Conservation ecology of Slater's skink *Liopholis slateri* in central Australia

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A thesis submitted for the degree of
Doctor of Philosophy

March 2017

Contents

Summary

The varied habitats of arid Australia have a diverse and specialised fauna that have evolved a range of life history strategies to persist in this arid environment. Desert river floodplain habitats are geomorphologically distinctive from their non-mesic counterparts. For surface-dwelling animals in these floodplain habitats, persistence is a trade-off between the advantages of relatively abundant food resources and the costs of episodic surface disturbances from infrequent, but unpredictable, rainfall. Riverine environments, as a whole, are threatened by invasive C4 grasses and dramatic changes in fire regimes. How terrestrial species persist in these high-risk habitats is not well understood. Slater's skink, *Liopholis slateri*, is a desert floodplain specialist, and it is endangered; the species has been recorded, relatively recently, at floodplain sites where it now no longer occurs. Land managers and ranger groups are investing in conserving *L. slateri*, but both ecological knowledge of, and survey protocols for the species are limited.

In this research, I sought to understand how *L. slateri* persists in disturbance-prone floodplain habitats. I focused my research on one population at Orange Creek. My aims were to (a) develop survey methods specific to *L. slateri*, and (b) use these methods to investigate the spatial dynamics, burrow occupancy, and fine-scale habitat use of a local population over four years.

I explored the use of photographic identification for *L. slateri* and compared the matching abilities of independent observers using a multi-choice key, with an automated computer algorithm, on a set of test photos. While neither independent observers nor computer matching had 100% accuracy, both systems sufficiently replicated my identifications, demonstrating the reliability of the technique for smaller populations. Future studies might consider using a combination of the two methods for individual identification of larger populations.

I investigated temporal activity patterns in *L. slateri*, with the aim of increasing detectability in observational surveys. Classification and regression tree (CART) models were used on repeated count data of individuals within a population, to correlate weather conditions with skink counts. Two weather variables, air temperature and humidity, influenced activity levels, but there was no consistent set of covariates that reliably explained surface activity. These data suggest that lizards respond to different weather conditions at different times of the day. I also found evidence of previously unreported nocturnal activity during the hottest months of the year.

In tracking the spatial dynamics of a population of over four years, I found evidence of a small and highly mobile, but site stable, population, with spatial clustering of burrows into local ‘neighbourhoods’. I observed both long-term residence of individuals and long-term use of burrows by multiple lizards at the site. Frequent movements within and among neighbourhoods, and regular new burrow construction, suggest a population capable of local dispersal in the event of high intensity disturbance. Dispersing individuals and some neighbourhoods may act as recolonization sources in the event of a flood extirpating the core population.

Using the spatial dynamics dataset, I characterised fine-scale habitat use of *L. slateri* within the broader floodplain. *Eremophila sturtii* and *Hakea leucoptera* were strong indicators of *L. slateri* occupancy. However, skink occupancy did not appear to be restricted to this vegetation assemblage, suggesting that *L. slateri* may be moderately flexible in its choice of habitat. Strong correspondence between buffel grass and lizard occupied areas, suggests that this invasive grass may be a considerable threat to *L. slateri* habitat.

The findings of this research have greatly increased our knowledge of the ecology of *L. slateri*, and our ability to effectively manage this endangered species.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university, and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person, except where due reference is made in the text.

Treilibs

Claire Treilibs

31 March 2017

Acknowledgements

This PhD research was funded by an ARC Linkage grant (LP1101000066) in collaboration with Flinders University, the Northern Territory Department for Land Resource Management, and the South Australian Museum. The NT Resources and Innovation Board contributed to funding for the field component. During my candidature, I was supported by an Australian Postgraduate Award from Flinders University. The NT Department of Land Resource Management and the CSIRO kindly provided facilities for me in Alice Springs.

Sincere thanks to Mike Bull and Chris Pavey for their supervision, guidance and encouragement through the project. I am grateful for their tuition and general wisdom, which they delivered with enthusiasm and good humour. Thanks also to Mike Gardner and Mark Hutchinson for advice and guidance, and Simon Ward and Alaric Fisher for supporting the project. I am profoundly grateful to Catherine Nano and Sathyamurthy Raghu, who generously contributed their time and statistical expertise to this research and to my education. Special thanks must go to Chris Pavey and Catherine Nano for their unstinting support and friendship throughout it all.

This project benefited greatly from early discussions with those with local knowledge about *Liopholis slateri,* and who took me out to population sites: Andrew Schubert, Gareth Catt, Peter McDonald, Aaron Fenner, Bill Low, Rachel Paltridge, Ltyentye Apurte rangers, Gerard Lessels, and the Tjuwanpa rangers.

I am grateful to those who helped with field work: Catherine Nano, Peter McDonald, Alistair Stewart, Grant Allen, Marg Friedal, Ben Kaethner, Marg Kaethner, Rose and Vern Treilibs, and Jordan de Jong. Peter Nunn, Rob Jansen and staff at the Alice Springs Desert Park generously collected data and shared observations from the *L. slateri* captive population. Thanks also to Aaron Fenner for many trips from Adelaide to demonstrate field capture techniques and procedures to me, and for kindly sourcing field equipment and materials.

Many other people contributed their technical capabilities. Andrew McGrath and Wolfgang Lieff from Airborne Research Australia diverted their flight path to acquire high resolution aerial imagery over the study site. Mina Ansari genotyped *L. slateri* scats. Sarah Pearson demonstrated DNA extraction and PCR procedures to me. Raphael Russel-Livingstone

donated hours of his time to my remote-student IT issues. Nigel Willoughby and Ken Clarke assisted with database design queries. Flinders University lizard lab mates Steph Godfrey, Aaron Fenner, Julie Schofield, Stephan Leu, Mehregan Ebrahimi, Jess Clayton, Leili Shamiminoori, Jordan de Jong, Jana Bradley, Dale Burzacott and Sarah Pearson offered helpful advice on technical aspects and on doctoral survival.

Many colleagues in Alice Springs contributed their expertise and assistance in various forms and at various stages of the project. In particular, thank you to: Simon Ward, Peter McDonald, Jane Brim Box, Jeff Cole, Alistair Stewart, Robyn Delaney, Jason Britten, Pat Hodgens, Jason Barnetson, Glenis McBurnie, Glenn Edwards, Jaimie Moore, Paul Box, Grant Allen, Coral Allen, Sheridan Martin, Bill Low, Lauren Young, Gary Bastin, Marg Friedal, Steve Morton, Christine Schlesinger, Rachel Paltridge and Wendy Stuart.

Thanks also to the skink identification observers for taking the test: Rose Treilibs, Ben Kaethner, Jess Harries, Alison Jackson, Peter McDonald, Simon Ward, Lisa Doucette, Natasha Cadenhead, Marica Rumbacic, Rebecca Glen, Caiti Read, Beth Woodward, Ellenie Pond, Chris Halacas, Nick Randles, Kirrily Douglas, Peter Nunn, Lauren Young, Alistair Stewart, Thalie Partridge, Jon Hodgetts, Lindsey Langford, Tristan Simons, and Michelle Armistead. Ellenie Pond requested that I mention her exceptionally high score (22/24) as a 'non-experienced' observer.

Finally, thank you to Anthea, Matt and Evie for keeping things in perspective, to Ben for being a patient, caring and supportive partner, and to my parents, for their love and support.

Candidate contribution to manuscripts

Four chapters of this thesis have been modified from manuscripts prepared for submission or published in peer-reviewed journals. Co-authors of these manuscripts have given their permission for the work to be included in this thesis. The relative contributions from each co-author are shown for each manuscript.

Chapter 5

Treilibs, C.E., Pavey, C.R., Hutchinson, M.N. & Bull, C.M. (2016). Photographic identification of individuals of a free-ranging, small terrestrial vertebrate. *Ecology and Evolution*, **6**, 800–809.

CET 75%; CRP 10%; MNH 5%; CMB 10%

CET collected the field data, developed the key, designed the identification test, analysed the data, and wrote the manuscript. CRP advised on test procedures and reviewed the manuscript. MNH advised on field work. CMB suggested analyses and reviewed the manuscript.

Chapter 6

Treilibs, C.E., Pavey, C.R., Raghu, S. & Bull, C.M. (2016). Weather correlates of temporal activity patterns in a desert lizard: insights for designing more effective surveys. *Journal of Zoology*, **300**, 281–290.

CET 70%; CRP 10%; RS 10%; CMB 10%

CET collected the field data, analysed the data, and wrote the manuscript. CRP suggested analyses and reviewed the manuscript. SR suggested and guided analyses. CMB reviewed the manuscript.

Chapter 7

Treilibs, C.E., Pavey, C.R., Gardner, M.G., Ansari, T.H., Johnston, A. & Bull, C.M. (2016). Spatial dynamics and burrow occupancy in a population of Slater's skink *Liopholis slateri*. (To be submitted.)

CET 60%; CRP 10%; MGG 5%; THA 10%; AJ 5%; CMB 10%

CET collected the field data, analysed the spatial data, and wrote the manuscript. CRP conceived conceptual basis, suggested analyses, and reviewed the manuscript. MGG analysed the molecular data and advised on the genetics sections of the manuscript. THA genotyped the scats and summarised the molecular data. AJ extracted the DNA from scats. CMB suggested analyses and reviewed the manuscript.

Chapter 8

Treilibs, C.E., Nano, C.E.M., Pavey, C.R., & Bull, C.M. (2016). Fine-scale habitat use of a terrestrial desert floodplain specialist. (In preparation.)

CET 60%; CEMN 25%; CRP 5%; CMB 10%

CET collected the field data, analysed the data, and wrote the manuscript. CEMN gave guidance on the experimental design, assisted with field work, ran the SIMPER analysis, and reviewed the manuscript. CRP reviewed the manuscript. CMB reviewed the manuscript.

Each of these four chapters includes broader introductions about the central themes and questions addressed, specific to each chapter. As such, the following general introduction is relatively brief, aiming to place the study system in context of wider arid Australia.

Chapter 1: General introduction

Australia's arid region covers 70% of the continent, and hosts a unique and highly diverse fauna (Barker & Greenslade 1982; Byrne *et al.* 2008). Recent aridification events, starting around 15 million years ago, created the conditions for the radiation and rapid diversification of Australian arid-adapted biota (Rabosky *et al.* 2007; Byrne *et al.* 2008). Some of the deepest divergences of arid taxa occurred at the onset of desiccation around 15 million years ago, while other lineages recently expanded during the cycling of climatic conditions over the last 0.8 million years (Byrne 2008). Phylogenetic studies of contemporary arid-adapted species suggest that they have evolved either from ancestors in mesic habitats, or by speciating rapidly within localised refugia (Byrne *et al.* 2008; Fujioka & Chappell 2010).

These evolutionary drivers have created an exceptional diversity of lizards in arid Australia (Pianka 1994; Rabosky *et al.* 2007; Cogger 2014). Compared with North American deserts that are similar in size, Australia has a much richer lizard fauna (Schall & Pianka 1978; Morton & James 1988). Australian sphenomorphine skinks, such as *Ctenotus* and *Lerista*, have one of the greatest vertebrate radiations of any continent (Rabosky *et al.* 2007). These extensive radiations have occurred despite a relatively uniform topography with few geographic barriers to promote speciation (James & Shine 2000). Lizard taxa occupy almost every desert habitat, but have highest species richness in non-mesic habitats, particularly spinifex (*Triodia*) grasslands (Pianka 1972; James & Shine 1988, 2000). The variety of different arid habitat types may partially explain the extensive speciation of lizards in arid Australia (Chapple *et al.* 2004; Shoo *et al.* 2008).

In contrast to the relatively uniform landscape, arid central Australia also contains several topographically complex formations of inland ranges. One of the most prominent formations is the Central Ranges, which comprise the MacDonnell, Petermann, Mann, Musgrave and Everard ranges of central Australia. During periods of aridification, these ancient landforms were centres of persistence for biota, isolated by the younger sandy deserts that formed less than 1 million years ago (Fujioka *et al.* 2009). These mesic ranges were evolutionary refugia that maintained a relatively stable thermal and hydric environment when climatic conditions were otherwise unsuitable (Hewitt 2004; Byrne *et al.* 2008; Pepper *et al.* 2011). Today, these inland ranges are refuges for relictual species that were once more widespread (Byrne *et al.* 2008; Fujita *et al.* 2010) (see below).

1.1 Persistence in arid environments

The varied habitats of arid Australia have a diverse and specialised fauna that have evolved a range of life history strategies to persist in these habitats. Spatial patterning in landform, soil, rainfall and vegetation, have created a mosaic of different habitat types that differ in reliability of resources for consumers (Shmida *et al.* 1986; Stafford-Smith & Morton 1990). Irregular resource supply has thus shaped a range of ecological strategies for fauna to persist in arid environments.

Evolutionary forces and environmental processes influence an organism's adaptations for survival. Arid-adapted fauna may be habitat generalists or specialists, have high or low vagility, have long or short generation times, or high or low reproductive rates (Southwood 1988). For example, fauna with irruptive strategies, such as desert rodents, are able to rapidly increase their numbers in times of uncertain resource supply, following rain (Pavey *et al.* 2014). In contrast to this irruptive strategy, nomadic strategies, used by migratory waterbirds, track resources as they change over space (Pedler *et al.* 2014b). In habitats where resources are relatively continuous and reliable, such as riverine channels, species may use a more persistent, stable strategy (Stafford-Smith & Morton 1990).

1.2 Diversification in arid-adapted *Liopholis*

The Australian scincid genus *Liopholis* (within the *Egernia* group) has 11 species widely distributed throughout the continent (Cogger 2014). Within this genus is a monophyletic clade of five burrowing species: *L. multiscutata*, *L. kintorei*, *L. inornata, L. striata*, and *L. slateri* (Chapple & Keogh 2004; Gardner *et al.* 2008). *Liopholis multiscutata* occupies a range of coastal to semiarid areas, but the other four species are adapted for arid environments; they burrow, have lower rates of water loss, and restricted diurnal exposure compared with congeners that occur in more temperate areas (Henzell 1982). The monophyletic structure of this group suggests these adaptations to aridity occurred only once within the group, probably from a *L. multiscutata*-like common ancestor from mesic areas (Chapple & Keogh 2004), which may have been ‘pre-adapted’ to arid conditions (Byrne *et al.* 2008). The divergence of arid and mesic lineages in *Liopholis* occurred around 4–8 million years ago (Chapple & Keogh 2004).

Of the four arid-adapted members of the burrowing *Liopholis* group, *L. inornata*, *L. striata* and *L. kintorei,* have large distributions within sandy deserts, and *L. slateri* is restricted to floodplains of the MacDonnell Ranges (Cogger 2014). The restricted distribution of *L. slateri* within the refugial area of the Central Ranges suggests it may be a relictual species that, like its arid group members, was once more widely distributed before periods of aridification (Henzell 1982; Chapple & Keogh 2004). Yet the strategies that this species has evolved to persist in these floodplain habitats of the Central Ranges are not well understood.

1.3 Slater’s skink *Liopholis slateri*

Liopholis slateri is a viviparous lizard (Scincidae) that occurs in floodplains of ephemeral rivers and low-order watercourses within the MacDonnell Ranges biogeographic region of the Northern Territory. The species has been recorded, relatively recently, at floodplain sites where it now no longer occurs. Consequently, in 2000, it was listed as Endangered under the Commonwealth *Environment Protection and Biodiversity Conservation Act* 1999. A recovery plan for the species was prepared in 2004 (Pavey 2004a).

As a nationally endangered species, awareness of, and concern for, *L. slateri* has grown. Dedicated efforts over the past decade have resulted in several new population discoveries (see Chapter 3). *Liopholis slateri* populations are found on Aboriginal Land Trusts, pastoral leases and government owned land in the MacDonnell Ranges bioregion, and monitoring of several populations is ongoing. Land managers and ranger groups are already investing in conserving *L. slateri*, but survey and monitoring protocols for the species are limited.

Multiple potential threats to *L. slateri* exist (Figure 1.1), but data demonstrating direct impacts to the species are few. At the Loves Creek population site, cattle severely trampled the channel banks and low vegetation of a tributary in early 2013, decimating *L. slateri* burrows that were established in the banks (pers. obs). After the trampling event, in April 2013, there

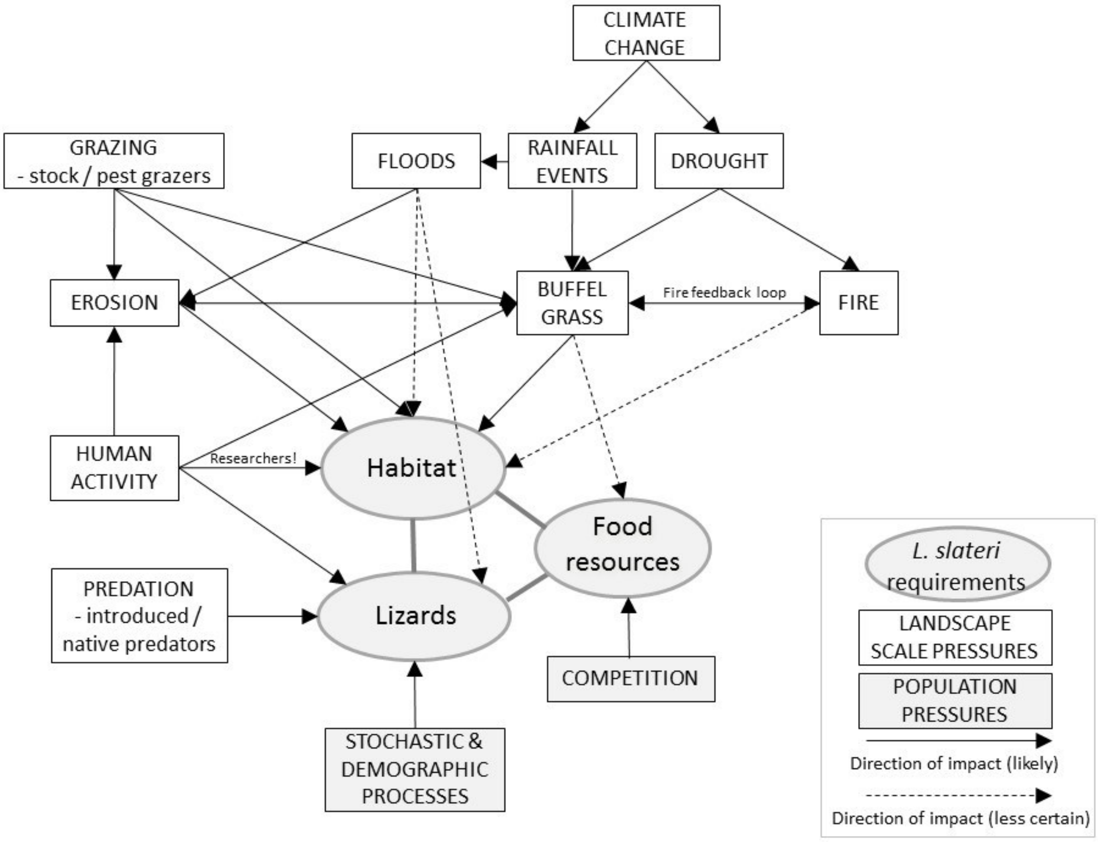


Figure 1.1. Conceptual model of potential influences on Slater's skink *Liopholis slateri.*

was no evidence of lizards occupying this area of the population where they had been recorded for the previous five years of monitoring (Paltridge 2013).

Flood events are concomitant with the flood-pulse dynamics of ephemeral desert rivers (Bunn *et al.* 2006), but how flooding affects *L. slateri* populations is unknown. Predictions for more frequent and intense rainfall events (Healy 2015), and more extreme La Niña events (Cai *et al.* 2015), may cause increased flooding disturbance to the species.

Buffel grass (*Cenchrus ciliaris*) has been identified as a particular threat to *L. slateri,* and a possible cause of decline (Pavey 2004a). The invasive pasture grass favours alluvial soils, and consequently has spread along water courses, establishing dense stands along river channels in central Australia (Griffin 1993). Fire has been shown to interact with buffel grass and alter the composition and structure of these alluvial habitats (Miller *et al.* 2010). Because of the requirement of *L. slateri* for foraging in open areas, buffel grass has been highlighted as a cause for concern by altering *L. slateri* habitat (McKinney *et al.* 2015). While the grass has encroached into areas where *L. slateri* is present, direct impacts of this vegetation change to these lizard populations remain unquantified.

1.4 Surveys and monitoring

Current *L. slateri* monitoring practices vary from annual to opportunistic surveys. Typically, surveys involve measuring abundance of active burrows, confirmed by the presence of scats, diggings, or sightings of the lizard (R. Paltridge pers. comm.). Previous sites and burrows are revisited, and new burrows are marked. In these surveys, population abundance and trends are inferred from the number of active burrows, but little is known about burrow occupancy of the species. For example: how many burrows an individual uses, if multiple individuals use the same burrow, or if lizards occupy the same site in sequential seasons.

Liopholis slateri can be difficult to detect. Like many arid reptiles, extremes of temperature and aridity constrain surface activity over extended periods of time (Whitford 2002), limiting opportunities for behavioural observation. For this reason, burrow surveys have been used in preference to observational surveys of lizards. However, for estimates of population abundance, surveys could be more effective if timed with environmental conditions, such as weather conditions, that increase the detectability of *L. slateri* (Sun *et al.* 2001; Brown & Shine 2002).

Two techniques for identifying individuals, which may be applicable to surveys of *L. slateri,* have been developed in recent years. One technique, photographic identification, has become increasingly popular, but limitations in both manual and computer-automated recognition of individuals are particularly problematic for smaller taxa (<500g) (Hartog & Reijns 2014). The natural history and varied scale markings of *L. slateri* make it a potentially suitable candidate for photo-identification, and this possibility is worth exploring. The other technique, distinguishing individuals from DNA in their scats, has recently been developing (Pearson *et al.* 2015). Most *Liopholis* species, and members of the *Egernia* group, pile scats outside of burrows or other shelter sites (Chapple 2003), thus providing a ready means of collecting molecular data. Identification of individuals from scat DNA has recently been used for investigating population structure in *Egernia stokesii* (S. Pearson unpubl.). This method could be potentially suitable for *L. slateri.*

1.5 Research objectives and scope

In this research, I sought to understand how *Liopholis slateri* persists in desert floodplain habitats. My aims were to (a) develop survey methods specific to *L. slateri*, and (b) use these methods to investigate the spatial dynamics, burrow occupancy, and fine-scale habitat use of a local population over four years. Specifically, my research objectives were to:

1. Trial non-invasive methods for identifying individuals;
2. Increase effectiveness of *L. slateri* surveys and monitoring programs;
3. Assess the ecological persistence strategy of *L. slateri* in floodplain habitats;
4. Characterise fine-scale habitats of occupied areas within the floodplain; and
5. Assess the threat of buffel grass to *L. slateri* habitat.

For this research, I focused on one population (at Orange Creek in Owen Springs Reserve) to study intensively over four spring-summer periods. Initially I considered studying multiple populations, but large distances to get to population sites, access restrictions, and extreme temperatures, meant that intensively observing more than one population was not feasible. While I had company in the field for a small proportion of surveys, I collected all field data, usually surveying two to three times a week during the activity season (Sept–May). Thus I chose to study the population at Orange Creek in Owen Springs Reserve because of its close proximity to Alice Springs (about 40 km), and relatively easy access. Compared with six other population sites that I inspected, Orange Creek appeared to be representative habitat from which my research conclusions might be extrapolated.

1.6 Thesis structure

The next three chapters present the background information for the subsequent research chapters. Chapter 2 provides a brief overview of the biology and ecology of *L. slateri*. In Chapter 3, I outline the history of population discoveries and summarise current known populations. Chapter 4 introduces the study site at Orange Creek.

In the research chapters following (Chapters 5–8), I present the studies on survey methods and sampling, and on the persistence and habitat use of *L. slateri*. In Chapter 5, I develop and test a photographic identification technique that I used for tracking individuals. Chapter 6 considers the issue of detectability and sampling effort in surveys and monitoring of *L. slateri*.

In Chapter 7, I investigate the spatial dynamics and burrow occupancy of the Orange Creek population using the photographic identification technique explored in Chapter 5. In Chapter 8, I look at the physical and vegetation patterns of the fine-scale habitat use derived from the movement and burrow use data. The final chapter (Chapter 9) is a general discussion of the findings and their contribution to ongoing conservation efforts.

Chapter 2: Biology and ecology of *Liopholis slateri*

Slater's skink *Liopholis slateri* (Scincidae) is a rare and globally endangered lizard that occupies floodplains of rivers and low-order tributaries within the MacDonnell Ranges biogeographic region of central Australia. Its current known range is restricted to 11 (meta-) populations, all located within an area of about 12,000 km^2 (see Chapter 3). The skink is a member of the *Liopholis whitii* species-group (formerly *Egernia*) that speciated into arid and temperate adapted lineages around 4-8 Myr (Chapple & Keogh 2004; Byrne *et al.* 2008).

Liopholis slateri is a mid-sized (85 mm snout-vent length) viviparous lizard. It is a smooth-scaled robust skink, with a deep head and a short snout (Storr 1968). The head and back are olive grey-brown, grading to a pale rufous brown on the flanks and limbs, and the underside is pale grey-white (Figure 2.1). Black or dark brown edges of some dorsal scales form longitudinal stripes. Similarly, dark-edged scales on the lateral side of the head form distinct markings, which may be a means of identifying individuals (see Chapter 5). While sample sizes are insufficient for a statistical analysis of sexual dimorphism in the species, slight variations are likely to be similar to those of its congeners *L. inornata* and *L. striata*; in these species, males have slightly larger heads, and females have more elongated bodies and shorter limbs (K. Aplin pers. comm.). However, in *Liopholis* species, sex cannot be reliably differentiated visually, but adults, sub-adults, and neonates can be told apart based on their relative size.

Liopholis species of this size probably take about two years to reach maturity and live for greater than five years. While age at maturity and longevity is unreported for *L. slateri* (Chapple 2003), estimates may be inferred from similar sized congeners. Specifically, *L. inornata* matures at two years (Daniel 1998). Mid-sized *Liopholis* species have been reported to live for over five years (*L. modesta*) or eight years (*L. whitii*) (Chapple 2003).

The eleven described species in the genus *Liopholis* are semi-fossorial, burrowing specialists that use an ambush foraging strategy (Wu *et al.* 2015). *Liopholis slateri* is a dietary generalist and individuals consume a range of invertebrate prey, mainly ants and termites (Pavey *et al.* 2010). The species is active predominantly during spring-summer, after over-wintering in burrows. This seasonal cycle of summer activity and winter inactivity is typical of many ectotherms in desert and temperate regions (James & Shine 1985; Adolph & Porter 1993).

Figure 2.1. Slater's skink *Liopholis slateri.*

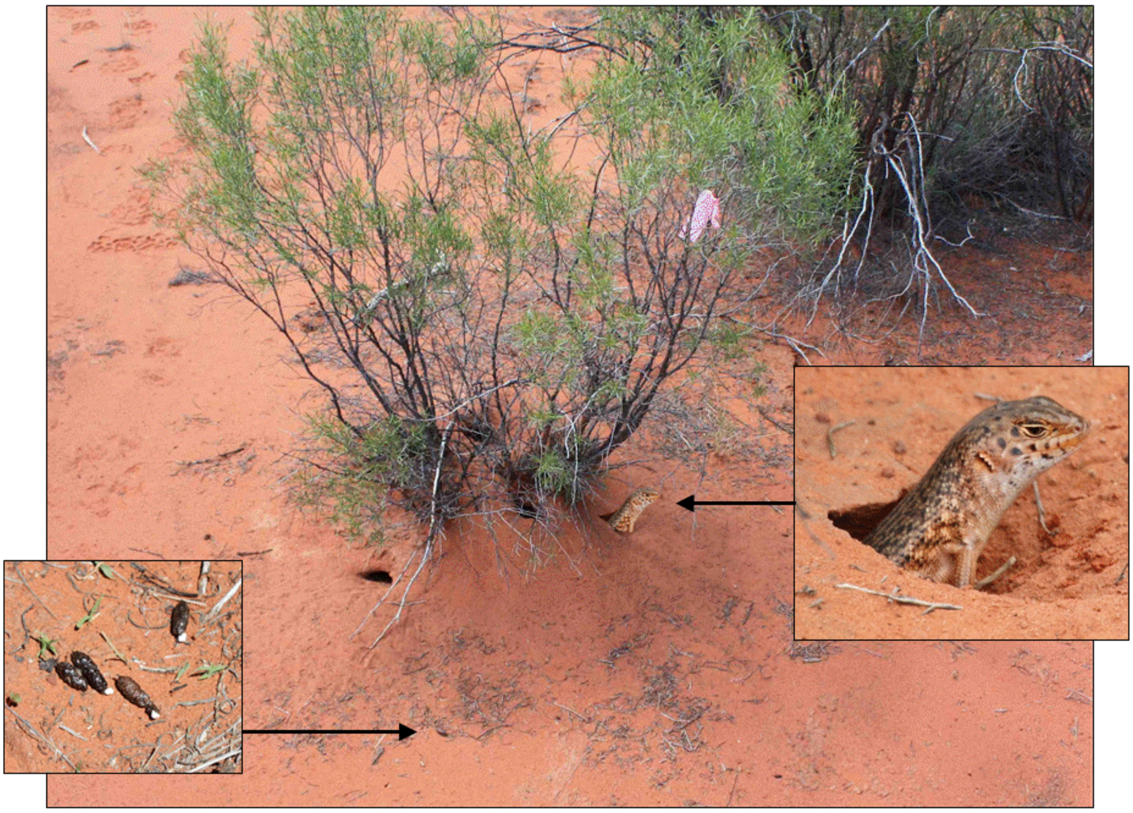


Figure 2.2. A typical active *Liopholis slateri* burrow: lizards maintain multiple entrances from which they bask and forage. Scat piles are commonly observed outside burrows.

Individuals of this species excavate multi-entrance burrows. After emergence, a lizard will sit at (or near) a burrow entrance basking and/or waiting for prey (Fenner *et al.* 2012a; McKinney *et al.* 2015) (Figure 2.2). This is their major daily activity, and movements away from the burrow are infrequent and rarely observed (see Chapter 6). Burrows are typically built into soil mounds at the base of shrubs, or into the banks of water courses. In both cases, burrow entrances are elevated above the surface. Entrances are connected by an internal tunnel system, often incorporating 'pop holes' – thinly concealed entrances – at higher elevations on the mound (Fenner *et al.* 2012b).

Lizards regularly maintain burrow entrances by casting sand from the entranceway with their fore limbs and distributing it in front of the entrance, forming a 'delta' shaped pattern of sand (pers. obs). Some authors have speculated that creating these sand fans, instead of sand piles, functions to conceal the burrow location from predators (Pianka & Giles 1982).Tracks and tail drag marks are often visible on the sand fan of active burrows. Scat piles, characteristic of the genus (Chapple 2003), are also commonly observed near active burrows (Figure 2.2). These features allow the ready identification of an active burrow within a population site, even when a lizard has not been seen.

Chapter 3: An historical overview and summary of current populations

3.1 Introduction

Slater's skink *Liopholis slateri* (Storr, 1968) is part of the former genus *Egernia* that was split into four genera in 2008: *Liopholis, Bellatorius, Egernia and Lissolepis* (Gardner *et al.* 2008). *Liopholis* is a clade of 11 burrow and rock dwelling species within which are seven temperate-adapted and four desert-adapted members, the latter group comprising *L. inornata, L. kintorei, L. striata* and *L. slateri* (Gardner *et al.* 2008). Of these, *L. slateri* and *L. kintorei* are listed as threatened under Commonwealth legislation.

Two subspecies of *L. slateri* have been described: *L. s. slateri,* from the Northern Territory and *L. s. virgata,* from northern South Australia. While the species is listed as Endangered under the Commonwealth *Environment Protection and Biodiversity Conservation* (EPBC) Act 1999, the Northern Territory subspecies is currently only listed as Vulnerable under Territory legislation (*Territory Parks and Wildlife Act* 2000*). Liopholis slateri virgata* is listed as Endangered in South Australia (*National Parks and Wildlife Act* 1972), although the subspecies has not been located for over 100 years (see below).

I documented the historical sequence of discovery of populations of *Liopholis slateri slateri* using local knowledge from researchers and land managers actively involved in discovering and monitoring populations of the subspecies (Figure 3.1). For older records of *L. s. slateri* (prior to 1980) and of *L. s. virgata*, I confirmed historical reports with specimen data lodged in the multiple-source national atlas, Atlas of Living Australia (ALA, http://www.ala.org.au, accessed 14 December 2015). I also used historical documents and anecdotal reports to expand on these discoveries and document other events of relevance to the persistence of the species, with particular reference to events that occurred at the type locality (summary in Table 3.1). Finally, I pooled occurrence data from the most recent surveys of extant sites of the Northern Territory subspecies *L. s. slateri* and summarised the status of currently known populations.

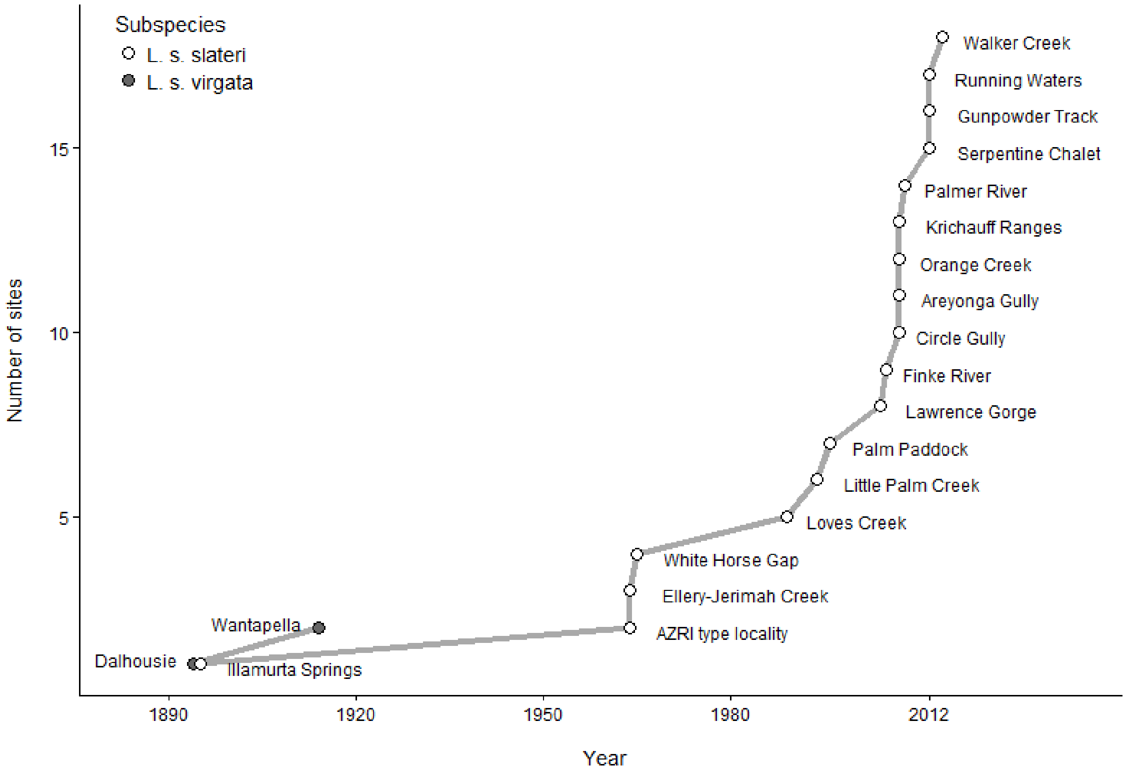


Figure 3.1. Cumulative number of *Liopholis slateri* sites (distinct from meta-population; see Table 3.2) detected since European settlement: Northern Territory subspecies *L. s. slateri* (white), South Australian subspecies *L. s. virgata* (grey). Exact localities of the 1894–1914 records are unknown. (Locations of each population are shown in Figure 3.6.)

Table 3.1. Timeline of events pertaining to Slater's skink *Liopholis slateri*, as documented since European settlement.

Year	Event	Source
1894	Horn Expedition	(Spencer 1896; Shea 2003)
1914	Captain S.A. White expedition	(White 1914)
1958–1966	Prolonged drought, top soil eroded by wind and grazing on Todd river floodplain – severe dust storms	(Keetch 1979; Albrecht & Pitts 2004)
1950s–1960s	Trials of mechanical planting for dust control; spiral tilling and sowing of buffel seed at AZRI block near type locality (see Figure 3.5)	(Keetch 1979; Albrecht & Pitts 2004)
1964–1965	65 specimens collected by K. Slater and D. Linder 8 km south of Alice Springs (AZRI type locality)	(Pavey 2004a; ALA 2015)
1964	32 specimens collected by K. Slater and D. Lindner at the junction of Ellery and Jerimah creek	(Pavey 2004a; ALA 2015)
1968	Both subspecies formally described	(Storr 1968)
1970s	Rapid spread of buffel grass with heavy rains – Alice Springs township and more distant drainage systems	(Griffin 1993)
2000	Listing of *L.slateri* as Endangered in the NT and Endangered nationally	(McAlpin 2000)
2004–2005	Recovery plan for 2005–2010 prepared and implemented	(Pavey 2004a)
2004–2010	Targeted searches to (re)discover populations	(Pavey 2004)
2008	*Egernia* split into four genera, including *Liopholis*	(Gardner *et al.* 2008)
2011	Revision of NT threatened species listing to Vulnerable following discovery of more populations	
2012	Incidental discovery of three more populations during biological surveys	

3.2 Population discovery

3.2.1 1894 – 1914

The first documented specimens of *Liopholis slateri* were from the 1894 Horn Expedition to central Australia. The expedition began at Oodnadatta in South Australia, followed the telegraph line into the Northern Territory and continued along the Finke River to the MacDonnell Ranges. Sir Walter Baldwin Spencer (1860 – 1929) and associates collected and documented herpetological specimens and other fauna along the way. While their collecting locations were recorded with considerably more precision than those from previous expeditions, exact localities of many of their specimens are unknown (Shea 2003). The expedition included specimens of *L. s. slateri* collected by E. C. Cowle from Illamurta Springs in the Northern Territory, and others less specifically from 'Central Australia' (Figure 3.2). They also collected specimens of *L. s. virgata* from northern South Australia (Figure 3.3) – the original labels in Spencer's hand writing are reported to read "Dalhousie" (source: ALA 2015).

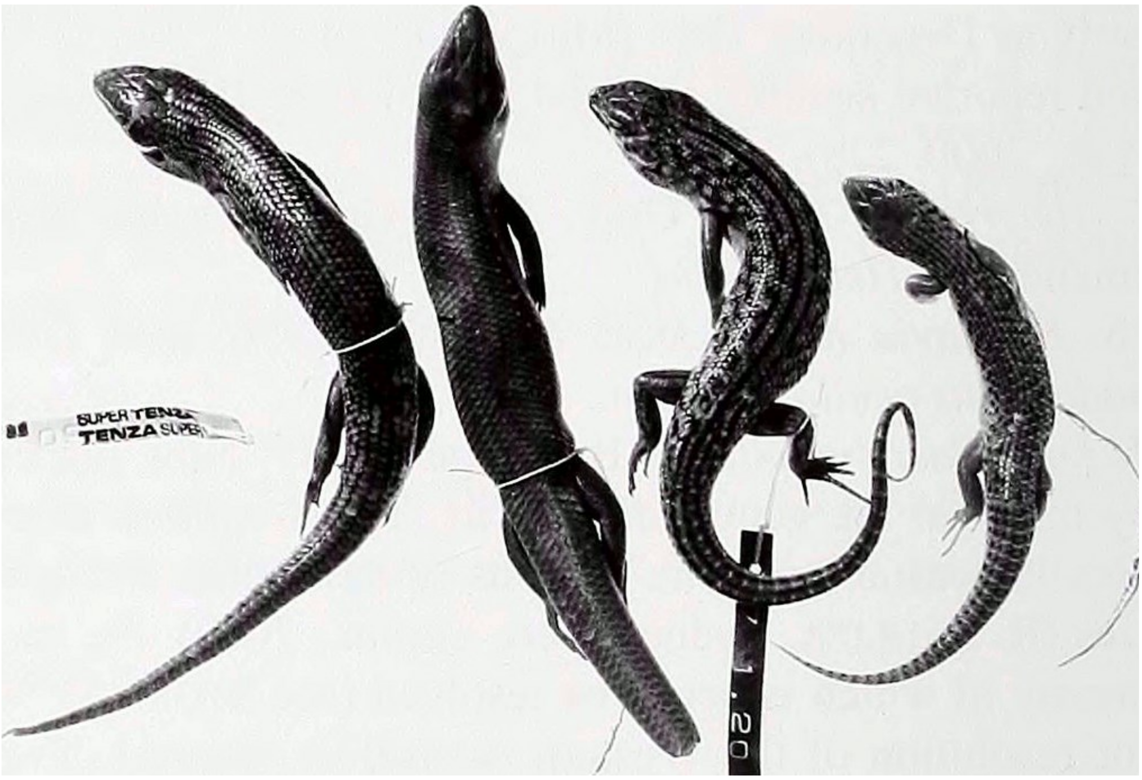


Figure 3.2. Specimens collected by the Horn Expedition in the Natural History Museum, London (Source: Internet archives, photo taken circa 1950). From left to right: *Liopholis slateri slateri, L. margaretae, L. s.virgata and L. inornata* (Shea 2003).

