

**Movement, home range and habitat use in leopard
tortoises (*Stigmochelys pardalis*) on commercial
farmland in the semi-arid Karoo.**

Martyn Drabik-Hamshare

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ABSTRACT

Given the ever-increasing demand for resources due to an increasing human population, vast ranges of natural areas have undergone land use change, either due to urbanisation or production and exploitation of resources. In the semi-arid Karoo of southern Africa, natural lands have been converted to private commercial farmland, reducing habitat available for wildlife. Furthermore, conversion of land to energy production is increasing, with areas affected by the introduction of wind energy, solar energy, or hydraulic fracturing. Such widespread changes affects a wide range of animal and plant communities.

Southern Africa hosts the highest diversity of tortoises (Family: Testudinidae), with up to 18 species present in sub-Saharan Africa, and 13 species within the borders of South Africa alone. Diversity culminates in the Karoo, whereby up to five species occur. Tortoises throughout the world are undergoing a crisis, with at least 80 % of the world's species listed at 'Vulnerable' or above. Given the importance of many tortoise species to their environments and ecosystems—tortoises are important seed dispersers, whilst some species produce burrows used by numerous other taxa—comparatively little is known about certain aspects relating to their ecology: for example spatial ecology, habitat use and activity patterns. Understanding an animal's use of an environment is important in learning more about certain ecosystem functions and offering information to guide future conservation management.

We studied spatial ecology and habitat use of the Leopard Tortoise, *Stigmochelys pardalis*: the largest and most abundant species in the region. Ten Global Positioning System (GPS) transmitters were placed eleven adult tortoises (one was redeployed following death of one individual), providing a minimum of 12 months of bihourly movement data. We used these data

to estimate home ranges, indicate important predictor variables to movement, and investigate differences in space use between seasons, sex, and time of day.

Using modern home range estimation techniques—such as GPS telemetry and Kernel Density Estimation (KDE)—we provided evidence that Leopard Tortoises have very large home ranges ($n = 9$, mean \pm SE: 121.86 ± 28.12 ha, range 40.53—258.52 ha), with no significant differences between males and females. This large home range size supports previous research in the region, and supports the theory that populations in more arid regions have larger home ranges than in regions of higher rainfall. However, we also found that some Leopard Tortoises do not hold a home range: site fidelity tests suggested that two individuals exhibited apparent nomadic behaviour (hence home range estimated for only 9 of 11 tortoises above). When investigating seasonal changes in home ranges with generalised linear mixed models (GLMMs), we found important individual (sex and body mass), and weather (temperature and rainfall) predictor variables.

We also used GLMMs to investigate bihourly and daily movement in Leopard Tortoises. Several important predictor models were identified, including temperature, rainfall, habitat type, availability of water, time of year, and time of day. We found a negative association for movement with distance from water sources, indicating that tortoises are more likely to move larger distances when closer to these resources. We attributed this behaviour to tortoises' ability to supplement much of their water intake from water-rich food resources (e.g. succulents, grasses and forbs), as shown in previous studies. In contrast, as tortoises are still required to drink water to maintain water balance and excrete electrolytes and nitrogenous wastes, tortoises that have knowledge of drinking water resources within their home range are likely to make regular long distance movements to these areas.

Movement data suggests that some Leopard Tortoises make nocturnal movements, despite tortoises being strongly diurnal animals. As adult Leopard Tortoises are large enough to avoid predation, are subject to reasonable night-time temperatures, and are able to maintain core temperatures above ambient temperatures, visibility of surroundings may be the largest limitation to movement. Further research is required, but we found higher nocturnal movement associated with periods of higher lunar illumination: e.g. full moon phase vs new moon phase.


As stated above, there is a great importance in learning more about tortoises and contributing to conservation. One of these areas is improving genetic contributions that assists in identifying species. However, previous genetic research using a common mitochondrial primer site—cytochrome c oxidase I (COI)—has shown poor success rates with respect to tortoises. Using all tortoise COI sequence information available on the Barcode of Life Database (BOLD), and six of our own Leopard Tortoise samples, we recommend primer sites for the production of a mini-barcode specific to tortoises. Such a mini-barcode can be used to improve success rates in identifying specimens based on DNA, and increase extraction success with degraded DNA: e.g. museum specimens or environmental DNA.

Information from this study can be used to further understanding of environmental and weather conditions that influence movement and space use in tortoises. We have identified several important resources and predictor variables which can affect how a tortoise uses its environment. Given that environments continue to be fragmented, degraded, or lost, better understanding of potential impacts on tortoises is required. We make recommendations on future research into fracking in the region, as well recommendations for use of electric fencing, which has been shown to cause large numbers of mortalities in Leopard Tortoises.

PREFACE

The data described in this thesis were collected in the Beaufort West / Nelspoort region, West Cape Province, Republic of South Africa from November 2014 to December 2015. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Prof. Colleen T. Downs.

This thesis, submitted for the degree of Master of Science in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.



Martyn Drabik-Hamshare

.....
 Martyn Drabik-Hamshare
 November 2016

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.




.....
 Professor Colleen T. Downs
 Supervisor
 November 2016

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DECLARATION 1 - PLAGIARISM

I, Martyn Drabik-Hamshare, declare that

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

Publication 1

M Drabik-Hamshare & CT Downs

Drabik-Hamshare, M. and Downs, C.T. In prep. Aspects of the home range ecology of the leopard tortoise in the semi-arid central Karoo: an area threatened with fracking.

Author contributions:

MDH conceived paper with CTD who sourced funding. MDH collected and analysed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 2

M Drabik-Hamshare & CT Downs

Drabik-Hamshare, M. and Downs, C.T. In prep. Movement of leopard tortoises in response to environmental and climatic variables in a semi-arid environment.

Author contributions:

MDH conceived paper with CTD who sourced funding. MDH collected and analysed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 3

M Drabik-Hamshare & CT Downs

Drabik-Hamshare, M. and Downs, C.T. In prep. Walking in the moonlight: nocturnal activity in leopard tortoises.

Author contributions:

MDH conceived paper with CTD who sourced funding. MDH collected and analysed data, and wrote the paper. CTD contributed valuable comments to the manuscript.


Publication 4

M Drabik-Hamshare, CT Downs & S Willows-Munro

Drabik-Hamshare, Downs, C.T., and Willows-Munro, S. In prep. Designing mini-barcodes for tortoises: Identification of important COI primer sites for tortoises (Testudinidae).

Author contributions:

MDH conceived paper with CTD and SWM. CTD and SWM sourced funding. MDH collected and analysed samples, and wrote the paper. CTD and SWM contributed valuable comments to the manuscript.



Martyn Drabik-Hamshare

Signed: _____

Martyn Drabik-Hamshare

November 2016

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Growing up as a tortoise enthusiast and otherwise keen herpetologist, there was always going to be one place that I wanted to carry out herpetological research. The opportunity to work in South Africa, and the numerous memorable adventures that have followed, will stay with me for a lifetime. None of this would have been possible without my supervisor, Prof Colleen Downs, to whom I owe the utmost gratitude, appreciation and respect. Thank you Colleen for everything you have provided me in these past two years; for taking me in, providing me with trust, belief, and your never-ending knowledge in biology. I also thank Dr Sandi Willows-Munro for allowing me access into her genetics lab, being patient, and allowing me the opportunity to conduct additional research and gain important new skills. Living in South Africa was also made a million times easier due to the love, care, and friendship of Colin and Sharon Boyes. I stayed with them for the majority of my stay in the country, and they made me feel part of their family.

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CHAPTER 1

INTRODUCTION

Effects of land use on biodiversity

Compared with mammals and birds, research and ecological knowledge of reptiles is relatively low, perhaps owing to the difficulties in locating, observing and collecting data on individuals (Ryan et al. 2002, Sutherland 2006). However, there is a global push towards furthering understanding and importance of reptile groups, most of which are declining (Gibbons et al. 2000). The trend for reptiles and amphibians species is that they are declining on a global scale (Baard and de Villiers 2000, Gibbons et al. 2000, Stuart et al. 2004, Gardner et al. 2007, Measey et al. 2009). In almost all cases, humans are the primary cause of these declines.

The global human population has increased by more than three orders of magnitude in fewer than 400 generations (Keinan and Clark 2012). Current estimates suggest that there are over 7 billion people on Earth, with numbers projected to surpass 8.3 billion by 2050 (Bradshaw and Brook 2014). Such rapid increases in size and distribution of human populations have contributed to a host of ecological factors, which are primary or secondary causes of global loss of biodiversity. Examples of such biological factors include, but are not limited to, climate change (Gibbons et al. 2000, Daszak et al. 2001, Giordani et al. 2002, Harvell et al. 2002, Reading et al. 2010), habitat destruction and alteration (Gibbons et al. 2000, Araújo et al. 2006, Gardner et al. 2007, Bickford et al. 2010, Reading et al. 2010), reduction in habitat connectivity (Reh and Seitz 1990, Blaustein et al. 1994, Araújo et al. 2006), and introduction of alien species and diseases (Rodda and Fritts 1992, Daszak et al. 2000, Mooney and Cleland 2001, Kambourova-Ivanova et al. 2012, Warnecke et al. 2012). Increased threats

to biodiversity are therefore expected with human population increases, especially considering 20 % of the human population exists in biodiversity hotspots (Cincotta et al. 2000).

Two of these ‘biodiversity hotspots’ exist in South Africa; the Succulent Karoo and the Cape Floristic Province (Myers et al. 2000). The Great Karoo (hereafter the Karoo), which is larger than the adjacent Succulent Karoo, is not listed as a biodiversity hotspot, but has a high level of endemism, particularly with birds and reptiles (Branch et al. 1995, Dean 1995, Branch 2014). However, plant endemism is typically low (Hilton-Taylor 1987). The Karoo is a semi-arid habitat that covers 37 million hectares: over 30 % of South Africa’s total area, covering the majority of the Northern, Eastern, and Western Cape Provinces (Vorster and Roux 1983). The region is typified by low, variable and unpredictable rainfall (van Rooyen 1999, Mucina et al. 2006), though ambient temperatures frequently exceed 30 °C through much of the year (Mucina et al. 2006).

Land use in the region has changed significantly since the arrival of Europeans in the mid-seventeenth century (Ross 2008). Prior to this, the Karoo was inhabited by Koi-San people, hunter-gatherers that survived primarily by hunting mammals, such as Springbok, *Antidorcas marsupialis* (Skead 1980, 1987, Dean 1995), though there is also evidence that humans have occupied the area for at least 3 million years (Smith 1999). Springbok herds once existed in their thousands, with legendary migrations known as ‘*Trekboke*’ (Roche 2005). Commercial farming—which now covers over 80 % of land in the Karoo (Hoffman et al. 1999)—using such large numbers of livestock (cattle, goats, sheep) is now commonplace, with most naturally-occurring ungulates restricted to game farms or small free-roaming populations. Changes in land use, and subsequent changes to vegetation structure and fire regimes are thought to have caused the removal of Wattled Cranes (*Bugeranus carunculatus*) and Southern Bald Ibis (*Geronticus calvus*) from the region (Manry 1985, Brooke and Vernon 1988). Cape Vulture (*Gyps coprotheres*) populations have also been extirpated from much of the Karoo,

with previously healthy colonies near Beaufort West long extinct (Jackson 1920). Extirpations are attributed to loss of scavenging opportunities, but it is also likely that these species were the unfortunate victims of poisoning events targeting Black-backed Jackal (*Canis mesomelas*) and Caracal (*Caracal caracal*), which are predators of livestock in the region. Conversely, other species have increased since European settlement. Chacma Baboons (*Papio ursinus*) and Vervet Monkeys (*Chlorocebus pygerythrus*) are both increasingly associated with urban environments and riverine ecosystems (Milton et al. 1999b, Pasternak et al. 2013). Tree-nesting raptors such as the Rufous-chested Sparrowhawk (*Accipiter rufiventris*) have expanded their distribution as a consequence of an increase in the planting of non-native trees where trees were previously sparse (Milton et al. 1999b).

Careful management is required to ensure there is no long-term damage to commercial land, especially as the Karoo has low annual rainfall and thus low primary production (Desmet and Cowling 1999). Poor land management and subsequent overgrazing have long-lasting damaging effects. Large livestock densities selectively remove palatable plant species, encouraging colonization of opportunistic unpalatable species, many of which are introduced, such as the prickly pear, *Opuntia ficus-indica* (Milton et al. 1999b). Past overgrazing events and subsequent changes in plant biodiversity affects animals at other trophic levels. Overgrazing affects behaviour, diversity and abundance of reptiles (Wasiolka et al. 2009, Cano and Leynaud 2010, Wasiolka et al. 2010). In extreme cases, overgrazing also contributes to wildfires, droughts and desertification (Dodd 1994), which has long-lasting effects on ecosystems (Castellano and Valone 2006). This is understood by modern-day farming, but many farms in the region are still suffering from past overexploitation (Conradie et al. 2013). As such, most landowners now incorporate holistic resource management and rotational grazing of mixed livestock, which reduces selective grazing (Savory 1991).

Habitat connectivity has also been negatively affected by changes in land use in the Karoo. Roads, fences, and other restrictive structures have been constructed with increased human populations in South Africa. Reptiles and amphibians are particularly susceptible to roads (Kabugumila 2001, Vijayakumar et al. 2001, Jochimsen et al. 2004, Andrews et al. 2008, Kambourova-Ivanova et al. 2012). Their small size, low speed and ectothermic biology can be detrimental to their survival when faced with the task of crossing a road. Animals move between different areas of habitat suitability for a number of reasons, usually determined by resources required: food, nutrients, shelter, mates, or egg-laying habitat (Boyce and McDonald 1999). In addition, roads are often used by reptiles and amphibians for basking behaviour, exposing themselves to traffic (Sullivan 1981, White and Burgin 2004, Meek 2009, Nafus et al. 2013). The Endangered Wildlife Trust (EWT), South Africa, are currently undertaking a Road Ecology project, which aims to “*mitigate the negative environmental impacts of transportation*” (EWT 2013). In addition to putting themselves at risk, use of roads by herpetofauna has potential of causing motor accidents (Langen et al. 2007).

While most fences serve simply as barriers to animal movement, some fences do cause mortalities, due to collisions or because animals are unable to free themselves after contact (Allen and Ramirez 1990, van der Ree 1999, Harrington and Conover 2006). Research has shown that electric fencing, in particular, has a negative effect on a number of different species. Tortoises (Testudinidae) and Ground Pangolins (*Manis temminckii*) are thought to be the most vulnerable species to electric fencing (Burger and Branch 1994, Beck 2010). Most animals that would come into contact with an electric fence will usually retreat after a shock. However tortoises and pangolins withdraw their limbs or curl up into a ball. Such a strategy, though effective in protecting against predation, causes multiple shocks over several hours or days; eventually leading to death (Burger and Branch 1994).

A more recent threat, of which potential impacts are still largely unknown is the practice of hydraulic fracturing (fracking). This is a process whereby a pressurised fluid mixture is injected deep into the earth in order to fracture rock formations, allowing collection of shale gas (De Wit 2011, Bažant et al. 2014). One worry is that increases in use of fracking will result in wastewater discharge entering natural water systems (Schmidt 2013), something which has been documented with a range of outcomes (McDermott-Levy et al. 2013). In 2012, plans were made to introduce fracking operations in several parts of South Africa (Roberts 2013). Operations are now expected to begin before the end of 2017 (De Wit 2011, Reuters 2016), with the Karoo being one of the regions targeted for shale gas exploration (Fig and Scholvin 2015). Given that much of South Africa is already water-scarce, especially in the semi-arid Karoo (Le Maitre et al. 2009), there is a valid concern that water contamination through accidental release of wastewater discharge (Schmidt 2013, Vidic et al. 2013) could severely affect the local human communities, and the native flora and fauna (Mash et al. 2014). Water demand in the Karoo currently exceeds availability (DWA 2003, Academy of Science of South Africa 2016), while projections show that demand will increase by up to 150 % by 2025 (Le Maitre et al. 2009). Despite fracking being used commercially in many parts of the world for several decades (Warner and Shapiro 2013, Academy of Science of South Africa 2016), potential effects on native fauna and flora are lacking. However, systematic surveying to fill biodiversity information gaps across the region by the South African National Biodiversity Institute (SANBI) hopes to support development decision making with regards to fracking operations (SANBI 2016).

It is of great importance to continue to study the above threats and roles they play in demise of reptiles and amphibians, not least because it can help develop effective strategies to combat these threats and understand the requirements to prevent further declines and possible extinctions of species (Gardner et al. 2007).

Tortoises of South Africa

The origins of the turtles (Order: Chelonii)—which includes all terrestrial tortoises, marine turtles and freshwater turtles—remains unclear, but it is generally believed that they are one of the oldest reptile lineages, evolving some 220 million years ago from Asia (Gaffney 1990). Two turtle-like sauropsids occurring 248 million years ago (Genus: *Anthodon*) and 230 million years ago (Genus: *Proganochelys*), appear to be the most likely ancestor sister taxa to modern turtles. These creatures had a very different appearance to modern-day turtles, with an incomplete shell and retention of teeth (Bonin et al. 2006). Through 220 million years of existence, movement, and evolution, turtles have evolved to fill niches in most environments, with species adapted to deserts, forests, lakes, and oceans. Such adaptations often relate to shape, structure and flexibility of turtles' shells. The order of Chelonii evolved in two main subgroups; Cryptodira and Pleurodira (Bonin et al. 2006). All tortoises and marine turtles, and some freshwater turtles, are cryptodires. Turtles are now found on all major continents (except Antarctica) and many islands (Bonin et al. 2006, Turtle Taxonomy Working Group 2014).

Tortoises belong to the family Testudinidae. Unlike all other turtles, tortoises are entirely terrestrial, though some do have a limited swimming ability (Boycott and Bourquin 2000). Tortoises are found across Europe, Asia, Africa and the Americas (Bonin et al. 2006, Turtle Taxonomy Working Group 2014). There are also several species that have evolved on isolated archipelagos and islands. Such examples include the Aldabra Giant Tortoise (*Aldabrachelys gigantea*) (Hansen et al. 2010) and the giant Galápagos tortoises (*Chelonoidis* spp.) (Bour 1980): both of which can have a body mass in excess of 300 kg (Bonin et al. 2006). In contrast, the world's smallest tortoise species, Speckled Padloper (*Homopus signatus*), has a maximum body mass of about 160 g (Boycott and Bourquin 2000, Loehr et al. 2007).

Tortoises are one of the most threatened taxa within the Animal Kingdom. A review in 2009 indicated that 63 % of the world's 317 species of non-marine turtles (i.e. tortoises and freshwater turtles) were listed as Threatened, with 10 % classified as Critically Endangered (IUCN 2008, Buhlmann et al. 2009). The numbers for tortoises are even more worrying, with 20 % of the world's 53 named species listed as critically endangered, and 80 % listed as at least Vulnerable (Turtle Taxonomy Working Group 2014).

Loss of tortoise populations can be particularly damaging, as tortoises are important to ecosystem health. For example, the Gopher Tortoise (*Gopherus polyphemus*) is considered to be keystone species, building burrows that are used by 60 vertebrates and 302 invertebrates (Eisenberg 1983, Jackson and Milstrey 1989). While tortoises in South Africa are not burrowing species, it is believed that they play an important role in seed dispersal of native flora in the harsh Karoo environment (Milton 1992, Mason et al. 1999, Loehr 2002). Tortoises' ability as seed dispersal can be particularly important given the removal of natural herd animals. Tortoises are threatened by human-mediated changes to habitats, overexploitation, and persecution (O'Brien et al. 2003, Young 2003, Ives et al. 2008, Lee and Smith 2010, Walker 2010, Perez et al. 2012). Many tortoise species have gone extinct in the last few centuries, with at least ten tortoise species extinct since 1700 AD (Turtle Extinctions Working Group 2015). Hansen et al. (2010) estimate that at least 36 large (> 30cm) species have experienced their demise since the Pleistocene; 14 since the Holocene. Many of these are the result of targeted hunting by humans. Larger species were seen as ideal food for early explorers, due to their relative ease of capture and containment, and their ability to survive for long periods of time without food or water (Dean 2009, Hansen et al. 2010). Archaeological evidence from hunter-gatherers also showed that tortoises may have played an important in early human life for food and trading in Europe and Africa (Klein and Cruz-Urbe 1983, Stiner et al. 1999, Grosman and Munro 2007, Thompson and Henshilwood 2014).

Sub-Saharan Africa contains the greatest diversity of terrestrial tortoises, with at least 18 species; approximately one-third of the world's 53 named species (Boycott and Bourquin 2000, Branch 2012, Hofmeyr et al. 2014, Turtle Taxonomy Working Group 2014). Not only does southern Africa have a high biodiversity of tortoises, it also has a high degree of endemism with three genera (*Chersina*, *Homopus*, *Psammobates*) and eleven species (*C. angulata*, *H. areolatus*, *H. boulengeri*, *H. femoralis*, *H. signatus*, *H. solus*, *Kinixys lobatsiana*, *K. natalensis*, *P. geometricus*, *P. oculiferus*, *P. tentorius*) endemic to the sub-region (Hofmeyr et al. 2014, Turtle Taxonomy Working Group 2014). Most notable are the Critically Endangered Geometric Tortoise (*P. geometricus*)—once considered extinct until its rediscovery in 1972 (De Villiers 1985)—and the Vulnerable Nama Padloper (*H. solus*), endemic to small regions in the south-western Cape and southern Namibia respectively (Greig and Burdett 1976, Baard 1993, Cunningham et al. 2002, Bonin et al. 2006, Branch 2007, Hofmeyr et al. 2014). There are more species of tortoise—thirteen in total—in South Africa than any other country in the world (Boycott and Bourquin 2000, Bonin et al. 2006, Hofmeyr et al. 2014, Turtle Taxonomy Working Group 2014), over half of which are endemic to the Karoo biome (Vernon 1999). In parts of southern Africa, multiple species exist sympatrically, with five species occurring within the Karoo National Park (approximate coordinates: -32° 21' 48", 22° 32' 19") (SANParks 2015). This is unique on a global scale, as regions are usually limited to one or two tortoise species (Luiselli 2006).

All but nine of the world's Testudinidae species are listed on The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II, meaning import and export of all tortoise species must be controlled in order to prevent negative impacts on wild populations (CITES 2014). The nine remaining tortoise species, including South Africa's Geometric Tortoise, are instead listed on CITES Appendix I, preventing commercial trade of wild specimens (CITES 2014). Each province within South

Africa has its own laws regarding keeping wild animals in captivity without the acquisition of a specific permit. For example, the Western Cape Province states that “*No person shall without a permit authorising him or her to do so, keep any wild animal in captivity*” (Western Cape Nature 2000). As such, these animals should only be obtained through means of captive breeding. Despite these laws, reptiles are kept illegally, consumed, used in traditional medicine (Young 2003, Smart et al. 2005, Wimberger et al. 2009), and are commonly found as important capital for the worldwide pet trade (Boycott and Bourquin 2000, Nijman and Shepherd 2015).

In the Karoo, the main threats to tortoise species are competition for food with livestock, habitat fragmentation, and electric fencing (Beck 2010). In previous decades, the species was also persecuted by man (Milton et al. 1999a). Farmers once believed that tortoises competed with livestock for food, while incidents of tortoises drowning within and subsequently poisoning dams, troughs, and watering holes was common (Grobler 1982). Tortoises have also been blamed for being vectors of tick-borne diseases, such as *Cowdria ruminantium* (heartwater), which negatively affects livestock health (Milton et al. 1999a, Peter et al. 2001). There is also a host of natural threats, which causes a low survivability to adulthood. Tortoises are predated on during incubation and soon after hatching (Epperson and Heise 2003, Smith et al. 2013). Examples of tortoise predators includes Pied Crows (*Corvus albus*), Verreaux's Eagles (*Aquila verreauxii*), Chacma Baboons, Honey Badgers (*Mellivora capensis*), Pale Chanting Goshawks (*Melierax canorus*), Mongooses and Rock Monitors (*Varanus albigularis*), as has been found in various tortoise species (Malan and Branch 1992, Butler and Sowell 1996, Lloyd and Stadler 1998, Hill 1999, Lovegrove 1999, Mason et al. 2000, Ramsay 2002, Alexander and Marais 2007, Branch et al. 2015). To reduce effect of predation on hatch success, some tortoise species in southern Africa lay multiple egg clutches throughout the year (iteroparity) (Jaques 1969, Cairncross and Greig 1977, Boycott and Bourquin 2000, Bonin et al. 2006, Loehr et al. 2011). Recent work has provided evidence that

predation on tortoises is higher on farmland due to reduced cover, increasing vulnerability of hatchlings (Milton et al. 1999a).

The leopard tortoise

The Leopard Tortoise (*Stigmochelys* (previously *Geochelone*) *pardalis*) (Bell 1828), also known as the Mountain Tortoise (due to appearance, rather than habitat) is currently the only named species within its genus (Hofmeyr et al. 2005, Bonin et al. 2006, Hofmeyr et al. 2014), but it is believed to be a sister clade to *Psammobates* (Tent and Geometric Tortoises). The species has one of the largest distributions of all tortoises in Africa; occurring as far north as South Sudan and Ethiopia, and as far south-west as Angola, Namibia, and South Africa (Greig and Burdett 1976, Bonin et al. 2006, Branch 2012, Hofmeyr et al. 2014). The Leopard Tortoise exists across a large range of habitats and climates, with populations present in the semi-arid Karoo, and various grasslands and savannah habitats (Boycott and Bourquin 2000). The species is, therefore, subject to a wide range of conditions. Therefore, it is naive to expect broad ecological statements to be associated with the species. Indeed, previous studies have shown that there are distinct differences in various aspects of the species' ecology in different regions: particularly home range, population size, movement patterns and feeding ecology. Despite being classified globally as a species of Least Concern (Turtle Taxonomy Working Group 2014) and the wide range in distribution, ecological understanding of the Leopard Tortoise remains low.

The Leopard Tortoise is the largest extant tortoise in southern Africa, with a maximum size that is only exceeded by the African Spurred Tortoise (*Centrochelys sulcata*) and the giant tortoises of the Galápagos and the Indian Ocean. Adult Leopard Tortoises usually have a mass of 10-20 kg, however, individuals of nearly 50 kg have been recorded (Boycott and Bourquin 2000). In southern Africa, individuals in the Karoo are much larger than conspecifics in

KwaZulu-Natal, Limpopo, and Swaziland (Boycott and Bourquin 2000, Branch 2012). Such geographic morphological variation suggested that the Leopard Tortoise may exist as two distinct subspecies; *S. p. pardalis*; and *S. p. babcocki* (Le et al. 2006). However, individuals further north in Africa (e.g. Somalia) also reach such large sizes. In addition, recent research into mitochondrial phylogeography of the species, suggests that the former distinction of subspecies has no foundation (Fritz et al. 2010).

Diet of tortoises

The majority of tortoises are herbivorous, though omnivorous species will opportunistically take insects, slugs, and other small prey items (Hailey 1997, Hailey et al. 2001, Loehr 2006). Tortoises also feed on bone fragments, soil, shells, and small stones to assist with intake of nutrients, such as calcium and magnesium, and to breakdown plant matter (Milton 1992, Esque and Peters 1994, Walde et al. 2007, Hazard et al. 2010, Moore and Dornburg 2014). Due to their long gut retention time (Hailey and Loveridge 1997, McMaster and Downs 2008, Sadeghayobi et al. 2011) and method of eating fruits and grasses, tortoises are recognised as important seed dispersers (Milton 1992, Mason et al. 2000, Strong and Fragoso 2006, Hansen et al. 2010, Blake et al. 2012, Falcón and Hansen 2014). This is especially the case in the tropics where most megaherbivores are now either extinct (Guimarães Jr et al. 2008, Gill 2014). Seeds remain undigested in the digestive tract of tortoises and are moved away from their parent plant. This distance may be further than seed dispersal by other animals due to low digestive rate and movement covered within this time. Most tortoises feed on a wide range of plant species throughout the year (Milton 1992, Joshua et al. 2010), often with dietary shifts based on food availability (De Neira and Johnson 1985). Many species are even able to tolerate various plants that are toxic to other reptiles (Meek 1985, Kabigumila 2001, Lagarde et al. 2003b, Henen et al. 2005, Del Vecchio et al. 2011). Leopard Tortoises are

one of a number of species that show dietary shifts throughout the year depending on food availability, switching from a grass-based diet to a succulent-based diet (Milton 1992, Rall and Fairall 1993). Such studies have suggested that the Leopard Tortoise is generally a specialist feeder when food is readily available, but switches to a generalist when availability decreases (Boycott and Bourquin 2000).

Due to their low metabolic rate and ability to store resources (Nagy and Medica 1986, Peterson 1996, Hailey and Loveridge 1997, McMaster and Downs 2008, Sadeghayobi et al. 2011), tortoises are able to overcome periods of droughts, travel long distances without food, and colonise regions with harsh climatic conditions, such as the semi-arid deserts of Africa and North America. Such characteristics also allowed species to cross oceanic barriers, as is believed to be the case for the Galápagos and Indian Ocean species, whereby individuals probably floated across the ocean to these islands (Caccone et al. 1999, Gerlach et al. 2006).

Tortoise home range and movement patterns

The term ‘home range’ was created by Burt (1943) to define the “*area traversed by the individual in its normal activities of food gathering, mating and caring for young*”. The definition has since been updated: in its simplest form, a home range relates to the estimated area of use across an environment based on locations in which an individual is commonly found (Powell and Mitchell 2012). As animals explore their environment they build and continuously update a cognitive map (Gautestad 2011). It has been proposed that the best estimate of animal’s home range are areas within this cognitive map that are updated more regularly (Powell and Mitchell 2012). As with many terrestrial animals, important resources and biological factors for survival are food and water, refugia, non-restrictive habitat and habitat that enables successful reproduction (e.g. egg-laying habitat) (Baard 1995).

Tortoise research with regards to home range patterns is easier in larger species, with restrictions placed on studies due to size, weight and battery life of telemetry units. A review of all home range studies for tortoises and turtles (Slavenko et al. 2016) found that size has not always prevented research on smaller species. In absence of such units, studies have used novel methods, such as thread trailing to conduct movement studies (Hailey 1989, Díaz-Paniagua et al. 1995, Hailey and Coulson 1996, Keller et al. 1997, Longepierre et al. 2001, Loehr 2004). Recent developments in technology have resulted in production of smaller transmitters, which are now used on smaller species, such as Geometric Tortoises (Hofmeyr et al. 2012) and Speckled Padlopers (Loehr 2014). In models created to predict home range size in turtles, Slavenko et al. (2016) concluded that home range size increases with body mass. Diet (omnivorous vs herbivorous), and habitat (aquatic vs semi-aquatic vs terrestrial) also influenced home range size, but not sex or social structure, though there are several examples where home range does differ between sexes (Mason and Weatherby 1996, McMaster and Downs 2009). It is important to note that studies reviewed varied greatly in relocation methods, study size, longevity, and home range estimation method.

Other studies have suggested that differences in home range estimates in tortoises could relate to seasonal fluctuations in availability of standing water, rainfall, food, and other resources (e.g. nutrients) (Marlow and Tollestrup 1982, Hailey and Coulson 1996, Van Bloemestein 2005, Monadjem et al. 2013). This has also been identified in various tortoise species, including Geometric Tortoises (Baard 1995, Van Bloemestein 2005, Hofmeyr et al. 2012) and Gopher Tortoises (Diemer 1992). Animals in areas of greater productivity may have a smaller home range than a conspecific in an area of lower productivity, as resource search efficiency is increased (Harestad and Bunnell 1979). Aldabra Tortoises migrate seasonally between coastal and inland areas in response to vegetative productivity (Swingland and Lessells 1979). However, this is not the only limiting resource for home range in tortoises:

seasonal movement fluctuations to reproductive movements, such as mate searching and egg-laying behaviour are also apparent (Gibbons 1986, Geffen and Mendelssohn 1988, Eubanks et al. 2003, Van Bloemestein 2005, Hofmeyr et al. 2012, Rozylowicz and Popescu 2013).

Expenses relating to transmitters is another limitation (Hebblewhite and Haydon 2010). As such, those studies that did use telemetry had a limited number of telemetry units, usually no more than ten (Slavenko et al. 2016). While detailed relocation data is useful in providing detailed estimates about an animals habitat use, Börger et al. (2006) believes that number of individuals tracked is more important. This is apparent when one considers that variation in home range size is often related to individual factors, such as the age, sex, and reproductive condition of the individuals being monitored (Rose and Judd 1975, Van Bloemestein 2005). Individual variation and body mass should be considered when investigating home range sizes, as studies have shown that larger individuals tend to cover larger areas (Gaston and Blackburn 1996). This is also shown in previous studies on Gopher Tortoises (Diemer 1992), Egyptian Tortoises (*Testudo kleinmanni*) (Geffen and Mendelssohn 1988), Geometric tortoises (Hofmeyr et al. 2012) and Horsfield's Tortoise (*Testudo horsfieldi*) (Lagarde et al. 2003a). Individual variation is also seen in Leopard Tortoises. For example, home range estimates for males in the Nama-Karoo ranged from 12.67 ha to 229.53 ha (McMaster and Downs 2009). To account for this, sufficient sample sizes must be considered before making home range estimates.

