Grazing as a management tool: Effects of varying intensity of sheep grazing on the endangered pygmy bluetongue lizard.

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"In a hole in the ground there lived a hobbit"

J.R.R. Tolkien, The Hobbit (1937)

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Abstract

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is an endangered scincid lizard only found in a few remaining patches of native grassland in the mid north region of South Australia. Pre-European settlement, these grasslands were a common habitat in the region and were grazed by native macropods. Today however, 98% have been converted into agricultural land and the remaining patches are primarily used for livestock grazing, which due to the high grazing intensity and the hard hooves of domestic ungulates can change both vegetation cover and composition in the grassland habitat.

The lizards inhabit spider burrows dug by lycosid or mygalomorph spiders and previous experiments with simulated grazing have shown that they prefer burrows with surrounding vegetation and that grazing leads to increased basking. It has also been shown that lizard burrows in bare ground deteriorate faster than lizard burrows surrounded by vegetation.

This thesis primarily investigated how different levels of sheep grazing affect the stability of lizard burrows and the availability of grasshoppers, the lizard's preferred prey. It also investigated how grazing affects the lizard's body condition and fecundity as well as their choice of burrows.

The results show that while burrows inhabited by lizards are more stable than empty burrows, increased grazing intensity makes all burrows more likely to deteriorate. Based on the studies in this thesis it is not clear if lizards prefer burrows with a particular vegetation cover. Generally, lizard burrows were found in less vegetated areas than the average of the paddock, but in the spring, when lizards are more likely to move between burrows, they showed a tendency to prefer burrows with more vegetation than the average. This indicated that burrow choices could be dependent on the availability of burrows and may not necessarily reflect what lizards prefer.

Grasshopper numbers were reduced by grazing, and lizard relative body mass also declined with increasing grazing intensity, although there did not seem to be a direct relationship between grasshopper numbers and relative body mass of the lizards, as the lizards were affected by grazing earlier in their activity season than the grasshoppers. Ultrasound scans of gravid female lizards showed a lower fecundity for lizards in hard grazed paddocks than for lizards in moderately grazed paddocks. This trend, which was not detected from observations of neonates in the burrows, is likely to be due to the neonates leaving their natal burrow before they were checked.

These results show that grazing affects the pygmy bluetongue lizard, and that an understanding of these effects is essential for the future management of the species.

Due to the lizard's cryptic lifestyle, finding their burrows in dense vegetation can be difficult and can cause large variations in monitoring surveys. A secondary aim of this thesis was to investigate the possible use of detection dogs in future surveys. It was found that one trained dog could detect pygmy bluetongue lizards in laboratory trials and during field training.

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.

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31/10-2016

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Thesis organization and candidate contribution

With the exception of a general introduction that sets the background for the thesis and a finalizing conclusion, this thesis consists of manuscripts that either have been published in peer review journals, are submitted to those journals, or are ready for submission. I conducted all the field work and most of the data organisation and analysis. My supervisor Prof. C Michael Bull reviewed and came up with helpful advice and editing suggestions to all manuscripts and chapters. Some manuscripts include other co-authors, who participated in various stages of the data analysis, and the editing process. In the list of manuscripts below, an estimate of the relative contribution of each co-author is given.

The thesis is organised into eight chapters with chapter one being the general introduction. Chapters two to five evaluate four different ways that pygmy bluetongue lizards were affected by different grazing intensities. The sixth chapter assesses the possibility of using trained sniffer dogs to survey pygmy bluetongue lizard populations. Chapter seven contains three manuscripts based on incidental behavioural and ecological observations, that may be of importance to the future management of this endangered lizard. Finally chapter eight contains a conclusion, including a short overview, an evaluation of the implications this thesis can have on the future management of the lizards and suggestions to future research derived from the work presented in the thesis.

Chapter 2:

Nielsen, TP, Fildes, S, and Bull, CM (2016) Does sheep grazing affect burrow choice of the pygmy bluetongue lizard (*Tiliqua adelaidensis*)?

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Chapter 5:

Nielsen, TP, and Bull, CM (2016) The impact of sheep grazing on the fecundity and timing of reproduction in the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*.

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Chapter 6:

Nielsen, TP, Jackson, G and Bull, CM " A nose for lizards; can a detection dog locate the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*)?"

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TPN 85%, CMB 15%

Nielsen, TP, Ebrahimi, M, and Bull, CM "A thirsty little lizard; Drinking by the pygmy bluetongue lizard"

Current status: Published, *Transactions of the Royal Society of South Australia* 2016. 140(1): 2-6

TPN 75%, ME 10%, CMB 15%

Chapter 1: Introduction

1.1 The loss of biodiversity

The exact number of non-microbal species on the earth is unknown but is estimated to between 2-50 million (Scheffers et al., 2012; Singh, 2002). Since life emerged on the planet, this number has constantly changed as new species evolved, while others became extinct. The current extinction rate is however probably 1000 - 10,000 times higher than the average background rate (Singh, 2002). This accelerated loss of biodiversity is impacting all the major ecosystems whether they are marine (Hall et al., 2000), freshwater (Dudgeon et al., 2006; Lemly et al., 2000) or terrestrial (Byrom et al., 2016; Kormann et al., 2015; Lion et al., 2016; Whitfield et al., 2016), and many species are likely to become extinct due to direct and indirect effects of human activities.

1.1.1 Fragmentation

Concurrently with the expanding human population, undisturbed natural habitats have become increasingly rare and habitat degradation and fragmentation have turned into one of the largest threats to biodiversity worldwide. Previous natural habitats are being converted into crop fields, grazing pastures or plantations (Lion et al., 2016; Liu et al., 2014), or in the case of wetlands and rivers, the water is being drained to be used for irrigation (Lemly et al., 2000). These activities leave only small fragments of natural habitat in a landscape dominated by humans. The effect of fragmentation is still to some degree unknown, even for some well studied or iconic animal groups like birds and many wild felids (Vargas et al., 2012; Zanin et al., 2015). Although some species seem unaffected by, or even thrive in the fragmented landscape (Lion et al., 2016), these fragments may not contain sufficient resources to sustain viable populations of many vulnerable species. This is the case for many large animals for example some of the large cats or the panda (Garbe et al., 2016; Zanin et al., 2015), but also smaller animals can be affected by fragmentation (Carvalho et al., 2008; Joern & Laws, 2013; Lion et al., 2016; Whitfield et al., 2016). These smaller species do not necessarily need large cleared areas to fragment their habitat and to separate populations. One study showed that a road through a forest habitat was enough to genetically separate two lizard populations (Tucker et al., 2014).

Loss of genetic variation and inbreeding is often a concern to small fragmented populations (Driscoll, 2004; Garbe et al., 2016; Mongue et al., 2016; Remón et al., 2013) although some species might be more susceptible to genetic effects than others. Studies of bats living in fragmented habitats indicate that mobile generalist species are less vulnerable to fragmentation induced genetic effects (McCulloch et al., 2013; O' Donnell et al., 2016). Some lizard species with natural low dispersal rates show no signs of inbreeding even in very small populations (Michaelides et al., 2015; Remón et al., 2013). It is suggested that these lizards have evolved to compensate for naturally low dispersal rates. They are more likely to mate with related individuals that are their neighbours, and to be able to persist as a species, have evolved fewer deleterious alleles and potentially a lower mutation load than species with higher dispersal rates and less chance of mating with related individuals (Remón et al., 2013). Alternatively these lizards may avoid inbreeding by choosing to mate with unrelated individuals (Remón et al., 2013).

The conservation value of a habitat fragment varies between species (Kormann et al., 2015; Lion et al., 2016) and to some species, particularly generalists, fragments of native habitat might be enough to sustain a population (Joern & Laws, 2013; Lion et al., 2016). Fragment size, shape, proximity to larger fragments or continuous habitat and quality of the surrounding habitat also affects which species utilize and inhabit a habitat fragment. Some species thrive better in square shaped fragments than in thinner strips of habitat of equivalent size (Driscoll, 2004; Lion et al., 2016; Stoner & Joern, 2004) while others can utilize fragments if they are connected to or in the proximity of a larger population, in a larger patch of habitat (Biaggini & Corti, 2015; Lion et al., 2016). Connections between fragments cannot however replace continuous habitat and Driscoll (2004) showed that despite apparent connectivity between the fragments, fragmentation of a woodland habitat still leads to a reduction in the local reptile populations.

1.1.2 Invasive species

"Exotic-", "Alien-" or "Invasive- "species are all terms that are used to describe species that have been introduced, intentionally or unintentionally, to an area where they are not native, and subsequently have established a population. These species may not have any natural predators, effective competitors or diseases in their new habitat and can in various ways cause serious loss of biodiversity (Byrom et al., 2016; Dudgeon et al., 2006; Whitfield et al., 2016). In Australia, well known invasive animal species include the red fox (*Vulpes vulpes*) and the feral cat (*Felis catus*). Both predate on native reptiles and small to medium sized mammals, and have had a huge negative impact on the populations of these species (Moseby et al., 2009; Moseby et al., 2011; Olsson et al., 2005; Read & Scoleri, 2015; Wayne et al., 2011). Invasive plants compete with natives and can locally change the micro habitat by creating a dense vegetation cover shading anything underneath (Carter et al., 2015; Hacking et al., 2014). Although these changes can create more cover and therefore less predation for small animals, some reptiles are excluded by these invasive plant species as the temperature underneath the vegetation gets too low for their ectothermic activity (Carter et al., 2015; Hacking et al., 2014). Pest control, targeting the invasive species can improve biodiversity (Byrom et al., 2016; Moseby et al., 2009; Olsson et al., 2005; Wayne et al., 2011), but as it is rarely possible to eradicate the pest species, this can be an ongoing and expensive effort. As many invasive species either provide an ecosystem function or assume the function of the native species they have suppressed, their removal might in some cases cause an immediate drop in biodiversity, as the ecosystem needs time to recover (Bateman et al., 2015).

1.2 Biodiversity in Grassland

Grassland and savannas used to be a common habitat type on most continents, covering 35-40% of the dry land (Joern & Laws, 2013). Prior to European settlement, large parts of Australia's southern temperate zone were covered with grassland or grassy woodlands (Dorrough et al., 2004; Lunt, 1991), but today human activities have greatly diminished this once vast habitat and only a few isolated fragments are left (Dorrough et al., 2004; Joern & Laws, 2013; Lunt, 1991; Prescott & Nicholls, 1998). In the mid-north region of South Australia it is estimated that only 2% of the original grassland is left and 98% of this remaining grassland habitat is privately owned (Prescott & Nicholls, 1998).

Native Australian temperate grassland is dominated by perennial grasses and forbs (Dorrough et al., 2004; Trémont & McIntyre, 1994) and although the majority of the biomass comes from grasses, a high proportion of the plant biodiversity comes from forbs (Trémont & McIntyre, 1994). Today most fragments of native grassland are however invaded by annual exotic grasses and forbs (Dorrough et al., 2004; Fleischner, 1994; Hacking et al., 2014; Trémont & McIntyre, 1994).

Most of the previous grassland has been converted into agricultural land, but farming also affects the few remaining patches of native grassland mainly through grazing from domestic live stock and fertilization (Dorrough et al., 2004; Fleischner, 1994; Prescott & Nicholls, 1998). A study by Kormann et al. (2015) showed that when the proportion of arable land in a grassland ecosystem was increased from 10% to 80%,

29% of plant and insect species were lost.

1.2.1 Effects of grazing

On a global scale, 25% of terrestrial ecosystems have been altered by grazing (Kay et al., 2016). Grazing has some ecological benefits and grazing by large native herbivores has always been one of the main drivers in renewing and creating variation in grassland ecosystems worldwide (Joern & Laws, 2013). However, grazing by domestic livestock often happens at much higher densities or frequencies than previous natural levels, and has the potential to cause dramatic negative changes to vegetation, soil structure and biodiversity in these ecosystems.

Before European settlement, the Australian grasslands and grassy woodlands were grazed by native macropods. These grazers probably had smaller population sizes in pre-European times, and their grazing, although it almost certainly had an important ecosystem function, would rarely have been intense (Lunt, 1991; Trémont & McIntyre, 1994). Today unintentional effects of farming, have resulted in a larger populations of some of these macropods (Lunt, 2005), but even though intensive grazing by kangaroos are known to be a danger to some endangered plants and animals, such incidents are rare (Howland et al., 2016; Lunt, 1991). Kangaroos and other native macropods also weigh less and have softer feet than hard-hoofed domestic ungulates, and the trampling effects of the native grazers are therefore smaller (Dorrough et al., 2004; Trémont & McIntyre, 1994). This lack of heavy ungulate grazers, might have made Australian ecosystems particularly susceptible to the impacts of introduced livestock (Lunt, 2005).

Since European settlement, grazing by domestic livestock has had a major impact on the remaining Australian grasslands and grassy woodlands (Trémont & McIntyre, 1994). But although grazing in general can change vegetation structure (Dorrough et al., 2004; Fleischner, 1994; Kahn et al., 2010; Lunt, 1991), remove soil litter (Fleischner, 1994; Germano et al., 2012), disrupt soil crust (Bertiller & Ares, 2011) and compact the soil structure, which can lead to increased erosion (Fleischner, 1994; Krümmelbein et al., 2006); different grazing regimes and livestock species have different impacts. Sheep are more selective grazers than cattle, and can therefore be more destructive to the plant species and communities they feed on (Lunt, 1991),. Different grazing regimes and resting times can also affect the impact of grazing (Kahn et al., 2010; McCosker, 2000). Some of the grazing regimes, used in Australia, and their possible effects are listed in Table 1.1.

The plant diversity of many grasslands are dependent on disturbances to create space for new and smaller plants and livestock grazing can reduce vegetation density and create open patches for smaller plants to grow in (Dorrough et al., 2004; Kahn et al., 2010; Lunt, 1991). In habitats where the large native herbivores have been displaced, domestic stock can in some cases provide a similar ecosystem function (Wright et al., 2010), which can maintain suitable habitats for birds, mammals and reptiles (Newbold & MacMahon, 2014; Rainho et al., 2010; Wright et al., 2010). In these cases, an absence of any domestic grazing would allow the vegetation density to grow to a thickness that could create unsuitable microhabitats (Hacking et al., 2014; Newbold & MacMahon, 2014) or prevent the small grassland animals from hunting, even when prey were abundant (Atkinson et al., 2005; Rainho et al., 2010; Wright et al., 2010).

Plants

In grasslands, grazing changes both vegetation structure and plant composition. While structural changes as a result of grazing are generally in the form of reduced vegetation cover and an increase in the amount of bare ground (Bertiller & Ares, 2011; Kahn et al., 2010; Trémont & McIntyre, 1994), the changes in plant diversity are far more complex and can happen without obvious effects on the dominant plant species(Fensham et al., 2014; Lunt, 1991). Some studies find that grazing reduces plant species diversity(Dorrough & Scroggie, 2008; Dorrough et al., 2004; Dorrough et al., 2007; Lunt, 1991; Romero-Schmidt & Ortega-Rubio, 1999; Stoner & Joern, 2004), others find that diversity is increased by grazing (Fensham et al., 2014; Pykälä, 2005; Rambo & Faeth, 1999) and others again find that the effect of grazing on plant species diversity depends on the interactions with other factors (Vesk & Westoby, 2001). When evaluating the effect of grazing on plant species diversity, it is important to take the scale of the observations into account. The increased variation in microhabitat caused by grazing might increase the number of available niches and therefore the diversity observed at a local scale, while at the same time, species vulnerable to grazing are becoming locally extinct, which lowers species diversity on a regional or global level (Landsberg et al., 2002).

At the species level, the effect of grazing is also context specific, as shown in a meta analysis of 35 published papers by Vesk and Westoby (2001), who found that some species showed positive responses to grazing in one or more studies but negative responses in others. With a few exceptions (Trémont & McIntyre, 1994), native Australian plant species do however, generally, seem to be more negatively affected by grazing than many exotic species, which is likely to be due to the evolutionary lack of adaptations among native plant species to any significant presence of large herbivores (Dorrough & Scroggie, 2008; Lunt, 2005). This has in many cases resulted in native perennial forbs and grasses being replaced with exotic annual plants (Dorrough et al., 2004; Lunt, 1991), which over the last 170 years has led to the elimination of grazing intolerant species in continually grazed areas (Dorrough et al., 2007).

Grazing regime	Definition	Effect
Continuous	Paddocks are continuously exposed to animals	Can cause both over and undergrazing dependent on stocking rate, which can lead to either increased erosion or increased fire risk respectively
Rotational resting	One or two more paddocks than there are herds. Rest may vary from weeks to years.	May defer effects of overgrazing by creating a reserve, but can lead to undergrazing
Rotational grazing	Three to seven paddocks per herd on fixed calendar based moves	Effects vary greatly with resting time and paddock/land quality
Multi-camp rotational grazing	More than 7 paddocks per herd. Paddocks are either grazed light or hard with calendar based moves.	Can reverse land degradation and increase the number of palatable plant species.
Time controlled grazing	Normally 20 to 40 paddocks per herd. Stocking rates are high and moves are not calendar based, but based on growth rate of the paddock and its physiological requirements for rest.	Can protect the plants and are in some instances based on the sustainability of the ecosystem.

Table 1.1 Summary of grazing regimes and some of their possible effects. Adjusted from (McCosker, 2000)

Insects

Grazing also affects the species diversity and abundance of insects and arachnids in grasslands (Fleischner, 1994; Joern & Laws, 2013; Kormann et al., 2015; van Klink et al., 2014), but neither group shows consistent responses to grazing over species or grazing regimes, and the grazing impact can interact with a large range of other factors (Joern & Laws, 2013). One of these factors is the species and even the breed of the domestic grazer. As different grazers cause different effects on the vegetation, they also affect arthrophods differently (Joern & Laws, 2013). Some studies of grasshoppers, find that certain species increase in abundance in grazed areas (O'Neill et al., 2003; O'Neill et al., 2010; Zhong et al., 2014), probably due to grazing livestock creating feeding opportunities that these grasshoppers can utilize (Zhong et al., 2014). Abundance of other insects and arachnids, including other grasshopper species, have however been shown to remain constant (Germano et al., 2012) or decrease by up to 10 times in areas used for grazing .(Dorrough et al., 2004; Kormann et al., 2015; O'Neill et al., 2003; O'Neill et al., 2010; Prieto-Benitez & Mendez, 2011; van Klink et al., 2014).

Reptiles

Although reptiles are rarely directly affected by grazing, the indirect effects of habitat changes can alter the local reptile species composition (Kay et al., 2016; Rotem et al., 2016; Zeng et al., 2014) and grazing can have great impact on individual species (Driscoll, 2004; Reading & Jofré, 2016). These indirect effects can include variation in food availability (Kormann et al., 2015; O'Neill et al., 2010; Prieto-Benitez & Mendez, 2011; Rambo & Faeth, 1999; Zhong et al., 2014), changes to ground temperature (Hacking et al., 2014; Newbold & MacMahon, 2014) and changes in predation pressure (Castellano & Valone, 2006) and parasite loads (Pafilis et al., 2013). Reptiles in different life stages can be affected differently by grazing and although individuals are found in an area, they are not necessarily able to successfully breed there (Reading & Jofré, 2016). As with plants (Landsberg et al., 2002) the increased heterogeneity in the vegetation structure, caused by grazing, can locally increase the diversity of reptiles (Rotem et al., 2016; Zeng et al., 2014) but might, on a regional scale, exclude species sensitive to grazing. Some generalist reptile species seem unaffected by grazing (Kay et al., 2016; Reading & Jofré, 2016; Wouters et al., 2012) or change their behaviour to suit the habitat changes (Wasiolka et al., 2010). Most reptile species are found to be more abundant in some specific grazing regimes than in other grazing affected habitats (Blevins & With, 2011; Driscoll, 2004; Kay et al., 2016; Reading & Jofré, 2016; Rotem et al., 2016; Waudby & Petit, 2015; Zeng et al., 2014). The effect of grazing, on a reptile species can be complex, as grazing can interact with climatic factors (Rotem et al., 2016). Some reptile species also respond to particular grazing parameters such as the duration, intensity and frequency of grazing, but different species in the same habitat can respond differently to these parameters (Kay et al., 2016), which makes it difficult to develop one grazing regime that will conserve the complete diversity of reptile species (Kay et al., 2016). Driscoll (2004) suggested that leaving unpalatable plants in a grazed area would reduce the impact of grazing on the reptile community, this could however divert more grazing pressure on to other adjacent plant communities and the reptiles that depend on these.

Future management

Different organisms and species all respond differently to grazing. Even within the same order or family, some species react positively and others react negatively (Fleischner, 1994; O'Neill et al., 2003; O'Neill et al., 2010). Species management gets even more difficult, when all the different factors that can interact with grazing gets included (Joern & Laws, 2013; Rotem et al., 2016; Vesk & Westoby, 2001; Zhong et al., 2014), which makes it impossible to manage any habitat to suit all species (Kay et al., 2016; Landsberg et al., 2002; O'Neill et al., 2003). It is therefore important that when a conservation management strategy is made, it is focusing on the specific needs of the species it is meant to conserve, and in order to conserve biodiversity of generalists as well as specialists a range of different management strategies will have to be applied to a number of different conservation areas(Kay et al., 2016; Landsberg et al., 2002).

This thesis focuses on one endangered reptile inhabitant of native grasslands in the mid-north region of South Australia, and asks what is an appropriate grazing regime to optimally manage its future persistence in the fragments of native grassland habitat that remain within its current distribution.

1.3 Biology and ecology of the pygmy bluetongue lizard

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) has an average snout-vent length (SVL) of 88.2 mm for males and 93.5 mm for females (Milne & Bull, 2000) and is the smallest member of the genus *Tiliqua*. The species was thought to be extinct until it was rediscovered in 1992 near Burra in the mid-north region of South Australia (Armstrong & Reid, 1992). Since then a number of populations have been found in this region and in 2012, 31 populations were known, all in isolated and privately owned patches of native grassland (Duffy et al., 2012). Habitat destruction is therefore considered the greatest threat to this species that is listed as endangered both Nationally under the EPBC Act and in South Australia under Schedule 7 of the National Parks and Wildlife Act 1972 (Duffy et al., 2012). Another risk to the lizard populations is genetic bottleneck effects in the small isolated populations, but although the populations show signs of isolation by distance, no signs of reduced genetic diversity that are symptomatic of bottlenecks have been found (Smith et al., 2009).

Pygmy bluetongue lizards live most of their lives in spider burrows dug by lycosid or mygalomorph spiders (Clayton & Bull, 2015; Fellows et al., 2009; McCullough, 2000; Milne & Bull, 2000; Milne et al., 2003b). They prefer burrows deeper than 30 cm and where the entrance width fits the size of their heads as closely as possible, but they can be found in burrows as shallow as 12 cm and deeper than 50 cm (Milne & Bull, 2000; Souter et al., 2007). A study by Fellows et al. (2009) showed that burrows occupied by lizards are significantly deeper than both empty burrows and burrows occupied by lycosid spiders, and the presence of deep burrows has been shown to be a determining habitat characteristic for the presence of pygmy bluetongue lizards (Souter et al., 2007).

