaves during the dry season.

Animals found in anthropogenically transformed landscapes showed higher nitrogen isotope ratios (and possible trophic level shifts in three species: *A. ineptus*, *L. rosalia*, and *M. namaquensis*) than those found in minimally disturbed landscapes. This increase in nitrogen in the diet may be linked with increased proximity to human settlements and consumption of abundant human-made food items. Carbon isotope ratios varied significantly across landscapes for *M. coucha*, which has been reported as a synanthropic species since the early 1900s and, is thus likely to have adapted to anthropogenic food resources, as seen in the consistently higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios relative to the other species (Skinner & Chimimba 2005). Carbon isotope ratios did not vary significantly for the other species, highlighting their generalist diet and their ability to adjust to consuming locally available food resources influenced by environmental conditions.

For *L. rosalia*, an opportunistic omnivore whose diet is made up of an equal proportion of seeds and herbage, land transformation and seasonal variation had no significant impact on carbon and nitrogen isotope ratios. The species has been reported to occur along the fringes of agricultural landscapes and regularly consumes planted crops and other anthropogenic food resources along with its natural diet (Monadjem & Perrin 1997, 2003). With wide-ranging diet and food types available year-round, seasonal and landscape changes appear to have little impact on this generalist species.

5.6 CONCLUSION

With continuous global changes in ecosystems, climates, and resource availability, rodents are better equipped, because their relatively small size and behavioural plasticity, to persist in anthropogenically transformed landscapes (Skinner & Chimimba 2005, World Wildlife Fund 2016). The study highlights the impact of land use on rodent diets and niches, along with evidence of behavioural shifts towards favourable feeding strategies. Generalist feeders are resilient and adaptive and can easily supplement their diet when resources are in short supply. In contrast, species that rely heavily on a specific type of diet must adapt their feeding patterns or risk population declines. Generalist and omnivorous species are more likely to persist in altered landscapes and increased human encroachment, whereas specialist species may struggle

to adapt and become locally extinct. Despite the behavioural plasticity seen in generalist species, prolonged or intense dry seasons can lead to potentially detrimental survival mechanisms. Uncharacteristically dry conditions can lead to urine retention in certain species, or in cases of extreme starvation, some species may begin to consume some of their own muscle tissue or exhibit cannibalistic behaviour (Canova & Fasola 1993, Skinner & Chimimba 2005, Fox 2011, Van Der Merwe & Hellgren 2016). These physiological responses can alter their nitrogen isotope ratios, while intense dry conditions may also result in more positive plant carbon isotope ratios (Fox 2011, Van Den Heuvel & Midgley 2014). Due to the dynamic nature of stable isotopes in both plants and animals, it is important to incorporate long-term dietary research in environmental impact and ecosystem health studies.

The data collected in this study can be useful for assessing environmental impacts that result from anthropogenic activities. It can also be used when implementing land use management strategies and determining suitable locations for land development with minimal risk to natural ecosystems and the wildlife occupying them. Rehabilitation managers and policy makers can use this information to identify the most vulnerable regions and create strategies specific to the landscape. Continued monitoring can inform the success or failure of management efforts.

Further research could include the calculation of discrimination factors for southern African rodent species because they are vital biological indicators of ecosystem health. Investigating the effects of land transformation and seasonality on other behavioural variables, such as exploration and boldness, can also be used to potentially link the personalities of individuals with their diet selection. Animals that exhibit adaptive exploratory behaviour may be more likely to venture further from their home ranges to find their preferred food resources, whereas more shy individuals may be more likely to forage for and consume available food resources, regardless of preference. Specialist species may also be valuable indicator species and require more intensive investigation because they may be less adaptive and more influenced by seasonal and landscape changes in their immediate environments. As human encroachment continues, specialist species numbers may decline if they lack the behavioural plasticity needed to forage for longer periods or adapt divergently from their obligate feeding like their generalist counterparts.

CHAPTER 6: GENERAL DISCUSSION

The present study aimed to investigate the effects of land transformation and seasonal variation on the population dynamics and community structure, stress-related physiology, and diet selection of small mammal species of the Magaliesberg region, North West province, South Africa. My study found that land transformation allows for homogenisation of ecosystems in various ways: the physical environment, available vegetation, small mammal presence and abundance, and animal behaviour. Seasonal and landscape variation influences small mammal community assemblages, resulting in fluctuations in population numbers, as well as temporal and spatial differences in species richness and diversity. There is homogenisation of small mammal community structures through reduced diversity and species richness, and population numbers tend to skew towards dominant, generalist species in anthropogenically disturbed areas. Some rodent species exhibit acclimatised behaviour in response to increased human encroachment, as seen in the homogenisation of stress-related physiological responses across minimally disturbed to highly disturbed landscapes. Generalist rodent species have also shown resilience and adaptive behaviour towards consuming a variety of food resources compared to more specialist feeders. As such, these generalists are more likely to continue being the dominant species in various ecosystems as s

An interesting finding in this study was the ability of small mammals to persist in environments subjected to varying levels of anthropogenic disturbance and transformation. This suggests that some rodents demonstrate conditional adaptability in areas where anthropogenic activities that change the physical landscape occur at moderate frequencies or with adequate temporal spacing. This adaptability likely allows rodents to adjust their reproductive cycles, behaviour, and dietary preferences in response to changing environmental conditions, ensuring their survival in altered landscapes (Townsend et al. 1997, Ferreira & Van Aarde 2000).

With their notable adaptability, it is important to collect data on small mammals across various habitats to better understand their ability to persist and the mechanisms that drive this behaviour. My study includes novel components that add to the local and global knowledge of rodent species in physiological and dietary aspects. The validation of assays that can reliably detect faecal GC concentrations in six southern African species and the successful quantification of adrenocortical activity using one of these assays can be seen as a worthwhile addition to the scientific study of small mammals and their behavioural ecology. In addition,

because most research conducted on rodent diet is either under strict laboratory conditions or requires euthanasia of species under study, my use of minimally invasive methods to investigate free-ranging southern African rodent diet can provide a suitable and sustainable pilot study towards larger environmental studies that focus on human-wildlife interactions and their effects on human food security and animal habitat and niche management in future.

6.1 SUMMARY OF FINDINGS AND IMPLICATIONS

In terms of diversity and community structure, my study objectives involved assessing the effects of season and land transformation on small mammal species abundance, species richness and diversity, and species composition. I predicted that species abundance, richness and diversity would be highest during the wet season and inversely proportional to the level of transformation in a landscape. I also predicted that community composition and evenness would be most balanced during the wet season across landscapes. My study found that seasonal and land-use variation influences small mammal community assemblages, resulting in fluctuations in population numbers, as well as temporal and spatial differences in species richness. Healthy and diverse wildlife populations in a changing world may rely on an environment that experiences some level of periodic disturbance (Magurran & Henderson 2018). Although relatively undisturbed landscapes are preferable, it is possible to manage transformed landscapes to safeguard the species richness and community diversity of the region. My study aligns with the findings of Magurran and Henderson (2018) suggesting that some rodent species are more successful in environments where human activity is not constant but rather intermittent, allowing them time to adjust their foraging strategies and reproductive behaviours. This flexibility in behavioural and physiological responses may explain why some dominant rodent species are commonly found in anthropogenic ecosystems, from agricultural landscapes to urban fringes. The ability of rodents to adapt to disturbances aligns with the concept of ecological resilience, where species exhibit flexibility in coping with disturbances without undergoing significant population declines (Richmond et al. 2005, Luo et al. 2018). My research supports this view, indicating that small mammals such as rodents can thrive in disturbed habitats when the intensity and frequency of disturbances are low enough to allow for ecological recovery between events. The presence of diverse food sources and suitable shelter in these altered environments may further facilitate their persistence. Therefore, it is important to carefully manage and monitor the frequency and intensity of anthropogenic disturbance to ensure that non-dominant native populations can re-establish and stabilise after

a disturbance event and continue to do so sustainably for extended periods. Considering the ecology, behaviour, and physiology of different species, we can implement careful land-use management strategies to reduce the homogenisation of natural landscapes and potentially slow the loss of species in southern African environments that are undergoing anthropogenic transformation.

I aimed to biologically and physiologically validate a suitable EIA for the quantification of fGCMs in southern African rodent species, determine baseline and peak fGCM hormone concentrations of captive rodent species, and assess the effects of season and land transformation on fGCM concentrations of rodent species. I predicted that captured murid species would have a common EIA that shows reliable sensitivity across an interspecific range. I also predicted that stress-related hormone responses would be directly correlated with the level of disturbance and human presence in a landscape and that fGCM concentrations of rodent species would be higher during the dry season. I found that the 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA can be used to monitor fGCM concentrations in selected southern African rodent species, with the potential for use in other yet untested species. However, it is important to avoid inter-specific comparisons of fGCM concentrations as hormone metabolism mechanisms may be species-specific. The findings on landscape comparisons inform on the resilience of dominant species in a world that is continuously changing by anthropogenic means. With increasing land transformation and human encroachment into natural landscapes, continued exposure to anthropogenic disturbance and dense human populations may lead to increased habituation by these and other indigenous species, as reported by Bashaw et al. (2016). Similarly, as seen with my study, an increase in human encroachment and land transformation may lead to some species showing reduced sensitivity to anthropogenic disturbance and densely populated human settlements and may become a cause for concern. Small mammals and other species may be forced to adopt an increasingly synanthropic lifestyle, which may lead to more occurrences of human-wildlife conflicts and potential classifications of indigenous species as pests (Monadjem & Perrin 1997, 2003). Conversations with local landowners suggest that this is already an issue, and few are using environmentally friendly pest-control methods. The majority prefer to eradicate the rodents in their areas with snap-traps or poison, which has adverse effects on small mammal populations, community structures, and their associated food webs across various ecosystems. Future studies should investigate whether behavioural habituation to transformed landscapes can be seen in other

indicator species, and whether we can quantify the extent of habituation in these species and use it as a minimally invasive indicator of ecosystem health in a fast-changing world.

In terms of diet-related responses, my objectives were to investigate minimally invasive methods of monitoring the diet of rodent species and assess the effects of season and land transformation on diet preference of rodent species. I predicted that a comparison of minimally invasive methods of data collection would reveal a method that can be used for multiple southern African rodent species, and the revealed method would show that carbon and nitrogen isotope values differ seasonally across rodent species occupying differently transformed landscapes. I found that carbon and nitrogen isotope ratios obtained from hair and nail samples of various species do not differ substantially. As such, it is recommended that hair samples be used for long-term diet studies of rodents as collection of this sample type is less invasive than nail sample collection, and samples are easier to prepare and analyse. I found that, with continuous changes in ecosystems, climates, and resource availability, rodents are better equipped, due to their relatively small size, to persist in anthropogenically transformed landscapes. My study highlights the impact of land use on small mammal diet and niches, along with evidence of behavioural shifts towards favourable feeding strategies. Generalist feeders are resilient and adaptive and can easily supplement their diet when resources are in short supply, whereas species that rely heavily on a specific diet must adapt their feeding patterns or risk population declines. Generalist and omnivorous species are more likely to persist in altered landscapes and increased human encroachment, while specialist species may struggle to adapt and become locally extinct.