Research on movement patterns and home range sizes in Leopard Tortoises has shown great variability in results. The mean home range estimate of adults in the Northern (205.41 ha) (McMaster and Downs 2013) and Eastern (57.56 ha) (Mason and Weatherby 1996) Cape Provinces were much larger than in KwaZulu-Natal (35.42 ha) (Wimberger et al. 2009), Swaziland (13.49 ha) (Monadjem et al. 2013) and Zimbabwe (26 ha) (Hailey and Coulson 1996). It is likely that resource availability accounts for some of this difference between

populations, though study longevity, survey methodology, and analyses are likely contributors. There also seems to be variability in terms of whether sex has an effect on home range size. Two of the previous Leopard Tortoise studies found home range sizes for females were significantly larger than home range sizes for males (Mason et al. 2000, McMaster and Downs 2009), though sex effect was not been observed in other populations (Monadjem et al. 2013).

Study Aims

This study aims to contribute to the growing knowledge and understanding of tortoise ecology in the Karoo. Data chapters provided in this thesis represent the most comprehensive and standardised dataset for movement ecology of Leopard Tortoises. We aim to examine relationships between movement ecology, habitat use and environmental variables, and show how home range on commercial private farmland in the Western Cape differs from that in previously studied populations in southern Africa. The study sites are located within one of the areas targeted for fracking. Due to the importance of tortoises to their environments (as seed dispersers and ecosystem engineers) and the current threats tortoises are facing, it is important to increase understanding of tortoise ecology in an environment that is already water scarce and heavily transformed by anthropogenic pressures related to commercial farming.

Understanding tortoise movement, including how patterns change daily and seasonally, can also assist with conservation efforts and reduce mortalities through guiding improved design for electric fences. We also aim to use such information to make recommendations for area available to, and movement between, populations. Such recommendations will help ensure that tortoises are able to move between populations and interbreed freely. All research from this study will not only benefit Leopard Tortoises but the wider ecological knowledge of tortoises in the region.

Contents of thesis

All data chapters within this thesis have been prepared as manuscripts to be submitted to international peer-reviewed journals. As such, it is an unavoidable circumstance that information provided in these chapters overlap in some areas. The various hypotheses and predictions for each of the major topics are presented in each chapter.

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CHAPTER 2

Aspects of the home range ecology of the leopard tortoise in the semi-arid central Karoo: an area threatened with fracking

Martyn Drabik-Hamshare, Colleen T. Downs*

School of Life Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville,

Pietermaritzburg, South Africa, 3209

**Corresponding author, email: Downs@ukzn.ac.za; m.drabik.hamshare@gmail.com*

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Corresponding Author: Professor Colleen Thelma Downs, PhD

Corresponding Author's Institution: University of KwaZulu-Natal

First Author: Martyn Drabik-Hamshare

Order of Authors: Martyn Drabik-Hamshare; Colleen Thelma Downs, PhD

Highlights

- Updated home range estimates given for leopard tortoises, *Stigmochelys pardalis*.
- Home ranges show individual and seasonal variation.
- Sex, biomass, temperature and rainfall significant predictors for monthly home range.
- Data recommended as baseline data in pre-fracking era for the Karoo.

Abstract

Whilst fracking is used globally, impact studies on wildlife are limited. The semi-arid Karoo, South Africa, a large ecosystem with a high degree of endemism, is targeted for fracking. We investigated how adult leopard tortoises (*Stigmochelys pardalis*) use their environment by determining individual and seasonal variation in home range and effects of weather factors on these pre-fracking. Data were obtained from Global Positioning System (GPS) transmitters placed on leopard tortoises ($n = 11$) on private livestock farms near Beaufort West, South Africa for a year. Kernel density estimation (KDE) was used to estimate home range. Individuals had a mean (\pm SE) home range of 121.86 ± 28.12 ha, (range 40.53—258.52 ha) with a core area of 76.55 ± 17.33 ha (range 21.22—83.89 ha). No difference was found between annual male and female home ranges. Two telemetered individuals were excluded from analysis because they exhibited apparent nomadic behaviour. Several individuals did not visit permanent water sources, possibly suggesting that dietary water intake was sufficient. Generalised Linear Mixed Models were used to explain monthly home range estimates (95% KDE_{href}) in regards to biologically significant predictor variables. A single top model ($\Delta AIC_c < 2$) was produced, indicating importance of individual variability (sex, body mass) and weather (temperature, rainfall) variables. Our results provide baseline data pre-fracking in the region, and as such, should be repeated following commencement of fracking.

1. Introduction

Most protected areas (PAs) are generally well-managed and well-researched in southern Africa (SANParks, 2013), however, there is an increasing need to further understand ecosystems that do not fall into PAs. The increase in human population has resulted in loss and degradation of naturally existing environments. Across the world, land is cleared to provide important human resources, including housing, agriculture, and energy production. The change in land use outside PAs for fuel and energy can heavily impact threatened species (Reimer and Snodgrass, 2009). Furthermore, interpretation of movement ecology and home range estimations of these species can be important in aiding successful management decisions, especially in areas of land use change (Hebblewhite and Haydon, 2010).

Burt (1943), originally defined ‘home range’ as the ‘*area traversed by the individual in its normal activities of food gathering, mating and caring*’, though further definitions and analytical methods have advanced our understanding of how animals occupy and use their spatial environment. As animals explore their environment they build and continuously update a cognitive map (Gautestad, 2011). It has been proposed that the best estimate of animal’s home range are areas within this cognitive map that are updated more regularly (Powell and Mitchell, 2012). Technological advances and improvements in statistical models have allowed a greater understanding of the utilisation of environments by animals. For example, Kernel Density Estimation (KDEs), a nonparametric statistical technique which utilises probability density functions to estimate home range (Worton, 1989), can also be used to investigate habitat use (Seaman and Powell, 1996).

South Africa, with its variety of habitats and ecosystems, is home to at least thirteen terrestrial tortoise species—~24% of the world’s 53 extant species (Hofmeyr et al., 2014, Turtle Taxonomy Working Group, 2014)—and is considered a centre of endemism for Testudinidae

(Branch et al., 1995). The Karoo biome, a semi-arid desert covering much of the Northern, Eastern and Western Cape Provinces, has a high tortoise diversity with eight species occurring in the region (Hofmeyr et al., 2014, Turtle Taxonomy Working Group, 2014). In some areas, up to five species coexist: leopard tortoise (*Stigmochelys pardalis*), angulate tortoise (*Chersina angulata*), tent tortoise (*Psammobates tentorius*), Karoo padloper (*Homopus boulengeri*) and greater padloper (*H. femoralis*) (Hofmeyr et al., 2014).

As with other major ecoregions, many parts of the Karoo are highlighted as potential sites for hydraulic fracturing (fracking) (Le Maitre et al., 2009): a process to collect shale gas using injection of a pressurised fluid mixture deep into the earth (Bažant et al., 2014; De Wit, 2011). Fracking has been used successfully in many parts of the world, however, studies on their implications on wildlife are limited. Potential impacts of fracking on the water-scarce Karoo ecosystem is currently unknown. Perhaps the greatest concern is the potential environmental contamination and degradation due to highly saline wastewater discharge entering into naturally-existing freshwater systems, as shown in North America (Schmidt, 2013). Many communities and ecosystems within the Karoo rely on existing water sources. Contamination of water sources could greatly impact local communities (Mash et al., 2014) and fauna and flora. This could reduce regional biodiversity, as the Karoo is a centre of endemism for birds and reptiles (Branch *et al.* 1995, Dean 1995). The South African National Biodiversity Institute (SANBI) is currently undertaking systematic surveying to fill biodiversity information gaps to support development decision making with regards to fracking operations (SANBI, 2016).

The leopard tortoise is listed as a species of “Least Concern” both internationally, by the International Union for Conservation of Nature (IUCN) (Turtle Taxonomy Working Group, 2014), and regionally, by the South African Reptile Conservation Assessment (SARCA) (Hofmeyr et al., 2014). The leopard tortoise is ecologically the best-studied African tortoise

species, owing to relative abundance, distribution, and relative ease of locating wild individuals. Previous home range studies have shown variability in results, with tracked individuals in the Nama-Karoo, Northern Cape (McMaster and Downs, 2009) and Addo Elephant Park region, Eastern Cape (Mason and Weatherby, 1996) showing much larger home range sizes than populations studied in Swaziland (Monadjem et al., 2013). This difference has previously been attributed to resource (vegetation/water) availability: animals are expected to travel further and cover larger areas when resources are scarce (Monadjem et al., 2013). The aforementioned studies also found variation between sexes. For example, females had significantly larger home ranges than males in the Northern and Eastern Cape Provinces (Mason and Weatherby, 1996; McMaster and Downs, 2009): a trait not shown in Swaziland (Monadjem et al., 2013). Individuals within these studies also displayed great variability in home range size and habitat use, regardless of sex or size. Such individual variation has been identified in other taxa with animals in similar environments exhibiting varying dietary preferences, sociality, and responses to environmental conditions (McMaster and Downs, 2013b; Pagani-Núñez et al., 2016; Pinter-Wollman et al., 2013). It is important to note that longevity of study, survey methodology, project funding and statistical techniques differed between these studies, and may account for some observed differences.

As with many taxa, home range analyses on tortoises have been conducted with the intent of further understanding their ecology (Slavenko et al., 2016). We investigated home range of leopard tortoises over 12 months on private livestock farmland in the Central Karoo, using Global Position System (GPS)-Global System for Mobile Communications (GSM) / Ultra High Frequency (UHF) telemetry techniques and estimation statistics. Such information is useful to guide management decisions for the species, whilst the methods and analysis are easily transferable to other tortoise species, including those that are currently listed as vulnerable (Hofmeyr et al., 2014, Turtle Taxonomy Working Group, 2014). Considering the

region is targeted as a centre for fracking, we present our methodology and results as baseline data in a pre-fracking era for the region. We predicted that home range estimates of leopard tortoises in the Central Karoo would be comparable to those in Nama-Karoo and the Addo region, due to similarities in habitat and average adult body mass of tortoise populations. We predicted that females would exhibit a larger home range size, as has been shown in previous studies in western populations, due to differing resource requirements (e.g. egg-laying habitat). As with previous studies of home range in tortoises (Hailey and Coulson, 1996; Mason and Weatherby, 1996; McMaster and Downs, 2009; Monadjem et al., 2013), we expected individual and seasonal variation. We expected this individual variation to reflect of individual habitat use and foraging.

2. Materials and methods

2.1. Study area

The Karoo is a large area covering approximately 37 million ha (Vorster and Roux, 1983), with northern and western areas typically arid, and remaining areas semi-arid. Rainfall in the Central Karoo is generally low, and unpredictable and unreliable in terms of quantity and timing (Mucina et al., 2006). Mean daily ambient temperatures frequently surpass 30 °C in summer, when plants and animals are under severe heat and desiccation stress (Mucina et al., 2006; Vorster and Roux, 1983). Furthermore severe frost events can occur during winter (Muller et al., 2016). The result is that vegetation is adapted, wide-ranging, and typically of low levels of endemism, with much of the flora also occurring in surrounding biomes (Hilton-Taylor, 1987).

The study area consisted of three private mixed livestock farms surrounding Nelspoort and Beaufort West, Central Karoo, Western Cape, South Africa: Baakensrug (32°13S, 23°11E), Kamferskraal (32°14S, 23° 2E), and Elandsfontein (32°18S, 22° 54E) (Fig. 2.1).

These farms utilise aspects of holistic resource management, using rotational intensive grazing of livestock (sheep, goats, cattle), aimed at reducing selective grazing and subsequent desertification of their lands (Savory, 1991). Distinct boundaries between the farms exist in the form of mountains, roads, and fencing (pers. obs.). Each farm uses gates and various types of agricultural fencing to separate pastures, which vary greatly in size. These fences have varying levels of restriction to tortoises; from little (e.g. low tensile wire fence) to full (e.g. chain-link fence).

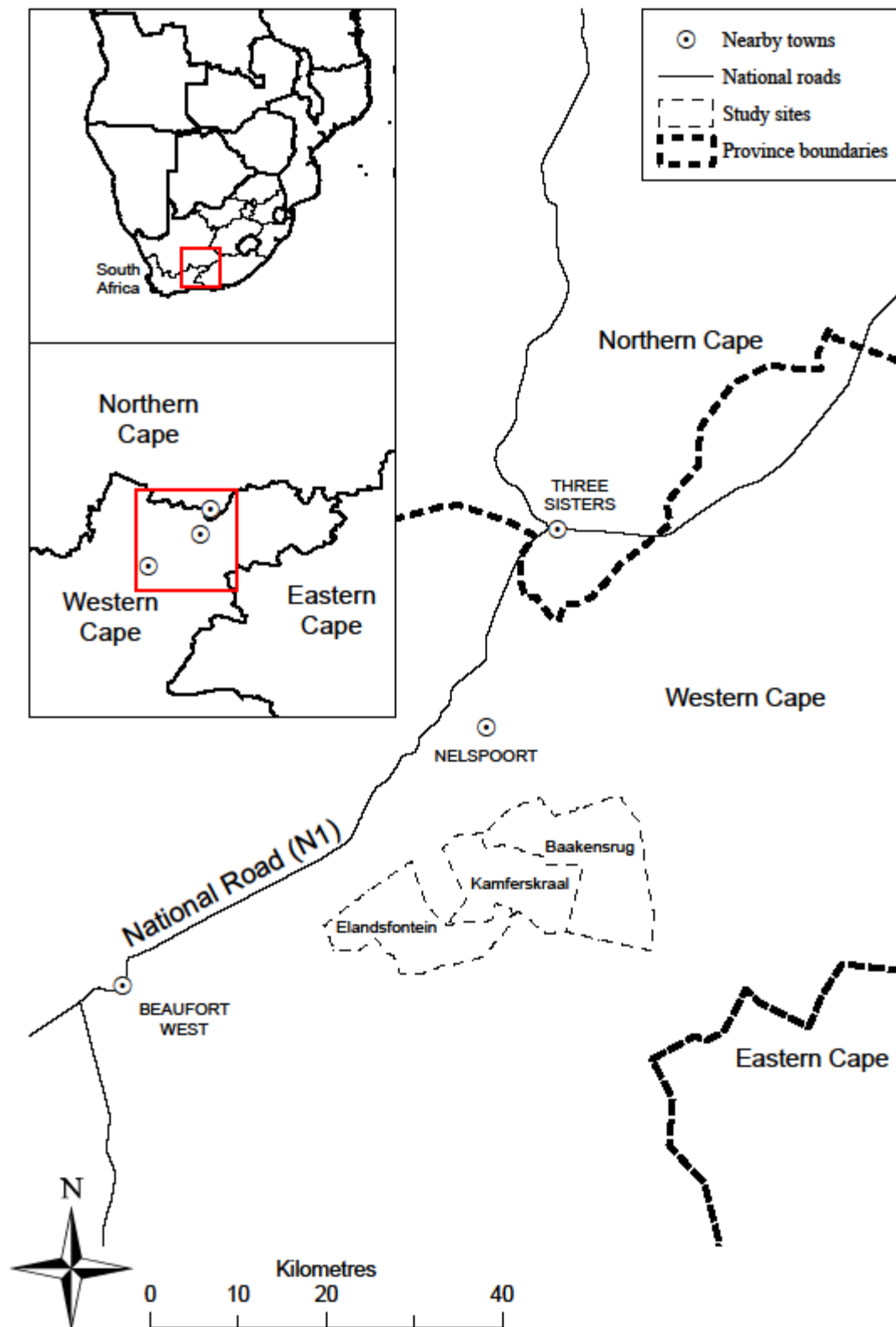


Fig. 2.1. Study sites. Local area map of study sites near Beaufort West, South Africa.

2.2. Fieldwork

Adult leopard tortoises were initially located by walking morning and evening transects within study areas during November and December 2014. Transect locations were determined using the 'Create Random Points' tool in ArcGIS 10.3.1 (ESRI, CA, USA). Most of these were away from croplands, buildings and manmade watering points, to buffer effects of anthropogenic environments. Haglöf Mantax Blue callipers (Haglöf, Långsele, Sweden) were used to measure straight carapace length (SCL), straight carapace width (SCW) and straight carapace height (SCH), whilst digital hanging scales (Pesola, Schindellegi, Switzerland) were used to measure mass (to nearest ± 0.1 kg). Geolocation was recorded using a Garmin eTrex 10 Worldwide Handheld GPS Navigator (Garmin, Schaffhausen, Switzerland). We had ethical clearance from the University of KwaZulu-Natal Ethics Committee (020/15/animal).

Unique GPS-GSM/UHF transmitters (Wireless Wildlife, Pretoria, South Africa) were attached to the carapace of adult tortoises ($n = 10$) in late 2014. These transmitters weighed 74g each, less than 1.05% mass of the smallest tortoise used, and much lower than the 5% suggested by Gursky (1998). To avoid inhibiting mating attempts, transmitters were attached to the front of carapace for females and the back of carapace for males. Care was taken to avoid placing transmitters across scutes to avoid problems relating to growth. Transmitters were programmed to receive positioning data at 2-h intervals throughout day and night with a minimum duration of 12 months, based on battery requirements.

The study areas were revisited four times throughout 2015 to download data via a portable solar-powered base station, and for telemetered tortoises to be physically located. Condition of tortoises (general activity, change in body mass, etc.) and status of transmitters were assessed on each occasion. The base station downloaded telemetry data and subsequently sent data via a cell phone network. A CSV file containing raw telemetry data was accessed and downloaded via Wireless Wildlife (<http://www.wireless-wildlife.co.za/>).

2.3. Climatic variables

Temperature and rainfall data were obtained for the region from the South African Weather Service (Pretoria, South Africa) (<http://www.weathersa.co.za/>), using the Beaufort-West weather station (station number: 0092081 5), which is the closest weather station (approximately 45 km). Mean temperature and total rainfall were collected for each month from December 2014 to November 2015. We also obtained long-term temperature and rainfall data to compare the study period to previous years. All available hourly temperature and rainfall data were collected from the same weather station (beginning September 1993). Mean temperature and total rainfall was collected for each month.

2.4. Data screening

As accuracy of home range estimators is affected by precision of GPS fixes (Laver et al., 2015; Lewis et al., 2007; Shimada et al., 2012), data were first screened to remove inaccurate data points. We screened data using the ‘adehabitatLT’ version 0.3.20, ‘adehabitatMA’ version 0.3.10, ‘ade4’ version 1.7-4 and ‘sp’ version 1.2-3 packages in R version 3.1.2 (Calenge, 2006; R Core Development Team, 2014), using RStudio version 0.98.1091 (RStudio Team, 2015). We incorporated aspects of work by Laver et al. (2015), whereby we eliminated data fixes based on z-coordinate error. We compared internal transmitter altitude estimate with approximate heights in digital elevation models (DEMs), freely available from the ‘raster’ version 2.5-2 package (Hijmans, 2015). Data were discarded for points whereby this z-coordinate error (transmitter altitude – DEM altitude) exceeded 100 m, selected based on DEM accuracy. We only used daytime fixes to reduce levels of autocorrelation, as tortoise movement is generally restricted during night-time hours (McMaster and Downs, 2006). Finally, we rejected fixes that were not approximate to

predefined time settings (e.g. > 120 s after intended fix), which would indicate likely error in transmitter functionality or inaccuracy based on receiving satellite data.

2.5. Home range estimation

Prior to carrying out home range estimations, site fidelity—using Mean Squared Distance from Centre of Activity (Laver and Kelly, 2008)—was tested for each individual using the ‘rhr’ version 1.2.909 package in R (Signer and Balkenhol, 2015). The input spatial reference system (EPSG 4326) was transformed to a more accurate spatial reference system for the survey area (EPSG 32734). Site fidelity was tested using 10,000 bootstrap replicates at 95% confidence level.

Reporting of home range estimation was carried out in line with Laver and Kelly (2008). We used KDE_{href} for our analyses (Walter et al., 2011), reporting 95% and 50% isopleths, and the individual’s core home range (Laver and Kelly, 2008; Samuel et al., 1985). Buffers and resolutions were selected based on visual assessment of data. We assessed each home range estimate using asymptote analyses to confirm that home range estimation represents animal’s space use (Laver and Kelly, 2008). For this we used a sampling interval of 50, 20 replications and a consecutive sampling regime. An asymptote was considered reached using a 95% confidence level. It is recommended to exclude individuals if an asymptote is not reached (Laver and Kelly, 2008). We also calculated monthly home range (December 2014 to November 2015) to display changes in area used by each tortoise throughout the year.

2.6. Statistical analyses

All statistical analyses were executed in R (R Core Development Team, 2014), using RStudio (RStudio Team, 2015). We tested for differences in mean body mass of males and females using independent Mann-Whitney U-test. Following confirmation there were no

significant differences between body mass of males and females, biometric data were pooled. We then tested for correlations between tortoise biometrics using Spearman's rank-order correlations for use in generalised linear mixed models (GLMMs). Prior to analyses on home range estimates, we tested for normality, and subsequently log-transformed the data, as recommended by Börger et al. (2006). The One-way ANOVA was used to test for effect of sex on overall home range size. Welch two sample t-tests were used to compare weather data for the study period to previous years. A repeated measures ANOVA (RMANOVA) was used to test effect of month on home range, both for 95% KDE_{href} and core KDE_{href}.

We used GLMMs specifying an identity link function and Gaussian response to produce models to predict effects of several variables on monthly home range size (95% KDE_{href}). Three continuous variables (body mass, mean monthly temperature (°C), and monthly rainfall (mm)) and one fixed variable (sex) were input. Tortoise ID was set as the random variable to account for pseudoreplication. Models to explain data were created and compared using 'MuMIn' version 1.15.6 package (Barton, 2016), which ranked models based on values for Akaike's Information Criterion (AIC_c). We selected all top models ($\Delta AIC_c < 2$) and used model averaging to determine relative importance of each predictor variable. Interaction effects for important predictor variables in were tested using analysis of deviance in 'phia' version 0.2-1 package (De Rosario-Martinez, 2015). All means are reported with standard error (\pm SE).

3. Results

Monthly weather data (mean temperature and total rainfall) were collected for each month from September 1993 to December 2015. Total rainfall during the study period (170.8 mm) was lower than mean yearly rainfall (259.7 mm). However, mean (\pm SE) monthly rainfall for the period (14.2 ± 2.65 mm) did not differ significantly from previous years (21.5 ± 1.43

mm) (Welch two sample t-test, $t_{(12)} = 0.4005$, $P = 0.696$). Mean monthly temperature (18.2 ± 1.36 °C) also did not significantly differ from previous years (17.9 ± 0.26 °C) (Welch two sample t-test, $t_{(12)} = -0.2096$, $P = 0.838$).

The smallest leopard tortoise tracked weighed 7.4 kg (345 mm carapace length) (Table 2.1). The largest individual tracked was a female of 26 kg (540 mm in carapace length). One transmitter was recovered from a dead individual (LPD006) and subsequently attached to a new individual. As such, location data were collected from 11 individuals: five males and six females. Mean (\pm SE) male body mass (11.9 ± 1.53 kg, $n = 5$) did not significantly differ from females (15.6 ± 2.52 kg, $n = 6$) (independent Mann-Whitney U-test, $U = 9$, $P = 0.329$). Biometric variables measured were found to be positively correlated (Supp. 2.1, Supp. 2.2) using Spearman's rank-order correlation ($P \leq 0.05$). As there was a significant correlation between biometric variables, body mass was used to represent tortoise size in subsequent analyses.

Table 2.1: Location, sex, biometrics (individuals' mass and straight carapace length (SCL)) and transmitter deployment information of leopard tortoise individuals in the current study on farmland in the vicinity of Beaufort West, South Africa.

| Individual | Farm | Sex | Mass (g) | SCL (mm) | Days deployed |
|------------|---------------|--------|----------|----------|---------------|
| LPD001 | Baakensrug | Female | 12000 | 368 | 375 |
| LPD002 | Baakensrug | Female | 12200 | 390 | 376 |
| LPD004 | Baakensrug | Male | 7500 | 359 | 377 |
| LPD006* | Baakensrug | Female | 9400 | 345 | 77 |
| LPD010 | Kamferskraal | Female | 24700 | 540 | 372 |
| LPD011 | Kamferskraal | Female | 18300 | 467 | 369 |
| LPD013 | Kamferskraal | Male | 12100 | 431 | 361 |
| LPD015 | Elandsfontein | Male | 14100 | 451 | 359 |
| LPD016 | Elandsfontein | Male | 14700 | 484 | 362 |
| LPD017 | Elandsfontein | Female | 17500 | 455 | 359 |
| LPD048 | Baakensrug | Male | 9300 | 391 | 283 |

Note: LPD006 was found dead through course of study. The GPS transmitter was recovered and reattached to a new individual (LPD048).

Leopard tortoise location data were obtained from November 2014 to December 2015 for a minimum of 359 days. Including recovery and redeployment of one transmitter, location data were analysed from 11 individuals. A total of 43,392 data points were collected (Supp. 2.3). After screening for suspected fix errors, time errors and daytime-only data, the dataset consisted of 12,090 data fixes. The data screening process removed an average of 69.4 % of fixes per individual. Data were collected for approximately one year for 9 individuals, with the remaining transmitter split between two individuals. We used site fidelity tests to confirm whether individuals qualified for reporting of home range analysis. Two female individuals

(LPD002 and LPD011) did not display site fidelity. The latter appeared to relocate during the first three months of the study.

Home range estimates were carried out for the five males and four females that met site fidelity tests. Estimates for home range size varied greatly between individuals, showing individual variability in home range estimates (Table 2.2). Mean home range was 121.86 ± 28.12 ha (range 40.53—258.52 ha) for 95% KDE_{href}, with a core area of 76.55 ± 17.33 ha (range 21.22—183.89 ha). The individual with the highest estimated home range was for a male tortoise, whilst the smallest estimated home range was for a female tortoise (Fig. 2.2). The mean (\pm SE) home range for males (95% KDE_{href}: 164.11 ± 41.98 ha, core area: 103.25 ± 25.4 ha) was higher than female home range (95% KDE_{href}: 69.04 ± 11.61 ha, core area: 43.17 ± 11.61 ha). However, no significance was found due to sex for estimated 95% KDEs (One-way ANOVA, $F_{(1,7)} = 3.439$, $P = 0.106$) or for core range (One-way ANOVA, $F_{(1,7)} = 5.054$, $P = 0.059$) (Fig. 2.3).

Table 2.2: Home range estimates of leopard tortoises on farmland in the vicinity of Beaufort West, South Africa using kernel density estimation with reference bandwidth, with 95% and 50% isopleths (values in hectares). Core home range and core home range isopleths are also shown.

| Individual | Sex | Bandwidth (h) | KDE _{href} (95%) (ha) | KDE _{href} (50%) (ha) | Core home range (ha) | Core range isopleth (%) |
|------------|--------|------------------|-----------------------------------|-----------------------------------|-------------------------|-------------------------------|
| LPD001 | Female | 101.57 | 84.36 | 18.85 | 50.23 | 82.30 |
| LPD004 | Male | 151.66 | 258.52 | 55.17 | 183.89 | 93.17 |
| LPD006 | Female | 82.61 | 40.53 | 8.79 | 21.22 | 86.56 |
| LPD010 | Female | 68.87 | 60.08 | 11.24 | 45.52 | 79.26 |
| LPD013 | Male | 156.84 | 229.43 | 43.02 | 124.97 | 89.19 |
| LPD015 | Male | 73.09 | 67.70 | 11.37 | 49.22 | 85.61 |
| LPD016 | Male | 175.38 | 205.79 | 45.51 | 109.45 | 84.79 |
| LPD017 | Female | 99.34 | 91.19 | 20.26 | 55.72 | 88.57 |
| LPD048 | Male | 77.32 | 59.10 | 9.64 | 48.72 | 79.69 |
| | | Mean | 121.86 | 24.87 | 76.55 | |

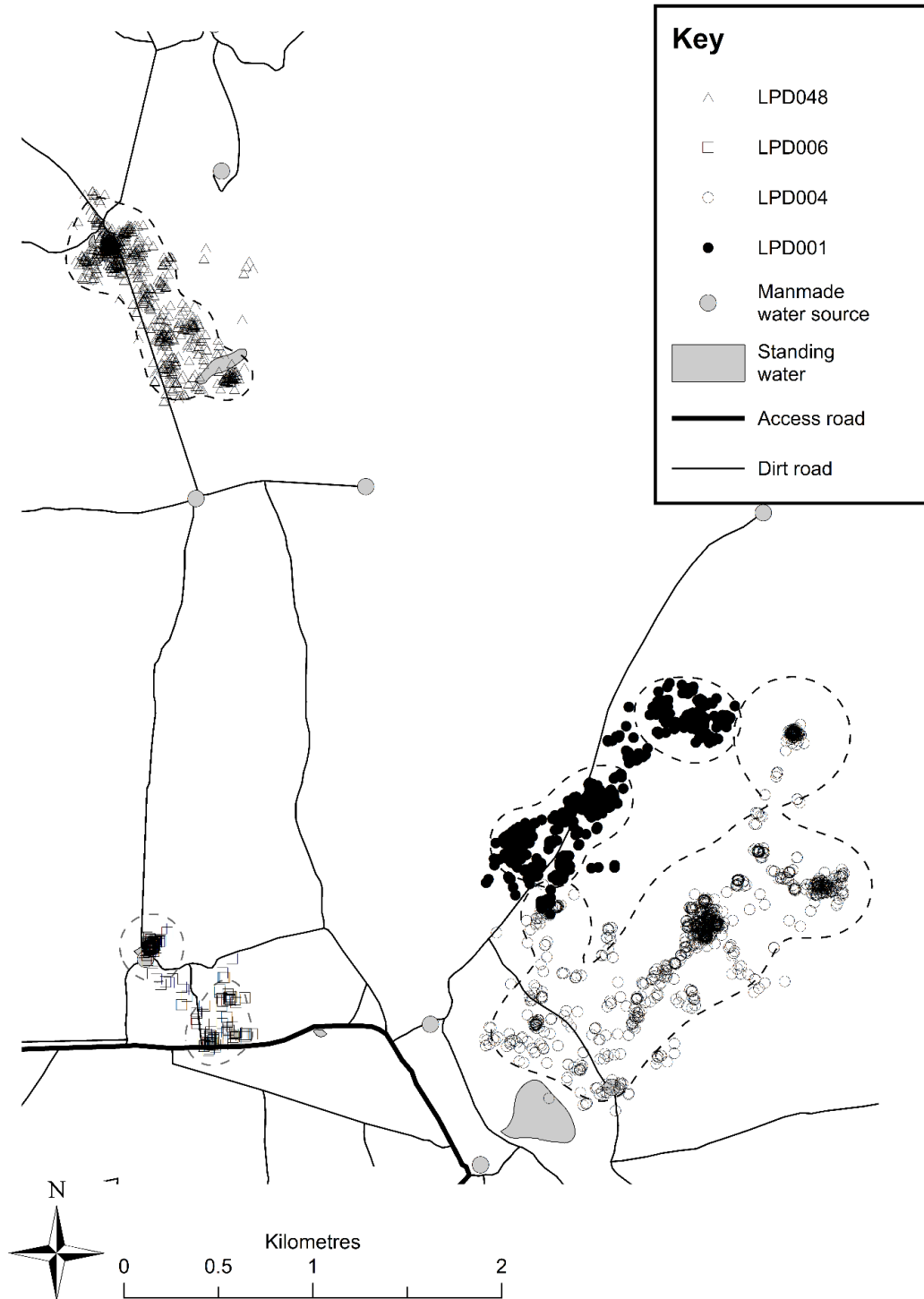


Fig. 2.2. Home range of Baakensrug individuals. Map of part of Baakensrug farm (near Beaufort West, South Africa), showing positions of data fixes and kernel density estimates (95% KDE_{href}) for four leopard tortoise adults.