The burrows serve as refuge from predators (Fenner et al., 2008; Milne et al., 2003b), extreme weather conditions (Milne et al., 2003b) and grassfires (Fenner & Bull, 2007) as well as ambush sites, from where the lizard can ambush passing insect prey (Ebrahimi et al., 2015b; Milne et al., 2003b). Lizards defend their burrows against conspecifics (Fenner & Bull, 2011b) and can stay in the same burrows for over a year (Bull et al., 2015). Several other reptile species also inhabit the grassland habitat, but the pygmy bluetongue lizards are unique in their use of spider burrows and are unlikely to compete with other reptiles for these refuges (Pelgrim et al., 2014).

While occupying a burrow, pygmy bluetongue lizards only leave briefly to stalk passing prey (Ebrahimi et al., 2015b) or to defecate, which is also likely to serve as territory marking (Ebrahimi et al., 2016; Fenner & Bull, 2011a). The lizards even stay in the burrow when it gets flooded by rain. During these conditions the lizards can stay submerged in the burrow with only the nostrils above the water (Ebrahimi et al., 2012). Burrows are only permanently vacated when outgrown by the lizard, deteriorating or during the mating season when males leave their burrows to find females (Fenner & Bull, 2011a; Milne et al., 2003b; Schofield et al., 2012).

The diet of the pygmy bluetongue lizard consists mainly of insects, but lizards have also been known to eat flowers of certain plants (Fenner et al., 2007; Milne et al., 2003b). Although the lizards prey on a wide variety of insects (Fenner et al., 2007), the main part of their food is grasshoppers (Ebrahimi et al., 2015b; Fenner et al., 2007) which are mainly caught so close to the burrow entrance that the lizard does not have to emerge completely from the burrow (Ebrahimi et al., 2015b). This strategy is most likely developed to avoid the risk of exposure to native predators such as eastern brown snakes (*Pseudonaja textilis*) and various birds of prey (Fenner et al., 2008), but introduced mammalian predators such as the red fox (*Vulpes vulpes*) and feral cats (*Felis catus*) are also likely to prey on the pygmy bluetongue lizards.

Pygmy bluetongue lizards are active during the Australian spring and summer (October - April) and spend the colder part of the year inactive in their burrows. The lizards are most active in spring (Milne et al., 2003b; Pettigrew & Bull, 2012) which is also their mating season (October - November) (Fenner & Bull, 2009; Schofield et al., 2014). During this season males move between burrows (Schofield et al., 2012) in search of females who are thought to lay out scent trails for the males to follow (Ebrahimi et al., 2014; Fenner & Bull, 2009). Once the male has found a female he tries to pull her out of her burrow and if successful, a short mating takes place on the ground next to the burrow (Ebrahimi et al., 2014; Fenner & Bull, 2009). Genetic analysis of neonates from the same litter has shown that a female can mate with several males during a mating season and that siblings can be fathered by different males (Schofield et al., 2014). From December until February lizards are less likely to move between burrows(Schofield et al., 2012) and the males body-condition decreases (Fenner & Bull, 2007; Shamiminoori et al., 2014). Females body-condition increases (Milne et al., 2003a) until, usually between late January and early March, they give birth to 1-4 live neonates inside the burrow (Milne et al., 2002; Shamiminoori et al., 2015). The neonates have a snout-vent length of 44-45 mm and weigh around 2 grams (Milne et al., 2002; Shamiminoori et al., 2015). The neonates only stay with the mother for a few weeks before they disperse to find their own burrows (Milne et al., 2002; Schofield et al., 2012).

Pygmy bluetongue lizards are endemic to their grassland habitat, but within this habitat they are not dependent on any specific plant or ground dwelling invertebrate community (Souter et al., 2007) with the exception of the burrow digging spiders (Fellows et al., 2009; Milne et al., 2003b). Many of the grassland patches that house pygmy bluetongue lizards are grazed and have been so for decades (Prescott & Nicholls, 1998). This in itself proves that pygmy bluetongue lizards can tolerate at least some level of grazing, although the grazing might affect the lizards in various ways. Some of these effects are negative. The lizards depend on spiders to dig their burrows and the number of existing and new spider burrows decreases in paddocks with grazing, compared to paddocks without grazing (Clayton & Bull, 2015). This effect seems to be the same in traditionally grazed and cell grazed paddocks (Sharp et al., 2010). It has also been shown that burrows with no surrounding vegetation tend to deteriorate faster than burrows with surrounding vegetation (Ebrahimi et al., 2012). Reduced vegetation cover has also been shown to affect both movement and hunting behaviour of the lizards. In simulated grazing experiments lizards avoided or spent less time near burrows without surrounding vegetation (Pettigrew & Bull, 2011). When there was less vegetation, they also moved around outside the burrows less often, but when they moved, they moved further away from their original burrow(Ebrahimi & Bull, 2015).

Basking and hunting behaviour were affected as lizards in simulated grazed burrows basked for longer, with a greater proportion of their body outside the burrow, and made more attempts to catch prey than lizards in ungrazed burrows (Ebrahimi & Bull, 2015; Pettigrew & Bull, 2012, 2014). However, in one experiment this difference in activity was likely to be the cause of a larger body mass increase in lizards from cages with grass than from cages without grass (Ebrahimi & Bull, 2015). Although lizards so far have been able to survive in grassland grazed by live stock, the long term effects of grazing, the effects of different intensities of grazing and how grazing interacts with environmental factors are still largely unknown. The area of habitat suitable for pygmy bluetongue lizard populations is predicted to shrink within the coming century (Delean et al., 2013) and in order to manage the lizard populations, relocations of lizards are very likely to be necessary (Fordham et al., 2012). Understanding the effects of grazing and how to manage current and coming habitats are therefore essential.

1.4 Study area and climate

The study took place at the "Tiliqua" property of the Nature Foundation of SA (Figure 1.1), near Burra, South Australia (33.67^{O}S; 138.93^{O}E). The surrounding area is characterized by rolling hills, and was predominantly native grassland previous to European settlement (Prescott & Nicholls, 1998). Today, the native grassland has largely been converted into cereal crop fields, with only a few isolated fragments of the native grassland left (Prescott & Nicholls, 1998; Smith et al., 2009) (including the Tiliqua site). These fragments are generally privately owned and almost all of them are used for live stock grazing (Prescott & Nicholls, 1998). A previous study of native grasslands in the mid-north region of South Australia (that includes Burra) showed that different grazing regimes can change both the vegetation cover and plant species composition (Kahn et al., 2010).

The study site is contained within one of the remaining fragments of native grassland, on a gently sloping east facing hillside (Figure 1.2). The vegetation is dominated by native grasses such as iron grass (*Lamandra spp.*), spear grass (*Austrostipa spp.*), and wallaby grass (*Danthonia spp.*) but is also heavily invaded by exotic species particularly wild oats (*Avena barbata*) and wild sage (*Salvia verbenaca*) (Souter, 2003). The property had been owned and run by several generations of the Reed family, before being set up as a conservation reserve. It has been subjected to sheep grazing for over a century, but has not been ploughed for decades (Chris Reed, personal communication). To allow different grazing regimes, six experimental paddock were created (size range 3.5-6.9 ha, Figure 1.1) each with similar elevation and slope

This study was conducted over three spring and summer lizard activity seasons starting in October 2012. From December 2012 temperature and rainfall at the study site were measured by an onsite weather station. Temperatures (Figure 1.3) varied between an average daily minimum temperature of 6.0°C in July and an average daily maximum temperature of 30.6°C in February. Monthly average rainfall was highest in June and lowest in March (Figure 1.4).

Figure 1.1. Map showing the location of the study site, and a satellite image of the study site with the six experimental paddocks.

Source: Area map adjusted from Google maps.

Figure 1.2. Top photo: Sheep grazing the field site in spring (early October 2013) when the vegetation is dominated by wild oats (Avena barbata). Bottom photo: Me working with Roxy the pygmy bluetongue lizard detection dog (late November 2014), in a hard grazed paddock where only a few of the native grass tussocks are left. In contrast, a moderately grazed paddock with dry wild oats is seen in the background.

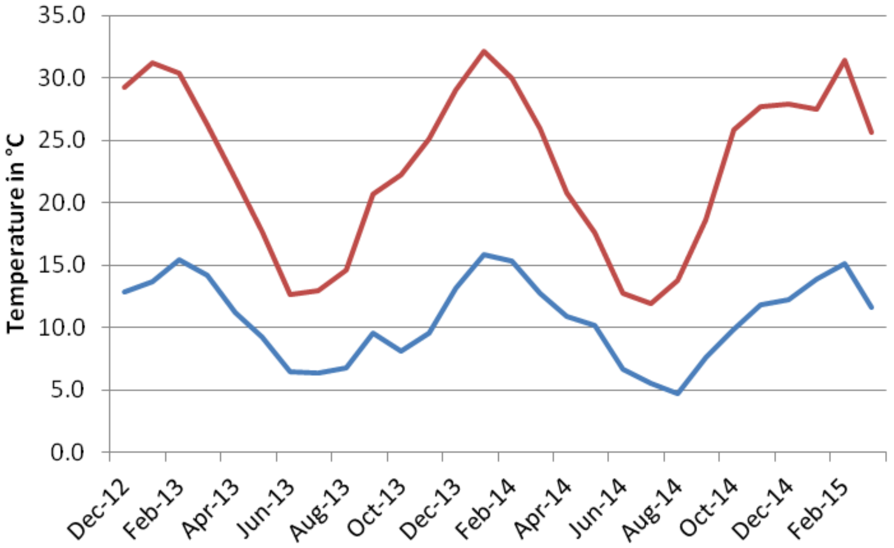

Figure 1.3. Mean monthly minimum (blue) and maximum (red) temperatures in degrees Celsius

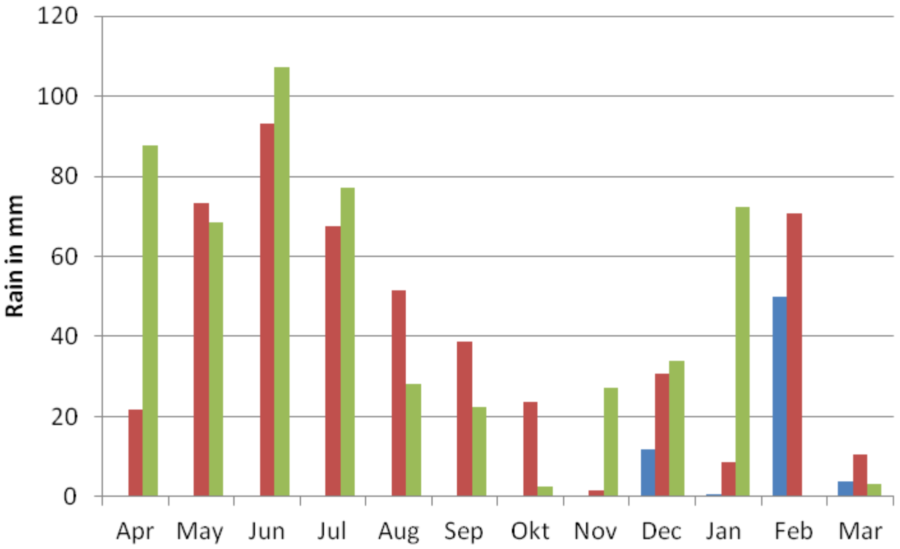

Figure 1.4. Rainfall in mm for each month between December 2012 and March 2015, divided into season 1 2012 – 2013 (blue), season 2 2013 -2014 (red) and season 3 2014 – 2015 (green).

1.5 Research aims and hypothesis

Previous studies of the effects of grazing on vegetation, insects and the pygmy bluetongue lizard have revealed some potentially detrimental effects, and some potentially beneficial effects for this endangered skink. However since grasslands were grazed by native herbivores prior to European settlement, some level of grazing is assumed to be necessary to maintain open spots in the vegetation cover that will allow the lizards to thermoregulate and hunt efficiently. Furthermore since lizards and grazing sheep have coexisted for probably over 100 years, some grazing is not fatal for lizard populations in some places.

The hypothesis of this thesis is therefore that an intermediate level of vegetation cover, following a moderate grazing intensity results in better conditions for the lizards than dense vegetation, resulting from no grazing, or very open vegetation with much bare ground, caused by very intense grazing.

Through experiments with different grazing regimes in six experimental paddocks, it is the aim of this thesis to investigate how the short term applications of moderate and hard/intense grazing regimes compare to no grazing, in relation to their effects on:

- Vegetation cover in the paddocks
- Availability of suitable lizard burrows
- Prey (grasshopper) availability to the lizards
- Lizard body condition
- Lizard fecundity

It is predicted that vegetation cover will decrease relatively to the increase in grazing pressure, leading to increased erosion and decreased durability of lizard burrows. The abundance of grasshoppers are also predicted to be reduced in response to the reduction of vegetation cover. Because lizards prey on grasshoppers but are likely to dependent on open spaces between grass tussocks to thermoregulate and hunt, it is predicted that lizards in paddocks with moderate grazing will have a better body condition and higher fecundity than lizards in paddocks with no grazing or hard grazing.

Through a better understanding of the positive and negative effects of different levels of grazing, this information should contribute to appropriate future management of the pygmy bluetongue lizards and their habitat, and hopefully help to insure the survival of this charismatic little lizard.

Chapter 2: Vegetation cover and burrow choice.

Today only a few percent of South Australia's native grassland is left, with the remaining parts of this once vast habitat being converted into agricultural land (Dorrough et al., 2004; Prescott & Nicholls, 1998). The remaining native grassland is heavily fragmented and almost all of these fragments are privately owned and used for live stock grazing (Prescott & Nicholls, 1998). Furthermore, the native grasslands that were once dominated by native perennial species are now heavily invaded by annual exotic species that change the vegetation composition (Dorrough et al., 2004; Lunt, 1991), and can create very dense vegetation, closing up open spaces between the native grass tussocks (Hacking et al., 2014; Trémont & McIntyre, 1994).

The native grassland was, pre-European settlement, grazed by native macropdos such as kangaroos and wombats (Lunt, 1991, 2005; Trémont & McIntyre, 1994). Grazing by domestic live stock is however occurring at much higher densities and frequencies than what used to be the case with the native animals (Trémont & McIntyre, 1994). As with exotic plant species, grazing also has the ability to change vegetation cover and composition in the native grassland (Kahn et al., 2010; Landsberg et al., 2002; Romero-Schmidt & Ortega-Rubio, 1999; Trémont & McIntyre, 1994).

Previous studies of other lizard species have shown that grazing and the subsequent changes to vegetation structure can be important habitat characteristics and can change the abundance and diversity of the local reptile community (Driscoll, 2004; Kay et al., 2016; Reading & Jofré, 2016; Rotem et al., 2016; Zeng et al., 2014). Vegetation cover also impacts the pygmy bluetongue lizard. A number of studies by Pettigrew and Bull (2011, 2012, 2014) showed that lizards preferred burrows with surrounding vegetation, but basked more in burrows where surrounding vegetation had been removed.

Knowing how different grazing intensities affect the grassland vegetation and how in turn this will affect the burrow choice of the pygmy bluetongue lizard is important, not only to ensure suitable habitat for the existing lizard populations but also to facilitate the establishment of future relocated lizard populations. This chapter aims to document the effects of three grazing regimes on the vegetation cover of the native grassland, and to investigate how these changes to vegetation cover affect the burrow choices of the pygmy bluetongue lizards.

2.1 Does sheep grazing affect burrow choice of the pygmy bluetongue lizard (*Tiliqua adelaidensis*)?

Abstract

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) inhabits burrows originally dug by spiders in a few remaining fragments of native Australian grassland. These fragments are typically invaded by exotic plant species and subject to livestock grazing. We predicted that lizards prefer burrows in areas with reduced vegetation cover that will allow them to bask and see their invertebrate prey. We applied alternative grazing regimes to six experimental paddocks and found that sheep reduced vegetation density proportionally to the grazing intensity. Within each paddock, independent of the level of grazing, and in seven of the eight surveys, lizard burrows were in locations with lower vegetation density than random points However lizards moving between burrows in spring showed a tendency to choose burrows with relatively more surrounding vegetation. Our initial hypothesis that lizards would prefer burrows with an intermediate level of grazing that produced some reduction of vegetation cover was not completely supported, probably because lizards often choose burrows early in spring when grasslands have denser vegetation cover, and then tend to remain in the same burrow as grazing or seasonal drying reduces vegetation cover. Changes in vegetation do not seem to induce lizard movement.

Introduction

Today, native grassland habitats often remain as isolated small fragments in an agricultural landscape (Dorrough et al., 2004). These habitat fragments are often grazed by domestic stock and invaded by exotic plant species (Dorrough et al., 2004; Fleischner, 1994; Germano et al., 2012), resulting in altered vegetative structures and a changed environment for native species (Carter et al., 2015; Castellano & Valone, 2006; Germano et al., 2012; Hacking et al., 2014). Invasive plants that increase vegetation cover can be advantageous by providing additional refuge and shelter for some small mammals (Torre et al., 2007), but disadvantageous to others (Romero-Schmidt & Ortega-Rubio, 1999) where more shaded habitat has lower ground temperature, and is unsuitable for reptiles (Carter et al., 2015; Hacking et al., 2014). This paper explores the impact of sheep grazing on the vegetation cover of a native grassland in South Australia, and how grazing induced changes influence the choice of burrow sites for the endangered pygmy bluetongue lizard.

Although grazing can be used to reduce overgrowth by exotic plants, and maintain native plant biodiversity within remnant grasslands (Dorrough et al., 2004; Rambo & Faeth, 1999), it can also change the plant species composition and facilitate the spread of the annual invasive plant species it is supposed to control (Bertiller & Ares, 2011; Dorrough et al., 2004; Fleischner, 1994). Additionally, grazing livestock may reduce plant litter, an important habitat structure for many animal species (Germano et al., 2012), increase erosion and soil compaction (Fleischner, 1994), and reduce the abundance of insects, an important food resource for many smaller vertebrates (Dorrough et al., 2004; Rambo & Faeth, 1999; van Klink et al., 2014).The response to grazing varies among lizard species. Some are more commonly found in open microhabitats created by grazing (Castellano & Valone, 2006; Germano et al., 2012; Newbold & MacMahon, 2014; Romero-Schmidt & Ortega-Rubio, 1999; Waudby & Petit, 2015), while others prefer the closed habitat of ungrazed areas (Blevins & With, 2011; Howland et al., 2014; James, 2003; Pafilis et al., 2013; Wouters et al., 2012). Those lizard species with lower preferred activity temperatures may tolerate the increased shade of non-grazed sites, and prefer the extra cover provided (Newbold & MacMahon, 2014), and the reduced risks of exposure to ectoparasite infection (Pafilis et al., 2013) and predation (Castellano & Valone, 2006). Lizards that occupy both grazed and ungrazed habitats, change their foraging behaviour and microhabitat preference in overgrazed habitat (Wasiolka et al., 2010).

The endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) occupies isolated patches of native grassland in the mid north region of South Australia. All known population sites are privately owned and used for livestock grazing. Adults of this moderate sized scincid lizard have a mean snout-vent length of 95mm (Milne, 1999). They inhabit single entrance burrows, originally constructed by lycosid or mygalomorph spiders (Milne & Bull, 2000; Milne et al., 2003b; Souter et al., 2007) and prefer burrows deeper than 15 cm (Milne & Bull, 2000). Although 12 other reptile species co-exist with them at our study site, pygmy bluetongue lizards are unique in their use of spider burrows and are unlikely to compete with other lizards for these refuges (Pelgrim et al., 2014).

Burrows are used as refuges from extreme climate and as ambush sites for passing insect prey (Fenner & Bull, 2007; Fenner et al., 2007; Milne et al., 2003b). The lizards are primarily insectivores, with grasshoppers being their major prey (Ebrahimi et al., 2015b; Fenner et al., 2007). They are solitary and territorial, and aggressively defend their burrows against conspecific rivals (Fenner & Bull, 2011b). Although they occasionally leave their burrows, briefly, to defecate (Ebrahimi et al., 2016) or to stalk prey for short distances (Ebrahimi et al., 2015b), burrows are only vacated permanently if they get too small, deteriorate, or in the spring mating season, when males move around between the burrows to find females (Fenner & Bull, 2011a; Milne et al., 2003b; Schofield et al., 2012). Previous experiments, simulating grazing by the removal of above ground vegetation from immediately around burrow entrances, showed pygmy bluetongue lizards preferred burrows with some surrounding vegetation (Pettigrew & Bull, 2011), but basked more and captured more prey in burrows with less surrounding vegetation (Pettigrew & Bull, 2012, 2014). Thus some reduction of vegetation by grazing might benefit lizards from increased opportunities to bask and to see and capture passing prey. Grazing costs might include increased exposure to predators, and reduced prey abundance.

In the current study we asked how the lizards choice of burrows was related to varying levels of vegetation cover resulting from sheep grazing. We hypothesised that lizards prefer to occupy burrows located in sites with an intermediate level of vegetation density. . This was investigated by manipulating levels of vegetation cover through varying sheep grazing pressure.

Methods

Study area

The study was conducted over three consecutive spring-summer lizard activity seasons (October-March; henceforth called season one, two and three) between October 2012 and March 2015, within the “Tiliqua” property of the Nature Foundation of SA, near Burra, South Australia (33.67^{O}S; 138.93^{O}E). The property contains an isolated habitat of native grassland, extensively invaded by exotic pasture plants. It is used for sheep grazing and has not been ploughed for cereal cropping for at least four decades. It also contains a population of pygmy bluetongue lizards, and has been the site of several previous studies of this species (Fenner et al., 2007; Milne & Bull, 2000; Pettigrew & Bull, 2011). The annual rainfall, measured from May to April with an onsite weather station, was 558 mm in season 2 and 528 mm in season 3, compared to an average annual rainfall at Burra of between 400-500 mm. A full 12 months of rainfall data from the field site were not available for season 1, as the weather station did not become operational until November 2012. The area has hot summers (average daily max = 30.6°C in Feb) and cool winters (average daily minimum of 6.0°C in July). In the analysis of vegetation cover, we focused on the four month period, December to March of each season, and temperature and rainfall in these periods are shown in Table 2. 1.

The study was conducted in six adjacent experimental paddocks (size range 3.5-6.9 ha) arranged in a north to south line along the eastern edge of the property, each with similar elevation and slope.

Grazing regimes

In each season we divided the six paddocks into two sheep grazing treatment groups, with different treatments applied to adjacent paddocks as shown in Table 2. 1. Kangaroos were rarely seen grazing in these paddocks, and we assumed their impact was limited and spread evenly across all paddocks.

In season one both treatment groups were left ungrazed. Over the winter between season one and season two, and then during all of season two, one group of three paddocks was left ungrazed and the other had moderate grazing that left some vegetation in the paddocks. This is the traditional way of grazing the area, and we relied on the advice of the former owner of the property to maintain the correct level of grazing. To do this, sheep had to be rotated in and out of paddocks at irregular intervals during the season. In season two the treatment group with moderate grazed paddocks were stocked at a mean of 2.70 (SE = 0.11) sheep per ha per day.

Then from early in season three, we retained moderate grazing in the treatment group that were moderately grazed in season two, but now with a mean of 1.54 (SE = 0.15) sheep per ha per day, to accommodate the farmer's perception of changed food conditions. We also applied hard grazing to the previously ungrazed treatment group, to simulate overgrazing or a drought scenario. In this treatment group the paddocks were grazed as hard as possible, without starving the sheep, with a mean of 2.69 (SE = 0.26) sheep per ha per day. Although this hard grazing treatment in season three was not much greater than the moderate grazing treatment in season two, it reflected a real increase in grazing pressure on the reduced amount of vegetation in that season. Grazing was applied during the wetter months of each year and infrequently during most of each lizard activity season from Oct – March.