6.2 FUTURE DIRECTIONS AND RESEARCH NEEDS

The data collected in the study can be useful for assessing environmental impacts that result from anthropogenic activities. It can also be applied when implementing land use management strategies and determining suitable locations for land development with minimal risk to natural ecosystems and the wildlife occupying them. Rehabilitation managers and policy makers can use this information to determine the most vulnerable regions and create strategies specific to the landscape. Continued monitoring can inform on the success or failure of management efforts.

It is essential to recognise the limits of the behavioural adaptability observed in some species. Extreme or continuous disturbance, such as habitat fragmentation, pollution, or urbanisation without adequate natural refuges, may eventually overwhelm these species' capacity to adapt. Future research should investigate the thresholds at which these adaptations no longer suffice and explore long-term consequences of ongoing environmental transformation on rodent populations. Future studies should also investigate whether behavioural habituation to transformed landscapes can be seen in other indicator species, and whether we can quantify the extent of habituation in these species and use it as a minimally invasive indicator of ecosystem health in a fast-changing world. Continued research that investigates adrenocortical activity in southern African rodent species should, ideally, avoid using Oxoetiocholanolone I and Cortisol assays due to their presence in trace amounts in rodent species of interest. The validated 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA can now be used to monitor fGCM concentrations in six southern African rodent species and future research may extend that list. This has various implications for small mammal research as researchers conducting captive studies can reliably quantify the impact of their experiments on their study animals and, where necessary, adjust their protocols to improve their ethical compliance. Some southern African murids can also be used as biological indicators of ecosystem health by monitoring their stress-related physiological responses to various anthropogenic disturbances and land transformations in a minimally invasive way.

In terms of diet, future research can include the calculation of discrimination factors for southern African rodent species, as these are vital biological indicators of ecosystem health. Additionally, further research can investigate the effects of land transformation and seasonality on other behavioural variables, such as exploration and boldness, as these may be used to link personalities of individuals with their diet selection. Animals that exhibit adaptive exploratory behaviour may be more likely to venture further from their home ranges to find their preferred food resources, while more shy individuals may be more likely to forage for and consume available food resources, regardless of preference. Further studies should also investigate specialist species more intensively, as they may prove less adaptive and more influenced by seasonal and landscape changes in their immediate environments. As human encroachment continues, specialist species numbers may decline if they lack the behavioural plasticity needed to forage for longer or adapt away from their obligate feeding ways like their generalist counterparts.

6.3 CONCLUSIONS

My study shows that dominant biological indicator species provide relevant information on ecosystem health and levels of disturbance in terms of their population dynamics, as their presence and relative abundance can indicate anthropogenic disturbance and disturbance frequency and intensity. However, behavioural responses of dominant indicator species are less informative as these species are more adaptive and resilient to environmental changes. These species are most abundant primarily because of the advantages associated with their behavioural plasticity. Some species can adapt to environmental variation by adjusting their physiological and behavioural responses over time. The dominant species in my study are classified as generalist feeders which may explain their ability to adapt to variable food resource types and availability throughout the year. Using rodents as biological indicators of ecosystem health in future research can be effective; however, research questions and objectives should highlight the appropriate variables (population, behavioural or physiological) and study species (abundant or scarce) to be investigated and incorporate temporal activity patterns as these may influence some observed behavioural and physiological responses. This will ensure that the data collected are accurate and can be used to make accurate inferences on wildlife responses to our changing world.

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APPENDIX A: CHAPTER 2 SUPPLEMENTARY MATERIALS

A.1 MOLECULAR METHODOLOGY

A.1.1 DNA extraction and molecular identification of cryptic *Aethomys* and *Mastomys* species

Genomic DNA extractions were performed using ear clip samples collected from cryptic *Aethomys* and *Mastomys* species. These samples were collected from live individuals, and immediately frozen and stored at -20°C, before transporting them to the University of Pretoria (UP) for processing and analysis. Following this, DNA extraction was performed at the UP molecular laboratory using the Roche® High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Germany), using the extraction protocol prescribed for mammalian tissue. Briefly, all samples underwent an overnight Proteinase K digestion at 56°C, the DNA was bound to a cartridge and was eluted in a final volume of 200 µL subsequent to a number of inhibitor removal and wash steps. Touchdown PCR reactions were performed in a final volume of 40 µl, which contained 1.5 units of DreamTaq (Thermo Fisher Scientific, USA), 2.5 mM dNTPs (Fermentas), 3 µl of template DNA and 0.4 µM of each of the murid rodent primers L14724 (TGAYATGAAAAAYCATCGTTG) and H15915 (CATTTTCAGGTTTACAAGAC). The aforementioned primers, which bind in regions flanking the cytochrome *b* (cyt *b*) gene, amplify a mitochondrial genome region of ~1,2 kbp. Thermal cycling was performed on an ABI 3500 (Applied Biosystems) PCR machine using the following conditions: an initial denaturation step at 96°C for 10s, annealing for 30s, and elongation at 70°C for 1 minute, with a final elongation step at 70°C for 1 minute. Following Bastos et al. [37], the annealing temperature for the first two cycles was 50°C, 48°C for the next three cycles, and 46°C for the final 35 cycles.

A.1.2 Nucleotide sequencing

The PCR products were separated using a 1.5% agarose gel electrophoresis and run against a 1kb DNA molecular weight marker (Thermo Scientific Fisher, United States) to estimate product sizes. Agarose gels were stained with Goldview and were visualized using UV irradiation. PCR products of the expected size were purified directly from the tube using the Roche PCR High Pure Product Purification Kit (Roche Diagnostics GmbH, Germany), according to the manufacturer's instructions. Cycle sequencing was performed using the BigDye v.3.1 terminator cycle-sequencing kit (Perkin Elmer, USA), and the external PCR

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primer L14724 at an annealing temperature of 48°C. Unincorporated primers, dNTPs and fluorescently-labelled ddNTPs were removed by sodium-acetate precipitation, and the purified products were then submitted to the Core Sanger sequencing facility of the University of Pretoria.

A.1.3 Sequence analysis

Sequence chromatographs were visualized and edited in the Chromas program embedded in MEGA 7 and used to generate contiguous sequences (contigs) [38]. Sequences were aligned using ClustalW, and each sequence was then used in a nucleotide blast search against the Genbank database (<https://www.ncbi.nlm.nih.gov/blast>) to identify the most closely related sequences (>98% identity) linked to published peer-reviewed papers, in the database. Reference and outgroup sequences were identified for the cryptic species that were sourced from the Genbank database and added to the final alignment for phylogenetic analysis.

Table A1. Gene regions, accession numbers, and publication sources obtained for reference sequences used in the construction of the Maximum Likelihood Tree.

Species	Gene region	Accession number	Publication
<i>Aethomys ineptus</i>	<i>cytb</i>	MW537307	Krásová et al. (2021)
<i>Aethomys ineptus</i>	<i>cytb</i>	MW537308	Krásová et al. (2021)
<i>Mastomys coucha</i>	<i>cytb</i>	MT524718	McDonough & Sotero-Caio (2019)
<i>Mastomys coucha</i>	<i>cytb</i>	MT524719	Eiseb et al. (2021)
<i>Micaelamys namaquensis</i>	<i>cytb</i>	GQ471959	Russo et al. (2010)
<i>Micaelamys namaquensis</i>	<i>cytb</i>	GQ472095	Russo et al. (2010)

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APPENDIX B: CHAPTER 3 SUPPLEMENTARY MATERIALS

Table B1. Summary of intra- and inter- assay variances with associated dry faecal mass sensitivities calculated for six Rodentia species (*A. ineptus*, *L. rosalia*, *M. coucha*, *M. namaquensis*, *O. irroratus*, and *S. pratensis*) captured in the Magaliesberg Biosphere, North West province, South Africa after experiencing a potential biological stressor (handling and temporary removal from enclosure), and a physiological stressor (ACTH administration) for each of the five enzyme immunoassays tested. Associated relative variation of slopes of the respective parallelism trendlines are also presented (%).

	Intra-assay variance (%)	Inter-assay variance (%)	Dry faecal mass sensitivity (ng/g DW)	<i>Aethomys ineptus</i>	<i>Lemniscomys rosalia</i>	<i>Mastomys coucha</i>	<i>Micaelamys namaquensis</i>	<i>Otomys irroratus</i>	<i>Steatomys pratensis</i>
				Parallelism					
5α-pregnane-3β,11β,21-triol-20-one (5α)	3.15 and 3.61	10.27 and 10.40	2.4	<3%	<3%	<5%	<4%	<1%	N/A
Oxoetiocholanolone I (Oxo I)	4.09 and 4.81	13.02 and 14.16	1.2	<3%	<5%			<5%	No parallelism
Oxoetiocholanolone II (Oxo II)	4.94 and 6.84	8.71 and 13.46	0.75	<1%			<5%	<5%	<5%
Corticosterone (CC)	4.57 and 5.74	8.34 and 11.72	2.4						
Cortisol (C)	4.42 and 6.24	11.46 and 13.29	0.75		<5%				<1%

Table B2. Baseline, biological and physiological peak faecal glucocorticoid metabolite (fGCM) concentrations, percentage increase and hours post-injection when peak fGCM concentrations were attained for six Rodentia species one female and one male *A. ineptus* individual in the Magaliesberg Biosphere, North West province, South Africa after perceiving three potential stressors (handling, weighing, and enclosure cleaning), and adrenocorticotrophic hormone (ACTH) administration for each of the five enzyme immunoassays tested. Highlighted cells indicate a validation with a 2-fold increase.

EIAs as follows: 5 α - 5 α -pregnane-3 β ,11 β ,21-triol-20-one; Oxo I - Oxoetiocholanolone I; Oxo II - Oxoetiocholanolone II; CC – Corticosterone; C – Cortisol.