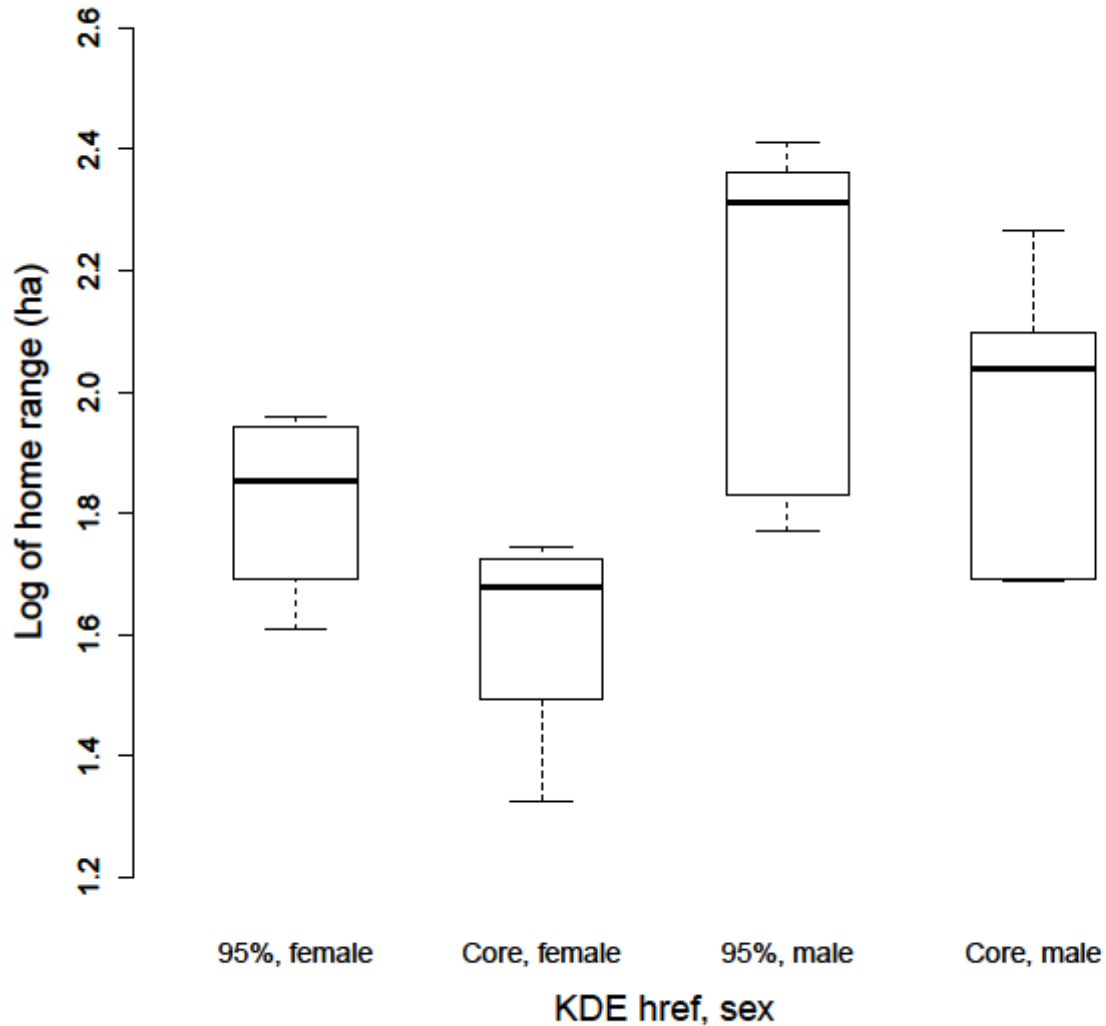


Fig. 2.3. Male vs Female home range estimates. Box plots of home range estimations (log x) for male and female leopard tortoises near Beaufort West, South Africa, using two estimators: 95% kernel density estimation with reference bandwidth (95% KDE_{href}), and core home range estimation using reference bandwidth (core KDE_{href}).

Home range differed significantly between months (RMANOVA, $F_{(11,99)} = 13.714$, $P < 0.001$), with mean July estimates (0.77 ± 0.21 ha) in particular differing significantly from other months (Table 2.3, Fig. 2.4). Individuals used a smaller area in general during winter months compared with other times of the year (Supp. 2.4). Other winter months of June (9.84 ± 4.41 ha)

and August (18.48 ± 10.80 ha) also had lower home range estimates than overall mean monthly home range (83.42 ± 15.32 ha).

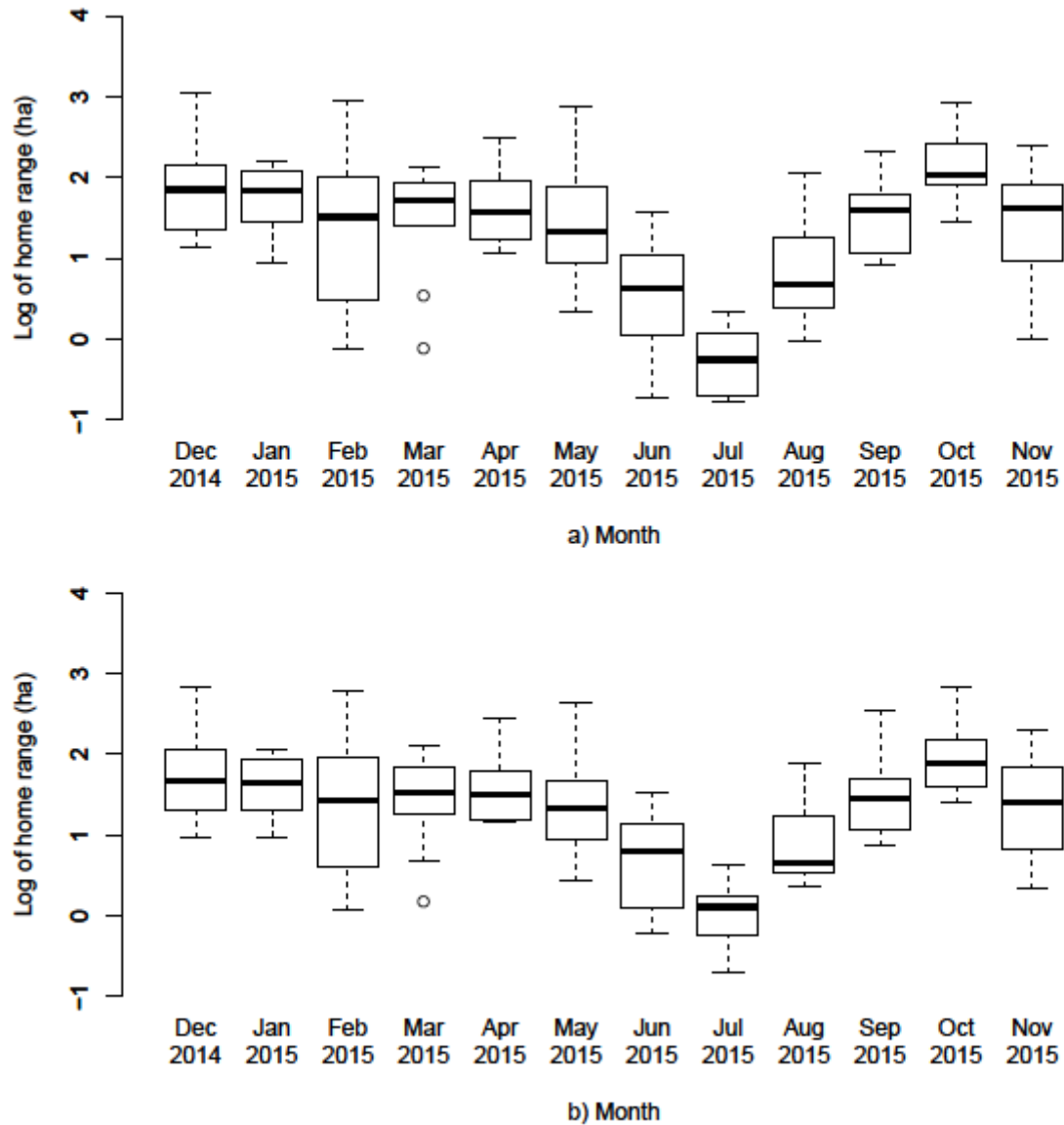


Fig. 2.4. Monthly differences in home range. Home range estimates (log x) comparing effect of month on a) 95% KDE_{href} estimates and b) core home range estimates for adult leopard tortoises tracked near Beaufort West, South Africa.

Table 2.3: Monthly home range estimates (KDE_{href} (95%) (ha)) for each leopard tortoise tracked. Also presented are mean monthly temperature

2 and monthly rainfall. Weather data were obtained from the South African Weather Service (Pretoria, South Africa) for the Beaufort West area, South Africa.

| Individual | Dec-14 | Jan-15 | Feb-15 | Mar-15 | Apr-15 | May-15 | Jun-15 | Jul-15 | Aug-15 | Sep-15 | Oct-15 | Nov-15 |
|-----------------|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| LPD001 | 22.95 | 63.50 | 11.71 | 57.39 | 37.37 | 59.56 | 0.47 | 0.40 | 17.81 | 29.71 | 57.81 | 1.18 |
| LPD002 | 144.50 | 114.39 | 261.19 | 136.69 | 300.62 | 736.02 | 4.36 | 1.52 | 8.73 | 60.65 | 857.86 | 252.17 |
| LPD004 | 143.48 | 121.36 | 64.23 | 66.99 | 91.04 | 19.48 | 36.72 | 0.46 | 113.10 | 9.23 | 101.64 | 225.91 |
| LPD006 | 13.78 | 9.49 | 0.88 | - | - | - | - | - | - | - | - | - |
| LPD010 | 27.60 | 28.25 | 0.75 | 0.78 | 17.06 | 8.80 | 4.06 | 0.18 | 0.92 | 40.81 | 110.45 | 46.14 |
| LPD011 | 1113.91 | 139.87 | 889.48 | 123.39 | 151.53 | 77.37 | 1.09 | 0.17 | 9.73 | 11.84 | 148.10 | 9.32 |
| LPD013 | 86.78 | 154.40 | 154.58 | 84.30 | 36.14 | 142.74 | 4.85 | 0.68 | 24.88 | 211.07 | 265.98 | 80.22 |
| LPD015 | 58.05 | 38.39 | 69.81 | 47.33 | 55.07 | 2.17 | 0.18 | 0.20 | 2.48 | 58.27 | 79.45 | 70.90 |
| LPD016 | 16.67 | 74.09 | 10.26 | 25.11 | 11.74 | 22.56 | 10.79 | 2.18 | 2.43 | 63.61 | 327.41 | 0.98 |
| LPD017 | 200.23 | 8.94 | 32.40 | 3.36 | 15.03 | 5.14 | 34.49 | 0.66 | 2.21 | 38.01 | 27.72 | 11.88 |
| LPD048 | - | - | 0.88 | 26.84 | 20.97 | 11.24 | 1.40 | 1.20 | 2.54 | 8.48 | 89.13 | 36.27 |
| Mean HR | 182.80 | 75.27 | 136.02 | 57.22 | 73.66 | 108.51 | 9.84 | 0.77 | 18.48 | 53.17 | 206.56 | 73.50 |
| Temp (°C) | 23.6 | 25.9 | 23.3 | 22.9 | 17.1 | 16.7 | 11.6 | 10.9 | 15 | 15.4 | 20.9 | 20 |
| Total Rain (mm) | 23.6 | 7 | 13.2 | 28.4 | 0 | 1.2 | 15.2 | 20.4 | 25 | 8.8 | 17.8 | 10.2 |

The GLMM analysis of monthly area used, as determined by 95% KDE_{href}, included just one top model ($\Delta\text{AIC}_c < 2$) (Table 2.4). The top model showed that all included predictor variables were important in predicting monthly home range: sex (males larger than females), body mass (negative effect), temperature (positive effect) and rainfall (negative effect) (Table 2.5). No significant interactive effects were found between predictor variables (Table 2.6).

Table 2.4: Results from top GLMMs comparing model fitness for home range (95% KDE_{href}) for 11 leopard tortoises over 12 months. Only one model was considered a top model ($\Delta\text{AIC}_c < 2$). Predictor variables include sex, body mass (g), mean temperature, and total rainfall. The temperature and rainfall variables were collected for each month, using data obtained from the South African Weather Service (Pretoria, South Africa).

| Model | df | Log likelihood | AIC _c | ΔAIC_c | w_i |
|--------------------|----|-------------------|------------------|----------------------|-------|
| Sex+mass+temp+rain | 7 | -741.337 | 1497.692 | 0.000 | 0.939 |
| Sex+mass+temp | 6 | -745.648 | 1504.054 | 6.361 | 0.039 |
| Sex+temp+rain | 6 | -746.800 | 1506.356 | 8.665 | 0.012 |
| Mass+temp+rain | 6 | -747.190 | 1507.137 | 9.445 | 0.008 |

Note: df = degrees of freedom, ΔAIC_c = deviation for AIC_c compared with top model, w_i = AIC_c weight.

Table 2.5: Fixed effects, coefficient estimates and confidence intervals for variables explaining variance in monthly home range (95% KDE_{href}) in leopard tortoises, based on top GLMM model. Predictor variables shown include sex, and standardized continuous variables of body mass, mean monthly temperature and monthly rainfall.

| | Fixed effects | Coefficient estimate | Coefficient SE | Confidence intervals | |
|--------------|---------------|----------------------|----------------|----------------------|---------|
| | | | | 2.5% | 97.5% |
| (Intercept) | 124.292 | 124.29 | 41.26 | 49.562 | 199.015 |
| Sex = male † | -80.283 | -80.28 | 64.29 | -196.733 | 36.143 |
| Mass | -56.129 | -56.13 | 64.51 | -172.957 | 60.728 |
| Temperature | 88.042 | 88.04 | 28.18 | 33.120 | 143.492 |
| Rainfall | -9.559 | -9.56 | 28.12 | -64.636 | 45.542 |

† = Females were used as reference for sex variable.

Table 2.6: Analysis of deviance table for predictor variables of monthly home range of leopard tortoises giving interactive effects of statistically significant predictor variables for predicting their monthly home range. Predictor variables are shown alone, and with potential interactive variables, along with likelihood ratio (LR) chi-squared statistic, degrees of freedom (df) and statistical significance (probability) values.

| Predictor variables | LR | df | Probability |
|--------------------------|-------|----|-------------|
| Mass | 2.059 | 1 | P = 0.151 |
| Sex | 5.095 | 1 | P = 0.024 |
| Temperature | 7.520 | 1 | P = 0.006 |
| Rainfall | 0.087 | 1 | P = 0.768 |
| Mass : Sex | 0.418 | 1 | P = 0.518 |
| Mass : Temperature | 0.005 | 1 | P = 0.944 |
| Mass : Rainfall | 0.824 | 1 | P = 0.364 |
| Sex : Temperature | 1.096 | 1 | P = 0.295 |
| Sex : Rainfall | 0.356 | 1 | P = 0.550 |
| Mass : Sex : Temperature | 0.002 | 1 | P = 0.962 |
| Mass : Sex : Rainfall | 0.414 | 1 | P = 0.520 |

4. Discussion

Due to use of GPS transmitters, our study represents the most standardised dataset on space use and home range of leopard tortoises thus far, therefore giving potentially more accurate results compared with previous studies (see Table 2.7). GPS telemetry is an excellent tool for detailed movement and home range studies as animal locations are recorded systematically and without visual interference to the animals. However, because of this, behaviour can only be estimated throughout this period, whilst fix accuracy and battery longevity is also reduced, limiting effectiveness of studies. With GPS telemetry, there can be a trade-off between battery life and fix frequency. Due to selected frequency of location fixes, internal batteries were not estimated to last beyond the survey period in the current study.

Our telemetry study of leopard tortoises produced a mean home range estimate of 121.86 \pm 28.12 ha (n = 9) using the 95% KDE_{href} estimation method, with estimates ranging from 40.53 ha to 258.52 ha. The range for estimated core area similarly varied, from 21.22 ha to 183.89 ha. These results emphasise the importance of individual variability. Differences in an animal's resource search behaviour, dietary preferences, and social ability are likely to affect area covered. This is shown further by two individuals that did not display site fidelity. In these instances, it appears that some individuals may be more nomadic, as they did not associate with specific areas. Nomadic behaviour has been identified previously in at least one yellow-footed tortoise *Chelonoidis denticulata* (Guzmán and Stevenson, 2008), whilst Rall (1985) noted two leopard tortoise individuals did not have a clearly defined area. Additional reports that Texas tortoises *Gopherus berlandieri* were primarily nomadic (Auffenberg and Weaver 1969) were disputed in subsequent studies where individuals were found to hold a home range (Kazmaier et al., 2002).

Table 2.7: Summarised results from previously published data on home range estimates for leopard tortoises in southern Africa.

| Reference | Location | Relocations | Home range (ha) | Tracking method | n | Duration | Home range estimator |
|---------------------------|-----------------------------|-------------|-----------------|-----------------|----|--------------|----------------------|
| Bertram, 1979 * | Tanzania | Unknown | 160 | Radiotelemetry | 1 | 10 months | Unknown |
| Rall, 1985 | Free State, South Africa | 8 to 14 | 13.07 | Radiotelemetry | 3 | 14 days | Unknown |
| Hailey and Coulson, 1996 | Zimbabwe | Continuous | 26 | Thread-trailing | 6 | < 10 days | MCP |
| Mason and Weatherby, 1996 | Eastern Cape, South Africa | Unknown | 57.56 | Radiotelemetry | 10 | 9 months | Unknown |
| McMaster and Downs, 2009 | Northern Cape, South Africa | > 200 | c. 350 | Radiotelemetry | 14 | 18 months | MCP |
| Wimberger et al., 2009 † | KwaZulu-Natal, South Africa | Unknown | 35.42 | Radiotelemetry | 15 | 10-25 months | MCP |
| Monadjem et al., 2013 | Swaziland | 65 | 13.49 | Radiotelemetry | 6 | 9 months | MCP |

* = Bertram (1979) was investigating homing ability of leopard tortoises after a relocation. † = Wimberger et al. (2009) concede that “tortoises had probably not yet developed a home range”, as tortoises were released into wild from captivity.

Guzmán and Stevenson (2008) proposed that such nomadic behaviour is occasionally expected in all individuals, as an animal uses nomadic behaviour to increase knowledge and awareness of surroundings and resource availability. Occasional nomadic behaviour serves to update an animal's cognitive map (Gautestad, 2011). Whilst occasional nomadic behaviour for purposes of updating cognitive maps is a reasonable explanation, it is also likely that individuals vary in their maintenance of home ranges. Individual variation in movement and migration patterns in Galápagos tortoises (*Chelonoidis* spp.) and Aldabra tortoises (*Aldabrachelys gigantea*) has been described, whereby prolonged sedentary phases in relatively small areas are interrupted by altitudinal migration in response to vegetation dynamics (Gibson and Hamilton, 1983, Blake et al., 2013). However, no information exists on whether giant tortoises use the same areas seasonally between migration events.

In the current study initial handling and transmitter deployment may have caused the two individuals to move away from the immediate area, however this behaviour was not seen in other individuals. The two nomadic individuals were both females. We propose that nomadic behaviour in leopard tortoises is a consequence of their searching for optimal conditions and resources, which is likely to vary based the individual, seasonal, and breeding requirements. It is unknown whether these two individuals mated or laid eggs during this period. However, one of the two (LPD011) appeared to stay within a small area (75 ha) for two months soon after the initial relocation, before moving on once more to an area with increased availability of food, shelter, and refugia (Supp. 2.5). Contrastingly, the other individual (LPD002) exhibited apparent nomadic behaviour for the entire study period (Supp. 2.6).

The large individual variability in our results was similar to those seen in previous leopard tortoise home range studies (Hailey and Coulson, 1996; Mason and Weatherby, 1996; McMaster

and Downs, 2009; Monadjem et al., 2013), and adds to the ever-increasing examples in the literature of ecological individual variation within populations and species. Such examples of individual variability include dietary preferences (Pagani-Núñez et al., 2016), social structure (Pinter-Wollman et al., 2013), and behavioural responses to environmental conditions (McMaster and Downs, 2013b).

The mean (\pm SE) home range estimate was smaller than leopard tortoises in the Northern Cape Province (205.41 ± 45.57 ha) (McMaster and Downs, 2009), but larger than those in the Eastern Cape (56.76 ± 79.17 ha) (Mason and Weatherby, 1996), Swaziland (13.49 ± 6.93 ha) (Monadjem et al., 2013), Zimbabwe (26 ha) (Hailey and Coulson, 1996) and Tanzania (160 ha) (Bertram, 1979). The previous maximum home range estimate for any individual tortoise is believed to be a female leopard tortoise, which covered an area of 1,247.51 ha (McMaster and Downs, 2009). However, tests for site fidelity and asymptote analyses were not carried out in any of the previous leopard tortoise studies. As such, it is not possible to rule out presence of nomadic behaviour. Home range size was also estimated for rehabilitated tortoises in KwaZulu-Natal (35.42 ha) (Wimberger et al., 2009), but it was conceded that released tortoises probably had not established home ranges within the study period. None of these aforementioned studies used KDEs for their estimates, and therefore comparisons between estimates may not be appropriate. We recommend that future tortoise home range studies (as with most other taxa) attempt to use kernels and follow recommendations by Laver and Kelly (2008) to ensure reproducibility, improvements in accuracy of home range estimation, and an increased ability to make comparisons between studies.

Variations in home range of tortoise species and populations have previously been attributed to variations in habitat and resource availability between study sites (Monadjem et al.,

2013; Van Bloemestein, 2005). An element of the metabolic theory of ecology (Brown et al., 2004), whereby larger animals utilise a larger area, can also explain this variation, and is apparently supported by multiple species of tortoises (Slavenko et al., 2016). However, the theory is not widely accepted, with some suggesting the theory is poorly tested and not supported by certain aspects of ecology and physiology (O'Connor et al., 2007). Resource availability and individual requirements are more likely to be important in variations in home range estimations.

The effect that individual size has in determining home range may also relate to effects of age. However, movement ecology of juvenile and sub-adult tortoises remains largely unknown. In addition, further research is required to investigate how site fidelity and home range are developed in individuals in these cohorts. This is especially important as juvenile tortoises—and adults of smaller species—are more susceptible to predation, as predators are usually better equipped to feed on smaller or younger individuals; e.g. monitors, raptors, corvids (Malan and Branch, 1992, Berry et al., 2013, Branch et al., 2015). It has also been suggested that land use change in some areas has further increased exposure and vulnerability of smaller individuals, due to reduced cover associated with increased livestock densities in commercial farmland (Milton et al., 1999). Further understanding of juveniles, coupled with knowledge of predator species can help direct conservation attention to these areas, and aim to reduce predation rates by controlling such predators.

Distinct differences in morphological features, including body mass and carapace shape, of eastern and western populations of leopard tortoises, has previously been identified, with individuals in western populations (e.g. Central Karoo and Namibia) growing to much larger sizes than those in eastern populations (e.g. KwaZulu-Natal, Swaziland) (Hofmeyr et al., 2014). Biometrics probably has an effect on differences in home range estimates between populations,

although resource availability is likely to be the primary reason. Our GLMMs identified just one top model, which indicated that all input predictor variables (sex, body mass, temperature, rainfall) had an effect on monthly home range estimates, whilst no statistically significant interaction effects were found. The top model suggests that females were more likely to have a larger monthly home range than males, although this was not identified for overall home range estimates. The top model also suggested that monthly home range decreased as an individual's mass increased. It is likely that age may be more important than size, as more mature individuals are likely to have stronger links to specific environments, and could, therefore, be more efficient at resource searching, resulting in smaller home range estimates. Larger sample sizes (10 of each sex) may be required to better quantify effect of sex on home range size, whilst individuals of known age may be required to test age effects.

Environmental conditions influence spatial and temporal habitat use, activity, diet and reproductive ability, particularly in ectotherms (Brown and Weatherhead, 2000; Currylow et al., 2015). The top model included both mean monthly temperature and monthly rainfall as important predictor variables. The model suggested that home range increases with temperature, and decreases with rainfall. Elevations in temperature increase an ectotherm's metabolic functions (Dunham et al., 1989), also increasing the individual's need to search for resources. Seasonal habitat use and site selection for ectotherms can be strongly determined by conditions of such animals (Dunham et al., 1989). Whilst leopard tortoises do not brumate, area used decreased in winter months, particularly June and July.

Conversely, increased rainfall decreases the need to search for resources, as water and food resources are likely to be more abundant. Whilst mean monthly temperature is relatively predictable for the Karoo, rainfall is sporadic and less predictable (van Rooyen, 1999). As there

were very few instances of significant rainfall, monthly responses to rainfall would be difficult to identify. Additionally, home ranges were estimated in a dry year. Whilst rainfall was not significantly lower than previous years, rainfall for 2015 was 114 mm lower than the preceding year, which had above mean rainfall (271.4 mm). Lower rainfall may have affected home range estimation, as it is well documented that tortoise movement is influenced by rainfall (Rose and Judd, 1975, Bertram 1979, Medica et al., 1980, Duda et al., 1999), although the home range response to rainfall is likely much more complex. For example, Bertram (1979) noted that during winter and spring, 57% of leopard tortoise movement occurred on the 14% of days with rainfall. As food resources would also be expected to decrease in drier years, tortoises may increase home range in response to fewer resources. For example, species in more arid areas, such as desert tortoises (*Gopherus agassizii*) (Duda et al., 1999), have larger home ranges than species in higher rainfall regions, such as gopher tortoises (*Gopherus polyphemus*) (Diemer, 1992). However, Duda et al. (1999) found that both male and female desert tortoises had smaller home ranges during drought years. In addition, previous studies on leopard tortoises found that activity (walking and feeding) and home range size were not correlated with rainfall (McMaster and Downs, 2013a, Monadjem et al., 2013). Ideally, home range studies should take place over multiple seasons, to improve the accuracy in predicting variables that affect home range. Additionally, other predictor variables that were not tested in our models may be important.

Several individuals did not visit any permanent or temporary water sources: out of the 11 individuals, only three visited such areas. This is in contrast to the study by McMaster and Downs (2009), where home range of several individuals overlapped at known watering points. Several non-telemetered individuals were located at watering points (Drabik-Hamshare, unpublished data). Telemetered individuals were initially captured away from such areas, although at least two

visited a water source once or twice during the year of study. Therefore, free standing water sources may be of little importance to leopard tortoises. Instead in the absence of these water sources, water intake may primarily be through dietary intake as preformed and metabolic water in ingested food. However, as stated above, the lack of rainfall during the year of study may have affected the association of tortoises with rainfall and water sources in the current study.

Conclusions

This study contributes to the understanding of home range in adult leopard tortoises on private livestock farmland in an area that is threatened by the introduction of fracking operations and continued habitat fragmentation. We found variability in individual home range and behaviour in terms of spatial use, with two individuals displaying apparent nomadic behaviour. We propose that nomadic behaviour in leopard tortoises is due to the search for optimal environmental conditions and resource accessibility, which varies based on individual, seasonal, and breeding requirements. Variability in spatial use emphasised the importance of tracking multiple individuals in home range studies, assisting in identifying spatial behaviour variability within populations (e.g. minimum and maximum areas used, or nomadic behaviour). Simply reporting of mean results may not account for the dynamic nature of home range estimates for populations. In addition to individual plasticity, our models indicate importance of sex, mass and weather variables, although further predictor variables, such as habitat and food availability, should also be investigated. The importance of our study is exemplified given the current unknown effects of fracking activities in the Karoo, and given the importance of tortoises to their environments. It is suggested that our study is used as a precursor for future leopard tortoise home range studies in areas where future fracking activities occur to investigate its possible effects.

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Legends for figures

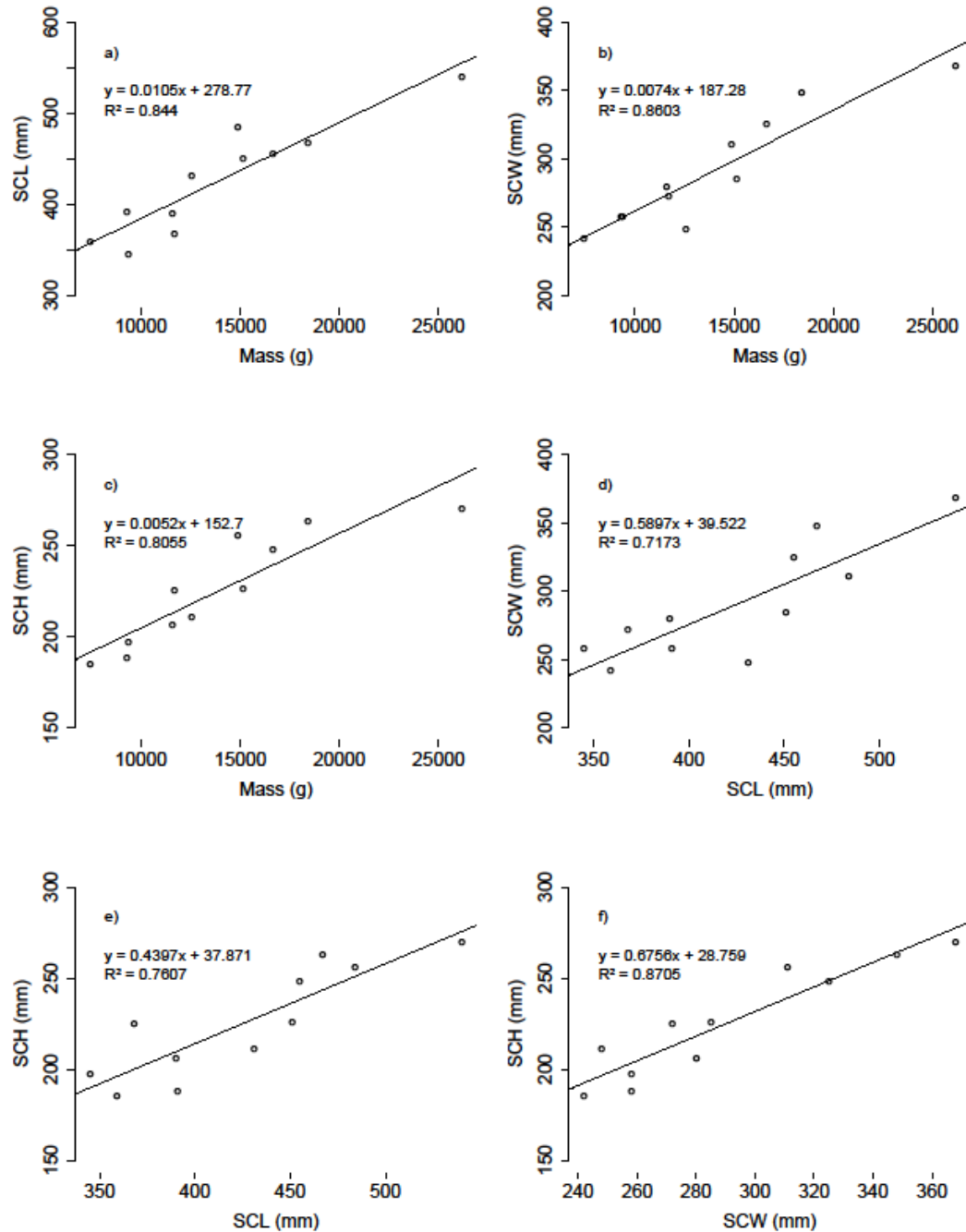
Fig. 2.1. Study sites. Local area map of study sites near Beaufort West, South Africa.

Fig. 2.2. Home range of Baakensrug individuals. Map of part of Baakensrug farm (near Beaufort West, South Africa), showing positions of data fixes and kernel density estimates (95% KDE_{href}) for four leopard tortoise adults.

Fig. 2.3. Male vs Female home range estimates. Box plots of home range estimations (log x) for male and female leopard tortoises near Beaufort West, South Africa, using two estimators: 95% kernel density estimation with reference bandwidth (95% KDE_{href}), and core home range estimation using reference bandwidth (core KDE_{href}).

Fig. 2.4. Monthly differences in home range. Home range estimates (log x) comparing effect of month on a) 95% KDE_{href} estimates and b) core home range estimates for adult leopard tortoises tracked near Beaufort West, South Africa.

Supplementary material



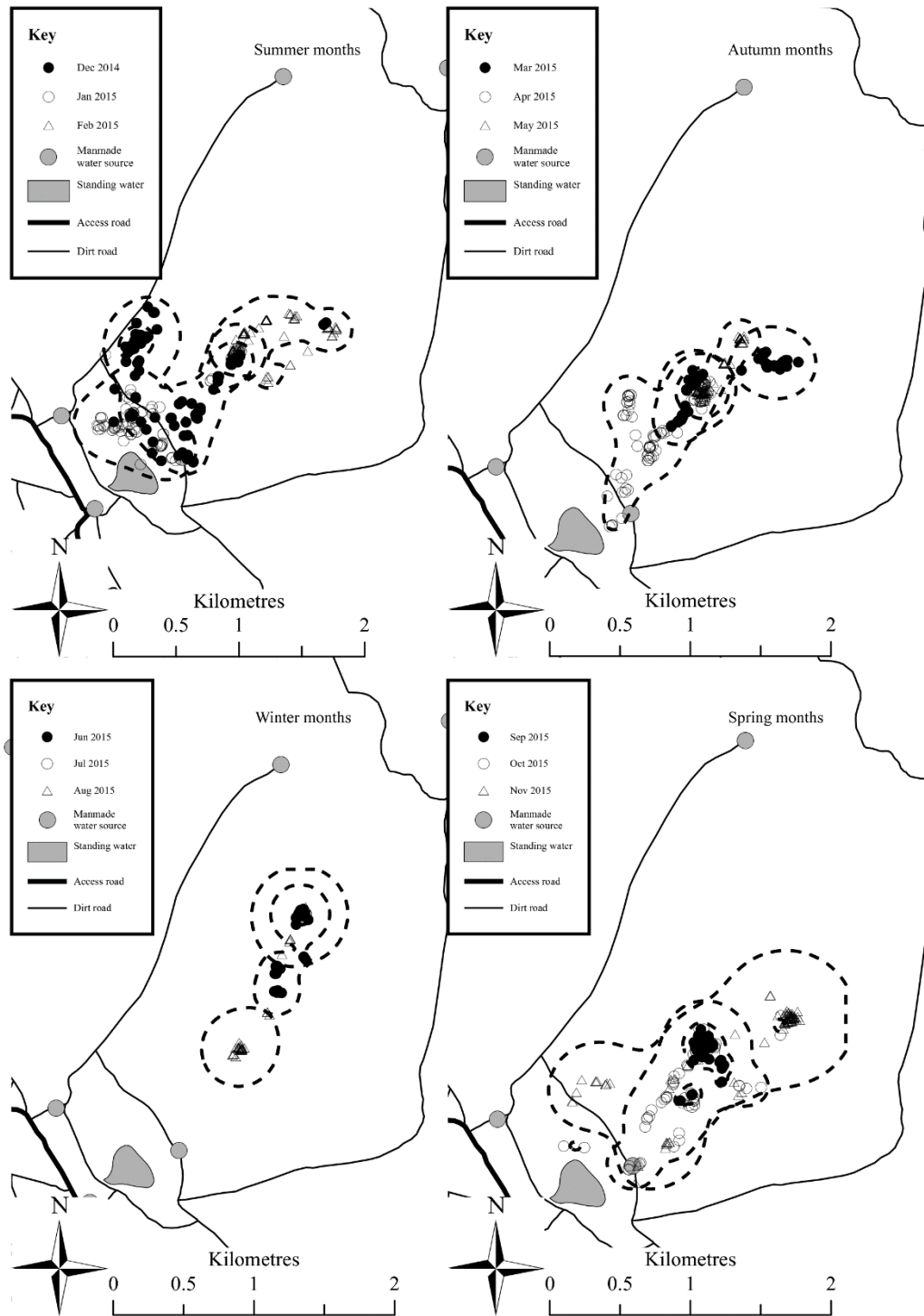
Supp. 2.1. Correlations in biometric measurements. Scatter plots for biometric measurements for telemetered leopard tortoises near Beaufort West, South Africa: a) Mass vs SCL, b) Mass vs SCW, c) Mass vs SCH, d) SCL vs SCW, e) SCL vs SCH, f) SCW vs SCH. SCL = straight carapace length, SCW = straight carapace width, SCH = straight carapace height.