Vegetation cover

We used multi-temporal high-resolution imagery acquired by the Pléiades satellite to map the variation in the proportion of ground cover in response to the different grazing regimes in the six experimental paddocks. Remotely sensed vegetation indices, such as the Normalised Difference Vegetation Index (NDVI), typically measure the contrast between the red and near-infrared reflectance of photosynthetically active live green plants. These so-called 'greenness' indices have been widely used to identify green vegetated areas and their condition (Kawamura et al., 2005; Tsalyuk et al., 2015). However, they are less able to measure variations in vegetation cover (and thus grazing impacts) in areas where non-photosynthetic vegetation ('dry' plants and plant litter) is present (Xu et al., 2014). This is typically a major component of vegetation structure in semi-arid pastoral lease areas of southern Australia, where both green plants and non-photosynthetic vegetation provide key habitat for a range of animal species.

Instead we used a ‘fractional ground cover’ index developed by Graetz et al. (1983) to measure the proportion of ground cover, taking account of the presence of both photosynthetic and non-photosynthetic vegetation. It is a simple but effective model when applied to a small area where the variation in soil type (and thus brightness) is unlikely to change or is at least minimal. Moreover, adapting this method to a range of image types, including the Pléiades satellite image data used in this study, is relatively straightforward.

Multi-temporal Pléiades satellite imagery was supplied as an orthorectified pan-sharpened product where the higher resolution panchromatic band was merged with the lower resolution multispectral image bands. This allowed high-resolution mapping with a pixel size of 50 x 50 cm and an accuracy of up to 8 m. Notably, the Pléiades revised pan-sharpening method almost completely retains the original spectral information yielding high-resolution multispectral images (Astrium, 2012). The pan-sharpened images were derived from calibrated top-of-atmosphere (TOA) reflectance, normalised for earth-sun distance and solar elevation changes across the time series. A total of 16 images were acquired at different dates across the study period, including three images in season one, six images in season two and seven images in season three (See Appendix A).

Pre-processing included minimising local geometric distortions in each image using sub-meter DGPS positions of landscape features visible in the satellite images to derive polynomial corrections in ArcGIS Version 10.1 (ESRI). Subsequent analysis of 47 control points, not used in the polynomial correction, showed a mean accuracy of 0.78 + 0.07(SE) m. A relative radiometric correction was also applied to each band of every image in the time series using linear regression of Pseudo Invariant Features (PIFs). This process adjusts the gain and offsets of each image against a reference image to normalise radiometry and allow relative comparisons of vegetation cover values between dates. Finally, roads and other artificial features not related to grasslands were identified and masked from further processing.

Following Graetz et al. (1983), a ground cover index layer was generated for each image date to allow comparison of vegetation cover response to different grazing treatments in individual pixels

(Figure 2. 1). Mean red and near-infrared reflectance values from several known bare soil (~0% ground cover) and heavily vegetated areas (~100% ground cover) were calculated to define the trajectory and length of the soil line upon which all pixels were projected to calculate relative vegetation cover.

To compare vegetation cover between the two grazing treatment groups at any one time, we selected 100 random points in each paddock and recoded the vegetation cover index value at each corresponding 50 x 50 cm pixel location. We used the same random point locations throughout the vegetation cover index time series in our analyses. We then used repeated measures ANOVA (Split plot design, SPSS ver. 23.0) on the vegetation cover index values during December, January and March, in each of the three seasons. We chose those three months because we had satellite imagery at those times in each of the three seasons. In these analyses, within-subjects factors were season and month, with grazing treatment as a between-subjects factor. We used separate analyses to compare season one with season two and season two with season three. We tested the data for sphericity and applied the Greenhouse-Geisser correction in cases where sphericity could not be assumed.

Burrow surveys

Eleven burrow surveys were conducted, three in season one and four in each of season two and three (Appendix B). Each survey took no more than three weeks in seasons two and three, although the first and second surveys in season one took about six weeks to complete. In the first survey (October-December, 2012) each paddock was searched systematically by walking straight transects across the paddock using landmarks such as poles and bare ground patches as reference points. Because burrows may have been harder to detect in areas with dense vegetation and reduced ground visibility, the distance between adjacent transects was kept smaller in these areas than in less vegetated areas. Paddocks were searched until at least 50 burrows (range 50 – 53), considered to be suitable for lizards were located. Burrows considered suitable were spider burrows at least 15 cm deep and with a narrowest entrance diameter greater than 1.2 cm (Milne & Bull, 2000). Each burrow was marked with a plastic peg placed 30-40 cm in a standard direction from the burrow entrance, and its GPS coordinates were recorded. An optic fiberscope was used to inspect the inside of the burrow, to determine if it had a pygmy bluetongue lizard occupant, following Milne & Bull (2000). In each of subsequent surveys, all previously identified suitable burrows were relocated, and checked for inhabitants and burrow condition. Some burrows had deteriorated in condition, often getting filled in with debris so that they were no longer lizard suitable. These were excluded from the following surveys and we aimed to locate an equivalent number of new lizard suitable burrows. The aim, to maintain a stable number of about 50 surveyed burrows in each paddock in each survey, was not always possible within the time limits of the surveys as lizard burrows are cryptic and difficult to find.

For analysis we defined burrows as occupied or not occupied by a lizard in each survey, and as used or not used if they were occupied by a lizard in any survey. In each survey the sample of “occupied” burrows was a subsample of the “used’ burrows.

To explore whether vegetation cover influenced burrow location, we used repeated measures ANOVA (Split plot design, SPSS ver. 23.0), with analyses run separately for each of the two treatment seasons (season two and three). We compared the mean vegetation cover index around "suitable" lizard burrows to the mean vegetation cover index of the 100 random points in each paddock. We used survey number and location category (burrows or random points) as within subject effects and grazing treatment as a between subjects effect. Greenhouse-Geisser correction was applied in cases where sphericity could not be assumed.

Similarly, to determine whether vegetation cover influenced the sub-set of suitable burrows that lizards chose to occupy, we compared the mean vegetation index of "used" burrows to random points. Because these two analyses (comparison of random points with all “suitable” burrows or with “used” burrows) resulted in identical results in regards to the significance of included factors, we only report on the comparison of “used” burrows and random points. We repeated the analysis again comparing “occupied” burrows in each survey with random points, and finally we compared vegetation cover of suitable burrows that were “occupied” or “not-occupied” in each survey.

Results

Grazing regime

Grazing had a significant impact on vegetation cover (Figure 2. 2). In comparisons between season one and season two, treatment group 1 (moderately grazed in season two) always had lower vegetation indices than treatment group 2 (ungrazed in both seasons), but that difference became greater in season two after the grazing was imposed (Figure 2. 3). This resulted in a marginally nonsignificant (P = 0.052) interaction between season and treatment, and a highly significant main effect of treatment (Table 2. 2). There was also a highly significant interaction between season and month. While the vegetation index decreased from December to March in season one, it increased over the same period in season two (Figure 2. 4).

In comparisons between season two and season three, treatment group 2 (ungrazed season two; hard grazed season three), went from having higher vegetation indices in season two, to lower vegetation indices in season three, when compared to treatment group 1 (moderate grazed in both seasons) (Figure 2. 3). This led to a significant interaction between season and treatment (Table 2. 2). There was also a significant main effect of season, with lower overall vegetation indices in season three when hard grazing was imposed, and a significant main effect of month with consistently lower vegetation in December of each of the two seasons (Figure 2. 4).

Burrow survey

In seven of the eight surveys, “used” burrows were located in less dense vegetation than random locations in all paddocks (Figure 2. 5). Our focus in the analysis was whether there were any significant differences (main or interaction effects) in vegetation density between the two location "categories": used burrows and random points. In both seasons we found a significant survey x category interaction (Table 2. 3). In season two, “used” burrows were located in denser vegetation than random in the first survey, but in less dense vegetation than random in the next three surveys (Figure 2. 5). In season three “used” burrows were always in less dense vegetation than random, but the difference was greater in the third survey of that season (survey 10) than in the other three surveys (Figure 2. 5). Additionally there was a significant three way interaction (survey x category x treatment) in season three (Table 2. 3). In all four surveys, points in hard grazed treatment group had less vegetation than in the moderately grazed treatment group (as expected), and in all four surveys lizard “used” burrows had less vegetation than the random points in the same treatment group, but the extent of those differences varied among the surveys (Figure 2. 6).

Importantly, for our hypothesis, there were no significant category x treatment interaction effects. That is, the difference in vegetation density between burrow locations and random points remained consistent across the two grazing treatments that were applied in each season.

Our second comparison, between the subset of “used” burrows that were “occupied” by a lizard in each survey and random points found significant survey x category interactions. In both seasons the trends were identical to those shown for “used” burrows (Appendix C).

Finally, whether lizards occupied or did not occupy a burrow had no detectable impact on the mean vegetation index around that burrow. The analysis showed vegetation was not significantly affected by any main effects of category (“occupied” or “not-occupied”) or by any interactions of other factors with category in either season (Appendix D).

Discussion

How does grazing affect vegetation cover?

We found two clear results. First, grazing reduced vegetation cover. This result is simple and predictable, but confirms that our assays of vegetation cover produced biologically sensible results. Second, in seven of the eight surveys, the locations of lizard burrows had less vegetation cover than random points in the paddocks, irrespective of the grazing treatment. Our results showed a significant impact of sheep grazing on the vegetation cover in the experimental paddocks. Within each of the two treatment seasons, the treatment with the highest grazing intensity had the lowest mean vegetation cover index value.

Season three had lower overall vegetation cover than season one and two, which most likely resulted from all of the paddocks being grazed in season three, and because three of them were hard grazed, compared to season one and two where all or some paddocks were left ungrazed. Lower rainfall in season three than in season two may also have contributed to the difference in vegetation cover density. Similarly, different rainfall patterns are a likely explanation of why vegetation cover decreased from December to March in season one, but increased over the same period in seasons two and three (Appendix A). There was less rain between December and March in season one compared to the following seasons (Table 2. 1). The decrease shown between January and March in season three, is likely to be caused by grazing applied to both treatments from mid-February 2015 and lack of rain after the sheep were put into the paddocks.

These results indicate that in a semi-arid grassland habitat, timing of the grazing in relation to rain and other environmental factors, could be just as important for the effect on vegetation cover, as the density of livestock. Our results do however confirm two major assumptions of the study. First, the alternative grazing treatments had the assumed outcome. Under similar environmental pre-conditions, more sheep ate more vegetation and resulted in lower vegetation density on the ground. Second, the satellite imagery, and the derived vegetation cover indices could be used at the 50 x 50 cm pixel level to document expected changes in vegetation cover. The trend for grazed paddocks to have lower vegetation density has been separately ground truthed by comparing dry weights of all above ground vegetation from small sample quadrates in each of the paddocks (Clayton & Bull, 2016).

Burrow choice

Our hypothesis was that lizards would prefer intermediate levels of vegetation around the burrows they chose to occupy. Thus in hard grazed paddocks we expected lizards would prefer burrows with a higher vegetation cover index than random, while in moderate grazing they would choose burrows with similar vegetation cover to random points, and lizards in ungrazed paddocks should prefer burrows with less vegetation cover than random. Although vegetation cover was found to be an important environmental factor in the habitat choice of the desert horned lizard (*Phrynosoma platyrhinos*) (Newbold & MacMahon, 2014), a lizard with a similar ecology to the pygmy bluetongue lizard. We did not find statistical support for the predicted outcomes in significant interaction effects between treatment and location category in each season. Instead we found a consistent trend over almost all surveys, in both season two and season three, for suitable burrows, used or unused by lizards, to be in places in paddocks where vegetation cover was lower than at random points in the same paddocks. The same trend was found in ungrazed, moderately grazed and hard grazed paddocks. Our analyses all showed similar results, whether considering all suitable lizard burrows, used lizard burrows, or just those burrows actually occupied by a lizard at the time of a survey.

There is a possible bias in our surveys as we may have under-sampled cryptic burrows in denser vegetation. However, we suggest the trends are biologically valid because we conducted closer searching in dense vegetation. Furthermore, in the first survey, when most of the burrows were initially identified, the locations of the burrows were in places with higher or equal vegetation density as random points. It was only in later surveys, after the burrow sites had been established, that significantly lower vegetation around burrows became apparent. That is we observed vegetation density becoming less dense than random points after we had established the burrows we were surveying.

One explanation of this trend is that pygmy bluetongue lizards choose burrows in sites that will become open habitat because they will provide better opportunities to thermoregulate and hunt. Reductions of vegetation cover, caused by large grazing mammals, have previously been shown to improve habitat quality by providing access to prey for both birds and mammals (Rainho et al., 2010; Wright et al., 2010). However, since the lizards are incapable of digging their own burrows, they are dependent on spider burrows and thus might not have access to burrows in their preferred habitat. Spiders are likely to choose open areas where they have access to bare ground and where the risk of vegetation overgrowing their burrows is smaller. A burrow can also be constructed by a spider months or years before a lizard moves in (Fellows et al., 2009), and in the meantime vegetation cover can change as a consequence of environmental conditions or sheep grazing. Our results might therefore not be explained by the preferences of the lizards, but more by the availability of burrows.

Although other and more mobile species such as the skylark (*Alauda arvensis*), have been shown to move between different farmland habitats, to suit their varying seasonal requirements (Hiron et al., 2012). An alternative explanation is that pygmy bluetongue lizards actively choose among available burrows and prefer those with intermediate levels of surrounding vegetation when they are searching for a new burrow, but that, once established in a suitable burrow, they will rarely vacate that burrow based on local changes to vegetation cover. For pygmy bluetongue lizards, suitable burrows can be a limiting resource that they defend against conspecifics (Fenner & Bull, 2011b), and lizards have been known to stay in the same burrow for more than two seasons (Bull et al., 2015). Even though previous experiments with altered vegetation immediately around burrows by Pettigrew and Bull (2011) indicated that vegetation cover affected the burrow choice of lizards, once they have become established, lizards commonly persist in their burrows through periods of drought, without the possibility of moving out of the dry areas. Therefore, a single season of grazing might not be enough to make them move.

In the first survey of season two, lizard burrows had higher vegetation cover than random points, but from the second survey and forward, this trend was reversed. A similar trend was found in season three, where the difference in vegetation cover between burrows and random points in hard grazed paddocks was smaller in earlier surveys (Figure 2. 6). This indicates that those lizards that move between burrows at the start of their activity season (October -November), may prefer burrows with higher levels of surrounding vegetation cover than random, but as lizard movement between burrows is infrequent between December and March (Bull et al., 2015; Schofield et al., 2012), these movements are an unlikely explanation to the difference in mean vegetation cover between burrows and random points in this late part of the activity season.

Pygmy bluetongue lizards might have burrow selection behaviour that leads them to find a suitable burrow in the beginning of their activity season, and then stay there as long as possible.

Moving at the beginning of the season when vegetation cover is denser, gives more cover from predators (Castellano & Valone, 2006) and, because it is cooler then, reduces the chance of getting exposed to extreme high temperatures outside the burrow. The way vegetation cover changes through the season in these grazed native grasslands might also be evolutionarily "novel" to the lizards. Most grassland areas are invaded by exotic plant species, which create different and denser vegetation cover than native plants (Hacking et al., 2014) and lizards may simply not have adjusted their burrow choice behaviour to this habitat change.

In summary, this study has shown a consistent trend for less vegetation around lizard burrows compared to random points in the same treatment groups. We suggest this may not necessarily be the preferred habitat for the lizards, but could result from the effects of invasive plant species or limits set by where the spiders choose to dig their burrows. Our simple comparison of locations of lizard burrows in treatment groups with different grazing regimes could not tell us the consequences of any constraints imposed on the lizards by the places where spider burrows become available. Future research should therefore focus on the fitness effects for lizards living in burrows with varying vegetation cover, or with different grazing regimes. If the natural habitat changes, more mobile species can move to different suitable habitats but pygmy bluetongue lizards are faced with habitat fragmentation and limited dispersal capability. As most of the surviving habitat of the pygmy bluetongue lizard is used for grazing, the results presented here, along with future research that will improve our understanding of how grazing affects the lizards, will be essential for the conservation and management of this endangered skink. Furthermore our results adds to the general understanding of the environmental factors involved in micro habitat selection of grassland fauna. Grazing are applied to a large part of the remaining grassland and have altered 25% of the terrestrial ecosystems (Kay et al., 2016), it does therefore affects the habitat choice of many grassland inhabitants (Castellano & Valone, 2006; Germano et al., 2012; Howland et al., 2014; James, 2003; Newbold & MacMahon, 2014; Rainho et al., 2010; Wright et al., 2010). Although the affect of grazing can be both positive and negative, it is still unknown exactly how different levels of grazing will affect many of the species that are dependent on the grassland habitat.

Acknowledgements

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	Mean daily air temperature °C	Rain (mm)	grazing pressure paddock 1,3,5	grazing pressure paddock 2,4,6
Season 1 2012/3	20.9	66.2	ungrazed	ungrazed
Season 2 2013/4	20.9	120.6	moderate	ungrazed
Season 3 2014/5	19.7	109.6	moderate	hard

Table 2. 1 Mean air temperature, rain (measured by an onsite weather station) shown for December to March, and grazing pressure (ungrazed, moderate grazing or hard grazing) in each of the two treatment groups, in each of the three seasons

	Factor	D.f.	F	P
Season 1,2	Season	1, 4	0.089	0.780
	Month	2, 8	15.128	**0.002**
	Treatment	1, 4	38.351	**0.003**
	Season*Treatment	1, 4	7.547	0.052
	Season*Month	2, 8	34.576	**< 0.001**
	Month*Treatment	2, 8	1.565	0.267
	Season*Month*Treatment	2, 8	0.547	0.599
Season 2,3	Season	1, 4	12.779	**0.023**
	Month	2, 8	16.115	**0.002**
	Treatment	1, 4	0.706	0.448
	Season*Treatment	1, 4	22.444	**0.009**
	Season*Month	2, 8	3.754	0.071
	Month*Treatment	2, 8	0.144	0.868
	Season*Month*Treatment	2, 8	1.243	0.339

Table 2. 2 Repeated measures ANOVA (Split plot design) comparing the average vegetation cover (derived from 100 random points) in each experimental paddock, between treatment groups for satellite images acquired during December, January and March of each season. Comparisons are made between the two first and the two last seasons separately.

	Factor	D.f.	F	P
Season 2	Survey	3, 12	24.890	**1.940E-5**
	Category	1, 4	0.191	0.685
	Treatment	1, 4	53.353	**0.002**
	Survey *Category	3, 12	3.961	**0.036**
	Survey *Treatment	3, 12	5.872	**0.010**
	Category*Treatment	1, 4	1.963	0.234
	Survey*Category*Treatment	3, 12	0.171	0.914
Season 3	Survey	3,12	5.701 ^{GG}	0.057
	Category	1, 4	14.998	**0.018**
	Treatment	1, 4	20.480	**0.011**
	Survey *Category	3, 12	5.480	**0.013**
	Survey *Treatment	3,12	0.712 ^{GG}	0.473
	Category*Treatment	1, 4	0.714	0.446
	Survey*Category*Treatment	3, 12	4.474	**0.025**

Table 2. 3 Repeated measures ANOVAs for season two and season three comparing the average vegetation cover index per paddock between random points and "used" lizard burrows (lizards observed in the burrow in at least one survey) for season two and season three separately. Degrees of freedom (d.f), F-value and P-value are shown for the within subjects effects, survey and category (lizard burrow or random point) and the between subjects effect treatment, as well as any cross effects. ^{GG} indicates that sphericity could not be assumed and the Greenhouse-Geisser correction has been used.

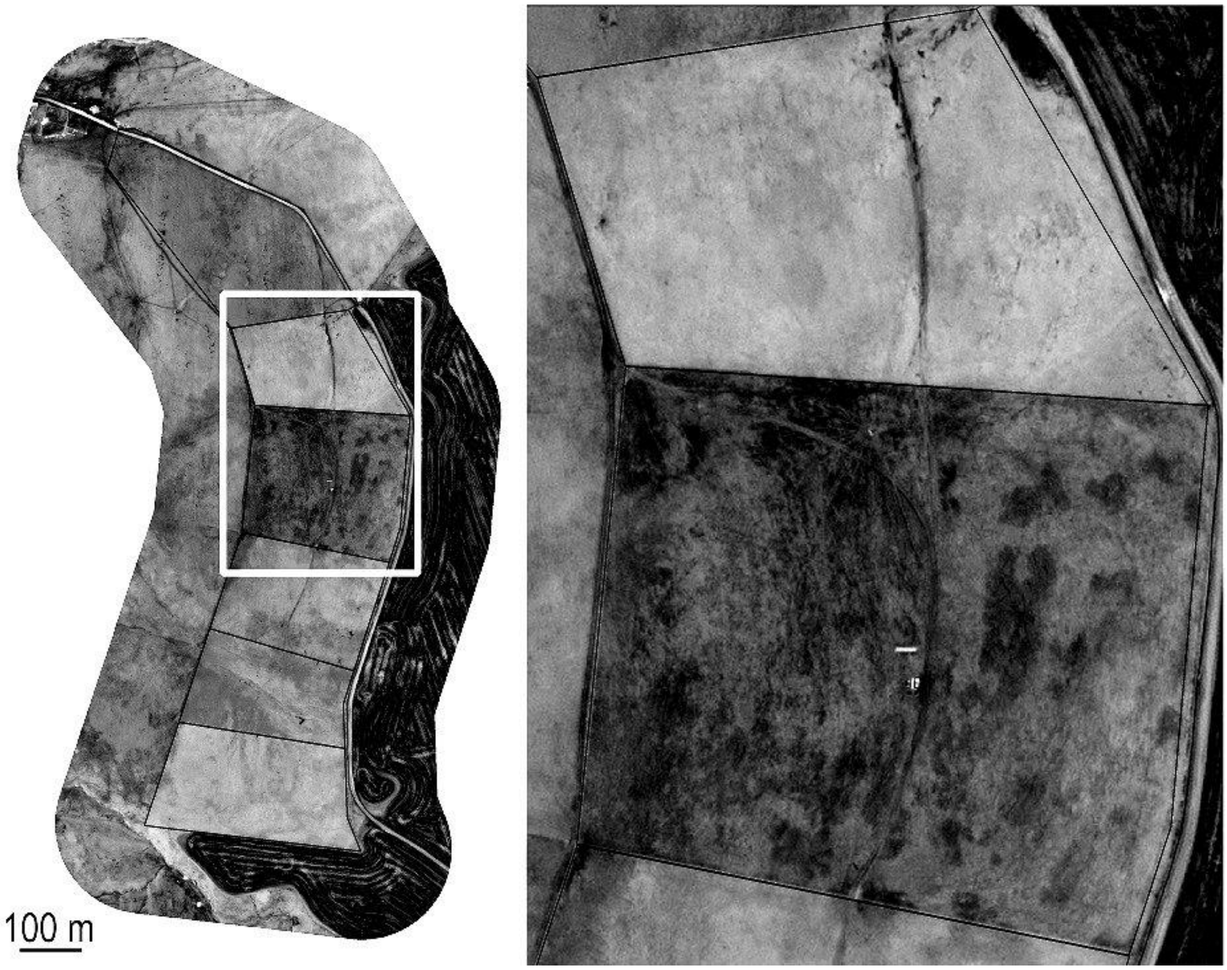


Figure 2. 1 Vegetation cover index image, generated for December 2014 (season three) and shown before artificial features were masked. The amount of vegetation cover is displayed by the relative grey-scale, with bare ground being dark grey/black turning lighter as more ground gets covered by vegetation. The study area with all six paddocks is shown on the left, with an enlargement on the right of paddock 4 (lower, hard grazed) and paddock 5 (upper, moderately grazed).