<i>Aethomys ineptus</i>										
Sex	Female					Male				
EIA	5 α	Oxo I	Oxo II	CC	C	5 α	Oxo I	Oxo II	CC	C
Baseline (ug/g DW)	4.61	0.05	0.76	1.49	0.11	3.31	0.13	0.89	0.85	0.05
Biological peak (ug/g DW)	9.81	0.16	1.06	2.73	0.29	5.23	0.17	1.41	0.98	0.07
Increase %	112.62	202.18	39.72	83.47	158.37	58.12	33.40	58.46	14.60	28.61
Fold increase	2.13	3.02	1.40	1.83	2.58	1.58	1.33	1.58	1.15	1.29
Physiological peak (ug/g DW)	10.90	0.11	1.26	2.29	0.11	8.52	0.34	2.60	1.54	0.08

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Increase %	136.25	98.54	65.09	53.92	-1.89	157.52	168.17	192.68	81.00	62.60
Fold increase	2.36	1.99	1.65	1.54	0.98	2.58	2.68	2.93	1.81	1.63
Sample number post-injection	2	2	2	2	2	7	7	7	7	7
Hours post defecation	5	5	5	5	5	17	17	17	17	17

Table B3. Baseline, biological and physiological peak faecal glucocorticoid metabolite (fGCM) concentrations, percentage increase and hours post-injection when peak fGCM concentrations were attained for six Rodentia species one female and one male *L. rosalia* individual in the Magaliesberg Biosphere, North West province, South Africa after perceiving three potential stressors (handling, weighing, and enclosure cleaning), and adrenocorticotrophic hormone (ACTH) administration for each of the five enzyme immunoassays tested. Highlighted cells indicate a validation with a 2-fold increase.

EIAs as follows: 5 α - 5 α -pregnane-3 β ,11 β ,21-triol-20-one; Oxo I - Oxoetiocholanolone I; Oxo II - Oxoetiocholanolone II; CC – Corticosterone; C – Cortisol.

<i>Lemniscomys rosalia</i>										
Sex	Female					Male				
EIA	5 α	Oxo I	Oxo II	CC	C	5 α	Oxo I	Oxo II	CC	C
Baseline (ug/g DW)	1.12	0.01	0.30	0.38	0.04	0.49	0.02	0.23	0.28	0.01
Biological peak (ug/g DW)	1.68	0.04	0.50	0.68	0.12	1.34	0.04	0.36	0.50	0.02
Increase %	49.90	349.66	67.91	77.59	186.22	174.39	112.53	59.36	77.42	28.35
Fold increase	1.50	4.50	1.68	1.78	2.86	2.74	2.13	1.59	1.77	1.28
Physiological peak (ug/g DW)	1.52	0.04	0.51	0.66	0.09	0.51	0.03	0.26	0.42	0.10
Increase %	35.39	311.62	69.35	71.34	112.82	5.11	73.52	12.53	48.33	727.26
Fold increase	1.35	4.12	1.69	1.71	2.13	1.05	1.74	1.13	1.48	8.27
Sample number post-injection	7	7	7	5	5	2	12	5	2	4
Hours post defecation	20	20	20	8	8	4	23	8	4	7

Table B4. Baseline, biological and physiological peak faecal glucocorticoid metabolite (fGCM) concentrations, percentage increase and hours post-injection when peak fGCM concentrations were attained for two male *M. coucha* individuals in the Magaliesberg Biosphere, North West province, South Africa after perceiving three potential stressors (handling, weighing, and enclosure cleaning), and adrenocorticotrophic hormone (ACTH) administration for each of the five enzyme immunoassays tested. Highlighted cells indicate a validation with a 2-fold increase.

EIAs as follows: 5 α - 5 α -pregnane-3 β ,11 β ,21-triol-20-one; Oxo I - Oxoetiocholanolone I; Oxo II - Oxoetiocholanolone II; CC – Corticosterone; C – Cortisol.

<i>Mastomys coucha</i>										
Sex	Male 1					Male 2				
EIA	5 α	Oxo I	Oxo II	CC	C	5 α	Oxo I	Oxo II	CC	C
Baseline (ug/g DW)	3.43	0.02	0.44	0.80	0.05	3.54	0.03	0.39	1.15	0.07
Biological peak (ug/g DW)	13.49	0.03	0.90	2.51	0.13	5.09	0.05	0.70	1.50	0.08
Increase %	292.98	88.35	105.06	215.61	142.74	43.71	43.84	79.84	29.61	16.61
Fold increase	3.93	1.88	2.05	3.16	2.43	1.44	1.44	1.80	1.30	1.17

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Physiological peak (ug/g DW)	6.14	0.02	0.73	1.49	0.09	4.96	0.04	0.72	1.66	0.09
Increase %	78.92	50.82	66.25	87.10	62.72	40.05	10.29	84.66	43.75	36.28
Fold increase	1.79	1.51	1.66	1.87	1.63	1.40	1.10	1.85	1.44	1.36
Sample number post-injection	5	5	5	5	5	2	2	2	2	2
Hours post defecation	10	10	10	10	10	5	5	5	5	5

Table B5. Baseline, biological and physiological peak faecal glucocorticoid metabolite (fGCM) concentrations, percentage increase and hours post-injection when peak fGCM concentrations were attained for two female *M. namaquensis* individuals in the Magaliesberg Biosphere, North West province, South Africa after perceiving three potential stressors (handling, weighing, and enclosure cleaning), and adrenocorticotrophic hormone (ACTH) administration for each of the five enzyme immunoassays tested. Highlighted cells indicate a validation with a 2-fold increase.

EIAs as follows: 5 α - 5 α -pregnane-3 β ,11 β ,21-triol-20-one; Oxo I - Oxoetiocholanolone I; Oxo II - Oxoetiocholanolone II; CC – Corticosterone; C – Cortisol.

<i>Micaelamys namaquensis</i>										
Sex	Female 1					Female 2				
EIA	5 α	Oxo I	Oxo II	CC	C	5 α	Oxo I	Oxo II	CC	C
Baseline (ug/g DW)	1.91	0.01	0.49	1.21	0.07	2.18	0.01	0.40	1.38	0.07
Biological peak (ug/g DW)	5.05	0.02	0.84	1.84	0.11	4.70	0.02	1.08	2.42	0.20
Increase %	164.43	63.98	71.15	51.46	54.34	115.31	21.92	173.12	75.73	193.78
Fold increase	2.64	1.64	1.71	1.51	1.54	2.15	1.22	2.73	1.76	2.94
Physiological peak (ug/g DW)	4.84	0.02	0.60	1.93	0.13	5.22	0.02	0.79	1.55	0.16
Increase %	153.31	97.81	22.02	58.76	83.68	139.24	19.22	100.47	12.10	130.65
Fold increase	2.53	1.98	1.22	1.59	1.84	2.39	1.19	2.00	1.12	2.31
Sample number post-injection	4	4	4	4	4	1	2	1	4	3
Hours post defecation	5	5	5	5	5	3	5	3	8	7

Table B6. Baseline, biological and physiological peak faecal glucocorticoid metabolite (fGCM) concentrations, percentage increase and hours post-injection when peak fGCM concentrations were attained for one female and one male *O. irroratus* individual in the Magaliesberg Biosphere, North West province, South Africa after perceiving three potential stressors (handling, weighing, and enclosure cleaning), and adrenocorticotrophic hormone (ACTH) administration for each of the five enzyme immunoassays tested. Highlighted cells indicate a validation with a 2-fold increase.

EIAs as follows: 5 α - 5 α -pregnane-3 β ,11 β ,21-triol-20-one; Oxo I - Oxoetiocholanolone I; Oxo II - Oxoetiocholanolone II; CC – Corticosterone; C – Cortisol.

<i>Otomys irroratus</i>										
Sex	Female					Male				
EIA	5 α	Oxo I	Oxo II	CC	C	5 α	Oxo I	Oxo II	CC	C
Baseline (ug/g DW)	2.46	0.05	0.76	0.69	0.07	1.55	0.03	0.89	0.97	0.07
Biological peak (ug/g DW)	5.30	0.32	2.40	1.41	0.24	6.16	0.09	2.42	1.58	0.29
Increase %	115.26	533.19	216.98	103.46	262.40	297.89	146.58	171.92	63.34	309.45

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Fold increase	2.15	6.33	3.17	2.03	3.62	3.98	2.47	2.72	1.63	4.09
Physiological peak (ug/g DW)	5.21	0.08	1.88	1.03	0.11	3.38	0.03	1.94	1.09	0.14
Increase %	111.73	50.91	148.84	49.45	61.82	118.13	-7.12	118.22	12.24	96.06
Fold increase	2.12	1.51	2.49	1.49	1.62	2.18	0.93	2.18	1.12	1.96
Sample number post-injection	3	3	3	3	3	3	2	1	2	2
Hours post defecation	1	1	1	1	1	5	3	2	3	3

Table B7. Baseline, biological and physiological peak faecal glucocorticoid metabolite (fGCM) concentrations, percentage increase and hours post-injection when peak fGCM concentrations were attained for one female and one male *S. pratensis* individual in the Magaliesberg Biosphere, North West province, South Africa after perceiving three potential stressors (handling, weighing, and enclosure cleaning), and adrenocorticotrophic hormone (ACTH) administration for each of the five enzyme immunoassays tested. Highlighted cells indicate a validation with a 2-fold increase.

EIAs as follows: 5 α - 5 α -pregnane-3 β ,11 β ,21-triol-20-one; Oxo I - Oxoetiocholanolone I; Oxo II - Oxoetiocholanolone II; CC – Corticosterone; C – Cortisol.

<i>Steatomys pratensis</i>										
Sex	Female					Male				
EIA	5 α	Oxo I	Oxo II	CC	C	5 α	Oxo I	Oxo II	CC	C
Baseline (ug/g DW)	2.06	0.06	0.98	0.51	0.06	1.73	0.05	0.85	0.44	0.06
Biological peak (ug/g DW)	4.92	0.06	2.07	0.72	0.09	5.16	0.34	1.21	1.33	0.13
Increase %	138.95	-0.56	110.61	41.51	36.33	197.44	616.15	42.27	206.06	120.68
Fold increase	2.39	0.99	2.11	1.42	1.36	2.97	7.16	1.42	3.06	2.21
Physiological peak (ug/g DW)	4.98	0.06	2.92	1.10	0.13	3.89	0.11	1.58	0.98	0.26
Increase %	141.91	2.52	197.38	116.32	106.06	124.31	138.66	85.79	124.31	343.31
Fold increase	2.42	1.03	2.97	2.16	2.06	2.24	2.39	1.86	2.24	4.43
Sample number post-injection	1	1	1	1	1	4	4	4	3	4
Hours post defecation	9	9	9	9	9	12	12	12	9	12

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APPENDIX C: CHAPTER 5 SUPPLEMENTARY MATERIALS

C.1 KERATIN SAMPLE COMPARISON OF HAIR AND NAIL CARBON AND NITROGEN ISOTOPE RATIOS

We compared collection protocols, preparation, and stable isotope analysis of two keratin sample materials, namely hair and nail clippings, from nine southern African rodent species. With this comparison, I aimed to determine whether the sample types have similar carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), and which sample type is the more animal- and researcher-friendly option for long-term diet studies.