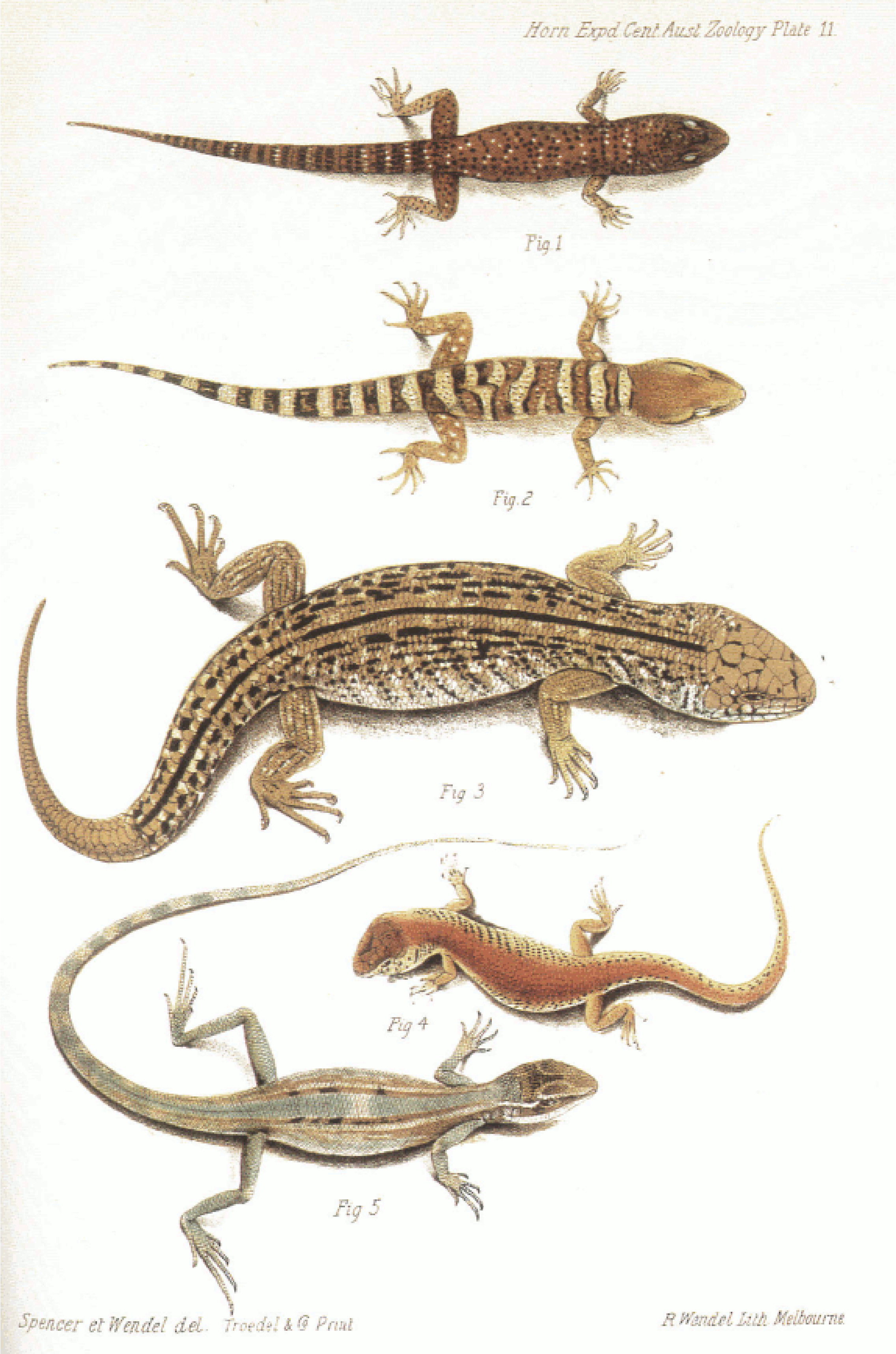


Figure 3.3. Drawings of specimens collected on Horn Expedition. ‘Fig 3’ is of *Liopholis slateri virgata* (Spencer 1896; Shea 2003).

The expedition of Captain S. A. White followed in 1914, collecting four specimens of *L. s. virgata* somewhere “between Oodnadatta and the Everard Ranges”, which may be around Wantapella swamp (White 1914; Storr 1968). The specimens collected on the Horn and White expeditions were not described and identified as *L. slateri* until 1968 (see below).

The specimens of *L. s. virgata* collected on Captain S. A. White’s 1914 expedition were the last specimens of the South Australian subspecies to be recorded. Targeted searches of northern South Australia were undertaken for four days in November 2004 (G. Fyfe *in. litt*.) and for two weeks in September 2012 searching the region of the early expeditions, but without success.

3.2.2 1964 – 1970

Half a century passed before a further record of *Liopholis slateri*. In September 1964 and March 1965, Ken R. Slater and David R. Lindner, from the Animal Industry branch of the Northern Territory Administration, collected 65 specimens on the Todd River floodplain 8 km south of Alice Springs [23°46’S 133°53’E] (Storr 1968), near the Arid Zone Research Institute (AZRI) block. The species appears to have been common in the area as Slater collected 27 of the skinks in one day (Finnane 2000). They also collected specimens from the junction of Ellery and Jerimah creeks (n=32) in November – December 1964, and White Horse Gap on Tempe Downs (n=1) in April 1965 (source: ALA 2015). As a percentage of the total number recorded in the national archive, Slater and Lindner’s collection over the seven month period (Sept 1964 – April 1965) makes up 59% of all *L. slateri* preserved specimens held in Australia (n=98/165, source: ALA 2015).

In 1968, G. M. Storr revised the *Egernia* group, and described for the first time the two subspecies now known as *Liopholis slateri virgata* and *L. s. slateri* (formerly *Egernia slateri virgata* and *E. s. slateri*). Storr used one of Captain S.A. White’s 1914 specimens as the holotype for the South Australian subspecies *L. s. virgata* and one of Slater’s collection from the AZRI site as the holotype for *L. s. slateri* (Storr 1968). Consequently, the AZRI site on the Todd River floodplain became the type locality for *L. s. slateri*.

Robert Henzell and associates collected an unknown number of specimens (but more than five) in 1969 for his ecophysiological PhD project at the University of Adelaide, without reporting any problems of finding animals. Ken Slater (in Henzell (1972)) observed that during the drought of the 1960s, *L. s. slateri* had a greater survival in areas where *Hakea divaricata* was abundant, but during the last year of the drought, burrows under *Hakea* were also empty (sample sizes and location unknown, but likely the AZRI type locality).

In the decades before and after Slater and Lindner's large collection at the type locality, the area changed rapidly. Overgrazing of lands around Alice Springs caused major dust problems, which escalated during the severe and prolonged drought of 1958–1966 (Keetch 1979). During the 1950s and 1960s, mechanical rehabilitation trials to ameliorate dust problems were underway on the AZRI and 'Butcher's' blocks (Albrecht & Pitts 2004) less than one kilometre from the type locality (Figure 3.4). CSIRO and the NT Agricultural Branch trialled spiral tilling, furrowing and other methods to sow Buffel grass *Cenchrus ciliaris* seed (Figure 3.5). Buffel grass was widely sown after the 1958–1966 drought and rapidly spread after heavy rains in the 1970s (Griffin 1993). The Todd River floodplain, which a 1962 land survey described as vegetated with sparse low trees, such as ironwood (*Acacia estrophiolata*) and whitewood (*Atalaya hemiglauca*), over short native grasses and forbs (Perry *et al.* 1962), changed to an understory of dense buffel grass in the following decades (Griffin 1993).

3.2.3 1975 – 2003

After the apparent former abundance of *L. s. slateri* at the type locality in 1964–1965, the subspecies was last detected at the site in 1975 when a single specimen was collected. The area was searched comprehensively in 1995, 2000 and 2003, with considerable survey effort put into trapping, active searching and burrow excavation, but with no sign of any live specimens (Pavey 2004a). Prior to this, however, NT Parks and Wildlife staff detected populations at three new sites. In 1989, a specimen was collected at a new site in the eastern MacDonnell Ranges at Loves Creek Station. In Finke Gorge National Park, specimens were collected at Little Palm Creek in 1994, and at Palm Paddock in 1996.

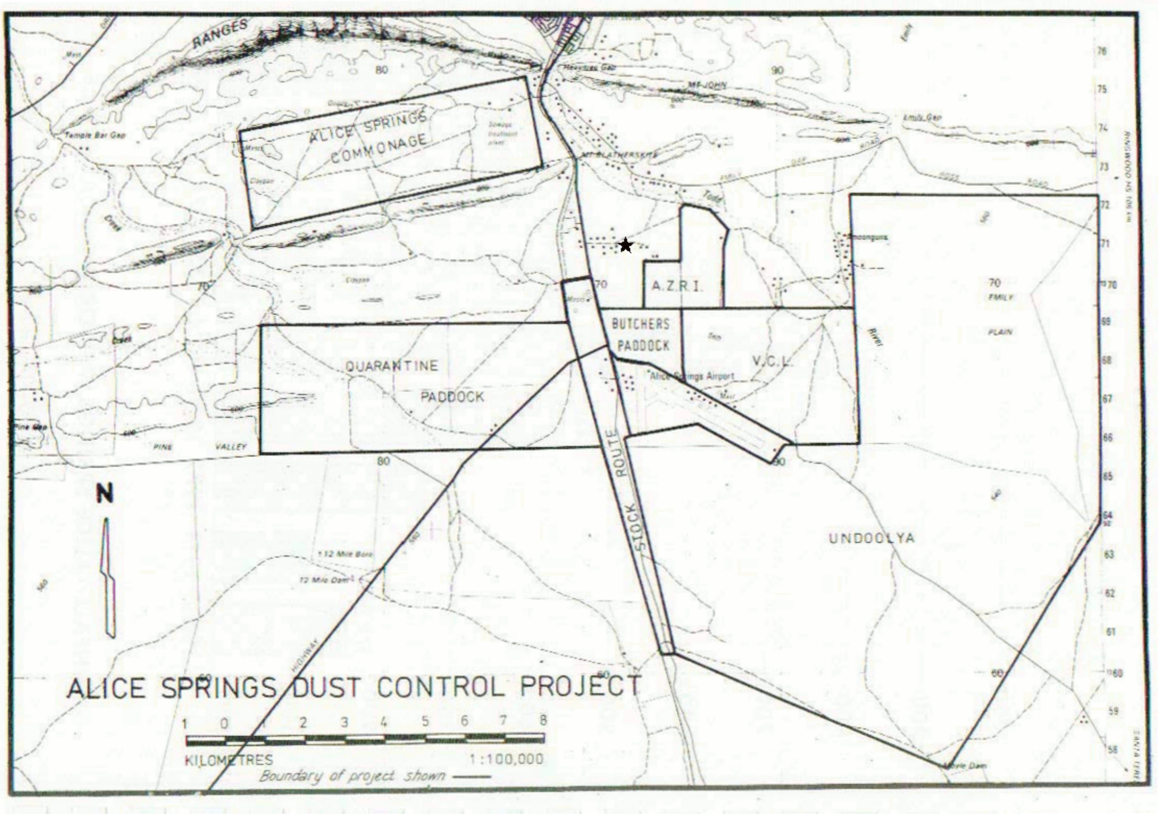


Figure 3.4. Land used for mechanical rehabilitation trials 1950–1970 (bold outlines). Site of the *Liopholis slateri slateri* type locality 1964 (star). Reproduced from Keetch (1979).

Figure 3.5. Spiral tilling used in mechanical land rehabilitation trials, 1966. Land Conservation Unit Archives.

3.2.4 2004 – 2010

The apparent decline of the species in the Northern Territory resulted in a nomination being made by Steve McAlpin for the subspecies to be listed nationally as Endangered under the EPBC Act. This nomination was accepted and the subspecies formally listed as Endangered on 4 April 2001 (Australian Government 2016). On the basis of this listing, the Northern Territory government sought and received funding from the Australian Government to prepare a recovery plan for the species (Pavey 2004a). The recovery plan was accepted in March 2005 for the period 2005–2010. As part of implementing the recovery plan, targeted surveys were undertaken by experienced observers from 2004 to 2010. In addition, inexperienced observers were trained in locating the species. These targeted surveys confirmed the presence of the species at some previously known sites and discovered several new sites. Previously known sites where the species was located were Illamurta Springs, White Horse Gap (on Tempe Downs Station), Ellery Creek, Loves Creek and a site at Circle Gully/Areyonga Valley in Finke Gorge National Park. Additional populations were located at multiple locations in Finke Gorge National Park, the Finke River north of Hermannsburg, Lawrence Gorge and Orange Creek on Owens Springs Reserve and Tempe Downs Station. Incidental observations were made at a site on the Krichauff Range (Bill Low pers. comm.). The Krichauff Range site was unusual in that lizards were located on a ridge top of the ranges, while all other populations were found in river floodplain habitat.

3.2.5 Post 2010

Three new sites have been located during routine biological surveys following the end of the life of the recovery plan. These sites are at Serpentine Chalet and Gunpowder Track in the MacDonnell Ranges, Walker Creek on Tempe Downs, and at Running Waters on Henbury Station.

3.3 Conservation status

Primarily due to the disappearance at the type locality, *L. s. slateri* was listed as a threatened species in 2000 (McAlpin 2000). The initial listing was for Endangered, which it qualified for under four IUCN criteria: extent of occurrence <5,000 km^2; area of occupancy <500 km^2; population size estimated to number fewer than 2,500 mature individuals; and inferred decline in extent of occurrence, area of occupancy, quality of habitat, and number of mature individuals. However, with the discovery of new populations after 2004, the species status in the Northern Territory was down-listed to Vulnerable in 2011.

3.4 Population estimates

Occurrence data from recent years show that populations of *L. s. slateri* usually occupy sites close to water courses along both major connector channels and other minor tributaries (Figure 3.6). I used an estimate of a maximum dispersal distance of 5 km, and then suggested a buffer zone of 5 km around known population sites to indicate likely site connectivity within a meta-population. In this interpretation, distances of more than 5 km between sites distinguish separate (non-mixing) populations. Limited data exist on dispersal distances for other *Liopholis* species, but those reported suggest maximum dispersal distances are less than a few hundred metres (Daniel 1998; Chapple 2003; Chapple & Keogh 2005). However, flood-assisted dispersal may feasibly increase this range to a few kilometres between sites that are located on a connecting watercourse. Therefore, an estimate of 5 km seemed a reasonable upper limit by which to distinguish non-mixing populations. Based on this criterion and corresponding assumptions, I concluded that the species persists in 11 currently known populations or meta-populations (Figure 3.6). Populations are associated with six major river systems within the Finke River and Todd River basins (Table 3.2).

Data on population sizes were lacking, but relative sizes could be coarsely estimated from the number of active burrows at a population site. At sites that I visited, I categorised relative size of populations by estimating burrow abundance: larger populations (>100 active burrows) or smaller (<50 burrows) (Table 3.2). Note, burrow estimates are from a single visit to the population site, not the cumulative number of active burrows over multiple visits.

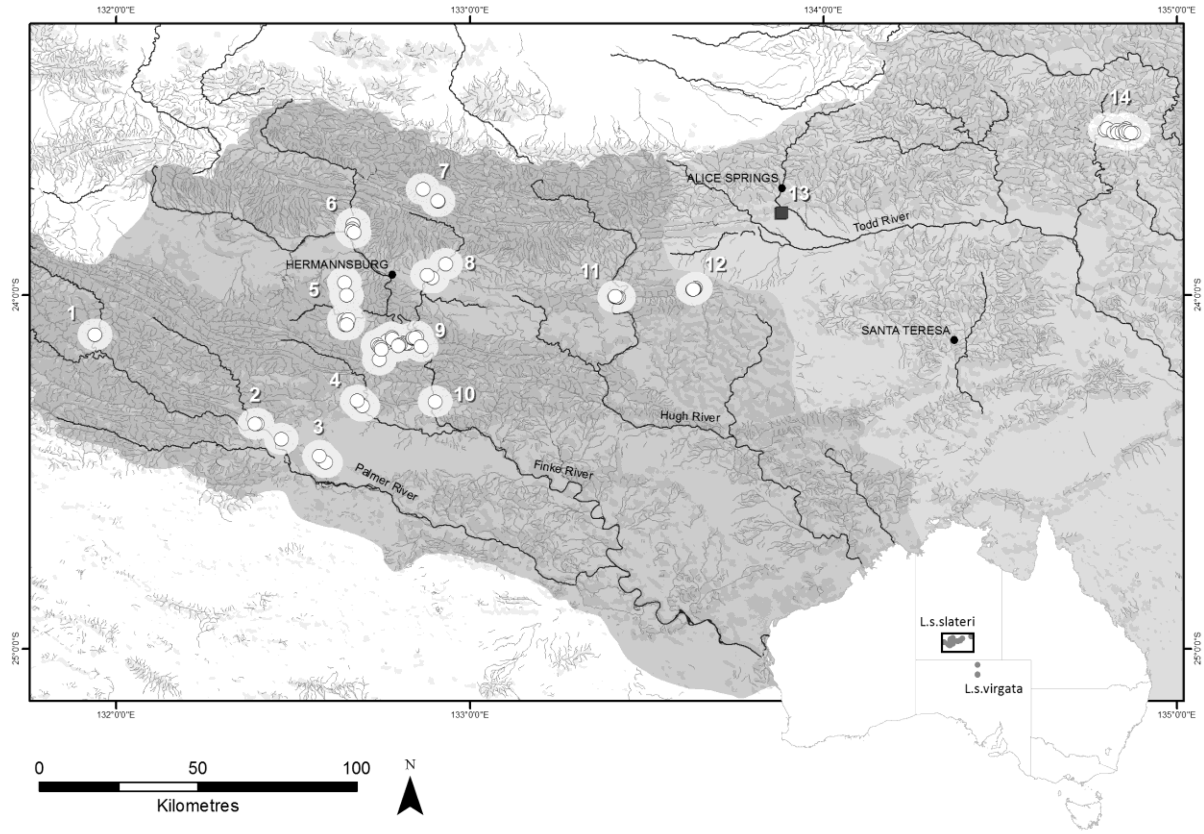


Figure 3.6. Currently known populations/meta-populations of *L. s. slateri* as of August 2016. Buffers around sites of known occurrence (5 km) delineate probable meta-populations with interchange between populations. Populations occur within the Finke River catchment (dark grey shading) and Todd River catchment (light grey): 1=Walker Creek, 2=Palmer River, 3=White Horse Gap, 4=Illamurta Springs (uncertain), 5=Palm Paddock-Krichauff Range, 6=Finke River, 7=Serpentine Chalet-Gunpowder Track, 8=Ellery-Jerimah Creek (uncertain), 9=Little Palm Creek, 10=Running Waters, 11=Lawrence Gorge, 12=Orange Creek, 13=AZRI type locality (extinct), 14= Loves Creek.

Table 3.2. Populations/meta-populations of *L. s. slateri* in major river systems within the MacDonnell Ranges biogeographic region. Relative size of populations is estimated from the number of active burrows on a single visit to the population site: larger population (>100 active burrows) or smaller (<50 burrows). The status of Ellery-Jerimah Creek and Illamurta Springs populations is uncertain (italics). Cross reference locations with map Figure 3.6.

River catchment	River system	Meta-population	Burrows	Reference
Finke River	Palmer River	Walker Creek	?	1
		Palmer River	?	2
		White Horse Gap	?	3
	Finke River	Palm Paddock-Krichauff Range	<50	5
		Finke River	>100	6
		Little Palm Creek	>100	9
		Running Waters	?	10
	(none)	Serpentine Chalet-Gunpowder	<50	7
	Ellery Creek	*Ellery-Jerimah Creek*	?	8
	McMinn Creek	*Illamurta Springs*	?	4
	Hugh River	Lawrence Gorge	<50	11
		Orange Creek	<50	12
Todd River	Todd River	Extinct: AZRI – type locality	-	13
	Giles Creek	Loves Creek	>100	14

3.5 Summary

From the currently known extant populations, I chose the Orange Creek population for a four-year intensive study of population spatial dynamics and burrow occupancy (see Chapter 7). In the next chapter, I describe the geomorphology of the study site to place the research in context. Note, hereafter, all references to *Liopholis slateri* refer to the Northern Territory subspecies *Liopholis slateri slateri*.

Chapter 4: Description of the Orange Creek study site

The study site was at Orange Creek (23°59’S, 133°37’E), 620 m ASL, 40 km south west of Alice Springs, within the MacDonnell Ranges bioregion of the Northern Territory, Australia. The climate is semi-arid with a mean annual rainfall of 283mm (Alice Springs airport; ID: 015590; Bureau of Meteorology [data 1941-2015]). Rainfall in the region is highly variable with most falling during the summer months (November–February). Daily temperatures range from a mean maximum of 36.4°C in January to a mean minimum of 4°C in July (Bureau of Meteorology [data 1941-2015 at Alice Springs airport]).

The Orange Creek site is on Owen Springs Reserve, which was formerly a pastoral property (1872 to 2003). The property was destocked after 2004 when it became part of the Northern Territory parks and reserve system. The reserve comprises a range of landforms and topography, including the Waterhouse Range Anticline in which the study site was located. The Waterhouse Range is a formation of sandstone geologies, including Mereenie sandstone, within the Amadeus basin (Nicoll *et al.* 1991). The range is a long and narrow formation, about 50 km long x 7.5 km wide, oriented roughly east-west along its longest axis (Figure 4.1). It is isolated from the MacDonnell Range and Chewings Range to the north, and the James Ranges to the south.

The Waterhouse Range is a site of national conservation significance for flora and fauna (Neave 2007). The site supports a number of species listed as threatened nationally and/or in the Northern Territory, including Minnie daisy (*Minuria tridens*), black-footed rock wallaby (*Petrogale lateralis*), Australian bustard (*Ardeotis australis*), and Slater’s skink (*Liopholis slateri*). The central rock-rat (*Zyzomys pedunculatus*) was reported from the range, but is now considered extinct at the site (Pavey 2004b). The rock-rat is, however, still found elsewhere within central Australia.

Both the Orange Creek and Lawrence Gorge *Liopholis slateri* populations are located in the Waterhouse Range pound, a long thin valley of about 30 x 2.5 km (Figure 4.1). The two *L. slateri* population sites are separated by 22 km. The Hugh River, part of the Finke River catchment, which starts its course in the Chewings Range, bisects the Waterhouse Range at roughly its central point (Lawrence Gorge). Two creeks flow laterally along the pound, both

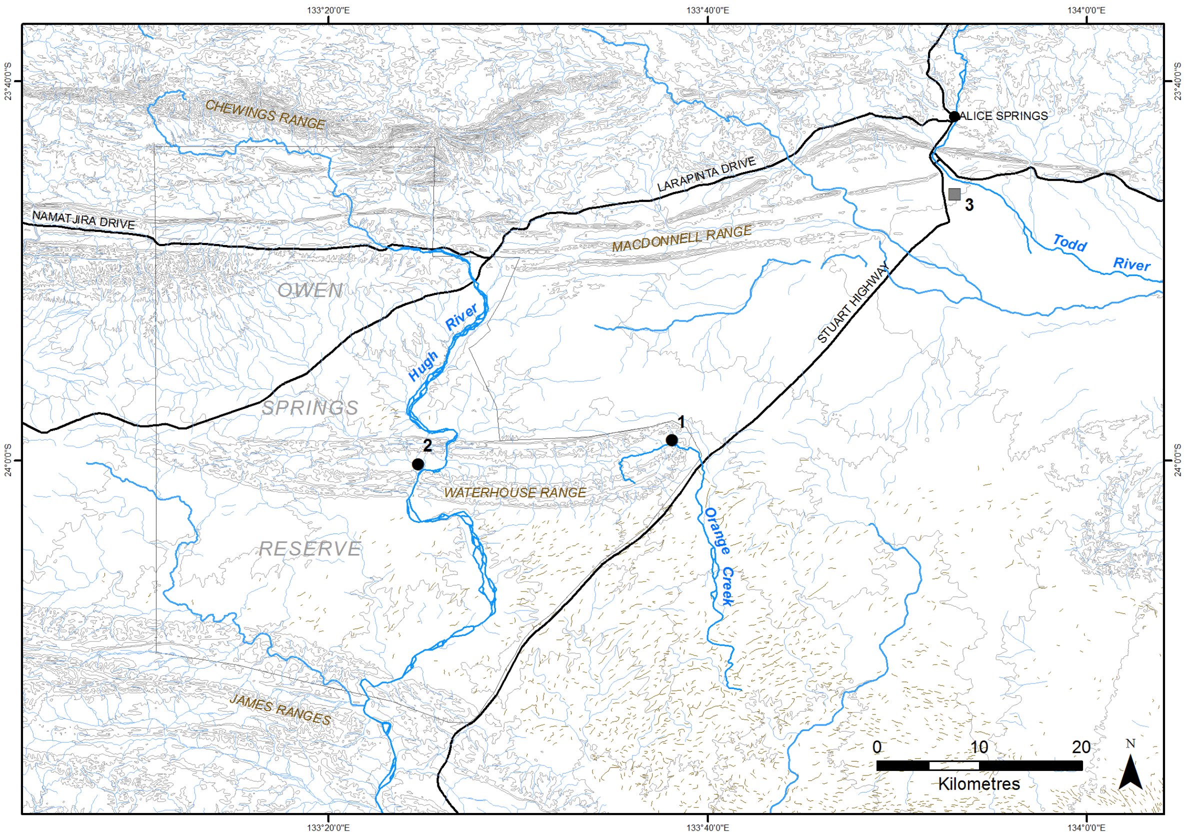


Figure 4.1. Map of the region showing the Orange Creek study site in relation to the topography and hydrology of the surrounding region, and nearest *Liopholis slateri* populations. 1=Orange Creek population and study site, 2=Lawrence Gorge population, 3=AZRI type locality (extinct). NB: Orange Creek is a minor watercourse compared with the Hugh River, but has been highlighted here for visibility.

starting their catchments at the eastern end of the pound, but flowing in opposite directions (Figure 4.1). Both creeks collect water from tributaries that run-off from the flanking sandstone ranges on the north and the south faces of the pound. Mueller Creek flows west and joins the Hugh River at Lawrence Gorge, to the east of the site of the Lawrence Gorge *L. slateri* population. Orange Creek flows east along the pound, toward the Orange Creek population site, before cutting southeast through the range. Orange Creek continues south and dissipates into sand ridge country (Figure 4.1). Orange Creek, Mueller Creek and the Hugh River are ephemeral, and normally dry.

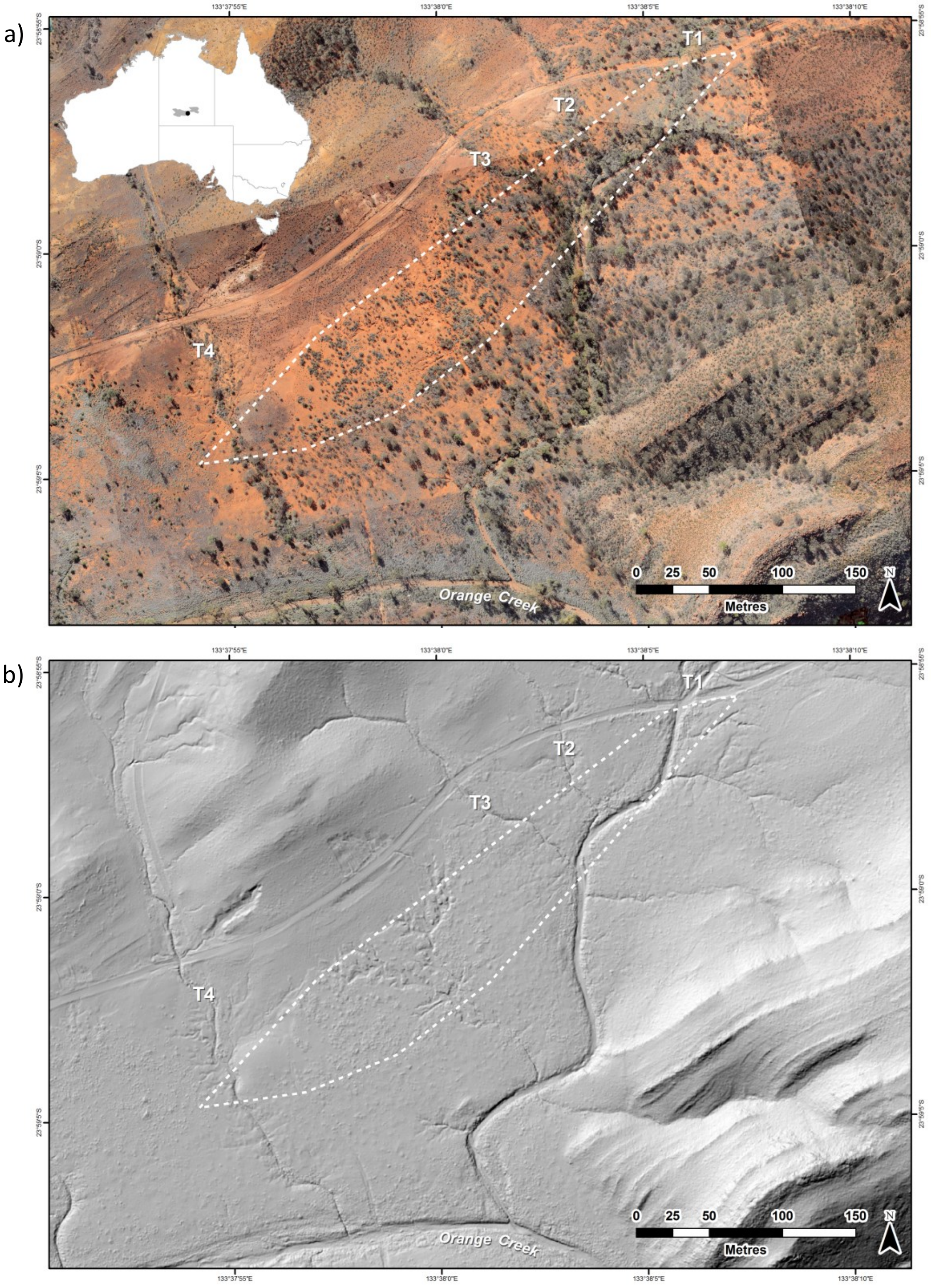


Figure 4.2. Aerial photograph and location of the Orange Creek study site in relation to the MacDonnell Ranges bioregion, central Australia. Dashed line shows the approximate area of occupancy as a minimum convex polygon around detected burrows. The area of occupancy is bisected by three low order tributaries (T2–T4) and one major tributary (T1) that flow south into Orange Creek. Tributaries T2 and T3 flow into T1 which then converges with Orange Creek at the south of the image. Site shown in (a) natural colour at 3–4 cm ground sample distance (GSD), and (b) topographic relief generated from LiDAR imagery at 25 cm GSD.

Figure 4.3. On-ground view of the Orange Creek study site, looking southeast to where Orange Creek passes through the Waterhouse Range. The photo is taken from the rocky upper slopes to the north of the site. Downslope is the area of occupancy of the study population, an *Eremophila*-dominated shrubland on red earth alluvium.

Figure 4.4. The area of occupancy showing the contiguous pattern of cleared patches and accumulated soil pedestals, here at the base of *Eremophila sturtii.* Grass tussocks in the foreground are buffel grass, *Cenchrus ciliaris.*

At the Orange Creek study site, during the study period of my research (see chapters following), a population of Slater’s skink occupied a 460 x 75 m (23,000 m^2) area of *Eremophila*-dominated shrubland on a red earth alluvial foot slope. The population site was about 100 m north of Orange Creek (Figure 4.2, Figure 4.3). After large but infrequent rainfall events, water run-off from the rocky upper slopes north of the site, is partly diverted by the vehicle track that crosses the slope (again to the north of the population site), but then flows into a network of erosion channels covering about 20% of the area of occupancy of the population. The channels, up to 5 m wide and 1 m deep, converge and flow into Orange Creek to the south of the site (Figure 4.2). In the non-channelled section, surface sheet flow after rain (flowing north to south) transports and deposits alluvial material, creating a contiguous pattern of cleared patches and accumulated soil pedestals, typically at the base of shrubs (Figure 4.4). The lizards dig their burrows both in these soil pedestals and in some banks of the erosion channels (see Chapter 8 for details of burrow sites).

Chapter 5: Photographic identification of individuals

My research required a non-invasive sampling method for *Liopholis slateri.* I evaluated methods of capture, such as noosing or trapping, but concluded that these methods were infeasible, inefficient, and far too time-expensive to be practical for a thorough survey. Frequent recapture of individuals over multiple years would have been unduly disruptive to the population, and therefore unsuitable for an observational study. Instead, I considered an alternative, non-invasive option. In this study, I explored the use of photographic identification for *L. slateri* and compared the matching abilities of independent observers with an automated computer algorithm. This chapter has been adapted from the publication: Treilibs, C.E., Pavey, C.R., Hutchinson, M.N. & Bull, C.M. (2016). Photographic identification of individuals of a free-ranging, small terrestrial vertebrate. *Ecology and Evolution*, **6**, 800–809.

5.1 Introduction

Recognition of individuals within an animal population is central to a range of estimates about population structure and dynamics. Estimates of population density and abundance rely on an ability to distinguish individual animals; estimates of life history parameters, such as growth rate and survival, require tracking those individuals through space and time. However, traditional methods of marking individuals, such as toe-clipping, may cause stress, injury or infection to the animal (Reisser *et al.* 2008; Sacchi *et al.* 2010) and are ethically questionable. Capture and handling, often required to apply marking, may also affect normal behaviour of an individual, at least in the short term (Rodda *et al.* 1988; Langkilde & Shine 2006). Such impacts are undesirable, particularly for threatened or rare species (Bradfield 2004), but also when the goal of research is to observe natural population processes and behaviour with minimal interference.

Photographic identification has become a popular, non-invasive alternative for recognising individuals from natural variation in their markings. The technique has typically been used for mark-recapture studies, which assume that a species displays sufficient phenotypic variation to distinguish among conspecific individuals, that the unique markings are constant through time, and that the markings can be recognised from photographs taken under different conditions (Pennycuick 1978; Bolger *et al.* 2012). Naturally variable phenotypic patterns on a wide range of taxa, from large mammals (Van Tienhoven *et al.* 2007; Anderson *et al.* 2010) to crustaceans (Frisch & Hobbs 2007), have been used for photographic identification of both free-ranging and captured animals.

In photographic mark-recapture, individuals are cross-matched in a library of photo capture histories. However, the time-expense of manually comparing photo pairs increases exponentially with sample size (Speed *et al.* 2007; Van Tienhoven *et al.* 2007; Bolger *et al.* 2012). One way to overcome the difficulty of cross-matching large datasets is by computer-assisted matching of photos of unknown individuals to a reference library. Many algorithms have been developed for this purpose, but many are highly specialised for particular species or for specific morphological features (Speed *et al.* 2007; Bolger *et al.* 2012; Town *et al.* 2013; Drechsler *et al.* 2015). A simple and freely available software package, *Interactive Individual Identification System*, I^{3}S Pattern v.4.0.2 (Hartog & Reijns 2014), is a pattern-matching algorithm that has the potential to be applied to any species with variable markings (Speed *et al.* 2007; Hartog & Reijns 2014).

Computer-assisted matching has often been used with large-bodied free-ranging marine mammals, where underwater views of the animal are usually unobstructed and evenly illuminated (Speed *et al.* 2007; Van Tienhoven *et al.* 2007; Hartog & Reijns 2014). However, even in these conditions, parallax effects of taking photographs at wide horizontal angles (>30°) to the subject can still be problematic for the automated matching process (Speed *et al.* 2007; Hartog & Reijns 2014). The greater the horizontal angle of deviation from 0° (perpendicular to the subject), the higher the likelihood of a low scoring match (Speed *et al.* 2007; Rocha *et al.* 2013).

For smaller taxa (<500g), parallax effects are likely to be exacerbated because of the comparatively small body areas being photographed. Most studies of smaller-sized fauna have controlled for the parallax problem by capturing the animal and manipulating it into a fixed position relative to the camera, photographing either in-hand or using a holding pen (Bradfield 2004; Frisch & Hobbs 2007; Sacchi *et al.* 2007; Hachtel *et al.* 2009; Kenyon *et al.* 2009; Knox *et al.* 2013; Rocha *et al.* 2013; Drechsler *et al.* 2015). This reintroduces the potential stress that the non-invasive technique is supposed to avoid, and involves a large effort to capture the animal for photography.

Because of the often inconspicuous or flighty nature of many herpetofauna, photo-identification has seldom been applied to free-ranging individuals of this group. One study showed photo-identification could be used to track movements of free-ranging eastern water dragon *Intellagama lesueurii* and calculate their home ranges (Gardiner *et al.* 2014). However, few reports have investigated the broader limitations of the technique or evaluated alternative ways of using the technique for a free-ranging reptile.

In this study, I explored the use of photographic identification for Slater's skink *Liopholis slateri* (mean snout-to-vent length (SVL) 85mm). The natural history of Slater's skink and its varied scale markings (see Chapter 2) make it a potentially suitable candidate for photo-identification. I assessed whether unique facial markings of Slater's skink could be used as a reliable means of distinguishing individuals from photographs using (a) an identification key or (b) the I^{3}S Pattern algorithm, and whether I could detect any temporal changes in these markings.

5.2 Methods

5.2.1 Study species

Slater's skink is a rare and globally endangered lizard that exists in small isolated populations within the MacDonnell Ranges bioregion of Central Australia, where it occupies burrow systems located in river floodplains (Pavey 2004a). The skink is a sit-and-wait forager, typically spending much of its active time sitting at, or close to, a burrow entrance to bask and ambush passing invertebrate prey (Pavey *et al.* 2010; Fenner *et al.* 2012a; McKinney *et al.* 2015). Individuals are easy to observe at these times from as close as 5 m from the burrow, but are difficult to catch without destroying their burrows into which they retreat when more closely approached. Of special relevance is that local population sizes are relatively small (Pavey *et al.* 2010), allowing the potential for reliable identification among resident individuals, and recognition of any new recruits into the population. Like several other species in the *Egernia* group, individuals have variable spots and facial markings which potentially could be used as unique natural markers (Pavey *et al.* 2010).

5.2.2 Study site

The study site was at Orange Creek, south west of Alice Springs in Central Australia (23°59’S, 133°37’E) (see Chapter 4 for details). At this site a population of Slater’s skink occupied a 500 x 200 m area of *Eremophila* shrubland on an alluvial flat. The lizards occupied burrows in soil pedestals that had formed at the base of shrubs by wind and water processes. Over four spring-summer seasons, I detected 104 burrows at the site with evidence of lizard occupation at some time during the period. No other burrows were detected within 5 km of the study site, allowing me to assume I had surveyed an entire population within the site.

5.2.3 Population survey – photographic mark recapture (PMR)

Over four spring-summer periods, from December 2011 to April 2015, I photographed all detected individuals during site visits, usually twice a week. At each visit, I scanned all entrances of each burrow with binoculars (Zeiss 10x40) from a distance of greater than 15 m from the burrow entrance. When a lizard was observed out of its burrow, or at the burrow entrance, I photographed it several times (a photographic capture) with a DSLR camera (Canon EOS 450D) and telephoto lens (Canon70-300mm). By moving slowly and quietly, I could normally approach to within 4 or 5 m without disturbing the lizard and I attempted to get lateral head photographs from both the left and right side. Each photographic capture was stored in a photo catalogue with assigned tags about date and burrow number.