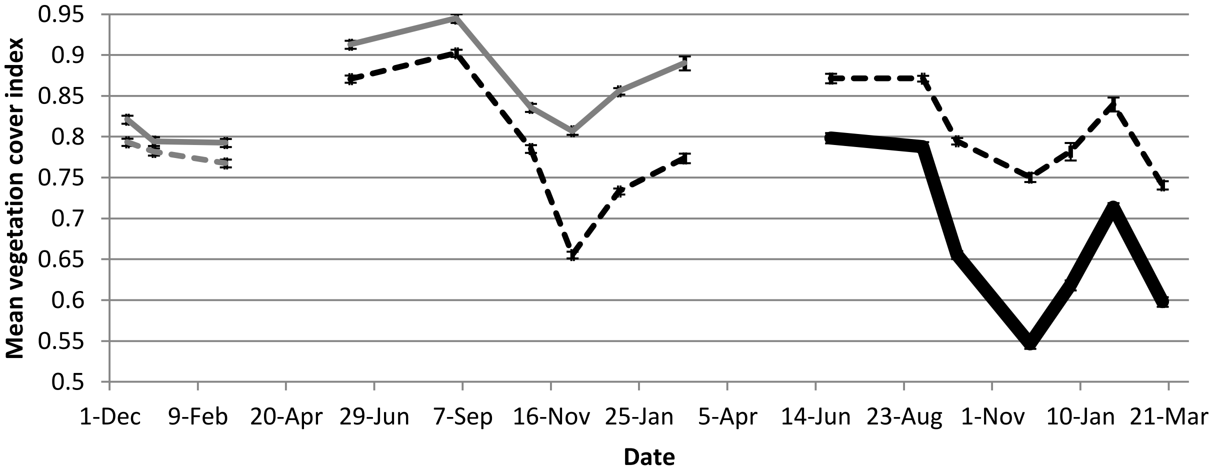


Figure 2. 2 Mean vegetation cover index (+ SE) for all three seasons (Dec 2012 - Mar 2015) derived from 100 random points within each of the six paddocks. In each season, dashed lines indicate paddocks 1, 3 and 5 (treatment group 1) and solid lines indicate paddocks 2, 4 and 6 (treatment group 2). Treatments are indicated by colour ungrazed (grey), moderate (black) and hard (bold black).

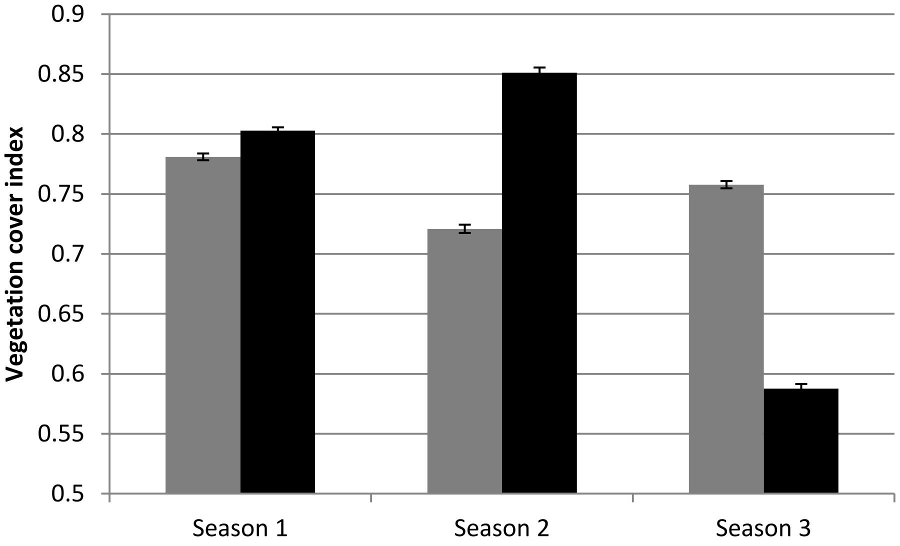


Figure 2. 3 Mean vegetation cover index + SE, for treatment group one (grey: In season one, this group was ungrazed and in season two and three, moderately grazed) and group two (black: this group was ungrazed in season one and two, but hard grazed in season three). Data used from December, January and March in each season.

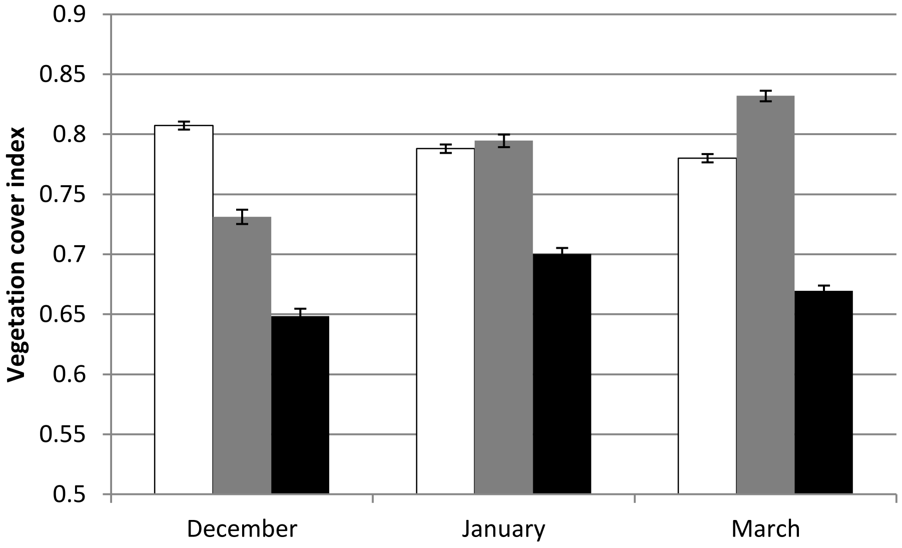
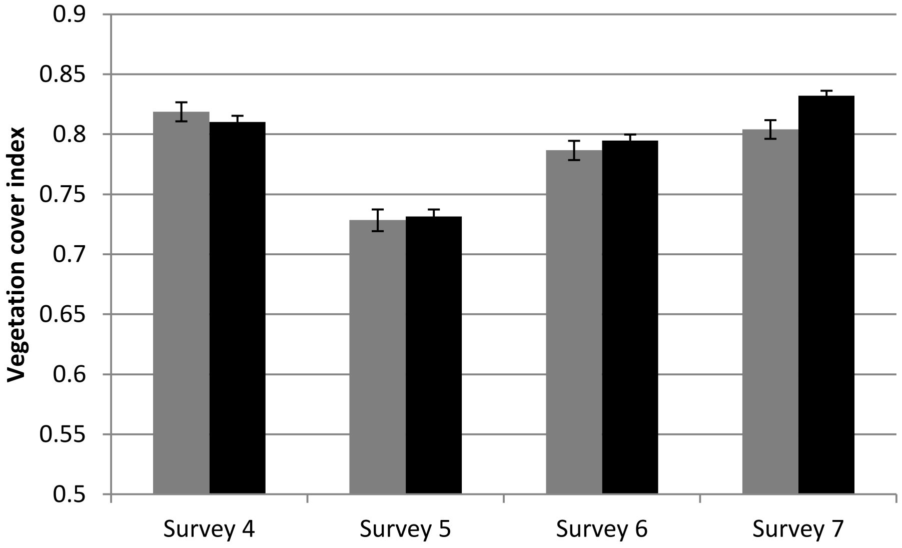
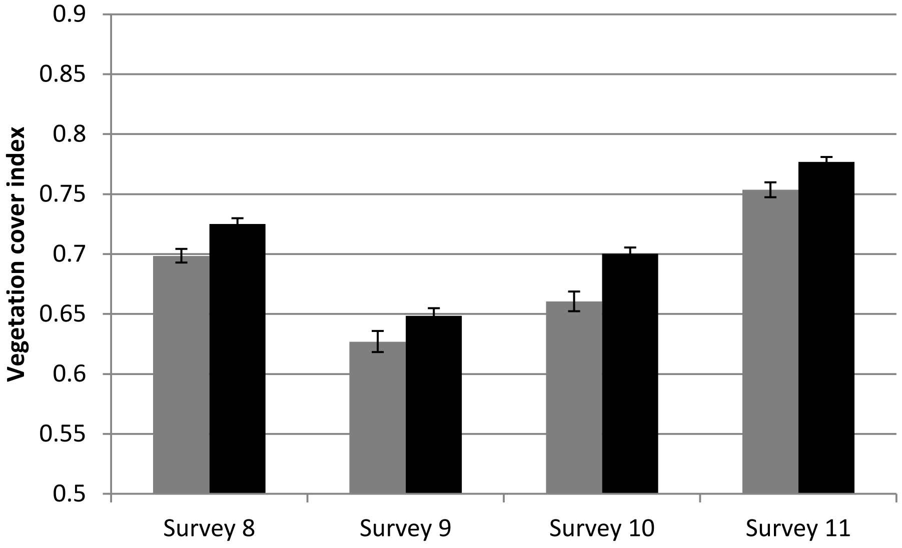


Figure 2. 4 Mean vegetation cover index + SE, for all six paddocks, for season one (white), season two (grey) and season three (black) in each of the three months December, January and March.



A



B

Figure 2. 5 Vegetation cover index + SE, shown in A) for season two and B) for season three, and divided into the two categories; "used" burrows (grey) and random points (black).

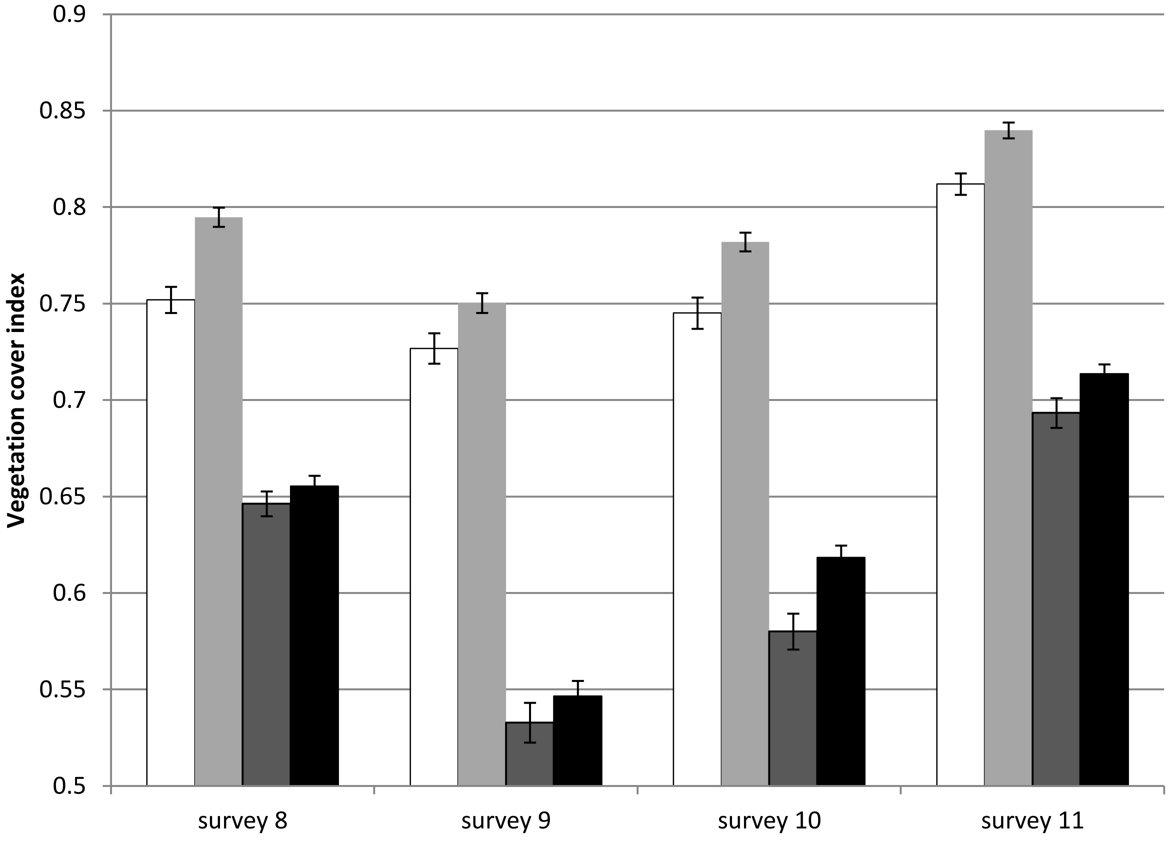


Figure 2. 6 Vegetation cover index + SE, shown for: “used” burrows in moderate grazing (white), random point in moderate grazing (light grey), “used” burrow in hard grazing (dark grey) and random point in hard grazing (black), all divided into surveys.

Chapter 3: Lizard burrow stability.

In this study sheep grazing affected the vegetation cover in the native grassland (chapter 2). This might be considered analogous to the effects of previous macropod grazers. But because domestic livestock are hard-hoofed and often much heavier than native macropods, their trampling effect is also much larger. This trampling of the ground can lead to changes in the biological crust (Bertiller & Ares, 2011), soil compaction and to a decreased ability for water to soak into the ground (Fleischner, 1994; Krümmelbein et al., 2006). These changes to soil structure can create increased runoff and lead to erosion (Fleischner, 1994; Krümmelbein et al., 2006).

Since the pygmy bluetongue lizard spend almost all of their lives in spider burrows, access to suitable burrows are a key habitat feature for the lizards and have been shown to be a potential limiting factor to the lizard populations (Souter et al., 2007). Lizards prefer deep burrows (Milne & Bull, 2000) but Ebrahimi et al. (2012) showed that during heavy rainfall, burrows without surrounding vegetation can become filled with debris and soil faster than burrows with surrounding vegetation. Pygmy bluetongue lizards are incapable of digging their own burrows, but it is unknown if their movement inside the burrows helps to maintain and stabilize the burrows.

As deep spider burrows are such an important habitat feature for the pygmy bluetongue lizards. knowing the effects of runoff and erosion on these burrows, as well as how resilient lizards are to these effects, is important to the management of the species. The aim of this chapter was to investigate the destructive effects of winter grazing and how deterioration of the burrows is affected by the presence or absence of a lizard occupant.

3.1 Winter durability of pygmy bluetongue lizard burrows is higher for occupied than unoccupied burrows and for those in less grazed neighbourhoods.

Abstract

Context. Many ectothermic animals survive winter by hibernating buried or in burrows. During their hibernation these animals are vulnerable to changes in soil structure and temperature, caused by the activity of grazing mammals. This may be a particular risk to ectotherms living in native grassland, as this habitat is often used for livestock grazing. The endangered pygmy bluetongue lizard lives in burrows in fragments of native grassland in South Australia, and these burrows are likely to be affected by sheep grazing while the lizards are hibernating.

Aims. The current study aimed to determine the effects of different levels of grazing, winter vegetation cover, burrow entrance size and whether a lizard was overwintering in the burrow, on the persistence of burrows suitable to the pygmy bluetongue lizard.

Methods. During two winters, we applied different grazing treatments to six experimental paddocks and determined whether suitable lizard burrows located in autumn were still suitable to lizards in the following spring. For each burrow we recorded whether a lizard was overwintering, the burrow entrance diameter and the vegetation cover around the burrow during the winter.

Key results. Increased grazing pressure led to decreased persistence of lizard burrows. We also found that burrows with an overwintering lizard had a greater chance of persisting, but we found no direct effect of winter vegetation cover or entrance diameter.

Conclusions. The results show that although pygmy bluetongue lizards may be able to stabilize their own burrows, the more intense the grazing the lower the chance of lizard burrow persistence.

Implications. Management of sheep grazing is an important component in future conservation of the endangered pygmy bluetongue lizard. Grazing on grassland with a lizard population should be kept at a moderate level and hard grazing should be avoided.

Introduction

In temperate ecosystems many ectothermic animals hibernate either buried (Currylow et al., 2013) or in burrows (Harris et al., 2015; Jacome-Flores et al., 2015; Williams et al., 2015; Zappalorti et al., 2014). In autumn, when ground temperature drops below their minimum activity temperature, these animals rely on the stable habitat underground, until temperatures rise and allow them to become active again.

Although some ectotherms can have limited activity inside their burrow (Harris et al., 2015), most are inactive during winter and can become passive victims to habitat changes that will render their hibernaculum unsuitable. Grazing mammals remain active over winter and can change vegetation and soil structure, through feeding activities and trampling of the ground (Fleischner, 1994; Krümmelbein et al., 2006). These changes can be a threat to hibernating ectotherms, as vegetation removal can change the temperature in the ground down to 30cm depth (Currylow et al., 2013) and changes to soil structure and rainwater runoff potentially cause burrows to be filled in or collapse (Ebrahimi et al., 2012).

Native grasslands and scrublands are found in many climates in southern hemisphere ecosystems. They are commonly used as grazing for introduced ungulate livestock (Dorrough et al., 2004; Fleischner, 1994; Krümmelbein et al., 2006; Rambo & Faeth, 1999), which are kept at higher rates than naturally occurring grazing mammals. This can potentially increase the threat to hibernating ectotherms living in the grassland habitat. It is therefore essential to the conservation of these species to know the mechanisms through which grazing mammals impact the overwintering survival rate of native ectotherms.

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is an endangered burrow dwelling skink, with a mean snout-vent length of 95mm (Milne, 1999). Although previously recorded as far south as Adelaide and Gawler, it is now only found in a few remaining patches of native grassland in the Mid-North region of South Australia. This region has an annual rainfall of 400-500 mm, but most of the rain falls in the cool winters when the lizards are inactive in their burrows. Lizards have never been observed to dig their own burrows, but use burrows dug by lycosid and mygalomorph spiders (Milne & Bull, 2000; Milne et al., 2003b; Souter et al., 2007). It is however possible that lizards moving inside their burrows in spring and summer help to stabilize and clear debris out of the burrows. Burrows are used as refuges against extreme weather and predators as well as ambush sites for passing insects (Fenner & Bull, 2007; Fenner et al., 2007; Milne et al., 2003a). Pygmy bluetongue lizards are solitary and spend most of their time in their burrows, which are defended against conspecifics (Bull et al., 2015; Fenner & Bull, 2011b). Burrows are left temporarily, when lizards stalk passing prey, and to defecate outside the burrow, but only vacated permanently if the burrows get filled in, get too small for a growing subadult lizard, or during the mating season where males move around on the surface to find burrows occupied by females (Ebrahimi et al., 2014; Fenner & Bull, 2011a; Milne et al., 2003a; Schofield et al., 2012).

Pygmy bluetongue lizards prefer burrows with a depth of more than 230 mm and an entrance diameter that fits the head width of the lizard as closely as possible (Milne & Bull, 2000; Souter et al., 2007). These criteria differ from the average spider burrow dimensions (Clayton & Bull, 2015; Souter et al., 2004) and suitable burrows can be a limiting factor in a lizard population (Souter et al., 2007). Previous experiments, conducted in the same area as the present study, have shown that the number of spider burrows decreased once grazing commenced, while the number of burrows in ungrazed paddocks increased in the same period (Clayton & Bull, 2015). It has also been shown that in enclosures with grass tussocks, fewer burrows were filled in by debris during a rainstorm, than in enclosures with bare ground (Ebrahimi et al., 2012). Furthermore, burrows with enlarged entrances due to foxes digging for the lizards quickly get filled with debris (Nielsen & Bull, 2016b).

Although lizards may be able to maintain their burrows during their spring-summer activity season, deteriorating condition of burrows over winter could trap hibernating lizards in these burrows and also impact population dynamics in the following activity season. In the current study we asked whether grazing treatment and vegetation cover affected the proportion of burrows destroyed over winter. We also investigated whether the presence of a lizard and the entrance diameter of the burrow affected the persistence of burrows. Our predictions were that increased grazing, reduced winter vegetation and enlarged burrow diameter would all increase the chance of a burrow deteriorating over winter, while the presence of a lizard was predicted to decrease the chance of a burrow deteriorating.

Methods

Grazing treatments

The study was conducted from mid autumn to mid spring in two successive years 2013 and 2014 (Table 3. 1), and included two austral winter periods (May-September) when cool temperatures keep pygmy bluetongue lizards inactive within their burrows. The study site was the 'Tiliqua' property of the Nature Foundation of SA, near Burra, South Australia (33.67^{O}S; 138.93^{O}E), an isolated native grassland habitat, used for sheep grazing for over a century, and unploughed for cereal cropping for at least four decades. It contains a population of pygmy bluetongue lizards, and has been the site of previous studies of this species (Clayton & Bull, 2015; Fenner et al., 2007; Pettigrew & Bull, 2011).

The region has hot dry summers and cool wet winters with an average annual rainfall at Burra between 400-500 mm. During the five winter months the field site received similar rainfall in 2013 (324 mm) and in 2014 (303 mm). The study site contained six adjacent experimental paddocks (size range 3.5-6.9 ha), arranged in a north to south line along the eastern edge of the property, each with similar elevation and slope. In those paddocks we applied two alternative sheep grazing regimes each winter, with adjacent paddocks having different treatments (Nielsen et al., 2016b). To maintain an adequate level of vegetation and feed for the sheep, we had to rotate the sheep in and out of the paddocks regularly rather than keep a standardised number in each paddock at all times. We registered the number and dates of sheep movements, and calculated an average number of sheep units (sheep/day/hectare) for each paddock over each winter. Very rarely we saw kangaroos, but their grazing impact was likely to be limited and spread evenly across all paddocks. In the 2013 winter three paddocks were left 'Ungrazed' where no sheep were allowed into the paddocks, while the other three had 'Moderate' grazing, at the traditional level of grazing for the area (3.15± 0.14 (SE) sheep/ day/ ha). We relied on the advice of a local farmer to keep a level of grazing that maintained some vegetation in the paddocks. In the 2014 winter, the same three paddocks were kept with a 'Moderate' grazing regime (2.67± 0.24 (SE) sheep /day /ha), while the previously 'Ungrazed' paddocks were exposed to 'Hard' grazing (almost double the grazing pressure of the Moderate grazing regime). This grazing load over winter (4.36± 0.42 (SE) sheep /day /ha) meant the paddocks were grazed as hard as possible, without starving the sheep. This treatment was intended to simulate an overgrazing or drought scenario.

We used pan-sharpened Pléiades satellite imagery and the method described in Nielsen et al. (2016b), to generate a ground vegetation cover index for images acquired in June and October for each of the two winters. We considered that these two months provided images representative of the vegetative growth over the early and later parts of each winter. The Pléiades revised pan-sharpening method almost completely retains the original spectral information yielding high-resolution multispectral images (Astrium, 2012). Mean red and near-infrared reflectance values from several known bare soil (~0% ground cover) and heavily vegetated areas (~100% ground cover) were calculated to define the trajectory and length of the soil line upon which all pixels (50 by 50 cm) were projected to calculate relative vegetation cover. To characterise overall winter vegetation cover, we calculated a mean vegetation cover index for each pixel, using the two index values from the June and October images.