Supp. 2.2. Spearman's rank-order correlation statistics for biometric measurements for telemetered leopard tortoises near Beaufort West, South Africa. SCL = straight carapace length, SCW = straight carapace width, SCH = straight carapace height. ρ represents statistical dependence between ranking of two variables.

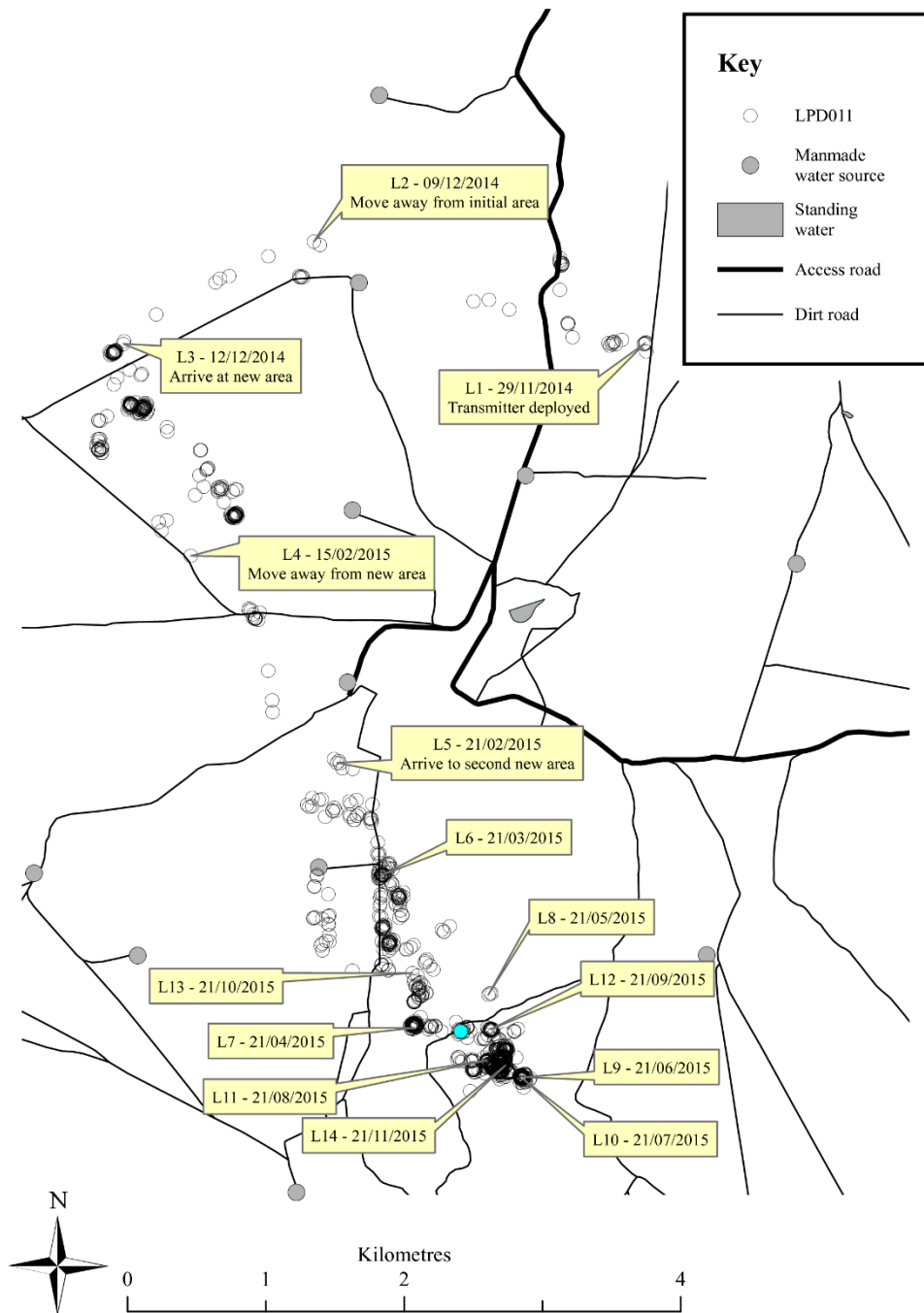
| Variable 1 | Variable 2 | Statistical dependence (ρ) | Probability |
|------------|------------|--------------------------------------|--------------|
| Mass | SCL | 0.8818 | $P < 0.001$ |
| Mass | SCW | 0.9431 | $P < 0.001$ |
| Mass | SCH | 0.9545 | $P < 0.001$ |
| SCL | SCW | 0.8200 | $P = 0.002$ |
| SCL | SCH | 0.8818 | $P < 0.001$ |
| SCW | SCH | 0.9157 | $P < 0.0001$ |

Supp. 2.3. Additional information on biometrics and telemetry data for all telemetered leopard tortoises. A total of 11 tortoises were tracked, with 10 transmitters used. One individual (LPD006) died of unknown causes. The transmitter was recovered and subsequently deployed on another individual (LPD048). Near Beaufort West, South Africa.

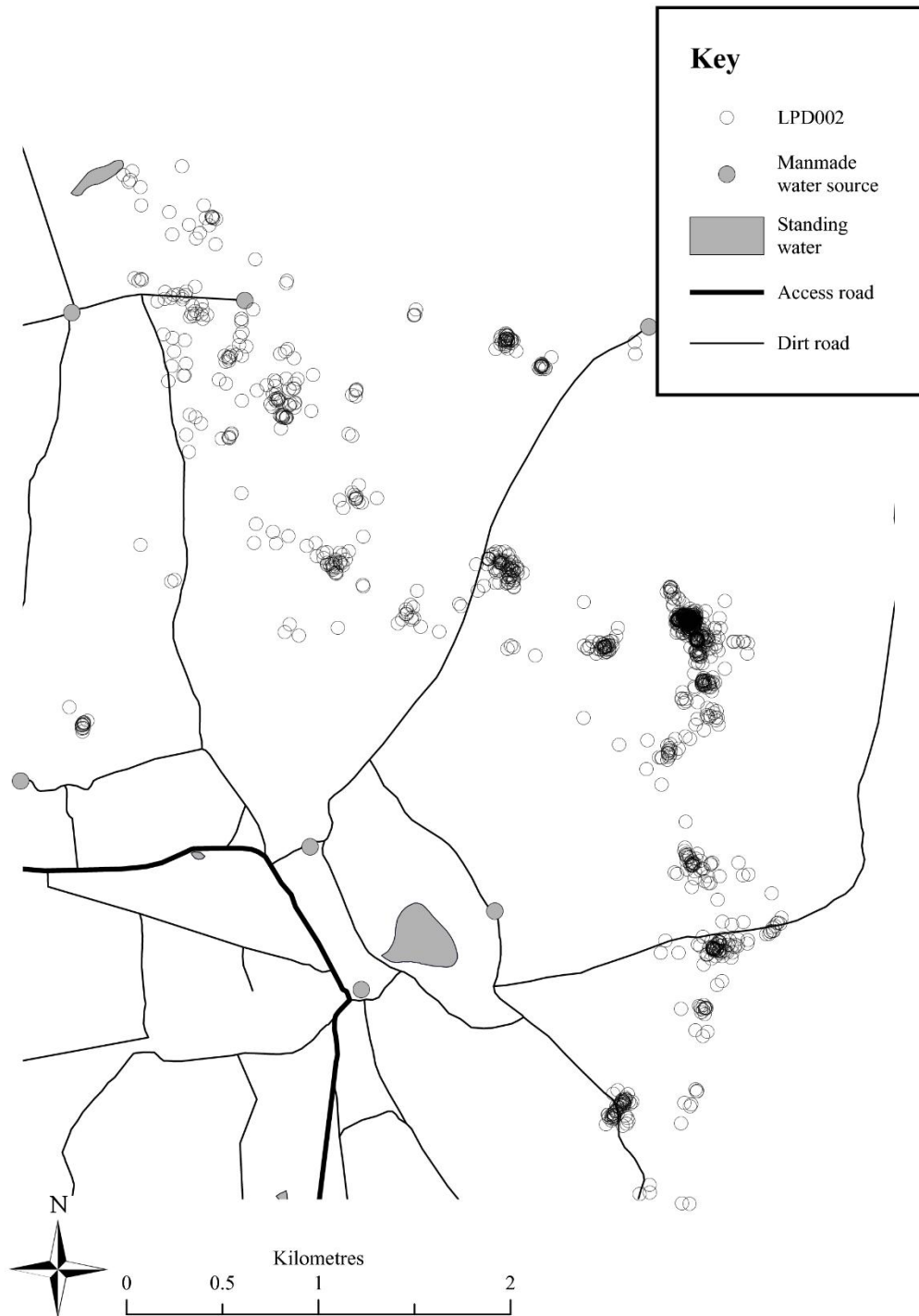
| Individual | SCW (mm) | SCH (mm) | Transmitter deployed | Final data date | GPS fixes pre- screening | Fully-screened data fixes |
|------------|-------------|-------------|-------------------------|--------------------|-----------------------------|------------------------------|
| LPD001 | 272 | 225 | 27/11/2014 | 07/12/2015 | 4486 | 1280 |
| LPD002 | 280 | 206 | 27/11/2014 | 08/12/2015 | 4496 | 1148 |
| LPD004 | 242 | 185 | 27/11/2014 | 09/12/2015 | 4498 | 1167 |
| LPD006 | 258 | 197 | 28/11/2014 | 13/02/2015 | 925 | 612 |
| LPD010 | 368 | 270 | 29/11/2014 | 06/12/2015 | 4447 | 1224 |
| LPD011 | 348 | 263 | 29/11/2014 | 03/12/2015 | 4191 | 1159 |
| LPD013 | 248 | 211 | 04/12/2014 | 30/11/2015 | 4282 | 1129 |
| LPD015 | 245 | 226 | 05/12/2014 | 29/11/2015 | 4173 | 1159 |
| LPD016 | 311 | 256 | 05/12/2014 | 02/12/2015 | 4214 | 1130 |
| LPD017 | 325 | 248 | 05/12/2014 | 29/11/2015 | 4294 | 1196 |
| LPD048 | 258 | 188 | 25/02/2015 | 05/12/2015 | 3386 | 886 |



Supp. 2.4. Seasonal changes in home range size. Monthly data fixes and kernel density estimation (95% KDE_{href}) for one leopard tortoise (Baakensrug farm, near Beaufort West, South Africa).



Supp. 2.5. Nomadic behaviour in LPD011. Map of one leopard tortoise (LPD011) displaying apparent nomadic behaviour (Kamferskraal farm, near Beaufort West, South Africa). The individual displayed no site fidelity. L1-L14 show locations with dates. Individual was initially located at L1, stayed between L3 and L4 for two months, and then used a more defined area from L5 to L14.



Supp. 2.6. Nomadic behaviour in LPD002. Map of one leopard tortoise (LPD002) displaying apparent nomadic behaviour (Baakensrug farm, near Beaufort West, South Africa). Individual displayed no site fidelity.

CHAPTER 3

Movement of leopard tortoises in response to environmental and climatic variables in a semi-arid environment

Martyn Drabik-Hamshare, Colleen T. Downs*

*School of Life Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville,
Pietermaritzburg, South Africa, 3209, Pietermaritzburg, KwaZulu-Natal, South Africa.*

**Corresponding author, email: Downs@ukzn.ac.za; m.drabik.hamshare@gmail.com*

Formatted for Movement Ecology (Provisionally accepted)

*** Corresponding Author:** Colleen T. Downs

Email: downs@ukzn.ac.za

Tel: +27 (0)33 260 5127

ORCID: <http://orcid.org/0000-0001-8334-1510>

Mailing address: University of KwaZulu-Natal, School of Life Sciences, Private Bag X01, Scottsville, Pietermaritzburg, South Africa, 3209

Other email: m.drabik.hamshare@gmail.com

Running header: Movement of leopard tortoises

Abstract

Background: Tortoises (Testudinidae) occur in a wide range of environments, providing important ecosystem functions, such as seed dispersal and refuge in the form of burrows. Tortoise movement has been previously shown to be related to resource availability, reproductive status and local environmental conditions. However, understanding of the variables that drive their movement remains comparatively poor.

Methods: We investigated aspects of movement in leopard tortoises *Stigmochelys pardalis*—the largest and most abundant tortoise species in sub-Saharan Africa—in response to environmental, climatic and individual variables. We used GPS telemetry to calculate bihourly and daily movement and used generalized linear mixed models (GLMMs) to ascertain important predictor variables.

Results: Temperature, distance from water sources, and month were important variables for predicting both bihourly and daily movement. Our results showed that movement increased when individuals were close to known water sources, indicating that individuals close to water resources make regular long distance movements. Movement showed a positive relationship for temperature in both models, whilst rainfall was an important predictor for bihourly movement. Our results displayed aspects of seasonality, with movement highest in spring months, likely related to reproductive activities, although no sex differences were observed.

Conclusions: We identified temporal and spatial conditions in which leopard tortoise movement increased. Our results further support the relationship between water as a resource and movement in leopard tortoises. Individuals used one of two basic movement behaviours in relation to water in this water scarce environment. Either an individual's home range and movements included

permanent water resources allowing internal water storage replenishment, or excluded these with reliance on food resources (such as grasses, forbs, and succulents) for water.

Keywords: Spatial ecology, water loss, Karoo, *Stigmochelys pardalis*, environmental variables, electric fencing.

Background

Continual growth of human population increases need to harvest and distribute essential resources, causing modifications to environments, and subsequent disturbance and contamination of local ecosystems [1]. Such land use change is a primary cause for damage to ecosystems and animal populations [2], as it directly relates to habitat loss, habitat defragmentation, and global warming [3]. It is of great importance to conduct systematic research with regards to potential effects of land use change, in order to produce effective decision-making and management for protection and conservation of endangered and threatened species and habitats. Land use change in the Central Karoo over the last few centuries has greatly affected animal populations, with the vast majority of the pre-existing lands now converted to private commercial farming. Introduction of livestock, building of roads and fences, and reliance of animal and human communities on already depleted water supplies, has negatively affected many animal and plant species. For example, wattled cranes (*Buggeranus carunculatus*), southern bald ibis (*Geronticus calvus*), and Cape vultures (*Gyps coprotheres*)—regionally common before the arrival of Europeans (c. 1650)—are now all but extinct regionally, partly due to changes in availability of water and natural food resources [4-6]. Changes in land use in the Karoo are expected to continue with the introduction of hydraulic fracturing (fracking) activities: a process whereby fuel is extracted from

deep within the Earth's surface following the injection of a highly pressurised liquid fluid [7]. Fracking operations are expected before end of 2017 [8, 9], despite worries about impacts on human and animal communities due to increasing water salinity and altering water quality through accidental release of water runoff [10, 11].

The Karoo is an important ecosystem, as it is seen as a centre for endemism in birds and reptiles [12, 13]. For example, of the 18 tortoise species in sub-Saharan Africa, at least eight species occur somewhere in the Karoo: up to five sympatrically [14-16]. Tortoises are of the most threatened animals, with as many as 80 % classified at least as 'Vulnerable', and 47 % at least as 'Endangered' by the International Union for the Conservation of Nature (IUCN) [14, 17]. The importance of tortoises to their environments is increasingly being understood. Tortoises provide an important ecosystem function in the form of seed dispersal [18, 19] promoted by periodical long distance movement and long gut retention time [20]. This function is particularly importance in xeric areas where natural herbivores are no longer present. Tortoises are considered keystone species in some regions. For example, burrowing species such as *Gopherus* spp. produce refugia used by multiple species to escape harsh environmental conditions [21]. It is important to improve understanding of tortoise spatial ecology.

Tortoises are able to tolerate imbalances in regards to their water:electrolyte ratio [22, 23], allowing a greater ability to survive drought conditions [23, 24]. However, drinking water remains necessary to facilitate urination to remove waste products, which otherwise can cause severe stress and mortality [24]. Several studies investigating spatial ecology of tortoises have identified the positive relationship between movement and water (e.g. permanent water sources or rainfall) with movement typically increased after periods of higher rainfall [22, 25-29]. Increased tortoise movement has also been related to elevated temperatures [30, 31], seasonality (e.g. higher in

spring) [31-34], and reproductive status (search for mates, egg-laying habitat and resources to feed increased energy demand) [32, 35-38]. Resource availability also appears to be of importance. For example, distribution and movement in Aldabra tortoises (*Aldabrachelys gigantea*) appears to be related to resources [39], whilst the Santa Cruz giant tortoise of the Galápagos archipelago (*Chelonoidis nigra*) undertakes seasonal altitudinal migrations in response to vegetation dynamics [40]. In contrast, most other tortoise species maintain home ranges, instead modifying home range size in response to resource availability [22, 34]. Further information is required to better understand interactions between tortoises and environmental conditions.

The leopard tortoise (*Stigmochelys pardalis*) is the largest tortoise species in sub-Saharan Africa, inhabiting a wide range of environmental conditions across the eastern and southern parts of the continent [14, 15]. The species is currently classified by IUCN as ‘Least Concern’ [14, 36], though they appear to be particularly vulnerable to electric fencing, which is common in Karoo farms to control predation on livestock by wild caracal (*Caracal caracal*) and black-backed jackal (*Canis mesomelas*) [41]. Leopard tortoises account for most (> 86 %) electric fencing related reptile mortalities [42, 43], likely related to their size and spatial ecology. As electric fencing is becoming more affordable in South Africa, tortoise mortalities by electrocutions is increasing. Further research is required to find appropriate solutions.

Previous leopard tortoise research has shown great variability in movement distances and home range sizes, likely related to seasonal temperature, food availability, rainfall, mean body mass, and access to other important resources [31, 33, 44]. For example, leopard tortoises were shown to move much larger distances in the Nama-Karoo (up to 8km per day) [33]—even displaying nomadic behaviour in some cases [26]—when compared with populations in valley thicket (up to 100 m per day) [45] and Swaziland (about 50 m per day) [31]. Karoo leopard

tortoises also have larger home range sizes, using areas upwards of 200 ha [33] compared with valley thicket (57.56 ha) and Swaziland (13.49 ha). These studies suggest that movement and home range is higher in areas where resource availability (e.g. food, water, and mates) is decreased. Despite several studies investigating movement of leopard tortoises, information on drivers of movement and habitat use is not fully understood.

Geolocation information helps to understand species interactions, identify important habitats, and quantify the relationship between behaviour and climatic and environmental variables [46]. Improving knowledge of spatial ecology is important to identify biotic and abiotic effects relating to land use, and to guide successful management decisions for species conservation [47]. Global positioning system (GPS) transmitters were deployed on ten wild-caught individuals. We set out to further investigate spatial ecology of leopard tortoises, to a) provide details on movement distances in relation to climatic, environmental and sex variables, b) highlight importance of water and food resources, and c) provide recommendations for electric fencing use in farmland where tortoises are abundant.

We predicted movement would be higher when closer to important resources (e.g. food and water) was reduced, as previous studies have shown increased activity with abundant resources [22]. We predicted climatic variables would influence elevated movement: a) higher temperatures causing increasing metabolic rate; b) higher rainfall, as we expected tortoises would seek natural water sources after rain events [22, 28]. Finally, we expected tortoises would make larger movements during the breeding season (September to November). However, given leopard tortoises can occur at very low densities (0.017 tortoises per ha) [25], we expected mate-searching behaviour by males would produce higher movements overall.

Methods

Study area

The semi-arid Karoo covers much of the Northern, Western and Eastern Cape Provinces of South Africa, covering an area of approximately 37 million ha [48]. Northern and western parts of the Karoo are typically arid, though even in eastern semi-arid areas, rainfall is both unpredictable and unreliable [49, 50]. During summer, daily temperatures of more than 30 °C are regularly recorded [49], whilst severe frost events are also not uncommon [51]. Plants in the region have adapted to such conditions—hairy cuticles, tannins and phenolic compounds [52]—to cope with severe stress and desiccation [48, 49, 53]. Due to common weather conditions, vegetation of the Central Karoo is highly homogenous with typically low levels of endemism [54].

The study was carried out on three private mixed livestock commercial farms in the Central Karoo, Western Cape Province, South Africa (Fig. 3.1). The farms used were Baakensrug, Kamferskraal, and Elandsfontein (approximately 32°15S, 23°E), which are part of the Nelspoort and Beaufort West communities. Each farm utilises aspects of holistic resource management, with rotational grazing of mixed livestock to reduce selective grazing and subsequent desertification [55]. Private hunting of free-roaming game is also present. Whilst the three farms are connected, roads, fences and mountain ranges form distinct boundaries (unpublished observations). These farms use various agricultural fencing to separate pastures of varying sizes and protect livestock. These fences have varying levels of restriction to tortoises; from little (e.g. low tensile wire fence) to full (e.g. chain-link fence). In some areas, farms also use electric fencing to prevent animals digging under agricultural fencing. These electric fences present a major mortality risk for tortoises [42, 43].

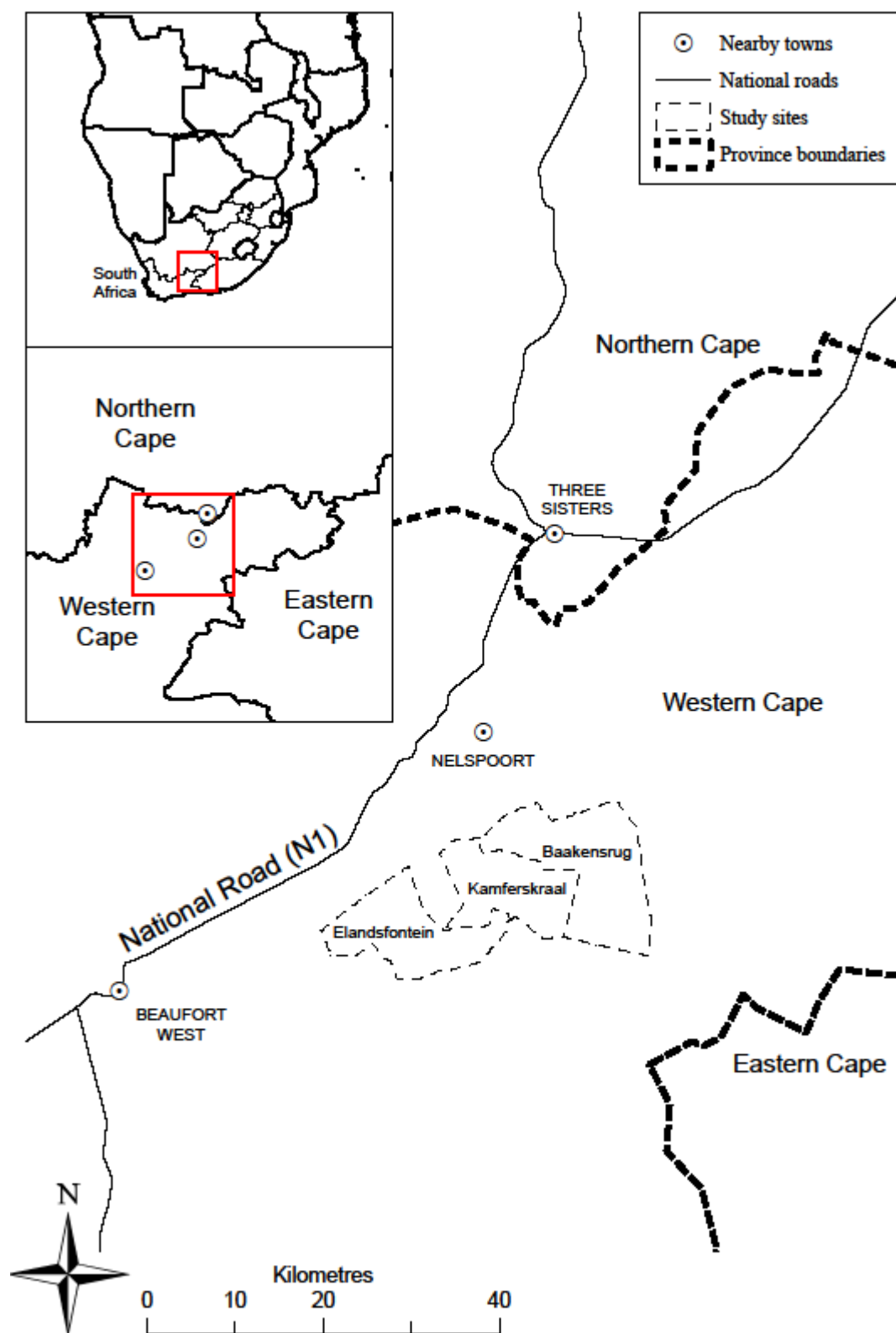


Fig. 3.1. Study sites. Local area map of study sites near Beaufort West, South Africa.

Fieldwork

As tortoises generally have a bimodal activity pattern, especially in spring and summer [28, 56, 57], wild-caught adult leopard tortoises were located by walking 2 km transects ($n = 20$) in mornings and evenings in November and December 2014. Transect locations on farms were determined using random points in ArcGIS 10.3.1 (ESRI, CA, USA). Upon locating each individual, digital hanging scales (Pesola, Schindellegi, Switzerland) were used to measure body mass (g).

Unique GPS-Global System for Mobile Communications (GSM) / Ultra High Frequency (UHF) transmitters (Wireless Wildlife, Pretoria, South Africa) were initially placed on adult leopard tortoises ($n = 10$). Care was taken to avoid placing transmitters across scutes to avoid problems relating to growth. Tortoises were selected based on body mass (mean: 13.92 kg, range: 7.43 to 26.27 kg) and sex. We determined sex of individuals based on plastral concavity, tail length, and shapes of anal scutes and supracaudal shield [58, 59]. The transmitter was placed appropriately on the carapace to avoid inhibiting potential mating events (e.g. front of carapace for females) using dental acrylic. Mass of transmitters (74 g) was much lower (range: 0.28 to 0.99 %) than the suggested 5 % body mass [60]. Transmitters were programmed to receive bihourly geolocation data for a minimum period of 12 months, based on expected internal battery life. Individuals were released at initial point of location within 30 min.

In order to download telemetry data, the study area was revisited four times (approximately every three months) throughout 2015. A base-station was used to communicate with the transmitters to download internally-stored data. The base-station was positioned at high elevations, as direct ‘line-of-sight’ between base-station and transmitters was required. Once downloaded, base-station sent data via a local cell-phone network. Raw telemetry data were downloaded as a

CSV file via Wireless Wildlife [61]. On each visit, attempts were made to physically locate telemetered individuals to assess condition of each tortoise, using recently downloaded data. Whilst no body condition index was used, we assessed condition based on levels of activity, general well-being, and changes to body mass. In instances where individuals could not be physically located, condition was assessed based on recent movement data via Wireless Wildlife. One individual (LPD006) was found to have died of unknown causes during February 2015 after only 88 days. The transmitter was redeployed on a new leopard tortoise individual.

Climatic variables

Hourly temperature and rainfall data were collected from the South African Weather Service (Pretoria, South Africa) [62], using Beaufort-West weather station (station number: 0092081 5), approximately 45 km west of study sites. Data were collected from September 1993 to end of study period (December 2015) to compare study period to previous years. Mean temperature and total amount of rainfall (mm) were calculated for three temporal scales for the study period: bihourly and daily for movement analysis, and monthly for long-term data comparisons.

Data screening

Screening of data were carried out to discard incorrect location fixes using ‘adehabitatLT’ version 0.3.20, ‘adehabitatMA’ version 0.3.10, ‘ade4’ version 1.7-4 and ‘sp’ version 1.2-3 in R version 3.1.2 [63, 64], using RStudio version 0.98.1091 [65]. Data were discarded based on values for extreme horizontal dilution of precision (HDOP) values, incorrect time zones, incomplete or

dubious transmitter data (e.g. negative activity), impossible and improbable movement distances, and z-coordinate error.

Habitat extraction and proximity

A 2014 South Africa land cover layer was downloaded from GEOTERRAIMAGE (Pretoria, South Africa). The land cover layer is a raster that categorises land area as a habitat; for example, grassland, low shrubland, or cultivated commercial fields. ArcGIS was used to crop raster to local area. Habitats were extracted from the raster layer to each GPS location, with extracted results saved as an Excel file. The land cover raster layer was converted to place a point for each 3 m x 3 m pixel.

In addition, two other important layers were also used; inland water areas, and manmade water source points; taken from a 1:50,000 topographical map of South Africa. These two layers represent potentially important water features that may not be recognised by the land cover layer, as the feature is within a forested area (and so would be classified as the top layer) or too small to be recognised in the South Africa land cover layer (manmade wells and feeding or drinking stations for livestock). We calculated an individual's Euclidean distance to water resources (inland water areas and manmade water source points) to create an additional predictor variable for generalized linear mixed models (GLMMs).

For the purpose of identifying associations with habitats that might supply more food resources, we grouped other habitat categories (dense bush, open bush, wetland, grassland, cultivated commercial fields) based on expectations compared to low shrubland and non-vegetated habitats (Table 3.1). We grouped the cultivated commercial field categories, which were previously separated into high, medium or low layers. Other habitat types were excluded, due to

no nearby tortoise location data. We used the proximity function to also calculate distances to a) water resources, b) increased food resources, and 3) cultivation areas.

Table 3.1: Habitat and resource groupings used in the current study. (Habitat classifications were from 2014 South Africa land cover layer, GEOTERRAIMAGE (Pretoria, South Africa). Additional layers include manmade water source points and inland water areas from a 1:50,000 topographical map of South Africa. Resource categories are based on expected access to increased food resources and water.)

| Land cover layer classification | Updated classification | Additional layers | Resource categories |
|---------------------------------|------------------------------|-------------------|---------------------|
| Water seasonal | Water seasonal | - | Water |
| Water permanent | Water permanent | - | Water |
| Wetlands | Wetlands | - | Food |
| Thicket /Dense bush | Dense bush | - | Food |
| Woodlan/Open bush | Open bush | - | Food |
| Grassland | Grassland | - | Food |
| Low shrubland | Low shrubland | - | None |
| Cultivated comm fields (high) | Cultivated commercial fields | - | Food |
| Cultivated comm fields (med) | Cultivated commercial fields | - | Food |
| Cultivated comm fields (low) | Cultivated commercial fields | - | Food |

| | | | |
|---------------------|---------------|-----------------------------|-------|
| Bare none vegetated | Non-vegetated | - | None |
| - | - | Manmade water source points | Water |
| - | - | Inland areas | Water |

Statistics

Prior to calculating distances between tortoise locations, transmitter fix error was quantified. We used Euclidean distances between fix locations and known transmitter locations in Pietermaritzburg, South Africa, prior to transmitter deployment. Test data had a mean (\pm SE) fix error of 17.01 ± 0.59 m (range: 1.78 to 134.78 m).

Distances between transmitter locations and subsequent statistical analyses were carried out in R [63] using RStudio [65]. Bihourly movement was calculated using ‘adehabitatLT’, ‘adehabitatMA’, ‘ade4’ and ‘sp’ [64]. We assumed each movement was Euclidean distance between successive locations [66]. We assumed each location fix was affected by a fix error. We ranked calculated distance for each movement and assumed larger distances were more likely to be due to larger fix errors. Therefore, we corrected each calculated distance by deducting inverse log of the quantile for the known error fixes (Equation 3.1), where d_{rank} is the d_{th} percentile from log transformed known error distribution, d_{est} is estimated distance between points, and d_{corr} is corrected distance.

$$\text{Equation 3.1: } d_{corr} = d_{est} - 10^{\log(d_{rank})}$$

In addition to the above, data was also screen based on z-coordinate error [67]. Internal transmitter altitude estimates were compared with approximate heights in digital elevation models (DEMs)—freely available from ‘raster’ version 2.5-2 package [68]—and discarded when z-coordinate error exceeded 100 m. Fixes were also discarded if time record was not approximate to predefined settings (e.g. > 120 s after intended fix), which would indicate error in transmitter functionality or inaccuracy based on receiving satellite data.

Cumulative distances were calculated for daily and monthly periods for all but one individual: LPD006 was excluded from analyses due to death and reduced amount of data. Bihourly and daily movement distances were tested for normality using a ‘quantile-quantile’ plot using ‘stats’ version 3.1.2 package in R [63]. As these data were heavily right-skewed, log transformations of both bihourly and daily datasets were carried out prior to analysis. As tortoise movement can be strongly affected by environmental conditions [22], we compared the study year to long-term data for the region. We used Welch two sample t-tests to compare monthly mean temperature and total rainfall data to previous years.