5.2.4 Spot development and stability

I documented ontogenic changes in facial markings in three ways. First, I compared the number of spots on temporal, subralabial and infralabial scales (see below) on six neonates at the end of a summer (early April when young are about 3-4 months old), with the patterns on 29 adult lizards photographically sampled at the same time of year. I assumed spot patterns on left and right sides were related and selected one side (right) to compare spot numbers of neonates and adults using t-tests of independence, followed by Cohen’s D index to evaluate the magnitude of effect size (Cohen 1988). Second, I inspected a four month summer time-sequence of repeated photos of six neonates first observed in December 2012 or in December 2014. I deduced they were the same individuals if they were repeatedly observed as the only juvenile lizard in the same burrow from December to March. Third, I examined photographs for longer term changes (> 12 months) in facial patterning in each of the 10 adult individuals that I was able to follow for the entire four-year duration of the study.

For these 10 mature adult lizards, other distinguishing features such as size, scale shape and arrangement, scars, and other markings, allowed me to be confident that photo sequences were of the same individual.

5.2.5 Developing a key

I used high quality images of 12 adults in the first spring-summer period to identify characteristics suitable for distinguishing individuals. I targeted the head region, as this is often the most exposed and most easily photographed body part, and within that region I examined ear lobules, melanic spots and scale patterns. In my initial inspection I found these characteristics differed between the left and right sides of an individual lizard. Among left and right profiles of the 12 lizards, I identified 11 characters, each with 2-3 alternative states, which might be used to differentiate among lizards (Figure 5.1). I then scored the frequency of each character state for a larger sample of 30 lizards (Table 5.1).

Using the selected characters, I developed an interactive, multi-choice key with character scores derived for the right and left sides for each of the 30 individual lizards. The key enables

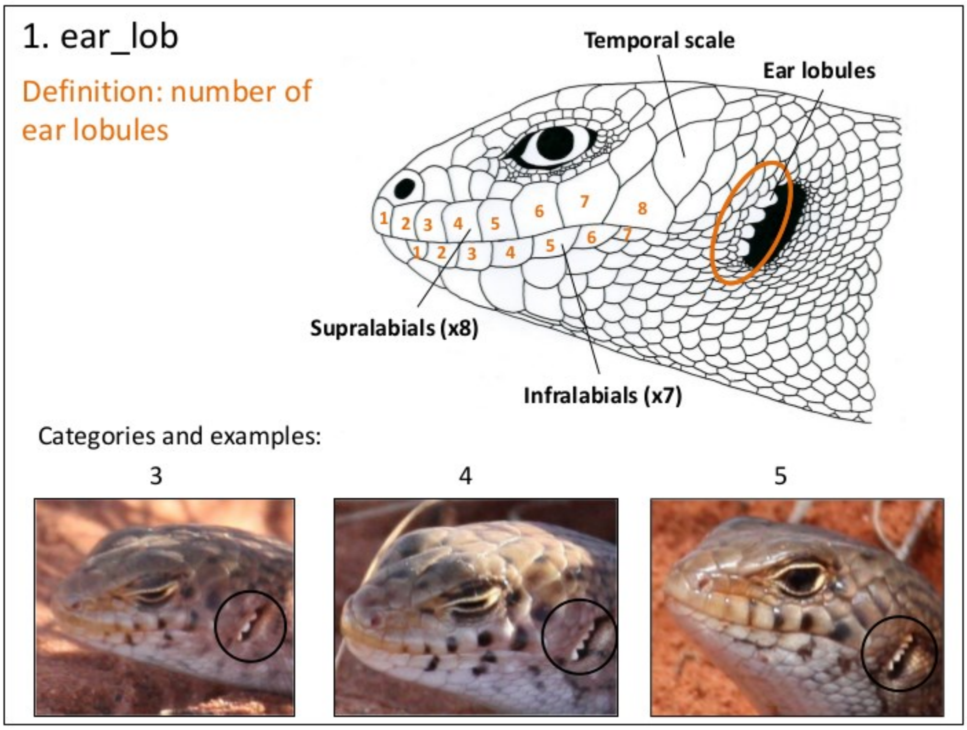


Figure 5.1. Information provided *a priori* to observers to enable identification of character 1, ear lobules (Table 5.1). Similar diagrams were presented for each of the 11 characters.

the user to select assessed character states in a spreadsheet, for comparison with a library of the previously scored individuals. As each character is scored, the key filters out known individuals in the population that do not display that character state. The user continues to select character states, in any order, either until the spreadsheet identifies a single individual, or until all 11 characters have been scored.

Table 5.1. The 11 characters used to distinguish individuals of Slater's skink, and frequencies of alternate character states, from 30 individuals.

Character		Value	Frequency	Description
1	Number of ear lobules	3	0.04	
		4	0.75	
		5	0.21	
2	Temporal scale marks	0	0.07	Number of discrete, dark markings on the largest temporal scale
		1	0.82	
		2	0.11	
3	Temporal scale marks	0	0.43	Discrete, dark markings on the largest temporal scale touch (1) or do not touch (0) the scale's edge
		1	0.57	
4	Supralabial scales	3	0.64	Number of discrete, dark markings on any of the eight supralabial scales
		4	0.27	
		5	0.09	
5	Infralabial scales	0	1.00	Presence (1) or absence (0) of discrete, dark markings on each of the seven infralabial scales
		1	0.00	
6		0	0.96	
		1	0.04	
7		0	0.66	
		1	0.34	
8		0	0.36	
		1	0.64	
9		0	0.55	
		1	0.45	
10		0	0.46	
		1	0.54	
11		0	0.88	
		1	0.13	

5.2.6 Testing the key

When testing started after the 2012-2013 spring-summer season, the photo database contained 1153 images from 314 photo-captures (mean 3.67 images per capture) of what I considered to be 30 different adult lizards. Matching of individuals to images was based not only on the character key, but also on other distinguishing features discussed above, and on the tendency of individual lizards to remain at the same burrow for extended periods of time. With continued exposure to the population I came to recognise individuals, but my question was whether I could develop a key that would allow others to identify individuals without that extended experience.

I predicted that images of skinks that were highly angled (>30°) or that had one or more key characters obscured would be more difficult for observers to identify. I tested this by classifying each image into one of three categories according to image viewing angle and the degree to which the key characters were obscured (Table 5.2). To determine image category, I estimated size of image viewing angle by measuring the angle between the line of sight and the line through the centres of the eyes (Figure 5.2) using Screen Protractor^{TM} software. Because facial profiles were not parallel to the mid-line of the body but tapered to the snout, I adjusted each measurement by subtracting 25° (Figure 5.2). I then randomly selected eight photos from each of the three image categories. The 24 photos were of 14 different individuals with four individuals represented twice and three individuals three times. The test sample included nearly half of the known population, with some individuals represented by

Table 5.2. The three photo categories used for testing an identification key for individuals of Slater's skink.

Category		Description
1	Full lateral image	Head profile at, or close to, right angles to camera (i.e. angle ≤30°). All characters visible.
2	Angled	Head profile at angle to camera (i.e. angle >30°). All characters visible.
3	Obscured characters	Characters partially obscured by vegetation/soil/scarring. Head profile at, or close to, right angles to camera (angle ≤30°).

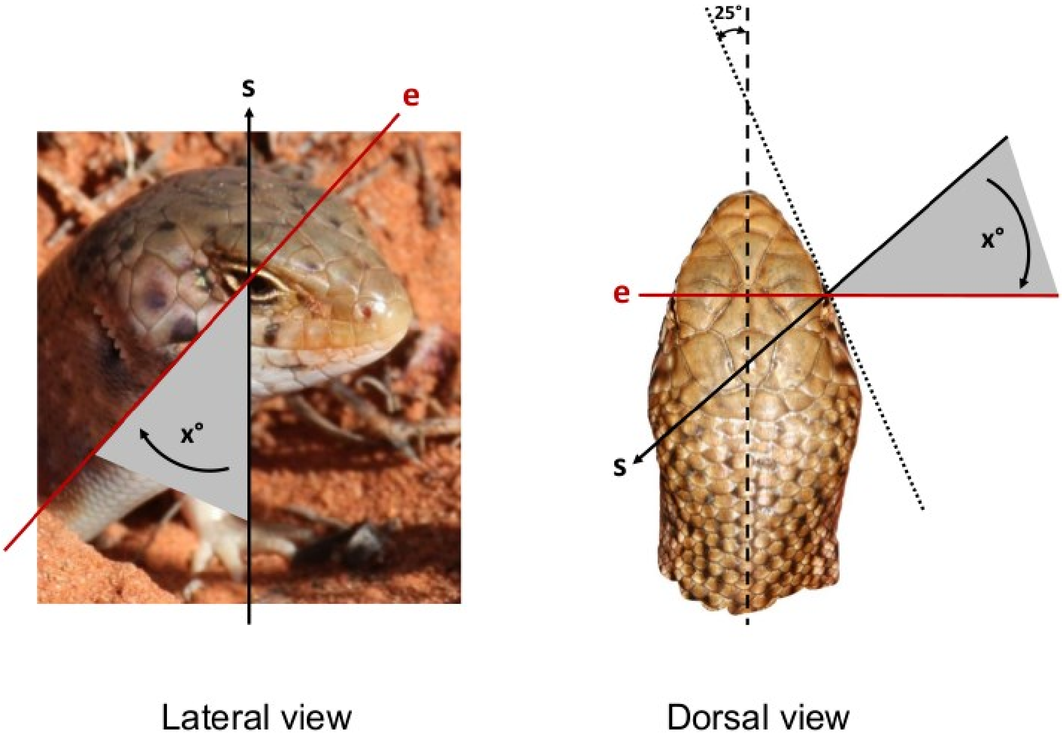


Figure 5.2. Size of image viewing angle (x°) was estimated by measuring the angle between the line of sight (s) and the line through the centres of the eyes (e), and then corrected (-25°) for head tapering; the facial plane (dotted line) tapers at an approximate angle of 25° from the mid-line of the body (dashed line).

two or three photographs taken at different times and in different conditions. The sample size was intended to reflect a typical survey period, without imposing too high a load on the volunteer observers. The photos were uploaded to a free online survey tool with response options in a multiple choice format.

I then asked 24 observers to use the key developed from the previous library to identify the lizards in each of the 24 test photos. I considered that previous experience working with wildlife might improve identification skills in these observers. To test this, I selected 12 observers with experience in wildlife survey and 12 observers with no experience, a sample size that I thought would be sufficient to detect any effect of previous experience. Comparable studies that included a human identification component used a range of three (Frisch & Hobbs 2007) to eight (Knox *et al.* 2013) observers (mean 5.6; n = 3 studies) with varying levels of experience. Each of the 12 experienced observers was a professional field biologist who specialised in plant or animal surveys, although none had specific experience with the study species. None of the 12 inexperienced observers had any advanced training in biology, or professional association with field biology.

The observers were given a 10 minute explanation with examples of each character state (Figure 5.1), and then worked independently and with no time limit. I allowed observers to select up to three responses if they were unable to narrow the field to a single candidate individual, since, in practice, the key is not always the ultimate identification step, but often the means to selecting a final few for photo-comparison. Responses were scored as either correct, if the correct individual was among the selection, or incorrect, for the wrong identification. Observers' test times were recorded by the survey tool, and average times for the two observer types compared with a Kolmogorov-Smirnov test. Times are reported as mean ± SD.

I used a repeated measures ANOVA to examine the effect of observer type (experienced vs. not experienced) and category of photograph (full lateral view vs. angled vs. obscured) on the proportion of correct identifications of the set of photographs. Since both observer types examined the same set of 24 photographs, observer type was a within-subjects factor, while category of photograph was a between-subjects factor. To ensure conformity with the assumptions of the analysis, the response variable was transformed using an arcsine square root transformation, and effect size calculated using partial eta-squared (Bakeman 2005). All statistical analyses were computed in R ver. 3.2.1 (R Core Team 2016).

5.2.7 I^{3}S Pattern

The *I^{3}S Interactive Individual Identification System*, originally developed to identify whale sharks (Van Tienhoven *et al.* 2007), now includes I^{3}S Pattern (Hartog & Reijns 2014), which uses photographs of natural body patterns. It calculates a set number of measurements based on differences in patterning after the user has identified three reference points on the photograph and has outlined the region of interest. While the reference points should correct for differences in viewing angle, rotation and scaling, Hartog and Reijns (2014) recommend that images should be taken perpendicular to the line of sight or no more than 30 degrees off that line. The software's key point extraction algorithm generates a 'fingerprint' file (a point cloud) for each image which can be compared with other files in the reference library to create a ranked list (Hartog & Reijns 2014). The key points in the fingerprint files are matched for sizes and separation distance to determine potential matching key point pairs. Then a distance metric is calculated by summing the distances between each point pair and dividing by the square of the number of key point pairs (Hartog & Reijns 2014). Lower scores indicate a better match.

Where available, I selected three high quality images of the left and right side of each of the 30 individuals used in the key (n = 98; for some individuals I only had one or two images per side profile) and loaded them into the I^{3}S database. I selected the region of interest to contain 10 of the 11 characters described in the written key; ear lobules were not included. I selected three reference points to form a triangle around the region of interest: outer edge of nasal hole, edge of eye-ring, and the bottom edge of the tympanum (Figure 5.3). Photos were annotated as left or right profile, and fingerprint files were created for each lizard. Then the same 24 test photos that had been used to test the key by independent observers were run through the I^{3}S software and matched to the database of the known 30 individuals. I recorded the score metric, rank, and the processing and matching time taken for each test photo.

To get a sense of how well the algorithm could match photos of the same individual with each other, I ran the in-built simple evaluation test. The entire database of 90 photos was matched

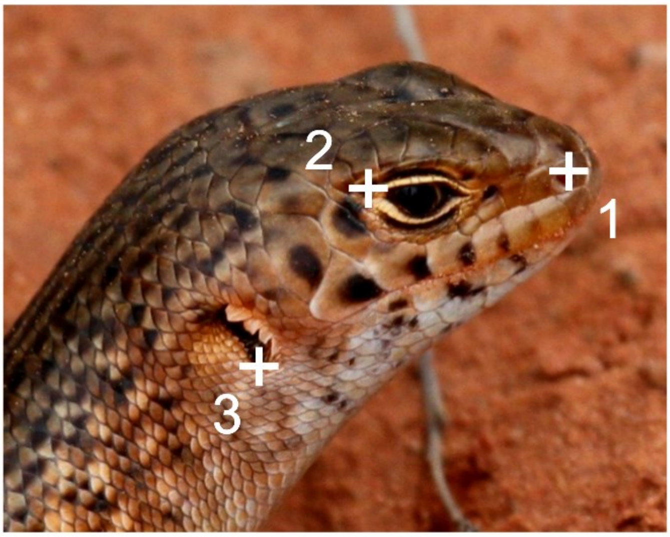


Figure 5.3. Three reference points selected by the user as required by the I^{3}S Pattern software: 1) outer edge of nasal hole, 2) upper corner of eye-ring, and 3) bottom edge of tympanum.

with itself, with 94 intra individual comparisons and 8010 comparisons overall. The evaluation test reported the number and percentage of comparisons in the top one to 20 matches (Hartog & Reijns 2014).

5.3 Results

5.3.1 Spot development and stability

Pigmentation spot patterns in Slater's skink developed during early growth. Right-side profiles of end-of-summer neonates (n=6) had a significantly lower mean spot count than right-side profiles of all adult skinks at the same time (n=29), on all scored characters: fewer marks on temporal scales (character 2, $t_{(5)=}2.74$, p<0.05, d=1.65), on supralabial scales (character 4, $t_{(28)}=3.82$, p<0.001, d=0.77) and on all infralabial scales (sum of characters 5-11, $t_{(13)}=4.41$, p<0.001, d=1.32). Repeat inspection of juvenile individuals over time showed that these spots appeared and then grew larger and darker over the first summer growth period (Appendix Table 1). In multiple images, over periods of 12–36 months, I found 10 mature adults retained identical spot patterns.

5.3.2 Testing the key

From the 24 test photos, 24 independent observers correctly identified a mean of 16.6 ± 0.77 SE (69%) of individuals. There was no significant effect of category of photograph, nor any interaction effect between category and observer type, but there was a significant main effect of observer type (Table 5.3). Observers experienced in wildlife survey identified a significantly

Table 5.3. Results of a repeated measures analysis of variance comparing effect of observer group (experience vs. no experience) and category of photograph (full lateral view vs. angled vs. obscured) on the proportion of correct identifications of each test photograph.

	F	d.f	*p*
Observer	7.66	1, 21	**0.01**
Category	0.01	2, 21	0.90
Category x Observer	0.73	1, 21	0.39

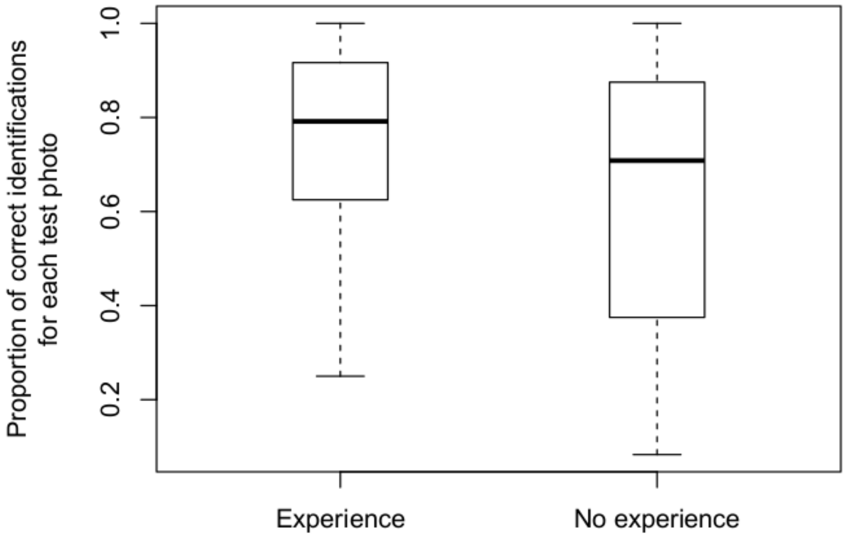


Figure 5.4. Proportion of correct identifications for each test photo by observers with experience and no experience.

higher proportion of photos correctly (74%) than observers without experience (64%; Figure 5.4, $\eta_p^2 = 0.25$). There was no significant difference in time taken between observer groups (two-sample Kolmogorov-Smirnov test D=0.269), with experienced observers taking an average time of 171.8 ± 35.8 s and inexperienced observers 176.5 ± 50.3 s per test image.

I did not quantify the nature of the errors made by the observers. However, for some photos the errors related to a variety of different 'key choices' by observers, while in other photos the errors were consistent. Consistent errors appeared to be caused by reflective shine on the subject, poor light exposure, poor focus, or a combination. Observers also appeared to find certain characters more difficult to inspect than others. In particular, markings on each of the infralabial scales (characters 5–11) seemed difficult for observers to distinguish. In one test photo, where the individual skink was isolated without observers needing to make a decision about the infralabial scales, 23 of 24 observers correctly identified the individual.

5.3.3 I³S Pattern

The I³S Pattern algorithm correctly matched each of the 24 test photos within the top 21 matches. Sixteen (67%) of the 24 test photos were matched as the number one rank, 20 (83%) in the first five ranks, and 22 (92%) in the first 10. Of the eight test photos in each category, I³S Pattern correctly matched as the top match, six in category 1 (full lateral image), five in category 2 (angled), and five in category 3 (obscured characters)(Figure 5.5). Closer inspection of the two test images for which the correct identity was ranked out of the top ten choices, revealed one of the most widely angled photos (70°) with high image contrast, and the other, a high percentage of vegetation cover over the region of interest. With I³S, I could match a test image in an average time of 39.9 ± 47.6 s, plus a processing time of 32.6 ± 4.3 s per image (total time: 73 s or about 40% of the time taken by human observers). The self-evaluation test calculated 75.5% of correct matches ranked as the number one choice, and 92.7% in the top 20 matches (Table 5.4).

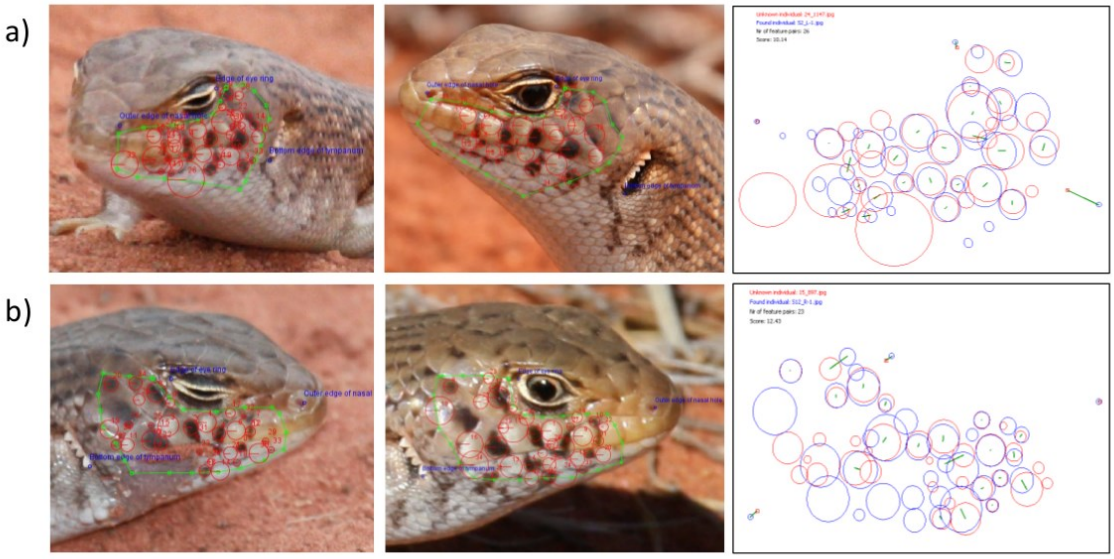


Figure 5.5. I³S Pattern comparisons for matching photographs of Slater’s skink. Test photos included (a) subject at extreme angles to camera, and (b) some obscured characters. Diagrams on the right are the corresponding ‘point cloud’ for the two images; green lines indicate distance calculations between matching key point pairs.

Table 5.4. Output from self-evaluation results of I^3S software for the database of 56* effective individuals of Slater's skink where the number and percentage of comparisons were calculated in the top #X rank. *30 individuals with unique left and right sides.

Rank	Number	Percentage
Top #1	41	74.5
Top #2	44	80.0
Top #3	45	81.8
Top #5	46	83.6
Top #10	48	87.2
Top #20	51	92.7

5.4 Discussion

This study has been one of the first to explore the use of photographic identification for individuals of a free-ranging, small terrestrial vertebrate. I showed that with careful examination of facial markings from good quality photos, developing an identification key for individuals is possible in a species that has stable facial markings. I also showed that observers can use the key to score poorer quality photos, whether the face was partly obscured or at wide horizontal angles to the camera. A key that discriminates on characteristics that can be objectively described (e.g. presence/absence of marking on a particular scale) can be used by any observer, regardless of their familiarity with the species, or their experience in wildlife survey. However, the key still requires a subjective assessment by the observer relative to the designer's assessment, and is therefore imperfect. The significantly greater performance by observers with experience suggests that less-experienced observers could achieve a greater accuracy with more training, time and effort.

Testing of the computer matching algorithm I^3S found the identification ability to be no better than human observers. In each photo category, the proportion of correct identifications with automated matching was comparable with that of the human observers'. The software was able to correctly match some individuals from photos that most observers incorrectly identified and vice versa. The software's self-assessment results showed matching rates below that of photo datasets from other taxa, and accordingly, the developers have concluded that this particular algorithm is not well suited to this species (J. Hartog and R. Reijns pers. comm. 2015). I suspect that flash on reflective scales, shadows, variable lighting, and other photo artefacts account for the low self-matching scores in this dataset. Epidermal shine is common in skinks (Scincidae), as determined by their relatively fine (smooth) microornamentation (Arnold 2002). In comparison, the eastern water dragon's coarse surface structure was not reported to cause reflective issues in photos or be problematic for the I^{3}S software (Gardiner *et al.* 2014). In this study, an insufficient number of high quality reference images most likely contributed to the low score in the self-evaluation results. Nevertheless, those lower quality images represent a typical sample in the study system. If this automated technique is to be more widely useful it may be that separate new algorithms will need to be developed to account for scale-shine and other species specific features, or that useable images will need to come from a narrower set of ambient conditions, such as cloudy days.

While automated computer-assisted identification had a clear time advantage, the higher percentage of correct identifications of experienced observers suggests a possible trade-off between time and accuracy. If there is some differential rate of misidentification between human and computer assisted techniques, then, particularly for smaller populations, the compromise of taking more time to achieve more reliable identification may be worthwhile.

I have shown that developing an identification key for human observers may be a viable and reliable technique, especially for a finite and small population. Where photographic images can be collected easily, and where there is sufficient variability in marking patterns among individuals, the technique can be used to assess identity without substantial impact on the observed population. While each of the alternative approaches, human or automatic identification, has its advantages, it may be possible to use a combination of the two. The key could be used by human observers to narrow the field to a group of individuals, which may then be separated based on other behavioural, spatial, or morphological features. In the latter case the computer system may be used.

The photographic key will be particularly valuable in the confident identification of previously recorded individuals, and of new adult entrants into the Slater's skink population, when candidate individuals from the key are combined with additional information from field observations, including spatial stability and other distinguishing features of individual lizards. For this endangered lizard species, the photo-identification key will be a valuable source of information about spatial structuring of individuals in a population within a season, about social interactions within a population, and about dynamical changes to population numbers across successive seasons (see Chapter 7). The key will also allow comparable monitoring programs by different personnel in the inevitable case of staff turnover in a conservation management program.

This technique may have wider direct benefit for camera traps, or motion-sensor cameras, which are becoming increasingly popular. While, at present, camera traps cannot focus or target a subject like a human operated camera, they have potential for individual identification of reptile taxa. For example, Welbourne (2013) incidentally observed that he could distinguish individuals of a small agamid lizard, *Amphibolurus muricatus* (body mass <60 g), on the basis of ornamental spots, from camera traps. Recognising trapped individuals of other species may depend on the resolution of the camera, the size of the animal and the proximity to the camera (Mendoza *et al.* 2011), and on acquiring multiple images to get the appropriate angle (Hohnen *et al.* 2013). The method I have developed here clearly has the potential to be applied to camera-trapping studies and thus a range of terrestrial wildlife monitoring and management applications.

5.5 Summary

I demonstrated here that independent observers were able to sufficiently replicate my selection of character states in the multi-choice key for an unknown individual. While the independent observers did not score perfect marks on the test, they were also not given the option of checking their selection against individuals in a reference library. In practice, this was my two-step identification method: narrowing the field with the key, and then checking the match against the reference database. Given the relatively small size of the Orange Creek population, I felt this study justified my use of photographic identification as a suitable technique for the subsequent research, presented in the following chapters. In the next chapter, I use photo-identification to examine the temporal activity patterns of *L. slateri.*

Chapter 6: Temporal activity patterns

Land managers and ranger groups in central Australia are invested in monitoring and managing populations of *L. slateri*. However, survey and monitoring protocols for the species are not fully developed. Here I address the issues of detectability and sampling effort in surveys of *L. slateri,* by investigating both seasonal and diel activity patterns in the species. This chapter has been adapted from the publication: Treilibs, C.E., Pavey, C.R., Raghu, S. & Bull, C.M. (2016). Weather correlates of temporal activity patterns in a desert lizard: insights for designing more effective surveys. *Journal of Zoology*, **300**, 281–290.

6.1 Introduction

Intraspecific variation in behavioural activity among individuals can be inherently difficult in surveys of animal populations, particularly of rare or inconspicuous taxa. In populations where individuals occupy independent shelter sites, variation in their times of activity influences which individuals are detected at a given time (Caughley 1977; Sun *et al.* 2001). The observational bias arising from this differential detectability among individuals may cause erroneous estimates of abundance or of other population metrics that assume an equal detection probability (Ramsey & Harrison 2004; Thompson 2013). However, detectability may be improved under particular environmental conditions, for instance seasonal or weather conditions, which increase detection among individuals in a population. Timing surveys to coincide with favourable conditions thus may help increase detectability (or 'observability') of the study species and optimise survey efforts (Sun *et al.* 2001; Brown & Shine 2002).

Low detectability is common in deserts where extremes of temperature and aridity constrain surface activity of many animals (and researchers) over extended periods of time, limiting opportunities for behavioural observation. Most desert organisms avoid extreme conditions by being nocturnal and selecting shelter sites, such as burrows, that buffer extreme temperature and relative humidity (Whitford 2002). When there are extreme conditions at the surface (e.g. where temperatures may exceed 60°C and humidity is <10%) animals can either move above (to perch sites) or below the surface (into burrows) to considerably reduce thermal and hydric stress (Whitford 2002). Many desert ectotherms burrow below the surface or use burrows of other animals, which affords thermal buffering, constant humidity, and a retreat from predators and extreme weather (Greer 1989; Körtner *et al.* 2008).

Exposure to air temperatures greater than 45°C can be lethal for most reptiles, with the critical thermal maxima for heliothermic, surface active skinks ranging from 38 to 45°C (Greer 1980; Bennett & John-Alder 1986). However, at 15cm below the surface, temperatures are consistently between 28 to 32°C, and humidity increases to 90-100% at 45cm depth (Henzell 1972; Whitford 2002; Körtner *et al.* 2008).

During periods when animals are not physiologically constrained by extreme temperatures, they can safely leave their burrows (Costa 1995; Bradshaw 1997). These operative conditions are effectively time 'windows' when conditions are suitable for surface activity such as foraging, basking, and mate-searching. For diurnal reptiles, this often results in a bimodal pattern of activity during hot summer months, with a peak in surface activity in the morning and late afternoon, and a single midday peak during cooler periods (Cloudsley-Thompson 1991; Adolph & Porter 1993; Dodd *et al.* 1994).

Within these time windows, activity patterns may be either fixed or opportunistically flexible over a 24-hour period with changing abiotic and biotic conditions (Gordon *et al.* 2010a). Activity may be influenced by a range of intrinsic and environmental factors including food resources (Gordon *et al.* 2010a), reproductive condition (Keogh *et al.* 2012), body size and age class (Carothers 1983), gender (Brown & Shine 2002, but see Huey & Pianka 2007), level of interspecific competition (Huey & Pianka 1983; Gordon *et al.* 2010a), energy budget (Adolph & Porter 1993; Kearney 2013) and water balance (Kearney *et al.* 2013). The level of activity will also differ among species depending on their foraging mode (Huey & Pianka 1981). In social species, social interaction between conspecifics also may influence patterns of activity (Qi *et al.* 2012).

Given the strong association between ectotherms and their thermal environment, air temperature typically has been considered the main influence on the surface activity of reptiles (Huey & Kingsolver 1989; Adolph & Porter 1993). However, empirical studies suggest that ambient temperature may have a greater influence on activity levels in temperate than in tropical species (Shine & Madsen 1996; Brown & Shine 2002; Price-Rees *et al.* 2014). While tropical species have been understudied compared with their temperate counterparts, there are still fewer data on desert species. Compared with the relatively stable ambient temperatures of tropical climates, the highly variable temperatures of desert environments may play a more significant role in the time of activity of desert ectotherms (Davis *et al.* 2008).

Slater's skink, *Liopholis slateri*, is an endangered desert reptile which, historically, has proved difficult to locate (Pavey 2004a). Contradictory statements have been published about its activity patterns, indicating they are not well understood. Wilson and Swan (2013) described the species as crepuscular to nocturnal while others reported a diurnal, bimodal activity, focused in the hours just after sunrise and just before sunset (Henzell 1972; Pavey *et al.* 2010; McKinney *et al.* 2015). While most congeners of *L. slateri* (formerly *Egernia,* Gardner *et al.* 2008) are diurnal, some (*L. kintorei, L. striata* and *L. inornata*) are crepuscular to nocturnal, with flexible activity patterns depending on environmental conditions (Pianka & Giles 1982; Pearson *et al.* 2001; Chapple 2003). *Liopholis slateri* may show similar flexibility, but no study has investigated its activity outside of daylight hours.

In this study, I explored the diel and seasonal activity patterns for *L. slateri* at both an individual and population level in relation to local weather conditions and time in the season. This study was a correlative one, without any attempt to deduce the causes of the patterns I observed. Instead, I asked broader questions about whether there was a particular set of conditions that were associated with greater levels of surface activity that would maximise the effectiveness of population surveys for this species and potentially for other desert reptiles.

Specifically I asked:

a. When is the best time to survey the population?
b. How many visits are needed to survey the majority of the population?
c. Is there variation in individual detectability?

I also asked whether this species was active at night and how the day-night activity pattern of an individual changes over a survey year.

6.2 Methods

6.2.1 Study species

Lizards in the genus *Liopholis* are semi-fossorial, burrowing specialists that use an ambush foraging strategy, staying in close proximity to the burrow entrance when they emerge (Wu *et al.* 2015). Individuals of *L. slateri* typically emerge from a burrow and sit at (or near) a burrow entrance to bask and wait for prey as their major activity (Fenner *et al.* 2012a;

McKinney *et al.* 2015). *Liopholis slateri* is active during the spring-summer period after over-wintering, typical of many ectotherms in desert and temperate regions (James & Shine 1985; Adolph & Porter 1993).

6.2.2 Study site

The study site was at Orange Creek (23°59’S, 133°37’E), 40 km south west of Alice Springs, within the MacDonnell Ranges bioregion of the Northern Territory, Australia (see Chapter 4 for details). The study was conducted over four austral spring-summer periods (hereafter referred to as ‘survey years’), from December 2011 to April 2015 (Figure 6.1). Over the entire study period, I detected 104 active burrows at the site, with usually 49 to 52 burrows occupied per survey year (see Chapter 7). No lizards or active burrows were located during intensive searches over several kilometres beyond the study site, leading me to conclude that my research effort encompassed all the individuals of the study population.

6.2.3 Population survey

I used photographic identification (see Chapter 5) to identify individuals within the population from a reference collection of their photographs. I surveyed those individuals as often as twice per week in each survey year (2011–12 = 20 surveys; 2012–13 = 37; 2013–14=7; 2014–15 = 62). Of the 126 surveys, 94 were in the morning soon after sunrise (between 0600-0830 hrs), while 32 were late in the afternoon, just before sunset (1700–1930hrs). On each visit all known burrows were surveyed twice—varying direction and survey sequence each time—and nearby areas, within and outside of the previous population extent, were searched for new burrow activity that may have occurred since the previous visit. The second survey usually started within 10 minutes of finishing the first. Typically, the same lizards were again seen on the second pass leading me to conclude that any observer influence was probably minimal. For each burrow on each visit, I recorded if a lizard was outside a burrow on at least one of the two surveys. On each survey visit, I derived an estimate of lizard activity from the number of lizards observed sitting at a burrow entrance. Data for all visits across years were collated and analysed together.

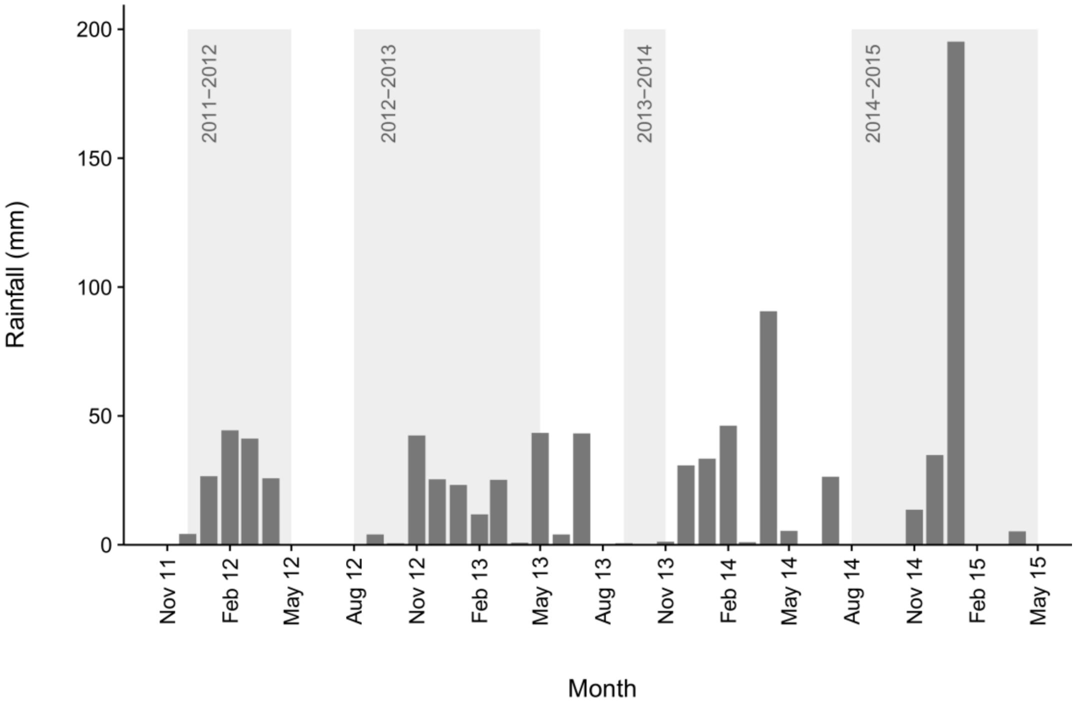


Figure 6.1. Monthly rainfall (dark grey bars) recorded at Alice Springs airport weather station (#015590) over the four survey years (light grey shading) of the study period December 2011-May 2015 (source: Bureau of Meteorology).

6.2.4 CART modelling

I explored the influence of weather conditions on *L. slateri* activity using classification and regression tree (CART) models. CARTs are a simple but analytically robust technique of describing variation in a single response variable through recursive binary partitioning of multiple explanatory variables (Breiman *et al.* 1984; De'ath & Fabricius 2000). CART algorithms work by selecting a single explanatory variable and a value for that variable that best splits the dataset into two mutually-exclusive groups (e.g. surveys with high or with low skink counts) that are most homogenous (pure); the splitting process is then repeated for each sub-group until an impurity threshold is reached (Therneau *et al.* 2015). CARTs can handle multiple types of covariates, collinearity, and missing values, and they are relatively simple to construct and interpret, often uncovering patterns that traditional linear models may not (De'ath & Fabricius 2000; Cutler *et al.* 2007). While CART modelling can be used for both exploration and prediction, I used the approach to explore weather conditions that might be optimal for survey when there was greatest lizard activity, using the count of individuals observed active per visit as the response variable. I used descriptive (unpruned) trees to examine activity patterns in relation to key weather variables (De'ath & Fabricius 2000). I used the 'rpart' (Therneau *et al.* 2015) and 'partykit' (Hothorn & Zeileis 2015) packages in R (R Core Team 2016) to generate the CART models and visualise the resulting trees.

I used daily observations from the nearest Australian Bureau of Meteorology weather station (Alice Springs Airport; ID: 015590; about 33 km from the study site) to generate 14 explanatory variables for use in the CART models (Table 6.1). I ran separate analyses for the morning and afternoon datasets, using the 09:00 observations of relative humidity, temperature, wind speed, atmospheric pressure and cloud cover for morning activity and the 15:00 observations of the same variables for afternoon activity. Other daily weather observations were used as covariates for both the morning and afternoon datasets, including pan-evaporation, minimum overnight temperature, rainfall in the 24 hours prior to 09:00, and five lagged rainfall-related variables (Table 6.1).

6.2.5 Survey effort

I generated rarefaction curves using the 'vegan' package in R (Oksanen *et al.* 2015) to assess the survey effort in each of the four years (Wanger *et al.* 2009; Gotelli & Colwell 2011) for both neonates (individuals born during the survey year) and other individuals at least in their second season. To assess the degree of individual variation in detectability, I also calculated a weighted sighting probability for each individual over a period R, their effective residency time in the population (between the first visit and the last visit when the individual was sighted) (Equation 6.1). For this assessment, I only considered individuals that had been resident for 10 or more visits to avoid skewing the data toward perfect sighting probabilities. Sighting probabilities between neonate and non-neonate groups were compared with a t-test.

$$\text{Sighting probability} = \frac{\text{number of visits sighted during R}}{\text{total number of visits during R}} \tag{6.1}$$

Table 6.1. Explanatory variables used in CART modelling of Slater's skink activity. Variables are weather observations recorded every 30 minutes from the Alice Springs weather station, or derived from rainfall observations. Mean and standard deviation (SD) for each covariate is listed for the corresponding morning (m) and afternoon (a) datasets.