To document the effect the different treatments had on the vegetation cover, we calculated mean winter vegetation index for each treatment in 2013 and 2014, based on 100 randomly selected pixels in each paddock. The same pixels were repeated in both years. We then used repeated measures ANOVAs (Split plot design, SPSS ver. 23.0) to compare the effect of year (within subjects factor) and treatment (between subjects factor) and the interaction effect between these factors.

Burrow surveys

To determine burrow persistence over each winter we conducted two burrow surveys each year, (Table 3. 1), one in autumn, when lizards first became inactive, and one early in the next spring, when the lizards started to become active again. During each autumn we surveyed 42-50 previously located lizard-suitable burrows in each paddock. Although lizards prefer deep burrows, these are not always available and lizards are often found occupying shallower burrows. A burrow was therefore considered suitable for a pygmy bluetongue lizard if it was at least 150 mm deep, and had an entrance diameter wider than 12 mm (Milne & Bull, 2000). The burrows were marked by plastic pegs placed 30-40 cm in a standard direction from the burrow entrance, and their GPS coordinates were recorded. We used an optic fiberscope to inspect the inside of each burrow during each survey and determined if there was a lizard occupant, following Milne & Bull (2000). We assumed that, because they had become inactive by autumn, a lizard found occupying a burrow in the autumn surveys overwintered in that burrow, although we recognize that some could have moved burrows after the autumn survey.

We then derived the mean winter vegetation cover index for each burrow from the pixel it was in, in the satellite images. For analysis purposes we define a suitable burrow that was occupied by a lizard in the autumn survey as an 'occupied burrow'. In the spring surveys, all previously identified burrows were relocated, and checked for inhabitants and burrow condition. Burrows were either still 'suitable' or had become 'not suitable' for lizards. Burrows that had become 'not suitable' had either filled with debris or had collapsed walls to become too shallow, or had entrances that had become too narrow.

Among the burrows located in autumn we compared those that remained suitable with those that had become not suitable over the winter, using binary logistic regression (SPSS ver. 23.0). In separate analyses for each winter, we considered four possible predictors of burrow deterioration. These were grazing treatment, burrow entrance diameter, winter vegetation cover index in the pixel containing the burrow, and burrow occupancy. The binomial response variable was whether an autumn suitable burrow was still suitable for lizards (0=remaining suitable; 1=destroyed/not suitable). We constructed 16 alternative models including each of the four factors alone and in various additive combinations, and compared those models by calculating the Akaike Information Criterion (AIC) of each. The models were then ranked based on ΔAIC, relative to the top model.

Results

Figure 3. 1 shows the mean winter vegetation cover index for each treatment in each of the two winters 2013 and 2014, with analysis shown in Table 3. 2. Mean winter vegetation cover index was significantly lower in 2014 than in 2013. There was also a significant interaction effect between

year and treatment. While one group of paddocks was moderately grazed in both seasons and therefore showed very little variation in vegetation cover, the paddocks that were ungrazed in 2013 were changed into hard grazed paddocks in 2014. This resulted in a reduction in vegetation cover meaning these paddocks went from having higher vegetation cover to having lower vegetation cover than the moderately grazed paddocks.

Of 297 burrows surveyed in autumn 2013, 210 (70.7%) were still suitable for lizards in the following spring while 87 (29.3%) were not. Of 285 burrows surveyed in autumn 2014, 204 (71.6%) remaining suitable by spring, while 81 (28.4%) did not.

In both years the top model to explain burrow persistence over winter included burrow occupancy and grazing treatment (Table 3. 3). Burrows that had a lizard occupant in autumn had much higher persistence over each winter than did those without a resident lizard (Figure 3. 2), and burrows in paddocks with a lower grazing pressure over winter had a higher persistence (Figure 3. 3).

Both entrance diameter and winter vegetation cover appeared as factors in combination with burrow occupancy and grazing treatment in some of the other significant models in each year, but neither of these factors made a significant individual contribution to any model.

Discussion

In this study we investigated four factors that we considered might have an impact on the probability of burrows remaining suitable for lizards over winter. Our results show that both grazing treatment and lizard use significantly increased the chance that a burrow remained suitable for lizards over winter.

The effect of grazing treatment can be related to several direct and indirect effects of grazing. The most direct effect is trampling and destruction of the burrow entrance. Although this has not been shown specifically for burrows used by pygmy bluetongue lizards, sheep grazing has been shown to change soil structure (Krümmelbein et al., 2006) and reduce the number of spider burrows that lizards rely on (Clayton & Bull, 2015). The lizards are not directly affected by changes in soil structure (Souter et al., 2007), as they do not dig burrows themselves, but the trampled, compacted ground might make it harder for spiders to replace any deteriorated burrows, even after the sheep have been removed from the area. Trampling of the ground also reduces the ability of the soil to absorb rainwater. This, combined with reduced vegetation cover, can cause increased surface runoff and erosion (Krümmelbein et al., 2006), leading to more debris and soil particles being washed into burrows.

Our study found that the mean vegetation cover was significantly lower in 2014 than in 2013. This is most likely due to all paddocks being grazed in 2014 while half of the paddocks were left ungrazed in 2013. A slight rainfall decrease in 2014 was also likely to be a contributing factor. We also showed that vegetation cover was reduced in accordance with grazing treatment, with the most intense grazing causing the largest reduction in vegetation cover. A previous study by Ebrahimi et al. (2012) found that burrows became filled with debris after heavy rain faster in enclosures without vegetation, than in enclosures with grass tussocks. However, in the current study, we found no significant individual effects of winter vegetation cover on the persistence of lizard burrows.

We suggest two possible explanations for why burrows used by lizards are less likely to deteriorate over winter than non-occupied burrows. The first is that lizards choose the most stable burrows to live in. This is supported by lizards preferring deeper burrows (Milne & Bull, 2000), often constructed by mygalomorph spiders. These spiders construct deep stable burrows that they can inhabit and continue to extend for up to 20 years (McCullough, 2000). We intended only to include spider burrows that lizards would themselves prefer, by selecting those with entrance diameter and depth, previously shown to be suitable as lizard burrows. It is however possible that lizards choose burrows based on some additional criteria, related to burrow stability, that have not yet been characterised.

The second explanation is that lizards maintain and stabilize their own burrows. Although lizards are presumed unable to dig burrows themselves, their movements inside the burrow might help to clear out debris and prevent deterioration. Although the lizards may not move much in the cool winter conditions, they may have started some movement and burrow maintenance by the time we conducted our early spring surveys. The two alternative explanations are not mutually exclusive and both mechanisms may be important.

As the pygmy bluetongue lizards are obligate burrow dwellers, without the ability to dig their own burrows, access to suitable burrows is essential for the lizards to inhabit an area (Souter et al., 2004; Souter et al., 2007). A better understanding of the factors affecting the number of suitable lizard burrows in grassland habitat is therefore important for future management of these endangered lizards. Although our results show that burrows with lizards are less likely to deteriorate, and therefore show some resilience in relation to impacts of grazing, they also show that grazing increases the number of burrows that become unsuitable for lizards during a winter. This negative impact of grazing must be balanced against possible benefits of grazing and vegetation cover removal on the lizards opportunity to bask and hunt. Grazing of pygmy bluetongue lizard habitat should be kept at moderate level, leaving enough vegetation to minimise erosion and soil structure changes. Future research should focus on the fine scale timing of grazing in relation to rain and lizard activity, to gain more exact knowledge of when grazing is most beneficial and least harmful.

Acknowledgements

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	Survey start	Survey finish	Number of burrows/ paddock	Burrows with lizards/ paddock	Satellite image date
Burrow survey	25-03-2013	04-04-2013			
autumn 2013			48-50	18-28	
Winter 2013					10-06-2013
Burrow survey	08-10-2013	24-10-2013			
spring 2013					31-10-2013
Burrow survey	10-03-2014	18-03-2014			
autumn 2014			42-50	13-35	
Winter 2014					26-06-2014
Burrow survey	14-10-2014	05-11-2014			
spring 2014					04-10-2014

Table 3. 1 Survey dates, range of the number of burrows, and of the number of burrows with lizards in each paddock, and date of the satellite images used to calculate vegetation cover index for each survey. The winter satellite image date indicates the date of the June image used with the October image to calculate mean winter vegetation cover index.

Factor	D.f.	F	P
Year	1,4	54.432	**0.002**
Treatment	1,4	3.477	0.136
Year*Treatment	1,4	62.668	**0.001**

Table 3. 2 Repeated measures ANOVA (Split plot design), comparing the average winter vegetation cover index of random points in each experimental paddock, between year and treatments.

Model	Chi-square	D.f.	P	AIC	ΔAIC
T;BO	51.43	2	**6.794E-12**	313.79	0.00
T;BO;ED	52.94	3	**1.886E-11**	314.28	0.49
T;BO;WV	51.67	3	**3.515E-11**	315.55	1.76
BO;WV	49.53	2	**1.755E-11**	315.69	1.90
BO	47.26	1	**6.209E-12**	315.96	2.17
T;BO;ED;WV	53.09	4	**8.153E-11**	316.13	2.34
BO;ED;WV	50.54	3	**6.127E-11**	316.68	2.89
BO;ED	48.33	2	**3.209E-11**	316.90	3.11
T;ED	7.35	2	**0.025**	357.88	44.08
T	3.82	1	0.051	359.41	45.61
T;ED;WV	7.51	3	0.057	359.72	45.92
ED;WV	4.99	2	0.082	360.23	46.44
ED	2.82	1	0.093	360.40	46.61
WV	2.05	1	0.152	361.18	47.38
T;WV	4.03	2	0.133	361.20	47.40
Null-model	N/A	N/A	N/A	361.22	47.43
A					
T;BO	31.19	2	**1.691E-07**	315.04	0.00
BO	27.76	1	**1.374E-07**	316.47	1.43
T;BO;WV	31.24	3	**7.580E-07**	316.99	1.95
T;BO;ED	31.19	3	**7.748E-07**	317.03	1.99
BO;WV	28.49	2	**6.501E-07**	317.73	2.69
BO;ED	27.94	2	**8.576E-07**	318.29	3.25
T;BO;ED;WV	31.24	4	**2.735E-06**	318.99	3.94
BO;ED;WV	28.64	3	**2.671E-06**	319.59	4.55
T	7.88	1	**0.005**	336.34	21.30
T;ED	9.48	2	**0.009**	336.75	21.71

T;WV	8.19	2	**0.017**	338.04	23.00
T;ED;WV	9.69	3	**0.021**	338.53	23.49
ED	3.28	1	0.070	340.95	25.91
ED;WV	4.23	2	0.121	342.00	26.96
Null-model	N/A	N/A	N/A	342.23	27.19
WV	1.12	1	0.290	343.11	28.07

B

Table 3. 3 Statistical values for binary logistic models based on the factors; (T)Treatment (2013; ungrazed/moderate, 2014; moderate/hard), (ED)entrance diameter, (WV)mean winter vegetation and (BO)burrow occupancy (whether a lizard were overwintering). Table A shows the 2013 winter while table B shows the 2014 winter. Models are ranked according to ΔAIC: increase in AIC compared to the top model.

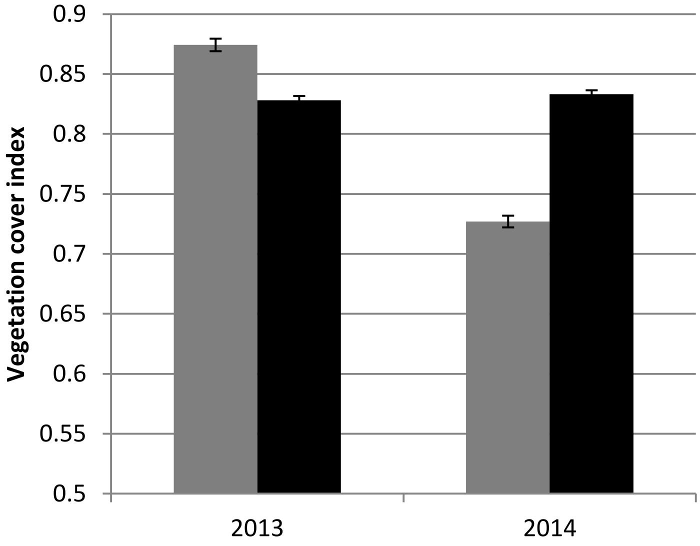

Figure 3. 1 Mean vegetation cover index ± SE, for the two treatments of the 2013 and 2014 winters. Grey; these paddocks were in 2013 ungrazed, and in 2014 hard grazed. Black; these paddocks were moderately grazed in both 2013 and 2014.

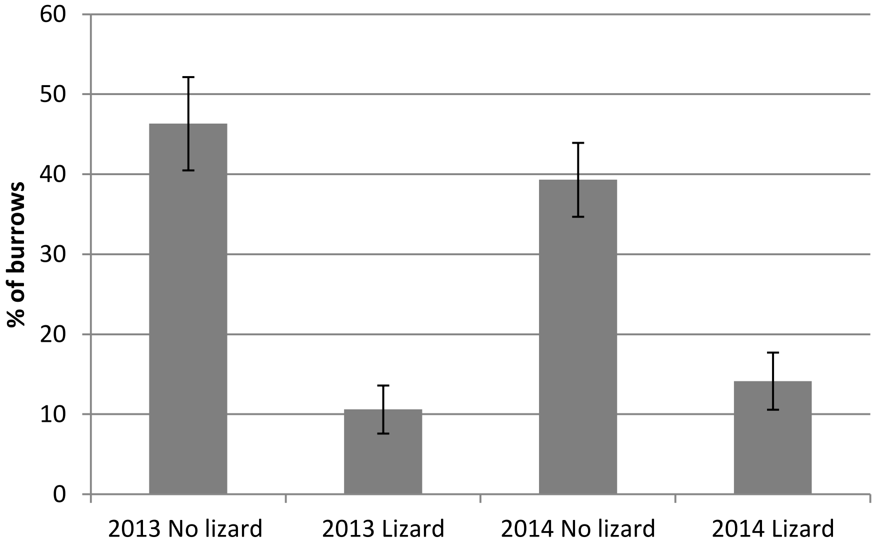

Figure 3. 2 Effect of lizard occupancy on burrow deterioration. Bars show the mean percentage (+ SE) of burrows that has deteriorated and are no longer lizard suitable, divided into burrows where a lizard was overwintering and burrows without a lizard in each of the two winters.

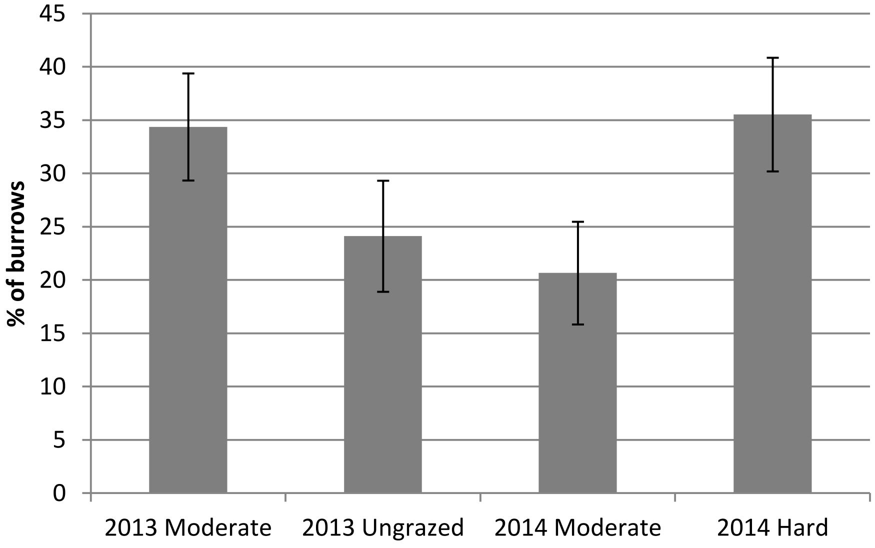

Figure 3. 3 Effect of grazing intensity on burrow deterioration. Bars show the mean percentage (+ SE) of burrows that have deteriorated and are no longer lizard suitable, divided into the two treatments of each winter season.

Chapter 4:Prey abundance and lizard body condition

Body condition is a measure of an individual's energy reserves and a low body condition can lead to reduced survival (Madsen & Shine, 1993; Sperry & Weatherhead, 2008). Changes in body condition can be caused by environmental factors such as drought (Sperry & Weatherhead, 2008) but are also often linked to changes in food availability (Forsman & Lindell, 1997; Shine & Madsen, 1997; Sperry & Weatherhead, 2008). To insectivorous lizards reduction of vegetation levels by mammalian grazing will not have any direct effects on their body condition, but is likely to have an indirect effect through changes in the diversity and abundance of insects.

The vegetation changes caused by grazing affect different insect species differently and can also have interactions with a wide range of other factors (Joern & Laws, 2013). Some studies have found that grazing increased the abundance of certain insects (O'Neill et al., 2010; Zhong et al., 2014), while others found that grazing reduced overall insect abundance by up to 10 times (Rambo & Faeth, 1999). Even among different species of grasshoppers found in the same area, some species thrive while others decrease as a consequence of grazing (O'Neill et al., 2003; O'Neill et al., 2010; Zhong et al., 2014).

Changes to food availability is an important management issue and pygmy bluetongue lizards are predominantly insectivores, with grasshoppers being their preferred prey (Ebrahimi et al., 2015b; Fenner et al., 2007). Changes to grasshopper abundance and size is therefore likely to cause an impact on their body condition.

It is the aim of this chapter to show any effects of grazing on the abundance and size of grasshoppers. Changes in the body condition of pygmy bluetongue lizards are also investigated to show any relationship between the effect on grasshoppers and the body condition of the lizards.

4.1 Grazing regime affects relative body mass of the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*)

Abstract

Food is a resource that, for many animal species, is accessible at irregular intervals, so when it is plentiful, excess energy can be stored as fat and used at a later stage. Many animals rely on fat reserves to keep them alive through extended periods of food shortage such as the winter and to provide additional energy for reproduction. The present study investigated how different levels of grazing by domestic stock in native grassland habitat affect the body condition of the pygmy bluetongue lizard (*Tiliqua adelaidensis*), and if these effects are related to changes in the abundance and size of grasshoppers which are the lizards primary source of food. The hypothesis was that lizards would have the highest body condition in moderately grazed paddocks, because those paddocks would have more grasshoppers than heavily grazed paddocks, and better visual conditions for lizards to catch those grasshoppers than in ungrazed paddocks. The results, however, showed that both lizard body condition and the abundance of grasshoppers increased with decreasing grazing intensity. The connection between lizard body condition and abundance of grasshoppers was complex. Within an activity season, lizard body condition generally declined from spring to summer, while the number of grasshoppers grew. The mean size of grasshoppers seemed to be more important, as lizard body condition increased in spring, the time of year with the largest grasshoppers. These results show that the intensity of grazing by domestic stock influences the body condition of pygmy bluetongue lizards, but that this effect is not entirely due to the reduction in the number of grasshoppers resulting from grazing.

Introduction.

When animals eat, the energy from the food can either be spent in daily activities or can be stored primarily as fat reserves. The level of these fat reserves is referred to as the animal’s body condition (Labocha et al., 2014). Body condition influences fitness and can vary naturally during ontogeny, as different life stages have different energy requirements. For example, in many reptile species, juveniles have relatively low body condition as they allocate energy to rapid growth, to quickly achieve a size where they can catch larger prey and become less vulnerable to predators, while adults, especially females, develop a higher body condition as they store energy for later mating and breeding (Madsen & Shine, 2002). Female body condition can relate to subsequent reproductive fitness as those with low body condition have reduced fecundity (Brown & Shine, 2007) or no reproduction at all (Aubret et al., 2002).

Body condition of individuals at the same life stage can also be affected by environmental factors such as drought, which leads to reduced body condition (Sperry & Weatherhead, 2008), and especially by the availability of food (Forsman & Lindell, 1997; Shine & Madsen, 1997; Sperry & Weatherhead, 2008). When food gets scarce, energy reserves and then body condition decline. Eventually reduced body condition leads to decreased survival rates, either directly through the risk of starvation (Sperry & Weatherhead, 2008) or indirectly by increased predation risk as individuals take larger risks to find food (Madsen & Shine, 1993).

The endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) is only found in a few remaining patches of native grassland in the mid north region of South Australia. These lizards have a mean adult snout-vent length (SVL) of 95mm (Milne, 1999), and inhabit burrows dug by lycosid or mygalomorph spiders (Fellows et al., 2009; Milne & Bull, 2000; Milne et al., 2003b; Souter et al., 2007). The burrows are used as refuges from extreme temperatures and grassfires as well as ambush sites from where the lizards prey on passing insects (Fenner & Bull, 2007; Fenner et al., 2007; Milne et al., 2003b). Pygmy bluetongue lizards defend their burrows against conspecific rivals (Fenner & Bull, 2011b) and have been reported to inhabit the same burrow for more than a year (Bull et al., 2015). The lizards only leave their burrows temporarily to defecate (Ebrahimi et al., 2016; Fenner & Bull, 2011a), to stalk insect prey over short distances (Ebrahimi et al., 2015b) or in the mating season where predominantly males move around to find potential mates (Milne et al., 2003b; Schofield et al., 2012). In spring, the lizards only emerge during warm days, but they are generally active from October to April, with the remaining cold part of the year spent inactive in their burrows. Mating occurs during October and November (Ebrahimi et al., 2014; Schofield et al., 2012) which is also the time of the year when the lizards are most often at their burrow entrances (Pettigrew & Bull, 2012). From mid January to mid February the females give birth to 1-4 live young, which are born inside the burrow (Milne et al., 2002; Schofield et al., 2014; Shamiminoori et al., 2015).

Previous studies have shown that grazing by domestic stock can affect the durability of the lizard burrows (Clayton & Bull, 2015; Ebrahimi et al., 2012; Nielsen & Bull, 2016c). Experimental removal of vegetation around burrows suggested grazing also affects the behaviour of the lizards. Pettigrew and Bull (2012, 2014) found that lizards basked for a longer time, moved further out of their burrows and made more attempts to capture prey from burrows with reduced levels of surrounding vegetation. They suggested that grazing might benefit the lizards by allowing them to see and capture invertebrate prey more easily. On the other hand, grazing might reduce the vegetation so that the number of grasshoppers, their primary food source (Ebrahimi et al., 2015b; Fenner et al., 2007), may be reduced, disadvantaging the lizards.

In the present study we extended those simulated grazing trials and investigated what effects different levels of grazing by sheep have, both on the body condition of pygmy bluetongue lizards and on the abundance and composition of their grasshopper prey. Local abundance of grasshoppers can vary from year to year, depending, among other things on both local and distant weather events, and on grassfires and the availability of vegetation for food (Branson, 2008;

Branson & Haferkamp, 2014; Jonas & Joern, 2007). Mammalian grazing can both reduce the amount of available vegetation, and stimulate the growth of nutritious new vegetation, and can lead either to grasshopper numbers increasing (O'Neill et al., 2010; Zhong et al., 2014) or decreasing (Dorrough et al., 2004; O'Neill et al., 2003; Rambo & Faeth, 1999) following grazing.