GLMMs were used to create and test models to compare effect of predictor variables on bihourly and daily movement. Predictor variables used were a mix of individual, environmental and weather variables; habitat, month, sex, time of day, distance from water source, mean temperature, and total rainfall. Tortoise ID was set as the random variable to account for pseudoreplication. To ensure data were standardised, we used the standardize function in ‘arm’ version 1.8-6 package in R [69]. For daily models, habitat type for each datapoint was determined as most common habitat type used by individual for each day. Time of day was not included in daily analysis, as hour-sensitive data were combined for each day. For the continuous predictor variables in daily models we took mean result for all locations during that day. Aside from

temperature, continuous predictor variables used in bihourly models did not use mean results. All possible combination models were tested using the ‘glmer’ function within ‘lme4’ version 1.1-10 package [70] and ‘dredge’ function using ‘MuMIn’ version 1.15.6 package [71].

Top candidate models ($\Delta AIC_c < 2$) were selected for bihourly and daily GLMMs, with models ranked based on values for AIC_c ; Akaike’s Information Criterion, adjusted for small sample size [72]. As both GLMMs provided more than one top model, model averaging was used to identify important predictor variables and model coefficients based on those variables. All distance moved calculations presented as means are reported with standard error (\pm SE). Interaction effects for important predictor variables in both models were tested using analysis of deviance in ‘phia’ version 0.2-1 package [73]. For bihourly models, we tested the effect of month and time on other variables, whilst month and habitat were tested for daily models. Predictor variables not identified as important were excluded from post-hoc analyses.

Results

Movement summary

Relocation data were collected from 10 telemetry transmitters on adult leopard tortoises from November 2014 to December 2015. LPD048 was tracked for only 283 days, as transmitter was redeployed following death of LPD006. All other individuals were tracked for a minimum of 359 days. In total, 42,467 data points were collected (Table 3.2). The data screening process removed 5,413 data points: a mean (\pm SE) of 541.3 (\pm 77.20) per individual. The final bihourly dataset consisted of 37,054 data points.

Table 3.2: Biological information for each telemetered individual leopard tortoise, along with the number of geolocation fixes used in final analyses for each.

| Individual | Farm | Sex | Biomass (g) | Screened fixes |
|------------|---------------|--------|-------------|----------------|
| LPD001 | Baakensrug | Female | 11,685 | 4017 |
| LPD002 | Baakensrug | Female | 11,580 | 3587 |
| LPD004 | Baakensrug | Male | 7,425 | 4122 |
| LPD006* | Baakensrug | Female | 9,360 | 751 |
| LPD010 | Kamferskraal | Female | 26,167 | 4159 |
| LPD011 | Kamferskraal | Female | 18,400 | 3647 |
| LPD013 | Kamferskraal | Male | 12,560 | 3790 |
| LPD015 | Elandsfontein | Male | 15,125 | 3941 |
| LPD016 | Elandsfontein | Male | 14,870 | 3330 |
| LPD017 | Elandsfontein | Female | 16,638 | 3884 |
| LPD048 | Baakensrug | Male | 9,275 | 2577 |

*Note: LPD006 was found dead through course of study. The GPS unit was recovered and reattached to a new individual (LPD048).

Bi-hourly and daily movement of leopard tortoises were calculated for each individual throughout course of the study period. Overall mean distance moved by leopard tortoises was $257.7 (\pm 3.64)$ m per day (range: 1.79 to 2611.24 m). Males (291.6 ± 6.00 m) appeared to move further than females (225.9 ± 4.11 m), although largest daily distance moved was by a female (2611.24 m). The largest daily distance by a male tortoise was 2477.31 m. Movement varied seasonally, with spring months of September (302.0 ± 14.68 m), October (471.7 ± 20.57 m), and November (295.6 ± 14.66 m) showing largest daily movement distances (Fig. 3.2, Table 3.3). Mean daily movement was consistently above 150 m per day throughout much of the year, but

winter months showed the shortest movement distances; June (162.1 ± 4.84 m), July (157.6 ± 4.09 m), and August (191.1 ± 6.46 m).

Table 3.3: Sex differences in daily movement of leopard tortoises for each month, along with weather conditions. (Weather data supplied by South African Weather Service (Pretoria, South Africa) for Beaufort West area, South Africa).

| Month | Daily distance moved (mean \pm SE) in metres | | | Mean | Total |
|-----------|--|--------------------|--------------------|---------------------|------------------|
| | Total | Male | Female | temperature (°C) | Rainfall (mm) |
| January | 256.02 ± 11.29 | 292.24 ± 18.99 | 227.51 ± 13.16 | 25.3 | 7.0 |
| February | 217.98 ± 9.71 | 200.92 ± 12.97 | 232.31 ± 14.08 | 22.7 | 13.2 |
| March | 248.19 ± 9.10 | 240.66 ± 12.40 | 255.58 ± 13.31 | 22.2 | 28.4 |
| April | 256.54 ± 9.59 | 291.65 ± 6.00 | 260.14 ± 16.05 | 16.8 | 0.0 |
| May | 218.17 ± 8.15 | 208.53 ± 10.14 | 227.82 ± 12.74 | 16.6 | 1.2 |
| June | 162.14 ± 4.84 | 175.43 ± 7.08 | 148.85 ± 6.44 | 11.5 | 15.2 |
| July | 157.56 ± 4.09 | 165.13 ± 6.23 | 149.98 ± 5.24 | 10.6 | 20.4 |
| August | 191.12 ± 6.46 | 212.18 ± 10.30 | 170.47 ± 7.54 | 14.7 | 25.0 |
| September | 302.03 ± 14.68 | 371.05 ± 26.15 | 233.00 ± 10.80 | 15.1 | 8.8 |
| October | 471.69 ± 20.57 | 624.08 ± 29.74 | 319.31 ± 22.64 | 20.7 | 17.8 |
| November | 296.56 ± 14.66 | 390.14 ± 24.41 | 207.24 ± 13.21 | 19.9 | 10.2 |
| December | 306.67 ± 14.31 | 349.76 ± 23.02 | 275.40 ± 17.89 | 23.3 | 23.8 |

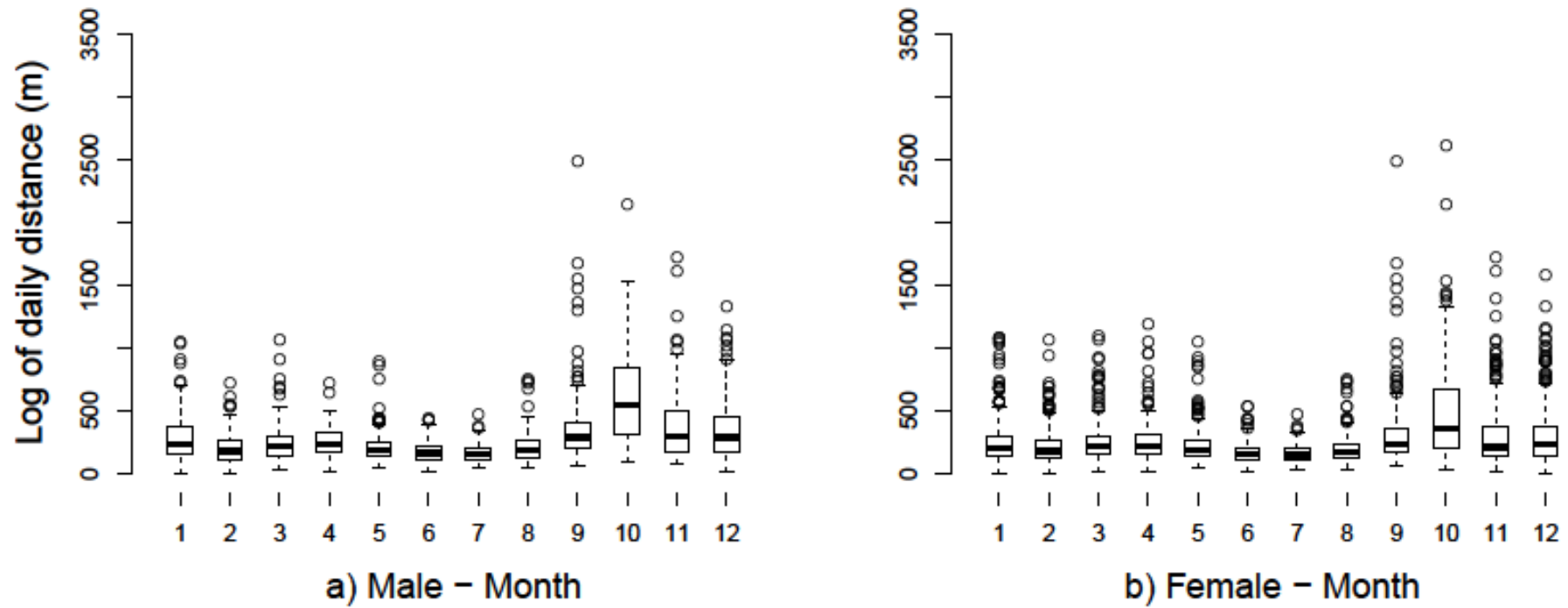


Fig. 3.2. Sex variation in daily movement. Daily movement in adult a) male ($n = 5$), and b) female ($n = 5$) leopard tortoises for each month of study period, near Beaufort West, South Africa. Numbers correspond to months; e.g. 1 = January, 2 = February, 3 = March, etc.

Habitat type associations

Habitat extractions showed variability between individual leopard tortoises. Whilst 85.1 % of all data points were within habitat classified as ‘low shrubland’, two individuals were found in low shrubland habitat less than 50 % of the time. In each, dense bush was an important habitat type, with over 30 % of data points. Use of wetlands (0.05 %), grassland (1.1 %), and non-vegetated (2.1 %) habitats were used infrequently, although amount of land covered by each of these was much lower than low shrubland. There were changes to habitat use throughout the year (Table 3.4), in particular during winter months (June to August), where individuals appeared to stay in low shrubland areas.

The above is also reflected by associations leopard tortoises had with features. Only one telemetered individual (LPD011) approached within 250 m of cultivated commercial fields. Majority of data points showed no association with water resources, with 77.2 % of data points away (> 250 m) from these areas. Only 47.2 % of data points were within close proximities to habitats listed as providing increased food resources.

Table 3.4: Leopard tortoise habitat types used throughout the year. (Numbers represent the number of data points for each habitat type for each month of the year.)

| Habitat type | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Mean (\pm SE) |
|------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|----------------------|
| Low shrubland | 2351 | 1999 | 2312 | 2241 | 2900 | 3019 | 3156 | 2963 | 2672 | 2634 | 2187 | 2659 | 2591.08 \pm 107.34 |
| Non-vegetated | 158 | 140 | 86 | 8 | 34 | 18 | 15 | 50 | 80 | 42 | 25 | 129 | 65.42 \pm 15.15 |
| Dense bush | 203 | 340 | 453 | 137 | 195 | 7 | 14 | 194 | 264 | 321 | 788 | 144 | 255.00 \pm 60.98 |
| Open bush | 120 | 4 | 102 | 191 | 67 | 41 | 20 | 19 | 115 | 174 | 121 | 31 | 83.75 \pm 18.08 |
| Grassland | 2 | 1 | 36 | 1 | 25 | 0 | 0 | 0 | 64 | 55 | 41 | 163 | 32.33 \pm 13.69 |
| Wetlands | 3 | 6 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 1.42 \pm 0.62 |
| Cultivated commercial fields | 0 | 31 | 127 | 3 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 16.75 \pm 10.78 |
| Water permanent | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.50 \pm 0.50 |
| Total | 2837 | 2521 | 3127 | 2581 | 3221 | 3085 | 3205 | 3226 | 3195 | 3268 | 3163 | 3126 | |

Weather comparison to previous years

Mean monthly temperature and total rainfall was calculated for the study period, and for the long-term data available. Mean monthly temperature during study period (18.2 ± 1.36 °C) did not significantly deviate from long-term (from September 1993) monthly temperature (17.9 ± 0.26 °C) (Welch two sample t-test, $t_{(12)} = -0.2096$, $P = 0.838$). Mean monthly rainfall was low (14.2 ± 2.65 mm) when compared with other years (21.5 ± 1.43 mm), though no significant difference was found ($t_{(12)} = 0.4005$, $P = 0.696$).

Bihourly movement

Bihourly movement behaviour of leopard tortoises showed a bimodal pattern during spring and summer, with highest movement during later morning and mid-afternoon. This bimodal pattern was more pronounced in summer (Fig. 3.3), whereby movement was highest around 10:00 and 18:00 and generally decreased at 14:00. A unimodal pattern is observed during autumn and winter. Movement was identified during night-time hours during all months of the year, though this was decreased in winter.

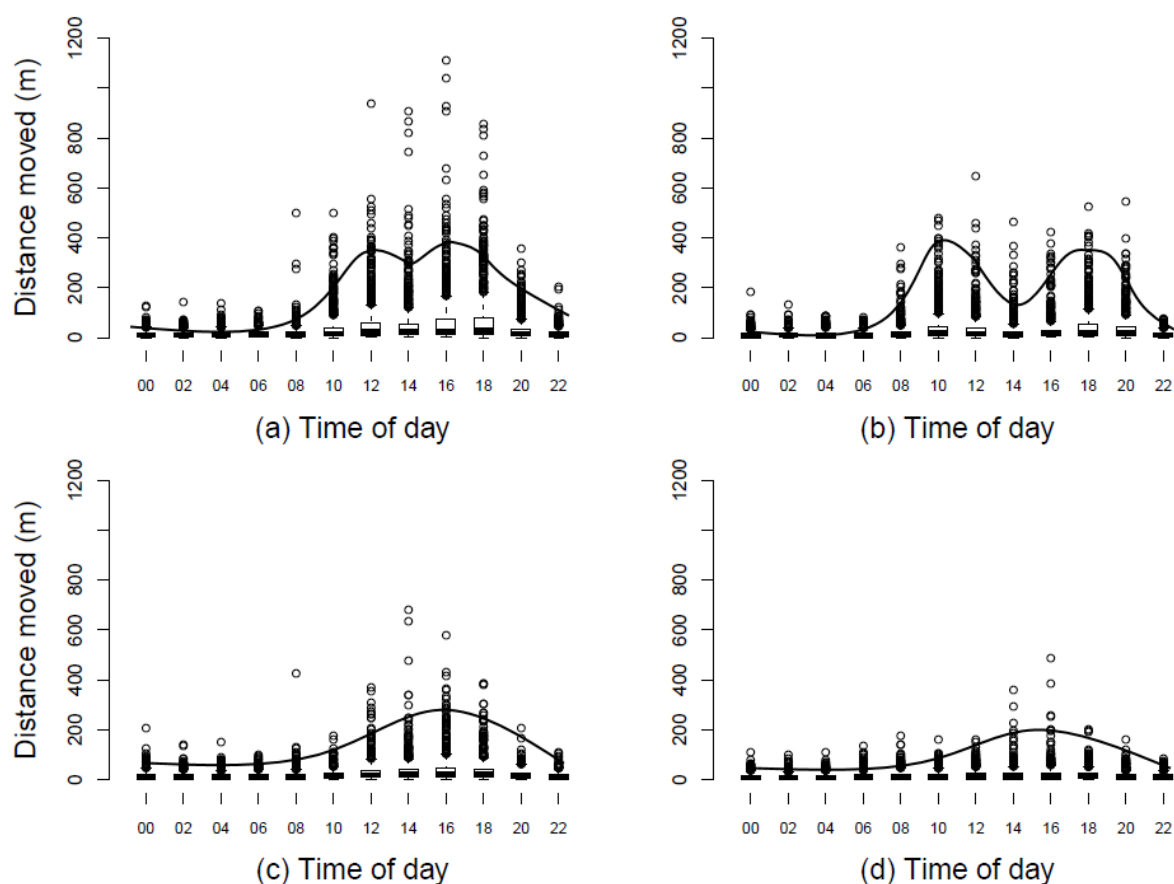


Fig. 3.3. Seasonal variation in daily movement. Bihourly movement of adult leopard tortoises ($n = 10$) throughout day and night in a) spring (September to November), b) summer (December to February), c) autumn (March to May), and d) winter (June to August), near Beaufort West, South Africa. Lines indicate general activity patterns for that season.

A total of 128 candidate models were tested to predict bihourly movement of leopard tortoises. We identified two top candidate models ($\Delta AIC_c < 2$) (Table 3.5). Model averaging highlighted five important predictor variables, based on relative importance (RI); month, time of day, distance from water source, mean temperature (all $RI = 1.00$), and total rainfall ($RI = 0.44$) (Table 3.6). Habitat type and sex were not significantly significant predictor variables in either top candidate model.

Table 3.5: Bihourly movement top models in the current study showing results from top GLMMs comparing model fitness for bihourly movement for leopard tortoises. (Predictor variables included habitat type, month, sex, time of day, distance from water source, mean temperature, and total rainfall. Rows shown in bold indicate top models ($\Delta AIC_c < 2$). Rainfall and temperature measurements were from the two hour period prior to positional fix, using data supplied by South African Weather Service (Pretoria, South Africa) for Beaufort West area, South Africa).

| Model | df | log.like | AIC _c | ΔAIC_c | w_i |
|--|-----------|------------------|------------------|----------------|--------------|
| month + time + water + temp | 27 | -23481.89 | 47017.82 | 0 | 0.521 |
| month + time + water + rain + temp | 28 | -23481.13 | 47018.30 | 0.48 | 0.409 |
| month + sex + time + water + temp | 28 | -23483.52 | 47023.10 | 5.28 | 0.037 |
| month + sex + time + water + rain + temp | 29 | -23482.77 | 47023.58 | 5.76 | 0.029 |
| habitat + month + time + water + temp | 34 | -23480.30 | 47028.67 | 10.86 | 0.002 |
| habitat + month + time + water + rain + temp | 35 | -23479.79 | 47029.65 | 11.83 | 0.001 |

Notes: df = degrees of freedom, log.like = log likelihood, ΔAIC_c = deviation for AIC_c compared with top model, w_i = AIC_c weight.

Table 3.6: Statistically significant predictor variables for bihourly movement in leopard tortoises. (Unconditional parameter estimates, standard error, confidence intervals and relative importance (RI) of tested predictor variables for bihourly displacement distances, using two top candidate models ($\Delta AIC_c < 2$). Predictor variables shown include month, time of day, distance from water source, total rainfall, and mean temperature).

| | β | SE | z | Confidence intervals | | RI |
|---------------|---------|-------|-------|----------------------|--------|------|
| | | | | 2.5 % | 97.5 % | |
| (Intercept) | 1.009 | 0.037 | 27.26 | 0.94 | 1.08 | - |
| Month * | | | | | | 1.00 |
| January | -0.010 | 0.013 | 0.72 | -0.04 | 0.02 | |
| February | -0.047 | 0.013 | 3.47 | -0.07 | -0.02 | |
| March | -0.018 | 0.013 | 1.46 | -0.04 | 0.00 | |
| May | -0.025 | 0.012 | 2.02 | -0.05 | -0.00 | |
| June | -0.086 | 0.013 | 6.76 | -0.11 | -0.06 | |
| July | -0.081 | 0.013 | 6.34 | -0.11 | -0.06 | |
| August | -0.057 | 0.037 | 4.66 | -0.08 | -0.03 | |
| September | 0.044 | 0.012 | 3.55 | 0.02 | 0.07 | |
| October | 0.0114 | 0.012 | 9.25 | 0.09 | 0.14 | |
| November | 0.010 | 0.012 | 0.79 | -0.01 | 0.03 | |
| December | -0.011 | 0.013 | 0.87 | -0.04 | 0.01 | |
| Time of day † | | | | | | 1.00 |
| 2am | -0.008 | 0.012 | 0.68 | -0.03 | 0.02 | |
| 4am | 0.015 | 0.012 | 1.27 | -0.01 | 0.04 | |
| 6am | 0.021 | 0.012 | 1.77 | -0.00 | 0.04 | |
| 8am | 0.058 | 0.012 | 4.86 | 0.03 | 0.08 | |
| 10am | 0.176 | 0.012 | 14.90 | 0.15 | 0.20 | |
| 12pm | 0.228 | 0.012 | 18.90 | 0.20 | 0.25 | |
| 2pm | 0.190 | 0.012 | 15.21 | 0.17 | 0.21 | |
| 4pm | 0.248 | 0.013 | 19.41 | 0.22 | 0.27 | |
| 6pm | 0.281 | 0.012 | 22.53 | 0.26 | 0.31 | |
| 8pm | 0.143 | 0.012 | 11.93 | 0.12 | 0.17 | |
| 10pm | 0.018 | 0.012 | 1.52 | -0.01 | 0.04 | |

| | | | | | | |
|---------------------|--------|-------|-------|-------|-------|------|
| Distance from water | -0.101 | 0.008 | 11.86 | -0.12 | -0.08 | 1.00 |
| Rainfall | 0.016 | 0.005 | 0.82 | 0.01 | 0.03 | 0.44 |
| Temperature | 0.072 | 0.008 | 8.48 | 0.06 | 0.09 | 1.00 |

Notes: * = April used as reference for month variable. † = 00am used as reference for time of day variable.

Results showed a positive relationship between movement distance of leopard tortoises and mean temperature, and rainfall (Fig. 3.4). There was a negative relationship for movement with distance from water source. Month as a predictor variable also showed that movement was expected to be highest in the spring months (September to November), with lowest movement predicted in winter (June to August).

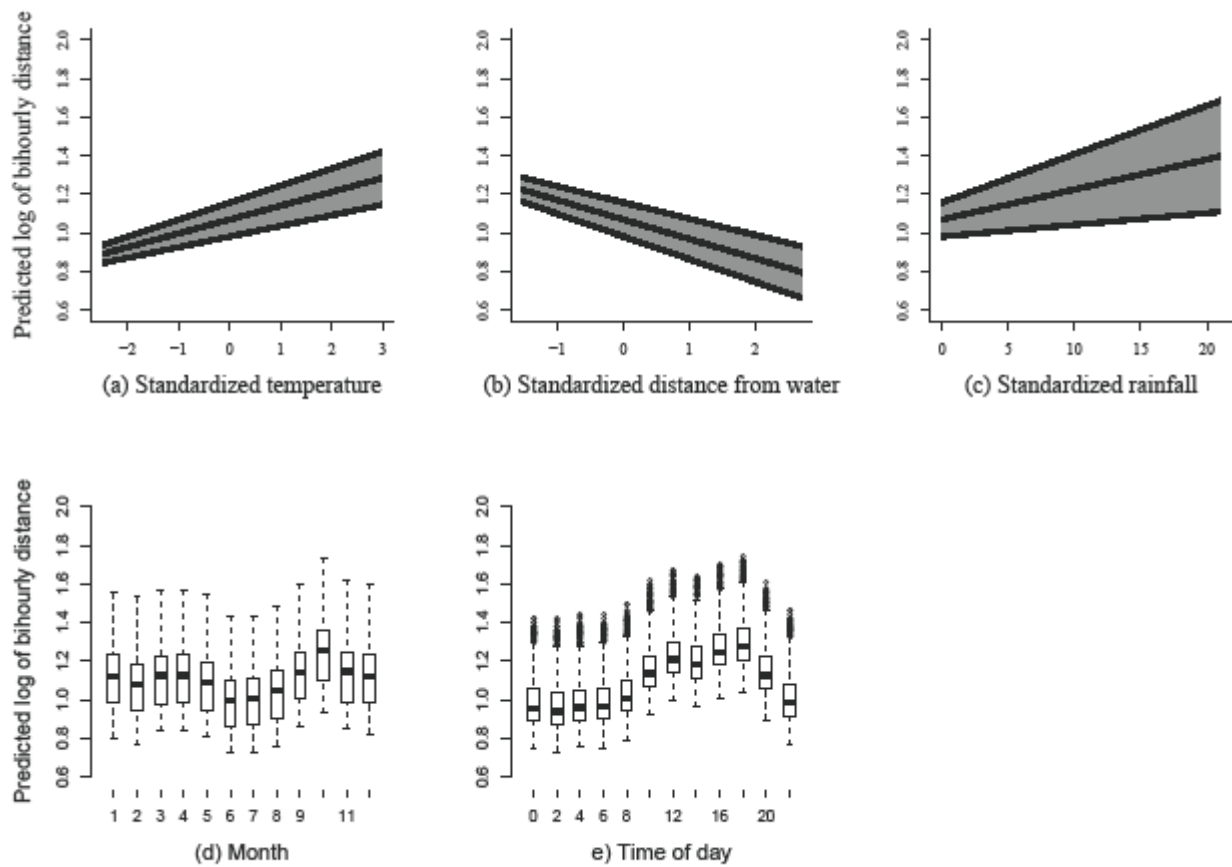


Fig. 3.4. Variables predicting bihourly movement. Bihourly movement for leopard tortoises, Central Karoo, South Africa, as predicted by model averaging using two top candidate generalized linear mixed models. Predictor variables with relative importance (RI) include a) standardized mean temperature (RI = 1.00), b) standardized distance from water source (RI = 1.00), c) standardized rainfall (RI = 0.44), d) month (RI = 1.00), and e) time of day (RI = 1.00). For month, 1 = January, 2 = February, 3 = March, etc.

A significant interactive effect was found for month and distance from water, indicating that effect of distance from water on bihourly movement is dependent on time of the year (Table 3.7). No other interaction effects were significant.

Table 3.7: Analysis of deviance table for predictor variables of bihourly movement. Interactive effect of statistically significant predictor variables for predicting bihourly movement in leopard tortoises. Predictor variables are shown alone, and with potential interactive variables, along with likelihood ratio (LR) chi-squared statistic, degrees of freedom (df) and statistical significance (P) values.

| Predictor variables | LR | df | Probability |
|------------------------------------|---------|-----|-------------|
| Time of day | 25962.2 | 11 | P > 0.001 |
| Month | 11721.4 | 11 | P > 0.001 |
| Temperature | 1496.8 | 1 | P > 0.001 |
| Rainfall | 32.5 | 1 | P > 0.001 |
| Distance from water | 1703.6 | 1 | P > 0.001 |
| Month : Temperature | 11.6 | 11 | P = 0.393 |
| Month : Rainfall | 1.9 | 10 | P = 0.997 |
| Month : Distance from water | 731.2 | 11 | P > 0.001 |
| Time of day : Month | 39.4 | 121 | P = 1.000 |
| Time of day : Temperature | 2.3 | 11 | P = 0.997 |
| Time of day : Rainfall | 1.2 | 11 | P = 1.000 |
| Time of day : Distance from water | 10.6 | 11 | P = 0.474 |
| Time : Month : Temperature | 19.4 | 121 | P = 1.000 |
| Time : Month : Rainfall | 6.8 | 49 | P = 1.000 |
| Time : Month : Distance from water | 46.7 | 121 | P = 1.000 |

Daily movement

When aggregating daily habitat type, only one location recorded wetlands as a habitat. This record was excluded from the dataset prior to GLMM analysis. A total of 64 candidate models were tested to predict daily movement distances. We identified two top candidate models ($\Delta AIC_c < 2$) (Table 3.8). The important predictor variables were habitat type, month, distance from water source (all $RI = 1.00$), and mean temperature ($RI = 0.70$) (Table 3.9).

Table 3.8: Daily movement top models. Results from top GLMMs comparing model fitness for daily movement for Leopard Tortoises. Predictor variables included habitat type, month, sex, distance from water source, mean temperature, and total rainfall. Rows shown in bold indicate top models ($\Delta AIC_c < 2$). Rainfall and temperature measurements were provided by South African Weather Service (Pretoria, South Africa) for Beaufort West area, South Africa.

| Model | df | log.likelihood | AIC_c | ΔAIC_c | w_i |
|---------------------------------------|----|----------------|---------|----------------|-------|
| habitat + month + temp + water | 21 | -429.80 | 901.87 | 0 | 0.653 |
| habitat + month + water | 20 | -431.64 | 903.52 | 1.65 | 0.286 |
| habitat + month + sex + temp + water | 22 | -431.70 | 907.69 | 5.83 | 0.035 |
| habitat + month + sex + water | 21 | -433.54 | 909.33 | 7.47 | 0.016 |
| habitat + month + temp + water + rain | 22 | -433.26 | 910.80 | 8.93 | 0.007 |
| habitat + month + water + rain | 21 | -435.33 | 912.93 | 11.06 | 0.003 |

Notes: df = degrees of freedom, log.likelihood = log likelihood, ΔAIC_c = deviation for AIC_c compared with top model, w_i = AIC_c weight.

Table 3.9: Statistically significant predictor variables for daily movement. Unconditional parameter estimates, standard error, confidence intervals and relative importance (RI) of tested predictor variables for daily movement, using two top candidate models ($\Delta AIC_c < 2$). Predictor variables shown include most common habitat type, month, mean temperature, and distance from water source.

| | β | SE | z | Confidence intervals | | RI |
|-------------------|---------|------|-------|----------------------|--------|------|
| | | | | 2.5 % | 97.5 % | |
| (Intercept) | 2.345 | 0.03 | 73.81 | 2.28 | 2.41 | - |
| Habitat type * | | | | | | 1.00 |
| Non-vegetated | -0.291 | 0.04 | 7.75 | -0.36 | -0.22 | |
| Dense bush | -0.138 | 0.02 | 6.81 | -0.18 | -0.10 | |
| Open bush | -0.137 | 0.03 | 4.15 | -0.20 | -0.07 | |
| Grassland | -0.119 | 0.05 | 2.39 | -0.22 | -0.02 | |
| Cultivated fields | -0.132 | 0.06 | 2.05 | -0.26 | -0.01 | |
| Month † | | | | | | 1.00 |
| January | -0.010 | 0.03 | 0.34 | -0.07 | 0.05 | |
| February | -0.077 | 0.03 | 2.81 | -0.13 | -0.02 | |
| March | 0.005 | 0.03 | 0.20 | -0.05 | 0.06 | |
| May | -0.056 | 0.02 | 2.48 | -0.10 | -0.01 | |
| June | -0.170 | 0.03 | 6.63 | -0.22 | -0.12 | |
| July | -0.172 | 0.03 | 6.55 | -0.22 | -0.12 | |
| August | -0.100 | 0.02 | 4.31 | -0.15 | -0.05 | |
| September | 0.087 | 0.02 | 3.76 | 0.04 | 0.13 | |
| October | 0.207 | 0.02 | 8.57 | 0.16 | 0.25 | |
| November | 0.027 | 0.02 | 1.14 | -0.02 | 0.07 | |
| December | 0.014 | 0.03 | 0.54 | -0.04 | 0.07 | |
| Temperature | 0.048 | 0.01 | 3.20 | 0.02 | 0.08 | 0.70 |

| | | | | | | |
|---------------------|--------|------|------|-------|-------|------|
| Distance from water | -0.147 | 0.02 | 8.86 | -0.18 | -0.11 | 1.00 |
|---------------------|--------|------|------|-------|-------|------|

Notes: * = Low shrubland used as reference for habitat type variable. † = April used as reference for month variable.

Temperature (positive relationship), distance from water source (negative relationship), and month variables presented similar results when compared with bihourly models (Fig. 3.5). Effect of habitat type on predicted movement was variable. Highest movement was predicted at low shrubland and cultivated commercial fields, whilst non-vegetated land predicted lowest movement. Sex and rainfall were not significantly significant predictor variables in either top candidate model predicting daily movement.

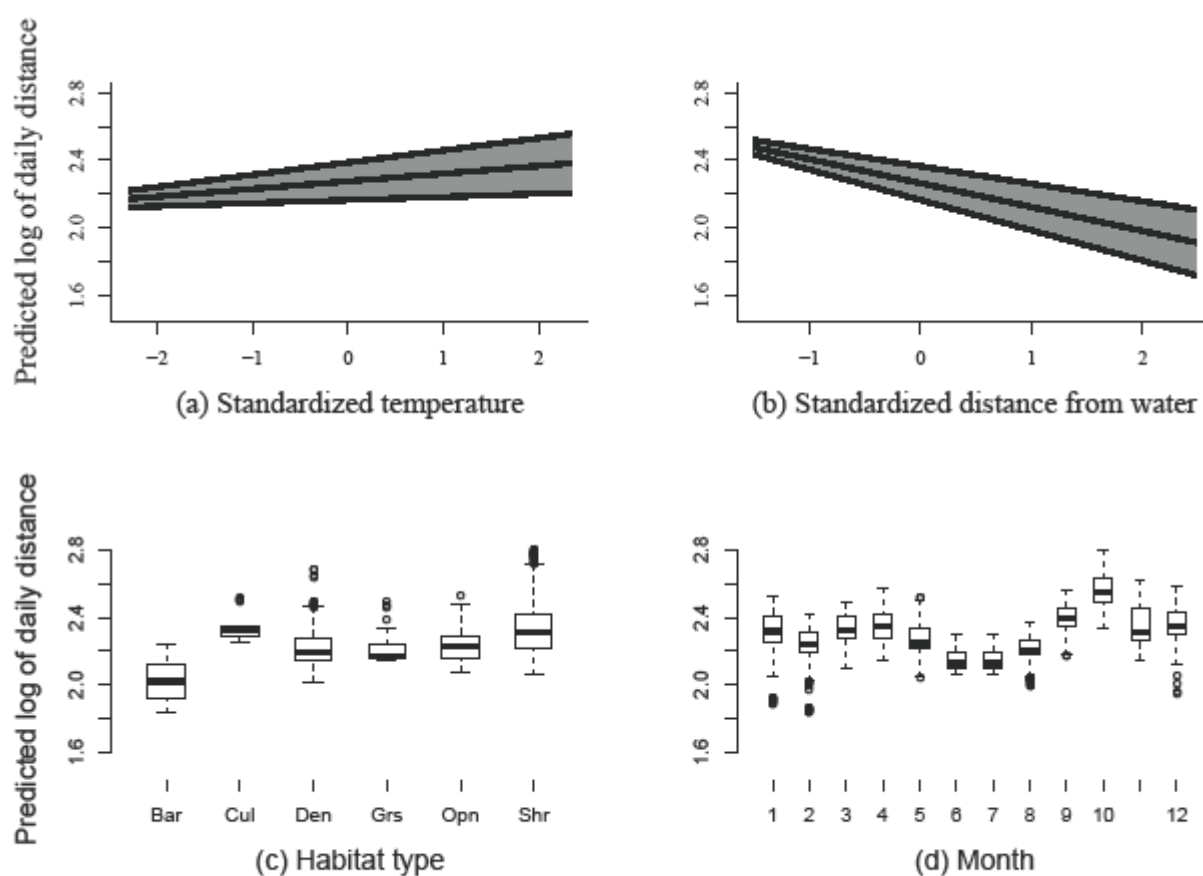


Fig. 3.5. Variables predicting daily movement. Daily movement for leopard tortoises, Central Karoo, South Africa, as predicted by model averaging using two top candidate generalized linear mixed models. Predictor variables with relative importance (RI) include a) standardized mean temperature (RI = 0.70), b) standardized distance from water source (RI = 1.00), c) habitat type (RI = 1.00), and d) month (RI = 1.00). Abbreviations include: Bar = non-vegetated, Cul = cultivated fields, Den = dense bush, Grs = grassland, Opn = open bush, and Shr = low shrubland. Spr = spring, Sum = summer, Aut =autumn, and Win = winter. For month, 1 = January, 2 = February, 3 = March, etc.