In terms of hair sample collection, the animal could be easily handled whilst in an upright position with its feet on the workbench, and its face temporarily covered by a steadying hand. This led to minimised movement and reduced visible signs of distress in the animal. Nail clippings were more difficult to collect as rodents were hyper-sensitive to the handling of their appendages. Nail sample collection proved more invasive as it required the use of a disinfectant due to the small size of the nails and potential nicking of blood vessels. Overall, hair sample collection was the simpler and more animal friendly method.

Statistical analyses showed that hair and nail samples have negligible variation in terms of carbon and nitrogen isotope ratios for six of the nine species. There was a high correlation between carbon and nitrogen isotope ratios of the species tested. In instances of high correlation for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios of a single species, hair and nails can be used interchangeably for long-term diet research using stable isotope analysis. However, for a more ethically compliant study, hair was used in my rodent diet research.

C.2 STABLE ISOTOPE DATA

Table C.2.1 Summary of stable isotope data for *Aethomys ineptus*. Abbreviations in the *Site* column denote anthropogenic disturbance (AD) and minimal disturbance (MD).

Season	Site	Sex	Age group	Mass (g)	N peak	$\delta^{15}\text{N}$	% N	C peak	$\delta^{13}\text{C}$	% C	Cover N
Dry	AD	Male	Adult	0.514	35.83	5.85	12.43%	42.82	-13.66	37.79%	3.55
Dry	AD	Male	Adult	0.54	43.53	7.52	14.37%	51.12	-17.4	42.94%	3.49
Dry	AD	Male	Adult	0.536	42.63	8.86	14.18%	48.76	-20.47	41.27%	3.4
Dry	MD	Female	Adult	0.635	52.23	4.41	14.74%	59.59	-22.77	43.38%	3.43

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Dry	MD	Male	Adult	0.527	42.26	4.55	14.36%	49.98	-23.22	43.84%	3.56
Dry	MD	Female	Adult	0.555	46.4	4.88	14.98%	53.37	-23.38	44.45%	3.46
Dry	MD	Male	Sub-adult	0.634	52.74	4.9	14.90%	60.6	-21.61	44.19%	3.46
Dry	MD	Female	Adult	0.596	50.31	5.18	15.12%	55.25	-20.56	42.85%	3.31
Dry	MD	Male	Sub-adult	0.577	46.15	5.31	14.33%	54.08	-22.89	43.33%	3.53
Dry	MD	Male	Adult	0.643	49.97	5.42	13.92%	57.65	-22.69	41.44%	3.47
Dry	MD	Female	Sub-adult	0.505	27.72	4.18	14.30%	20.41	-21.62	44.06%	3.6
Wet	AD	Male	Sub-adult	0.527	38.14	5.43	14.81%	67.56	-21.08	44.69%	3.52
Wet	AD	Male	Adult	0.537	38.63	5.71	14.72%	68.73	-18.56	44.62%	3.54
Wet	AD	Male	Adult	0.515	38.35	6.95	15.14%	63.85	-21.41	43.09%	3.32
Wet	AD	Male	Adult	0.579	72.99	4.81	14.54%	186.49	-22.94	40.87%	3.28
Wet	AD	Male	Adult	0.564	72.01	6.86	14.86%	190.12	-19.21	42.78%	3.36
Wet	AD	Female	Adult	0.575	70.17	7.22	14.36%	188.35	-17.03	41.57%	3.38
Wet	AD	Female	Adult	0.577	72.2	9.41	14.59%	199.82	-21.59	43.95%	3.51
Wet	AD	Male	Juvenile	0.543	68.86	9.71	14.67%	189.99	-19.5	44.40%	3.53
Wet	AD	Male	Adult	0.572	72.2	9.97	14.66%	196.3	-21.54	43.55%	3.47
Wet	AD	Female	Adult	0.597	72.03	5.24	13.91%	187.73	-21.52	39.91%	3.35
Wet	AD	Male	Adult	0.556	70.24	5.62	14.55%	180.98	-22.12	41.31%	3.31
Wet	AD	Female	Adult	0.532	65.37	7.43	14.16%	187.01	-15.21	44.61%	3.68
Wet	MD	Male	Adult	0.576	39.13	3.97	13.81%	68.3	-20.93	41.21%	3.48
Wet	MD	Male	Adult	0.581	41.3	4.27	14.45%	73.59	-21.57	44.02%	3.55
Wet	MD	Male	Sub-adult	0.576	40	5.75	14.12%	71.2	-14.7	42.96%	3.55
Wet	MD	Female	Adult	0.536	37.08	6.78	14.15%	66.58	-21.27	43.31%	3.57
Wet	MD	Male	Adult	0.567	72.47	5.29	14.75%	191.51	-21.85	42.86%	3.39
Wet	AD	Female	Adult	0.543	65.03	9.09	14.08%	181.3	-22.77	42.37%	3.51

Table C.2.2 Summary of stable isotope data for *Lemniscomys rosalia*. Abbreviations in the *Site* column denote anthropogenic disturbance (AD) and minimal disturbance (MD).

Season	Site	Sex	Age group	Mass (g)	N peak	$\delta^{15}\text{N}$	% N	C peak	$\delta^{13}\text{C}$	% C	Cover N
Dry	AD	Male	Adult	0.507	38.76	8.78	13.70%	45.16	-14.25	41.18%	3.51
Dry	AD	Male	Adult	0.578	37.37	5.94	11.58%	43.99	-14.77	35.18%	3.54
Dry	AD	Male	Adult	0.367	27.08	6.09	13.15%	31.55	-14.11	39.00%	3.46
Dry	AD	Male	Adult	0.63	50.75	4.46	14.36%	59.00	-11.82	42.49%	3.45
Dry	AD	Female	Sub-adult	0.519	37.51	4.51	12.88%	43.48	-11.54	38.01%	3.44
Dry	AD	Female	Sub-adult	0.567	43.26	5.64	13.60%	51.19	-10.38	40.96%	3.51
Dry	AD	Male	Adult	0.508	40.94	6.03	14.36%	47.54	-18.01	42.45%	3.45
Dry	AD	Female	Adult	0.545	44.10	7.26	14.42%	50.32	-17.07	41.89%	3.39
Dry	AD	Male	Adult	0.542	41.93	7.38	13.79%	48.63	-15.73	40.70%	3.44

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Dry	AD	Female	Sub-adult	0.558	43.38	7.39	13.86%	50.93	-16.34	41.40%	3.49
Dry	AD	Female	Adult	0.557	31.68	8.11	10.14%	36.30	-18.79	29.56%	3.40
Dry	AD	Male	Adult	0.676	53.89	8.15	14.21%	63.12	-16.73	42.36%	3.48
Dry	AD	Female	Sub-adult	0.562	31.49	7.16	14.59%	23.52	-13.00	45.62%	3.65
Dry	MD	Male	Adult	0.571	49.73	5.40	15.60%	48.64	-15.68	39.37%	2.94
Dry	MD	Male	Adult	0.594	46.73	5.62	14.09%	53.90	-16.75	41.95%	3.47
Dry	MD	Male	Adult	0.539	42.77	5.63	14.14%	48.88	-15.30	41.14%	3.39
Dry	MD	Male	Adult	0.545	42.82	5.64	14.01%	49.61	-18.67	41.29%	3.44
Dry	MD	Female	Sub-adult	0.62	52.68	5.78	15.22%	61.54	-12.37	45.88%	3.52
Dry	MD	Male	Adult	0.541	37.06	5.84	12.27%	43.28	-12.08	36.98%	3.52
Dry	MD	Male	Adult	0.561	48.14	5.86	15.30%	52.85	-14.53	42.74%	3.26
Dry	MD	Male	Adult	0.315	26.83	6.50	15.26%	28.73	-11.45	42.16%	3.22
Dry	MD	Male	Adult	0.363	30.83	6.76	15.22%	36.31	-13.70	46.23%	3.54
Dry	MD	Male	Adult	0.534	43.72	7.12	14.67%	50.88	-15.65	44.04%	3.50
Dry	MD	Male	Adult	0.582	40.48	7.18	12.46%	46.63	-12.20	37.03%	3.47
Dry	MD	Female	Adult	0.679	53.37	8.77	14.08%	62.20	-14.76	42.34%	3.51
Dry	MD	Male	Sub-adult	0.678	56.99	3.47	15.06%	62.15	-10.78	42.37%	3.28
Dry	MD	Female	Sub-adult	0.51	35.48	3.56	12.46%	42.38	-10.73	38.41%	3.60
Dry	MD	Female	Adult	0.555	40.78	3.80	13.10%	48.21	-10.25	39.41%	3.51
Dry	MD	Male	Adult	0.519	38.55	3.87	13.24%	45.04	-12.06	39.37%	3.47
Dry	MD	Male	Sub-adult	0.581	48.46	4.34	14.94%	48.40	-19.28	38.50%	3.01
Dry	MD	Male	Adult	0.574	41.03	4.81	12.81%	45.93	-20.92	36.99%	3.37
Dry	MD	Female	Sub-adult	0.646	54.06	5.04	14.99%	61.24	-20.46	43.82%	3.41
Dry	MD	Female	Adult	0.686	55.58	5.37	14.51%	65.81	-21.15	44.34%	3.56
Dry	MD	Male	Sub-adult	0.488	39.75	5.57	14.41%	43.92	-18.61	42.34%	3.43
Wet	AD	Female	Juvenile	0.57	39.53	8.35	14.19%	73.62	-12.08	45.03%	3.70
Wet	AD	Male	Adult	0.529	66.57	6.34	14.48%	173.23	-21.15	41.56%	3.35
Wet	AD	Male	Adult	0.537	65.01	7.65	13.90%	180.66	-16.21	42.69%	3.58
Wet	AD	Female	Adult	0.564	67.32	7.85	13.90%	187.01	-19.78	42.08%	3.53
Wet	AD	Male	Adult	0.576	64.66	9.23	13.12%	175.88	-18.62	38.75%	3.45
Wet	AD	Female	Adult	0.569	71.68	11.61	14.73%	189.98	-21.75	42.37%	3.36
Wet	AD	Male	Adult	0.554	37.21	5.69	13.74%	68.49	-13.29	43.10%	3.66
Wet	AD	Female	Adult	0.584	35.08	6.09	12.29%	61.83	-11.92	36.91%	3.50
Wet	AD	Female	Sub-adult	0.589	46.02	6.35	15.99%	76.38	-12.92	45.21%	3.30
Wet	AD	Female	Sub-adult	0.494	34.66	7.14	14.26%	62.09	-10.85	43.68%	3.57
Wet	AD	Female	Adult	0.507	37.70	7.20	15.12%	67.02	-12.72	45.94%	3.55
Wet	AD	Female	Adult	0.518	36.98	7.37	14.61%	62.74	-14.78	42.23%	3.37
Wet	AD	Male	Sub-adult	0.541	32.83	5.18	12.34%	59.30	-16.04	38.10%	3.60
Wet	AD	Female	Adult	0.604	43.78	5.70	14.74%	74.87	-12.12	43.08%	3.41
Wet	AD	Female	Sub-adult	0.55	38.94	5.96	14.49%	73.73	-13.61	46.74%	3.76