Covariate		**Units**	**Mean (m)**	**SD (m)**	**Mean (a)**	**SD (a)**
Observations at 0900 hrs local time						
X9am.RH[1]	Relative humidity at 9am	percent	12.1	16.6		
X9am.temp[1]	Temperature at 9am	degrees Celsius	25.9	5.6		
X9am.wind[1]	Wind speed averaged over 10 minutes prior to 9am	kilometres per hour	16.9	8.1		
X9am.pressure[1]	Atmospheric pressure reduced to mean sea level at 9am	hectopascals	1014.1	4.8		
X9am.cloud[1]	Fraction of sky obscured by cloud at 9am	eights	2.2	2.7		
Observations at 1500 hrs local time						
X3pm.RH[1]	Relative humidity at 3pm	percent			16.2	8.9
X3pm.temp[1]	Temperature at 3pm	degrees Celsius			30.8	5.5
X3pm.wind[1]	Wind speed averaged over 10 minutes prior to 3pm	kilometres per hour			19.6	6.8
X3pm.pressure[1]	Atmospheric pressure reduced to mean sea level at 3pm	hectopascals			1011.6	4.6
X3pm.cloud[1]	Fraction of sky obscured by cloud at 3pm	eights			3.2	2.5
min.temp[1]	Minimum temperature in the 24 hours to 9am	degrees Celsius	15.8	6.4	14.5	6.3
evap[1]	Class A pan evaporation in the 24 hours to 9am	millimetres	11.1	3.5	10.2	3.7
rainfall[1]	Precipitation in the 24 hours to 9am	millimetres	0.4	1.7	0.3	1.8
dsrain[2]	Days since rainfall	days	34.0	37.8	44.3	37.9
dsrain2mm[2]	Days since rainfall greater than 2 mm	days	53.8	38.5	39.4	43.3
dsrain5mm[2]	Days since rainfall greater than 5 mm	days	64.7	54.1	73.9	52.0
dsrain10mm[2]	Days since rainfall greater than 10 mm	days	70.6	56.0	73.9	52.0
X7day.rain[2]	Cumulative rain over the past 7 days	days	2.1	5.6	8.1	34.1
X14day.rain[2]	Cumulative rain over the past 14 days	days	7.0	17.9	10.1	35.1

Sources:

[1] Bureau of Meteorology daily data for Alice Springs airport (purchased)

[2] Derived from Bureau of Meteorology rainfall observations

6.2.6 Diel activity survey

To further understand how individual activity might vary over a day-night cycle at a higher temporal resolution, I gathered activity data from individual lizard burrow entrances. I positioned time-triggered cameras (Reconyx Hyperfire HC500) focused on one single-entrance burrow (OC99) from November 2012 to May 2013, and on three other burrows (OC72, OC57 and OC118) for two-week periods during April 2013 (OC57), November 2013 (OC57, OC72), August 2014 (OC118), and September 2014 (OC118). I secured the cameras on 900 mm aluminium fence spacers set at 2m from the burrow, angled downward toward the burrow entrance. I set the cameras to trigger every 30 minutes because motion detection triggers were unreliable for this mostly sedentary lizard. I assessed activity based on resident lizard presence or absence at the burrow entrance in each of 6420 half-hourly photos, and coded these by tag in the photo metadata. I then extracted date, time, temperature and tag from the metadata with ExifTool (ver 9.99) (Harvey 2015) and used listed sunrise and sunset times for Alice Springs (source: Geosciences Australia 2012-2014) to classify activity into day and night observations.

I also sourced half-hourly weather observations from the Alice Springs airport weather station for the period of November 2012 to May 2013 to match with the seven month set of half-hourly photo data (Appendix Table 2). I constructed CARTs using presence/absence data as the response variable with 24 half-hourly weather observations as the explanatory variables, and ran separate analyses for the day and night datasets.

6.3 Results

6.3.1 Population survey

During the 94 morning surveys I recorded a mean of 6.0 (± 0.4 SE) lizards at their burrow entrances per visit. CART modelling showed the highest counts (mean 10.8 ± 0.5 SE) occurred during the eight surveys when rainfall in the previous week was greater than 1.1 mm and when the relative humidity at 09:00 on the survey day was greater than 47.5% (Figure 6.2). The fewest lizard counts (1.8 ± 0.5 SE) were on the 11 survey mornings when the rainfall in the previous week was less than 1.1 mm, the 09:00 relative humidity was less than 22.5%, the 09:00 air pressure was less than 1014 hectopascals, and there had been more than 23 days without rain greater than 2 mm.

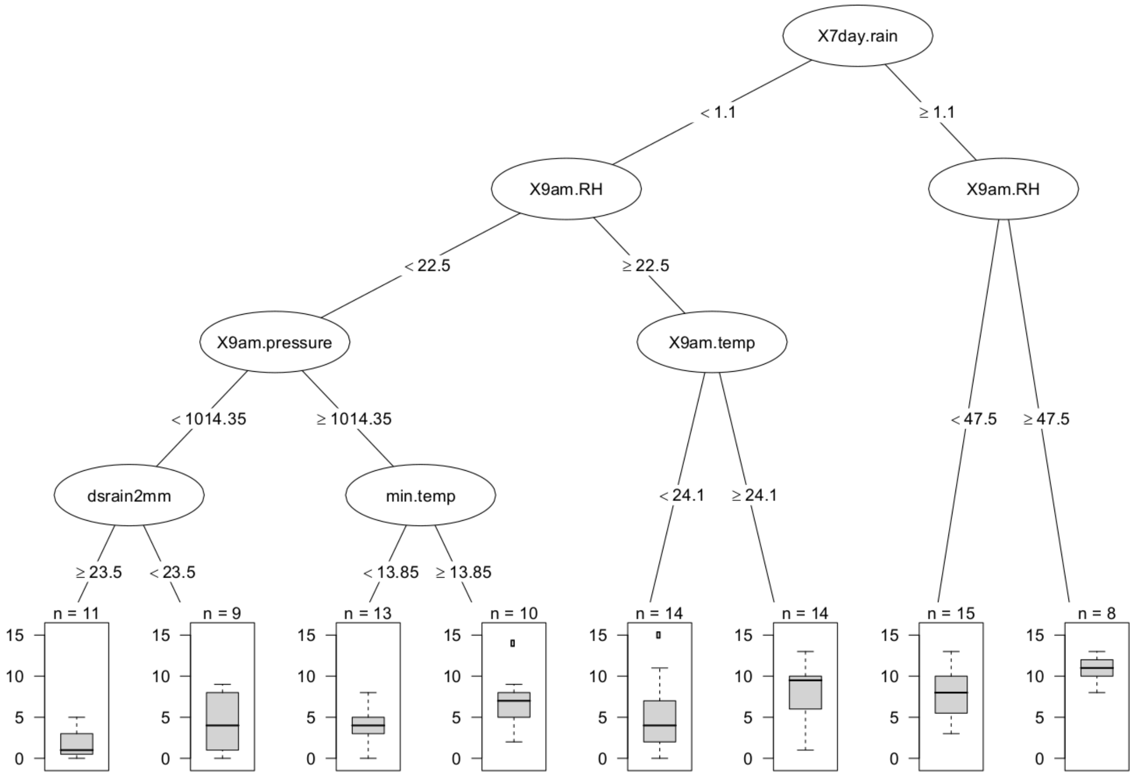


Figure 6.2. Regression tree for skink counts on morning visits (n=94). Data are partitioned by daily weather observations from the Alice Springs airport (Table 6.1). Each split (nonterminal node) is labelled with the node number (square box), the variable (circled) and the variable values (on the lines below) that determine the split. Terminal nodes are labelled with the number of visits for the corresponding set of conditions, and display the distribution of observed values (i.e. skink counts) in a box plot. For example, reading from the top to the right side of the figure, the highest mean skink counts were observed when the rainfall over the seven days prior to the field visit (X7day.rain) was greater than or equal to 1.1 mm and the relative humidity at 09:00 h on the day of the field visit (X9am.RH) was greater than or equal to 47.5 %.

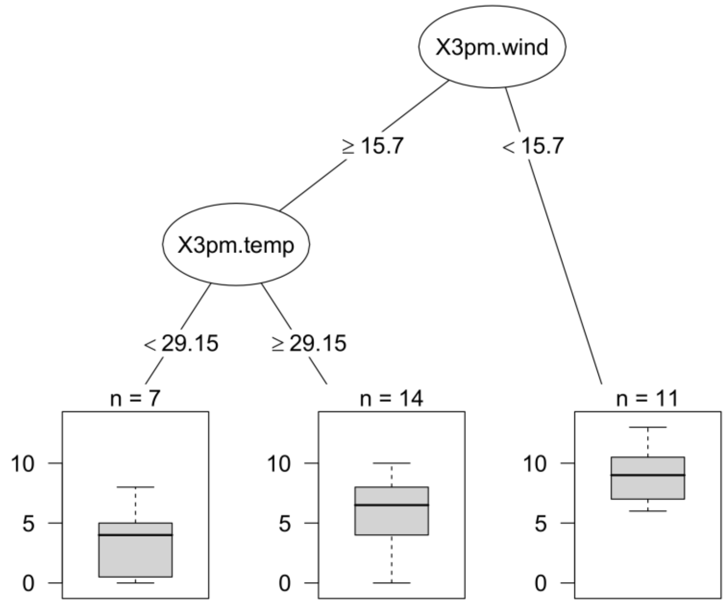


Figure 6.3. Regression tree for skink counts on afternoon visits (n=32). Data are partitioned by daily weather observations from the Alice Springs airport (Table 6.1). Terminal nodes are labelled with the number of visits for the corresponding set of conditions, and display the distribution of observed values (i.e. skink counts) in a box plot.

During the 32 afternoon surveys, I recorded a mean of 6.3 (± 0.6 SE) lizards at their burrow entrances per visit. I observed highest skink counts (mean 9.0 ± 0.7 SE) in relatively windless conditions on 11 afternoons when the wind at 15:00 was less than 15.7 km/hr (Figure 6.3). On windier days I observed more lizards when the 15:00 temperature was more than 29.1°C (14 afternoons, mean 5.7 ± 0.7 SE) than when it was cooler (seven afternoons, mean 3.2 ± 1.1 SE).

6.3.2 Survey effort

Rarefaction curves indicated that observers required about 24 visits to sight all individuals of the population (Figure 6.4a). Fewer surveys were required to sight all neonates (about 14 visits, Figure 6.4b) than non-neonate individuals (about 20 visits, Figure 6.4c). Individual neonates had a higher mean ($t = 3.86$, d.f. = 9.7, $p < 0.005$) and lower variance of sighting probability than non-neonate individuals (Figure 6.5). There was no difference in sighting probability between morning or afternoon visits ($t = 0.14$, d.f. = 34, $p = 0.88$).

6.3.3 Diel activity survey

One visually distinct individual lizard, S22, was resident in burrow OC99 throughout the seven month period of half-hourly camera data. This individual appeared in 34.5% of all of my photographic observations (n=2214/6420). I regularly observed long sequences of 10 to 29 consecutive images (= 5–14.5 h) with the lizard remaining 'sitting' outside the burrow. During those sequences, the lizard seldom changed its general position, although there were occasional small adjustments in posture from one image to the next. The motion detection trigger showed 14 foraging bouts each of less than 30 minutes within the seven month filming period, when the lizard moved away and then reappeared at the burrow entrance. I cannot be confident if there were any more of these, or if other absences from the burrow entrance meant the lizard had retreated into the burrow (inactive) or had left to forage (active). Nevertheless, I assumed that I could use sitting at the burrow entrance as an approximation of time spent "active" on the surface (i.e. emerged from burrow).

I found shifts in the same individual's (S22) day-night activity patterns among months over the course of one survey year (Figure 6.6a). This lizard regularly exhibited nocturnal activity, which peaked from December to March, the hottest months of the year. In those months more than 50% of its emergences were at night. Occupants of two other burrows (OC57 and OC72) also showed nocturnal activity in November (Figure 6.6b), suggesting that the nocturnal behaviour of S22 was not unique among *L. slateri*.

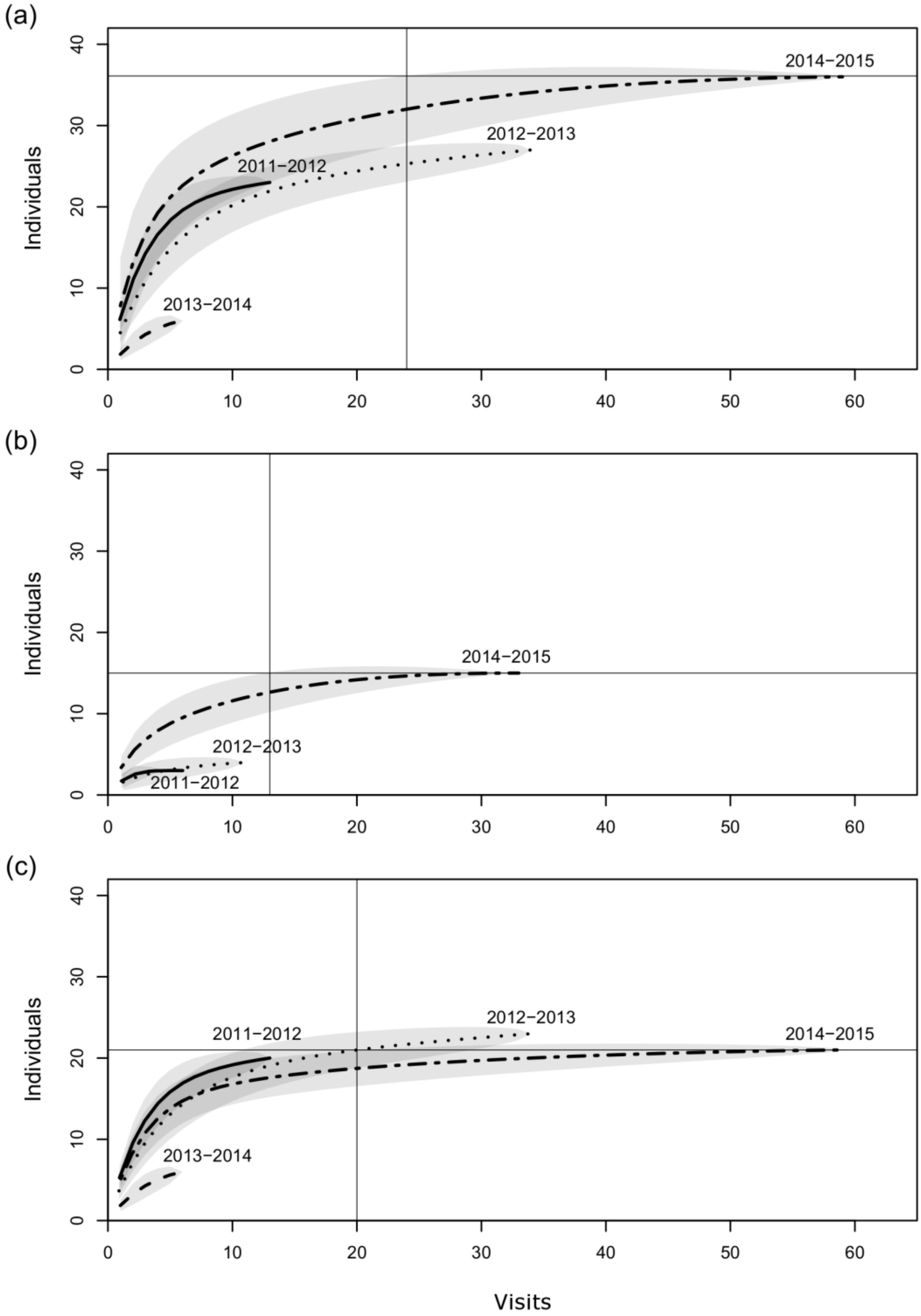


Figure 6.4. Rarefaction curves for the number of individuals detected per number of visits to the population over four survey years for (a) all individuals, (b) neonates, and (c) non-neonates. Cross-hairs mark the intercept of where the asymptotic line leaves the 95% C.I. interval for the 2014-2015 rarefaction curve i.e. greatest survey effort (n=62; 2014-2015). These cross-hair points approximate the number of visits required to survey all members of the respective group e.g. in (a) about 24 visits are required to sight all individuals.

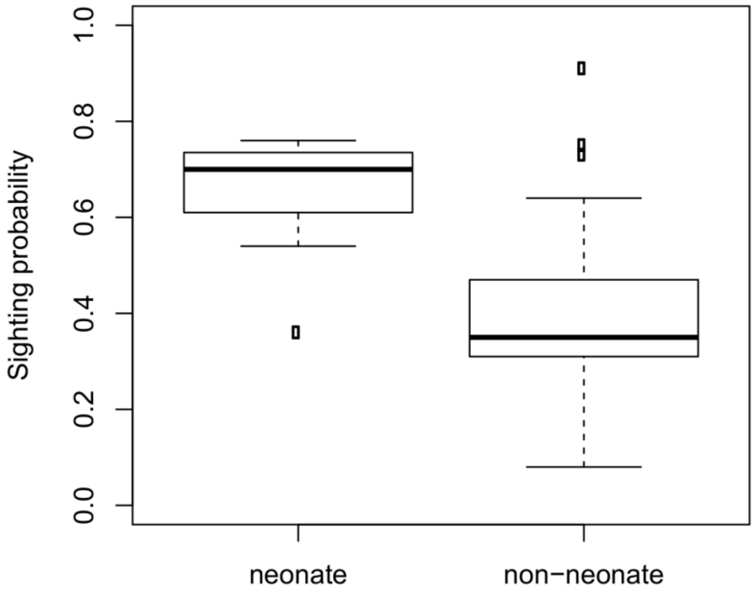


Figure 6.5. Sighting probabilities for neonate and non-neonate individuals for those resident in the population for 10 or more visits.

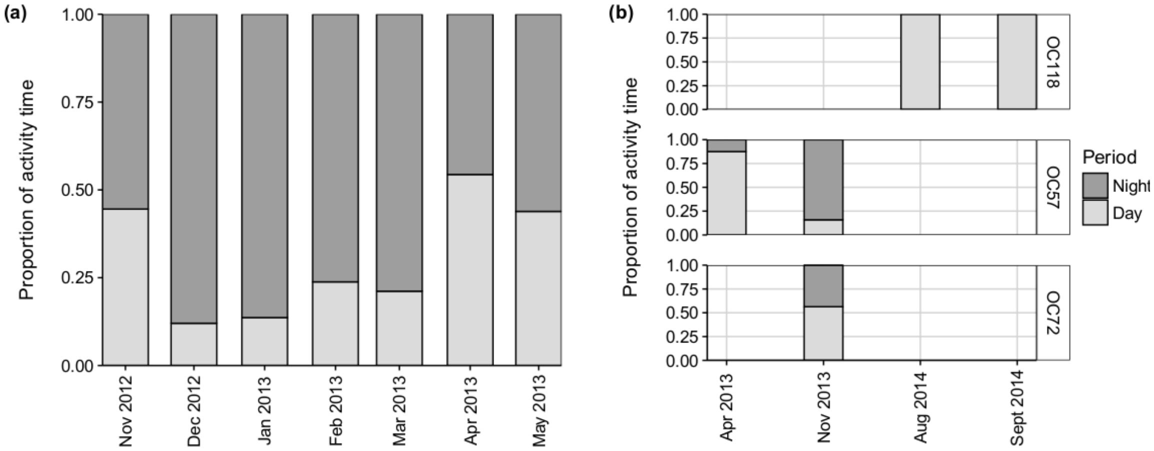


Figure 6.6. Diel activity as defined by presence outside the burrow, recorded every 30 minutes by a time-triggered remote camera, for (a) one individual (S22) from November 2012 through May 2013, and (b) occupants of three geographically separate burrows over 2-week periods in April 2013, November 2013, August 2014 and September 2014.

S22 was visible in the half-hourly images (‘present’) for 20% of observations during daylight hours (n=643/3141) and 48% of observations during the night (n=1571/3279). CART modelling of the daylight dataset showed temperature at the time of the photographic image was the primary variable in splitting the dataset (Appendix Figure 1). Absences were more likely when air temperatures were greater than or equal to 30°C (n=2189/2492: 87% probability). Presences were most likely (n=122/139: 87% probability) when the air temperature was less than 30°C, the dewpoint was greater than -4.1°C, and there had been fewer than 16 days since rain.

For the night dataset, CART models showed the main variable influencing presence or absence of lizard S22 was rainfall in the 14 days preceding the observation (Appendix Figure 2). Absences were more likely when there had been relatively heavy rainfall (more than 24 mm) in the previous 14 days (n=307/323: 95% probability). Presences were more likely when there had been some rain, but not a lot (21–24 mm) in the previous 14 days (n=437/527: 82% probability).

6.4 Discussion

In this chapter, I explored the weather conditions that correlate with surface activity of a desert ectotherm. Key explanatory variables that were featured in the CART models showed that, in general, lizards were more active in non-desiccating conditions: higher humidity, more recent rainfall, lower evaporation rates, and lower wind speed. Air temperature and humidity had an influence on surface activity but their influence varied between the morning and afternoon datasets. Although the afternoon sample size was smaller, the data appear to suggest that lizards respond to different weather influences at different times of the day. This pattern was consistent whether I compared morning and afternoon surveys, or daytime and nighttime photographic records. I also found evidence of previously unreported nocturnal activity during the hottest months of the year.

The results support the findings of another correlative study of reptile encounter rates (Brown & Shine 2002) that found no strong association of a single covariate, or set of covariates, with activity levels. In this study, the broad range of conditions in which high lizard counts occurred – not exclusive to any particular set of weather conditions – suggests that other factors, such as individual variability in both activity patterns and foraging success, may be involved. The large variation in my calculation of individual sighting probabilities supports this interpretation to some extent. Other studies have reported that diel cycles of behavioural thermoregulation in lizards follow a circadian rhythm (Kerr *et al.* 2008) that may be out of synchrony among different individuals. Additionally, a growing body of literature shows lizards have individual preferences and intra-individual variability in behavioural type affecting their activity levels (Stamps *et al.* 2012; Spiegel *et al.* 2015). These studies demonstrate that the factors influencing activity patterns of an individual are complex.

The findings of nocturnal activity, and of considerable flexibility in day-night activity patterns, suggest that *L. slateri* may have a greater daily time window for foraging than previously considered in climate change models. *Liopholis slateri* and *L. kintorei*, were identified as two of the lizard species in Australia at high risk of extinction from climate change under a warming-induced activity restriction scenario (Sinervo *et al.* 2010). However, the modelling behind this prediction considered *L. slateri*, along with *L. kintorei*, to be diurnal (Kearney 2013). Opportunistic nocturnal activity has been demonstrated in the usually diurnal lizards *Ctenotus pantherinus* (Gordon *et al.* 2010b) and *Tiliqua rugosa* (Kerr & Bull 2004) and should be considered for other lizards when modelling impacts of climate change.

CART models are a simple but powerful means of searching for patterns in datasets with a large number of covariates. Compared with data-reduction techniques, the output models are relatively easy to interpret (De'ath & Fabricius 2000). With relatively small data sets, as in this study, we can only explore the trends without an assessment of the rigour of the interpretation. With larger data sets, CARTs can be developed for a subset of the data (i.e. a training set) that can then be tested on the remainder to assess the model fit.

In the case of environmental variables, it is clearly desirable to have measurements taken from as close to the study species as possible. In this study, the use of data from a weather station 33 km away is likely to have decreased the spatial and temporal precision of the observed correlation, particularly with patchy, localised rainfall, but I was expecting strong regional correlations with the other variables. Future studies may consider installing a local weather station or data loggers, but this will be a trade-off between enhanced local accuracy and a reduced number of environmental variables compared with those that a Bureau of Meteorology weather station can offer. Nevertheless, the method provides a useful guide to advise on optimal times to monitor activity in species that are not regularly active. As more surveys on this endangered skink are completed, we may be able to more precisely model the occupancy of this species across space and in relation to key biotic and abiotic factors (Welsh *et al.* 2013).

6.5 Summary

The results I presented here will enable monitoring rounds and surveys to be targeted to the environmental conditions that increase detectability for *L. slateri*. Specifically, monitoring success might be increased by timing surveys to non-desiccating weather conditions. Repeating survey visits to a population will also increase accuracy of abundance estimates. In the next chapter, I investigate the persistence strategy of *L. slateri* by tracking the spatial and temporal dynamics of the Orange Creek population.

Chapter 7: Spatial dynamics and burrow occupancy

Liopholis slateri is a floodplain habitat specialist; populations occur along both major river channels and minor watercourses within the MacDonnell Ranges bioregion (see Chapter 3). Desert floodplains are high-risk habitats, where persistence is a trade-off between the advantages of relatively abundant food resources, and the costs of episodic surface disturbances from infrequent, but unpredictable, rainfall events. In central Australia, there are few non-flying, terrestrial vertebrates that are specialised floodplain occupants, and their persistence strategies are not well understood. In this study, I examined how a population of *L. slateri* persists in these disturbance-prone habitats. This chapter has been adapted from the manuscript: Treilibs, C.E., Pavey, C.R., Gardner, M.G., Ansari, T.H., Johnston, A. & Bull, C.M. (2016). Spatial dynamics and burrow occupancy in a population of Slater's skink *Liopholis slateri*. (To be submitted.)

7.1 Introduction

Water poses two contrasting challenges for species in arid environments. For extended periods, water availability is limited, and then, infrequently, flooding flows from heavy rainfall can dramatically disrupt ecosystem stability (Schwinning & Sala 2004). Water from intermittent precipitation (rainfall, hail, snow) is redistributed through the landscape by run-off, or stored in the soil, depending on rainfall intensity, antecedent moisture, slope, aspect, soil texture, biocrust, micro-climate, and intercepting features (Noy-Meir 1973; Ludwig *et al.* 2005; Collins *et al.* 2014). As a consequence, soil water in a given arid habitat is not directly related to the amount of rainfall, but influenced by the topographic and edaphic properties of the habitat's position in the landscape (Ayyad 1981). Positive feedbacks between plants and available soil moisture mean that soil texture and landform also largely determine the structural stability and productivity of the habitat (Noy-Meir 1974; Crawford & Gosz 1982; Peters & Havstad 2006; Nano & Pavey 2013).

The variable topography and rainfall of arid landscapes creates a mosaic of habitats that differ in productivity and physical stability. As an example, sand ridges are more structurally stable and productive than adjacent gibber-clay habitats because moisture can infiltrate further into sand, promoting deeper rooted perennial plants (Nano & Pavey 2013). In contrast, rocky range habitat is stable physically and provides thermal buffering from environmental variation in temperature, but resource availability is comparatively low (e.g. Gilfillan 2001a; Geiser & Pavey 2007). Desert riverine habitats (ephemeral rivers and floodplains) provide a contrast to the remainder of the arid landscape. Because of the greater availability of moisture, often in the form of groundwater, these riverine areas are persistently resource rich, relative to adjacent arid areas (Pickup 1991; Kingsford 2006). These areas have reliable soil moisture, nutrients, and shelter and food resources for consumers (Stafford-Smith & Morton 1990; Gilfillan 2001a; Free *et al.* 2013). However, the high resource availability is associated with unpredictable, and usually infrequent, periods of high levels of disturbance from heavy rainfall. These events cause river flows, inundation, lateral overflow and major sediment erosion and deposition. Terrestrial organisms that inhabit riverine environments and exploit the high resource availability in periods of low rainfall, need to have evolved mechanisms for persisting there, despite the intermittent pulses of disturbance when there are major rainfall events.

How animal populations persist in these productive but high-risk environments is not well understood. In fertile environments with reliable resources, life-history strategies of higher order consumers tend towards persistent, rather than opportunistic strategies (Gilfillan 2001b; Morton *et al.* 2011). In these areas, long duration of high levels of resource availability will result in relatively small increases in resource abundance in response to large rainfall events (Morton *et al.* 2011). Thus I predict that species in these habitats would persist at relatively stable and persistent abundances in contrast to the irruptive dynamics that are a feature of populations in nearby, more resource poor, arid environments after rain. The question is how species persist to exploit these stable resources despite occasional disturbance. Shmida *et al.* (1986) postulated that organisms colonising areas with episodic surface disturbances should have fast, long-range dispersal mechanisms and/or abilities to cope with surface disturbances. These might include the ability to fly, live in trees or to dig burrows where flood damage is least likely. In areas of high flood frequency, individuals of a floodplain species without these abilities may periodically be extirpated following flood events, with persistence reliant upon recolonization occurring from adjacent upland areas (see McDonald *et al.* 2012 for an example).

Understanding the mechanisms by which terrestrial species persist in desert riverine habitats is of some urgency, given the prediction for an increase in frequency and intensity of extreme rainfall events in the latest climate modelling for arid Australia (Healy 2015). Increased rainfall events may be an issue, particularly for species that currently occur in small isolated populations, which are already subject to new environmental pressures from invasive species and human habitat alteration. In central Australia, although a number of species occupy floodplains as well as other habitats, specialised terrestrial floodplain occupants are rare. Slater's skink, *Liopholis slateri*, is one such desert floodplain specialist, and it is endangered. The species has previously been recorded, relatively recently, at floodplain sites where it now no longer occurs. This pattern of disappearance implies relatively recent local extinction (Pavey 2004a). It also indicates that population sites are ephemeral, although a critical question in the conservation management of this species is whether there are source populations that can recolonise these sites. The small body size of these lizards means they do not have high mobility, and they live in burrows rather than in the trees, so they would appear to be particularly vulnerable to flooding disturbances. In this study, I investigated what adaptive strategies this species has adopted to persist in this habitat, despite the periodic disturbances. I observed a population of Slater's skink over four years, to understand the spatial and temporal patterns of individuals within a population site in this dynamic landscape.

I predicted that this lizard species would respond to the reliable resources in its floodplain habitat by showing high site fidelity and relatively low fluctuations in population size among years. I predicted that it would have evolved a relatively mobile strategy, with individuals exploring alternative burrows within the population site to allow them to rapidly adjust to any flooding induced local burrow destructions. I also predicted some dispersal related dynamical adjustment to the population boundaries, as individuals continually explored new site opportunities, allowing them the flexibility to adjust if parts of the site were made inhospitable by flooding damage.

7.2 Methods

7.2.1 Study site

The study was at the Orange Creek population site (see Chapter 4 for details of hydrology at the study site). The population was first located in 2008. A previous study in the 2010–2011 spring-summer recorded 16 individuals and 16 active burrows at the site (A. Fenner pers. comm., Fenner *et al.* 2012). My study was conducted over the austral spring and summer of the four following seasons, 2011–2012 to 2014–2015 (hereafter referred to as 'survey years') from December 2011 to April 2015. Although I incorporate the 2010–2011 results in this study, in that first season, only seven individuals were captured and photographed to a resolution that could be used for individual photo-identification (see below). Additionally, I had limited opportunity for surveys in the 2013–2014 survey year due to chronic illness. Rainfall over the study fell predominantly during the summer months, with extensive periods of several winter months with no rain at all. During the study there was one four-day period of heavy rainfall (186 mm) in January 2015 (Figure 7.1).

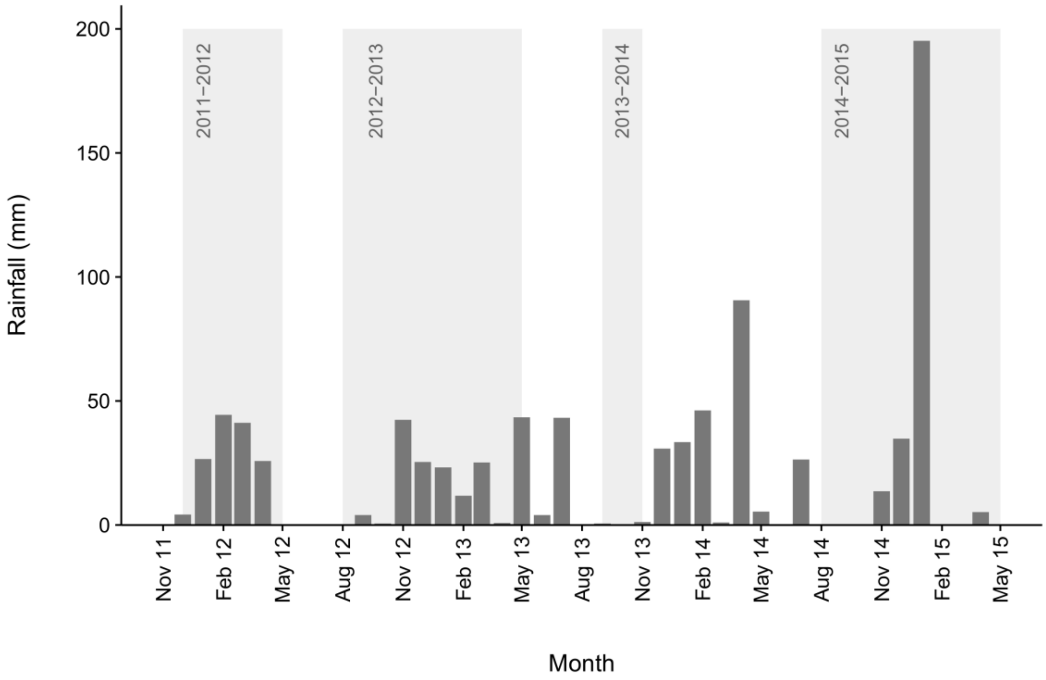


Figure 7.1. The periods of study in each of the four survey years (light grey shading) in relation to total monthly rainfall (dark grey bars) recorded at Alice Springs airport weather station (#015590) December 2011 – May 2015 (source: Bureau of Meteorology). Heavy rainfall (186 mm) in January 2015 fell over 4 days.

7.2.2 Population survey

I used a photographic based mark-recapture method to track individuals over time (see Chapter 5). I surveyed the lizard individuals in the population, as described in Chapter 5, as often as twice per week in each survey year (2011–12 = 20 lizard surveys; 2012–13 = 37; 2013–14=7; 2014–15 = 62). Of the 126 surveys, 94 were in the morning soon after sunrise (between 0600-0830 hrs), while 32 were late in the afternoon, just before sunset (1700–1930hrs). Lizards were always sighted sitting in the entrance or immediately outside of their burrows, and could be photographed for subsequent identification. As previously described, I was confident I could re-identify adults and sub-adults from their spot patterns across years, but ontogenetic changes in markings during early growth made it difficult to determine if apparently new adults in one year were new immigrants or recruited neonates from a previous year. I could more confidently link neonates in one year to sub-adults in the next when they appeared next spring in their natal burrow system (see Chapter 5).

On each visit all identified burrows were surveyed twice, varying direction and survey sequence each time. Additionally, on each site visit, nearby areas within and outside of the previous population extent, were searched for new burrow activity that may have occurred since the previous visit. Each site visit took about 2.5 h to complete. For each burrow, I recorded the identity of each lizard sighted on at least one of the two surveys during that visit. No lizards or active burrows were located during intensive searches over several kilometres beyond the study site. These external searches took about 2 – 3 hours and were repeated twice each survey year. In these searches, I particularly focused on floodplain habitat along Orange Creek and associated tributaries, both up and downstream from the study site, and found no evidence of any burrow structures. While it is possible that some isolated and obscure burrows may have been overlooked, I concluded that my research effort encompassed all the burrows and their individual occupants in the study population.

7.2.3 Burrow status

I recorded a burrow as having occupancy status if I made at least one visual sighting of skinks using that burrow at least once during the study. In addition to the skink sighting surveys, each month of each survey year, I recorded the activity level status of each identified burrow (2011–12 = 5 monthly burrow status surveys; 2012–13 = 9; 2013–14=2; 2014–15 = 7). Burrow activity was assessed in the morning, usually between 0900–1200 hrs, with about three hours

Table 7.1. Burrow activity level recorded each month during each survey year.

Activity level	Criteria
Active	Animal sighted OR fresh scats OR fresh tracks/digging
Recently active	Burrow entrances intact, clear, but no sign of current activity
Not active	Burrow entrances intact but debris or cobwebs block entry way
Derelict	Burrow entrances partially collapsed, but an entryway is detectable
Long derelict	Burrow entrances barely discernible from surrounding surface

required to survey all burrows. The activity level of each burrow on each survey was categorised according to descriptive criteria using the presence of skink signs and the structural state of burrow entrances (Table 7.1). Burrows were classed as active for that month if a skink was observed at the burrow, or the burrow had fresh scats, tracks, tail drag marks, or signs of recent burrow maintenance. In contrast, the entrance structures of derelict or long derelict burrows were partially or wholly collapsed (Table 7.1). Note that burrows that had been abandoned at some time during the study could become re-occupied at a later time.

7.2.4 Spatial dynamics

Although I observed skinks away from their burrows only four times in 300 h of survey observation, I recorded regular changes between visits, of the burrows that individual skinks occupied. If an individual was sighted at a different burrow on successive visits, I calculated the net displacement between the two burrows as a straight-line distance and classed this as a movement. Movement distances were calculated using the Geospatial Modelling Environment (Beyer 2012; R Core Team 2016). I generated kernel density maps from these movements and other spatial figures in ArcGIS 10.2.2. I used chi-square tests to look for differences in the proportion of movements (moved vs. not moved) and movement distance (long > 50 m vs. short < 50 m) across months. Means are presented ± standard error.

7.2.5 Genetic analysis

I collected fresh scats from outside burrow entrances, with the aim of identifying the individuals occupying those burrows using molecular genetic analysis. I collected 113 scats opportunistically throughout the four survey years, sampling from scat piles outside of 31 burrows, and with 3–8 scats from each scat pile.

Scats were completely desiccated to preserve DNA following the two-step protocol for scat preservation in Nsubuga *et al.* (2004). Scats were placed individually in cotton cloth 'tea' bags, saturated with 95% Ethanol (through the bag), and then stored on a bed of silica beads in an air-tight container. Silica beads were monitored for moisture by checking the colour indicator dye, and replaced as needed. Lizard DNA was extracted from the scats by A. Johnston at Flinders University, Adelaide, using the method described in Pearson *et al.* (2015).

Individuals were genotyped by T. H. Ansari at Flinders University, Adelaide, for 14 *L. slateri* specific microsatellite loci following Gardner *et al.* (in prep., see Appendix 2). The resulting genotypes were checked against each other in GENALEX v. 6.5 (Peakall & Smouse 2006, 2012) to identify genotypes from different scats of the same individual. When alleles at all loci were identical or only mismatched at a single locus, they were considered to be the same individual, and a consensus of the individual's genotype was obtained to include in the final data set. These genotypes were used as input to COANCESTRY v1.0.1.5 (Wang 2011). Pairwise relatedness was estimated between individuals using the QuellerGt estimator (Queller & Goodnight 1989), rather than the Wang estimator, as QuellerGt estimates were closest to the simulated true values in COANCESTRY.

Spatial autocorrelation analyses were run by M. G. Gardner at Flinders University, Adelaide, using GENALEX, to determine if the related individuals in the population were spatially clustered. Here the input was individual pairwise shared allele distances and geographic distances calculated from the burrow location XY information. Analyses were conducted at two distance intervals, five and 20 m, each for five distance classes, to explore the spatial auto correlation at a fine and larger population scale. These distances relate to the distance between burrows and encompass the spatial distance across neighbourhoods (see below). For each of these analyses, values were permuted (10,000 times) to test for significance (heterogeneity test); confidence intervals for the correlograms were generated by bootstrapping 9999 times.

7.3 Results

7.3.1 Population survey

Across four survey years, I collected 796 photographic captures including 731 recaptures of 65 individuals. I detected a total of 20–23 adult and sub-adult individuals in the population

Table 7.2. Number of different individuals observed in the population each survey year. Numbers in brackets are the numbers of non-neonates new for that survey year. Note: some of the 10 new individuals in 2014–2015 are likely to have been born in the population the previous year (data from survey year 2013-2014 were not complete because of insufficient sampling). *Data from A. Fenner (pers. comm.). **Neonates from two breeding events in November–December 2014 (n=10) and March 2015 (n=5).

Survey year	Non-neonate	Neonate	Total
2010–2011	13	3	16*
2011–2012	20	3	23 (11)
2012–2013	23	4	27 (4)
2013–2014	6	-	6 (0)
2014–2015	21	15**	36 (10)

in each of the three comparable survey years (data from survey year 2013-2014 were not complete because of insufficient sampling) (Table 7.2). In the two consecutive survey years that could be compared for survey effort, four ‘new’ adults identified in 2012-2013 had not been previously detected in 2011–2012. Two of these adults were first observed in October 2012 and then remained in the population through to the end of the study in March 2015. The other two were each observed once, one in January 2013 and the other in March 2013 and then not seen again for the remainder of the study (Figure 7.2). These four new adults may either have been previous neonates, now reaching maturity, or immigrants from another population, although, as previously mentioned, intensive searching had failed to locate any potential source populations in suitable habitats within 20 km of the site.