In this study we predicted that grasshopper numbers might decrease with increasing grazing pressure, but that lizards would have the best body condition in paddocks with moderate grazing. We predicted that a moderate grazing regime would maintain a habitat with enough open spaces for lizards to bask and hunt, but also enough grasshoppers and sufficient cover from predators. Ungrazed paddocks might support more grasshoppers but vegetation may be too dense for the lizards to effectively hunt them. Hard grazed paddocks might be open enough for effective prey capture, but will have fewer grasshoppers to catch. We compared how three different grazing treatments (ungrazed, moderately grazed and hard grazed) affected the number and size of grasshoppers in experimental paddocks, and how this impacted the body condition of the lizards. Understanding the impact of grazing on lizard body condition has important implications for the management of the small fragments of native grassland where this endangered lizard species currently persists.

Methods.

The study took place in two successive lizard activity seasons (year 1, Oct 2013- Mar 2014; year 2, Oct 2014- Mar 2015), in a 30 ha area of native grassland in the Tiliqua property of the Nature Foundation of South Australia, near Burra, South Australia (33.67^{O}S; 138.93^{O}E). This area has been used for sheep grazing for over a century, contains a stable population of pygmy bluetongue lizards, and has previously been described in other studies (Bull et al., 2015; Fenner et al., 2007; Milne & Bull, 2000; Schofield et al., 2014; Shamiminoori et al., 2014). The climate in the region is characterized by hot dry summers and cool wet winters, with an annual rainfall in Burra of 400-500 mm. Rainfall during the spring and summer of each of the two years of the study, measured by an on-site weather station, is shown in Table 4.1.

The study site was divided into six experimental paddocks (size range 3.5-6.9 ha) arranged in a line along the eastern edge of the property, so that each paddock had similar elevation and slope. Commencing in the winter before each field season we applied two different sheep grazing treatments to these paddocks, with three replicates of each treatment. Treatments were applied in an alternate pattern so that adjacent paddocks had different treatments. Due to variations in vegetation growth and climate, sheep numbers were adjusted between years, based on local farming advice, to allow comparable reductions in vegetation cover (Nielsen et al., 2016b). In year one (October 2013 - March 2014) three paddocks were ungrazed, while three had a moderate level of grazing (2.70 ± 0.11 sheep/hectare/day). In year two the same three paddocks had moderate grazing (1.54 ± 0.15 sheep/hectare/day), while the previously ungrazed paddocks were hard grazed (2.69 ± 0.26 sheep/hectare/day). Kangaroos were rarely seen in the paddocks and their impact was assumed to be limited and evenly distributed across the paddocks.

Lizard survey

Initially, in October 2013, each of the six experimental paddocks was searched systematically for 2-4 days to create a base of 13-25 inhabited lizard burrows in each paddock from which resident lizards could subsequently be caught. The GPS location of each burrow was recorded and it was marked by a plastic peg placed 30-40 cm and in a standard direction from the burrow entrance. In subsequent surveys, if burrows became lost or vacated, new inhabited lizard burrows were located in the same paddock to retain about the same sample size in each paddock each time.

For this study, two lizard surveys were subsequently conducted in each year, one shortly after the mating season, and before significant embryonic development is likely in females, in late November - early December and the other after the breeding season, when females were no longer gravid, in late February - mid March. In each survey 7-10 lizards per paddock were caught from the known burrows by luring them out with live mealworms, as described by Milne and Bull (2000). The aim, to catch the same lizards repeatedly across all surveys, was frustrated when some lizards were not re-located in surveys of the same burrows. Other lizards were not sampled at each survey time because they could not always be lured from their burrows.

Lizards were individually marked by toe clip, and their snout-vent length (SVL) and mass were measured to the nearest mm and tenth of a gram. Relative body mass (weight divided by SVL) was used as a proxy for body condition in this study as it is a simple index that has been used in previous reptile studies (Battles et al., 2013; Forsman & Lindell, 1997; Madsen & Shine, 2002). This index is correlated with SVL but only adult lizards with a small range of SVL values (84-117 mm) were used for analysis, reducing any skewing effect of the correlation (Labocha et al., 2014). Previous studies of this species have used a body condition index based on the residuals of the regression of mass and SVL (Fenner & Bull, 2007; Shamiminoori et al., 2014) but this regression based index is not suitable for data from the same individuals repeated over time (Green, 2001).

Analyses used the mean values of the relative body mass per paddock in each survey, rather than the individual values per lizard.

To determine the impact of grazing regime, we used a repeated measures ANOVA (Split plot design, SPSS ver. 23.0) with year (1 and 2) and survey time (spring or autumn) as within subject factors and treatment group (group 1 = moderately grazed in each year; group 2 = no grazing year 1, hard grazing year 2) as a between subject factor. Because of a significant three way interaction, data from each survey time (spring and autumn) were subsequently analysed using separate repeated measure ANOVAs to investigate the effects of year and treatment group. Since one set of paddocks retained the same moderate grazing regime, while the other was switched from no grazing to hard grazing between years, we expected to detect an impact of grazing in the analysis by the treatment and year interaction at each survey time.

Grasshopper index.

Three grasshopper surveys were conducted each year. One was in mid November, when video recorded lizards had the highest rate of predation attempts (Ebrahimi et al., 2015b), one was in January, and the third was in late February - early March after litters of neonates had been produced (Milne et al. 2002). Within each two to three day survey period, the six paddocks were surveyed for grasshoppers in a random order, between 1000 – 1800 h. In each paddock, around each of ten predetermined, computer generated, random GPS positions (ArcGIS by Esri ver. 10.1), ten, arm’s length, half circle sweeps were made at ground level through the vegetation with a 38 cm diameter butterfly net. Each survey used a different set of random positions in each paddock. The grasshoppers from the ten sweeps at each position were collected into a single plastic bag (ten bags per paddock in each survey) and frozen for later sorting, counting and weighing. We did not attempt to identify individual grasshopper species in the analyses.

Grasshopper abundance and mean grasshopper weight per paddock were compared to assess the impact of grazing regime, using similar repeated measures ANOVA designs as described above for mean lizard body condition.

Results.

Lizard survey.

In year one (2013/14), 79 (62 females/16 males/1unknown) different lizards were caught and 77 (61 females/ 16 males) lizards were caught in year two, 24 of these lizards were recaptures from year one (2014/2015).The repeated measures ANOVA (Table 4.2) showed a significant three way interaction between year, survey time and grazing treatment. Figure 4.1 shows the mean relative body mass of lizards in the two treatment groups, in each survey time, in each year. Relative body mass was considerably lower in March than December, but the differences between treatment groups from year one to year two were less in March than December.

A separate repeated measures ANOVA, based on the December surveys only, showed a significant interaction effect between year and treatment (Table 4.3) in the direction predicted to indicate a negative impact of grazing on lizard body condition. Lizards in ungrazed paddocks in year one had a higher relative body mass than lizards in moderately grazed paddocks. When the ungrazed paddocks in year one were changed to hard grazed paddocks in year two, the lizards changed to having lower relative body mass than lizards in the moderately grazed paddocks (Figure 4.1). Data collected in March, towards the end of the activity season when lizards had generally lower relative body mass (Figure 4.1), showed no significant treatment and year interaction (Table 4.3). The significant main effect of year reflected an overall lower relative body mass in March in year two, when hard grazing had been applied (Figure 4.1), although the decline was not significantly greater in the paddocks where grazing pressure had been increased than in the paddocks where grazing stayed the same.

Grasshopper index.

The repeated measures ANOVA on the mean number of grasshoppers in the paddocks also showed a significant three way interaction between the three main factors (Table 4.4). Figure 4.2 shows that there was a tendency for there to be more grasshoppers in the ungrazed paddocks than in the moderately grazed paddocks in year one but the reverse tendency in the last two surveys of year two where ungrazed paddocks had been changed to hard grazed paddocks. This tendency was stronger in the last survey (Feb-March) than in earlier surveys each year. Individual repeated measures ANOVAs analysing the effects of year and treatment within each of the survey times (Table 4.5) revealed no significant year and treatment interaction effects in November or January, but a significant interaction in Feb/ March, with fewer grasshoppers in the moderate grazed treatment in year one (when the other paddocks were ungrazed) and more grasshoppers in the moderately grazed treatment in year two (when the other paddocks were hard grazed) (Figure 4.2). That indicates a significant impact of grazing treatment on the number of grasshoppers. In the last two surveys more grasshoppers were caught in year two than in year one (Figure 4.2).

The analysis of mean weight of sampled grasshoppers showed survey time within a year as the only significant main factor, and there were no significant interaction effects (Table 4.6). A Bonferroni post hoc test showed that grasshoppers were consistently larger and heavier in November than in the following two surveys (Figure 4.3).

Discussion.

Lizard survey

Our initial hypothesis was that pygmy bluetongue lizards in moderately grazed paddocks should have a better body condition than lizards in ungrazed or hard grazed paddocks. The results from the December surveys confirm that lizards in moderately grazed paddocks had higher relative body mass than in hard grazed paddocks, but, contrary to the hypothesis, lizards in ungrazed paddocks had a higher relative body mass than lizards in moderately grazed paddocks. Among our three grazing treatments, these results indicate that grassland without grazing is the best habitat for the lizards, a similar conclusion to what was found in a study of the effects of human land use on the green anole lizard (*Anolis carolinensis*) (Battles et al., 2013). This conclusion should be considered in the context of the single year over which each treatment was applied. Over a longer time, paddocks entirely without grazing would probably develop very dense vegetation and a substantial thatch of dead material from annual grasses, allowing limited bare ground and little sunlight reaching the ground. This could prevent lizards from basking and detecting prey insects near their burrows. It could also prevent males from moving easily between burrows to find females during the mating season. The ungrazed paddocks in this study had probably not been left ungrazed for long enough to reach the high vegetation density threshold implied in our hypothesis, where the vegetation would become a problem to the lizards. Reduced habitat use in relation to dense vegetation caused by invasive exotic plant species, have however been shown in both the rainbow skink (*Carlia schmeltzii*), the copperhead snakes (*Agkistrodon contortrix* Linnaeus) and the greater mouse-eared bat (*Myotis myotis*) (Carter et al., 2015; Hacking et al., 2014; Rainho et al., 2010).

Although the results from the end of spring (December) suggested an influence of grazing treatment, by the end of summer (March) the mean relative body mass for lizards showed no significant effect of grazing treatment, or any interaction between treatment and year (Figure 4.1). That is, in paddocks that went from no grazing to hard grazing, the change in lizard relative body mass was no different from those in paddocks that retained a moderate grazing regime. Thus impacts of grazing, on the relative body mass of lizards in spring, did not appear to be sustained over summer. One possible explanation is that the high number of grasshoppers late in year 2, combined with lizards being more likely to attempt catching prey when vegetation around the burrows is sparse (Pettigrew & Bull, 2014) may have offset any negative effect of the hard grazing. An alternative explanation is that any surplus energy stores accumulated during spring and shown by higher relative body masses in paddocks with lower grazing pressure in December are invested by the females in offspring that are born in January - February, and that females in each grazing treatment only retain enough energy reserves (as measured in March) to survive the following winter. This explanation is supported by results which show that both offspring and litter size of the aspic viper (*Vipera aspis*)are related to short term energy acquisition during the breeding season (Bonnet et al., 2001), this relation between energy intake and reproductive output is however not present in all snakes (Shine & Madsen, 1997).

In each treatment group and each of the two years lizards had consistently higher mean relative body mass in spring (December) than later in summer (March), which is likely to be a result of energy invested between the two survey times in mating and the production of offspring. By December (late spring) of year two, the relative body mass was higher than in March (late summer) of the previous year. As the lizards are mainly inactive after the surveys in March and throughout the winter (Pettigrew & Bull, 2012, 2014), this build up in body mass and energy reserves is most likely to happen during early spring. These results are consistent with patterns previously reported by Shamiminoori et al. (2014), who suggested higher prey abundance and more feeding opportunities during spring, as an explanation for higher body condition in the early part of the spring- summer activity season. Our results show that grasshoppers, which are a major part of the lizard diet, are less abundant but significantly larger in spring than later in the activity season, indicating that it is the quality and size, and not the abundance of prey, that enable the lizards to build up energy stores. Consumption of high quality food to enable build up of energy stores are also known in some hibernating mammals, among these the fat-tailed dwarf lemur (*Cheirogaleus medius*) (Fietz & Ganzhorn, 1999). Future research should focus on the factors that influence how quickly the lizards can build up their energy stores in early spring, a vital determinant of lizard survival and reproduction.

In both years we found proportionally more female lizards than male lizards. This could be caused by a real bias towards females in the population, but could also be because most of the lizards initially caught and then followed in this study were found in their burrows in early spring, a time when males are moving between burrows (Ebrahimi et al., 2014; Schofield et al., 2012) to find females. Thus even with an equal sex ratio males were less likely to be included in the study.

Grasshopper index

The surveys showed that grasshoppers were present and available for lizards to prey upon throughout the lizards activity season and in all grazing treatments. Overall grasshopper numbers were however highest in early summer (January) in year 1 and in late summer (February/ March) in year 2. The analysis of grasshopper numbers showed a significant three-way interaction resulting from large variation among the three sampling times in their responses to different grazing treatments across the two years. Because we were principally interested in the effect of changing grazing regime, and because we could detect that by the year and grazing treatment interaction, we considered the effects of grazing treatment and year, separately at each survey time.

Those analyses only showed an impact of the level of grazing in the last surveys, late in the summer (February/March), when the numbers of grasshoppers were higher in ungrazed than moderately grazed paddocks in year 1, but then higher in moderately grazed than hard grazed paddocks in year 2. Note that this is close to the time when the lizard surveys showed no effect on the level of grazing on lizard relative body mass. A possible explanation for why grazing did not influence grasshopper numbers in the November and January surveys may be the relatively small sample size combined with fewer grasshoppers particularly in November. Alternatively, the small paddock size, and the relatively high mobility of grasshoppers may have allowed individuals from paddocks with one grazing treatment to move into paddocks with another grazing treatment and mask a real impact on grasshopper numbers. This could particularly be the case in the November where the grasshoppers were large and very mobile.

The abundance of grasshoppers was significantly higher in season two in both the January and February/March surveys although not in the November survey. This increase in grasshopper numbers in season two could be caused by a higher rainfall in November-December (Table 4.1), providing more plant food and better conditions for the grasshoppers. A more in-depth discussion of the factors causing fluctuations in grasshopper populations is however outside the scope of this paper. Theincrease in grasshoppers between the two years also poses a possible explanation for the surprising main effect of treatment in the February/March surveys. Although the number of grasshoppers in year one was higher in ungrazed paddocks than in moderately grazed paddocks, that difference was much smaller than the large difference between moderate and hard grazed paddocks in year 2 (note the log scale in Figure 4.2), leading to an overall higher abundance in the moderate/moderate grazed paddocks than the no grazing/ hard grazed paddocks.

The results only include two lizard/ grasshopper activity seasons, and have to be interpreted with caution. In the November surveys, there was no influence on grasshopper numbers detected from either year or grazing treatment, even though the ungrazed paddocks of year one had been changed into hard grazed paddocks for much of the winter before the surveys of year two. This indicates that the numbers of grasshoppers in spring of year two may have depended on the grazing level of the previous year, or may have been completely independent of local grazing pressure.

In contrast to the number of grasshoppers, patterns of the mean weight of the grasshoppers were much simpler, being only affected by survey time. Grasshoppers in November (late spring) were significantly larger than those caught in January or February/March (summer), and were completely unaffected in weight by the local grazing regime. A probable interpretation is that some large species may overwinter as adults or sub-adults and have their largest adult stage in spring, the period of peak activity and mating of the lizards.

Conclusion

The present study shows significant effects of grazing treatments on both the relative body mass of the pygmy bluetongue lizards and on the numbers of grasshoppers. The grazing effect was however not present throughout all of the lizard activity season. Lizard body condition was only affected in spring while grasshopper abundance was mainly affected in late summer. This suggests there was no direct relation between grasshopper numbers and the relative body mass of the lizards. In fact the mean relative body mass of lizards declined through their activity season, while grasshopper numbers were increasing.

It is likely that the scales of this study both in time and size were insufficient to detect the true causal links between grazing, grasshoppers and relative body mass of the pygmy bluetongue lizards. A single season without grazing is unlikely to have allowed the vegetation to build up sufficiently to disadvantage the lizards in their ability to capture invertebrate prey. And the relatively small experimental paddocks probably allowed the mobile grasshoppers to move between paddocks, masking any difference between grazing treatments. Furthermore the grasshopper numbers were very low at the start of the activity seasons, making it difficult to detect minor treatment effects at these survey times.

For the lizards, contrary to our initial hypotheses, mean relative body mass in ungrazed paddocks was higher than in moderately grazed paddocks. This was however likely to be a consequence of lack of time for vegetation to build up, rather than lizards gaining an advantage from living in ungrazed paddocks. Future research should therefore focus specifically on what impact, living in high density vegetation, has on the lizards.

Through this study, it has been shown that the relative body mass of the pygmy bluetongue lizards is affected by different levels of grazing, with lowest relative body mass values during hard grazing. The specific factors causing the grazing effect, and the impacts of changes in relative body mass on lizard fitness is however unclear. It will therefore be important to the future management of this species to determine how grazing affects survival and fecundity of the lizards and to establish what level of grazing becomes harmful to the lizard populations. The study broadens our general understanding of the factors through which grazing affects local insectivore species. It also adds to our knowledge of the importance of food quality and accessibility to species that depends on internal energy stores for survival.

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	Year 1 (2013-2014)	Year 2 (2014 – 2015)
September-October	62	25
November-December	32	61
January-February	79	73

Table 4.1 Rainfall in mm rain, during September to February in each of the two years

Factor	D.f.	F	P
Year	1,4	6.193	0.068
Survey	1,4	876.290	**<0.001**
Treatment	1,4	1.098	0.354
Year*Survey	1,4	22.307	**0.009**
Year*Treatment	1,4	19.814	**0.011**
Survey*Treatment	1,4	3.668	0.128
Year*Survey*Treatment	1,4	17.728	**0.014**

Table 4.2 Results of repeated measures ANOVA comparing mean relative body mass of lizards per paddock in each grazing treatment group, in the December and March surveys of each year.

Factor	D.f.	F	P
Year	1,4	2.496	0.189
Treatment	1,4	0.048	0.837
Year*Treatment	1,4	43.445	**0.003**

A

Factor	D.f.	F	P
Year	1,4	22.573	**0.009**
Treatment	1,4	7.200	0.055
Year*Treatment	1,4	0.081	0.790

B

Table 4.3 Results of repeated measures ANOVAs comparing mean relative body mass of lizards per paddock in each grazing treatment group, separately in the A: December and B: March surveys of each year.

Factor	D.f.	F	P
Year	1,4	151.675	**<0.001**
Survey	2,8	105.943	**<0.001**
Treatment	1,4	45.407	**0.003**
Year*Survey	2,8	95.512	**<0.001**
Year*Treatment	1,4	43.593	**0.003**
Survey*Treatment	2,8	40.801	**<0.001**
Year*Survey*Treatment	2,8	37.917	**<0.001**

Table 4.4 Grasshopper numbers ^{GG} indicates that sphericity could not be assumed and the Greenhouse-Geisser correction has been used.

Factor	D.f.	F	P
Year	1,4	1.966	0.234
Treatment	1,4	3.670	0.128
Year*Treatment	1,4	0.003	0.956
A			
Year	1,4	13.182	**0.022**
Treatment	1,4	1.121	0.349
Year*Treatment	1,4	0.946	0.386
B			
Year	1,4	176.262	**<0.001**
Treatment	1,4	60.024	**0.001**
Year*Treatment	1,4	62.918	**0.001**
C			

Table 4.5 Results of repeated measures ANOVAs comparing mean grasshopper numbers per paddock in each grazing treatment group, separately in the A: November, B: January and C: late February to early March surveys of each year.

Factor	D.f.	F	P
Year	1,4	0.005^{GG}	0.947
Survey	2,8	19.682^{GG}	**0.010**
Treatment	1,4	0.330	0.596
Year*Survey	2,8	1.002^{GG}	0.374
Year*Treatment	1,4	0.006^{GG}	0.940
Survey*Treatment	2,8	1.426^{GG}	0.299
Year*Survey*Treatment	2,8	0.584^{GG}	0.490

Table 4.6 Result of repeated measures ANOVA considering effects on mean weight of grasshoppers in each paddock, of factors grazing treatment, survey time and year. ^{GG} indicates that sphericity could not be assumed and the Greenhouse-Geisser correction has been used.

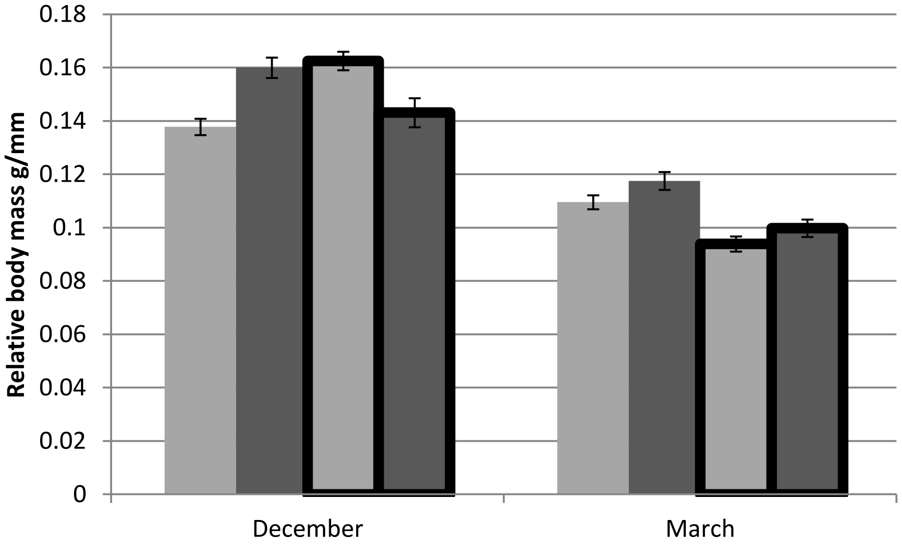

Figure 4.1 Mean (SE) relative body mass of adult lizards sampled in each grazing treatment group in each year and in each survey time: Non framed bars= year 1, Framed bars= year 2; Light grey=Moderate/Moderate grazing Dark grey=No grazing/Hard grazing

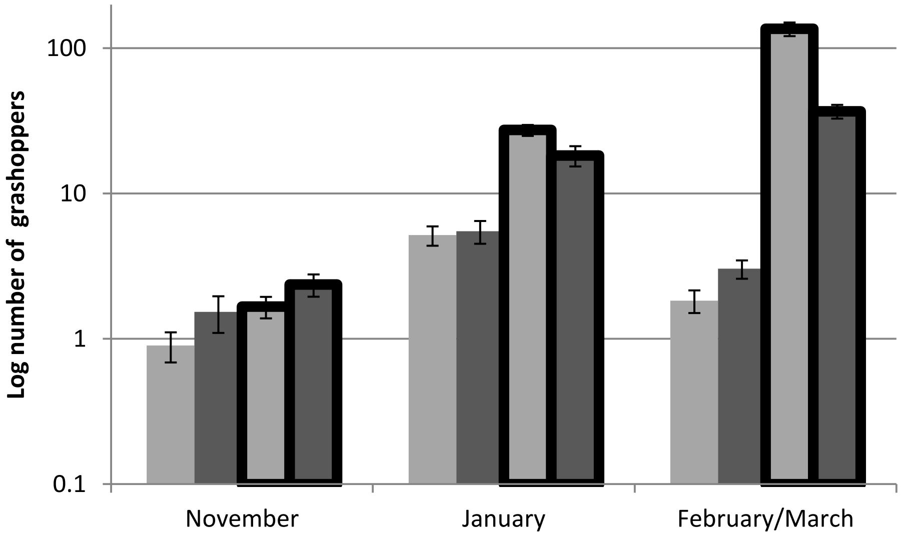

Figure 4.2 Mean (SE) number of grasshoppers (log transformed) per paddock for each treatment group, survey time and year. Non framed bars= year 1, Framed bars= year 2; Light grey=Moderate/Moderate; Dark grey=Non/Hard

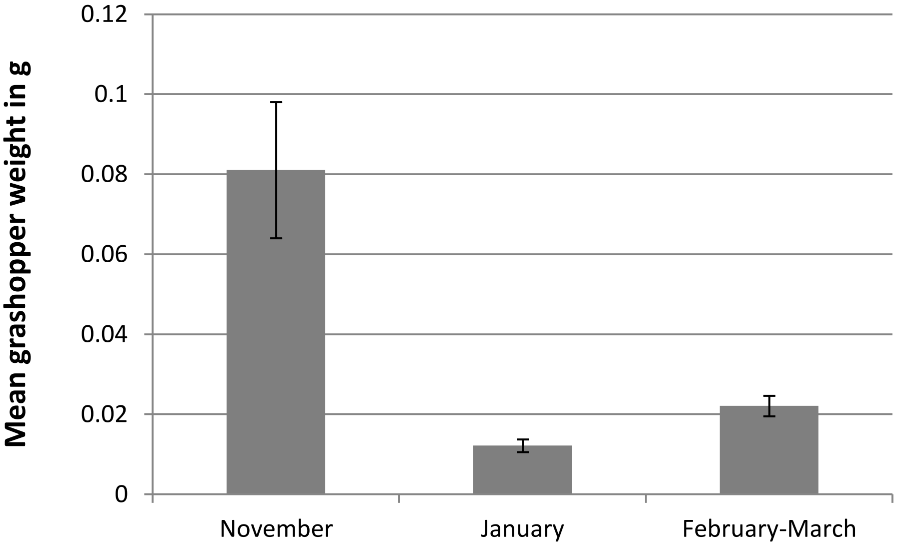

Figure 4.3 Mean grasshopper weight of both years, divided into surveys

Chapter 5: Fecundity

Low body condition and insufficient food availability can reduce the reproductive output of a reptile population. This can happen as a consequence of longer time between breeding events (Shine & Madsen, 1997) or in some cases no breeding activity at all (Aubret et al., 2002; Naulleau & Bonnet, 1996), but can also be caused by fewer offspring per reproductive event (Bonnet et al., 2001; Madsen & Shine, 1999).