Significant combination effects for daily movement were shown for month, habitat type, and distance from water, indicating that effect of these variables on daily movement is

affected by each other (Table 3.10). Temperature did not show any interactive effects with other important predictor variables.

Table 3.10: Analysis of deviance table for predictor variables of daily movement. Interactive effect of statistically significant predictor variables for predicting daily movement in leopard tortoises. Predictor variables are shown alone, and with potential interactive variables, along with likelihood ratio (LR) chi-squared statistic, degrees of freedom (df) and statistical significance (P) values.

| Predictor variables | LR | df | Probability |
|---------------------------------------|--------|----|-------------|
| Month | 8292.0 | 11 | P < 0.001 |
| Habitat | 3938.9 | 5 | P < 0.001 |
| Temperature | 98.6 | 1 | P < 0.001 |
| Distance from water | 810.5 | 1 | P < 0.001 |
| Month : Habitat | 120.9 | 29 | P < 0.001 |
| Month : Temperature | 3.0 | 11 | P < 0.001 |
| Month : Distance from water | 126.4 | 11 | P < 0.001 |
| Habitat : Temperature | 0.4 | 5 | P = 0.990 |
| Habitat : Distance from water | 14.7 | 5 | P = 0.012 |
| Month : Habitat : Temperature | 2.9 | 26 | P = 1.000 |
| Month : Habitat : Distance from water | 136.3 | 26 | P < 0.001 |

Discussion

Movement and activity in tortoises is influenced by life history, resource availability, thermoregulatory necessities, habitat fragmentation, and reproductive requirements [74]. Although daily movement in leopard tortoises is generally affected by season, daily movement

is generally short. Previous estimations of daily movement of leopard tortoises (usually < 100 m) [31, 33, 45] were much lower than present study (256.97 ± 3.56 m per day). Sporadic large movements by individuals (up to 8 km) have been recorded [33], although most other studies show a maximum long distance movement of leopard tortoises is approximately 4 km [26, 31]. Movement in more arid environments of the Nama-Karoo [33] was higher than in Eastern Cape, South Africa [45], Swaziland [31], and Zimbabwe [44]. Variation in movement distances of the above studies has been attributed to seasonal temperature, availability of food resources, rainfall, differences in mean body mass, and need to ingest key isolated resources (e.g. sodium) [31, 33, 44]. In our study GLMMs identified multiple important climatic, environmental, and individual predictor variables on two temporal scales (bihourly and daily). Three variables (mean temperature, distance from water resource, and month) were important predictor variables in top candidate models for both GLMMs. Three additional predictor variables were also important: rainfall and time of day (bihourly movement), and habitat type (daily movement).

Male leopard tortoises moved further than females overall, and in seven individual months, including each of the spring months (September to November) which is when breeding activity (reproduction and egg-laying) in leopard tortoises is typically elevated [75, 76]. However, sexual differences in movement were not highlighted in either GLMM. This is contradictory to the majority of published tortoise movement ecology studies which show that male movement is significantly higher than females [22, 32, 36, 37]. Peak movement in leopard tortoises of both sexes occurred in spring (September to November). There was a female lag behind males for peak movement: male movement began to increase in September, whilst female movement increased in October. October was the peak month of movement for both sexes. This supports previous research on leopard tortoises [56]. These peaks could be related to individual reproductive status. Mate-searching in tortoises, conducted primarily by males,

generally occurs in spring when resource availability and climatic conditions are suitable [56]. Female movement may increase following fertilisation in mid-spring, as search for suitable egg-laying habitat begins [36]. As leopard tortoises can occur at very low densities (e.g. 0.017 tortoises per ha) in some parts of the Karoo [25], it can be expected that males will make much larger movements to find mates compared with other species and other regions. This is supported by research on *Gopherus* tortoises, where males made larger daily spring movements (up to 500 m) in areas of lower burrow (and therefore population) density in search of mates [37]. Associated with reproduction is an increase in energy costs: especially for females with regards to producing eggs [32, 35]. Tortoises of both sexes generally increase activity, not only to search for mates and egg-laying habitat, but also for increased demand for food intake and, in case of females, other important resources [35, 37]. No specific instances of reproductive behaviour was observed, though one male (LPD013) was observed alongside several non-telemetered females at a watering point during December 2015.

Habitat type was found to be an important predictor variable for predicting daily leopard tortoise movement. Daily movement was shown to be highest in low shrubland habitat, the most-used habitat type. Cultivated commercial fields also predicted high movement distances, although only one individual used this habitat. We classified multiple habitat types as providing an expected higher supply of food resources (compared with low shrubland and non-vegetated). However, only two individuals remained in these areas throughout the majority of the study. The results showed that non-vegetated habitat type was predicted to have lowest movements by daily models, which supports previous research that shows that activity is decreased when resources are low [22]. Our classification for higher food resources was based on expected resources from a land cover layer. However, no surveys were conducted for these habitat types and diet in leopard tortoises is extremely adaptable. Diet-switching behaviour has been identified in leopard tortoises whereby they feed on different plants through year, depending

on resource availability [18]. In addition, they will feed on a wide variety of foods, including grasses, forbs, fruits, and succulents [18]. Succulents are even avoided by livestock [18], and are sometimes present in over-grazed areas, such as non-vegetated habitat (unpublished observations). Therefore, smaller movements by individuals in non-vegetated habitat may be due to a higher food searching efficiency by leopard tortoises.

Distance from known water sources was an important predictor in both GLMMs for leopard tortoise movement. Contrary to our predictions, movement decreased as individuals moved away from water resources. As forbs (74.5 %) and succulents (51.0 %) generally represent a large percentage of their diet [77], it is likely that high water content of these plants could supplement water intake for individuals for much of the year, especially in such a water scarce habitat [31]. In addition, leopard tortoises are able to adapt digestive parameters (food intake, water loss and urine osmolality) in response to diet to maintain body mass and water balance [20]. This could make them even more resilient to lack of water associated with arid environments [22, 24]. Despite their ability to obtain much of their water requirements from food intake and metabolic water, they may need to drink free standing water so supplement their water budget demands and restore osmotic homoeostasis, as high electrolyte contents can cause severe stress and sometimes death [22-24].

It appears leopard tortoise movement increased when individuals were closer to water resources, perhaps because of knowledge of resource localities: animals maintain and continually update a cognitive map [78]. Whereas tortoises further away from permanent water appear to rely on food resources for water intake, if known water sources exist within an animal's home range, individuals may make regular movements to maintain internal water balance, though water balance was not measured. Most telemetered individuals had little or no association with known water resources. However, many non-telemetered individuals were frequently observed congregated around manmade watering points and dams (unpublished

observations). Such observations have been previously reported, whereby home range of several individuals overlapped at manmade water sources [25, 26]. This presents a potential issue, considering the upcoming introduction of fracking activities in the Karoo (expected before end of 2017) [8, 9, 79, 80], as contamination of these water sources through increased water salinity and decreased water quality [10, 11] could adversely affect a large number of individuals that rely on these permanent water sources. Demand for water in the region already exceeds availability [81, 82], with demand projected to increase by up to 150 % by 2025 [79]. Up to 90 % of water use in South Africa is supplied from surface resources [82], yet infrequent rains in the Karoo rarely reach rivers and cannot supply demand [79]. Whilst it appears that tortoises are able to use food sources for water, it is unknown how fracking will impact these food sources. Further research is required to assess how fracking will affect local human, animal, and plant communities.

The adaptations to water scarce environments are especially important due to unpredictable and infrequent nature of rainfall in the Karoo [49, 50]. Increased tortoise activity is usually found to be associated with rainfall [22, 27-29], with several species having physiological and behavioural adaptations to facilitate drinking rainwater [76, 83]. Our results support these previous findings, with bihourly movement showing a positive relationship with rainfall. This is in contrast to lack of correlation between activity and rainfall found by McMaster and Downs [56] in a similar region. However, one must be cautious when interpreting our results. Whilst no significant difference was found between monthly rainfall during the study year and previous years, rainfall was lower. The mean daily rainfall was 0.44 mm, although over half of rainfall days yielded less than 2 mm of rain. Rainfall also did not fall in any one particular season; 12 days in spring, 15 days in summer, 6 days in autumn, and 20 days in winter. Tortoises have the ability to use their bladders as water reservoirs [23]. As such, early rains may be more important, and could explain why rainfall was not shown as an

important predictor variable in daily models. Such unpredictability in rainfall increases importance of permanent water resources. Movement studies should ideally be conducted over several seasons, though financial, battery life, and time restrictions vary.

Whilst rainfall is unpredictable, temperature is less so, and has been shown as important in dictating movement in tortoise studies previously [30, 31]. Tortoises are ectothermic, and so activity is directly related to local environmental conditions to support metabolism [56]. As such, tortoises generally move more in spring and summer, with movement decreased in winter [31-34], though patterns are likely more complex and related to specific environments and climatic conditions. Behaviour is also important: tortoises bask in morning sun prior to becoming active during the day [56]. Temperature and month were important predictor variables in both GLMMs. Our bihourly data also showed a basic bimodal movement pattern in warmer seasons of the year, when maximum daily temperatures frequently exceeded 30 °C. This bimodal activity pattern (with movement higher during mornings and evenings) is a behavioural adaptation that allows individuals to avoid extreme temperatures, which may cause severe stress or death [26, 28, 36, 56, 57]. Indeed, hours of activity restriction due to increased temperatures associated with global warming is believed to be a main predictor for local extinctions of yellow-footed tortoises, *Chelonoidis denticulata* [84]. Some species (e.g. *Testudo* spp.) reduce activity in summertime to avoid extreme temperatures [30], whilst others (e.g. *Gopherus* spp., African spurred tortoise, *Centrochelys sulcata*) remain in burrows over many weeks [76]. Leopard tortoises are not known to dig burrows, but will use shade of bushes and boulders to shield themselves from sun [56, 85].

Due to the close relationship between temperature and activity, leopard tortoise movement is generally restricted in cooler temperatures, such as during winter months and during night-time hours. In more moderate climates, tortoises brumate to avoid cold conditions [22, 30, 34, 76]. However, mean winter (June to August) temperatures in the Karoo are still

warm enough to facilitate movement: over 1/3 of winter days had maximum temperatures exceeding 20 °C. Mean daily movement of leopard tortoises during winter months exceeded 150 m. Leopard tortoises do not typically brumate [26, 31], although isolated records do occur [25]. In contrast to bimodal activity patterns in warmer months, a unimodal activity pattern was observed in autumn and winter, as described previously by McMaster and Downs [56]. McMaster and Downs [56] also noted leopard tortoises are generally inactive during night-time. However, our results show night-time movement does occur, especially in summer and autumn months. Night-time foraging in leopard tortoises has been reported in one individual previously [75]. It is currently unknown what may facilitate night-time movement, although it appears that night-time temperatures are often non-restrictive during these periods. More research is required to ascertain variables enabling this night-time movement. Other potentially important variables, such as environmental illumination, may also affect movement ability during night-time hours when temperatures are non-restrictive.

Information regarding drivers of movement, and periods in which movement is highest, can be used to mitigate against other threats to tortoises. For example, electric fencing is used in much of the Karoo as a means to control predation on livestock by wild caracal (*Caracal caracal*) and black-backed jackal (*Canis mesomelas*) [41]. This electric fencing causes mortalities in a number of mammals, reptiles, and amphibians [42], though fatalities are highest with respect to tortoise species and ground pangolin (*Smutsia temmincki*) [42]. Leopard tortoises account for most (> 86 %) electric fencing related reptile mortalities [42, 43], likely related to their size and spatial ecology. Whilst it has been recommended that raising the electric line to a minimum height of 250 mm could reduce mortalities [43], strategic planning can also be incorporated into operations by reducing use of electric fencing when and where tortoises are most active: in mornings and evenings, mating season, and nearer to water sources.

Conclusions

Our results further display the relationship between water as a resource and movement in leopard tortoises. We provide evidence individuals can use either one of two basic movement behaviours in relation to water sources in water scarce environments: either an individual's home range and movements is such that it includes permanent water resources, allowing regular long-distance movements to replenish internal water storage; or these are excluded and there is a reliance on food resources (such as grasses, forbs, and succulents) as the primary source of water. It is known from previous research that multiple tortoise species are able to tolerate high internal electrolyte concentrations, though drinking water is a requirement for urination and restoration of internal water balance. Further research should be carried out on the potential impacts of fracking activities, as contamination and increased salination of groundwater may affect ability to restore water balance. Subsequent dehydration could cause severe stress and possible mortality.

In particular our research identified temporal and spatial conditions in which leopard tortoise movement increased. Such information can be used to guide designs, constructions and operations of electric fencing. As leopard tortoise movement is higher in areas closer to water resources, we advise that electric fencing does not occur within close proximities to these areas. We also advise that electric fencing should not operate during spring and summer months, whereby reproductive and general activities are increased. However, our data shows tortoises move throughout the year, and even during night-time hours. Whilst it may not be possible to avoid all mortalities related to electric fencing, we hope that the above suggestions could reduce impacts. Increasing time between shocks, or alternating in electric fence functionality at intervals may also enable shocked individuals to escape should contact occur. We also support previous suggestions whereby the electrified line is raised to a minimum height of 250 mm.

Abbreviations

AIC: Akaike's information criterion; GLMM: generalized linear mixed model; GPS: global positioning system; GSM: global system for mobile communications; HDOP: horizontal dilution of precision; RI: relative importance; UFH: ultra high frequency.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available due to them being part of a current postgraduate study but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Author details

¹*School of Life Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville, Pietermaritzburg, South Africa, 3209, Pietermaritzburg, KwaZulu-Natal, South Africa.*

**Corresponding author, email: Downs@ukzn.ac.za; m.drabik.hamshare@gmail.com*

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Author's contributions

MDH collected, analysed and interpreted the telemetry data. All authors read and approved the final manuscript.

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CHAPTER 4

Walking in the moonlight: nocturnal activity in leopard tortoises

Martyn Drabik-Hamshare, Colleen T. Downs*

School of Life Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville,

Pietermaritzburg, South Africa, 3209

**Corresponding author, email: Downs@ukzn.ac.za; m.drabik.hamshare@gmail.com*

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Abstract

Activity in tortoises is mostly restricted to daytime hours, with movement in many species associated with multiple variables, including temperature, rainfall, and seasonality. However, using GPS telemetry to investigate patterns of leopard tortoise (*Stigmochelys pardalis*) movement in the semi-arid Karoo of South Africa identified the presence of nocturnal movement. We isolated nocturnal movement and explored the significance of several variables; ambient temperatures, sex, seasonality and moon phases. Seasonality was shown to be important, with nocturnal movement lower in winter, though sex had no effect. Regarding overnight ambient temperatures, mean nocturnal temperatures appear to be more important than minimum temperatures. It was found that nocturnal movement was significantly increased in days either side of a full moon, suggesting that visibility is one of the key factors in restricting tortoise movement. We suggest that ability to maintain core temperatures above ambient temperatures allows leopard tortoises to move nocturnally, as night-time temperatures are not restrictive throughout the year.

Text

Our understanding of habitat use and spatial ecology has improved and amended management regimes^{1,2}. Such research has also identified how activity fluctuates daily and seasonally, depending on species' life history in multiple taxa: e.g. in seals³, turtles⁴, and fish⁵. Animals are generally classed into one of several terms depending on period of primary activity; diurnal (during the day), nocturnal (during the night), matutinal (at dawn), verspertine (dusk), and crepuscular (dawn and dusk). Nocturnal activity is affected by the lunar cycle in many species; e.g. terrestrial mammals⁶, birds^{7,8} and reptiles⁹. In most cases, lunar illumination

affects predator-prey relationships, either by altering prey-searching ability^{8,10} or predator avoidance^{6,11}.

Lunar illumination is also used as a tool for navigation¹² and detection of appropriate habitat¹³. In addition, there is evidence that leatherback turtles (*Dermochelys coriacea*) use the lunar cycle to facilitate nesting behaviour¹⁴. Studies on various nocturnal rodents show that perceptual abilities are increased during higher levels of lunar illumination¹³, though some species increase their use of cover¹⁵, showing a trade-off between resource search efficiency and evading predation by nocturnal predators¹¹.

Tortoises (Family: Testudinidae) are strongly diurnal, though they express bimodal levels of daily activity in areas of extreme ambient temperature¹⁶, whereby activity levels peak during mornings and evenings. Understanding of movement in tortoises has shown great variability, depending on climate¹⁷, species¹⁸, sex^{17,19}, and resource availability²⁰. For example, movement in some tortoise species is increased in spring when males are searching for mates¹⁹ and females are dealing with increased energy requirements related to egg production²¹. Nocturnal activity has been identified previously in gopher tortoises (*Gopherus polyphemus*)²² and leopard tortoises (*Stigmochelys pardalis*)²³. However, nocturnal activity is considered to be low. To our knowledge, no study on tortoises has specifically investigated nocturnal movement in tortoises.

During our study investigating movement response of leopard tortoises to environmental and weather variables, nocturnal movement was evident²⁴. Leopard tortoises are the largest and most wide-spread tortoise species in sub-Saharan Africa, occurring in a wide range of ecosystems²⁵. Previous studies have identified that leopard tortoise movement is related to temperature, resource availability, and reproductive requirements^{20,24,26}. As tortoises can provide important ecosystem functions, such as seed dispersal²⁷ and habitat engineering²², and many (> 80 %) are listed as ‘Vulnerable’ or above by International Union for Conservation

of Nature (IUCN)²⁵, it is important to further understand their spatial ecology for application to conservation.

Due to their size, adult leopard tortoises are unlikely to be concerned by predators during night-time hours: almost all reports of predation on leopard tortoises are on juveniles or sub-adults (e.g. by rock monitors, *Varanus albigularis*²⁸). We predicted that night-time movement of leopard tortoises could be related to ambient temperature, seasonality and lunar illumination. As the semi-arid Karoo (South Africa) presents a warm climate especially in summer, even during night-time hours, and cloud cover is generally low, ability to recognise surroundings could be the main limitation to nocturnal movement. We used 12 months of movement data and compared night-time movement with season, overnight ambient temperatures, and levels of lunar illumination. We predicted movement would be increased during higher lunar illumination periods (full moon \pm 5 days) compared with lower illumination (new moon \pm 5 days). We also predicted that movement would be increased with overnight ambient temperatures, and during spring and summer due to reproductive activities (e.g. mating and searching for egg-laying habitat)²³.

Overall mean (\pm SE) overnight movement in leopard tortoises ($n = 11$) was 63.4 ± 0.68 m ($n = 1198$, range: 0.58 to 347.59 m) (Table 4.1, example: Fig. 4.1). As predicted, a significant difference was found (RMANOVA, $F_{1,9} = 6.037$, $P = 0.036$) between distance moved during full moon phase (64.27 ± 1.08 m, $n = 1361$) compared with new moon phase (58.98 ± 1.06 m, $n = 1291$). There was no significant effect of sex ($F_{1,7} = 0.024$, $P = 0.880$) and no interactive effect between sex and moon phase ($F_{1,9} = 0.002$, $P = 0.97$).

Table 4.1: Telemetered tortoises. Telemetered leopard tortoise individual, sex, body mass, number of geolocation fixes used in the final analysis of their nocturnal movement, and overall individual mean nocturnal movement, Beaufort West, South Africa in 2015.

| Individual | Sex | Body mass (kg) | Overnight fixes | Mean \pm SE movement (m) |
|------------|-----|-------------------|--------------------|-------------------------------|
| LPD001 | F | 11.69 | 1,608 | 55.89 \pm 1.49 |
| LPD002 | F | 11.58 | 1,543 | 83.56 \pm 2.67 |
| LPD004 | M | 7.43 | 1,680 | 55.25 \pm 1.71 |
| LPD006* | F | 9.36 | 328 | 91.66 \pm 4.56 |
| LPD010 | F | 26.17 | 1,765 | 55.33 \pm 1.58 |
| LPD011 | F | 18.40 | 1,443 | 50.86 \pm 1.74 |
| LPD013 | M | 12.56 | 1,603 | 56.73 \pm 1.79 |
| LPD015 | M | 15.13 | 1,645 | 56.26 \pm 1.98 |
| LPD016 | M | 14.87 | 1,300 | 69.05 \pm 2.54 |
| LPD017 | F | 16.64 | 1,684 | 60.24 \pm 1.72 |
| LPD048 | M | 9.28 | 1,079 | 89.66 \pm 3.28 |

*Note: Transmitter recovered from dead tortoise and redeployed.

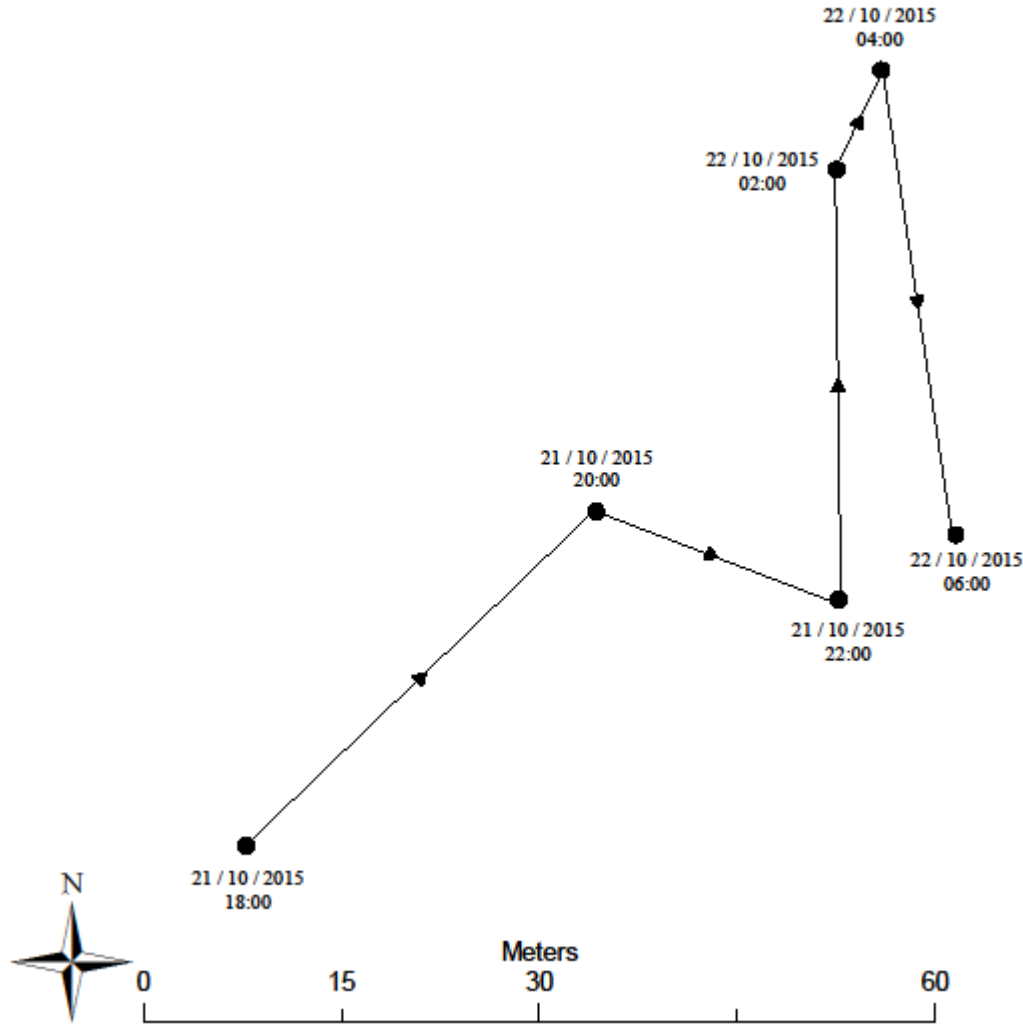


Fig. 4.1. Example of nocturnal movement in one leopard tortoise during a single night in spring.

Note: The midnight fix was removed in the data screening process.

Season also had a significant effect on nocturnal movement of leopard tortoises ($F_{3,2624} = 6.513$, $P < 0.001$), although there was no interactive effect between season and moon phase ($F_{3,2624} = 0.279$, $P = 0.840$) (Fig. 4.2). Leopard tortoises moved at night in all seasons, however they moved further in spring (67.7 ± 1.26 m, $n = 915$, range: 5.21 to 286.88 m), summer (62.1 ± 1.34 m, $n = 880$, range: 0.78 to 294.61 m), and autumn (65.1 ± 1.49 m, $n = 873$, range: 0.58 to 347.59 m) compared with winter (58.7 ± 1.16 m, $n = 913$, range: 0.95 to 269.92 m). In addition, night-time

movement was influenced by mean night-time temperatures (Pearsons's product moment correlation, $\rho = 0.041$, $df = 3579$, $p = 0.015$) but not minimum night-time temperatures ($\rho = 0.030$, $df = 3579$, $p = 0.072$).

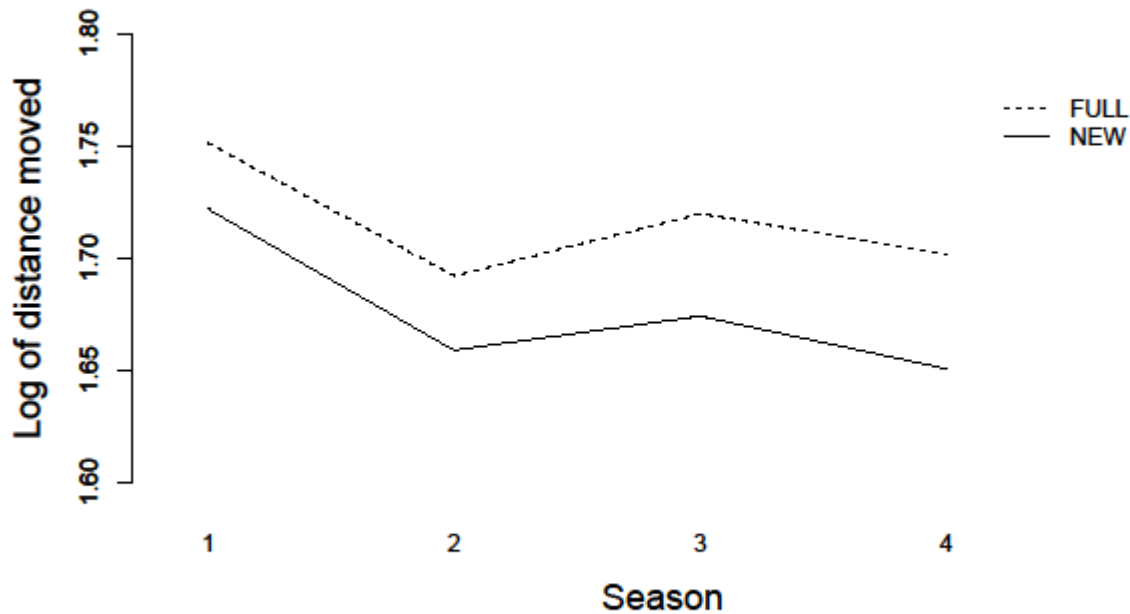


Fig. 4.2. Interaction plot of log transformed nocturnal movement in adult leopard tortoises *Stigmochelys pardalis* ($n = 11$), with moon phase and season. Moon phase is determined as a 5 day period for both full moon (± 5 days) and new moon (± 5 days) lunar phases.

As mentioned, terrestrial tortoises are considered diurnal, with the majority of their activity occurring during mornings and evenings, depending on species, climate and season. However, data here show that nocturnal movement does occur in leopard tortoises. Whilst studies have reported differences in activity of males and females during the day^{17,19}, no differences were found during the night. Therefore, we do not believe there is a relationship between night-time movement and sexual activity. A positive correlation between mean temperatures and nocturnal movement was observed, however there was no correlation with minimum temperatures. Seasonal variations in

night-time movement was observed. The majority of this nocturnal movement occurred during the breeding season (spring and summer), and decreased in winter. This generally matched up with periods of seasonal day-time movement observed previously^{19,20,24,26}. As day-time ambient temperatures fluctuate much more than night-time ambient temperatures, it is possible that night-time movement might instead be influenced by temperature absorbed earlier in the day. Given that larger animals have a smaller surface area:volume ratio, maintaining internal body temperatures is easier²⁹. As such, internal body temperatures of adult leopard tortoises may be higher than ambient temperatures for much of the night. This is supported by previous research showing core body temperatures in leopard tortoises ($> 20\text{ }^{\circ}\text{C}$) being maintained above ambient night-time temperatures, even when temperatures dropped to $10\text{ }^{\circ}\text{C}$ ³⁰.

As predicted, moon cycle was shown to have a significant effect on night-time movement. Given the moderate temperatures, tortoises' ability to maintain core temperatures, and protection from predation due to size, we suspect that movement in tortoises is limited mostly by visibility. If there are less restrictions to visibility, and environmental conditions support activity, tortoises could be able to sufficiently use their local environment.

Data presented here are from Global Position System (GPS) data only, and therefore may not fully represent behaviour. We believe that GPS fix error could account for some apparent movement, but this still would not explain observed differences between different periods of the lunar cycle. Accurate information on cloud cover was also unavailable and may have affected results. However, as the region experienced lower than average rainfall during 2015, we do not expect that cloud cover had a significant impact. Regardless of the above issues, further investigation is necessary to understand reasons and behaviour of night-time activity.

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Figure legends

Fig. 4.1. Example of nocturnal movement in one leopard tortoise during a single night in spring.

Note: The midnight fix was removed in the data screening process.

Fig. 4.2. Interaction plot of log transformed nocturnal movement in adult leopard tortoises *Stigmochelys pardalis* (n = 11), with moon phase and season. Moon phase is determined as a 5 day period for both full moon (± 5 days) and new moon (± 5 days) lunar phases.

Methods

Study area

The research was carried out in the Karoo; a unique ecosystem of approximately 37 million ha that covers much of the Northern, Western and Eastern Cape Provinces, South Africa³¹. The

Karoo varies in aridity, with northern and western parts typically arid and eastern parts typically semi-arid, though rainfall throughout region is unpredictable and unreliable^{32,33}, and summer daily ambient temperatures regularly exceed 30 °C³². Three private mixed livestock farms (Baakensrug, Kamferskraal, and Elandsfontein, approximate coordinates 32°15S, 23°E) in the Central Karoo, Western Cape Province, South Africa were used. These farms, which are part of the Nelspoort and Beaufort West communities, use aspects of holistic resource management. Private hunting of free-roaming game—e.g. springbok (*Antidorcas marsupialis*), greater kudu (*Tragelaphus strepsiceros*), and blue wildebeest (*Connochaetes taurinus*)—is also present.

Fieldwork

Wild-caught adult leopard tortoises (n = 11, mean: 13.92 kg, range: 7.43 to 26.27 kg) were captured during late 2014. The body mass for each individual was recorded using digital hanging scales (Pesola, Schindellegi, Switzerland). Sex was determined by examining the tortoises' plastral concavity, tail length, and shapes of anal scutes and supracaudal shield³⁴.

Unique GPS-Global System for Mobile Communications (GSM) / Ultra High Frequency (UHF) transmitters (Wireless Wildlife, Potchefstroom, South Africa) were placed directly on carapaces using dental acrylic: front for females, back for males, to avoid inhibiting mating attempts. Transmitter mass (74 g) ranged from 0.28 % to 0.99 % of tortoise body mass; much lower than the 5 % suggested for telemetry studies³⁵. Individuals were released at their initial point of capture within 30 min of capture. Bihourly geolocation information was collected for a minimum period of 12 months.