Three adults were resident in the population throughout the four-year study period. One was photographed in November 2010 before the study started (A. Fenner; pers. comm.), and was last observed in September 2014, a residence duration of at least 47 months. Twenty two individuals were observed in two or more survey years. However, with insufficient sampling in the 2013–2014 survey year, I cannot be certain how many of the new individuals, first detected in 2014–2015, had been present, perhaps as neonates, in the previous year. Of the neonates, three individuals detected in the first two survey years were probably detected again in the following survey year as sub-adults. One of them was first detected as a neonate in December 2012, and remained in the core area through to the end of the study (Figure 7.2).

Neonates were usually first seen at burrow entrances, often with an adult that I assumed was their mother, in November–December, and were detected in every survey year, except in 2013–2014 when there was insufficient sampling during the November–December period. I also observed second litters of neonates from burrows in three neighbourhoods (see below) in the 2014–2015 survey year, first appearing in March 2015, following the large rainfall event in January 2015. Ten neonates were detected in November–December 2014 and five neonates in March 2015.

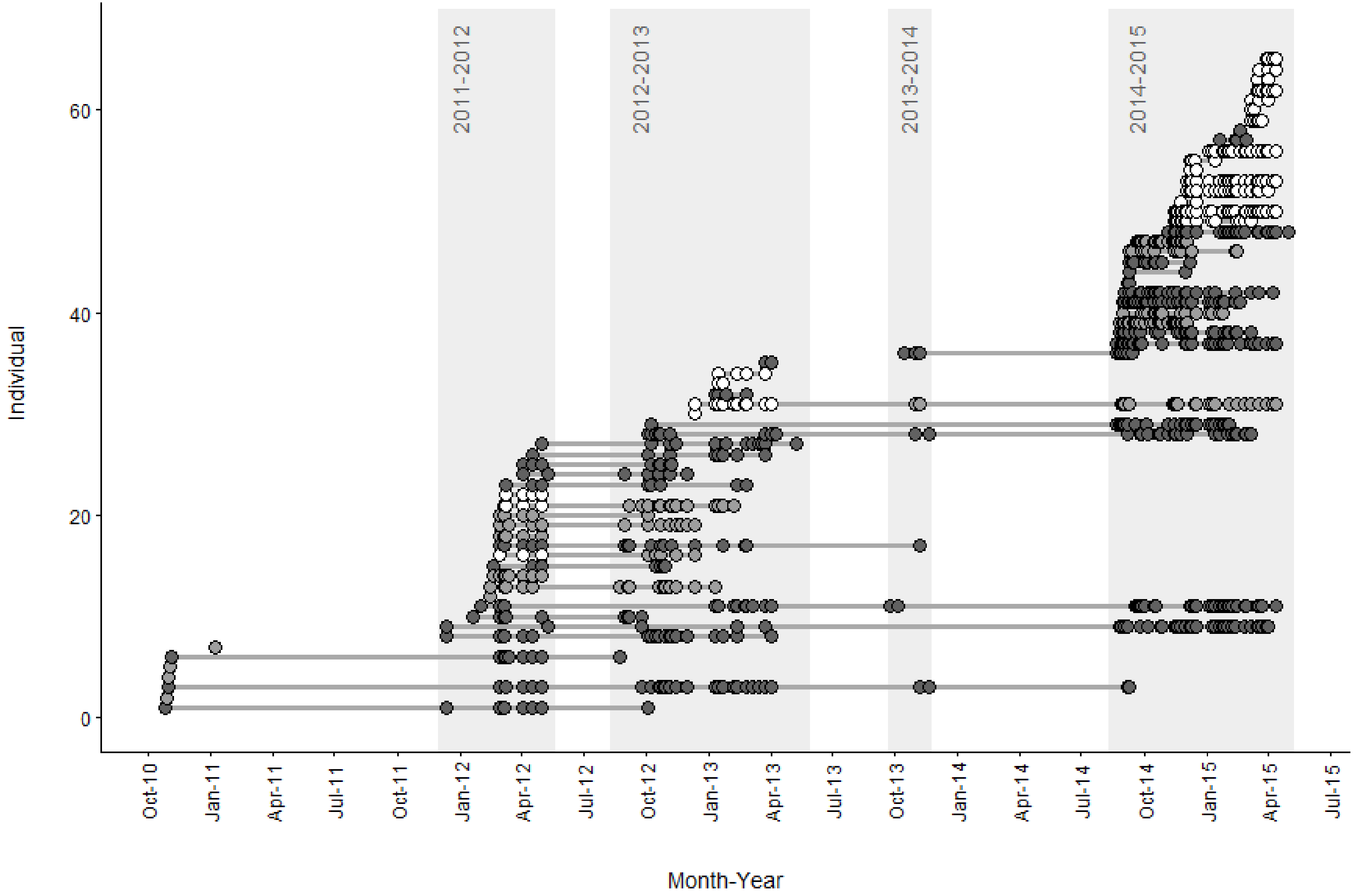


Figure 7.2. Residency time of 65 individuals recorded within the population over four survey years. Adults (dark grey), sub-adults (light grey) and neonates (white). Seven individuals were photographed in the 2010-2011 field season before the start of the study period.

7.3.2 Burrow status

Over the study period, I detected a total of 104 burrows at the site that were occupied in at least one of the survey years, with 52 to 62 burrows (mean 58 ± 3.1 SE) active on at least one survey month in each survey year (Table 7.3). Among these, 28 burrows were constructed during the study period and only ever used for periods of two months or fewer. Without regular burrow maintenance, burrows could change from active to derelict in a period of about 3–5 months. I also observed derelict burrows become active. About 20% of the burrows were used for three or more survey years, but discontinuously, with alternating phases of use and dis-use over periods of a few months. Fourteen burrows were used for some periods in each of the four survey years.

After the 186 mm rain event in January 2015, I observed sediment and debris accumulation in open areas at the site from sheet flow across the surface. However, I saw no evidence of burrow entrances destroyed or damaged by rainfall or surface flows during that period, or at any other time in the study.

Skinks were often detected in multiple burrows, asynchronously. Some individuals tended to stay for long periods of several weeks at one burrow and then they switched to another, while others alternated back and forth between neighbouring burrows. Up to five different individuals (including neonates) used the same burrow over the course of a survey year but were usually sighted at that burrow at different times. I observed 19 instances of burrow

Table 7.3. Frequency distribution of the 104 active burrows detected during the survey period (2011–2015). 2010–2011 data are from A. Fenner, with no formal survey.

Survey year	No. of surveys	No. of active burrows	No. new burrows	Proportion reused
2010–2011		16	16	
2011–2012	5	52	42	19%
2012–2013	9	62	18	71%
2013–2014	2	32	1	97%
2014–2015	7	60	33	45%

co-occupancy, where two individuals were sighted together at the same burrow on the same survey day. Some cases involved the same two lizards observed together more than once. There were 13 different pairs of lizards, five adult pairs, eight adult-neonate combinations, and one case of two neonates. The five adult pairs were observed co-occupying burrows at different times of the season. One pair was detected in early September, and was subsequently observed mating. The other four adult pairs were observed in late September, November, December, and in April–May. I observed adult-neonate and neonate co-occupancy from November to March.

7.3.3 Spatial dynamics

Of the 731 photographic recaptures, 61% of recaptures (n=449) were of an individual at the same burrow as the previous capture. I recorded 283 cases of lizards moving between burrows. The proportion of recaptures in which a skink had moved or not moved differed across months of the activity season (pooled across survey years into months September–April) ($\chi^2 = 16.1$, df = 7, $p < 0.05$). Lizards were more likely to have moved early in the spring (Sept–Dec: 0.42 ± 0.03 SE) than later in the summer (Jan–Apr: 0.34 ± 0.03 SE).

The distribution of movement distances between sequential visits was unimodal and skewed to the right, with a median distance of 13.1 m (Figure 7.3). I selected a 50 m threshold to define short distance movements which separated 92.5% of the observations from the skewed tail. I defined the remaining 7.5% of recorded moves as long distance movements. The proportion of long (>50 m) vs short movements (<50 m) did not differ among months ($\chi^2 = 5.34$, d.f. = 9, $p = 0.80$).

I found distinct geographic and temporal clustering of skink activity within the population. I generated a density map of the short distance movements, using the kernel line density function (cell size 0.5m^2, search radius 9.3m^2) to delineate eight discrete areas of high-use (A–H) that I termed ‘neighbourhoods’ (Figure 7.4). While individuals often moved short distances between burrows, my observations showed they usually occupied burrows in only one neighbourhood for most of a survey year. Thus I assigned individuals to a neighbourhood per survey year based on the greatest number of observations, and used this to estimate neighbourhood abundance (Table 7.4).

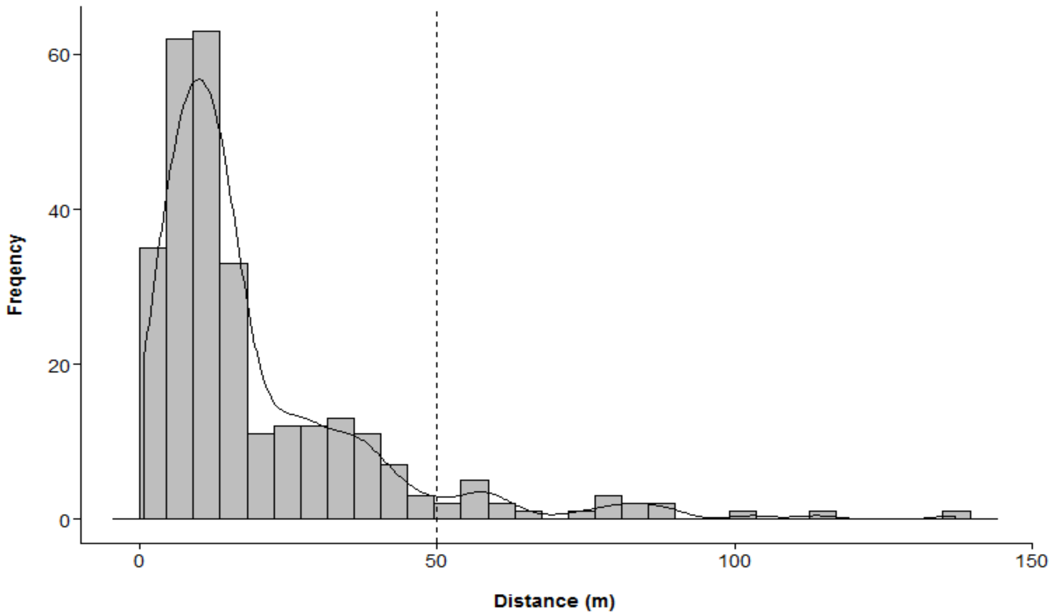


Figure 7.3. Distribution of movement distances of Slater's skink (n=732), calculated as net displacement between capture locations on sequential visits. The histogram is overlaid with a density curve (solid line) scaled to bin width (4.5 m). A threshold of 50 m (dashed line) was used to distinguish short distance and long distance movements.

Table 7.4. Number of individuals detected in the population each survey year. Estimate for the 2013–2014 survey year not included because of insufficient sampling.

Neighbourhood		2011–2012	2012–2013	2014–2015
A	Adults	2	3	
	Sub-adults			
	Neonates		1	
B	Adults			2
	Sub-adults	1		1
	Neonates			2
C	Adults	5	5	4
	Sub-adults	2	2	
	Neonates	2		3
D	Adults	3	4	
	Sub-adults	3	2	
	Neonates		1	
E	Adults	1		1
	Sub-adults		1	
	Neonates	1		
F	Adults	3	5	2
	Sub-adults			1
	Neonates		2	
G	Adults			3
	Sub-adults			1
	Neonates			5
H	Adults			2
	Sub-adults			
	Neonates			5
Outliers			1	4
Total		23	27	36

Short distance movements within the eight neighbourhoods ranged from a density level of 0.38 lines (movements) per 0.5 m^2 up to 2.48 lines per 0.5 m^2 (Figure 7.4). Five of the neighbourhoods (B–F) were more closely grouped (within 110 m of each other), and three of these (B–D) were adjacent. Neighbourhood A was located 40 m to the west of neighbourhood B across the dry bed of a creek tributary, and neighbourhoods G and H were 48 m and 172 m to the north east of neighbourhood F. Neighbourhoods ranged in area from 270 m^2 (E) to 970 m^2 (C), and in elevation from 623.00 m ASL (burrows in the south of neighbourhood G) to 625.22 m ASL (burrows in the north of neighbourhood C).

Maps of burrow occupancy and movement over consecutive survey years showed significant shifts in neighbourhood use over time (Figure 7.5). In the 2011–2012 survey year, neighbourhoods C and D formed the core area of the population, used by about 65% of detected individuals (Table 7.4). The following year (2012–2013) neighbourhoods A and F were also used. In the 2014–2015 survey year, neighbourhoods B, C, G and H were the main centres of activity, used by about 77% of the population, and neighbourhood A was unused.

Three outlier burrows, outside of the neighbourhood structure, and occupied by previously unknown individuals (outliers in Table 7.4) were also detected in the 2014–2015 survey year. Two were located 30 m and 50 m to the east of neighbourhood H and were occupied by a sub-adult and adult respectively (see Figure 7.5e). The third burrow was located 462 m to the northeast of neighbourhood H and was occupied by the same single adult individual for all of the 2014–2015 survey year.

Individuals used 1–12 (mean 2.4 ± 0.2 SE) burrows per season. Some longer term residents moved between neighbourhoods within and between survey years (see examples Appendix Figure 3).

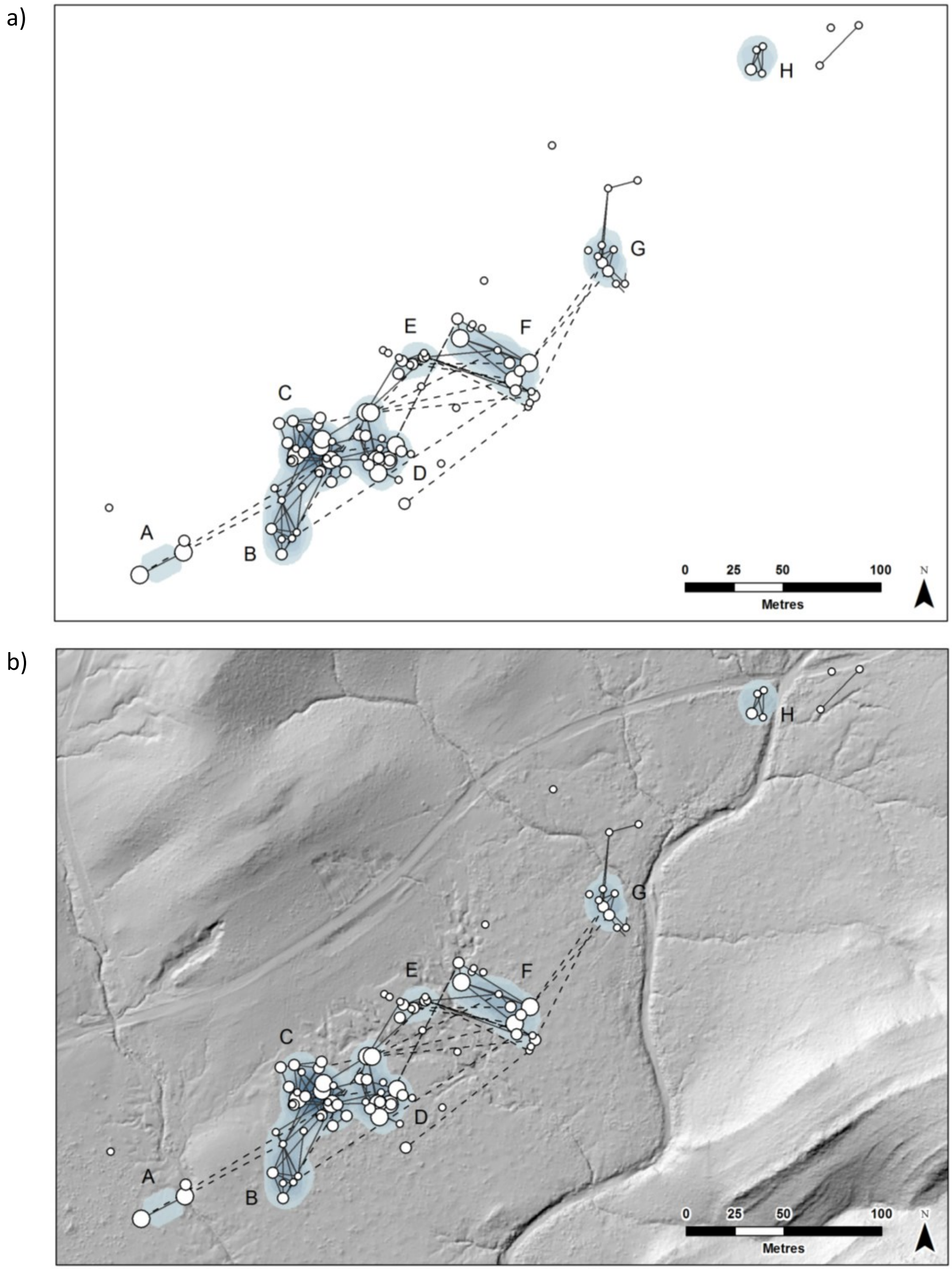


Figure 7.4. Neighbourhoods, burrows and movements within a population of Slater’s skink over four survey years (2011–2015). Lines are movement distances less than 50 m (solid lines) and greater than 50 m (dashed lines). Graduated circles are burrows of varying activity: 12–19 months active (large circles), 6–11 months active (mid-size), and 1–5 months active (small). Polygons are areas of frequent movements between burrows (<50 m) and delineate areas of high use (neighbourhoods A through H) with movement distances of 0.38 lines/0.5m^2 (lightest shading) to 2.48 lines/0.5m^2 (darkest shading). The pattern is shown with (a) no background and (b) with a digital terrain model (25 cm ground sample distance).

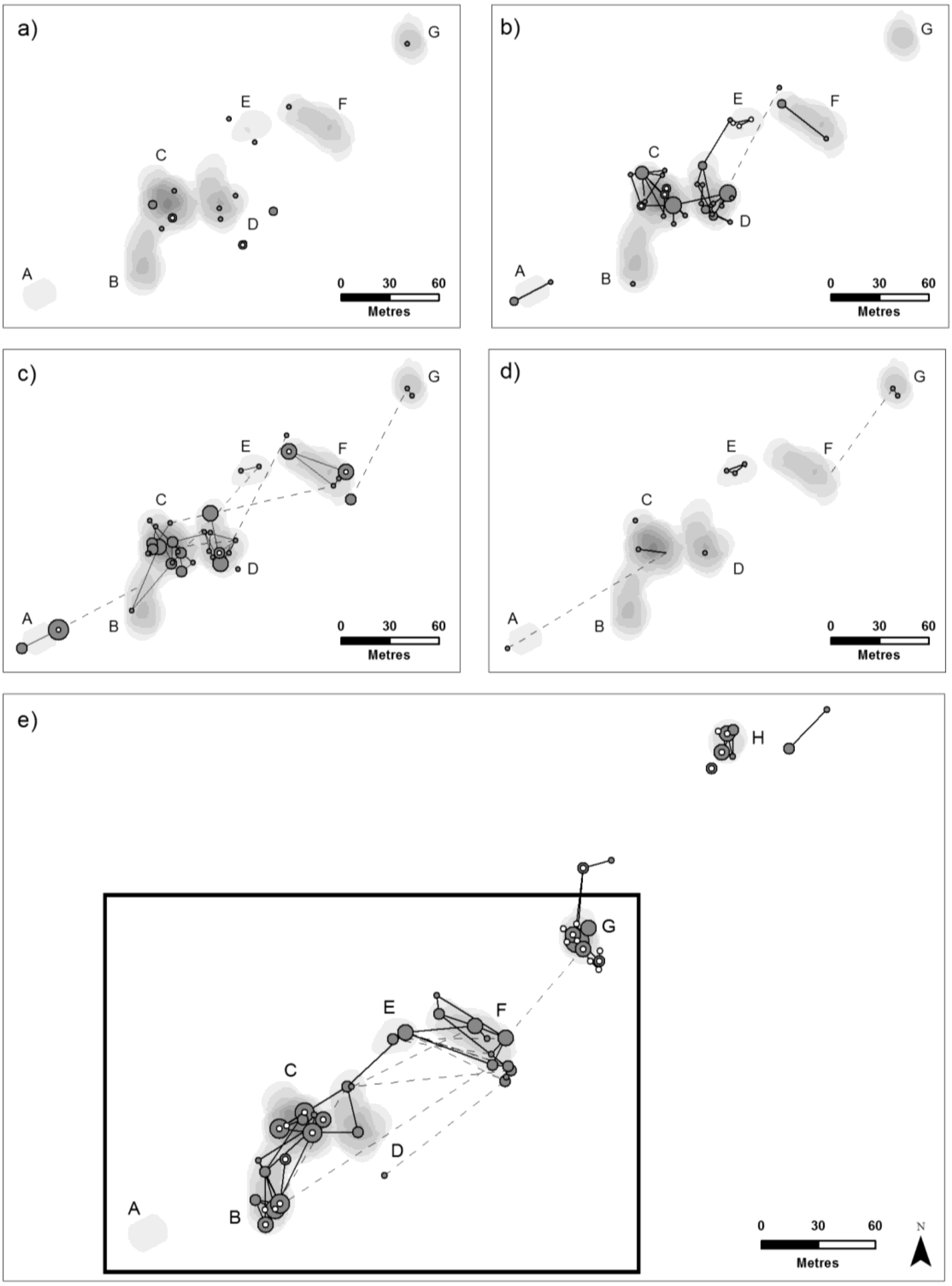


Figure 7.5. Spatial dynamics in a population of Slater's skink over consecutive survey years: (a) 2010–2011 (A. Fenner pers. comm.), (b) 2011–2012, (c) 2012–2013, (d) 2013–2014, and (e) 2014–2015 showing short distance movements < 50m (solid lines) and long distance movements >50m (dashed lines) between burrows and neighbourhoods. Graduated circles indicate number of skinks using a burrow for the survey year: small (1 individual) to large (5 individuals); white circles represent locations of neonates. Two occupied outlier burrows are not shown: 27 m to the northwest of neighbourhood A (for a short duration in 2012–2013), and 462 m to the northeast of neighbourhood H, occupied by the same individual for all of the 2014–2015 survey year.

7.3.4 Genetic analysis

Samples from 76 scats were successfully genotyped for up to 14 loci and 28 unique individuals were identified. In all instances, scats identified from the same individual were found at the same burrow.

Twenty-five pairwise combinations of 17 individuals that were genotyped from their scats were identified as possible siblings or as parent offspring combinations, using the QuellerGt estimator of greater than 0.35 (Figure 7.6). These highly related combinations were dispersed across burrows and across neighbourhoods (Figure 7.6). In four cases, two different individuals were identified from scats at the same burrow. Two of these four cases were of scats from individuals that were highly related (QuellerGt r = 0.58, 0.47) and two were not (both QuellerGt r = -0.05). Scat collection at one burrow in the latter group coincided with the co-occupancy sighting of the adult pair observed in April–May, reported above.

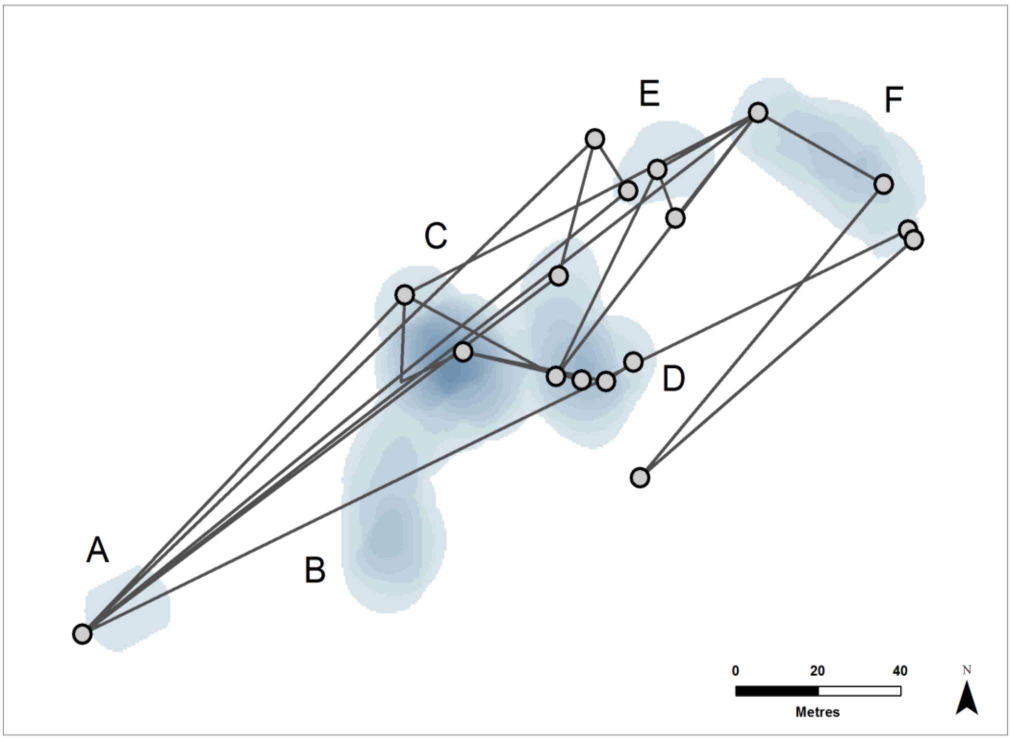


Figure 7.6. Paired individuals that are potentially full siblings or parent offspring combinations (n=25 paired comparisons among 17 individuals) based on a QuellerGt relatedness estimate of greater than 0.35. Straight lines connect geographic locations of paired individuals.

7.3.5 Spatial autocorrelation of genotype data

Individuals within the population were not significantly spatially structured at either the 5 m or the 20 m scale (Figure 7.7 a, b) (5 m intervals: Omega = 20.34, p = 0.028; 20m intervals: Omega = 14.93, p = 0.127). The analysis followed Banks & Peakall (2012) where a value of $p < 0.01$ is required for significance of the Heterogeneity Test.

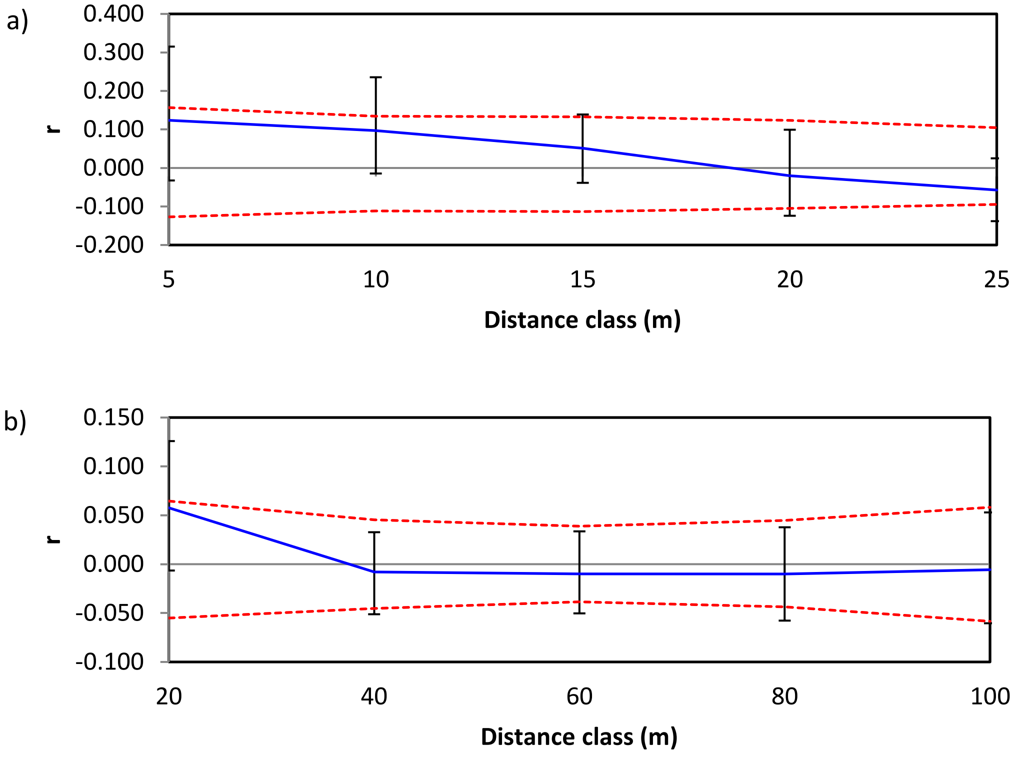


Figure 7.7. Correlograms of spatial autocorrelation analyses at (a) five m and (b) 20 m distance classes (y axis) from pairwise individuals shared allele distance (x axis).

7.4 Discussion

In this study, I sought to understand how a lizard species that is a floodplain specialist persists in a resource rich, but high risk, habitat. I predicted that populations of a species adapted to this unique habitat might combine high site fidelity and relatively low fluctuations in population size among years. I also predicted a relatively mobile strategy, with individuals exploring alternative burrows within the population site, to allow them to rapidly adjust to any flooding-induced local burrow destructions.

7.4.1 Population size

Over the study period, I found a stable population of 20–23 adults and sub-adults, with reproductive recruitment observed in each completely observed season. There were no dramatic fluctuations in abundance during the study period. However, observations of two litters from some burrows in the 2014–2015 survey year suggests an ability to capitalise on longer periods of resource availability, but resume a steady reproductive output in low rainfall years. Interestingly, the second breeding event was almost synchronous with a captive population of *Liopholis slateri* at the Alice Springs Desert Park (P. Nunn pers. comm.). While members of the *Egernia* group generally produce one litter each season (Chapple 2003), the desert dwelling *Liopholis inornata* has also been reported producing two litters in one season (Pianka & Giles 1982). This may be an adaptation of desert *Liopholis* to respond to resource pulses in arid environments, which has been retained in *L. slateri* in its river floodplain habitat. In contrast, lizards of the genus *Ctenotus*, another scincid lineage with a large radiation into arid habitats, have an opportunistic strategy of irregular reproduction in a resource poor spinifex habitat, with no young produced in years of uncertain resource supply, but a large reproductive effort in high rainfall years (James 1991).

7.4.2 Site stability

I observed that the *L. slateri* population occupied the site for the duration of the four-year study. Site fidelity was suggested by both long-term residence of some individuals and by long-term use of some burrows at the site. This in turn suggested that the resource supply in the river floodplain habitat was sufficient and stable over time, and there was no need to move from the site. There are few data on the spatial stability of other desert lizard populations to compare these findings. Family groups of *Liopholis kintorei* have been found to occupy the same burrow for up to seven years (McAlpin *et al.* 2011).

7.4.3 Spatial dynamics and dispersal

The data showed a highly spatially dynamic and mobile population at the site with frequent movements between burrows and neighbourhoods. The genetic data showed that highly related individuals were often spaced apart within the population site, and spatial autocorrelation analyses of scat genotype data were also consistent with high levels of movement. I observed individuals moving up to 120 m within the population between survey visits. Similar observations of mobility were reported in populations of the closely related *Liopholis inornata* in the Middleback Ranges, South Australia, where individuals regularly moved among multiple burrows during an activity season (Daniel 1998; Bourne 2011). While some *L. inornata* co-occupied burrows, most burrow sharing was asynchronous (Bourne 2011). Frequent changing between burrows may provide more foraging sites from which to ambush prey, may be driven by social interactions between conspecifics, may reduce risk from predators, or may be a means of reducing parasite load (Fenner *et al.* 2011). In this study of *L. slateri*, I suggest that regular movements from burrow to burrow about the population site might additionally provide the flexibility to respond to substantial flooding of the site. Without growing, the population changed its area of highest density from year to year, so although it was broadly stable, the population made small shifts of the local boundaries. Both individuals, and the population as a whole, had the flexibility to respond to any partial destruction of their population site.

Overall, the data indicated a stable, persistent strategy for *Liopholis slateri*. This strategy is different from those of other arid-adapted fauna. For example, a nomadic strategy used by more mobile species, such as some migratory waterbirds, tracks resources as they change over space (Pedler *et al.* 2014b), and an irruptive strategy, used by some desert rodents, tracks resources in time (Pavey *et al.* 2014).

7.4.4 Management, monitoring and conservation

My method of monitoring individuals in the population by locating burrows and observing the occupants was non-invasive and relatively simple to operate (see Chapter 5), but had limitations. As I was unable to track known individuals that disappeared from the population, I can infer only that individuals either remained in the population but were missed in my searches, dispersed out of the population, or had died, possibly from predation. Every effort was made to search for new burrows, but it is possible that some, and their occupants, went undetected in the heterogeneous floodplain habitat. Although my study did not quantify predation pressure on *Liopholis slateri*, occasional signs of feral house cat (*Felis catus*), dingo (*Canis dingo*) and sand goanna (*Varanus gouldi*) were observed at the site. The detection of occupied outlying burrows (one 462 m to the northeast of the population), suggests that this species may have a high dispersal capability, unrecorded among my records of movements within the population site.

Although the entire floodplain habitat is the result of flooding along the normally dry creek and river beds, flood pulses resulting in inundation of the floodplain are infrequent in central Australia (Pickup 1991). The large rainfall event I observed in 2015 produced local sheet flows that did not destroy any burrows. Although I have not witnessed this phenomenon at the Orange Creek site, a more substantial flood is likely to cause catastrophic damage to *L. slateri* burrows and individuals. Elevated burrow entrances in soil pedestals above the surrounding soil surface may be a strategy to safely position lizards above the level of sheet flow in a less severe flood or large rainfall event. Under an extreme flood scenario, it is possible that enough lizards may survive immersion and get to safety and colonise new areas. Another scincid lizard, the pygmy bluetongue *Tiliqua adelaidensis*, survived periods of burrow flooding while almost completely submerged (Ebrahimi *et al.* 2012).

Neighbourhoods and satellite burrows may be a model of spreading the flood risk. The bronze back (Pygopodidae) *Ophidiocephalus taeniatus* is another lizard species that occupies areas prone to disturbance along minor drainages of river catchments (McDonald *et al.* 2012; Pedler *et al.* 2014a). In that species, it has been proposed that survivors of floods will be found in litter mats positioned higher up in the catchment, and these could be used as a source for re-stocking destroyed populations lower down in the catchments (McDonald *et al.* 2012). In *L. slateri* populations, dispersed individuals, or some neighbourhoods, may be the source for the species to recolonise after a catastrophic flood pulse that extirpates some neighbourhoods.

The species’ current distribution is along low order water courses and floodplains of main channels within the MacDonnell Ranges bioregion. In the past, the species has probably been maintained as a series of temporal meta-populations at these sites, with local density foci shifting spatially over time, particularly following large flood events. This process requires that a series of suitable connected sites be available for existing populations to expand into, and to use in dispersal to recolonise previous locations. New threats to the species include anthropogenic changes that are reducing the availability of those sites. This occurs through processes such as the trampling of burrows and alluvial floodplain structures by cattle (Paltridge 2013), and invasion by exotic plants, like buffel grass *Cenchrus ciliaris,* encroaching and making habitats less suitable (Pavey 2004a). Superimposed on these threats are predictions of more frequent and intense rainfall events, increasing the likelihood of disturbance from flooding. Collectively, these changes may result in dispersers failing to establish refuge populations because suitable sites are few and increasingly hard (for the lizards) to locate. This complexity needs to be given consideration when developing regional conservation strategies for this and similar species.

7.5 Summary

In this study, I found a small and highly mobile, but site stable, population, with spatial clustering of burrows into local ‘neighbourhoods’. Are there fine-scale habitat differences between neighbourhoods and adjacent non-occupied areas that are creating these centres of lizard activity? To address this question, in the next chapter, I investigate how the Orange Creek population uses the floodplain environment at the site and burrow scales.

Chapter 8: Fine-scale habitat use

How *Liopholis slateri* populations use floodplains is largely unknown. Arid floodplains are heterogeneous habitats which are highly variable. Therefore, understanding which elements of the floodplain lizards are using, may help identify other available suitable habitat and priority areas for conservation. This knowledge might also be used in correlative models for predicting future species distributions under various climate scenarios. In this study, I investigated the fine-scale habitat use of the Orange Creek study population. This chapter has been adapted from the manuscript: Treilibs, C.E., Nano, C.E.M., Pavey, C.R., & Bull, C.M. (2016). Fine-scale habitat use of a terrestrial desert floodplain specialist. (To be submitted.)

8.1 Introduction

Extant *Liopholis slateri* populations occupy the channels and floodplains of lower order streams and the floodplains away from main channels (see Chapter 3). Past reports of the habitat have described the presence of soil mounding at the base of shrubs at both existing and historic sites (Henzell 1972; Pavey 2004a). Subsequent studies have shown these soil pedestals, or mounded shrubs, to be an important feature for burrow construction (Pavey *et al.* 2010; Fenner *et al.* 2012b). Slater (in Henzell (1972)) observed *L. slateri* burrows under *Hakea divaricata* and *Eremophila sturtii* at the type locality in the 1960s. A habitat study at the Lawrence Gorge population on Owen Springs Reserve reported *L. slateri* burrowing in mounded shrubs of *Eremophila maculata*, *E. sturtii*, *Acacia victoriae* and *Senna artemisioides* (Pavey *et al.* 2010).

Open areas are thought to be a requirement for the ambush foraging behaviour of these lizards since bare ground increases visibility of both prey and predators (McKinney *et al.* 2015). For this reason, buffel grass *Cenchrus ciliaris* has been identified as a potential threat to *L. slateri* (Pavey 2004a). This invasive pasture grass favours alluvial soils, and consequently has spread along water courses and established dense stands along river channels in central Australia (Griffin 1993). While the grass has encroached into areas where *L. slateri* is present, direct impacts of this vegetation change to these skink populations remain unquantified.

The four-year dataset of the spatial and temporal dynamics of the Orange Creek population (see Chapter 7) showed sub-areas (neighbourhoods) within the area of occupancy that had higher use than other areas. Are these neighbourhoods ‘minor habitat centres’ (Elton 1949) which are driving skink occupancy, or are other factors, such as social interaction effects creating these hubs of activity? The spatial dynamics data also showed that burrows varied in length of tenure. Of the 104 burrows that the population used over the course of the study, 22% (n=23/104) were used for periods of less than two months, while 13% (n=14/104) were reused for periods of time in each of the four survey years. Do long-term burrows have different attributes to short-term burrows?

In this study, I investigated how the Orange Creek population of Slater’s skink uses the floodplain at the site and burrow scales. I used aerial surveys, an on-ground description of burrow attributes, and a broader vegetation survey of areas inside and outside of the area of occupancy, to ask questions about the fine-scale use of habitat at the site. Specifically, I asked:

1. Does *L. slateri* occupy a discrete landscape position at the site?
2. How does buffel grass cover vary across the broader landscape and within the site?
3. Is burrow site selection exclusive to shrub-mounds?
4. Do mound physical attributes correlate with *L. slateri* occupancy time?
5. Can neighbourhoods and area of occupancy habitats be distinguished from each other and from neighbouring external habitat according to vegetation type (species assemblages) and/or dominant plant species i.e. are vegetation elements useful indicators of *L. slateri* habitat/occupancy?

8.2 Methods

8.2.1 Study site

The study site was at Orange Creek (23°59’S, 133°37’E), 620 m ASL, and 40 km south west of Alice Springs. At this site, during the study period December 2011 to April 2015, a population of Slater’s skink occupied a 460 x 75 m (23,000 m^2) area of *Eremophila*-dominated shrubland on a red earth alluvial foot slope within the Waterhouse Range (see Chapter 4 for detailed site description). In this habitat study, I used a combination of survey methods to answer my research questions. Each of the three components, aerial surveys, burrow attribute surveys, and on-ground vegetation surveys, are described below.

8.2.2 Aerial surveys

I acquired high resolution aerial imagery over the study site to describe its topographic position in the broader floodplain landscape and assess fine-scale spatial variation within the area of occupancy. Specifically, I used high resolution aerial photography to assess the cover of buffel grass *Cenchrus ciliaris* in the surrounding landscape, and LiDAR (Light Imaging, Detection and Ranging) to investigate the terrain profile across the floodplain and assess whether I could use the imagery to detect shrub mounds. Aerial photography was acquired at a ground sample distance (GSD) of 3–4 cm resolution, flown on 25 May 2013 at 14:45 hrs. A LiDAR (Light Imaging, Detection, and Ranging) sensor was flown over the site on 1st September 2013 to produce a digital terrain model (DTM) at 25 cm GSD, and vertical resolution of about 3 cm (Source: Airborne Research Australia, A. McGrath pers. comm.). A DTM is the land surface with the vegetation digitally removed. I used the profile tool and other classification tools in ArcGIS 10.2.2 for DTM analysis.