As shown in chapter 4 grazing leads to a reduction in body condition of the pygmy bluetongue lizard, it is therefore likely that grazing also reduces the fecundity of this endangered species. This can have detrimental consequences, not only for the sustainability of current populations, but also because relocations have been predicted to be a necessary management initiative to protect the lizards from the effects of climate change (Fordham et al., 2012). As the success of captive breeding has so far been limited, future relocation efforts might have to depend of surplus individuals from existing populations.

Reliable survey methods are necessary to correctly assess the fecundity of the lizards and to determine population sustainability and whether individuals are available for relocation. During their breeding season (mid January to mid March) pygmy bluetongue lizards give birth to 1-4 live neonates, which are born inside their mother's burrow (Milne et al., 2002; Shamiminoori et al., 2015). Previous fecundity surveys have relied on observing and counting the neonates inside the burrow, but this is often made difficult by the female occasionally blocking the burrow and neonates dispersing shortly after birth (Milne et al., 2002). Ultrasound scanning of pregnant females of other lizard species has, although not perfect, proven quite accurate in determining the number of offspring, particularly in lizards with small litter sizes (Gartrell et al., 2002; Gilman & Wolf, 2007; Sacchi et al., 2012). Ultrasound scans could therefore be an alternative to observations in the burrows and could eliminate the risk of neonates being missed due to early dispersal from the maternal burrow.

This chapter aim to assess the effect of the different grazing regimes on the fecundity of the lizards. This is investigated using both traditional observations of neonates in the burrows and ultrasound scans of gravid female lizards, in order to compare the two methods.

5.1 The impact of sheep grazing on the fecundity and timing of reproduction in the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*.

Abstract

The endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) is only found in a few remaining patches of South Australian native grassland, all of which are privately owned and most of which are used for live stock grazing. The lizards occupy spider burrows, they mate in October - November and females produce litters of 1-4 live born neonates in mid January - mid March. Neonates are born inside their mother's burrow but within a few weeks they disperse to find their own burrows.

In this study we use ultrasound scans of females and observations of neonates in their maternal burrows to investigate how grazing affects the fecundity of the pygmy bluetongue lizard. We predicted that lizards in moderately grazed paddocks would have a higher reproductive output than lizards in hard grazed paddocks. Our results confirmed this prediction as ultrasound scans showed a higher mean number of yolk sacs in females from moderately than from hard grazed paddocks. Females from moderately grazed paddocks also gave birth significantly earlier than females in hard grazed paddocks. The higher number of yolk sacs did not result in a significantly higher number of neonates observed in the burrows, which indicates that the weekly burrow observations used in this study may underestimate true fecundity.

Understanding how grazing affects the fecundity of the pygmy bluetongue lizard is essential to the future management of this endangered species. This is not only because grazing is used to manage the habitat of all currently known lizard populations, but also because successful reproduction will be needed in those populations to supply the "surplus" individuals predicted to be essential for relocation programs to ensure the survival of the species.

Introduction

Intensive grazing by domestic livestock presents a challenge to many native species (Blevins & With, 2011; Howland et al., 2014; James, 2003; Pafilis et al., 2013; Wouters et al., 2012) as the grazing has the potential to change vegetation and soil structure (Bertiller & Ares, 2011; Dorrough et al., 2004; Fleischner, 1994). A reduction in the vegetation cover by grazing animals means that ground living animals such as lizards have less cover, and this could lead to a higher predation pressure (Castellano & Valone, 2006) and potentially change lizard behaviour (Wasiolka et al., 2010). Less vegetation could also reduce the density of herbivorous insects (Dorrough et al., 2004;

Rambo & Faeth, 1999; van Klink et al., 2014) and thereby reduce food availability to insectivorous lizards.

Increased predation stress and reduced food levels can decrease reproductive output, either through reduced fecundity, seen as fewer offspring per reproductive bout (Bonnet et al., 2001; Madsen & Shine, 1999) or as longer time spent to produce the offspring (Shine & Madsen, 1997). Thus in conservation management of native grasslands it is important to consider how grazing influences fecundity of the resident animal species.

The pygmy bluetongue lizard (Tiliqua adelaidensis) is an endangered scincid lizard only found in a few remaining patches of native grassland in the mid north region of South Australia. The lizards have a mean adult snout-vent length of 95mm (Milne, 1999) and live in spider burrows made by lycosid or mygalomorph spiders (Fellows et al., 2009; Milne & Bull, 2000; Milne et al., 2003b; Souter et al., 2007). The burrows function both as a refuge from predators, extreme weather conditions, and grass fires, and as an ambush point from where the lizards can prey on passing insects (Fenner & Bull, 2007; Fenner et al., 2007; Milne et al., 2003b). Lizards have been known to live in the same burrow for more than a year (Bull et al., 2015) and apart from short trips to defecate or stalk passing prey (Ebrahimi et al., 2015b, 2016; Fenner & Bull, 2011a), lizards normally only leave their burrows if they have become unsuitable and need to be replaced or during the October-November mating season (Milne et al., 2003b; Schofield et al., 2012), when males leave their burrows to find burrows with females, while the females lay out scent tracks to lead the males to their burrows(Ebrahimi et al., 2014). From mid January to mid March females give birth to litters of 1-4 live neonates (Milne et al., 2002; Shamiminoori et al., 2015). The neonates are born in their mother's burrow and then disperse to find their own burrows, usually within one to six weeks (Milne et al., 2002).

For any endangered species, understanding human impact on fecundity is essential for conservation management. Almost all of the native grassland habitat of the pygmy bluetongue lizard is now used for livestock grazing. Understanding the impact this grazing has on lizard reproduction is essential for the future management of the species. Grazing could increase the fecundity of pygmy bluetongue lizards by clearing the spaces between the grass tussocks, thereby enabling the lizards to thermoregulate and hunt more efficiently. Pettigrew and Bull (2012) showed that lizards living in burrows where the surrounding vegetation had been removed basked for longer times and made more predation attempts than lizards in burrows with denser surrounding vegetation. Overgrazing can however lead to lizards with lower body condition (Nielsen & Bull, 2016a) and reduced local abundances of grasshoppers (Nielsen & Bull, 2016a) their most common prey (Ebrahimi et al., 2015b; Fenner et al., 2007). We hypothesized that lizards would have higher fecundity in paddocks with a moderate level of grazing and an intermediate vegetation density, than in paddocks with more severe grazing pressure.

In this study we also compared two methods of assessing lizard fecundity. We used both ultrasound scans of wild caught female lizards, and optic fiberscope assisted observations of neonates in the lizard burrows. Ultrasound scanning can be used to confirm whether female reptiles are gravid and to provide an estimate of clutch sizes for both oviparous and viviparous species (Gartrell et al., 2002; Gilman & Wolf, 2007; Sacchi et al., 2012). This method has been successfully used on lizards as small as 40-50mm snout-vent length (SVL) (Gilman & Wolf, 2007; Sacchi et al., 2012). Early in gestation, fluid filled foetal membranes or yolk sacs can be seen in the scans as round or elongated structures, while embryonic movement, heart beat and skeletal features are visible during later stages (Gartrell et al., 2002; Gilman & Wolf, 2007). Maternal stomach, gut and other intestinal or skeletal structures can sometimes obscure the scanning image or appear similar to yolk sacs and embryonic membranes, but can in most cases be distinguished as they are more continual structures (Gartrell et al., 2002; Gilman & Wolf, 2007). Estimates of clutch size become harder, with a tendency to overestimate, as clutch size increases(Gartrell et al., 2002; Gilman & Wolf, 2007) but Sacchi et al. (2012) reported that 20 out of 22 common wall lizards (*Podarcis muralis*) with a mean clutch size of 3.6, laid exactly the same number of eggs as predicted by ultrasound scans. Gillman and Wolf (2007) found a highly significant correlation between the clutch size predicted by ultrasound scans and the number of eggs laid in five other species of lizards. In some viviparous lizards infertile yolk sacs are retained throughout gestation, so that scans overestimate the actual litter size (Gartrell et al., 2002).

For the pygmy bluetongue lizard, previous estimates of fecundity have been derived from observations of neonate lizards in the maternal burrows (Milne et al., 2002; Shamiminoori et al., 2015). Two previous studies using that method have reported different mean numbers of neonates per litter (Milne et al. (2002): 3.23 neonates per burrow; Shamiminoori et al. (2015): 1.58 neonates per burrow). This difference may have reflected real fecundity differences resulting from different ecological conditions during the two studies. Alternatively the estimates may have been differently affected by two sources of possible bias. First female lizards occasionally block the burrow, remaining above their offspring and preventing reliable fiberscope observations. Second, some neonate lizards can disperse within their first week (Milne et al., 2002), so that a survey that first detects a litter a week after it is produced, may only detect a portion of the original litter members.

In this study we investigated how different grazing regimes affected the fecundity of the pygmy bluetongue lizards, and we compared fecundity estimates from ultrasound scans and from burrow observations. We considered the implications of these results on future methodology for fecundity estimates and on conservation management.

Methods

The study was conducted during two lizard activity seasons (October to March 2013-2014 and 2014 - 2015) at the 'Tiliqua' property of the Nature Foundation of SA, near Burra, South Australia (33.67^{O}S; 138.93^{O}E) that has been previously described (Clayton & Bull, 2015; Fenner et al., 2007; Pettigrew & Bull, 2011). The study site is in an isolated patch of native grassland, that has not been ploughed for at least four decades, but has been used for sheep grazing for over a century. The region has hot dry summers and cool wet winters with an average annual rainfall at Burra of between 400-500 mm. The study site was divided into six adjacent experimental paddocks (size range 3.5-6.9 ha), arranged in a north to south line along the eastern edge of the property, each with similar elevation and slope. During the study alternative sheep grazing regimes were applied to these paddocks. We took advice on stocking rates from the property manager, but the number of sheep applied to a particular grazing regime varied between the two seasons as different rainfall patterns led to variation in vegetation growth. During the first season (2013 – 2014) half of the six paddocks were left ungrazed, while moderate grazing (2.70 ± 0.11(SE) sheep/ day/ ha) was applied to the other half. In the second season (2014 – 2015), moderate grazing pressure (1.54 ± 0.15(SE) sheep/ day/ ha) was maintained on the same three paddocks, while the three previously ungrazed paddocks were changed to a hard grazing pressure (2.69 ± 0.26(SE) sheep/ day/ ha). The two grazing treatments each year were applied to the six paddocks in an alternate pattern and details of the treatments have been previously described (Nielsen et al., 2016b). We previously reported a significant greater reduction in vegetation levels with increased grazing pressure (Nielsen et al., 2016b). Very rarely we saw kangaroos in the paddocks, and their grazing impact was considered to be limited and spread evenly across all paddocks.

Season 1 2013-2014

During September and October 2013, the six experimental paddocks were searched systematically for burrows, resulting in the location of between 14 and 26 inhabited lizard burrows in each paddock. These burrows were each marked by a plastic peg placed 30-40 cm in a standard direction from the burrow entrance and their GPS locations were recorded. Although we did not determine the sex of the occupying lizard at that stage, we assumed some were adult females. Many female lizards remain in the same burrows for an entire activity season or longer (Bull et al., 2015), allowing reliable relocation and observation of those females later in the season after they have mated. Mating takes place in late October or early November (Ebrahimi et al., 2014; Fenner & Bull, 2009; Milne et al., 2003a).

Between January 15 and February 13 2014, 342 burrows were inspected, and in 39 of those burrows clutches of neonates were recorded. The natal burrows were inspected to count the number of neonates that could be seen associated with each female, using an optic fiberscope as described by Milne, Bull and Hutchinson (2002). The mean litter size was compared between moderately grazed and ungrazed paddocks. Adult lizards were not extracted from their burrows, and sex could not be determined from the fiberscope images. Thus females without litters could not be told apart from males, and therefore, in the first season, the proportion of females producing litters could not be determined.

Season 2 2014-2015

In the second season, adults were located in their burrows as before during the 2014 spring. Then, in December 2014, following the mating season and probably over one month into their three month gestation, 38 females (Dec 3:14 lizards ; Dec10:16 lizards ; Dec 12:8 lizards) were caught by luring them out of their burrows with live mealworms, following the method described by Milne and Bull (2000). There were 19 females from the moderately grazed and 19 from the hard grazed paddocks. The lizards were placed in individual calico bags and transported, on their day of capture to a nearby veterinary clinic at Clare (33.8^{O}S; 138.6^{O}E). There each lizard was scanned with a 7mHz ultrasound scanner (Honda Electronics, HS-2000Vet), using a flat transducer that covered the length of the lizard from the anterior of the thorax to the cloacal vent. Lizards were scanned laying on their side, as the vertebral column obstructed the scanned view when the lizards were laying upright or on their back. Round structures (up to 8mm in diameter) within the female body cavity were detected and presumed to be developing yolk sacs (Figure 5.1). These yolk sacs were occasionally hidden behind each other or obscured by other structures. All lizards were therefore scanned from both sides and the yolk sacs were counted as a combined effort between the veterinary and the researcher to increase the reliability of the count. If the counts from each side were different, usually by one, the larger number was taken as the more accurate number of yolk sacs. In 13 cases, it was possible to see individual embryonic movements of a small white structure in the middle of the yolk sac, and these were classed as live embryos. Thus we had two measures of fecundity, the number of developing embryos as indicated by the yolk sacs, and the number of live embryos as indicated by movement. Following the ultrasound scans, each lizard was returned to her burrow within 10 hours of initial capture.

From mid January 2015, all 38 burrows of the ultrasound scanned lizards were checked for neonates weekly. When these surveys started three of the female lizards were no longer in their original burrows and they were excluded from any future surveys and analysis. The first neonates were detected on January 21 2015, and weekly inspections were made over the next five weeks, with a final inspection in the seventh week.

For each burrow in each survey we recorded if the adult female was still present and the number of neonates we could see. Adult mothers sometimes blocked the burrow so that the fiberscope probe could not pass, in which case only neonates that were above the adult could be recorded. Thus the number counted may be lower than the actual litter size. The maximum number of neonates recorded over consecutive inspections was used as an estimate of the litter size. Neonates typically leave their natal burrow at some time over the six weeks after birth (Milne *et al.* 2002), so subsequent surveys are also likely to underestimate actual litter size. Day of birth was calculated from January 21, the first observation of neonates in the second season. We assumed either a neonate or an adult female had dispersed from the burrow if it was not detected there on two consecutive surveys. A separation time was estimated for each burrow, from the birth date to the first time when only one lizard (either the female or a single neonate) was left in the burrow, or the burrow had been completely abandoned. Neonates are never found with adult lizards in the following spring and so adults and neonates must all separate sometime before they become inactive in winter. In this study any mothers and neonates that had not yet separated by the last survey in week seven (March 9), were assumed to have separated in the following week.

We tested whether fecundity, as indicated by ultrasound scans or by visual counts of litters, date of birth, and separation time differed between moderate and hard grazing treatments, and whether there were differences among the various methods of measuring fecundity. For most parameters, the data were not normally distributed, and nonparametric tests were used to compare reproductive outputs between grazing treatments. Fisher's exact test was used to compare proportions. Mann-Whitney tests (GraphPad InStat ver. 3.06) were used to compare fecundity measures, day of birth and separation times, between grazing treatments. Repeated measures Wilcoxon signed-rank tests (SPSS ver. 23.0) were used to test the difference between yolk sac counts, live embryo counts and neonates observed in the burrows, where data for each parameter were available from the same female individuals.

Results

Season 1 2013-2014:

In burrows where neonates were detected in season one, there were no significant difference (Mann-Whitney U-statistics= 149.0 P= 0.29) between the mean number of neonates counted in the burrows from ungrazed (2.29 ± 0.29 SE; N = 17) and moderately grazed paddocks (1.86 ± 0.22 SE; N = 22)

Season 2 2014-2015

Ultrasound scans detected visible yolk sacs in 35 of the 38 females that were scanned in the second season. In one of those females, the scans were less clear, but the female was later confirmed to be gravid by visual observation of neonates. The last three females (one in moderate and two in hard grazed paddocks) showed no signs of reproduction either by ultrasound scan or by subsequent neonate observation. Thus 92.1% of sampled females showed evidence of being gravid in that season. Among the gravid females, live embryos were detected in nine of 18 (50%) in moderately grazed paddocks, and in four of the 17 (23.5%) in hard grazed paddocks. The difference in proportion of females with live embryos between grazing treatments was not significant (Fisher's exact test P= 0.16).

The mean number of yolk sacs detected by ultrasound scans of the 35 gravid females differed significantly between the two grazing treatments (Mann-Whitney U-statistics= 89.5 P= 0.036) with more detected in females from moderately grazed paddocks than from hard grazed paddocks (Figure 5.2).

Among the 13 females with live embryos detected, there was no significant difference (Mann-Whitney U = 12.5; P= 0.44) between the mean number of live embryos in females from moderately (n = 9; 2.78 ± 0.32 SE) and hard grazed (n = 4; 2.25 + 0.48 SE) paddocks.

Neonates were subsequently detected in nine burrows in moderately grazed paddocks (50% of the gravid females) and seven burrows in hard grazed paddocks (41% of the gravid females). There was no statistically significant difference in the proportions of females with detected litters between grazing treatments. We first compared mean detected litter sizes between grazing treatments, using all 35 females that had been ultrasound scanned and had remained in their burrows. This analysis included females with no detected neonates (i.e. a litter size of zero) and showed no difference in mean detected litter size (Mann-Whitney U = 143.5; P= 0.76) between burrows in moderately (n = 18; 0.83 ± 0.23 SE) and hard (n = 17; 0.82 ± 0.29 SE) grazed paddocks. In a second analysis only burrows with observed neonates were compared. Again there was no statistically significant difference in mean detected litter size (Mann-Whitney U = 27.5; P= 0.71) between females from moderately (n = 9; 1.67 ± 0.24 SE) and hard (n = 7; 2.00 ± 0.44 SE) grazed paddocks .

Females gave birth significantly earlier (Mann-Whitney U = 7.5; P= 0.007) (Figure 5.3) and there was a non-significant tendency for females and neonates to stay together for a shorter time (Mann-Whitney U= 18; P= 0.092) (Figure 5.4) in moderately grazed than in hard grazed paddocks.

Among the 13 females with live embryos observed through ultrasound scans, there were significantly more live embryos than detected neonates from the same females (Wilcoxon signed-rank Z= -2.791; P= 0.005) (Figure 5. 5). We also found a non-significant tendency among the 17 females with yolk sacs detected during the ultrasound scans and with subsequent observations of neonates in the burrows, for a higher mean number of yolk sacs (2.29 ± 0.56 SE) than observed neonates (1.82 ± 0.65 SE) per female (Wilcoxon signed-rank Z= -1.554; P= 0.12).

When comparing between the two seasons, the mean observed litter size, only in burrows with neonates detected, was not significantly different (Mann-Whitney U = 283.5; P= 0.60) between season one (n = 39; 2.05 ± 0.18 SE) and season two (n = 16; 1.81 ± 0.23 SE).

Discussion

Our analyses found no significant effect of grazing treatment on the mean number of neonates observed in litters inside female burrows in either of the two seasons. In season two, when we could identify burrows with resident females, litters were detected in 46% of those burrows. In contrast, ultrasound scans of the same females from earlier in that season showed that 92% were gravid and that females from moderately grazed paddocks carried significantly more yolk sacs than females from hard grazed paddocks. The larger number of yolk sacs, as well as the significantly earlier litter production in moderately grazed paddocks indicate a higher reproductive fitness in these paddocks compared to hard grazed paddocks. This increased reproductive fitness is likely to be related to the previously reported higher body condition of lizards in moderately than in hard grazed paddocks (Nielsen & Bull, 2016a). These results are similar to results from studies of the aspic viper (*Vivipara aspis*), which show that mating activity as well as litter and neonate size are affected by body condition and prey availability (Aubret et al., 2002; Bonnet et al., 2001).

We suggest two different explanations for why the ultrasound scans produced higher estimates of both the proportion of gravid females, and the mean litter sizes of those females, than the field observations of females with their litters. The first is that not all of the observed yolk sacs were fertile, or resulted in live neonates, so the count of yolk sacs overestimates the actual number of live births. We only found evidence, from movements, of live embryos in 13 females and it is possible that some of the yolk sacs without movement did not support a live embryo, as it has been shown that some viviparous lizards retain infertile yolk sacs all through the gestation (Gartrell et al., 2002). But it is also possible that the scanned yolk sacs may have all been alive but at different stages of development, so that only a proportion of them were sufficiently advanced to show movement. In ultrasound scans performed on a larger member of the tiliqua family, the blotched blue-tongued lizard (*Tiliqua nigrolutea*), identifiable features developed gradually as the gestation went along, and were not all visible in the early stages (Gartrell et al., 2002). The optic fiberscope inspections of burrows never found any infertile yolk sacs or dead neonates in the burrows. However, other reptile species have previously been reported to ingest dead neonates and other embryonic material soon after parturition (Lanham & Bull, 2000; Rivas, 2000), and it is therefore possible that infertile yolk sacs were present at birth but not detected. Future studies should focus on developing reliable methods to estimate fecundity of these endangered lizards, if possible by doing both ultrasound scans and burrow observations in captive caged populations where the number of live neonates produced at parturition can be more reliably determined. Then the relationship between ultrasound scan counts and actual litter sizes could be more rigorously established.