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Wet	AD	Male	Sub-adult	0.57	40.26	7.33	14.45%	76.87	-17.34	47.02%	3.80
Wet	AD	Male	Adult	0.585	72.30	4.37	14.40%	197.44	-14.07	42.83%	3.47
Wet	AD	Male	Adult	0.545	63.84	5.77	13.62%	177.62	-21.66	41.36%	3.54
Wet	AD	Female	Adult	0.523	64.82	6.03	14.44%	176.51	-15.00	42.83%	3.46
Wet	AD	Female	Adult	0.538	64.90	6.20	13.97%	172.82	-14.61	40.76%	3.40
Wet	AD	Female	Adult	0.59	73.04	6.50	14.32%	201.82	-14.00	43.41%	3.54
Wet	MD	Male	Adult	0.645	43.07	5.78	13.57%	75.18	-17.83	40.51%	3.48
Wet	MD	Male	Adult	0.569	28.62	6.03	10.29%	49.40	-17.37	30.27%	3.43
Wet	MD	Female	Adult	0.542	37.82	6.80	14.19%	65.72	-16.21	42.14%	3.47
Wet	MD	Female	Adult	0.533	67.31	5.54	14.58%	181.94	-13.49	43.32%	3.47
Wet	MD	Male	Adult	0.575	72.52	6.27	14.70%	200.74	-14.45	44.30%	3.52
Wet	MD	Female	Adult	0.564	70.41	6.77	14.49%	187.28	-13.36	42.14%	3.39
Wet	MD	Male	Adult	0.574	75.40	7.31	15.30%	194.89	-20.00	43.09%	3.29
Wet	MD	Female	Adult	0.554	67.20	7.51	14.14%	184.09	-19.47	42.17%	3.48
Wet	MD	Female	Juvenile	0.52	64.64	8.02	14.34%	180.40	-12.26	44.02%	3.58
Wet	MD	Male	Adult	0.539	65.79	9.24	14.21%	182.46	-11.76	42.96%	3.53

Table C.2.3 Summary of stable isotope data for *Mastomys coucha*. Abbreviations in the *Site* column denote anthropogenic disturbance (AD) and minimal disturbance (MD).

Season	Site	Sex	Age group	Mass (g)	N peak	$\delta^{15}\text{N}$	% N	C peak	$\delta^{13}\text{C}$	% C	Cover N
Dry	AD	Male	Adult	0.629	50.67	8.08	14.43%	59.87	-16.06	43.99%	3.56
Dry	AD	Female	Sub-adult	0.638	51.54	8.89	14.40%	60.88	-12.66	43.29%	3.51
Dry	AD	Male	Sub-adult	0.604	49.35	13.34	14.64%	58.76	-18.42	44.97%	3.58
Dry	AD	Female	Sub-adult	0.516	42.25	6.32	14.59%	50.09	-12.05	44.04%	3.52
Dry	AD	Male	Sub-adult	0.508	41.37	6.42	14.52%	48.37	-11.95	43.20%	3.47
Dry	AD	Male	Sub-adult	0.601	47.33	6.43	14.04%	57.13	-14.17	43.13%	3.58
Dry	AD	Male	Sub-adult	0.552	42.35	6.49	13.68%	51.43	-15.79	42.27%	3.61
Dry	AD	Male	Adult	0.572	41.13	6.62	12.82%	50.86	-19.05	40.34%	3.67
Dry	AD	Male	Sub-adult	0.521	40.80	6.64	13.96%	49.77	-13.00	43.34%	3.62
Dry	AD	Female	Sub-adult	0.519	40.75	6.71	14.00%	49.13	-15.38	42.95%	3.58
Dry	AD	Female	Sub-adult	0.559	44.01	6.77	14.04%	50.22	-12.22	40.76%	3.39
Dry	AD	Female	Sub-adult	0.548	43.99	6.92	14.31%	52.50	-13.11	43.47%	3.54
Dry	AD	Male	Sub-adult	0.563	41.35	7.00	13.09%	50.64	-15.51	40.81%	3.64
Dry	AD	Male	Sub-adult	0.522	41.80	7.05	14.35%	50.49	-12.27	44.71%	3.64
Dry	AD	Male	Sub-adult	0.563	43.90	7.28	13.97%	53.51	-15.91	43.93%	3.67
Dry	AD	Male	Adult	0.507	38.47	7.30	13.53%	46.73	-16.18	41.81%	3.61
Dry	AD	Male	Adult	0.556	41.51	7.36	13.31%	51.57	-10.09	42.08%	3.69

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Dry	AD	Male	Sub-adult	0.358	28.89	7.60	14.46%	33.99	-15.08	43.89%	3.54
Dry	AD	Male	Sub-adult	0.54	42.89	8.12	14.16%	51.18	-13.89	43.00%	3.54
Dry	AD	Male	Sub-adult	0.519	38.08	8.74	13.15%	45.15	-14.16	40.21%	3.57
Dry	AD	Female	Adult	0.571	47.15	8.89	14.72%	55.14	-12.45	43.81%	3.47
Dry	AD	Male	Juvenile	0.515	43.27	11.70	14.98%	49.17	-12.34	43.31%	3.37
Dry	AD	Male	Sub-adult	0.584	31.83	9.17	14.19%	23.81	-12.37	44.43%	3.65
Dry	AD	Male	Adult	0.56	44.95	4.74	14.31%	53.50	-12.55	43.34%	3.53
Dry	AD	Male	Adult	0.504	40.37	7.15	14.28%	48.40	-17.04	43.57%	3.56
Dry	AD	Female	Adult	0.578	46.24	7.26	14.26%	55.10	-18.90	43.25%	3.54
Dry	AD	Male	Adult	0.556	43.96	7.38	14.09%	53.63	-17.51	43.76%	3.62
Dry	AD	Male	Sub-adult	0.536	37.84	7.58	12.59%	45.44	-14.54	38.46%	3.56
Dry	AD	Male	Adult	0.512	40.49	7.64	14.10%	48.42	-15.05	42.90%	3.55
Dry	AD	Male	Adult	0.539	40.89	7.81	13.52%	49.29	-14.13	41.48%	3.58
Dry	AD	Male	Adult	0.523	39.89	7.92	13.60%	47.91	-12.56	41.56%	3.57
Dry	AD	Female	Sub-adult	0.517	40.70	8.15	14.03%	49.29	-14.96	43.25%	3.60
Dry	AD	Female	Sub-adult	0.597	45.93	8.90	13.71%	56.37	-14.66	42.84%	3.64
Dry	AD	Male	Sub-adult	0.514	26.50	6.49	13.43%	19.36	-15.86	41.05%	3.57
Dry	AD	Male	Sub-adult	0.527	25.33	7.44	12.52%	19.28	-16.79	39.88%	3.72
Dry	MD	Male	Sub-adult	0.513	39.52	5.55	13.73%	47.98	-21.54	42.43%	3.61
Dry	MD	Male	Juvenile	0.516	41.48	5.77	14.40%	45.60	-11.34	40.85%	3.31
Dry	MD	Female	Adult	0.517	41.69	6.04	14.38%	48.14	-19.08	42.24%	3.43
Dry	MD	Male	Sub-adult	0.639	49.94	6.20	14.00%	60.08	-21.84	43.46%	3.62
Dry	MD	Male	Adult	0.586	46.98	6.20	14.29%	54.89	-17.21	42.50%	3.47
Dry	MD	Female	Sub-adult	0.573	43.53	6.21	13.54%	53.55	-21.61	42.40%	3.65
Dry	MD	Female	Sub-adult	0.527	41.11	6.33	13.90%	49.22	-13.60	42.37%	3.55
Dry	MD	Female	Sub-adult	0.531	40.91	6.34	13.80%	48.39	-20.12	42.13%	3.56
Dry	MD	Female	Adult	0.581	48.18	6.45	14.78%	55.37	-17.69	43.23%	3.41
Dry	MD	Female	Adult	0.516	39.87	6.49	13.77%	46.92	-20.31	41.25%	3.49
Dry	MD	Male	Sub-adult	0.547	43.35	6.51	14.13%	52.31	-20.08	43.39%	3.58
Dry	MD	Female	Sub-adult	0.631	50.49	6.55	14.34%	59.78	-20.52	43.79%	3.56
Dry	MD	Male	Sub-adult	0.516	36.51	6.58	12.67%	43.55	-20.02	39.01%	3.59
Dry	MD	Female	Sub-adult	0.529	41.94	6.67	14.13%	49.92	-18.67	42.81%	3.53
Dry	MD	Female	Sub-adult	0.532	40.60	6.68	13.60%	49.16	-19.79	41.92%	3.60
Dry	MD	Male	Sub-adult	0.547	46.89	6.69	15.36%	55.86	-14.82	47.20%	3.59
Dry	MD	Male	Sub-adult	0.647	52.41	6.79	14.51%	61.60	-20.61	44.01%	3.54
Dry	MD	Male	Sub-adult	0.576	45.67	6.86	14.13%	55.23	-21.64	43.50%	3.59