GPS data were downloaded using a base-station that sent data to an online server via a cell-phone network. Raw telemetry data were then downloaded via the Wireless Wildlife website

(<http://www.wireless-wildlife.co.za/>) in a CSV format. Attempts were made to locate telemetered individuals using most recent location data during each season in 2015. One telemetered individual was found dead. The transmitter was recovered and redeployed. As such, telemetry data was collected for 11 individuals.

Temperature and moon phase variables

Hourly temperature data (November 2014 to December 2015) were obtained from South African Weather Service (Pretoria, South Africa) (<http://www.weathersa.co.za/>), using Beaufort West weather station 0092081 5 (~ 45 km west of study sites). Mean ambient temperatures were calculated for each 2 h period. Moon phase information was downloaded from the Astronomical Applications Department for the U. S. Naval Observatory (USNO) (<http://aa.usno.navy.mil/>), using data for Gaborone, Botswana (closest capital city in southern African, based on longitude).

Data screening and Euclidean distances

Prior to analysis, location data were screened to remove likely incorrect location fixes, using ‘adehabitatLT’ version 0.3.20, ‘adehabitatMA’ version 0.3.10, ‘ade4’ version 1.7-4 and ‘sp’ version 1.2-3 in R version 3.1.2^{36,37}, using RStudio version 0.98.1091³⁸. Data were discarded based on values for extreme horizontal dilution of precision (HDOP), incorrect time zones, incomplete or dubious transmitter data (e.g. negative activity), impossible and improbable movement distances, and z-coordinate error. Each movement was assumed to be the Euclidean distance between successive locations, which represents the smallest possible distance moved³⁹.

We tested transmitters for fix error by calculating Euclidean distances between GPS fix locations and known locations in Pietermaritzburg, South Africa, prior to deploying transmitters.

We calculated error using ‘Generate Near Table’ in ArcGIS. Mean (+ SE) fix error was found to be 17.0 ± 0.59 m ($n = 384$, max error = 134.78 m). Calculated error from known distances was positively skewed. As such, errors were corrected to fit a normal distribution using a logarithmic transformation.

We assumed each calculated distance was affected by a fix error. We ranked calculated distance for each fix and assumed that larger distances were more likely to be due to larger errors. Therefore, we corrected each calculated distance by deducting inverse log of the quantile for known error fixes (Equation 4.1). The equation for corrected distances is as follows, where d_{rank} is d_{th} percentile from transformed known error distribution, d_{est} is estimated distance between points, and d_{corr} is corrected distance between points. Following corrections for fix errors, we isolated night-time movement (8pm to 6am). Cumulative distances were calculated for each overnight period for each individual.

$$\text{Equation 4.1: } d_{corr} = d_{est} - 10^{\log(d_{rank})}$$

Statistics

We used ‘quantile-quantile’ plot using ‘stats’ version 3.1.2 package in R³⁶ to test for normality. As night-time movement data were positively skewed, a logarithmic transformation was carried out. We assigned each date as “full moon” (date of full moon ± 5 days), “new moon” (date of new moon ± 5 days), or “none” (neither of the above). To test for effect of lunar cycle, we eliminated data assigned as “none”. A repeated measures ANOVA was used to test for effect of sex and moon phase on nocturnal movement.

We assigned seasons as spring (September to November), summer (December to February), autumn (March to May), and winter (June to August). We tested interactive effects of moon phase and season using a Two-way ANOVA. To ascertain whether temperature had an effect, we tested for a relationship between night-time movement distance and temperature—using both mean and minimum overnight temperatures—using Pearson’s product moment correlation.

CHAPTER 5

Designing mini-barcodes for tortoises: Identification of important COI primer sites for tortoises (Testudinidae)

M Drabik-Hamshare, CT Downs*, S Willows-Munro

*School of Life Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville, Pietermaritzburg,
South Africa, 3209, Pietermaritzburg, KwaZulu-Natal, South Africa*

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***Corresponding author:** Colleen T. Downs

Email: downs@ukzn.ac.za

Tel: +27 (0)33 260 5127

Mailing Address: University of KwaZulu-Natal, School of Life Sciences, Private Bag X01, Scottsville, Pietermaritzburg, South Africa, 3209

Running header: Primer sites for tortoises for designing mini-barcodes

Abstract

Tortoises (Family: Testudinidae) are one of the most threatened taxa, with over 80 % of all species listed as Vulnerable or above. Previous attempts to use reptile cytochrome oxidase c subunit I (COI) primers to sequence genetic information in tortoises has had little success. No COI primers have been designed to work with tortoises, resulting in many studies using other primer sites. Given the worldwide decline of tortoises, the overexploitation of tortoises within the illegal pet and food trades, and the lack of clarity in differentiating species, specific COI primers are required to increase amplification success and application. Consequently we identified important COI primer sites for tortoises for designing mini-barcodes. A total of 69 tortoise sequences were downloaded from the Barcode of Life Database (BOLD), and additional sequences from wild-caught specimens, to create a sequence database of 14 of the 17 tortoise genera. We used sliding window analyses and maximum likelihood trees to identify important COI primer sites to design a mini-barcode. A 210 bp fragment was shown to be the optimal region within the tortoise COI sequence. Future work will test the use of this region in mini-barcodes, which can be applied to improve success rates in molecular studies.

Keywords: Chelonia, conservation genetics, COI, barcode of life database.

Introduction

Tortoises (Family: Testudinidae) are one of the most threatened animal taxa globally, with as many as 80 % species classified as at least ‘Vulnerable’ (Turtle Taxonomy Working Group 2014). According to the Turtle Taxonomy Working Group, 13 species are listed as Least Concern

(or Not Listed or Data Deficient), three as Near Threatened, 16 as Vulnerable, 7 as Endangered, and seven as Critically Endangered (Turtle Taxonomy Working Group 2014). However, since the recent International Union for Conservation of Nature (IUCN 2008) listings, newer assessments have proposed updated listings (Turtle Taxonomy Working Group 2014). Of the changes, two species are down-listed (e.g. Yellow-footed Tortoise, *Chelonoidis denticulata*; Vulnerable to Near Threatened), whilst 20 species were up-listed (e.g. Geometric Tortoise, *Psammobates geometricus*; Endangered to Critically Endangered). In addition, the status of seven new or previously unevaluated species, have been drafted or proposed by the South African Reptile Conservation Assessment (SARCA) committee and the IUCN Tortoise and Freshwater Turtle Specialist Group (TFTSG). Assuming these proposed listings are accepted, there will be seven species listed as Least Concern, three as Near Threatened, 17 as Vulnerable, 13 as Endangered, and 12 as Critically Endangered (Hofmeyr *et al.* 2014; Turtle Taxonomy Working Group 2011, 2012, 2014). A further, newly described species, *Gopherus evgoodei*, still requires classification (Edwards *et al.* 2016), but is likely to be listed at least as Vulnerable.

Threats to tortoises are numerous, but most are related to anthropomorphic pressures that are affecting other reptiles (Gibbons *et al.* 2000), including habitat loss, fragmentation and degradation (Anadon *et al.* 2007; BenDor *et al.* 2009; Edwards *et al.* 2004), land use change (Lovich *et al.* 2011), and climate change (Fernandez-Chacon *et al.* 2011; Lovich *et al.* 2014). Despite presence of all tortoise species on either Appendix I or II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (<https://www.cites.org/>) (CITES 2014), wild tortoises are still readily collected and traded illegally (Lau & Shi 2000; Nijman & Shepherd 2015; O'Brien *et al.* 2003).

To aid with current and future conservation and management, it is important to further develop appropriate genetic markers (barcodes) that can facilitate molecular identification. Such techniques have become vital in species identification and conservation (Francis *et al.* 2010; Klippel *et al.* 2015; Murphy *et al.* 2013; Nagy *et al.* 2012). Genetics studies can use a host of different gene regions, nuclear or mitochondrial, for species identification. Such techniques have been used in studies with vertebrates; e.g. birds (Hebert *et al.* 2004), fish (Ward *et al.* 2005), frogs (Fouquet *et al.* 2007); and invertebrates; e.g. ants (Smith *et al.* 2005), arachnids (Barrett & Hebert 2005), beetles (Monaghan *et al.* 2005). Among the many molecular markers readily used in research is cytochrome oxidase c subunit I (COI), a 648 base pair (bp) region of the mitochondrial gene (Ratnasingham & Hebert 2007). The creation of a DNA barcode database, the Barcode of Life Database (BOLD), using the COI gene for a large range of taxa is made freely available to researchers (Ratnasingham & Hebert 2007). This resource allows comparison of genes with over 1.3 million reference records on the BOLD website (<http://www.boldsystems.org/>). Using the website to compare sequences to reference barcodes gives a list of results showing the most likely species matches, with results ordered by a similarity rating.

Whilst there are several examples of projects using a COI gene for non-avian reptiles, their use and success with tortoises (and turtles) is relatively low. For example, in a study comparing the success of DNA amplification for a host of different reptile taxa using a reptile COI gene, success in Testudinidae (20 %) and Pelomedusidae (60 %) were lowest (Nagy *et al.* 2012). All other reptile taxa had a success rate over 70 % (Nagy *et al.* 2012). Other studies have shown similar results with amplifying turtle DNA with COI primers (Jeong *et al.* 2013). Studies have shown that misidentification of samples using COI gene and BOLD are mostly due to mistaken reference samples, lack of suitable reference samples, or poor primer specificity (Dawnay *et al.* 2007;

Wilson-Wilde *et al.* 2010). Poor primer specificity could be because most commonly used primers are created from non-turtle reptiles (Nagy *et al.* 2012). Given estimated time of divergence of turtles and tortoises—260 million years ago (mya), based on recent fossil evidence found in the Karoo basin, South Africa (Lyson *et al.* 2016)—primers specifically designed for tortoises are required to increase success rates.

To our knowledge, there are no specific COI primers for Testudines (tortoises) (Murphy *et al.* 2013), and no single pair of COI primers guarantee success across or within reptile taxa (Vences *et al.* 2012). The two most commonly-used reptile primers are for lizards (REPTBC) or all reptiles (RepCOI) (Castañeda & de Queiroz 2011; Murphy *et al.* 2013; Nagy *et al.* 2012), though primers for non-tortoise Testudines do exist; (e.g. M72/M73 for two families of Australian side-necked turtles, sub-order: Pleurodira (Georges *et al.* 1999) and multiple primers for the Asian box turtles, Genus: *Cuora* (Parham *et al.* 2004; Stuart & Parham 2004)), the success is still low compared with other gene regions (e.g. *16S*) (Georges *et al.* 1999).

Reduced amplification success is also associated with situations where DNA has become degraded or fragmented due to exposure to naturally-occurring environmental conditions (e.g. high temperatures) or internal digestive processes (Bär *et al.* 1988). In such cases, the use of mini-barcodes—shorter sequences with an increased specificity—can aid in accurate identification (Hajibabaei *et al.* 2006; Meusnier *et al.* 2008; Shokralla *et al.* 2011). Mini-barcodes improve success rates in genetics studies incorporating historical samples, allowing species identification of museum specimens (Shokralla *et al.* 2011), and prey specimens in faeces (Zeale *et al.* 2011). Although the mini-barcode is generally much shorter than the standard DNA barcoding fragment, it is possible to reconstruct the full barcode by using overlapping mini-barcode regions (Van Houdt *et al.* 2010).

We aimed to identify the most informative region of COI in tortoises using all COI tortoise records available on the BOLD database and our own tortoise COI sequences. We aimed to recommend a region for the creation of mini-barcodes, based on *in silico* analyses including comparisons of maximum likelihood trees of mini-barcodes with full sequence data. Identification of a smaller, most informative region of COI for tortoises will aid in future tortoise conservation and identification research projects.

Materials and methods

Data acquisition

A total of 69 tortoise (Testudinae) COI sequences were downloaded from Barcode of Life Database (BOLD) on 10/05/2016. An additional six sequences were also added to this database from our own collection of leopard tortoise (*Stigmochelys pardalis*) blood samples. The final database represented 14 genera and 26 species (Appendix 5.1). The only genera missing from the database were *Agrionemys*, *Malacochersus*, and *Chersina*; three single species genera (Turtle Taxonomy Working Group 2014). Three of the downloaded samples were listed simply as *Chelonoidis nigra*, representing Galápagos giant tortoises. However, *Chelonoidis nigra* has since been reclassified into ten species, of which three—including the floreana giant tortoise (*Chelonoidis nigra*)—are listed as extinct. The species origins of the three *C. nigra* sequences are unknown; therefore, we listed them simply as *Chelonoidis spp.* Clustal W Multiple Alignment (Thompson *et al.* 1994) in BioEdit 7.2.5 (Hall 1999) was used to modify and align the 620 bp sequences (n = 75). A neighbour-joining tree in MEGA 7.0.14 (Kumar *et al.* 2016) was used to confirm success of sequence alignment based on grouping of known related genera.

Creation of mini-barcode markers

Manually-created fragments

Nine manually-designed mini-barcode fragments were created from the full 620 base pair (bp) length alignment; six fragments of 103 bp in length; and three fragments of 206 bp in length were created. MEGA 7.0.14 (Kumar *et al.* 2016) was then used to obtain descriptive summary statistics from the 9 fragments, and the full 620 bp sequence alignment.

Sliding window analysis

A sliding window analysis using the ‘SPIDER’ package in R (Brown *et al.* 2012; R Core Development Team 2014) was used to create and test possible mini-barcodes. Fragments varying in size from 20 bp to 210 bp were tested (10 bp intervals). Each model showed the optimal position within the sequence of 10 fragments for each fragment length. In total, values and positions of 200 fragments were collected. The top four mini-barcode fragments were selected for each fragment based on high values for mean Kimura 2-parameter (K2P) distance, low values for least proportion of zero non-conspecific K2P distance and highest proportion of congruence of neighbour-joining trees; e.g. clade composition. A total of 80 mini-barcode fragments were created using BioEdit 7.2.5 (Hall 1999) based on optimal length and position of each fragment in the sliding window analysis.

Maximum likelihood trees

The 90 datasets (80 created from optimal sliding window analysis, 9 manually-designed, 1 reference dataset) were converted to a Phylip (.phy) format using Mesquite (Maddison & Maddison 2001). Maximum likelihood trees of these data were created in Garli v.0.951 (Zwickl 2006) using the *Kimura 2-parameter+gamma* (K2P+G) model for sequence evolution (Kimura

1980). Maximum likelihood trees were exported to Nexus (.nex) format using Figtree (Rambaut 2014). The maximum likelihood trees obtained from the truncated datasets were then compared with the reference tree obtained from the full dataset using Ktreedist (Soria-Carrasco *et al.* 2007). The Ktreedist calculated K-scores and Robinson-Foulds symmetric difference (R-F score); two statistical methods that compare topology and branch lengths of trees. For both of these metrics, lower values indicate a higher degree of similarity between reference tree (full sequence) and comparison tree (fragmented sequence).

Results

Creation of fragments

The total COI dataset with a length of 620bp was manually divided into mini datasets using two approaches. Six datasets of 103 bp each were created; and three datasets of 206 bp each were created. An additional 80 datasets of different fragment lengths (20 bp to 210 bp) were created using the sliding window analysis (Appendix 5.2). The full reference sequence had 238 parsimonious sites (38.4%) and consisted mostly of thymine/uracil (29.1 %), cytosine (26.2 %), and adenine (28.7 %) (Appendix 5.3). Guanine was represented the least in the reference sequence (16 %). The entire dataset consisted of a mean of 42.9 % parsimonious characters, 31.0 % thymine/uracil (range: 16.3 to 47.6 %), 26.6 % cytosine (range: 19.5 to 32.5 %), 27.5 % adenine (range: 17.0 to 33.9 %), and 14.9 % guanine (range: 4.9 to 34.4 %).

Maximum likelihood trees

Maximum likelihood trees for 90 sequence alignments were estimated. The tree for the full sequence alignment was compared with maximum likelihood trees for each of the 89 newly-

created alignments. K-scores and R-F scores were calculated for each comparison. Each score has been given a ranking based on comparisons with other sequences (Table 5.1).

Whilst many of the smaller fragments generated higher scores for mean distance to K2P, and lower scores for proportion of zero non-conspecific in the K2P distance matrix, larger fragments accumulated the best scores for congruence of neighbor joining trees. In addition to this, larger fragments also generated lower K-scores and R-F scores when compared with the reference tree (Appendix 5.4).

Of the ten best comparison trees, three were created from the three 206 bp sequences. The remaining seven best comparison trees were from fragments of between 140 bp and 210 bp, created using the sliding window analysis. The two best sequences based on K-score and R-F score rankings were 'fragment210bp_a' and 'fragment210bp_c'. We selected 'fragment210bp_a' as the optimal mini-barcode, based on a slightly higher mean (K2P) distance. The top ten fragment sequences consisted of a mean 40.1 % parsimonious characters, 31.2 % thymine/uracil, 26.8 % cytosine, 28.1 % adenine, and 13.9 % guanine.

Table 5.1. K-scores and Robinson-Foulds (R-F) scores for the ten best comparison trees against the full length alignment. Comparison trees were ranked based on ascending scores for each of the 89 comparison trees.

| Overall rank | Comparison tree | Position | K-score | Scale factor | R-F score | K-score rank | R-F rank |
|--------------|-----------------|----------|---------|--------------|-----------|--------------|----------|
| 1= | fragment210bp_a | 319 | 0.1726 | 0.866 | 56 | 2 | 1 |
| 1= | fragment210bp_c | 316 | 0.1715 | 0.844 | 60 | 1 | 2 |
| 3 | fragment206_1 | 0 | 0.2009 | 0.967 | 64 | 5 | 4.5 |
| 4 | fragment206_2 | 207 | 0.1903 | 0.840 | 66 | 3 | 7.5 |
| 5 | fragment200bp_d | 322 | 0.1928 | 0.820 | 66 | 4 | 7.5 |
| 6 | fragment180bp_b | 322 | 0.2056 | 0.826 | 66 | 6 | 7.5 |
| 7 | fragment190bp_c | 337 | 0.2216 | 0.702 | 62 | 13 | 3 |
| 8 | fragment140bp_b | 478 | 0.2115 | 0.905 | 68 | 7 | 11.5 |
| 9 | fragment206_3 | 413 | 0.2158 | 0.806 | 68 | 9 | 11.5 |
| 10 | fragment150bp_d | 457 | 0.2213 | 0.758 | 68 | 12 | 11.5 |

Discussion

Creation of smaller mini-barcode primers improves success and sequencing of markers from degraded molecular information (Hajibabaei *et al.* 2006), which is often the case for ancient and otherwise degraded specimens. DNA can become degraded due to natural environmental conditions and internal digestive processes (Bär *et al.* 1988; Janjua *et al.* 2016). In such cases, it is important to develop successful DNA amplification techniques, such as mini-barcodes. The ability to amplify degraded DNA has enabled scientists to amplify genetic information from museum

specimens (Parham *et al.* 2004; Shokralla *et al.* 2011), and prey items within stomachs and pellets of multiple taxa; e.g. bats (Zeale *et al.* 2011), birds (Joo & Park 2012), and rodents (Latinne *et al.* 2014).

Other sources of degraded DNA exists in the natural environment. Amplification of this terrestrial (e.g. soils) and aquatic environmental DNA (eDNA) allows scientists to conduct presence/likely absence surveys on ecosystems without witnessing, capturing or collecting specimens (Rees *et al.* 2014; Robe *et al.* 2003). This has been used to detect invasive, endangered, and difficult to find species (Rees *et al.* 2014). For example, in Europe, eDNA surveys are now widely used to identify whether the European Protected Species (EPS) Great Crested Newt (*Triturus cristatus*) is present in ponds, with methodology now used by Natural England and citizen scientists to identify potentially important sites (Biggs *et al.* 2015).

Using short DNA sequences to develop mini-barcodes can also identify regions that allows sufficient differentiation of genetically different species (Hajibabaei *et al.* 2006; Meusnier *et al.* 2008; Shokralla *et al.* 2011). Mini-barcodes make it possible to identify species based on genetic information rather than identifiable physical characteristics (morphospecies). In some cases, there are differences between the number of molecular species identified compared to morphospecies, leading to use of mini-barcodes to assess diversity (Osmundson *et al.* 2013).

Given the status of tortoises throughout the world (Turtle Taxonomy Working Group 2014), and the poor success rate of traditional sequencing attempts on turtles and tortoises (Jeong *et al.* 2013; Nagy *et al.* 2012), the identification of important sites for mini-barcodes can improve the acquisition of molecular data which would have direct impact on conservation efforts. This study adds to the molecular tools available to study and identify tortoise species around the world.

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Appendices

Appendix 5.1: Details of 75 barcode sequences analysed, with BOLD process ID, genus and species name. The six additional *Stigmochelys pardalis* sequences have been added without a BOLD process ID.

| Database ID | BOLD process ID | Genus | Species |
|-------------|-----------------|----------------------|--------------------|
| TORT029 | BENT131-08 | <i>Aldabrachelys</i> | <i>gigantea</i> |
| TORT044 | GBGC11210-13 | <i>Aldabrachelys</i> | <i>gigantea</i> |
| TORT005 | BENT308-09 | <i>Astrochelys</i> | <i>radiata</i> |
| TORT043 | GBGC11209-13 | <i>Astrochelys</i> | <i>radiata</i> |
| TORT025 | REPT315-12 | <i>Astrochelys</i> | <i>radiata</i> |
| TORT053 | BENT309-09 | <i>Astrochelys</i> | <i>yniphora</i> |
| TORT019 | GBGC11208-13 | <i>Astrochelys</i> | <i>yniphora</i> |
| TORT051 | BENT134-08 | <i>Centrochelys</i> | <i>sulcata</i> |
| TORT041 | GBGC11202-13 | <i>Centrochelys</i> | <i>sulcata</i> |
| TORT026 | BENT128-08 | <i>Chelonoidis</i> | <i>chilensis</i> |
| TORT068 | GBGC11207-13 | <i>Chelonoidis</i> | <i>chilensis</i> |
| TORT027 | BENT129-08 | <i>Chelonoidis</i> | <i>denticulata</i> |
| TORT067 | GBGC11206-13 | <i>Chelonoidis</i> | <i>denticulata</i> |
| TORT030 | BENT132-08 | <i>Chelonoidis</i> | spp |
| TORT062 | GBGC11168-13 | <i>Chelonoidis</i> | spp |
| TORT042 | GBGC11205-13 | <i>Chelonoidis</i> | spp |
| TORT028 | BENT130-08 | <i>Geochelone</i> | <i>elegans</i> |
| TORT066 | GBGC11204-13 | <i>Geochelone</i> | <i>elegans</i> |

| | | | |
|---------|--------------|--------------------|------------------------|
| TORT001 | BENT133-08 | <i>Geochelone</i> | <i>platynota</i> |
| TORT018 | GBGC11203-13 | <i>Geochelone</i> | <i>platynota</i> |
| TORT006 | BENT310-09 | <i>Gopherus</i> | <i>agassizii</i> |
| TORT032 | BENT311-09 | <i>Gopherus</i> | <i>agassizii</i> |
| TORT040 | GBGC11200-13 | <i>Gopherus</i> | <i>agassizii</i> |
| TORT017 | GBGC11201-13 | <i>Gopherus</i> | <i>agassizii</i> |
| TORT054 | BENT312-09 | <i>Gopherus</i> | <i>berlandieri</i> |
| TORT007 | BENT313-09 | <i>Gopherus</i> | <i>berlandieri</i> |
| TORT033 | BENT314-09 | <i>Gopherus</i> | <i>berlandieri</i> |
| TORT055 | BENT315-09 | <i>Gopherus</i> | <i>berlandieri</i> |
| TORT034 | BENT316-09 | <i>Gopherus</i> | <i>berlandieri</i> |
| TORT065 | GBGC11199-13 | <i>Gopherus</i> | <i>berlandieri</i> |
| TORT008 | BENT317-09 | <i>Gopherus</i> | <i>flavomarginatus</i> |
| TORT056 | BENT318-09 | <i>Gopherus</i> | <i>flavomarginatus</i> |
| TORT035 | BENT319-09 | <i>Gopherus</i> | <i>flavomarginatus</i> |
| TORT009 | BENT320-09 | <i>Gopherus</i> | <i>flavomarginatus</i> |
| TORT057 | BENT321-09 | <i>Gopherus</i> | <i>flavomarginatus</i> |
| TORT016 | GBGC11198-13 | <i>Gopherus</i> | <i>flavomarginatus</i> |
| TORT002 | BENT139-08 | <i>Gopherus</i> | <i>polyphemus</i> |
| TORT039 | GBGC11197-13 | <i>Gopherus</i> | <i>polyphemus</i> |
| TORT058 | BENT322-09 | <i>Homopus</i> | <i>signatus</i> |
| TORT064 | GBGC11196-13 | <i>Homopus</i> | <i>signatus</i> |
| TORT061 | GBGC10760-13 | <i>Indotestudo</i> | <i>elongata</i> |

| | | | |
|---------|--------------|--------------------|---------------------|
| TORT014 | GBGC10761-13 | <i>Indotestudo</i> | <i>elongata</i> |
| TORT022 | GBGC11422-13 | <i>Indotestudo</i> | <i>elongata</i> |
| TORT045 | GBGC11423-13 | <i>Indotestudo</i> | <i>elongata</i> |
| TORT046 | GBGC11424-13 | <i>Indotestudo</i> | <i>elongata</i> |
| TORT047 | GBGC11796-13 | <i>Indotestudo</i> | <i>elongata</i> |
| TORT049 | GBGC1466-06 | <i>Indotestudo</i> | <i>elongata</i> |
| TORT020 | GBGC11411-13 | <i>Indotestudo</i> | <i>forstenii</i> |
| TORT021 | GBGC11412-13 | <i>Indotestudo</i> | <i>forstenii</i> |
| TORT023 | GBGC11797-13 | <i>Indotestudo</i> | <i>forstenii</i> |
| TORT031 | BENT150-08 | <i>Indotestudo</i> | <i>travancorica</i> |
| TORT015 | GBGC11195-13 | <i>Indotestudo</i> | <i>travancorica</i> |
| TORT003 | BENT153-08 | <i>Kinixys</i> | <i>homeana</i> |
| TORT038 | GBGC11194-13 | <i>Kinixys</i> | <i>homeana</i> |
| TORT036 | BENT323-09 | <i>Kinixys</i> | <i>natalensis</i> |
| TORT037 | GBGC11193-13 | <i>Kinixys</i> | <i>natalensis</i> |
| TORT060 | GBGC10759-13 | <i>Manouria</i> | <i>emys</i> |
| TORT024 | GBGCR177-10 | <i>Manouria</i> | <i>emys</i> |
| TORT052 | BENT159-08 | <i>Manouria</i> | <i>impressa</i> |
| TORT011 | GBGC10437-12 | <i>Manouria</i> | <i>impressa</i> |
| TORT012 | GBGC10438-12 | <i>Manouria</i> | <i>impressa</i> |
| TORT013 | GBGC10439-12 | <i>Manouria</i> | <i>impressa</i> |
| TORT063 | GBGC11192-13 | <i>Manouria</i> | <i>impressa</i> |
| TORT010 | BENT324-09 | <i>Psammobates</i> | <i>geometricus</i> |

| | | | |
|---------|-------------|---------------------|--------------------|
| TORT004 | BENT183-08 | <i>Pyxis</i> | <i>arachnoides</i> |
| TORT050 | REPT314-12 | <i>Pyxis</i> | <i>arachnoides</i> |
| TORT059 | BENT325-09 | <i>Pyxis</i> | <i>planicauda</i> |
| TORT071 | - | <i>Stigmochelys</i> | <i>pardalis</i> |
| TORT072 | - | <i>Stigmochelys</i> | <i>pardalis</i> |
| TORT073 | - | <i>Stigmochelys</i> | <i>pardalis</i> |
| TORT074 | - | <i>Stigmochelys</i> | <i>pardalis</i> |
| TORT075 | - | <i>Stigmochelys</i> | <i>pardalis</i> |
| TORT076 | - | <i>Stigmochelys</i> | <i>pardalis</i> |
| TORT069 | GBGC1207-06 | <i>Testudo</i> | <i>graeca</i> |
| TORT048 | GBGC1203-06 | <i>Testudo</i> | <i>hermanni</i> |

Appendix 5.2: Summary statistics of the sliding window analysis for four selected fragments of each fragment length, showing potential segments for mini-barcodes and their position within the full alignment. Statistics include mean Kimura 2-parameter (K2P) distance, proportion of zero non-conspecific K2P distance, proportion of zero cells in K2P distance matrix, and congruence of neighbour joining trees (clade composition and clade composition shallow).

| Fragment length/name | Position | K2P dist | Zero non con dist | Zero dist | Clade comp | Clade comp shallow |
|-------------------------|----------|-------------|----------------------|-----------|------------|-----------------------|
| fragment20bp_a | 478 | 0.279 | 0.333 | 0.040 | 0.397 | 0.548 |
| fragment20bp_b | 31 | 0.252 | 0.333 | 0.044 | 0.493 | 0.595 |
| fragment20bp_c | 34 | 0.249 | 0.373 | 0.048 | 0.397 | 0.571 |
| fragment20bp_d | 37 | 0.246 | 0.320 | 0.070 | 0.438 | 0.595 |
| fragment30bp_a | 334 | 0.214 | 0.160 | 0.053 | 0.630 | 0.786 |
| fragment30bp_b | 31 | 0.207 | 0.080 | 0.033 | 0.616 | 0.786 |
| fragment30bp_c | 469 | 0.204 | 0.200 | 0.036 | 0.493 | 0.643 |
| fragment30bp_d | 22 | 0.204 | 0.320 | 0.042 | 0.507 | 0.595 |
| fragment40bp_a | 322 | 0.194 | 0.147 | 0.049 | 0.671 | 0.810 |
| fragment40bp_b | 565 | 0.193 | 0.213 | 0.045 | 0.589 | 0.762 |
| fragment40bp_c | 13 | 0.192 | 0.160 | 0.034 | 0.630 | 0.810 |
| fragment40bp_d | 22 | 0.192 | 0.080 | 0.031 | 0.671 | 0.810 |
| fragment50bp_a | 448 | 0.192 | 0.093 | 0.031 | 0.699 | 0.833 |
| fragment50bp_b | 478 | 0.185 | 0.147 | 0.034 | 0.712 | 0.905 |
| fragment50bp_c | 319 | 0.178 | 0.120 | 0.042 | 0.671 | 0.786 |
| fragment50bp_d | 562 | 0.178 | 0.187 | 0.034 | 0.630 | 0.810 |

| | | | | | | |
|-----------------|-----|-------|-------|-------|-------|-------|
| fragment60bp_a | 34 | 0.185 | 0.187 | 0.036 | 0.726 | 0.881 |
| fragment60bp_b | 37 | 0.184 | 0.187 | 0.035 | 0.767 | 0.929 |
| fragment60bp_c | 31 | 0.176 | 0.067 | 0.030 | 0.726 | 0.929 |
| fragment60bp_d | 334 | 0.174 | 0.120 | 0.042 | 0.644 | 0.762 |
| fragment70bp_a | 31 | 0.182 | 0.067 | 0.029 | 0.699 | 0.881 |
| fragment70bp_b | 25 | 0.180 | 0.067 | 0.029 | 0.685 | 0.857 |
| fragment70bp_c | 34 | 0.179 | 0.187 | 0.035 | 0.685 | 0.857 |
| fragment70bp_d | 37 | 0.177 | 0.187 | 0.035 | 0.671 | 0.833 |
| fragment80bp_a | 13 | 0.174 | 0.067 | 0.028 | 0.767 | 0.952 |
| fragment80bp_b | 22 | 0.173 | 0.067 | 0.027 | 0.781 | 0.952 |
| fragment80bp_c | 334 | 0.173 | 0.080 | 0.035 | 0.685 | 0.833 |
| fragment80bp_d | 16 | 0.173 | 0.067 | 0.027 | 0.795 | 0.952 |
| fragment90bp_a | 13 | 0.166 | 0.067 | 0.027 | 0.781 | 0.952 |
| fragment90bp_b | 22 | 0.164 | 0.067 | 0.027 | 0.767 | 0.952 |
| fragment90bp_c | 7 | 0.164 | 0.067 | 0.027 | 0.740 | 0.905 |
| fragment90bp_d | 334 | 0.163 | 0.080 | 0.033 | 0.685 | 0.857 |
| fragment100bp_a | 13 | 0.165 | 0.067 | 0.027 | 0.753 | 0.881 |
| fragment100bp_b | 478 | 0.164 | 0.000 | 0.029 | 0.699 | 0.810 |
| fragment100bp_c | 334 | 0.162 | 0.053 | 0.029 | 0.767 | 0.929 |
| fragment100bp_d | 313 | 0.161 | 0.053 | 0.031 | 0.740 | 0.881 |
| fragment110bp_a | 28 | 0.157 | 0.067 | 0.029 | 0.753 | 0.905 |
| fragment110bp_b | 466 | 0.157 | 0.000 | 0.029 | 0.726 | 0.857 |
| fragment110bp_c | 319 | 0.157 | 0.067 | 0.031 | 0.712 | 0.881 |

| | | | | | | |
|-----------------|-----|-------|-------|-------|-------|-------|
| fragment110bp_d | 322 | 0.157 | 0.067 | 0.031 | 0.699 | 0.857 |
| fragment120bp_a | 379 | 0.159 | 0.000 | 0.027 | 0.781 | 0.929 |
| fragment120bp_b | 382 | 0.156 | 0.000 | 0.027 | 0.726 | 0.881 |
| fragment120bp_c | 478 | 0.155 | 0.000 | 0.029 | 0.712 | 0.833 |
| fragment120bp_d | 484 | 0.155 | 0.000 | 0.029 | 0.712 | 0.881 |
| fragment130bp_a | 448 | 0.159 | 0.000 | 0.029 | 0.753 | 0.881 |
| fragment130bp_b | 478 | 0.158 | 0.000 | 0.028 | 0.753 | 0.929 |
| fragment130bp_c | 475 | 0.156 | 0.000 | 0.029 | 0.685 | 0.833 |
| fragment130bp_d | 445 | 0.155 | 0.000 | 0.029 | 0.712 | 0.857 |
| fragment140bp_a | 466 | 0.157 | 0.000 | 0.029 | 0.753 | 0.881 |
| fragment140bp_b | 478 | 0.156 | 0.000 | 0.028 | 0.712 | 0.905 |
| fragment140bp_c | 463 | 0.156 | 0.000 | 0.029 | 0.712 | 0.833 |
| fragment140bp_d | 358 | 0.154 | 0.000 | 0.027 | 0.822 | 0.952 |
| fragment150bp_a | 349 | 0.158 | 0.000 | 0.027 | 0.767 | 0.905 |
| fragment150bp_b | 454 | 0.154 | 0.000 | 0.029 | 0.699 | 0.833 |
| fragment150bp_c | 337 | 0.153 | 0.000 | 0.027 | 0.726 | 0.857 |
| fragment150bp_d | 457 | 0.153 | 0.000 | 0.028 | 0.808 | 0.952 |
| fragment160bp_a | 340 | 0.158 | 0.000 | 0.027 | 0.808 | 0.929 |
| fragment160bp_b | 334 | 0.158 | 0.000 | 0.027 | 0.808 | 0.881 |
| fragment160bp_c | 337 | 0.156 | 0.000 | 0.027 | 0.822 | 0.905 |
| fragment160bp_d | 343 | 0.156 | 0.000 | 0.027 | 0.822 | 0.952 |
| fragment170bp_a | 328 | 0.158 | 0.000 | 0.027 | 0.740 | 0.881 |
| fragment170bp_b | 334 | 0.158 | 0.000 | 0.027 | 0.740 | 0.857 |

| | | | | | | |
|-----------------|-----|-------|-------|-------|-------|-------|
| fragment170bp_c | 331 | 0.158 | 0.000 | 0.027 | 0.740 | 0.857 |
| fragment170bp_d | 448 | 0.153 | 0.000 | 0.028 | 0.767 | 0.905 |
| fragment180bp_a | 319 | 0.157 | 0.000 | 0.027 | 0.767 | 0.881 |
| fragment180bp_b | 322 | 0.156 | 0.000 | 0.027 | 0.767 | 0.905 |
| fragment180bp_c | 349 | 0.154 | 0.000 | 0.027 | 0.808 | 0.929 |
| fragment180bp_d | 325 | 0.153 | 0.000 | 0.027 | 0.740 | 0.881 |
| fragment190bp_a | 334 | 0.157 | 0.000 | 0.027 | 0.781 | 0.905 |
| fragment190bp_b | 331 | 0.154 | 0.000 | 0.027 | 0.753 | 0.881 |
| fragment190bp_c | 337 | 0.154 | 0.000 | 0.027 | 0.781 | 0.905 |
| fragment190bp_d | 343 | 0.154 | 0.000 | 0.027 | 0.753 | 0.881 |
| fragment200bp_a | 334 | 0.156 | 0.000 | 0.027 | 0.808 | 0.929 |
| fragment200bp_b | 328 | 0.154 | 0.000 | 0.027 | 0.781 | 0.905 |
| fragment200bp_c | 325 | 0.153 | 0.000 | 0.027 | 0.808 | 0.929 |
| fragment200bp_d | 322 | 0.153 | 0.000 | 0.027 | 0.808 | 0.929 |
| fragment210bp_a | 319 | 0.153 | 0.000 | 0.027 | 0.795 | 0.905 |
| fragment210bp_b | 325 | 0.152 | 0.000 | 0.027 | 0.781 | 0.905 |
| fragment210bp_c | 316 | 0.152 | 0.000 | 0.027 | 0.822 | 0.929 |
| fragment210bp_d | 331 | 0.151 | 0.000 | 0.027 | 0.808 | 0.929 |