I visually inspected terrain profile cross-sections of the DTM to compare how the slope changed across the area of *L. slateri* occupancy, and in adjacent areas of the floodplain where skinks are absent. To assess whether I could distinguish mounds or mound clusters from the surrounding surface, I applied slope and hillshade algorithms to the DTM. Given the vertical resolution of the LiDAR imagery was about 3 cm, it was likely that only mounds higher than 3 cm could be detected. In a previous study (Fenner *et al.* 2012b), shrub mounds used as burrow sites had been measured at mound heights of 4.5–33 cm (mean 14.90 ± 1.10 cm), so I assumed most mounds would be detected on the DTM.

Following Marshall *et al.* (2014), I used visual estimates from the aerial photography, rather than unsupervised classification or other digitising techniques, to estimate buffel grass cover at the site. I used five classes to estimate buffel cover in a 10 x 10 m grid: absent, 0%; low, 0–25 %; moderate–low, 25–55 %; moderate– high, 55–85 %; and high, 85–100% projected cover (Marshall *et al.* 2014). Rainfall in the 14 days prior to acquiring the aerial photography was moderate (43.4 mm), with 15.2 mm falling four days before. This amount of rain resulted in buffel grass tussocks having some new green shoots at their centres, but also residual longer dried leaves and seed heads. Consequently, in the aerial photography captured on 25 May 2013 in natural colour, buffel grass appeared as blue-grey, rather than as the bright green hue that it typically acquires following large rainfall events. Nevertheless, buffel grass was easily distinguished from other vegetation in the image by colour and texture.

8.2.3 Burrow attribute survey

Each month during the four year study period, I recorded the activity (occupancy) status of each burrow (from actively occupied to long-derelict) and recorded any new burrows that had been constructed since the last monthly survey (see Chapter 7 for details). For each of the 104 burrows detected at the site over the four survey years, I documented the habitat type of its position (shrub-mound, log-mound, channel bank, other), and the associated main shrub species or structure. I also estimated each month the cover of buffel grass on the shrub-mound, if applicable, or within a 1 m radius if the burrow was not in a soil mound. I established photo points at each burrow and took photographs each month. A single measure of vegetation cover was derived from these images. These temporal data were not analysed as I did not detect any significant change in buffel grass cover, or other vegetation, at any burrow during the four year study.

To assess whether there were differences in burrow occupancy, I qualitatively compared the habitat type of burrows that were only ever used for two months or fewer during the study period (‘short-term’, n=28) with those that were used for periods in all four survey years (‘long-term’, n=14). As burrows can be abandoned and then re-used later (Chapter 7), I defined a long-use burrow as one in which there was evidence of lizard occupancy on at least one survey in each of the four years.

To test whether burrow occupancy time was correlated with other burrow attributes, I randomly selected 26 burrows in shrub-mounds that lizards had selected to occupy, but that were used in only one survey year (n=5), two survey years (n=5), three survey years (n=6) or four survey years (n=10). Following Fenner *et al.* (2012), for each of the 26 burrows, I measured 15 mound attributes that could potentially influence the burrow’s microclimate and microhabitat (Table 8.1). I checked the data for outliers, homogeneity and collinearity and then used Analysis of Variance (ANOVA) to test whether any combination of these attributes could explain the duration of burrow occupancy (with survey year as a factor), and thus differentiate between more or less suitable burrow sites. All statistical testing was done in R (R Core Team 2016).

Table 8.1. The 15 variables recorded at a sample of burrows of short, mid and long term occupancy (n=26), mean, standard deviation (SD), and range shown for continuous (Con) variables. Cat=categorical variable.

Variable	Units	Type	Mean	SD	Range
Single shrub mound or cluster		Cat			
Dominant shrub species		Cat			
Total ground cover of other species on mound	%	Con	14.52	17.09	0–80
Cover of *C. ciliaris*	%	Con	1.06	2.58	0.00–12.85
Shrub height top	m	Con	1.69	0.50	0.54–2.8
Shrub height bulk	m	Con	1.26	0.39	0.42–1.90
Shrub foliage length	m	Con	2.14	0.90	0.0–4.1
Shrub foliage width	m	Con	1.70	0.90	0.0–3.9
Vertical distance between lowest live foliage and top of mound	m	Con	0.38	0.23	0.0–1.1
Vertical distance between lowest dead foliage and top of mound	m	Con	0.10	0.14	0.00–0.52
Mound height	m	Con	0.20	0.09	0.05–0.40
Mound basal circumference	m	Con	7.75	3.18	1.98–18.10
Distance to nearest mound	m	Con	0.52	0.45	0.0–1.5
Distance to nearest cover (>15cm)	m	Con	0.51	0.42	0.00–1.82
Distance to nearest *C. ciliaris*	m	Con	1.43	1.52	0.0–6.1

8.2.4 Vegetation survey

I recorded information about the vegetation at the site in May–June 2015, at the end of the four year study of the spatial dynamics of the lizard population (see Chapter 7). With this time frame I had the advantage of knowing the history of skink movements at the site and could plan the vegetation sampling design according to skink occupied areas.

To assess fine-scale spatial differences in vegetation at the site scale, I surveyed plant taxa in three habitat zones: neighbourhoods (Ne), outside of neighbourhoods but within the area of population occupancy (Ao), and external to the area of occupancy (Ex). Neighbourhoods (Ne) were identified using a kernel density of observed movements (see Chapter 7); the area of occupancy (Ao) was constructed from a minimum convex polygon that included all burrows occupied at least once; and an external zone (Ex) was created from a 50 m wide buffer outside

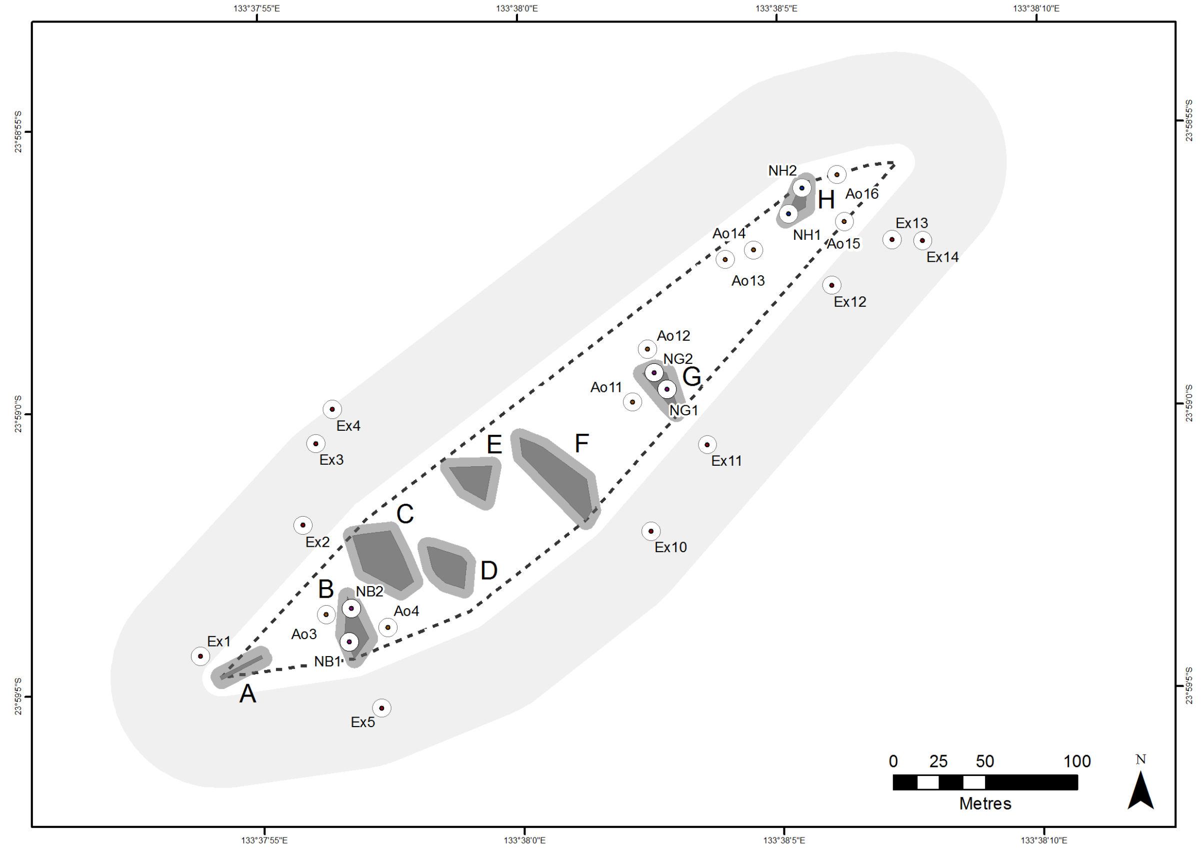


Figure 8.1. Sampling design for the vegetation survey. Sampling zones were: neighbourhoods A-H (dark grey polygons), area of occupancy (dashed line) and area external to area of occupancy (light grey). 10 x 10 m plots were randomly sampled within these zones: neighbourhoods B, G and H (Ne: n=6), area of occupancy (Ao: n=8), and external area (Ex: n=10).

of the area of occupancy (Figure 8.1). Within each zone, I randomly sampled 10 x 10 m circular plots: Ne (n=6), Ao (n=8) and Ex (n=10) (Figure 8.1). Three neighbourhoods (B, G and H) were surveyed based on their geographical separation across the area of occupancy, and I surveyed two replicate plots within each of those neighbourhoods. Neighbourhood B contained occupied mounds throughout the four years, neighbourhood G was occupied for three of the four years, and neighbourhood H only in the last year of the study (see Chapter 7). Ao sites were selected adjacent to the three sampled neighbourhoods, two next to each of neighbourhoods B and G, and four around neighbourhood H. I was particularly interested in why lizards had selected to move into neighbourhood H in year 4, and not into adjacent habitat. I used ArcGIS 10.2.2 for all spatial analyses in the survey design.

Within each circular 10 x 10 m plot, I recorded all plant taxa and estimated projected foliage cover using the classes: 1 (<5% cover, 1–5 individuals), 2 (<5% cover, 6–50 individuals), 3 (<5% cover, >50 individuals), 4 (5–9%), 5 (10–30%), and 6 (>30% cover), following Pavey *et al.* (2012). The scale allowed me to account for plant species that were frequent between sites but had a low overall cover, particularly annual or short-lived species.

Floristic differences among sampling zones were inspected with nonmetric multidimensional scaling ordination (NMDS) using a Bray-Curtis dissimilarity coefficient. I excluded non-representative species that had a cover class of 1 (<5% cover, 1–5 individuals), and ran analyses on all taxa, and then on perennial grasses and shrubs, as those two plant groups were likely to be present for most of the time and thus have more influence on *L. slateri* habitat.

To assess whether Ne habitats could be distinguished from Ao habitats by vegetation assemblage, and if there were distinguishing dominant species representative of each group of habitats, I used a similarity percentage analysis SIMPER (Clarke & Gorley 2006). The SIMPER procedure ranks species by their similarity to others within groups. I ran pair-wise comparisons to identify plant species contributing to 70% of the within-group similarity and dissimilarity for Ne, Ao and Ex habitats.

8.3 Results

8.3.1 Aerial surveys

The 50 cm resolution digital terrain model showed the area of occupancy had a slope of 0–2 % (Figure 8.2b). This was in contrast with the rocky slopes to the north of the zone (5–14% slope). There were no obvious topographical changes in slope or elevation to the south of the area of occupancy. Within the area of occupancy, the DTM showed approximately 150 shrubs were mounded, covering roughly 30% of the occupancy zone. Mounds formed around single shrubs (circular features) and multiple shrubs into mound clusters (larger elliptical features up to 10 m long; Figure 8.2a). Mound clusters were generally oriented cross-slope. Shrub mounds were predominantly situated in the south-west of the area of occupancy, with neighbourhoods C and D encompassing the areas of highest mound density. However, there were areas of shrub mounds outside of neighbourhoods, but still within the area of occupancy, mostly to the south of neighbourhood C. Some DTM derived ‘mounds’ outside of the area of occupancy coincided with the location of visual images of buffel grass, and were assumed to be buffel grass tussocks. These inferences were confirmed by ground trothing, where no soil mounds, or *L. slateri* burrows, were located within the dense cover of buffel grass.

Buffel grass was present in high densities (> 85% projected cover) along Orange Creek to the south and south-east, and also in areas beyond the creek bed to the west of the area of occupancy (Figure 8.3). Within the area of occupancy, buffel grass cover was generally low (0–25%), but there were dense stands of moderate–high cover (55–85%), particularly to the east of the main erosion gully, and along tributaries T1–T3 (refer to Figure 4.2, Chapter 4). Buffel grass was not present on the rocky slopes to the north of the area of occupancy.

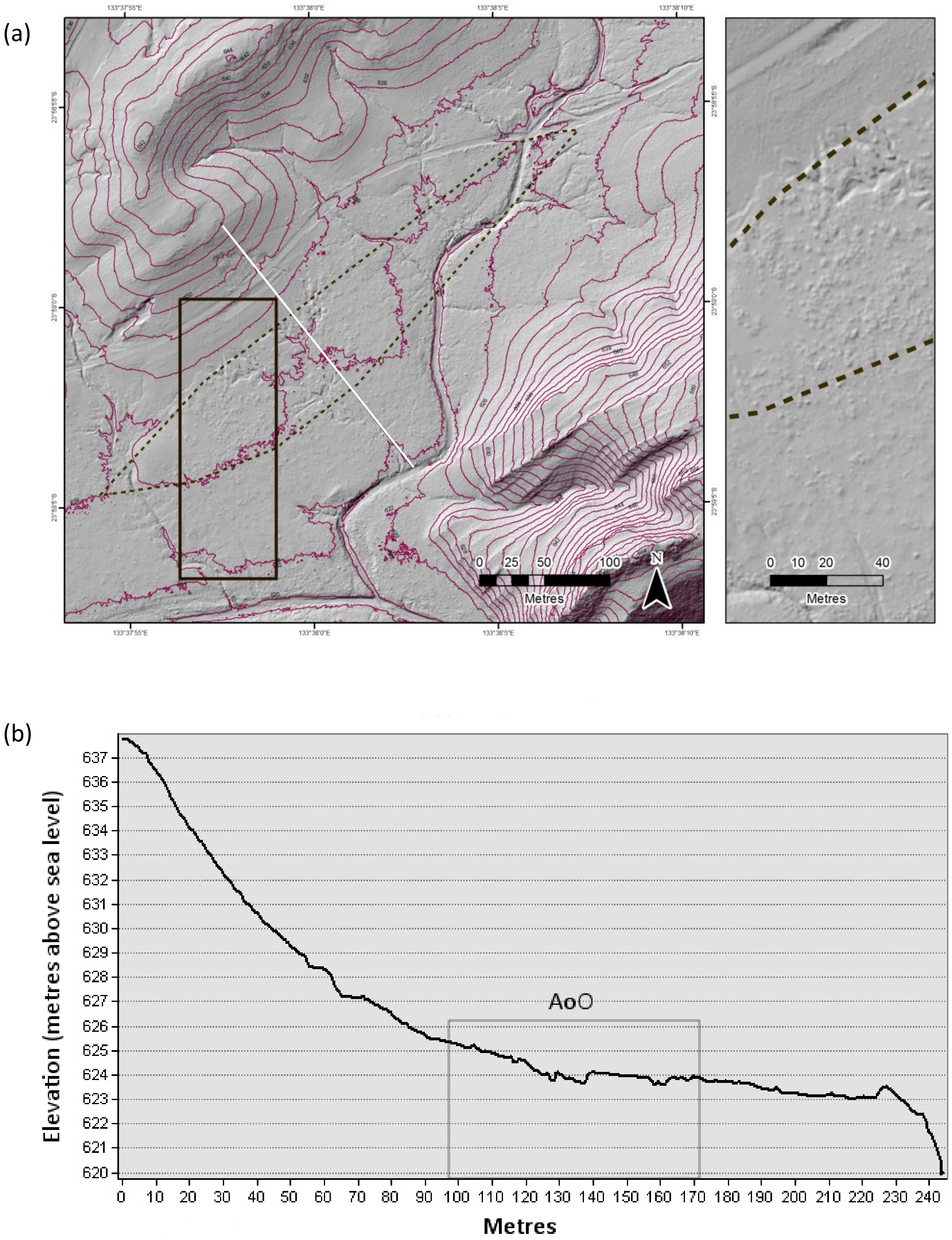


Figure 8.2 (a) Digital terrain model of the Orange Creek study site showing 2 m contours derived from LiDAR imagery (source: ARA March 2013). The area of occupancy is indicated by a dashed black line. Inset (rectangle) shows mounds and mound clusters visible within the occupancy zone. White line represents sample cross section (left to right) used for (b) terrain profile. *L. slateri* area of occupancy (AoO) occurs along the profile 97–171 m.

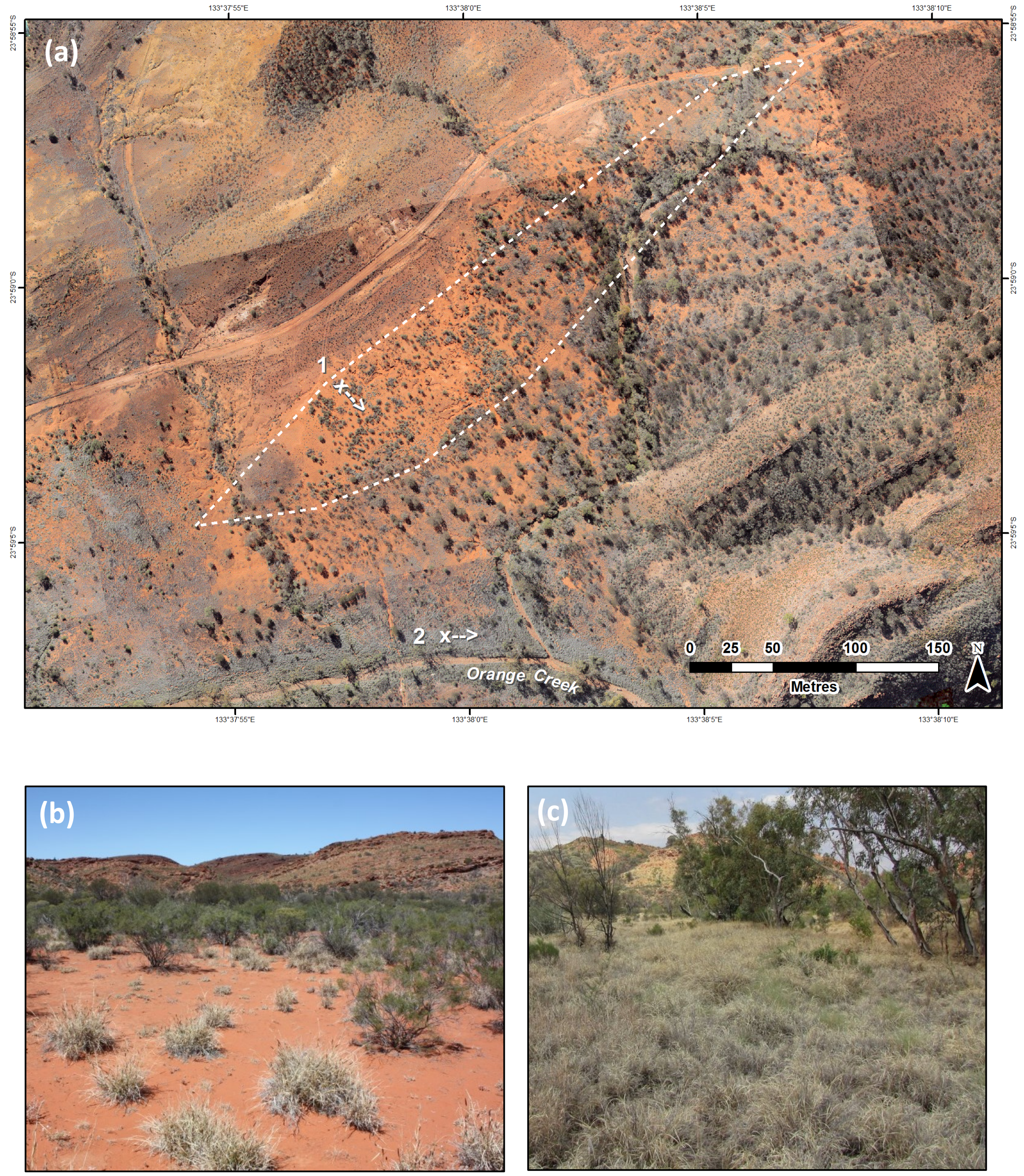


Figure 8.3. (a) Aerial image of the Orange Creek study site showing buffel grass *C. ciliaris* cover (blue-grey) in the broader landscape around the area of occupancy (white dashed line). Photopoints at locations (x) and direction of photo (indicated with arrow) for on-ground examples of (b) low 0–25% cover (photopoint 1), and (c) high > 85% cover (photopoint 2).

8.3.2 Burrow attribute survey

Of the 104 burrows, 85% (n=88/104) were located in shrub-mounds, 7% in mounds under logs, 6% in channel banks (though still beneath shrubs), one was under a rock, and two were under buffel grass tussocks. Of the shrub mounds, 78% (n=69/88) of burrows were in mounds around the base of live *Eremophila sturtii,* 11% in mounds around live *Senna artemisioides* subsp. *filifolia*, and 7% in mounds around dead shrubs, both standing and collapsed. In 26% of cases (n=23/88), burrows were located under grasses (*Enteropogon acicularis, Eragrostis eriopoda*) or sub-shrubs (e.g. *Enchylaena tomentosa, Sclerolaena sp.*) that had established on the pedestal but away from the trunk of the main shrub around which the soil had formed.

Of the 14 long-term burrows (used at least once in each of the four survey years), 12 were located in shrub mounds, and two burrows were at the base of deep drainage tributary banks (approximately 30 cm high). Of the 23 short-term burrows (only ever used for fewer than two months), 18 were in shrub mounds, and under other structures (log mounds, a large rock, shallower bank ledges and buffel grass tussocks).

In most cases, buffel grass cover on occupied burrow mounds was low, with 90% of those mounds having less than or equal to 10% cover (Figure 8.4). Over all measured burrows, buffel grass cover ranged from 0–30% cover (mean 3.3% ± 0.6%). Two burrows that were directly under buffel grass tussocks had 50% and 60% cover of buffel grass within a 1 m radius of the burrow, and both burrows were only occupied for about three months.

The sample of 26 occupied shrub mounds ranged in size from 1.98–18.10 m in circumference, and had formed around shrubs with heights ranging from 0.54–2.8 m (Table 8.1). The nearest buffel grass tussock was never more than 6.1 m away from the edge of a mound. ANOVA analyses showed that none of the 15 measured mound attributes differed significantly with occupancy duration.

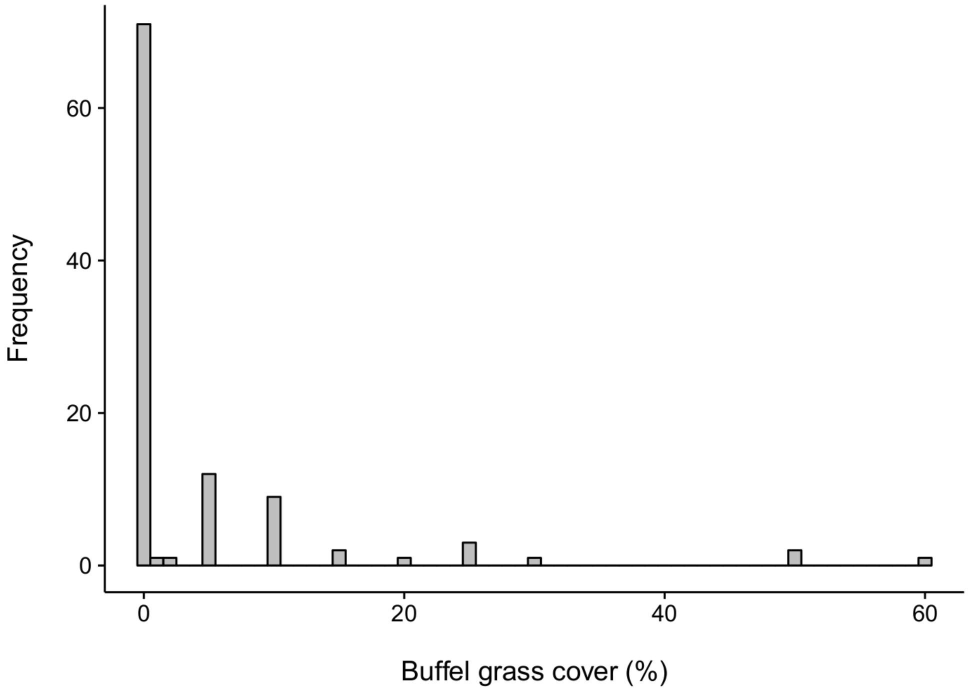


Figure 8.4. Percentage cover of buffel grass *Cenchrus ciliaris* around occupied *Lioholis slateri* burrows (n=104). For burrows located in shrub-mounds, buffel grass cover was estimated as percentage cover of the mound, otherwise within a 1 m radius of the burrow.

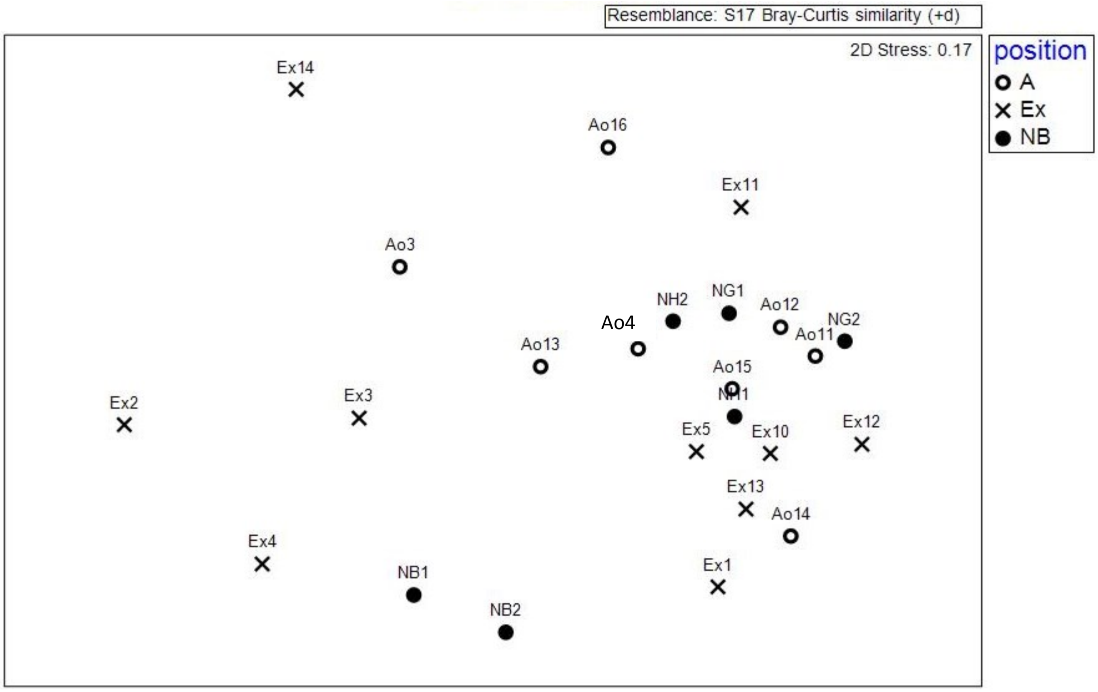
8.3.3 Vegetation survey

Analysis of floristics at the site scale showed considerable variation within both the external (Ex) and neighbourhood (Ne) habitats, and between area of occupancy (Ao) and Ne habitats (Figure 8.4). When considering shrubs and perennial grasses, most Ex plots were well outside the ranges of the Ne plots, and the two neighbourhood B (NB) plots were clearly distinct (Figure 8.4b). Further inspection of vegetation associations showed distinct differences between neighbourhood B (NB) and a cluster of plots from neighbourhoods G (NG) and H (NH), and Ao habitats (Figure 8.4). That cluster was characterised by consistent presence of *Acacia aneura*. Consequently, I ran the SIMPER analysis on neighbourhood NB separately from neighbourhoods NG and NH (hereafter NGH).

External (Ex) sites had lowest within-group similarity because of different vegetation types upslope and downslope from the area of occupancy (Table 8.2). Buffel grass *Cenchrus ciliaris* was not a defining characteristic of areas outside of the area of occupancy. In fact it was less prevalent 50m outside (Ex habitats) with an average cover class of 1.8, than inside the area of occupancy (Ao habitats). However, within the area of occupancy, lizards seemed to avoid buffel grass concentrations as *C. ciliaris* characterised Ao habitat; it was the first-order diagnostic species (average cover class 3.25). Compared with Ao sites, *C. ciliaris* was less dominant in Ne neighbourhood habitats, and was a higher-order contributor to within-group similarity of NGH more than NB (average cover class was 3 compared with 1.50).

Area of occupancy (Ao) and neighbourhood NGH habitats were most similar, and external (Ex) habitats and neighbourhood NB habitats least similar (Table 8.3). The older neighbourhood NB was distinctly different from its adjacent sites Ao3 and Ao4, whereas more recently occupied neighbourhoods NG and NH were more similar to their adjacent plots Ao11–Ao16 (Figure 8.4). Though both rare, *Eremophila sturtii* and *Hakea leucoptera* subsp. *leucoptera* were highly predictive of NB habitat only.

(a)



(b)

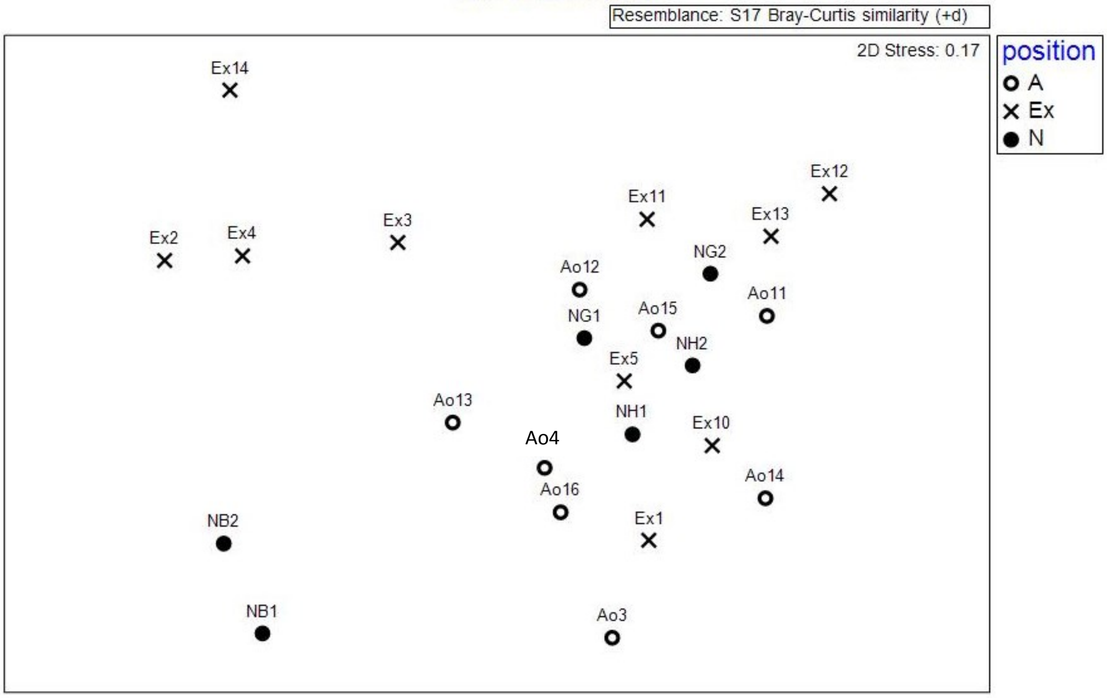


Figure 8.5. NMDS ordination of floristics within each of the habitat zones: area of occupancy (Ao), neighbourhoods (Ne), and external area (Ex), for (a) all plant species, and (b) perennial grasses and shrubs.

Table 8.2. Diagnostic species for the four habitat types that were monitored for *L. slateri*, and species contributing up to 70% of the average Bray-Curtis within-habitat similarity index (defined by SIMPER analysis). Numbers in bold indicate diagnostic species (perennial grasses and shrubs). Species with a cover class of <1 were excluded.

Taxon	NB	NGH	Ao	Ex
Acacia aneura	0	**1.32**	**0.59**	**0.68**
Brachyachne ciliaris	0.48	0	0	0
Cenchrus ciliaris	**1.47**	**1.14**	**1.41**	**0.45**
Enchylaena tomentosa	0	0	1.58	0
Enneapogon polyphyllus	0	0	0	0.38
Enteropogon acicularis	**0.26**	0	0	**0.51**
Eragrostis eriopoda	0	**10.12**	0	**0.77**
Eremophila sturtii	**0.6**	0	0	0
Eriachne helmsii	0	0	**0.91**	0
Fimbristylis dichotoma	0	2.5	0	0.69
Ptilotus gaudichaudii subsp_ parviflorus	0	0	0	0.26
Sclerolaena diacantha	0	0	0	0.46
Senna artemisioides subsp_ filifolia	0	**3.33**	**0.92**	0
Sida platycalyx	0	3.22	1.42	0.52
Tripogon loliiformis	0.78	0	0	0.67

Table 8.3. Diagnostic species for the four habitat types that were monitored for *L. slateri*, and species contributing up to 70% of the average Bray-Curtis between-habitat dissimilarity index (defined by SIMPER analysis). Numbers in bold indicate diagnostic species (perennial grasses and shrubs). Species with a cover class of <1 were excluded.

Taxon	Ao & Ex	Ao & NB	Ex & NB	Ao & NGH	Ex & NGH	NB & NGH
Acacia aneura	**1.12**	**0.99**	**1.06**	**1.16**	**1.11**	**1.39**
Aristida holathera var_ holathera	0	0	0	1.22	1.68	0
Brachyachne ciliaris	0.73	0	3.43	0.78	0.72	0
Cenchrus ciliaris	**1.23**	**1.02**	**1.9**	**1.24**	**1.29**	**1.15**
Dissocarpus paradoxus	0.44	0	0	0.37	0	0
Enchylaena tomentosa	1.27	1.06	1.38	2.04	1.1	1.6
Enneapogon polyphyllus	0.94	1.06	1	0.58	0.79	0.93
Enteropogon acicularis	1.13	3.19	2.86	0	0.96	4.82
Eragrostis eriopoda	1.18	0.76	1.14	1.19	1.19	1.77
Eremophila duttonii	**0.56**	0	0	0	**0.59**	0
Eremophila freelingii	**0.97**	0	**0.71**	0	**0.96**	0
Eremophila latrobei subsp_ glabra	**1.03**	0	0	**1.46**	**1.38**	0
Eremophila sturtii	0	**5.46**	**6.16**	0	0	**8.9**
Eriachne helmsii	**1.18**	**1.24**	0	**1.21**	**0.71**	0
Fimbristylis dichotoma	1.12	1.22	1.16	2.03	1.58	0
Grevillea striata	0	0	0	0	0.87	0
Hakea leucoptera subsp_ leucoptera	0	**0.95**	**0.96**	0	0	**0.93**
Lepidium muelleriferdinandi	0.7	0	0	0	0	0
Monachather paradoxus	0	0	0	1.05	0	0
Ptilotus aervoides	0	0	0	0.56	0	0
Ptilotus gaudichaudii subsp_ parviflorus	0.63	0	0.63	0	0.64	0
Ptilotus polystachyus	0.97	0	0	1.06	0	0
Sclerolaena convexula	1.05	0	0	1.13	1.07	0
Sclerolaena cuneata	0.69	0	0	0	0	0
Sclerolaena diacantha	0.9	1.08	1.07	0	0.82	0.93
Sclerolaena lanicuspis	0	0	2.39	0	0	2.33
Sclerolaena patenticuspis	0	0.99	0.95	0.92	0.88	1.24
Senna artemisioides subsp_ filifolia	**1.19**	**1.07**	**0.67**	**1.15**	**1.4**	**1.44**
Sida ammophila	0	0	0	0.86	0	0
Sida platycalyx	1.14	2.34	1.88	0.66	1.21	3.11
Sporobolus actinocladus	0.63	0	0	0	0	0
Tripogon loliiformis	1.1	1.93	1.12	1.01	1.26	1.24
Ventilago viminalis	0	0	0	**0.56**	**0.56**	0

8.4 Discussion

Does *L. slateri* occupy a discrete landscape position at the site?

The area of occupancy occupied a distinct topographic position within the landscape. The shallow gradient run-on area has created a depositional area where transported sand particles have slowed and been intercepted by larger structures (i.e. shrubs or logs). The large erosion channels (see Chapter 4) also indicate that water processes have played a prominent role in shaping landform and micro topography of the site. The area downslope from the occupancy zone had a shallower gradient, with little soil mounding, a taller canopy and generally higher ground cover of native and buffel grasses. One, possibly coincidental, benefit for lizards of habitat with a low shrubland compared with a tall woodland is fewer raptor perches and therefore decreased risk of predation from encamped aerial predators.

How does buffel grass cover vary across the broader landscape and within the site?

Photopoints showed no changes in buffel grass cover at the site over the four year study, at burrow mounds in the neighbourhoods. However, this study period is probably insufficient to detect minor changes in projected foliage cover. Dense buffel grass cover to the west of the area of occupancy, but outside of the 50 m external zone, suggests different edaphic attributes and slower sediment transport. Buffel grass characterised all habitat types within the study site (Ne, Ao and Ex habitats), but was most prevalent within the area of occupancy. *Liopholis slateri* may be avoiding patches of high density of buffel grass, or selecting burrow sites or shrub mounds with lower buffel grass abundance. Distribution modelling studies on the nature of buffel grass expansion have reported that areas in which buffel grass is already present are likely to maintain or increase buffel grass cover under future climate scenarios (Healy 2015). The dense stands of buffel grass already established within the area of occupancy suggest that the grass may increase in abundance over time.

Is burrow site selection exclusive to shrub-mounds?

Shrub mounds were used for burrow sites in the majority of cases. Shrub soil pedestals are likely to provide many benefits including root structure for stability of tunnel systems, increased humidity from transpiration from roots (Henzell 1972) and hydraulic lift of water (Horton & Hart 1998). Further, moisture infiltrates faster under shrubs than in open areas, shrub-mounds thus earning the name, ‘islands of fertility’ (Crawford & Gosz 1982). The long-term use of burrows in deeper channel banks may be a functional analogue of a shrub mound.

The use of mounds around dead shrubs and logs for some burrow sites suggests that the benefits induced by a live root system are not critical for burrow site selection, however, burrows located under these other structures were occupied only for short periods (less than one survey year).

As burrows can be abandoned and then re-used later (Chapter 7), I considered it misleading to collect data on attributes of mounds that were not ever occupied during the study, as I could not confidently assess whether the shrub mound (or other location) had been used as a burrow site in the past or would be used in future. Thus I can make no claims about burrow site availability.

Do mound physical attributes correlate with *L. slateri* occupancy time?

Fenner *et al.* (2012) found no difference in burrow attributes between occupied and unoccupied burrows at the Lawrence Gorge and Orange Creek populations. Given that data from Chapter 7 showed burrows can have discontinuous use, it is perhaps not surprising that that earlier study found no differences between burrow attributes of the occupied and unoccupied groups.

With the four year dataset of burrow occupancy time in the current study, I also found that vegetation and mound characteristics did not explain why some burrow sites selected for occupancy were used for shorter periods than others. However, these were only a small sample of attributes of a burrow, and there may be a suite of other attributes that are important for site selection of *L. slateri* but that I did not measure. Then either, this study has overlooked critical components of burrow suitability, my sample size was not large enough to detect any differences, or burrow attributes are independent of occupancy time, and other factors, such as conspecific interactions, may be influencing burrow use dynamics.

The low correlation of buffel grass cover at burrows with occupancy time could be because most occupied burrows only had a low cover of buffel grass. *Liopholis slateri* may be avoiding mounds with higher cover of buffel grass.