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Dry	MD	Female	Sub-adult	0.681	55.76	6.90	14.67%	64.01	-13.68	43.45%	3.46
Dry	MD	NA	Sub-adult	0.565	44.24	7.20	13.96%	53.16	-19.37	42.69%	3.57
Dry	MD	Female	Sub-adult	0.552	45.08	7.23	14.56%	51.09	-15.91	41.99%	3.37
Dry	MD	Male	Sub-adult	0.617	48.93	7.25	14.21%	57.81	-15.05	43.31%	3.56
Dry	MD	Female	Sub-adult	0.579	41.82	7.29	12.88%	50.02	-12.47	39.19%	3.55
Dry	MD	Male	Adult	0.579	48.02	7.42	14.79%	54.83	-14.62	42.96%	3.39
Dry	MD	Female	Sub-adult	0.587	48.03	7.56	14.59%	56.19	-11.02	43.43%	3.47
Dry	MD	Female	Adult	0.558	42.51	7.74	13.65%	50.49	-20.58	41.82%	3.58
Dry	MD	Male	Sub-adult	0.505	39.73	8.33	14.09%	47.16	-13.53	43.17%	3.57
Dry	MD	Female	Adult	0.588	46.40	8.41	14.07%	56.80	-13.16	43.82%	3.63
Dry	MD	Male	Sub-adult	0.507	40.44	8.77	14.22%	48.36	-14.11	43.27%	3.55
Dry	MD	Male	Sub-adult	0.652	52.07	10.43	14.31%	63.38	-12.91	44.93%	3.66
Dry	MD	Male	Sub-adult	0.504	40.25	11.64	14.31%	48.01	-11.49	44.04%	3.59
Dry	MD	Male	Sub-adult	0.514	39.58	12.21	13.73%	48.64	-17.78	42.93%	3.65
Dry	MD	Female	Adult	0.536	27.85	3.67	13.53%	21.10	-13.11	42.91%	3.70
Dry	MD	Male	Sub-adult	0.517	27.52	4.77	13.86%	20.37	-15.28	42.94%	3.61
Dry	MD	Male	Sub-adult	0.502	27.14	6.31	14.08%	20.31	-13.87	44.11%	3.66
Dry	MD	Male	Sub-adult	0.564	30.67	6.49	14.16%	23.14	-17.74	44.73%	3.69
Dry	MD	Female	Sub-adult	0.59	26.52	6.49	11.71%	20.01	-20.12	36.97%	3.68
Dry	MD	Female	Sub-adult	0.507	26.64	6.56	13.68%	19.96	-17.09	42.91%	3.66
Dry	MD	Male	Sub-adult	0.546	28.76	6.97	13.72%	21.90	-15.54	43.71%	3.72
Dry	MD	Male	Adult	0.565	29.68	7.01	13.68%	22.67	-12.11	43.74%	3.73
Dry	MD	Male	Sub-adult	0.571	30.86	7.48	14.07%	23.16	-13.25	44.21%	3.66
Dry	MD	Female	Sub-adult	0.593	31.25	7.48	13.72%	24.22	-13.45	44.51%	3.78
Dry	MD	Female	Sub-adult	0.581	31.01	7.67	13.90%	22.93	-16.49	43.02%	3.61
Dry	MD	Female	Sub-adult	0.535	28.87	7.67	14.05%	21.75	-10.33	44.31%	3.68
Dry	MD	Female	Sub-adult	0.582	46.89	3.27	14.36%	55.45	-10.11	43.22%	3.51
Dry	MD	Female	Sub-adult	0.585	47.25	3.58	14.47%	56.49	-9.84	44.63%	3.60
Dry	MD	Male	Sub-adult	0.637	51.13	3.65	14.38%	61.77	-10.02	44.82%	3.64
Dry	MD	Male	Sub-adult	0.507	41.90	3.76	14.73%	50.16	-10.45	44.88%	3.55
Dry	MD	Female	Sub-adult	0.523	41.75	4.22	14.23%	50.34	-9.96	43.67%	3.58
Dry	MD	Female	Sub-adult	0.551	42.68	4.32	13.88%	51.45	-10.17	43.17%	3.63
Dry	MD	Male	Sub-adult	0.554	29.37	3.93	13.81%	21.91	-11.07	43.11%	3.64
Dry	MD	Female	Sub-adult	0.565	30.13	3.98	13.89%	22.52	-11.05	43.45%	3.65

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Dry	MD	Male	Sub-adult	0.535	29.21	4.17	14.22%	21.66	-10.54	44.13%	3.62
Dry	MD	Female	Juvenile	0.564	30.04	4.84	13.87%	22.94	-13.24	44.34%	3.73
Dry	MD	Female	Sub-adult	0.501	31.01	7.93	16.12%	23.61	-18.49	51.37%	3.72
Wet	AD	Female	Sub-adult	0.576	40.44	4.90	14.37%	74.05	-11.72	44.82%	3.64
Wet	AD	Male	Sub-adult	0.593	41.05	6.73	14.16%	76.10	-21.29	44.74%	3.69
Wet	AD	Male	Adult	0.597	42.34	6.97	14.42%	73.95	-20.35	43.05%	3.48
Wet	AD	Male	Sub-adult	0.593	41.26	7.38	14.14%	72.87	-21.71	42.71%	3.52
Wet	AD	Male	Adult	0.519	38.20	8.87	15.06%	59.68	-19.05	40.09%	3.11
Wet	AD	Female	Adult	0.512	34.39	9.86	13.74%	61.68	-16.48	42.00%	3.57
Wet	AD	Male	Juvenile	0.626	42.86	11.56	13.92%	80.73	-13.25	44.82%	3.76
Wet	AD	Female	Adult	0.522	63.46	5.74	14.12%	168.98	-13.37	41.08%	3.40
Wet	AD	Female	Adult	0.545	68.56	6.89	14.65%	183.97	-17.96	42.84%	3.41
Wet	AD	Female	Adult	0.579	69.99	9.75	14.08%	195.38	-12.49	42.82%	3.55
Wet	AD	Male	Sub-adult	0.501	33.56	5.51	13.71%	69.83	-13.57	48.59%	4.14
Wet	AD	Male	Sub-adult	0.524	34.20	5.69	13.35%	63.70	-11.11	42.38%	3.70
Wet	AD	Male	Sub-adult	0.547	39.57	5.74	14.80%	74.04	-12.18	47.19%	3.72
Wet	AD	Male	Sub-adult	0.544	39.48	5.74	14.75%	68.02	-11.65	43.46%	3.44
Wet	AD	Female	Sub-adult	0.51	34.14	5.84	13.70%	71.57	-13.59	48.93%	4.17
Wet	AD	Female	Adult	0.589	39.67	5.87	13.78%	73.66	-11.24	43.60%	3.69
Wet	AD	Female	Sub-adult	0.539	39.33	5.88	14.84%	66.51	-11.09	42.89%	3.37
Wet	AD	Male	Sub-adult	0.56	41.09	5.89	14.92%	68.56	-12.00	42.55%	3.33
Wet	AD	Female	Sub-adult	0.589	42.93	5.92	14.91%	78.05	-11.12	46.20%	3.61
Wet	AD	Male	Juvenile	0.541	38.93	5.95	14.72%	70.84	-12.45	45.65%	3.62
Wet	AD	Male	Sub-adult	0.514	35.30	6.00	13.96%	64.18	-11.73	43.39%	3.63
Wet	AD	Male	Sub-adult	0.525	36.85	6.03	14.27%	65.25	-12.32	43.19%	3.53
Wet	AD	Female	Sub-adult	0.537	37.02	6.12	14.01%	65.54	-11.96	42.42%	3.53
Wet	AD	Male	Sub-adult	0.638	44.48	6.13	14.17%	81.53	-11.60	44.41%	3.66
Wet	AD	Male	Sub-adult	0.544	36.20	6.16	13.53%	64.09	-11.93	40.94%	3.53
Wet	AD	Male	Sub-adult	0.509	33.68	6.20	13.45%	62.09	-11.47	42.40%	3.68
Wet	AD	Male	Sub-adult	0.595	41.74	6.22	14.35%	78.22	-11.30	45.83%	3.73
Wet	AD	Male	Sub-adult	0.56	38.31	6.23	14.00%	72.82	-13.19	45.34%	3.78
Wet	AD	Female	Sub-adult	0.622	43.62	6.27	14.25%	79.47	-11.62	44.40%	3.63
Wet	AD	Male	Sub-adult	0.598	42.10	6.28	14.31%	75.41	-9.65	43.82%	3.57
Wet	AD	Male	Sub-adult	0.527	37.41	6.28	14.43%	65.83	-12.21	43.41%	3.51
Wet	AD	Male	Adult	0.546	38.35	6.30	14.28%	68.23	-11.32	43.43%	3.55

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Wet	AD	Female	Sub-adult	0.533	35.24	6.34	13.44%	63.26	-11.49	41.25%	3.58
Wet	AD	Male	Sub-adult	0.596	41.86	6.41	14.37%	74.83	-10.39	43.77%	3.55
Wet	AD	Male	Sub-adult	0.523	37.85	6.41	14.81%	69.46	-11.57	46.30%	3.65
Wet	AD	Male	Sub-adult	0.58	40.92	6.41	14.44%	75.65	-11.44	45.48%	3.67
Wet	AD	Female	Sub-adult	0.505	35.58	6.45	14.42%	66.03	-11.85	45.59%	3.69
Wet	AD	Female	Sub-adult	0.621	43.52	6.45	14.25%	78.98	-11.61	44.20%	3.62
Wet	AD	Female	Sub-adult	0.594	42.86	6.47	14.76%	79.57	-11.46	46.70%	3.69
Wet	AD	Female	Sub-adult	0.563	39.86	6.48	14.39%	70.17	-11.29	43.32%	3.51
Wet	AD	Female	Sub-adult	0.559	39.10	6.53	14.31%	72.97	-11.76	45.51%	3.71
Wet	AD	Male	Sub-adult	0.593	37.87	6.54	12.98%	68.32	-11.36	40.04%	3.60
Wet	AD	Female	Juvenile	0.551	36.84	6.70	13.68%	66.96	-12.10	42.37%	3.61
Wet	AD	Male	Sub-adult	0.524	37.00	6.72	14.45%	70.68	-13.17	47.03%	3.80
Wet	AD	Male	Adult	0.544	38.26	6.73	14.30%	68.98	-12.28	44.07%	3.60
Wet	AD	Female	Sub-adult	0.526	37.52	6.74	14.60%	69.77	-11.76	46.25%	3.70
Wet	AD	Female	Juvenile	0.526	36.15	6.78	14.06%	68.09	-12.27	45.13%	3.74
Wet	AD	Male	Sub-adult	0.534	38.22	6.85	14.55%	68.47	-13.96	44.56%	3.57
Wet	AD	Male	Sub-adult	0.567	39.24	6.88	14.07%	71.80	-11.43	44.01%	3.65
Wet	AD	Male	Sub-adult	0.599	40.71	6.91	13.82%	73.02	-10.53	42.37%	3.58
Wet	AD	Male	Sub-adult	0.589	42.88	6.92	14.80%	72.07	-11.60	42.52%	3.35
Wet	AD	Male	Adult	0.568	39.80	7.06	14.24%	65.91	-17.42	40.33%	3.30
Wet	AD	Male	Sub-adult	0.589	41.43	7.11	14.39%	78.01	-12.27	46.17%	3.74
Wet	AD	Male	Sub-adult	0.589	40.90	7.21	14.12%	75.76	-11.29	44.70%	3.69
Wet	AD	Female	Adult	0.509	36.84	7.22	14.81%	66.99	-16.38	45.89%	3.61
Wet	AD	Male	Sub-adult	0.554	39.03	7.36	14.41%	72.41	-11.60	45.57%	3.69
Wet	AD	Male	Juvenile	0.541	36.57	7.39	13.83%	71.78	-12.68	46.26%	3.90
Wet	AD	Male	Sub-adult	0.555	38.32	7.45	14.04%	70.23	-10.90	43.98%	3.66
Wet	AD	Male	Sub-adult	0.589	40.96	7.87	14.14%	72.83	-11.45	42.97%	3.55
Wet	AD	Female	Sub-adult	0.551	37.54	7.95	13.85%	67.87	-11.15	42.81%	3.61
Wet	AD	Male	Adult	0.566	70.99	4.34	14.48%	193.82	-14.51	43.46%	3.50
Wet	AD	Female	Adult	0.595	72.97	5.86	14.41%	196.88	-15.46	41.99%	3.40
Wet	AD	Male	Adult	0.595	74.10	6.01	14.66%	181.96	-14.67	38.81%	3.09
Wet	AD	Male	Adult	0.564	69.87	6.16	14.50%	187.37	-12.18	42.16%	3.39
Wet	AD	Female	Adult	0.53	66.54	6.31	14.48%	178.67	-23.79	42.78%	3.45
Wet	AD	Female	Adult	0.583	86.65	6.32	17.10%	187.25	-11.65	40.10%	2.74
Wet	AD	Male	Adult	0.597	76.99	6.82	15.17%	187.10	-13.75	39.77%	3.06
Wet	AD	Male	Adult	0.506	62.03	7.02	14.31%	165.78	-17.92	41.58%	3.39