Appendix 5.3: Summary descriptive statistics for the full-length aligned sequence, nine manually-created mini-barcode fragments (six 103 bp fragments and three 206 bp fragments), and 80 mini-barcode fragments created using the sliding window analysis. Summary statistics include number of base pairs, number of variable sites, number of parsimonious information sites, and average nucleotide composition (%) of each alignment.

| Dataset | Base pairs | Variable sites | Parsimonious information sites | Nucleotide Composition (%) | | | |
|----------------|---------------|-------------------|--------------------------------------|----------------------------|------|------|------|
| | | | | T/U | C | A | G |
| Ref-sequence | 620 | 250 | 238 | 29.1 | 26.2 | 28.7 | 16.0 |
| fragment103-1 | 103 | 46 | 41 | 29.1 | 24.8 | 29.2 | 16.9 |
| fragment103-2 | 103 | 38 | 37 | 28.6 | 20.4 | 33.9 | 17.1 |
| fragment103-3 | 103 | 37 | 36 | 29.4 | 19.5 | 33.9 | 17.1 |
| fragment103-4 | 103 | 42 | 41 | 32.2 | 26.8 | 24.6 | 16.5 |
| fragment103-5 | 103 | 43 | 42 | 30.6 | 29.2 | 28.8 | 11.3 |
| fragment103-6 | 103 | 46 | 42 | 28.7 | 25.8 | 30.8 | 14.7 |
| fragment206-1 | 206 | 83 | 77 | 29.2 | 22.2 | 31.6 | 17.0 |
| fragment206-2 | 206 | 77 | 76 | 28.5 | 28.7 | 24.8 | 18.0 |
| fragment206-3 | 206 | 89 | 84 | 29.7 | 27.5 | 29.8 | 13.0 |
| fragment20bp_a | 20 | 12 | 12 | 47.6 | 30.5 | 17.0 | 4.9 |
| fragment20bp_b | 20 | 12 | 12 | 18.9 | 30.4 | 20.3 | 30.5 |
| fragment20bp_c | 20 | 11 | 11 | 22.7 | 21.7 | 21.1 | 34.4 |
| fragment20bp_d | 20 | 11 | 11 | 21.2 | 28.8 | 25.6 | 24.4 |
| fragment30bp_a | 30 | 14 | 14 | 36.7 | 32.5 | 18.3 | 12.5 |

| | | | | | | | |
|----------------|----|----|----|------|------|------|------|
| fragment30bp_b | 30 | 16 | 15 | 19.6 | 26.6 | 29.5 | 24.3 |
| fragment30bp_c | 30 | 16 | 16 | 43.6 | 28.3 | 21.4 | 6.8 |
| fragment30bp_d | 30 | 16 | 16 | 16.7 | 30.0 | 28.9 | 24.3 |
| fragment40bp_a | 40 | 18 | 18 | 36.4 | 32.3 | 19.1 | 12.1 |
| fragment40bp_b | 40 | 22 | 21 | 23.0 | 28.2 | 28.1 | 20.7 |
| fragment40bp_c | 40 | 20 | 19 | 16.3 | 31.1 | 26.9 | 25.7 |
| fragment40bp_d | 40 | 21 | 20 | 20.0 | 27.6 | 31.2 | 21.2 |
| fragment50bp_a | 50 | 25 | 24 | 42.1 | 24.8 | 21.5 | 11.6 |
| fragment50bp_b | 50 | 23 | 23 | 35.8 | 29.4 | 22.3 | 12.5 |
| fragment50bp_c | 50 | 21 | 21 | 36.3 | 26.4 | 17.2 | 20.2 |
| fragment50bp_d | 50 | 25 | 22 | 26.2 | 25.8 | 27.1 | 20.9 |
| fragment60bp_a | 60 | 29 | 26 | 31.8 | 22.3 | 26.3 | 19.7 |
| fragment60bp_b | 60 | 29 | 26 | 32.9 | 23.0 | 27.7 | 16.3 |
| fragment60bp_c | 60 | 29 | 26 | 28.7 | 25.3 | 27.1 | 19.0 |
| fragment60bp_d | 60 | 23 | 23 | 37.9 | 25.7 | 22.1 | 14.3 |
| fragment70bp_a | 70 | 33 | 30 | 31.3 | 23.6 | 28.0 | 17.1 |
| fragment70bp_b | 70 | 34 | 31 | 27.9 | 25.9 | 27.7 | 18.6 |
| fragment70bp_c | 70 | 32 | 29 | 34.4 | 22.0 | 26.8 | 16.8 |
| fragment70bp_d | 70 | 32 | 29 | 36.7 | 22.7 | 26.6 | 14.0 |
| fragment80bp_a | 80 | 38 | 34 | 25.0 | 26.8 | 27.9 | 20.3 |
| fragment80bp_b | 80 | 37 | 34 | 30.2 | 24.3 | 29.0 | 16.5 |
| fragment80bp_c | 80 | 33 | 33 | 34.8 | 25.3 | 27.7 | 12.3 |
| fragment80bp_d | 80 | 37 | 34 | 26.1 | 25.8 | 29.1 | 19.0 |

| | | | | | | | |
|-----------------|-----|----|----|------|------|------|------|
| fragment90bp_a | 90 | 40 | 36 | 28.7 | 25.2 | 28.1 | 18.0 |
| fragment90bp_b | 90 | 40 | 37 | 32.9 | 23.4 | 27.9 | 15.9 |
| fragment90bp_c | 90 | 42 | 37 | 28.0 | 24.7 | 29.1 | 18.1 |
| fragment90bp_d | 90 | 37 | 37 | 33.3 | 26.6 | 29.1 | 11.1 |
| fragment100bp_a | 100 | 44 | 40 | 31.0 | 24.4 | 27.2 | 17.4 |
| fragment100bp_b | 100 | 43 | 42 | 30.6 | 28.2 | 27.0 | 14.3 |
| fragment100bp_c | 100 | 42 | 42 | 32.4 | 26.6 | 29.7 | 11.2 |
| fragment100bp_d | 100 | 41 | 40 | 33.2 | 26.6 | 24.3 | 15.9 |
| fragment110bp_a | 110 | 47 | 43 | 33.8 | 22.4 | 26.8 | 17.0 |
| fragment110bp_b | 110 | 48 | 47 | 30.9 | 28.6 | 27.3 | 13.2 |
| fragment110bp_c | 110 | 45 | 45 | 31.5 | 26.4 | 27.5 | 14.6 |
| fragment110bp_d | 110 | 45 | 45 | 32.6 | 26.2 | 28.2 | 13.0 |
| fragment120bp_a | 120 | 52 | 51 | 31.6 | 26.6 | 31.5 | 10.3 |
| fragment120bp_b | 120 | 52 | 51 | 31.2 | 28.2 | 31.9 | 8.7 |
| fragment120bp_c | 120 | 54 | 52 | 29.8 | 27.6 | 28.1 | 14.5 |
| fragment120bp_d | 120 | 55 | 53 | 28.5 | 28.1 | 29.5 | 13.9 |
| fragment130bp_a | 130 | 56 | 54 | 32.4 | 26.5 | 26.4 | 14.7 |
| fragment130bp_b | 130 | 61 | 57 | 30.1 | 28.0 | 28.2 | 13.7 |
| fragment130bp_c | 130 | 59 | 57 | 28.8 | 28.0 | 28.8 | 14.4 |
| fragment130bp_d | 130 | 56 | 54 | 32.2 | 28.0 | 26.7 | 13.1 |
| fragment140bp_a | 140 | 65 | 63 | 29.9 | 27.7 | 28.7 | 13.6 |
| fragment140bp_b | 140 | 64 | 60 | 31.3 | 27.2 | 27.5 | 14.0 |
| fragment140bp_c | 140 | 65 | 63 | 29.4 | 27.7 | 28.7 | 14.2 |

| | | | | | | | |
|-----------------|-----|----|----|------|------|------|------|
| fragment140bp_d | 140 | 58 | 57 | 31.4 | 25.9 | 30.5 | 12.2 |
| fragment150bp_a | 150 | 63 | 62 | 32.1 | 26.9 | 29.7 | 11.4 |
| fragment150bp_b | 150 | 68 | 66 | 30.3 | 27.0 | 27.8 | 14.9 |
| fragment150bp_c | 150 | 61 | 60 | 32.7 | 26.0 | 29.8 | 11.5 |
| fragment150bp_d | 150 | 69 | 60 | 30.5 | 26.7 | 28.5 | 14.4 |
| fragment160bp_a | 160 | 68 | 67 | 33.9 | 26.3 | 29.0 | 10.8 |
| fragment160bp_b | 160 | 67 | 66 | 33.2 | 27.0 | 29.0 | 10.8 |
| fragment160bp_c | 160 | 67 | 66 | 33.0 | 26.6 | 29.5 | 10.8 |
| fragment160bp_d | 160 | 68 | 67 | 32.9 | 27.4 | 28.9 | 10.8 |
| fragment170bp_a | 170 | 72 | 71 | 33.2 | 26.5 | 28.9 | 11.4 |
| fragment170bp_b | 170 | 72 | 71 | 32.8 | 27.5 | 28.8 | 10.9 |
| fragment170bp_c | 170 | 72 | 71 | 32.8 | 26.9 | 29.4 | 10.9 |
| fragment170bp_d | 170 | 77 | 72 | 32.6 | 26.1 | 27.0 | 14.4 |
| fragment180bp_a | 180 | 75 | 74 | 33.4 | 26.7 | 27.4 | 12.5 |
| fragment180bp_b | 180 | 75 | 74 | 32.9 | 27.8 | 27.9 | 11.4 |
| fragment180bp_c | 180 | 74 | 73 | 31.4 | 27.2 | 29.1 | 12.4 |
| fragment180bp_d | 180 | 75 | 74 | 33.1 | 27.1 | 28.3 | 11.4 |
| fragment190bp_a | 190 | 79 | 78 | 32.6 | 27.6 | 27.9 | 11.8 |
| fragment190bp_b | 190 | 79 | 78 | 32.3 | 26.9 | 28.4 | 12.4 |
| fragment190bp_c | 190 | 78 | 77 | 32.4 | 26.8 | 28.9 | 11.9 |
| fragment190bp_d | 190 | 79 | 78 | 32.7 | 27.3 | 28.1 | 11.8 |
| fragment200bp_a | 200 | 83 | 82 | 32.7 | 27.4 | 28.5 | 11.3 |
| fragment200bp_b | 200 | 83 | 82 | 32.4 | 26.8 | 28.4 | 12.3 |

| | | | | | | | |
|-----------------|-----|----|----|------|------|------|------|
| fragment200bp_c | 200 | 82 | 81 | 32.4 | 27.3 | 28.0 | 12.3 |
| fragment200bp_d | 200 | 82 | 81 | 32.5 | 27.1 | 27.6 | 12.8 |
| fragment210bp_a | 210 | 86 | 85 | 32.6 | 27.0 | 27.2 | 13.2 |
| fragment210bp_b | 210 | 86 | 85 | 32.5 | 27.6 | 28.1 | 11.8 |
| fragment210bp_c | 210 | 86 | 84 | 32.4 | 27.1 | 26.8 | 13.6 |
| fragment210bp_d | 210 | 86 | 85 | 31.4 | 27.3 | 29.0 | 12.3 |

Appendix 5.4: Summary statistics for comparison trees of all 89 fragments. K-scores and Robinson-Foulds (R-F) scores are used to identify best comparison trees. Each score is ranked based on the dataset in ascending order. The top 10 comparison trees are shown in bold (also shown in Table 5.1).

| Overall rank | Comparison tree | K-score | Scale factor | R-F score | Partitions | K-score rank | R-F rank |
|-----------------|----------------------|----------------|-----------------|--------------|------------|-----------------|-------------|
| 39 | fragment103-1 | 0.24678 | 0.87069 | 74 | 147 | 42 | 37 |
| 64 | fragment103-2 | 0.27108 | 0.8072 | 90 | 147 | 53 | 73 |
| 60 | fragment103-3 | 0.26087 | 0.82158 | 88 | 147 | 50 | 69.5 |
| 27 | fragment103-4 | 0.21482 | 0.68058 | 76 | 147 | 8 | 44 |
| 56 | fragment103-5 | 0.27354 | 0.82409 | 78 | 147 | 55 | 50.5 |
| 65= | fragment103-6 | 0.30291 | 0.79214 | 84 | 147 | 65 | 62.5 |
| 3 | fragment206-1 | 0.20094 | 0.9669 | 64 | 147 | 5 | 4.5 |
| 4 | fragment206-2 | 0.19034 | 0.84009 | 66 | 147 | 3 | 7.5 |
| 9 | fragment206-3 | 0.21576 | 0.80556 | 68 | 147 | 9 | 11.5 |
| 83 | fragment20bp_a | 0.35114 | 0.64249 | 104 | 147 | 81 | 84.5 |
| 88= | fragment20bp_b | 0.40206 | 0.44079 | 108 | 147 | 89 | 88 |
| 86 | fragment20bp_c | 0.38333 | 0.48779 | 104 | 147 | 87 | 84.5 |
| 88= | fragment20bp_d | 0.39341 | 0.41183 | 114 | 147 | 88 | 89 |
| 80= | fragment30bp_a | 0.36311 | 0.39759 | 94 | 147 | 85 | 76.5 |
| 84= | fragment30bp_b | 0.36215 | 0.76528 | 98 | 147 | 84 | 82.5 |
| 75= | fragment30bp_c | 0.33102 | 0.80768 | 96 | 147 | 73 | 79.5 |
| 87 | fragment30bp_d | 0.37757 | 0.5584 | 106 | 147 | 86 | 86.5 |

| | | | | | | | |
|-----|----------------|---------|---------|-----|-----|----|------|
| 77 | fragment40bp_a | 0.34179 | 0.43022 | 92 | 147 | 79 | 75 |
| 84= | fragment40bp_b | 0.35004 | 0.61333 | 106 | 147 | 80 | 86.5 |
| 82 | fragment40bp_c | 0.35287 | 0.64708 | 96 | 147 | 83 | 79.5 |
| 80= | fragment40bp_d | 0.35154 | 0.65574 | 96 | 147 | 82 | 79.5 |
| 61 | fragment50bp_a | 0.30265 | 0.87323 | 82 | 147 | 64 | 58.5 |
| 73 | fragment50bp_b | 0.30936 | 0.65295 | 90 | 147 | 68 | 73 |
| 75= | fragment50bp_c | 0.33671 | 0.63644 | 94 | 147 | 76 | 76.5 |
| 78 | fragment50bp_d | 0.3395 | 0.75783 | 96 | 147 | 77 | 79.5 |
| 74 | fragment60bp_a | 0.33266 | 0.65669 | 88 | 147 | 74 | 69.5 |
| 65= | fragment60bp_b | 0.31417 | 0.62815 | 82 | 147 | 69 | 58.5 |
| 79 | fragment60bp_c | 0.34117 | 0.66059 | 98 | 147 | 78 | 82.5 |
| 67 | fragment60bp_d | 0.29322 | 0.47134 | 88 | 147 | 61 | 69.5 |
| 69 | fragment70bp_a | 0.33632 | 0.55357 | 84 | 147 | 75 | 62.5 |
| 68 | fragment70bp_b | 0.3169 | 0.71889 | 86 | 147 | 70 | 66 |
| 72 | fragment70bp_c | 0.31885 | 0.62721 | 88 | 147 | 71 | 69.5 |
| 70 | fragment70bp_d | 0.32273 | 0.64757 | 86 | 147 | 72 | 66 |
| 62= | fragment80bp_a | 0.29994 | 0.7918 | 84 | 147 | 63 | 62.5 |
| 51 | fragment80bp_b | 0.29074 | 0.72778 | 74 | 147 | 60 | 37 |
| 50 | fragment80bp_c | 0.26643 | 0.53905 | 76 | 147 | 52 | 44 |
| 62= | fragment80bp_d | 0.30922 | 0.68848 | 82 | 147 | 67 | 58.5 |
| 71 | fragment90bp_a | 0.30372 | 0.61406 | 90 | 147 | 66 | 73 |
| 58 | fragment90bp_b | 0.27603 | 0.7927 | 80 | 147 | 56 | 55 |
| 44 | fragment90bp_c | 0.29754 | 0.71542 | 72 | 147 | 62 | 27.5 |

| | | | | | | | |
|----------|------------------------|----------------|----------------|-----------|------------|----------|-------------|
| 59 | fragment90bp_d | 0.2611 | 0.72518 | 86 | 147 | 51 | 66 |
| 53 | fragment100bp_a | 0.28702 | 0.74831 | 76 | 147 | 59 | 44 |
| 18= | fragment100bp_b | 0.23218 | 0.81564 | 70 | 147 | 24 | 17.5 |
| 55 | fragment100bp_c | 0.27267 | 0.5602 | 78 | 147 | 54 | 50.5 |
| 43 | fragment100bp_d | 0.2328 | 0.65243 | 84 | 147 | 26 | 62.5 |
| 48 | fragment110bp_a | 0.28651 | 0.76285 | 74 | 147 | 58 | 37 |
| 46 | fragment110bp_b | 0.25482 | 0.63197 | 76 | 147 | 48 | 44 |
| 47 | fragment110bp_c | 0.25037 | 0.73225 | 78 | 147 | 44 | 50.5 |
| 49 | fragment110bp_d | 0.25072 | 0.74156 | 78 | 147 | 45 | 50.5 |
| 57 | fragment120bp_a | 0.2816 | 0.63065 | 78 | 147 | 57 | 50.5 |
| 54 | fragment120bp_b | 0.2599 | 0.70862 | 80 | 147 | 49 | 55 |
| 52 | fragment120bp_c | 0.24621 | 0.71337 | 82 | 147 | 41 | 58.5 |
| 45 | fragment120bp_d | 0.24183 | 0.78967 | 80 | 147 | 35 | 55 |
| 42 | fragment130bp_a | 0.25372 | 0.64351 | 74 | 147 | 47 | 37 |
| 36 | fragment130bp_b | 0.23495 | 0.78533 | 76 | 147 | 31 | 44 |
| 34 | fragment130bp_c | 0.23674 | 0.75893 | 74 | 147 | 33 | 37 |
| 23= | fragment130bp_d | 0.22977 | 0.75105 | 72 | 147 | 19 | 27.5 |
| 33 | fragment140bp_a | 0.23608 | 0.71893 | 74 | 147 | 32 | 37 |
| 8 | fragment140bp_b | 0.21147 | 0.90464 | 68 | 147 | 7 | 11.5 |
| 37 | fragment140bp_c | 0.23232 | 0.76219 | 78 | 147 | 25 | 50.5 |
| 40 | fragment140bp_d | 0.24904 | 0.69966 | 74 | 147 | 43 | 37 |
| 30 | fragment150bp_a | 0.24446 | 0.71499 | 70 | 147 | 39 | 17.5 |
| 26 | fragment150bp_b | 0.23001 | 0.75948 | 72 | 147 | 22 | 27.5 |

| | | | | | | | |
|-----------|------------------------|----------------|----------------|-----------|------------|-----------|------------|
| 23= | fragment150bp_c | 0.23366 | 0.70972 | 70 | 147 | 29 | 17.5 |
| 10 | fragment150bp_d | 0.22133 | 0.75834 | 68 | 147 | 12 | 11.5 |
| 18= | fragment160bp_a | 0.23386 | 0.70151 | 68 | 147 | 30 | 11.5 |
| 35 | fragment160bp_b | 0.2512 | 0.61926 | 72 | 147 | 46 | 27.5 |
| 41 | fragment160bp_c | 0.24338 | 0.6427 | 76 | 147 | 38 | 44 |
| 28 | fragment160bp_d | 0.24263 | 0.66838 | 70 | 147 | 37 | 17.5 |
| 32 | fragment170bp_a | 0.24558 | 0.63732 | 72 | 147 | 40 | 27.5 |
| 38 | fragment170bp_b | 0.23747 | 0.66394 | 76 | 147 | 34 | 44 |
| 31 | fragment170bp_c | 0.24214 | 0.64342 | 72 | 147 | 36 | 27.5 |
| 20 | fragment170bp_d | 0.2252 | 0.74531 | 72 | 147 | 15 | 27.5 |
| 29 | fragment180bp_a | 0.23357 | 0.6797 | 72 | 147 | 28 | 27.5 |
| 6 | fragment180bp_b | 0.20562 | 0.82576 | 66 | 147 | 6 | 7.5 |
| 17 | fragment180bp_c | 0.22988 | 0.67309 | 70 | 147 | 21 | 17.5 |
| 16 | fragment180bp_d | 0.22054 | 0.7698 | 72 | 147 | 10 | 27.5 |
| 13 | fragment190bp_a | 0.22176 | 0.65477 | 70 | 147 | 14 | 17.5 |
| 14 | fragment190bp_b | 0.23285 | 0.64758 | 66 | 147 | 27 | 7.5 |
| 7 | fragment190bp_c | 0.22157 | 0.7017 | 62 | 147 | 13 | 3 |
| 21 | fragment190bp_d | 0.22701 | 0.68441 | 72 | 147 | 16 | 27.5 |
| 11 | fragment200bp_a | 0.23031 | 0.64735 | 64 | 147 | 23 | 4.5 |
| 22 | fragment200bp_b | 0.22712 | 0.67601 | 72 | 147 | 17 | 27.5 |
| 25 | fragment200bp_c | 0.22981 | 0.66693 | 72 | 147 | 20 | 27.5 |
| 5 | fragment200bp_d | 0.1928 | 0.81952 | 66 | 147 | 4 | 7.5 |
| 1= | fragment210bp_a | 0.17262 | 0.86644 | 56 | 147 | 2 | 1 |

| | | | | | | | |
|-----------|------------------------|---------------|----------------|-----------|------------|----------|----------|
| 15 | fragment210bp_b | 0.22962 | 0.67296 | 70 | 147 | 18 | 17.5 |
| 1= | fragment210bp_c | 0.1715 | 0.84436 | 60 | 147 | 1 | 2 |
| 12 | fragment210bp_d | 0.22119 | 0.7026 | 70 | 147 | 11 | 17.5 |

CHAPTER 6

CONCLUSIONS

Overview

In recent history, there have been great increases in urbanisation, dynamic land use changes, and removal of naturally occurring habitats. This is mostly caused by increases in human populations (Cincotta et al. 2000), which has increased human demand for space, food, and energy resources. The human-wildlife conflict has caused major declines in many taxa (Gibbons et al. 2000), contributing to a number of conservation programmes that aim to reduce and prevent animal extinctions and declines.

Declines in some animal taxa can have drastic implications for ecosystems, as reductions in important organisms (e.g. keystone species) can affect populations of dependent species. Tortoises are increasingly being recognised as important ecosystem engineers, as they contribute to seed dispersal dynamics (Jerozolinski et al. 2009, Blake et al. 2012, Falcón and Hansen 2014), and—in the case of burrowing species (e.g. *Gopherus* spp.)—can provide refugia for many animals from dehydration and predation (Eisenberg 1983).

Data provided in this thesis has contributed to the understanding of spatial ecology in leopard tortoises (*Stigmochelys pardalis*) in the semi-arid Karoo. Global Positioning System (GPS) telemetry has been used to estimate species home range sizes (Chapter 2), movement patterns and habitat use (Chapter 3), and nocturnal activity (Chapter 4). Prior to this work, information on the importance of various resources was poorly understood. In addition, we have identified a cytochrome oxidase c subunit I (COI) region that may be important for species identification in tortoises (Chapter 5).

Contribution to tortoise ecology and management

Ten GPS transmitters were used to record bihourly movement in wild-caught adult leopard tortoises, from November 2014 to December 2015. A total of eleven tortoises were used in the study, after the redeployment of one transmitter following the death of one animal. Of these, two individuals expressed apparent nomadic behaviour, which is unusual or uncommon behaviour in tortoises, with only a few previously reported incidents (Chapter 2). The remaining nine tortoises had a mean home range of 121.86 ± 28.12 ha, based on kernel density estimation (KDE): larger than the majority of tortoise species' home ranges (Slavenko et al. 2016). Using generalised linear mixed models (GLMMs), we showed the importance of individual variability (sex, body mass) and weather (temperature, rainfall) variables for predicting monthly home range patterns.

These weather variables were also important in predicting movement patterns in leopard tortoises (Chapter 3). Using GLMMs once more to find important predictor variables for bihourly and daily movement, we found that tortoises moved further in warmer conditions and after periods of increased rainfall with mean daily movement of 256.97 m per day over the study period (Chapter 3). This supports previous findings on multiple species (Duda et al. 1999, Henen 2002, Eubanks et al. 2003, McMaster and Downs 2009, Guyer et al. 2012). However, we found that leopard tortoises were less reliant on permanent water resources than expected, with some individuals appearing to use food resources and rainfall to supplement water balance. Leopard tortoise movement patterns support previous knowledge on seasonality (movement increased in spring) and sexual differences (increased activity in males) (McMaster and Downs 2013a).

It is especially important to learn more about movement and seed dispersal potential in tortoises in ecosystems in the Karoo as most naturally-occurring fauna have been removed or

severely reduced, replaced by commercial livestock. However, leopard tortoises are still relatively common. Given the large home range sizes (Chapter 2), periodic long-distance movements (Chapter 3), and their slow gut transit times of 8.75 days (McMaster and Downs 2008), it can be expected that leopard tortoises could greatly contribute to seed dispersal in the Karoo.

Information provided by data from this research should be used to help guide management decisions in the region. The semi-arid Karoo has been targeted by energy companies for the introduction of hydraulic fracturing (fracking) activities in search of shale gas (De Wit 2011, Serrai and Corrigan 2015) despite potential salinisation and contamination of water (Schmidt 2013, Vidic et al. 2013). The results presented should be used as baseline data for a pre-fracking era in the region.

This research can also be used to advise suitable use of electric fencing, which causes mortalities in tortoises (Burger and Branch 1994, Beck 2010). Movement data shows that whilst tortoises may not be reliant on permanent water sources, movement is increased closer to these areas (Chapter 3), likely due to the knowledge of the existence of these resources in the local area. As animals maintain and continually update a cognitive map (Gautestad 2011), if a tortoise is aware of a permanent water resource, they are likely to make regular, long-distance movements. As such, we advise against the use of electric fencing near permanent water areas.

Tortoise activity is generally believed to be absent during night-time hours, though we have identified periods of nocturnal activity in leopard tortoises (Chapter 4). This is a previously undocumented behaviour, which we believe could be due to several factors, including lack of predation in adult leopard tortoises, generally warm night-time temperatures, and tortoises' ability to maintain core temperatures above ambient temperatures (McMaster and Downs 2013b). Further

research is required to ascertain what other variables contribute to night-time movement in tortoises.

Suggestions for future research

We believe that aspects of our research should be incorporated into future studies. As shown in a literature review on turtle home range sizes by Slavenko et al. (2016), the majority of previous studies use minimum convex polygons to estimate home range size. However, this methodology for estimating home range has been criticised for producing overestimations (Börger et al. 2006, Laver and Kelly 2008), whilst the method offers a lower level of information on habitat use within the area, unlike KDEs (Seaman and Powell 1996). One of the main reasons for this is that the most commonly used method of estimating home range in tortoises—minimum convex polygons (MCPs)—are very sensitive to extreme outliers, which can drastically increase estimations. We recommend that future tortoise home range studies attempt to use 95% KDEs, and use modern systematic home range estimation to evaluate core areas; e.g. *rhr* (Signer and Balkenhol 2015).

Despite providing detailed ecological information about leopard tortoises, data regarding other aspects of their ecology will be prepared for publication.

1. In addition to telemetry work presented in this paper, information was collected on biometrics of leopard tortoises on the study sites. A total of 99 individuals were found during systematic transects and opportunistically whilst moving around the study sites. Data, including location, sex, life stage, body mass, and carapacial measurements, will be tested to compare to other leopard tortoise populations elsewhere in southern Africa.
2. As well as collecting data on leopard tortoises, other tortoise species were also found in the field. Live and dead Angulate Tortoise (*Chersina angulata*) and Tent Tortoise

(*Psammobates tentorius*) specimens (n = 29) were located. Biometric information (sex, body mass, carapace measurements) can contribute to the understanding of these two species in the Karoo.

3. The Tent Tortoise exists as three sub-species (Hofmeyr et al. 2014, Turtle Taxonomy Working Group 2014); Northern Tent Tortoise (*P. t. veroxii*), Southern Tent Tortoise (*P. t. tentorius*), and Western Tent Tortoise (*P. t. trimeni*), with Northern and Southern populations overlapping in the study area. Counts of vertebral and marginal scutes (which can be used to identify species), and blood and/or scute samples (where possible), were collected for 26 Tent Tortoise individuals. As the subspecies can interbreed, genetic information can identify interbreeding between populations.
4. Despite recommending sites for designing mini-barcode primers, work is required to show that these mini-barcodes a) increases success rates of amplifying DNA sequences, and b) that the mini-barcode can correctly identify tortoise species. Mini-barcode primers will be produced and tested with known and unknown blood, flesh, and faeces samples.
5. It is well known that tortoises are important seed dispersers in some ecosystems. A large number of faeces samples were collected in the field. Mini-barcodes will be used to identify species (though many were easily identified as leopard tortoise faeces due to size and content), whilst germination trials will allow assessments of which plant species tortoises can aid with seed dispersal. Using germination trials along with the information collected on movement, it is possible to estimate the distance that seeds can be dispersed from parent plants.

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