Can Ne and Ao habitats be distinguished from each other and from neighbouring Ex habitat according to vegetation type?

I found that neighbourhoods appeared to be associated both with mounded *Eremophila* habitat (NB) and with other species assemblages (NGH). While *Eremophila sturtii* and *Hakea leucoptera* subsp. *leucoptera* were indicators of *L. slateri* occupancy in this system, skink occupancy did not appear to be restricted to this vegetation assemblage. Recently colonised neighbourhood H showed little difference from its adjacent plots. These data imply there may be a stochastic element to colonisation and that *L. slateri* may be moderately flexible in its choice of habitat.

8.5 Summary

While most known *L. slateri* population sites are broadly similar in occupying river floodplain habitat, this study described the habitat use at the site occupied by one population. These findings may assist in broadly classifying habitat for the species.

Chapter 9: General discussion

In this thesis, I have presented a sequence of research studies that, in combination, have greatly increased our knowledge of the ecology of the endangered Slater's skink *Liopholis slateri*, and our ability to effectively manage it. In particular, my research has developed survey methods specific to *L. slateri*, and gained insight into the species' persistence in river floodplain habitats of central Australia. Here I present a summary of the key findings in relation to each of the five research objectives, discuss broader implications of the research, and suggest directions for future research and conservation efforts.

9.1 Key research findings

Trial non-invasive methods for identifying individuals

My study (Chapter 5) has been one of the first to explore the use of photographic identification for individuals of a free-ranging, small terrestrial vertebrate. I showed that with careful examination of facial markings from good quality photos, developing an identification key for individuals is possible in a species in which those markings are retained over long time periods. I showed that independent observers could use the key even when the face was partly obscured or at wide horizontal angles to the camera. In its current stage of development, the I^{3}S Pattern algorithm appeared to be a poor match for the species compared with some other taxa (J. Hartog and R. Reijns pers. comm. 2015). However, the software was able to correctly match some individuals from photos that most observers incorrectly identified and vice versa. While automated computer-assisted identification has a clear time advantage, the higher percentage of correct identifications from visual inspection by experienced observers suggested a possible trade-off between time and accuracy, particularly when the population size is relatively small. Use of photo-identification in future studies might consider using a combination of the two methods, especially for larger populations.

Scat DNA appears to be a convenient and non-invasive means of identifying individuals (Chapter 7). Following Pearson *et al.* (2015), members of the genetics lab at Flinders University were able to extract DNA from the 113 scats I collected. Samples from 76 scats were successfully genotyped for up to 14 loci and 28 unique individuals were identified. With relatedness estimates from these molecular data, I showed that individuals were mixing within the population, which supported the findings of my field observations. This DNA analysis may be a viable survey technique for future studies of the species.

Increase effectiveness of *L. slateri* surveys and monitoring programs

In exploring the weather conditions that correlated with surface activity in *L. slateri* (Chapter 6), I found that, in general, lizards were more active in non-desiccating conditions: higher humidity, more recent rainfall, lower evaporation rates, and lower wind speed. Air temperature and humidity had an influence on surface activity but their influence varied between the morning and afternoon datasets. Although the afternoon sample size was smaller, the data appeared to suggest that lizards respond to different weather influences at different times of the day. This pattern was consistent whether I compared morning and afternoon surveys, or daytime and night time photographic records. Using rarefaction curves, I also calculated how many visits were required to sample a given proportion of the population.

These results may enable monitoring rounds and surveys to be targeted to the environmental conditions that increase detectability for *L. slateri*. Specifically, monitoring success might be increased by surveying on warm, humid mornings, with low wind, in the days following a rainfall event. If the aim was to count non-neonate individuals in the population, survey groups might strive for approximately 20 visits (or fewer if surveys were timed with favourable weather conditions) to ensure they had sampled the majority of individuals. The finding of nocturnal activity might also be used for surveys on warm nights, and would be worth investigating further.

In investigating burrow occupancy dynamics in the Orange Creek population over four years (Chapter 7), I found that individual burrow use varied widely. On average, individuals used 1–12 (mean 2.4 ± 0.2 SE) burrows per season. Therefore, as a rough index, a count of active burrows in a population might be divided by 2.5 to coarsely estimate the number of individuals at the time of survey. However, this calculated result should be interpreted cautiously.

Assess the ecological persistence strategy of *L. slateri* in floodplain habitats

At Orange Creek, I found evidence of a small, highly mobile, but site stable population, with spatial clustering of burrows into eight local 'neighbourhoods' (Chapter 7). I found relatively low fluctuations in population size among years, with a single reproductive event in three years, but a second seasonal breeding event following heavy rain in January 2015. I observed both long-term residence of individuals and long-term use of burrows at the site. Frequent movements within and among neighbourhoods, and regular burrow construction, suggest a dynamically structured population capable of dispersal in the event of high intensity disturbance. In *L. slateri* populations, either dispersed individuals or residents in some neighbourhoods may be the source for the species to recolonise after a catastrophic flood pulse that extirpates some neighbourhoods.

In the past, the species has probably been maintained as a series of temporal meta-populations, with local density foci shifting spatially over time, particularly following large flood events. This process requires that a series of suitable connected sites be available for existing populations to expand into, and to use in dispersal to recolonise previous locations. New threats to the species include anthropogenic changes that are reducing the availability of those sites. This occurs through processes such as the trampling of burrows and alluvial floodplain structures by cattle (Paltridge 2013), and invasion by exotic plants, like buffel grass *Cenchrus ciliaris,* encroaching and making many potential habitats less suitable (Pavey 2004a). Superimposed on these threats are predictions of more frequent and intense rainfall events (Healy 2015), increasing the likelihood of disturbance from flooding. Collectively, these changes may result in dispersers failing to establish refuge populations because suitable sites are few and increasingly hard (for the lizards) to locate. This complexity needs to be given consideration when developing regional conservation strategies for *L. slateri*.

Characterise fine-scale habitats of occupied areas within the floodplain

The four-year dataset on spatial dynamics of the Orange Creek population allowed me to characterise the physical and vegetative features of areas of high lizard use within the broader floodplain (Chapter 8). I found that neighbourhoods appeared to be associated both with mounded *Eremophila* habitat (neighbourhood NB), and with other plant species assemblages (neighbourhoods NGH). *Eremophila sturtii* and *Hakea leucoptera* subsp. *leucoptera* were strong indicators of *L. slateri* occupancy in this system. However, skink occupancy did not appear to be restricted to this vegetation assemblage. The more recently colonised neighbourhood H showed little difference from its adjacent plots. These data suggest there may be a stochastic element to colonisation and that *L. slateri* may be moderately flexible in its choice of habitat.

Assess the threat of buffel grass to *L. slateri* habitat

The data indicate that buffel grass (*Cenchrus ciliaris*) may be a considerable threat to *L. slateri* habitat (Chapter 8). Buffel grass was present in all habitat types within the study site, in neighbourhood (Ne), area of occupancy (Ao) and external (Ex) habitats. Buffel grass was the first-order diagnostic for area of occupancy (Ao) habitats, and while less dominant, was still a high-order contributor to neighbourhood (Ne) habitats. On mounds, buffel grass cover was generally low, with 90% of those mounds having less than or equal to 10% cover. *Liopholis slateri* may be avoiding patches of high density of buffel grass*,* or selecting burrow sites or shrub mounds with lower buffel grass abundance. Distribution modelling studies on the nature of buffel grass expansion have reported that areas in which buffel grass is already present, are likely to maintain or increase buffel grass cover under future climate scenarios (Healy 2015). The dense stands of buffel grass already established within the area of occupancy suggest that the grass may increase in abundance in this *L. slateri* population site, and presumably in others, over time.

9.2 Broader implications of research findings

I found evidence of previously unreported nocturnal activity during the hottest months of the year (Chapter 6). The findings of nocturnal activity, and of considerable flexibility in day-night activity patterns, suggest that *L. slateri* may have a greater daily time window for foraging than previously considered in climate change models. *Liopholis slateri* and *L. kintorei*, were identified as two of the lizard species in Australia at high risk of extinction from climate change under a warming-induced activity restriction scenario (Sinervo *et al.* 2010). However, the modelling behind this prediction considered *L. slateri*, along with *L. kintorei*, to be diurnal (Kearney 2013). Opportunistic nocturnal activity has been demonstrated in the usually diurnal lizards *Ctenotus pantherinus* (Gordon *et al.* 2010b) and *Tiliqua rugosa* (Kerr & Bull 2004) and should be considered for other lizards when modelling impacts of climate change.

The photographic identification method (Chapter 5) has the potential to be applied to the developing field of camera-traps for wildlife survey and thus a wide range of survey and monitoring applications. Camera traps, or motion-sensor cameras, are becoming increasingly popular in wildlife surveys. While, at present, camera traps cannot focus, or target a subject like a human operated camera, they have potential for individual identification of reptile taxa. Rapid advances in computer matching algorithms may make this a completely automated and reliable method in future.

High levels of genetic mixing in the population (Chapter 7) imply that *L. slateri* is not aggregating in kin groups like other members of the *Egernia* group. This has implications for studies in the evolution of sociality (Gardner *et al.* 2015).

9.3 Future research and conservation efforts

My research has highlighted the importance of dispersal and resilience of *L. slateri* in relation to new threats, such as encroaching buffel grass and altered flood-pulse patterns under changing climates. Given the predictions of increased frequency and intensity of extreme rainfall events in arid Australia (Healy 2015), research in dispersal, and of the threats of flooding and buffel grass, should be given priority for conservation management of the species. Although more population sites are being detected than lost (Chapter 4), *L. slateri* is a species with a restricted range and small population sizes, and conservation efforts to ensure its persistence should be ongoing. In this section, I put forward suggestions for management actions, and future research and monitoring.

My data suggest that monitoring and follow-up management of buffel grass should be a priority for conserving *L. slateri* populations. While buffel grass was characteristic of occupied neighbourhoods at the Orange Creek site and dense stands of the grass were present, most of the area had low cover (0–25 %) and greater than 90 % of burrow sites had less than 10% cover, suggesting lizards may be avoiding areas of high grass density (Chapter 8). Buffel grass should be monitored broadly at population sites, and specifically at some burrow sites, to assess whether the grass is increasing in cover and may therefore require control measures. In the first instance, buffel grass management could be focused at current population sites within the areas of occupancy. Subsequently, buffel grass could be controlled at previously occupied sites, and along watercourse corridors connecting extant population sites, to ensure opportunity for recolonisation and dispersal.

Regular monitoring of the number of active burrows at population sites is important to assess the status of the species over time. An instantaneous count of active burrows may give a coarse estimate of population numbers (see in Section 9, a roughly estimated ratio of 2.5 burrows: 1 individual). I suggest an annual monitoring program of all historically or currently monitored populations. A currently-used practice of permanently tagging burrows allows a direct comparison of burrow use between years, and ensures previously used burrows are not overlooked in searches. The end of the activity season (March-April) could be a suitable time as the number of active burrows is potentially greatest (after neonates appear), and individuals are not disturbed during the breeding period. Months of more moderate temperatures also increase the likelihood of sighting animals later in the day, although this has no bearing on burrow counts.

Understanding the severity of the threat of changing flood-pulse dynamics will be important for managing populations effectively. Future studies might assess the extent of flooding impact by installing remote cameras at population sites. During periods of high rainfall (usually the summer months), most population sites are inaccessible due to road closure. Remote cameras would provide an on-ground view of flood levels, over-surface flow, and the extent of burrow damage during these rainfall events. Although experimental manipulation of population sites (for example, simulating a flood event), offer a rapid and controlled way to address this issue, the small population size and threatened status of this species make such approaches unfeasible and ethically unpalatable.

To predict the location of other potentially suitable population sites, future studies might build on the habitat research by characterising the fine-scale habitat at other population sites. This would assist with refining search efforts and provide a comprehensive dataset for spatially-predictive analyses, such as species distribution models (SDMs). Predictive models may then incorporate potential flooding impacts on *L. slateri* populations under various climate and rainfall scenarios.

Scat DNA provides a ready and non-invasive means of investigating dispersal ability in the species. By collecting scats, there is opportunity to further examine dispersal within some of the larger meta-population sites, such as at Loves Creek and Little Palm Creek, with apparent geographical barriers between occupied sites. There is also scope for using scats for phylogenetic analyses to assess connectivity and relatedness among populations, and identifying genetic units for conservation.

Future studies might also investigate what is occurring during nocturnal activity in *L. slateri*. Are skinks foraging during the night or regulating their temperature outside the burrow on hot nights? These questions may have implications for the species’ adaptation to a changing climate. New technologies in infrared video cameras, or perhaps night vision scopes, might be sufficient for this investigation. Additionally, short-term night time investigations might try using fluorescent powder to track individuals by UV light, if a reliable means of application could be found.

9.4 Conclusion

In the Anthropocene (Corlett 2015), there is an increasing number of species under threat from anthropogenic impacts, including environmental change. Historically, biota persisted in evolutionary refugia during periods of extreme aridification (Byrne *et al.* 2008; Pepper *et al.* 2011). In a modern context, arid-adapted fauna also survive in refuges, waiting out local catastrophic events, or unfavourable conditions. Anthropogenic changes to these refuges may be compromising the persistence of some of these species. This is particularly relevant to habitats of the Central Ranges, where buffel grass and fire interactions are dramatically modifying floodplain systems, and changing climates are altering flood regimes. Detailed autecological studies will be important for our understanding of species behaviour and the ecology of natural population dynamics, and integrating this information into management and monitoring protocols. This thesis has provided some of that background for one endangered species.

Chapter 10: Bibliography

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Appendices

Appendix 1: Supplementary tables and figures

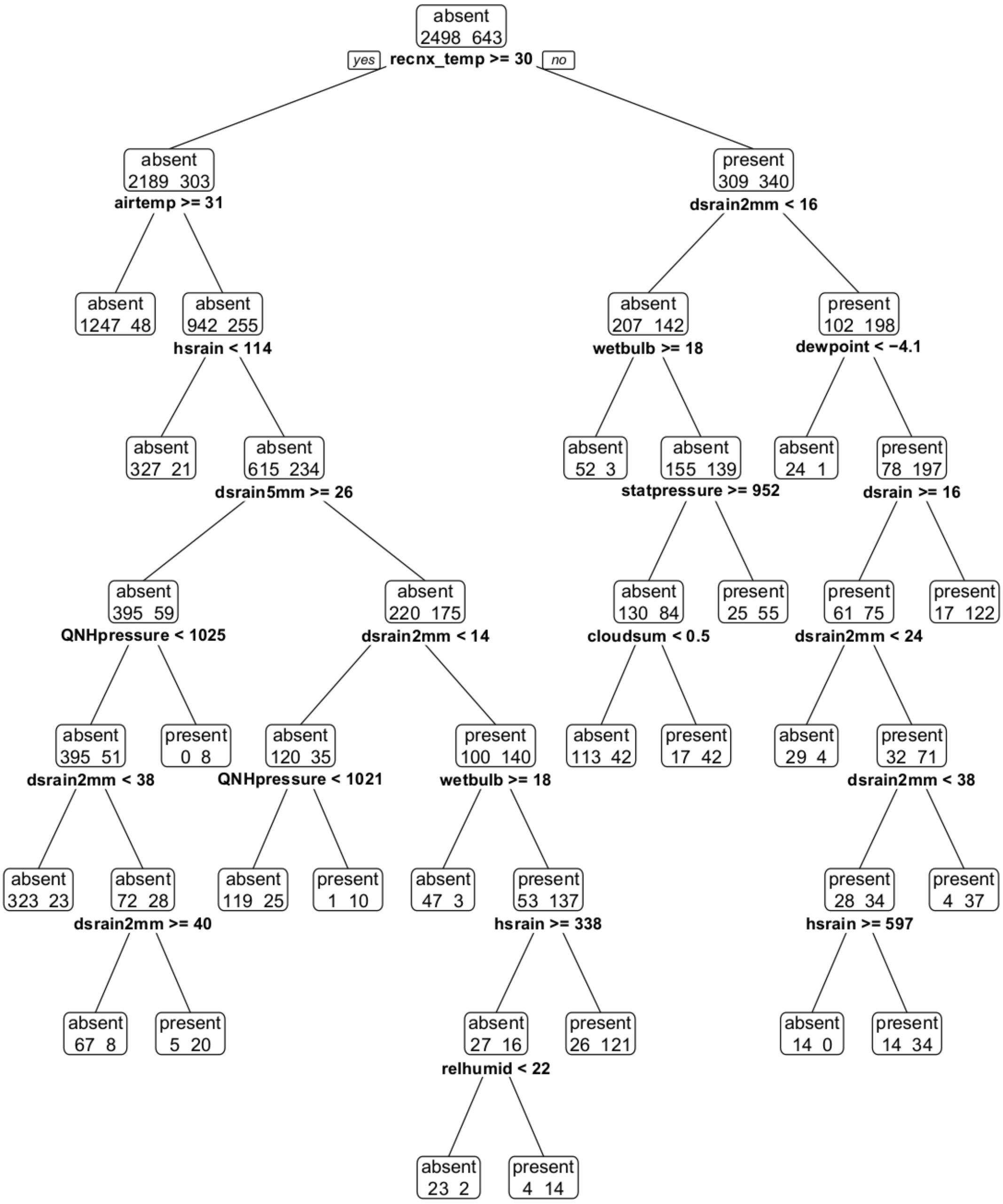
Appendix Table 1 (Chapter 5). Temporal sequence of the development of facial markings for an individual of *L. slateri* (S39). Spots have been scored according to the characters used in the key (Table 5.1). Days are days elapsed since first observing the neonate, and observations thereafter. Grey shading denotes row descriptions of the head profile on the right side.

Days	**Profile**	**ear_ lob**	**no_temp _marks**	**temp_ touch**	**no_ supralab**	**7_ infra**	**6_ infra**	**5_ infra**	**4_ infra**	**3_ infra**	**2_ infra**	**1_ infra**	**Description**
	R	5	0	0	3	0	0	0	0	0	0	0	three faint spots on supralabial scales
31	R	5	0	0	3	0	0	0	0	0	0	0	supralabial spots darker and more defined
31	L	4	0	0	3	0	0	0	0	0	0	0	three markings on supralabial scales, faint marking on lower jaw
42	R	5	0	0	3	0	0	0	0	0	0	0	faint marks developing on infralabial scales
57	L	4	0	0	3	0	0	0	0	0	0	0	spots darker, infralabial outline developing
77	L	4	0	0	3	0	0	0	0	0	0	0	spots more defined
105	L	4	0	0	3	0	0	0	1	0	0	0	fourth supralabial spot developing
114	R	5	0	0	3	0	0	1	1	0	0	0	faint spot developing on temporal scale, infralabial markings darkening
323	R	5	1	1	3	0	0	1	1	0	0	0	other markings developing on lower jaw, temporal scale spot darkening
323	L	4	0	0	3	0	1	0	0	0	0	0	supralabial markings darker and more defined, temporal scale spot darkening
332	L	4	0	0	3	0	1	0	0	0	0	0	other infralabial marks darkening
627	R	5	1	1	4	0	0	1	1	1	0	0	facial markings darker and more defined
627	L	4	1	1	4	1	1	1	1	1	0	0	facial markings darker and more defined, more marks on lower jaw
638	L	4	1	0	4	1	1	1	1	1	0	0	no change
638	R	5	1	1	4	0	0	1	1	1	0	0	no change

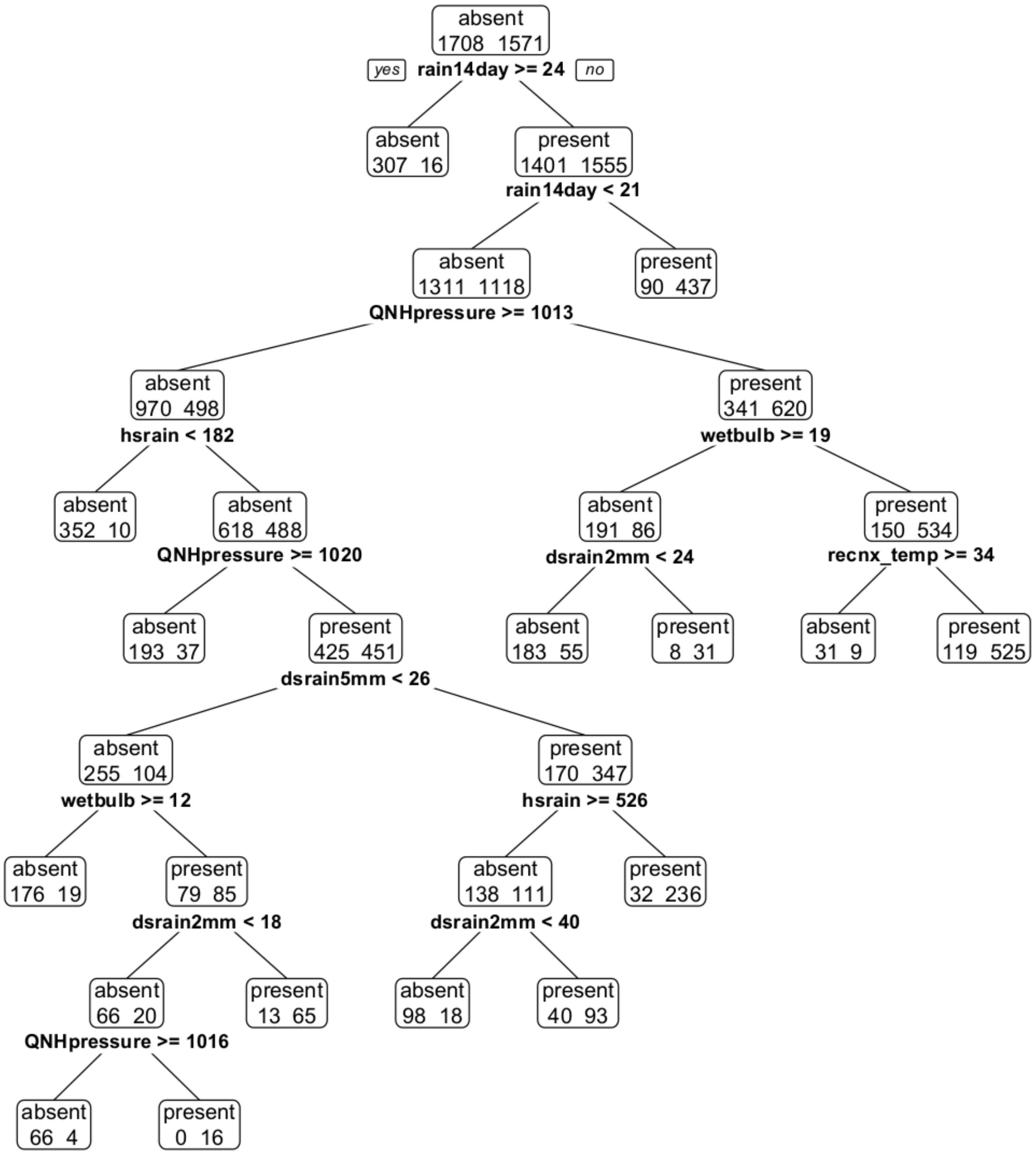
Appendix Table 2 (Chapter 6). Explanatory variables used in CART modelling of presence/absence of an individual Slater's skink (S22) from November 2012 to March 2013. Variables are weather observations recorded every 30 minutes from the Alice Springs weather station, or derived from these observations.

Covariate		**Units**	**Mean**	**Range**
recnx_temp	Air temperature recorded by the Reconyx camera	degrees Celsius	30.1	4–57
airtemp[1]	Air temperature	degrees Celsius	26.1	6.6–43.3
wetbulb[1]	Wet bulb temperature	degrees Celsius	15.3	2.2–25.1
dewpoint[1,3]	Dewpoint temperature	degrees Celsius	5.1	-18.8–21.5
relhumid[1]	Relative humidity	percent	30.6	3–98
windspeed[1]	Wind speed	kilometres per hour	13.2	0–42
windgust10min[1]	Speed of maximum wind gust in last 10 minutes	kilometres per hour	18.6	0–70
rain10min[1]	Precipitation in last 10 minutes	millimetres	0.003	0.0–5.6
rain9am[1]	Precipitation since 9am local time	millimetres	0.1	0.0–20.4
cloud1[1]	Cloud amount (of first group)	eights	2.3	1–7
cloud2[1]	Cloud amount (of second group)	eights	4.3	1–8
cloud3[1]	Cloud amount (of third group)	eights	5.2	1–8
cloud4[1]	Cloud amount (of fourth group)	eights	1.3	1–3
cloudsum[4]	Sum of cloud1, cloud2, cloud3 and cloud4	eights	0.4	0–16
mslpressure[1]	Mean sea level pressure	hectopascals	1011.4	997.7–1025.4
statpressure[1]	Station level pressure	hectopascals	950.8	940.6–961.4
QNHpressure[1]	QNH pressure	hectopascals	1014.9	1004.1–1026.1
hsrain[2]	Hours since rainfall	hours	276.8	0–817
dsrain[2]	Days since rainfall	days	11.0	0–34
dsrain2mm[2]	Days since rainfall greater than 2 mm	days	17.4	0–43
dsrain5mm[2]	Days since rainfall greater than 5 mm	days	21.1	0–44
dsrain10mm[2]	Days since rainfall greater than 10 mm	days	21.1	0–44
rain7day[2]	Cumulative rain over the past 7 days	millimetres	3.5	0.0–35.6
rain14day[2]	Cumulative rain over the past 14 days	millimetres	8.4	0.0–35.6

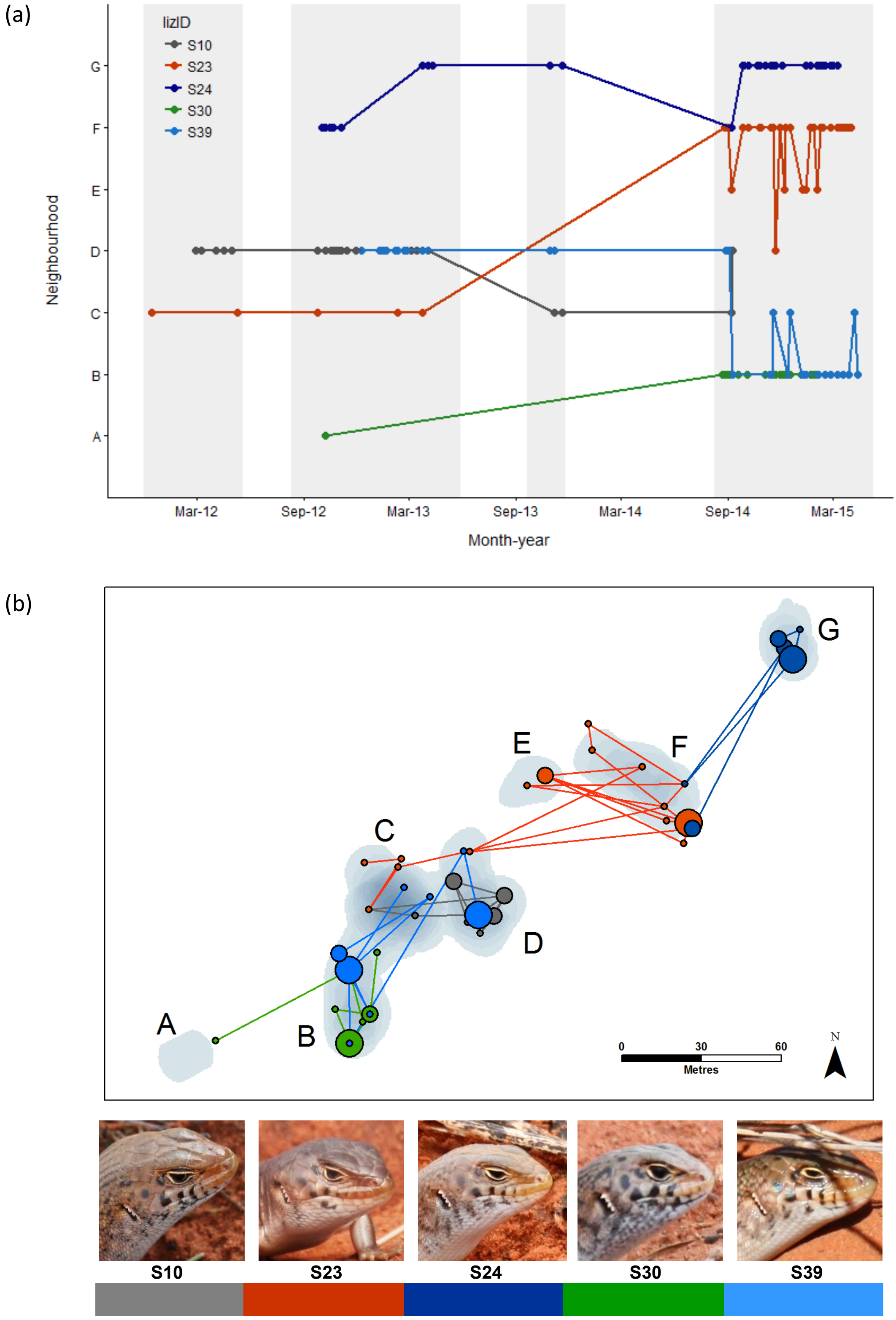
Sources: [1] Bureau of Meteorology daily data for Alice Springs airport (purchased), [2] Derived from Bureau of Meteorology rainfall observations, [3] Only available after 22/02/2013 for 43% (n=2753) of observations, [4] Derived from Bureau of Meteorology cloud observations.



Appendix Figure 1 (Chapter 6). Classification tree for the presence or absence of one individual outside the burrow during daylight hours, recorded every 30 minutes by a time-triggered camera (n=3141). Data are partitioned according to half-hourly weather observations from the Alice Springs airport weather station (Appendix Table 2). Each node shows the number of photos for which the lizard was present (right number) or absent (left number) for the corresponding conditions, and labelled according to the higher number. To read, follow the left branch from each node if the condition is met (yes) or the right branch if the condition is not met (no).



Appendix Figure 2 (Chapter 6). Classification tree for the presence or absence of one individual outside the burrow at night, recorded every 30 minutes by a time-triggered camera (n=3279). Data are partitioned according to half-hourly weather observations from the Alice Springs airport weather station (Appendix Table 2). Each node shows the number of photos for which the lizard was present (right number) or absent (left number) for the corresponding conditions, and labelled according to the higher number.



Appendix Figure 3 (Chapter 7). Movements of five individuals resident in the population over three or more survey periods. Movements are shown between neighbourhoods (a) temporally, (b) spatially, and colour-coded to individual: S10 = dark grey, S23 = red, S24 = dark blue, S30 = green and S39 = light blue. Shaded grey regions in (a) are survey years. Graduated circles in (b) represent number of photographic captures for each individual at the burrow: small circles (1–3 captures), medium (4–7 captures), and large (8–14 captures).

Appendix 2: Isolation and characterisation of microsatellites for the endangered Slater's skink, *Liopholis slateri* (Squamata: Scincidae), via next generation sequencing.

(Manuscript in preparation)

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Abstract

We characterised 14 new polymorphic microsatellite loci for the endangered lizard, *Liopholis slateri.* Initially, 454 shotgun sequencing was used to identify 46 loci which were trialled for amplification. Subsequently, 14 of these loci were screened for variation in 21 individuals from scat-derived DNA samples collected from Owen Springs Reserve in central Australia. All 14 loci were polymorphic with observed heterozygosity ranging from 0.19 to 0.86 and the number of alleles per locus ranging from 2 to 10. These loci will be useful in understanding the genetic variation and connectivity within and among extant *L. slateri* populations.

Keywords: *Liopholis slateri*; Slater's skink; scat DNA; microsatellites; 454 GS-FLX; shotgun sequencing.

Slater's skink, *Liopholis slateri* (Storr 1968), is a mid-sized (85 mm snout-vent length) viviparous scincid lizard that occupies floodplains of rivers and low-order tributaries within the MacDonnell Ranges IBRA region of central Australia. *Liopholis slateri* is an endangered species for which few data are available. Scat piles are characteristic of the genus (and this particular species) and provide a non-invasive way of collecting molecular data. We developed microsatellites to investigate fine-scale spatial structuring within the species.

Genomic DNA (5 µg) was isolated from tail tip tissues of two individuals of *Liopholis slateri* (ABTC114727 and ABTC114722) using a modified GENTRA tissue extraction. The DNA was then sent to the Australian Genomic Research Facility (AGRF) in Brisbane, Australia for shotgun sequencing on a Titanium GS-FLX (454 Life Sciences/Roche FLX), following Gardner *et al.* (2011). The sample occupied 6.25% of a plate and produced 47,825 individual sequences, with an average fragment size of 318. We used the program QDD v. 2 (Meglécz *et al.* 2010) to screen the raw sequences with ≥ eight di-, tetra- or penta-base repeats, remove redundant sequences, and design primers (automated in QDD using Primer3, (Rozen & Skaletsky 2000)) for 502 loci with PCR product lengths of 80-480 base pairs, and of a suitable quality for further development (classified as "A" in QDD see Meglécz *et al.* (2010)).

We followed the procedure outlined in Gardner *et al.* (2011), and 46 loci were chosen for further development (Supp. Material Table 1). In line with the MRT (Multiplex Ready Technology) method (Hayden et al. 2008), each forward primer and reverse primer was synthesized with the following bases: ACGACGTTGTAAAA or CATTAAGTTCCCATTA at the 5' end before the locus specific primer sequence, respectively. Initially, the loci were trialed for amplification in the two individuals used for the shotgun sequencing, and also for determining the best molarity of the primers (i.e. choosing the amplification that produced the clearest fragments on an agarose gel) for amplification following the MRT method (Hayden *et al.* 2008). For all amplifications, each 10µl reaction contained 1X MRT buffer, incorporating 0.5 U Immolase DNA polymerase (Bioline, Alexandria, NSW, Australia), 2.5 mm $MgCl_2$, 0.2 mM of each dNTP, with 75 nM of each forward and reverse MRT tag primer, between 10 and 60nM of each locus-specific primer and 10-50 ng gDNA. The 5' end of the forward MRT tag primer incorporated a florescent tag (Table 1). The PCR conditions were: 95°C for 10 min followed by 5 cycles at 92°C for 60 sec, 50°C for 90 sec, and 72°C for 60 sec; 20 cycles at 92°C for 30 sec, 63°C for 90 sec, and 72°C for 60 sec; then 40 cycles at 92°C for 15 sec, 54°C for 60 sec, and

72°C for 10 min and a final elongation step at 72°C for 10 min. PCR products were visualised on a 1.5% agarose gel stained with ethidium bromide. All loci amplified and were subsequently trialed on 10 individuals. As tissues were not available for primer trials, we first extracted DNA from 10 collected and dried *L. slateri* scats following Pearson *et al.* (2015).We assessed loci for amplification; loci were included for routine genotyping if they amplified in at least five scats. We subsequently extracted DNA from 76 scats from a single population at Owen Springs Reserve (23°59'S, 133°37'E) (Treilibs *et al.* 2016), and amplified these for 14 loci (Table 1). Each DNA was amplified between three to five times in order to obtain consistent genotypes. We compared the genotypes of individual scats using GENEALEX v 6.5 (Peakall & Smouse 2006, 2012) and pooled those with matching profiles. Consequently, we obtained 26 unique genotypes. From these genotypes, we calculated Quellar and Goodnight relatedness values using COANCESTRY v 1.0.1.7 (Wang 2011), and removed one of a pair when relatedness values were above 0.35 and potential first order relatives, which would bias determination of Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium. The final data set used for these tests, which were conducted in GENEPOP web (http://genepop.curtin.edu.au/) (Raymond & Rousset 1995; Rousset 2008), were the genotypes from 21 individuals. Two pairs of loci (Lst38 with Lst40; Lst35 with Lst41) showed significant linkage equilibrium after applying Holm's sequential Bonferroni corrections (Holm 1979) for multiple tests. Four loci also deviated from HWE (Table 1). The deviations were not large. Considering the small number of individuals used and the potentially restricted distribution of the species, and that scats were used as the starting material, it is not clear if the deviations are indicative of a real disequilibrium or other factors. Likely further testing on robust DNA samples, and for a larger number of individuals, would clarify the situation. For each locus, we calculated the number and range of alleles, observed and expected heterozygosity, and estimated null allele frequencies using GENALEX.

These markers will be used to document the genetic diversity in *L. slateri* and to investigate the fine-scale spatial structuring within populations of the species. This will be useful for understanding the spatial dynamics and dispersal within and among extant populations.

Acknowledgements

Funding was provided by the Australian Research Council (LP1101000066), the South Australian museum, the Northern Territory Department of Land Resource Management.

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Table 1. Characterisation of polymorphic loci. Primer sequences, GenBank accession number, repeat motif, and diversity characteristics of 14 microsatellite loci from *Liopholis slateri.* Primer molarity indicates the optimised primer molarity used (see text) N indicates the sample size; Na indicates number of alleles; *Ho* and *He* indicate observed and expected heterozygosity respectively; ND indicates not done; PIC indicates polymorphic information content; * indicates significance after corrections for multiple tests; HWE indicates Hardy-Weinberg Equilibrium. Superscripts F, N, V, and P indicate loci were amplified with MRT primers labelled with the dyes 6-FAM, NED, VIC and PET respectively. **Bold** *P* values are <0.05.