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Wet	AD	Male	Adult	0.578	70.40	7.03	14.13%	197.39	-14.72	43.34%	3.58
Wet	AD	Male	Adult	0.558	67.68	9.03	14.21%	188.75	-12.36	42.93%	3.52
Wet	AD	Male	Sub-adult	0.543	67.01	9.05	14.30%	192.66	-12.41	45.02%	3.67
Wet	AD	Female	Juvenile	0.551	65.75	9.20	13.94%	187.68	-12.69	43.22%	3.62
Wet	AD	Male	Juvenile	0.572	68.79	9.22	14.08%	193.21	-12.26	42.87%	3.55
Wet	AD	Female	Juvenile	0.568	68.51	9.24	14.11%	193.80	-12.13	43.30%	3.58
Wet	AD	Female	Juvenile	0.536	65.46	9.25	14.27%	183.39	-12.41	43.42%	3.55
Wet	AD	Female	Sub-adult	0.541	67.11	9.32	14.40%	190.35	-12.44	44.65%	3.62
Wet	AD	Male	Adult	0.591	40.92	5.04	14.17%	74.25	-10.82	43.80%	3.61
Wet	AD	Male	Sub-adult	0.564	39.19	5.89	14.12%	71.44	-19.16	44.02%	3.64
Wet	AD	Male	Sub-adult	0.572	40.62	5.97	14.53%	77.34	-16.72	47.14%	3.79
Wet	AD	Female	Sub-adult	0.589	42.35	6.27	14.71%	79.40	-12.60	47.00%	3.73
Wet	AD	Male	Juvenile	0.582	47.40	6.28	16.67%	69.04	-16.16	41.36%	2.90
Wet	AD	Male	Sub-adult	0.507	35.90	6.39	14.39%	64.25	-12.84	44.04%	3.57
Wet	AD	Female	Sub-adult	0.516	35.11	6.57	13.92%	72.41	-16.02	48.92%	4.10
Wet	AD	Male	Sub-adult	0.554	37.35	6.62	13.70%	69.82	-15.06	43.80%	3.73
Wet	AD	Male	Sub-adult	0.514	35.86	6.63	14.27%	65.99	-11.93	44.76%	3.66
Wet	AD	Female	Sub-adult	0.513	36.54	7.13	14.57%	71.33	-12.96	48.47%	3.88
Wet	AD	Male	Sub-adult	0.512	34.14	7.30	13.56%	63.49	-15.34	43.10%	3.71
Wet	AD	Male	Adult	0.563	42.42	7.60	15.32%	69.65	-16.21	42.99%	3.27
Wet	AD	Male	Sub-adult	0.51	34.71	7.78	13.84%	64.83	-14.34	44.18%	3.72
Wet	AD	Male	Sub-adult	0.557	37.09	8.29	13.63%	69.97	-11.95	43.80%	3.75
Wet	AD	Female	Adult	0.542	39.72	9.51	15.00%	70.61	-12.49	45.42%	3.53
Wet	AD	Male	Adult	0.593	71.28	6.43	13.82%	203.07	-17.46	43.46%	3.67
Wet	MD	Male	Adult	0.587	40.31	5.88	14.05%	73.45	-12.97	43.62%	3.62
Wet	MD	Female	Sub-adult	0.578	41.09	6.52	14.45%	74.72	-16.33	44.93%	3.63
Wet	MD	Male	Sub-adult	0.511	36.17	6.67	14.48%	66.58	-12.01	45.42%	3.66
Wet	MD	Female	Adult	0.538	38.13	6.74	14.50%	67.52	-20.82	43.76%	3.52
Wet	MD	Female	Sub-adult	0.533	37.22	6.90	14.29%	68.60	-12.29	44.87%	3.66
Wet	MD	Male	Sub-adult	0.554	41.46	6.91	15.31%	75.67	-12.73	47.62%	3.63
Wet	MD	Male	Sub-adult	0.583	43.69	6.97	15.23%	72.57	-13.37	43.26%	3.31
Wet	MD	Female	Sub-adult	0.52	38.95	7.00	15.33%	69.06	-12.07	46.30%	3.52
Wet	MD	Female	Sub-adult	0.576	41.20	7.14	14.63%	76.52	-11.97	46.32%	3.69
Wet	MD	Female	Sub-adult	0.595	42.71	7.19	14.69%	81.40	-13.30	47.69%	3.79
Wet	MD	Female	Juvenile	0.558	41.72	7.78	15.30%	76.16	-15.07	47.58%	3.63
Wet	MD	Male	Juvenile	0.56	40.37	7.79	14.75%	74.43	-12.03	46.34%	3.67

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Wet	MD	Male	Sub-adult	0.519	35.06	7.81	13.73%	63.74	-11.78	42.68%	3.63
Wet	MD	Male	Juvenile	0.585	41.71	8.00	14.59%	77.82	-15.09	46.38%	3.71
Wet	MD	Female	Juvenile	0.56	40.22	8.03	14.70%	75.46	-15.06	46.98%	3.73
Wet	MD	Male	Juvenile	0.556	38.18	8.09	14.05%	71.58	-14.29	44.89%	3.73
Wet	MD	Male	Sub-adult	0.59	39.92	8.38	13.84%	73.61	-13.65	43.50%	3.67
Wet	MD	Male	Juvenile	0.521	36.22	9.41	14.13%	65.64	-11.89	43.79%	3.61
Wet	MD	Male	Sub-adult	0.559	39.35	9.49	14.31%	71.76	-11.33	44.61%	3.64
Wet	MD	Male	Adult	0.578	72.59	6.06	14.77%	188.67	-14.01	41.42%	3.27
Wet	MD	Female	Adult	0.511	63.46	6.06	14.12%	172.56	-15.03	42.16%	3.48
Wet	MD	Female	Adult	0.509	64.09	6.25	14.42%	172.25	-17.41	42.94%	3.47
Wet	MD	Male	Adult	0.57	70.83	6.66	14.27%	195.40	-20.98	43.50%	3.56
Wet	MD	Female	Adult	0.517	64.98	6.98	14.45%	172.16	-21.94	42.26%	3.41
Wet	MD	Male	Adult	0.55	66.20	7.38	14.12%	179.00	-21.54	41.30%	3.41
Wet	MD	Female	Adult	0.528	64.45	7.68	14.23%	177.60	-12.49	42.68%	3.50
Wet	MD	Female	Adult	0.517	62.36	7.76	14.12%	167.57	-17.73	41.13%	3.40
Wet	MD	Male	Adult	0.566	68.61	7.86	14.16%	182.13	-16.65	40.83%	3.37
Wet	MD	Male	Adult	0.527	33.08	11.07	7.05%	88.16	-19.03	21.23%	3.52
Wet	MD	Male	Sub-adult	0.572	40.49	4.95	14.49%	77.10	-22.14	47.00%	3.79
Wet	AD	Female	Adult	0.551	64.16	6.56	13.69%	178.60	-18.57	41.13%	3.51
Wet	AD	Male	Adult	0.522	61.25	7.46	13.74%	173.59	-21.94	42.20%	3.58
Wet	AD	Male	Adult	0.544	66.61	8.45	14.41%	179.99	-21.79	41.99%	3.40
Wet	AD	Male	Adult	0.538	63.75	6.75	13.90%	170.85	-15.03	40.30%	3.38
Wet	AD	Male	Adult	0.575	70.99	7.23	14.56%	189.84	-18.69	41.90%	3.36
Wet	AD	Female	Adult	0.597	72.63	10.05	14.36%	201.72	-12.10	42.88%	3.48

Table C.2.4 Summary of stable isotope data for *Micaelamys namaquensis*. Abbreviations in the *Site* column denote anthropogenic disturbance (AD) and minimal disturbance (MD).

Season	Site	Sex	Age group	Mass (g)	N peak	$\delta^{15}\text{N}$	% N	C peak	$\delta^{13}\text{C}$	% C	Cover N
Dry	AD	Female	Adult	0.592	48.59	7.00	14.70%	56.43	-21.08	44.06%	3.50
Dry	AD	Female	Adult	0.606	50.25	7.31	14.78%	58.98	-19.67	44.16%	3.49
Dry	MD	Female	Adult	0.555	38.88	2.77	12.49%	43.04	-19.57	35.18%	3.29
Dry	MD	Male	Adult	0.551	42.33	3.09	13.76%	48.27	-19.15	40.50%	3.43
Dry	MD	Male	Adult	0.523	43.44	3.42	14.88%	48.87	-16.68	43.19%	3.39
Dry	MD	Male	Adult	0.61	47.34	3.45	13.83%	57.31	-9.96	42.62%	3.59
Dry	MD	Male	Adult	0.616	51.57	3.53	14.92%	57.48	-16.03	42.33%	3.31
Dry	MD	Male	Adult	0.581	46.52	3.71	14.34%	54.61	-18.61	43.45%	3.53
Dry	MD	Female	Sub-adult	0.514	43.77	3.74	15.18%	48.66	-17.09	42.95%	3.30
Dry	MD	Male	Adult	0.591	48.02	3.86	14.48%	52.27	-13.30	40.12%	3.23
Dry	MD	Male	Adult	0.545	43.51	3.87	14.30%	47.78	-17.38	40.53%	3.31
Dry	MD	Male	Adult	0.675	53.54	4.27	14.21%	61.44	-23.61	42.08%	3.45
Dry	MD	Female	Sub-adult	0.542	45.55	4.33	14.98%	52.44	-17.68	43.89%	3.42