Locus		Primer sequence (5'-3')	Primer molarity (nM)	GenBank accession no.	Repeat motif	N	Allele size range	Na	*Ho*	*He*	Null allele freq	HWE *P*
Locus Lsl11^{N}	F:	TTGTTTGTGATGCTCGTTCG		to be lodged	(AAAT)$_8$	18	190-202	4	0.444	0.449	0.064	0.319
	R:	TTTGTGAGCTGCCTTGAGTG	40									
Locus Lsl137^{F}	F:	GCACTTTATTCGCACCAAGG		to be lodged	(AAAT)$_9$	21	233-249	6	0.375	0.717	0.255	**0.002**
	R:	GTGGGTCCTGGACGCTAAAT	20									
Locus Lsl23^{V}	F:	CACTAGGGAATGTCCTGCATTT		to be lodged	(AG)$_8$	20	197-278	4	0.190	0.219	0.120	0.145
	R:	TTCCTGCATGTCAGCAACAG	40									
Locus Lsl29^{P}	F:	CAGGCATCCCAGGTCCTT		to be lodged	(AAC)$_{12}$	21	160-202	10	0.750	0.814	0.057	0.539
	R:	CATACCATGGTCGGATTGAAA	40									
Locus Lsl31^{F}	F:	GGAAAGCTGCCTCTTGGGTA		to be lodged	(AC)$_{10}$	16	167-237	4	0.524	0.452	0.000	1
	R:	TTGCAGAAGGAACATGAGCTT	40									
Locus Lsl21^{V}	F:	TCACATCGCATGATTTGCTA		to be lodged	(AC)$_9$	21	171-177	4	0.857	0.706	0.113	0.373
	R:	GGCTCAAACCTTGCCTCACT	60									
Locus Lsl36^{P}	F:	AGCTCTCAGAAGTGCTTGCC		to be lodged	(AC)$_{10}$	12	191-207	4	0.583	0.736	0.309	**0.01**
	R:	CCTGGCAATTAGGCTTTCAG	60									
Locus Lsl38^{N}	F:	CAGAACACAAACCGGCTCTC		to be lodged	(AG)$_8$	18	193-208	4	0.667	0.718	0.402	**0.006**
	R:	CCACCTTTGGCAAGACTCTG	60									

Locus Lsl42^{F}	F:	TTGCATGCTGCAGTGAGG		to be lodged	(AAAT)$_{10}$	12	165-203	8	0.583	0.847	0.289	0.117
	R:	TGGGAGGGATTAGAACTTTGG	20									
Locus Lsl12^{F}	F:	GCTTGAACACTGTGAGCCCT		to be lodged	(TGAT)$_{12}$	20	233-261	8	0.550	0.838	0.294	**0.006**
	R:	ATGTGATCCCTGACTGGGTG	20									
Locus Lsl35^{P}	F:	TTTAAAGTGAAAGTACTGCTGGC		to be lodged	(AAAT)$_{7}$	20	180-199	7	0.600	0.650	0.183	0.24
	R:	CATCAGTGGCATACCAAGGG	40									
Locus Lsl40^{F}	F:	CAGAGTCCCATCTCATGTCAAA		to be lodged	(AC)$_{9}$	20	175-177	2	0.450	0.439	0.671	1
	R:	TTGTTGGAGCCCAAAGAAGA	60									
Locus Lsl41^{N}	F:	TTGAAATGCTCGACATGCAG		to be lodged	(AC)$_{14}$	18	176-199	9	0.778	0.856	0.100	0.087
	R:	TGCATGTCTAAAGAGGAACGC	60									
Locus Lsl46^{V}	F:	AAGTTGAGTCATGGAGGGCA		to be lodged	(AC)$_{8}$	19	176-208	6	0.421	0.438	0.043	0.679
	R:	TCTGATGGGCATGGGTACTA	20									

Supplementary Material Table S1 for Gardner *et al.* Microsatellite loci for the endangered lizard *Liopholis slateri.* All trialled loci - output from QDD v.2.0 from 454 shotgun sequence run

SEQUENCE_CODE	LOCUS NAME	MOTIF	PCR_PRODUCT_SIZE	REPEAT_REGION_SEQ	PRIMER_LEFT_SEQUENCE (FORWARD)	PRIMER_RIGHT_SEQUENCE (REVERSE)
cons_gr129_2	Lst1	AGC	152	AGCAGCAGCAGCAGCAGCAGCAGC	GTATTCGGCAGCCAAGCC	CTGCACGACTCTCCACTCCT
cons_gr163_2	Lst2	AC	281	TGTGTGTGTGTGTGTGTGTGTG	CCTGTATGCATTAGACAACAGACA	TGTAGCACAGCACCAGGCTA
cons_gr204_2	Lst3	AG	146	AGAGAGAGAGAGAGAGAG	GCAGAAAGCAAACATCCACC	CAGAGCAGTGATGCCACAAT
cons_gr242_3	Lst4	AGT	209	TAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAG	CCAGGTCCTTGGGAAGAACT	TGTGGCTTGTTGCAATTGTT
cons_gr292_2	Lst5	AGAT	185	TATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATC	TGAACATAAGTTGCCATTCTGC	TCAAAGGCAAATGACTCCAA
cons_gr294_4	Lst6	AC	143	TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG	CTGAGGGCAACATCTGCAAG	CACCTAGGCCCATCACTGTT
cons_gr302_4	Lst7	AG	173	CTCTCTCTCTCTCTCTCT	AGCTGTCCAGGTCCTCCTTC	GCATCCAATATGCTTCCTGC
cons_gr344_2	Lst8	AACT	190	TGATTGATTGATTGATTGATTGATTGATTGATTGATTGAT	GGAGTCTCCTCAGACTTGCG	CAGCTCATACATGTTGCACACT
cons_gr345_2	Lst9	ACT	247	GATGATGATGATGATGATGATGATGATGATGATGATGAT	TGTGTCAGAACCACCATCCA	ATCCTATGCCAGGAGCTGTG
cons_gr392_2	Lst10	AC	193	ACACACACACACACACACACACACACAC	TTGCTAATTCAAAGGATGCAGA	GCCCATTAGGGTAAACGTGA
cons_gr74_2	Lst11	AAAT	158	TTTATTTATTTATTTATTTATTTATTTATTTA	TTGTTTGTGATGCTCGTTCG	TTTGTGAGCTGCCTTGAGTG
cons_gr78_4	Lst12	AACT	230	TGATTGATTGATTGATTGATTGATTGATTGATTGATTGATTGATTGAT	GCTTGAACACTGTGAGCCCT	ATGTGATCCCTGACTGGGTG

cons_gr95_5	Lst13	AATC	153	CAATCAATCAATCAATCAATCAATCAATCAATCAAT	TCATGCTATGTCAGGTTTCTCG	GCTCCTGTGCAACTCCAAAT
HKTG2QA01AID6Z	Lst14	AAC	350	AACAACAACAACAACAACAACAACAAC	GCCTTAAGAACAATGCCTTCC	ATCCTATGCCAGAATGTGCG
HKTG2QA01AKEOU	Lst15	AATG	169	GAATGAATGAATGAATGAATGAATGAATGAATGAAT	GAATTTGTGACTTTGGGCCTT	CTCCCTCCTTCCCTACAGGA
HKTG2QA01B0JXN	Lst16	AAAC	348	AAACAAACAAACAAACAAACAAACAAACAAACAAAC	GTGCAATAGGTGGCCTGAAA	TCACGACCCACAGTTTGAGA
HKTG2QA01B0UCP	Lst17	AC	196	GTGTGTGTGTGTGTGTGTGT	CTGTGCAGGAGCACAGTGTA	ACATATGTGGCCAAAGCACC
HKTG2QA01BJPEE	Lst18	AAAT	143	TAAATAAATAAATAAATAAATAAATAAATAAA	GTACACCGCCCTGAGCTCTT	TTTAGTGAGACTGTCAACCTATTGAA
HKTG2QA01BXCT9	Lst19	AC	141	CACACACACACACACACACACACA	CTTTCCAGTGGGCTGTTCAC	TGTGACTGTGCAGATTGGAAC
HKTG2QA01BZVC0	Lst20	AC	185	CACACACACACACACA	TGTCGCTAAGTCAGTGCTGG	GCAAAGAGGGCTCCTCAACT
HKTG2QA01C5MOH	Lst21	AC	145	CACACACACACACACACA	TCACATCGCATGATTTGCTA	GGCTCAAACCTTGCCTCACT
HKTG2QA01DBIA3	Lst22	AC	151	ACACACACACACACAC	TGAAAGTCACAGAAATGGGTTG	GAATCTAGAAAGCACTGAATTGTCA
HKTG2QA01DBX9G	Lst23	AG	168	GAGAGAGAGAGAGAGA	CACTAGGGAATGTCCTGCATTT	TTCCTGCATGTCAGCAACAG
HKTG2QA01DU3DD	Lst24	AC	156	ACACACACACACACACACACACACACAC	CTGGATCATGTGGATCTGC	ATAGCTTGGCCATGTTGTCC
HKTG2QA01EI471	Lst25	AC	183	CACACACACACACACACACACACACACA	AGAGGAGAACCAAACCACCTT	TCCACAGATTTCTGCAAAGTT
HKTG2QA01EIENH	Lst26	AAAT	143	TTATTTATTTATTTATTTATTTATTTATTTATTTATTTATTTAT	CATTAATGGTGTGCCCAAA	CCTTGGGCATCTACTCCAGA
HKTG2QA02F1V91	Lst27	AG	175	CTCTCTCTCTCTCTCTCTCTCT	ATTAGGGCCTGTGGATTGCT	AACGTCGTACAGGAGCCTTG
HKTG2QA02F3BYU	Lst28	AAT	340	AATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAAT	TGCTAAAGCTTAGTTAGGATTGGG	AGTTGTTTAGTCCAGCAAGGG

HKTG2QA02F9HYS	Lst29	AAC	140	AACAACAACAACAACAACAACAACAACAACAACAAC	CAGGCATCCCAGGTCCTT	CATACCATGGTCGGATTGAAA
HKTG2QA02FOBMV	Lst30	AG	191	AGAGAGAGAGAGAGAG	CAAATGCCCACCAAAGTCTC	GAGGTTGGATGGATTCATGG
HKTG2QA02FWCB7	Lst31	AC	140	ACACACACACACACACACAC	GGAAAGCTGCCTCTTGGGTA	TTGCAGAAGGAACATGAGCTT
HKTG2QA02FZRSY	Lst32	AAT	179	TAATAATAATAATAATAATAATAATAA	ATCCCTTCCTGCTGTTACCC	GAGGACCAAGAAAGGACGGT
HKTG2QA02GKT7H	Lst33	AGG	148	AGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	TTGGACCCAAAGAGTTCTTCC	GAGTGTCAATGACTTCCGTGG
HKTG2QA02GN555	Lst34	AC	194	CACACACACACACACACA	CAATGAGATGGAGACAGAAGGG	CCCTCAGGTCATGGTTTGAA
HKTG2QA02GS3ER	Lst35	AAAT	161	TTATTTATTTATTTATTTATTTATTTATTTAT	TTTAAAGTGAAAGTACTGCTGGC	CATCAGTGGCATACCAAGGG
HKTG2QA02HL6JK	Lst36	AC	160	TGTGTGTGTGTGTGTGTGTG	AGCTCTCAGAAGTGCTTGCC	CCTGGCAATTAGGCTTTCAG
HKTG2QA02HLJ81	Lst37	AAAT	207	TATTTATTTATTTATTTATTTATTTATTTATTTATTTATT	GCACTTTATTCGCACCAAGG	GTGGGTCCTGGACGCTAAAT
HKTG2QA02HN287	Lst38	AG	180	CTCTCTCTCTCTCTCT	CAGAACACAAACCGGCTCTC	CCACCTTTGGCAAGACTCTG
HKTG2QA02HRDYZ	Lst39	AG	145	TCTCTCTCTCTCTCTCTCTCTC	TCTTTAAGAAACCTATGAAACCTGG	TGCTTTGCAAATGAAGTATTTGAG
HKTG2QA02IILC5	Lst40	AC	145	GTGTGTGTGTGTGTGTGT	CAGAGTCCCATCTCATGTCAAA	TTGTTGGAGCCCAAAGAAGA
HKTG2QA02IP3J1	Lst41	AC	146	TGTGTGTGTGTGTGTGTGTGTGTGTGTG	TTGAAATGCTCGACATGCAG	TGCATGTCTAAAGAGGAACGC
HKTG2QA02IYDGL	Lst42	AAAT	162	AATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATA	TTGCATGCTGCAGTGAGG	TGGGAGGGATTAGAACTTTGG
HKTG2QA02JAQVE	Lst43	AG	144	GAGAGAGAGAGAGAGAGAGA	CAGTATCTGACACCGTTGGG	GGTTAGGTCAACTACGCCTCC
HKTG2QA02JJLR7	Lst44	AATC	228	TTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAG	AGAGCCTCAGTTAGGGACGG	GGCCTCCTCAAAGTAAGGGA

HKTG2QA02JYVPY	Lst45	AAT	241	AATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAATAAT	TTGTCTTCCAACTCCATTCTTTG	TGGATAACGGCTGAAATAAGA
HKTG2QA02JZNIS	Lst46	AC	148	ACACACACACACACAC	AAGTTGAGTCATGGAGGGCA	TCTGATGGGCATGGGTACTA

LOCUS NAME	COMPLETE SEQUENCE
Lst1	GGNAAAAGcccccGGCTCTTCCCAAGCctctctGAAGCCGTATTCGGCAGCCAAGCCGCAGGCTGCAAACCGCGGGGATAAAATGCCGCCTGCAGGGACGCTGGTGACTTGGCGTTTGCAGGGACAAGagcagcagcagcagcagcagcAACGGAGTGGGNATGTGCAAGGAGTGGAGAGTCGTGCAGGTTCCA
Lst2	AAGCTAACTACCTGTAATTAAACCTGTATGCATTAGACAACAGACACTTGTTTTGTTTAGAAAACTGTTTGGAATTGAGGGGTGTATGTACCtgtgtgtgtgtgtgtgtgtgtgCAAGCATGTTGAAGCTTATCACTTCCTACAGGTTCTCAAATCCAAATTCCACTTTACCAAATTACCTTTTNGCAATAACTGGCTTTAGGACCATTATACCTGTATTTACCCCATCAATTAATTTTNCTAAAGAGCACAGTACCAGACAGTGGCATAGCTAACAACCATGTAGCCTGGTGCTGTGCTACAAAATGATCGCCC
Lst3	TGTTTATGTGACTGTAATaaaaaaNNTGAAATAATGCAGAAAGCAAACATCCACCTTTGCCCTGTCACTCTTAATCAACAGAAACAATTATTGTGGCagagagagagagagagagAACATGTACTTACTNGGAAATCTCTGATGNATTTCTTCTGTTGTTTATTGTGGCATCACTGCTCTGGNATTTACTAGGTTAAAATTAAGTCATCCATCCTGAATCAGAGGAATCCATCCTGTCACCATCAATGAGATGTACATTNCCAAATAAGGCTGCAATCCTAAGAacacacCTCCTAGAAACGTAAACCCATTGAACTGAATAGGACCTACTTTGGATAGGCAGTAGATGACAAACAAGTGGAAGTACTTCTTCATGCAGCAGTAA
Lst4	gtagtagtagtagtagtagtagtagtagtaGTGAGTCAGGCATCCCAGGTCCTTGGGAAGAACTCTATCTCTGGAGAAAACAAACCAGTCAGTAACACCTATCTGACCGTGCAAAACAAtagtagtagtagtagtagtagtagtagtagtagtagtagtagtagtagtagtagtagTAATAAGGTCCTTTAAGAAATATCTGGTCAGTATAGGCATTGACAAGATAACAGTTAAACAATTGCAACAAGCCACACTGCNTAGGGAACAATAAAATATGTTGTATTTCatatgatatgatatgGCGAGATNNTNNNNAGTTNCTTAAANCTCTTAGAGTTCAAGCAAGTGA
Lst5	TTAAAATCTTGCCTCAGGCATTGTCTCACAAATGGAATGAAGAGTCTGATGGTCAGAATTAATTTTACAAAGGGGCAGACTTACAAGGTtgtgtgAGTTAATTTCTTTGTTAAGAAGATTATTGACATTGAAGATGTTTTGAAAATTCTGCCCCAGTTGATATAAAACTGTGGATTTAGATTTCAGATGCAGATCATCTTTaaaaaCGTGAACATAAGTTGCCATTCTGCTTTTATTCTCGGGCTAATTACCTtatctatctatctatctatctatctatctatctatctatctatctatctatctatctatctatctatctatctatcGATCTATCTCCTAGAACTTCATGAATATGAAAGATGTTGGAGTCATTTGCCTTTGACTTAC
Lst6	CATAACCCAAAAGTAAGTCACAAACAATAAACTAAGAAGACAAAGCAAAGGGAGAAAATGAACCCAGTTGATATCCAAGACATATAGaaaaaTACACAAATTTAATTCAGTTATAacacacCTGAGGGCAACATCTGCAAGCAAACACAGGAGAAACtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgATGCATNNNNCAANNNNNAGGACTGTATGATGGTACAAATNATTGCATAGCTAACAGTGATGGGCCTAGGTGGCTTTTAGATACTATTTGGTTATTAAGAAAATCCATGAAGgagagaTCTGTTGGCAGCCAGAAGCC
Lst7	ATGTGAGgagagaGCAACCGAAAGGAGCTGTCCAGGTCCTCCTTCCCTTCTGTGTTCTTCCTCTCAAACCTGAGGGctctctctctctctctCAGCCTCTCAAGTGAGGGCTGTCAATGGGTGGACCGATTCCCCAGGTGGGTTGAAGGTTTGTAATGTGGCCTTAAATTCAGATTGCAGGAAGCATATTGGATGCAGGTATGAAAGAGCATATGGTTTGTCTATGTTTCTAT

GATCAGAGGTTAGGGGCATTCACATTCACAGAGATGAATGGGGTCCTTCTTAGCAGTACCCCAATTGCTAATCTTAGCAACTTATTGCTGAGCAAATACTACTATTATAATAAGT
ATAAATTCTTTGTTAATACAAACATTTCAATTAATAAATACAAATACTTGTATTAATTACTTTTACAAACTaaaaaaaTGTTACAACTAAAGGAGATTACGTAGGTGACCAGATACA
ATGGAGGACAGAGT

Lst8 AGGGAGAGGGCCGAGAGCTGATCTTGGTGGCAGTGGTATGTGCTGATATCCTATcccccTTCAGAGCCTGGACCTGcccccTGGAGTCTCCTCAGACTTGCGCCAGCAAACATGC
ATGCCTAGATTTGAACAGTGGCATACCTAAGGTATCTACTATCCAGGGATCAAAGAAGATTTTGTAACCCTCCNACGAAGGAATCATACCtgattgattgattgattgattgattgattgatt
gattgatTTAAAAGTGTGCAACATGTATGAGCTGaaaaaTTTATTTGAAATAAAGAAGTGATATGAACCTTTATTAGCTGATGACTGAAATTCTAACTCATTTAATCTTAATTC

Lst9 GAAGCGTTTGTCACTCCCAAATTGGACTTTTCAGCCACACTAGACGATGTGTCAGAACCACCATCCAGAGCGCGATACCAAGGTTGCCAATGAgatgatgatgatgatgatgatgatgat
gatgatgatgatTTTATNAGTGACCTACAGNCTTTCCTTCTAGATGTGAAGGATATGACATCATAGAAGCATTATGCCTGTCATGTTTTGATCCTTTCTGTAGCTATGTTATCTCTGAC
ATCACTTCCTATAACTGGAGGGATGAACCCTATTTCACAGCTCCTGGCATAGGATTATTGGTTCTTTgggggggTGCGATATGCTACTTCATCAGCAAGTCAGAGTTCTGATGCATG
GTATATCTTTGTATTCACCAGATGCCAAG

Lst10 CCAAACTACATGTTTGGTCTTCTTGCTAATTCAAAGGATGCAGATCTTTTCCCCACTACTAAGAGAAACCATGTGGGGTCTCACAGCATGCAGGGNAGGAGGGATTAacacacaca
cacacacacacacacacTGCATACACCAATTCTACCAATCTAGTAAAGTTTTCATACCACCACTTGCCTATTTACCTGTTCACGTTTACCCTAATGGGCTATTGGTgtagtagtagtaTTTTG
AAGTTCAGAAGTCTCATTATGACATAATATTCGATTAGTGacacacacacacacacacacacacATACGacacacCGACCCANACACGCC

Lst11 AACAAATTTAAAAGTCACACTTTGGACAAGGACCACtttttCTCCAAATGCATTGGGACTTTTGTAAGTACTCCTTACATGCACTTAGGTATTGTTTGTGATGCTCGTTCGGTCCTTT
TCTGAATATGATTTATTCGATTGCTTTTCAGAAGGAAAAGCGAACTGTTTGtttatttatttatttatttatttatttatttatttaTATGCTGCCTTTCTATCCCTATAGAGGGCACTCAAGGCAGCTC
ACAAATAAAACCATCATGAAACATAAAATATGTTTNgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgt

Lst12 CTGAGTTTTCCCAAGTTTCAAGTGATGCAAAAGTTGAATTTTGCAGGAGGAAGGGTCTTCAGAAGGTCTTCTTTTCTGCATGTTTTGTTCATAGACAGTGTTTGCTAGTCTGATG
CTGCTTTTGGCACCATTTTCAACTTGAAAGGTGGTTATGTGCTGgtgtgtTTTTCCTGCTTGAACACTGTGAGCCCTTTTGGAATAGGCAACCACtgattgattgattgattgattgattgattga
ttgattgattgattgatTTATTGATTTTGCTATGAAACCACTTTTGGCTATAATAATTATCAACAATATCTATATGCCACTTTTCTACAAAGTGGTTTATAAAAGTTGTTGCTCTTAACAAT
ATTGTTCACGGTGAGGTCCCACCCAGTCAGGGATCACATCTTTAACAGGATGATGTCACAAcatcatcatCACCT

Lst13 TTGCTATCATGCTATGTCAGGTTTCTCGAGATCCCCAGTCAATGATTAGcaatcaatcaatcaatcaatcaatcaatcaatcaatCACCTCAGGGTGAACAGCCTCTATGGGGTAGGACAGG
CTTTTCTAGTCCCTATTATTTGGAGTTGCACAGGAGCaaaaaaGATACAGTACATGCAGTTCTAGGAAGTAACTGGtatatataTCCTAGATGGAGCTCTAGGAAATATCTGGTGGG
GCTTTTCTaaaaaaGGTTAGGCAAATATTAATTTTATGACCTAACTTTAAAACGTTAAATGCCTGATAATAGTCTGCTTGGAACATTATACCTAGTACTGATGATCCAAATTTCAGA
AGGATGAATTCAGATGAGGACAAGTAATCACAACCACAGATAGCCAAC

Lst14 GTACTTGTTCAAAAGGGAGAGTATGTGCTTCATTTAGCCTTAAGAACAATGCCTTCCTTCTAATAAAGTAAACTCCACAGTAACAGTCAGTGCAAAGAGCAATTCTTCCTCACAG
AACCACACCTTTGTTCCTTACAACTAAGATTCTTTTCCTTAATCACCTACTTTGTTTTCTTCTTAATGTTTTATCTTCTCTGAACTATAAGGAATTCTGAGAAAAGGGAAGAACTCAC
TACACAAAACAGAGAAAGTAAaacaacaacaacaacaacaacaacaacAGCTCCCCACATGCACAATTAAACCACCATCAAGGTTTCCTTAAAATACTGTGGTTCAAGATCACAAACAG
CCCAATGCTAAAACCGCACATTCTGGCATAGGATAGGGACTTTCCCAGACTATGCTGTTTGAGGAG

Lst15 CTGTATTTTATATTTGAATTTGTGACTTTGGGCCTTTTATTGTAAAAGGGAGAGGTGgaatgaatgaatgaatgaatgaatgaatgaatgaatGATATGGCCATAAGGGCCATAAGATAAAG
ATGGTTCCCAATGTCAGATTTCTTCGCTTAAATCTTGAAGGTTCCTGTAGGGAAGGAGGGAGGAAATAGTTGAAAGATACAATGGTTTATACAACCTGAATGGACAGTTACAGA

	TgacgacgacAAATGATTTCTTAGGAGCTCTGCAGTCACATGTTCATACCTGAGGCCTAAACTGCACATTACAGGGAAAATGTAGGGCTCTGCCAAACCGTATACTCTCCAGTAATCTTCTCTTACATCTGGAGAGCATCGCATTTCATCTCAGGGA
Lst16	GATGTTTGCTTTAAACAAAACAGAAGAATAGGGATTCTCAGGATGCTGAATTCAATACATCAGGGTGCAATAGGTGGCCTGAAAATGTTAACAACTAGGACATCAAGATTCCTTTCACAGGACTTCATGGTGTCAGCAACAGTGGGAGCTGTGCAGATTCTACAGTCCCAGAAAACATGGATGGGGCACTGAATCAGCTTCTTGAGAATCTCTGTGTTCCTTGaaacaaacaaacaaacaaacaaacaaacaaacTCCTAGTCCCTGAGGCTGCATTGAAGGCACATGGGCAAAAGTCTCAGCAGCTGGAGAGGTGGCTGAATAAGATTTCTTAATGCAAACAAGTGGCATTATGCCAAAATTCACTCAGTTTGTCTGACGCAGTGTTTCTCAAACTGTGGGTCGTGAGCCAATTTCAGGTGGGTCCCCATTCATTTTCAATAT
Lst17	AGTGCAATTTGAGATGATCCATATGATTGCAATCAGATTAtttttGTGTTGTTTTATCACTGTGCAGGAGCACAGTGTACATCgtgtgtgtgtgtgtgtgtgtGCATGTGTAGTAAAATACATGCACAGATCTCTTCCCCAGAGCTGTTATTTCCTGCAACTATATTTGATTCAAAAGAGACCTGGTCAGTCCTAAACACTTTGAGGAATGCTTTGAGGTGCATTTGAATTAGCAGGTGCTTTGGCCACATATGTGAGTCaggaggaggaggTATTTAATATCTGTTGAGTGATACTC
Lst18	GTGTACACCGCCCTGAGCTCTTTGGAGGAAGGGCGGTATACAAATGTAGtaaataaataaataaataaataaataaaTACTCCTGGGGTAAACGTACCCAGGCTGGGAATCACTGTTCAATAGGTTGACAGTCTCACTAAATCctctctCCAGAGTCATTGTAATTAAGATGACAACCTGCTGTGCATGTACAGAATTTGGCAAAGACAGGCAACCGCTACAATGCTAAGTGCCTTTCTGCCAGTCCTTGCATTTGTCCTATCGACACAGCGCTTACGGTTTTCCCACACCATATGCAGACTTGCCGAGGTCAGCTTCCGTAAATGAAGCAGACACCACACCTCTGCTGAAGCTGCATACAAGCTTAAATGATGCTGCATATCACTGTCTAATGCACAC
Lst19	CAATcccccAGCCTCATATACTAGCCTATGTAAACCTACATTAAAAGCCCAGCCTGTTTCACCAGGGTAATCAGAGGCATTAGTGGACTGCGGCATCACCTAGTGAACTGTTtgtgtgAATGACCCATTCTGAGAATGCAGTGCTTTCCAGTGGGCTGTTCACACTTGGGTCAGTCTcacacacacacacacacacacacaGGGTCCCTCCTCACTATAAGCATCCAAATTTGTCTTTCCTCGAGGGGACCGCTTTCATAGATGTTCCAATCTGCACAGTCACAGTTATTACC
Lst20	GTGCCAATGTGGCGTGCACTGCACCCTGCAATGGGGAGGCAGTCAAgggggCCTCCTCAAGGTAAGGGTATGCTTGTTACCTGACATTgggggCTGCATTGTCGCTAAGTCAGTGCTGGAAAGTTGATTAGGATTGTGCCCTAAAGTTCCTTGAAAGAGCTCATGACCTAGAGAAGACTCTGGGGTATTTTCTCAGTGCATCCTGCCTCCTTCTGCCCATCACTGCCTAACTCCCCcacacacacacacacaCCTCATTCCAAAAGTTGAGGAGCCCTCTTTGCTAGAAAAGAAAACAATCGCTGGGGTGACCATTGATCCTATGCAGCTCTGCCTAT
Lst21	AATATCCAGCCATGAGATACATTGTTATGTACCTActctctTCACATCGCATGATTTGCTAATGCTAACAGcacacacacacacacacaCTGAAAGTTACCCCAAATTCTTAGCAATATAAAGGGATTAATTGTCAGATACATACAGGTCTCTTACCAGATTAGTTAGTGAGGCAAGGTTTGAGCCCCACTTCCTAGAAAGGAAGTTCAGAACCCTGAATAGCACCTTTCTTTGCTAttttttAAAGACAGATCTCTAACAAAGAAACACTTTAAGGACCTGTATTGAACTGAgggggCAGAATTAAAATGAACTATTTCATAGCTCTTGATGAGG
Lst22	CCAAGAACAGTACAGTGCACAGCTAAAATGAAAGTCACAGAAATGGGTTGTAGAACCATCAACAAGCAAGGTGATGACCAAAGGCGGCATTAGACAATTTATAGATGGCTTTAAAGTTCAacacacacacacacacAGAATTGTGGTCATTTAATGACAATTCAGTGCTTTCTAGATTCTATCATAATGacacacAACAGATACATTAAAATTTTAAAATTAACATGCAGATTCAGGAAGAAATAGCAGTGCAATTAAAAGATTAGTATATTACATTATAGCAAACCTGAATTACAAGTGTGCCCTTACACCTCTGCGAGATCTGTTCTacacacacacacacacacacacacacacacA
Lst23	ACACAGTTCTTTTGCTTTTCATTCCCGTGGTGTGCAAtttttaaaaaGGAAAACACTAGGGAATGTCCTGCATTTAGAGCAATGTCTAGTTTGACACTGAGAGTGACTGGAATGGGgagagagagagagagaGCTGATAGCCAGTGGCTGATCAAAGTTGTCTTTGGGTGCAAGGCTGCTTTAGTGGGGACTGGATGTGATCTGTTGCTGACATGCAGGAATGTtgtgtgGCTTACTTCCACCTGATGACCCAAAGGTAAATAAAGCAGATAGTACAAAGACTCTATCT

Lst24 aaaaaTAGCATGGTGTATGTCAGACCCAATGCAGAAATGTTTCTGAAAAGGACAAGCACCAGTTTCAGAAAAGTAACAAAGAACTTACTTAAATCGTTCACTTAATTCCAGCAAG
GCTCCAGGATTTTCTGTAGCATGGaaaaaaCTGGATCATGTGGATCTGCAGATGTTTACAAATGTTTAGTGTGCAAAATAACAAGGCTTTAAATacacacacacacacacacacacaca
cGAGGAGAAGGAGCTGGATATAGAAACTGTAGTTGGCCAGCTTGTGGACAACATGGCCAAGCTATTTGAAATCTCTTTAGGGAGCAATAGTTTAGATAAACAAACTTTTAGGC
ACTACGGTAA

Lst25 CCTTAAAATAGACCAGGCATGTTGTGTTAGTAACAGCAAATACTATAACAACAGGTTCCTAGATGTGATATTTGAAGGATGTCTGTCATACGCCTGGTCTTATTTTACACCCCTTC
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GAACCAAACCACCTTAAATAGCATTGATGACACTGAAATCTTTACAAGCTTGACTCCCTTCCACATCCAACAGCAGTACAATAAAGTACATTTGCAGCCTCCCTATTCCCCAACTc
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Lst26 ATCCCATTAATGGTGTGCCCAAAATGAAAGTGAACGACAAGTAGATTTTAAATAATTCCTGTTTTCTAttatttatttatttatttatttatttatttatttatttatTCCCACCTTTCCTCTTCTG
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GAGTTcccccAGTAGGGAGTTCCATAACTGTGATGCCACTCCCGAAAAGGCTCA

Lst27 ATGCTGCCTTACTGTCCCTGTGATTAGGGCCTGTGGATTGCTGTAGCCActctctctctctctctctctctGGCCGAGCTACAGAGCACGAGGGGATACTCCTATAATTATTAGCATTCA
TTTTGCTGCATACCAAGGATGGGTACAAGTTAGTTCAGACATTATGATTATGACACAAGGCTCCTGTACGACGTTCCCTTGCGTACCAACTAGTTTAAAAC

Lst28 GCTCCGAGATTACAAAACTGCTGAAAATGCTAAAGCTTAGTTAGGATTGGGAAGTTCATTTGTAATTTTACTGCAATAATTTTCTACTGCTCTCCAAATGCTTGAGGAGGATTAC
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Lst29 ATAACAAAATACAAAGATTTACAAGTAGAAATTGAAAGGCTGTGGCagaagaagaCTAAAAGTGTTCCCAGTGGTGACTGCGCCCTCGGTGCTATTCCAAAACACCTTGAAGAGC
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Lst30 CTGGCCCACCAAATGCCCACCAAAGTCTCTTGTTGTCAGGGAGCACCCAAGACAAGAGGCTTGTACCCTGCAGCCACTCCCTTGCATCTGACATTCagagagagagagagagGCTAC
CTACAAAATCCTGAAGGTTGCATATAGTCTTCACGGTTTGTATCTCATGCTGGTACTTTTCCTCCATGAATCCATCCAACCTCCTTTAAAAGCATCACAACATTCTGTGGCAAGGT
AGTTTCACAGATTATTTAAATGCTAGGGTAAAGAAATTAtttttCTTTTGTAtttttCTAACTCCCTCAACTTCAGAGGATGTTCCCTAGTTCTGGTGTTATGTGATTCACTCTATCCATC
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Lst31 CCCTCTTTTCCCTTGCCTGTACTGGAAAAGGCAGGGAAAGCTGCCTCTTGGGTACAAAacacacacacacacacacacCCCTCCTTTTCAATTCATTCCTCAAAACCAGGTAAGGCCCC
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Lst32	CTGTGACAATCATGTACGCCTCTTGGTTTTCCCCTGCCTCCATCCCTTCCTGCTGTTACCCTCCCTTTGTGTTTAGATTGTAAGCCCGTTTGGGGCAGGGACCTGGTTCATAATTCTGTGAGTTCAGCAGTCACCAACCTCCTTTTCTCTACATTtaataataataataataataataataaTAGTACAGGTATTTCTATACCGTCCTTTCTTGGTCCTCAGTATTT
Lst33	GAGAAAGTTCTGTTAGGAGGGAAAGGGACCCCTTTGCATCTAGTCCAACAATACTAatgatgatgATAAGTTGGACCCAAAGAGTTCTTCCTTCTGAAAGGACAAGTTGGAGAAAGAGTCTCTTTTCTTGAaggaggaggaggaggaggaggaggaggaggaggGTAACCTTTAGATCCAACCAGATAATGATAATACCACGGAAGTCATTGACACTCCTTCCTATTTTCCCACTGAagagagCTATCATTGTTTATGAGATTGGAAGCACAGTAACTTTGCATAAATCTAAGGTCTGCAGCATGGGCCATTTGTGTTGAAATTTGTCtttttttGTCATTGCTCAAATGCAGCCTGAAAGTCTTTGATTCAACaaaaaaGCCTCCAAATGGTGGAGTgagagagagaAagagagCgagagaACCACTACAGTCTTGTACATCAGCCATTGATGACAAAA
Lst34	CACCTTGTTTACCAATGAGATGGAGACAGAAGGGGAGTGTTTAAAGAAAACTcacacacacacacacacaCCGGCAGCAGCCCCGTTAAAACTCCCCACAATCTGGCAATGGCCCAGGGTTTTGGGACACTTTTGGGAAGAGCTCAGCCAATTTTGTGCTCTGGTTAGTCTTCTAGAACAGGGGTCTTCAAACCATGACCTGAGGGCTGGATCCAGCTCACCAAGAAAAGCTTGTCAGGCACAGCGTGGA
Lst35	CATTaaaaaCATGAATTGGCACCCAACTAATAGAAGACTGACCAAACTTGCAGCCACTCCAGGCGCAATTGTGCGCTGcccccTCCATCAGGAGGAGTGGttttttCCTTTTAAAGTGAAAGTACTGCTGGCttatttatttatttatttatttatttatttatTTAAAGATAAATGGGCAGGAGGGAGGGGTGAGGGTGGCAGTGCTGCCCTCACAGGTGCGGCACCCTGGGCAATTTGCCCTTGTTGCCCCTTGGTATGCCACTGATGTATATGGGATAGTTCACAGATCCATATTAATCATTCACTGACTATTATCAAGCCATTCAGTTTGAttttttttCTGTAAA
Lst36	AAATAACCTTCACTGAAACCCTTCCAGGCAAAAGACGTCAATTAGCAGAAATTACCTGAATGACATATTGGCGGGGTgggggggAGGAGGATTTGCATGCAAAGCAGAGCCCCTAAATCACTATTAGGATTTTGGCTCTTTGAAAATATCCTTAATGGTCTCTTCCACCTCTAAAGAGCTCTCAGAAGTGCTTGCCAGTGGCGCACTATGCACAGTTTGTCAGGGCTCCTTCAAACGTGATGCACATGCTAAAACAGGAACATGCGCAAGACAAGAAGCAACCGTGTCTGCCTCtgtgtgtgtgtgtgtgtgtgATCTGAAAGCCTAATTGCCAGGCAGCGAGCTGG
Lst37	GAAGAAAACAGaaaaaGAAGGGTGTAAaggtgaggtgaggtgaggtgATGGCCTAGAGCTAGGATGCCAAATTTGTTTtatataGTGGGCTGAAGTTAGCATTTATGGCATCTGCTGAGGGCCaaaaaGTGATGTCATTAAGCAGGAAGTGATGTCATTAAACAGGTGGTGACCaaaaaTAAGCACTTTATTCGCACCAAGGAATTCACTTGGATCATATGTTTCAATGAAACtatttatttatttatttatttatttatttatttattACCTACTTACTTAATTTCTATCCCGCCTTTCTCCCCAAAGGGACCTAAGGCGTTTTGGGATATCAAAAGGATAGACTCTACAAAAGTGGTTCTCACATTTAGCGTCCAGGACCCACtttttAGAATGAGAATCTGTCAGGACCCACCGGAAGTGATGTCATGACCGGAAGTGACATCAT
Lst38	CAAGGGCAAGTGACGGACAAGCAGACTGTAACTGCCTCCTTTTATGTCCTGTTCTGAAGCAGAACACAAACCGGCTCTCAGGCACCCCAAGTGGACCAGGCAGCCAActctctctctctctctGCATATGAGACTTACCAGGAAGCCAAGCTGTGCGTAGGCCAGAGTAAGGATGGCGAAGGCCAGCGCTGCGCCAAAGAGCTCCTTTGCAGATCTCTGCAGAGTCTTGCCAAAGGTGGACCACTGACGGATGAAGCGCAGCTGCTGGGAGGCCtttttGGGAGCAACAGACAGGGAGGGAGTTGGACGGGGAACCCCAATGCCACCTTCCCAGCTCCACACCCGAAGGGACCAGTCAAGGTCCTGAGATGTATTTTCCACAAGACAGAAGACAGACAAGCTGTGAGTGG
Lst39	cacacaAAGACTTTTCTTTCTTTAAGAAACCTATGAAACCTGGTTTCATCTTTCACTTCTATCTAAGAGGGTATATGCAAATGAACTTACTGATAGCGTGCACTCCTTCCAAtctctctctctctctctctcTTTGTCTCAAATACTTCATTTGCAAAGCATGTTTTCCCCAgggggAGCATGACAGGATTTGATTCCCTTATTTTCTTCAATTAAATTAAACGGAGTGGTCACATCACGcccccAACGGAAGGAGGGAGGTCTTCTAGATCCTCGAAAGGAAGTGTAGTGGTCGTTACTTTACCaaaaaCGAAACGAACGTTaaaaaCTTTaaaaaaaaTTaaaaaaaTaaaaaTTaaaaaaTaaaaaTTTAAGGTAACGTAAC
Lst40	agagagTTCCCTTGAAACTAAACTGAGTTGTAAAGTTCATCTCAAAAGGGCATGGAAGCTGTGGTTgggggCAGAGTCCCATCTCATGTCAAATCAAGCAGATTGATTGTGGGgtgtgtgtgtgtgtgtgtGCTCACGTGCTATTTTGCATGTATGGATTTCTTTCCTTCAGTAGGGCAAGAAATGCAGCAAGTCCTCTTCTTTGGGCTCCAACAAGATGTAGCACATGGTAACTTCCCAAAGTACATTATCTGCTGTGCATCAGTGAGAAGTCCTAATAAGTAAATTTTCTTTTC

Lst41	ATAGATTGCTATGTGGGGATTTTGCTGGTGGAAACCATAAATTGTATCTGACTGGCACTCTCATATTAACAATTGTAATGGGAGCATTTTATGGCCGTGATATTTCCatatatTGCACTCAGGAATGATTTGAAATGCTCGACATGCAGCTAGCACATTtgtgtgtgtgtgtgtgtgtgtgtgtgtgACAAAGCATAATGTCAGTCTTTTAATTGCAGTCCCGGATAGCTCATTTGATACAAAATGTAAAGTCTGCGTTCCTCTTTAGACATGCAGAAATTATTTCCATGTGCTCTCATTTACCACTA
Lst42	GAGGCGGAGGGGCCGCTTGCATGCTGCAGTGAGGGTCACTGCAAAACTTAGTGCTTTGCAGGCTGTGCATATTCAGCTGCAGTGACAGAAAGGGGaataaataaataaataaataaataaataaataaataaataAGCTGTTGTAGCTGTTCTCCAAAGTTCTAATCCCTCCCAAAacacacTTCAAGGCACAC
Lst43	ACCTCTGGTAGGTCTGTGATGCAGAGCAGATACAGTATCTGACACCGTTGGGTTTCAACTAGTGAGTCTTGCCCTAACCTTCCAGTGGTACTAGCAACACCACTGCTATTTTCCTTAGTTTGTGTTTCTTTGTTTgagagagagagagagagagaGGAGGCGTAGTTGACCTAACCAAAAGACCTAACCaaaaaCGAAAACCTAACCTTCctctctCGTTCTT
Lst44	GTGCATAGAATTAGAGCCTCAGTTAGGGACGGCTGAGTTCTCCCTCACATTttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagttagTGCACAATCCTAACCCCTTATGTCAGTGCTTTCCAGCACTGACATAAGGACAATGCAGCTCTGAGGTAAGGGAACAAACATTCCCTTACTTTGAGGAGGCCTCTGTGAGTGACACCCAGCTGCAGGATGCAGCACACGTCCCATTGGC
Lst45	CCAATGTATCCTACTGTACTACATCACGTATATGCTGTTTTGCACAAGGAATGTTTATTTGTCTTCCAACTCCATTCTTTGATATTGGAAAATAACTTCTTCCTGATGTTGGTATTCTGCATTACAAGTTTTGCTTTGGGTGATAGTACTATTAACAAGTGTTTCTAGGAGTAATAAATCGTAATAAATTGTAATAaataataataataataataataataataataataataataataataataataataataataataataataataataatTAATAAAACAATTTACTCTTATTTCAGCCGTTATCCATAGAGTAAT
Lst46	CGGTATGCtttttAAAgggggggACGACTTGACTCCACTCCTCTACGGTCACTAAAAGGTACTTGGTGACTCAGAAAGTGcccccccATGATGGAGGGTCAAGTTGAGTCATGGAGGGCAGTGACTTGACAATTCAACTTGAGTCAAGCCATGCTGGACTTGCCCATCCCTGGTGTATacacacacacacacacAGAAGCGGCATCAGAGGTTAGGCAAGTCAGGCACTAGTACCCATGCCCATCAGA