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Dry	MD	Male	Adult	0.632	51.69	4.60	14.65%	59.64	-23.07	43.62%	3.47
Dry	MD	Female	Adult	0.508	27.65	3.94	14.17%	20.00	-12.98	42.92%	3.53
Dry	MD	Male	Adult	0.501	27.60	4.30	14.35%	20.17	-17.36	43.88%	3.57
Dry	MD	Male	Adult	0.53	32.80	4.40	16.12%	24.03	-12.35	49.42%	3.58
Dry	MD	Female	Sub-adult	0.544	29.12	4.49	13.94%	21.24	-12.37	42.56%	3.56
Dry	MD	Female	Adult	0.559	30.29	4.61	14.11%	22.00	-18.09	42.89%	3.55
Dry	MD	Male	Adult	0.593	32.35	4.63	14.21%	23.53	-20.57	43.25%	3.55
Dry	MD	Female	Adult	0.598	29.44	4.74	12.82%	21.41	-14.29	39.03%	3.55
Dry	MD	Male	Adult	0.527	29.10	4.77	14.38%	20.71	-14.00	42.84%	3.48
Dry	MD	Male	Adult	0.584	31.70	4.93	14.14%	23.00	-19.74	42.93%	3.54
Dry	MD	Male	Adult	0.551	30.87	5.57	14.59%	23.33	-23.11	46.16%	3.69
Wet	AD	Male	Adult	0.508	35.87	6.23	14.36%	62.91	-19.32	43.04%	3.50
Wet	AD	Male	Juvenile	0.566	39.21	6.51	14.08%	71.35	-21.37	43.81%	3.63
Wet	AD	Male	Juvenile	0.545	37.27	6.92	13.90%	66.61	-16.55	42.48%	3.56
Wet	AD	Female	Adult	0.58	71.24	5.76	14.12%	199.52	-21.05	43.65%	3.61
Wet	AD	Male	Juvenile	0.562	40.56	5.86	14.67%	72.24	-11.53	44.67%	3.55
Wet	AD	Male	Adult	0.589	45.93	5.89	15.96%	72.98	-14.77	43.20%	3.16
Wet	AD	Male	Juvenile	0.549	40.29	6.38	14.92%	67.95	-11.59	43.01%	3.36
Wet	AD	Female	Adult	0.586	43.09	6.73	15.05%	74.17	-16.46	44.13%	3.42
Wet	AD	Male	Juvenile	0.504	31.36	6.80	12.73%	64.61	-13.86	44.69%	4.10
Wet	AD	Female	Adult	0.527	38.54	7.27	14.96%	65.92	-14.35	43.61%	3.40
Wet	AD	Female	Adult	0.57	41.57	7.41	14.92%	74.69	-14.86	45.69%	3.57
Wet	AD	Male	Juvenile	0.536	37.16	5.26	14.18%	67.17	-20.22	43.69%	3.59
Wet	AD	Female	Sub-adult	0.511	34.27	6.02	13.63%	60.42	-14.10	41.09%	3.52
Wet	AD	Female	Adult	0.563	37.78	6.53	13.73%	68.25	-17.25	42.26%	3.59
Wet	AD	Female	Juvenile	0.567	37.70	6.66	13.52%	67.71	-14.01	41.50%	3.58
Wet	AD	Female	Adult	0.592	41.58	7.01	14.28%	73.37	-23.23	43.07%	3.52
Wet	AD	Male	Juvenile	0.569	39.18	7.05	14.09%	72.61	-13.38	44.49%	3.68
Wet	AD	Female	Juvenile	0.592	39.80	7.12	13.67%	72.34	-13.38	42.47%	3.63
Wet	AD	Female	Adult	0.534	35.94	7.31	13.77%	64.12	-15.33	41.86%	3.55
Wet	AD	Male	Adult	0.534	36.40	7.36	13.95%	64.91	-14.29	42.38%	3.54
Wet	AD	Female	Adult	0.525	35.16	7.73	13.62%	62.08	-18.16	41.10%	3.52
Wet	AD	Female	Juvenile	0.578	38.31	8.02	13.56%	72.62	-15.81	43.80%	3.77
Wet	AD	Male	Adult	0.537	68.41	5.33	14.71%	189.86	-21.73	44.87%	3.56
Wet	MD	Female	Sub-adult	0.507	37.61	5.91	15.18%	64.63	-20.59	44.44%	3.42
Wet	MD	Female	Sub-adult	0.537	37.44	6.11	14.27%	66.76	-18.34	43.34%	3.54
Wet	MD	Male	Sub-adult	0.572	41.10	6.26	14.61%	69.68	-16.74	42.33%	3.38
Wet	MD	Female	Juvenile	0.55	36.78	7.16	13.60%	67.74	-12.43	42.80%	3.67
Wet	MD	Male	Sub-adult	0.528	35.83	7.67	13.80%	66.40	-13.77	43.71%	3.70
Wet	MD	Male	Juvenile	0.556	40.11	7.91	14.76%	76.62	-15.07	48.04%	3.80
Wet	MD	Male	Juvenile	0.581	40.07	8.33	14.02%	72.36	-14.47	43.29%	3.60
Wet	MD	Female	Sub-adult	0.543	38.71	10.27	14.49%	67.26	-20.05	43.05%	3.47

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Wet	MD	Male	Adult	0.595	42.09	3.97	14.48%	73.07	-17.99	42.82%	3.45
Wet	MD	Female	Adult	0.553	36.91	4.09	13.66%	70.32	-21.48	44.33%	3.79
Wet	MD	Female	Adult	0.525	35.19	4.36	13.62%	62.30	-21.95	41.24%	3.53
Wet	MD	Male	Sub-adult	0.58	40.45	4.57	14.18%	72.72	-20.54	43.58%	3.59
Wet	MD	Male	Juvenile	0.539	37.81	4.87	14.36%	69.69	-21.29	45.08%	3.66
Wet	MD	Male	Juvenile	0.506	35.77	4.90	14.37%	64.12	-21.16	44.04%	3.58
Wet	MD	Female	Adult	0.537	36.01	5.01	13.72%	66.47	-22.23	43.15%	3.67
Wet	MD	Male	Sub-adult	0.586	40.76	5.08	14.23%	74.28	-22.89	44.19%	3.62
Wet	MD	Male	Juvenile	0.541	37.16	5.15	13.96%	66.56	-20.80	42.76%	3.57
Wet	MD	Male	Adult	0.577	41.48	5.15	14.71%	74.89	-18.00	45.25%	3.59
Wet	MD	Female	Adult	0.548	40.17	5.47	14.90%	68.57	-20.97	43.49%	3.40
Wet	MD	Female	Sub-adult	0.573	41.43	5.80	14.70%	71.70	-20.41	43.49%	3.45
Wet	MD	Female	Adult	0.541	33.76	7.55	12.77%	60.50	-17.93	38.99%	3.56
Wet	MD	Female	Adult	0.546	36.78	7.83	13.70%	68.11	-20.83	43.36%	3.69
Wet	MD	Female	Adult	0.503	62.09	4.60	14.45%	163.79	-20.59	41.32%	3.34
Wet	MD	Male	Juvenile	0.503	61.10	5.33	14.22%	167.24	-20.02	42.19%	3.46
Wet	MD	Female	Adult	0.524	64.30	5.36	14.19%	176.77	-20.29	42.81%	3.52
Wet	MD	Female	Adult	0.534	68.10	5.50	14.78%	187.92	-19.49	44.66%	3.52
Wet	MD	Female	Adult	0.533	72.11	6.40	15.56%	168.82	-15.16	40.19%	3.01
Wet	MD	Female	Adult	0.577	73.34	7.14	14.72%	193.82	-16.50	42.63%	3.38
Wet	MD	Female	Adult	0.538	63.71	4.43	13.92%	177.34	-19.76	41.83%	3.51

C.3 PLANT MATERIAL DATA

Table C.3.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of plant samples collected across minimally and anthropogenically disturbed landscapes in the Magaliesberg Biosphere, North West province, South Africa.

Type	Species	N	Common name	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
				Mean	SD	Mean	SD
Artificial feed	<i>Medicago sativa</i>	2	Lucerne (Alfalfa) pellets	-17.12	9.13	0.44	1.75
Artificial feed	-	1	Mixed grains	-12.95	-	2.31	-
Bulb	<i>Cyperus spp.</i>	7	Sedge	-13.19	5.94	0.23	1.50
Flower	<i>Helianthus annuus</i>	1	Sunflower	-27.50	-	2.54	-
Flower	<i>Protea caffra</i>	1	Common sugarbush	-26.54	-	2.36	-
Fruit	<i>Solanum lycopersicum</i>	3	Tomato	-25.71	9.54	5.58	4.84
Grain	<i>Zea mays</i>	3	Maize	-20.46	8.17	5.40	3.46
Grass	<i>Alloteropsis semialata</i>	4	Black-seed grass	-10.72	1.05	0.69	0.84
Grass	<i>Aristida congesta</i>	14	Three-awn grass	-11.83	1.84	0.65	1.26
Grass	<i>Chloris gayana</i>	3	Rhodes grass	-11.27	0.70	1.28	0.95
Grass	<i>Cymbopogon pospischilii</i>	4	Turpentine grass	-11.04	1.08	-0.81	0.67

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Grass	<i>Digitaria eriantha</i>	9	Pangola grass	-10.95	0.75	-1.15	1.08
Grass	<i>Diheteropogon amplexans</i>	11	Broad-leaved bluestem	-15.24	7.53	1.09	2.33
Grass	<i>Elionurus muticus</i>	4	Wire lemongrass	-10.67	0.75	-0.21	0.873
Grass	<i>Eragrostis chloromelas</i>	13	Blue lovegrass	-14.18	5.60	2.32	1.78
Grass	<i>Eragrostis superba</i>	6	Saw-toothed lovegrass	-16.53	8.02	-2.07	0.98
Grass	<i>Heteropogon contortus</i>	19	Spear grass	-17.72	7.16	1.44	3.06
Grass	<i>Hyparrhenia hirta</i>	6	Common thatching grass	-10.75	1.08	2.26	1.89
Grass	<i>Hyperthelia dissoluta</i>	13	Yellow thatching grass	-17.29	7.89	3.33	3.65
Grass	<i>Melinis repens</i>	14	Natal red top grass	-15.56	7.96	0.09	1.21
Grass	<i>Panicum maximum</i>	11	Guinea grass	-17.76	6.78	-0.13	2.03
Grass	<i>Pogonarthria squarrosa</i>	3	Sickle grass	-10.84	0.82	0.21	0.82
Grass	<i>Setaria sphacelata</i>	15	Golden bristle grass	-11.25	0.70	0.61	1.98
Grass	<i>Tristachya leucothrix</i>	3	Trident grass	-10.70	0.99	-1.23	0.91
Grass	<i>Urochloa mosambicensis</i>	3	Herringbone grass	-12.01	1.61	8.01	1.61