An alternative explanation for the difference between yolk sac counts and observed litter sizes is that we did not record all of the litter during observations of neonates in the burrows. There are two possible reasons for this. The first is that females with neonates physically block the burrow so that the optic fiberscope cannot pass, and observers are prevented from detecting neonates that are deeper in the burrow. In season two of this study, 21 of the 35 surveyed females (60%) blocked their burrows in this way during one or more of the weekly surveys. The second is that neonates may have already dispersed from their natal burrows before the litters have been detected in a weekly survey. Milne et al. (2002) showed that 34.4% of burrows with litters had no neonates left after one week. We performed weekly surveys during the birth period of the second season, and it was likely that some neonates would have left their natal burrow between the time of their birth and the time of the next survey. These results therefore indicate that studies based on weekly surveys of lizard burrows could underestimate the true reproductive output of pygmy bluetongue lizard populations.

In summary our study suggests that, although ultrasound scans seem more reliable than observed litter counts in the field, both estimates of fecundity come with assumptions and potential biases. Nevertheless, those biases may be tolerated in a comparison between alternative treatments, such as the grazing treatments in this study, if fecundity estimates in each treatment have similar biases, or if any differences in the biases can be accounted for. For instance any observed difference in neonate numbers between grazing treatments would have been skewed towards higher numbers in hard grazed paddocks, as the neonates from these paddocks tended to stay longer in their burrows and were therefore more likely to be detected and counted in the weekly surveys.

Although the ultrasound scans may overestimate the final neonate outcome, the significantly larger number of yolk sacs produced by females in moderately grazed paddocks, suggests that these females produce more neonates than females in hard grazed paddocks. We also showed that parturition happens earlier in moderate than hard grazed paddocks and since the neonates need to establish their own burrow refuges, and develop energy reserves during the late summer to allow them to survive the coming winter, an earlier birth is likely to give them an advantage. Our conclusion is that hard grazing probably reduces fecundity, and delays the time of birth of litters of the pygmy bluetongue lizard. This probably arises because excessive grazing reduces levels of vegetation to a degree that either significantly impacts the abundance of grasshoppers and other insects that the lizards feed on, or significantly increases the level of predation related stress because of lowered cover. The outcomes of this study are entirely consistent with a parallel set of analyses that showed lizard body condition was relatively lower in the hard grazed paddocks (Nielsen & Bull, 2016a). It is also consistent with studies of aspic vipers (*Vivipara aspis*) and water pythons (*Liasis fuscus*)showing that the proportion of reproducing individuals is positively correlated to prey abundance and body condition (Aubret et al., 2002; Bonnet et al., 2001; Naulleau & Bonnet, 1996; Shine & Madsen, 1997).

Understanding the factors that impact fecundity is important to the future management of the pygmy bluetongue lizard. The effects of grazing are particularly important as all currently known populations are on grassland used for grazing purposes, and pygmy bluetongue lizards and sheep have probably co-existed in these sites for more than 100 years. In these habitats invasive plant species tend to overgrow the open spaces between native grass tussocks, which possibly makes basking and hunting harder for the lizards (Pettigrew and Bull 2012). Thus conservation managers are likely to depend on some form of grazing to control the vegetation cover. A critical question is how much grazing is acceptable, and this study indicates that too much grazing can become detrimental for the future recruitment into the population. Relocations have been predicted to be part of the future management effort of this endangered species (Fordham et al., 2012). If these relocations rely on "surplus" individuals from current populations, then we need reliable indicators of fecundity and recruitment, and reliable information about the grazing regimes that maximise reproductive output.

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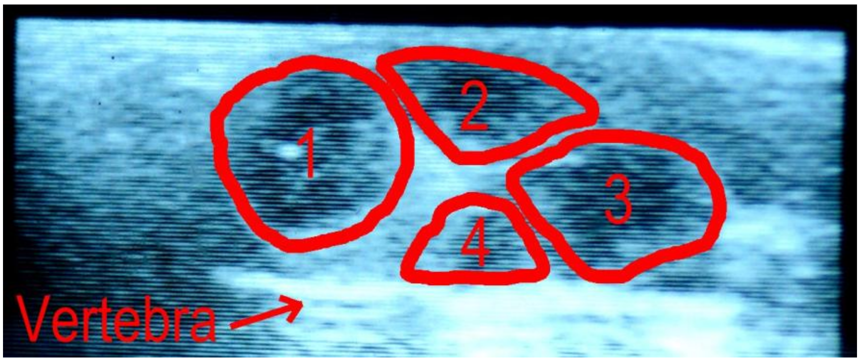

Figure 5.1 Ultrasound image showing four yolk sacs two slightly behind the other two. The female lizard was scanned from the side (ventral surface up, dorsal surface down on the image) so that the image shows the length of the lizard from the thorax (left) to the cloacal vent (right) with her vertebral column seen as a white line underneath the yolk sacs.

Figure 5.2 Number of yolk sacs per female found by ultrasound scans in gravid females caught in moderately (N=18) and hard (N=17) grazed paddocks. Boxes show the 1st quartile median and 3rd quartile (1st quartile and median are identical in moderately grazed paddocks). Whiskers show the 10th and 90th percentile and outliers outside these percentiles are shown with a dot.

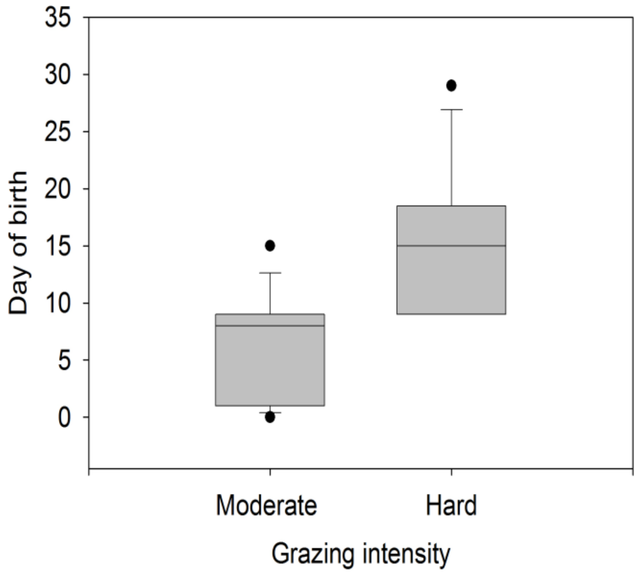

Figure 5.3 Day of birth, after Jan 21 for litters observed in burrows. Data are shown for females in moderately (N=9) and hard (N=8) grazed paddocks. Data displayed as in figure 2.

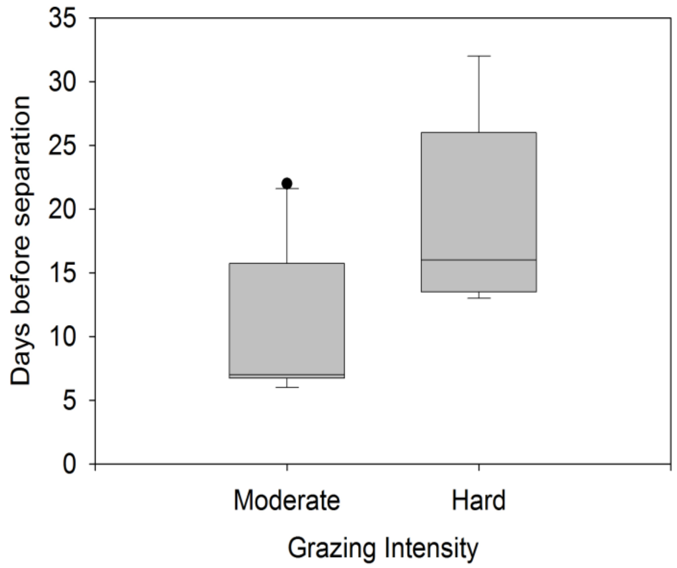

Figure 5.4. Separation times for observed litters in moderately (N=9) and hard (N=8) grazed paddocks. Data displayed as in figure 2.

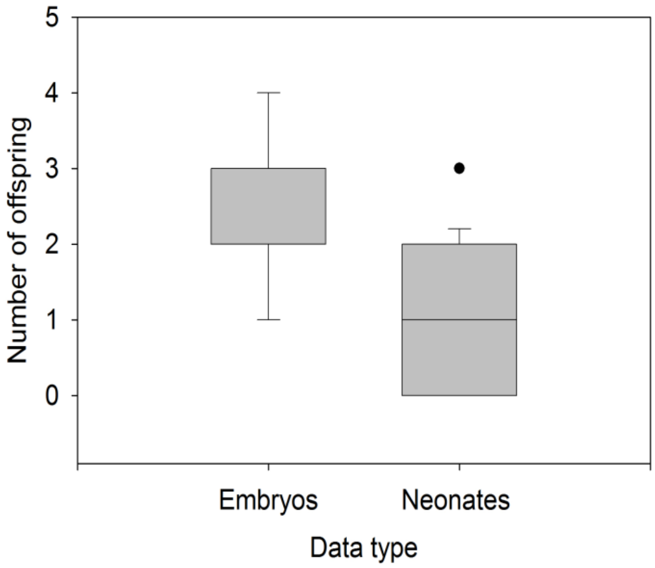

Figure 5. 5 Number of live embryos found by ultrasound scans compared to the number of neonates observed in the burrows of the same females (N=13). Data displayed as in figure 2.

Chapter 6: Assessment of the possible use of detection dogs in pygmy bluetongue lizard surveys.

The ability to reliably detect either an animal, its tracks or feces is often the first condition that needs to be met when a study on that animal species is being planned. This can be a problem with species that are either cryptic or rare (Cablk & Heaton, 2006; Davidson et al., 2014; Woollett et al., 2015). Trained detection dogs can in many cases be used to track animals and their whereabouts, and have been used for conservation work at least since the 1890s (Woollett et al., 2015).

Experiments have shown that dogs can discriminate between related species (Browne et al., 2015) and can generalize, which means they can be trained on captive individuals while still being able to detect wild ones (Cablk & Heaton, 2006). Certain environmental conditions are however harder for the dogs to work in than others. Since dogs cannot sniff and pant at the same time, warm weather or strenuous exercise can reduce the dog's detection rate (Gazit & Terkel, 2003; Smith et al., 2003). Some species are naturally harder for dogs to detect (Miller et al., 2015; Woollett et al., 2015), while others have behavioural traits such as living in deep burrows, so that odours are less widespread on the surface, that makes the dogs less likely to find them (Duggan et al., 2011).

Pygmy bluetongue lizards spends most of their lives in spider burrows, which is likely to be part of the reason why they were thought to be extinct for 32 years (Armstrong & Reid, 1992). The burrows have diameters less than 2cm and can be more than 50cm deep (Milne & Bull, 2000). Situated in grassland with a variety of native and exotic grasses and forbs, finding the burrows by visual detection can be challenging and the detection probability varies with the height and density of vegetation. Since the rediscovery of the pygmy bluetongue lizards in 1992 and until 2008 the population of pygmy bluetongue lizards in a one hectare square at the original rediscovery site was surveyed, but the reported population size varied greatly from year to year (Julie Schofield, personal communication).

The use of detection dogs trained to find pygmy bluetongue lizards in their burrows, could potentially provide a more reliable alternative to the present surveys based on visual detection. The detectability of the lizards is however unknown and both the warm climate and the potentially deep burrows that the lizards live in poses potential problems for the use of detection dogs. Based on the training of one pygmy bluetongue lizard detection dog, the aim of this chapter is to provide knowledge about the potential for the use of detection dogs in future surveys of these cryptic little lizards. Note that although this chapter suggests the value of using a dog for lizard surveys, the dog was not properly trained until the end of the project and so was not actually used to detect lizards for much of the project time.

6.1 A nose for lizards; can a detection dog locate the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*)?

Abstract

Environmental detection dogs have been used in various research and conservation projects. By detecting scent, dogs are capable of finding animals or scats that are virtually undetectable to humans. The endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) is endemic to heavily fragmented patches of native grassland in the mid-north region of South Australia. The lizards live in spider burrows with entrance diameters of 10-20 mm, which makes them difficult to find. This study presents the first data showing that a dog can detect pygmy bluetongue lizards. The dog showed the ability to distinguish between pygmy bluetongue lizard scent and the scent of sleepy lizard (*Tiliqua rugosa*) and bearded dragon (*Pogona barbata*). The dog also found more than 30 occupied lizard burrows in the field. Up until now surveys of this endangered lizard have relied on human visual detection of burrows, which is a very labour intensive process. Although the climate and the ecology of the lizards pose a challenge to detection dogs, this study showed that their use in surveys of pygmy bluetongue lizards could potentially increase efficiency and accuracy, while lowering the cost and the workload.

Introduction

Environmental detection dogs are increasingly being used for research and conservation projects. If an animal species is rare or cryptic, visual searching by humans to monitor and assess populations can be time consuming and unrewarding. Dogs have much greater olfactory sensitivity and, if trained to recognise and respond to the olfactory cues of a species of interest, are sometimes used to supplement human searching. They have been used to detect mammals (Brook et al., 2012; Cristescu et al., 2015; Davidson et al., 2014; de Oliveira et al., 2012; DeMatteo et al., 2014; Gsell et al., 2010; Smith et al., 2003), birds (Wasser et al., 2012; Woollett et al., 2015) and reptiles (Browne et al., 2015; Cablk & Heaton, 2006) as well as various invertebrates (O'Connor et al., 2012; Woollett et al., 2015). Dogs are used to detect either the animal itself, or scats of the animal, which indicate the species is present and can be used for DNA analysis (Davidson et al., 2014; DeMatteo et al., 2014; Pearson et al., 2014).

Some studies have found that searching with dogs was cost effective (Davidson et al., 2014), while others have not (O'Connor et al., 2012). However, most find that dogs search an area faster and more effectively than humans (Cablk & Heaton, 2006; Cristescu et al., 2015; de Oliveira et al., 2012; Smith et al., 2003). In some cases, dogs can find targets that are virtually undetectable to humans because of their small size or cryptic location (Cablk & Heaton, 2006; de Oliveira et al., 2012). Although effectiveness of searching by dogs can vary among target species (Miller et al., 2015), dogs can be used to survey areas where other methods are impractical, such as areas where camera traps are at high risk of being stolen (DeMatteo et al., 2014), or where animal movement is too infrequent for regular trapping methods to provide a representative sample of the population. This paper reports investigations into the practicality of using a dog to help monitor populations of the endangered Australian pygmy bluetongue lizard (*Tiliqua adelaidensis*).

The pygmy bluetongue lizard is an endangered skink endemic to a few remaining patches of native grassland in the mid-north region of South Australia (Souter et al., 2007). The climate in this region is characterised by cool wet winters and hot dry summers with frequent daytime temperatures above 30°C. Pygmy bluetongue lizards are only active during spring and summer, with their peak activity season being October to December (Ebrahimi & Bull, 2014; Pettigrew & Bull, 2012; Schofield et al., 2012). Effective conservation management of this species requires a clear understanding of the local population dynamics in these patches and monitoring of representative subsets of the populations (Fellows et al., 2009; Pelgrim et al., 2014).

Finding pygmy bluetongue lizards for effective monitoring can be very difficult as they are relatively small (mean adult snout-vent length: 95 mm) and spend most of their time in cryptic single entrance spider burrows with entrance diameters between 10 and 20 mm, and an average depth of 23 cm (Milne & Bull, 2000; Milne et al., 2003b). The burrows function as shelters from extreme weather, predators and bushfires, and as ambush sites to capture passing insects (Milne & Bull, 2000; Milne et al., 2003b; Pettigrew & Bull, 2014; Souter et al., 2007). Lizards may temporarily leave their burrow to defecate (Fenner & Bull, 2010) or to stalk passing prey (Ebrahimi et al., 2015b), but apart from this they only leave to find a new burrow, or during the spring mating season, when predominantly males move about outside their burrows looking for mates (Ebrahimi et al., 2014; Fenner & Bull, 2011b; Milne et al., 2003b; Schofield et al., 2012). Therefore, drift fences with pitfall or box traps that rely on lizards moving about will sample only a small portion of the pygmy bluetongue lizard population during limited periods.

Detection dogs could potentially be an effective tool to monitor known lizard populations and to survey areas for new populations. However, both the habitat and the lizard ecology (living predominantly underground) pose challenges to a detection dog. Using dogs to detect ground squirrels was less successful if squirrels were in natural or long artificial burrows, than if they were in short artificial burrows or if scat could be detected on the ground surface (Duggan et al., 2011). We were, therefore, unsure if a dog could reliably locate a lizard that was deep in a burrow.

Other problems concerned working in high summer temperatures. Dogs detect scents by sniffing, but cannot sniff and pant at the same time. In other studies, search duration increased and detection efficiency decreased when dogs were panting frequently, following exercise or during searches in high temperatures (Gazit & Terkel, 2003; Smith et al., 2003).

In this study, we investigated whether a detection dog can (1) reliably identify pygmy bluetongue lizard scent, and distinguish the target scent from scent from other lizards found in the same habitat; and (2) find naturally occupied pygmy bluetongue lizard burrows in their grassland habitat.

Methods

One mixed breed adult female dog was selected for training in February 2013 on the basis of an observed persistence in searching for food and toys. Laboratory training and trials with the dog were conducted in the animal housing facility at Flinders University, Adelaide, South Australia. Field training and one trial were conducted in native grassland habitat at the "Tiliqua" property of the Nature Foundation of SA, near Burra, South Australia (33.67^{O}S; 138.93^{O}E).

Dog training in the laboratory

From March to October 2013, the dog was trained to recognise pygmy bluetongue lizard scent. Lizard scent was derived from a captive colony of nine pygmy bluetongue lizards held in the animal housing facility at Flinders University. Lizards in the colony were held in individual cages with a sand substrate, and each lizard was provided with a 30 cm PVC pipe (diameter 20 mm) which it used as a refuge burrow. To collect scent samples we wiped a filter paper around the dorsal and ventral part of the front half of a lizard, avoiding the cloaca and, thus, any faecal contamination. Alternatively, we left a piece of filter paper inside the lizard burrow for 24 – 48 hours and allowed refuging lizards to leave their scent on it. Since lizards leave their burrows to defecate (Fenner & Bull, 2010) we assumed these samples would also not contain the scent of scats. Scat scent was avoided, to train the dog to find lizards rather than scats dropped by lizards away from the burrows. During training we used scent samples that were less than 24 hours old.

The dog was conditioned to associate pygmy bluetongue lizard scent with a reward. Conditioning sessions were conducted by one person (TN). Sessions were normally conducted daily and each lasted 5-10 minutes. In each session the dog was presented with a target 5-10 times. Targets consisted of a scent sample in a plastic vial with a mesh lid, and the dog was rewarded with food if she sniffed at the target. Once the dog started to respond to the scent, she was taught to indicate her finding by sitting and, on command, putting her nose on the target.

Dog training in the field

Field training sessions were conducted 4-6 times a week, between November 2013 and March 2014 and again during October - December 2014. During training, the dog was fitted with a harness and a 10 m leash, to allow unhindered search effort, while the handler still maintained control over the dog. Initially, the dog was led right up to an occupied burrow and was rewarded with her ball for sniffing at the burrow. She was then taught to indicate the burrow location by sitting and, on command, by pointing to the entrance with her nose. Following initial conditioning to the lizard scent and the Tiliqua site, the dog was allowed to search independently. During this phase the distance between the dog release site and a known occupied burrow, and the session length, were both gradually extended. At the beginning of the independent search training the distance to the burrow was 6-8 m and the maximum search time allowed was 3-4 minutes. By December 2014 the handler allowed the dog a search track of up to 100 m, and search sessions lasting up to 10 minutes. Before these free searches, we selected a target burrow, known to be occupied by a pygmy bluetongue lizard, and inspected that burrow and several unoccupied burrows in the immediate vicinity using the same techniques. This was done to avoid the dog simply responding to a burrow with scent from the handler.

Effectiveness of training; lab trials

Two laboratory trial series were conducted between 16 November 2013 and 11 January 2014. Trials were run in a 2.5 x 15 m corridor in the animal holding facility at Flinders University. In each trial, three plastic vials, as used in the training sessions, were placed in a line 3 m apart, along the corridor floor, at 3, 6 and 9 m from the point where the dog was released. In the first trial one vial contained a pygmy bluetongue lizard scent and the other two contained clean filter paper controls. In the second trial the two controls were replaced by filter papers containing scents from other lizards: a bearded dragon (*Pogona barbata*) and a sleepy lizard (*Tiliqua rugosa*). The three vials were placed in a new random order by an assistant before each search, with neither the dog nor the trainer present. The dog was then allowed to search the vials, and we recorded the first vial where she sat down. Each search took less than 30 seconds. Each trial consisted of 30 searches, performed in blocks of five searches in quick succession on each of 6 days.

Effectiveness of training; field trial

The dog became sick in the summer 2015 so the field trial could not be conducted until late February. In this trial, one search per day was conducted on four days between 23 and 26 February 2015. One search was however excluded and will not be mentioned any further, as it was impossible to establish if a lizard was in the target burrow. Each search was conducted in a previously unsearched area of the Tiliqua property. Prior to each search, the trainer, without the dog, located apparently suitable burrows, but remained more than 1 m from the entrances. One marker peg was placed at a random distance between 3 and 8 m from the burrows. A second marker peg was placed at the same distance from the burrow in an adjacent quadrant. To avoid human scent trail cues, the handler walked in a pattern, to make a five pointed star (this pattern was not marked by pegs), centred around the burrow, and with a 5 m tip to tip distance.

During searches the dog was then brought to the first marker peg, and allowed to start searching. If the dog showed signs of losing interest in the search, handler and dog moved to the second marker peg and the dog was sent off searching again. Each search was run for 10 minutes, or until a lizard was found, or until the dog showed obvious signs of not searching. Following each search, the burrow was checked with an optic fiberscope (Milne et al., 2003a, 2003b) to establish if there was a lizard present in the burrow.

To determine if the amount of time that lizards were at their burrow entrances might influence detectability, we measured lizard activity at the burrow entrance from 2 to 19 March 2015, by filming the lizard burrows using methods previously reported for the 2013-2014 field season (Nielsen et al., 2016a). During the time of the day when lizards were most likely to be active, video cameras were placed 1 m from the burrow entrance of confirmed lizard burrows. The recordings were started and the researcher left the area to avoid disturbing the lizard. Subsequently the recordings were analysed and the amount of time the lizard spent at the entrance or outside the burrow was recorded. Lizard activity in March 2015 was compared to lizard activity measurements from the same area but at different burrows, from the previous field season, using Mann-Whitney tests (GraphPad, InStat 3).

Results

Laboratory trials

In each of the two trials the dog found the pygmy bluetongue lizard target in 28 of 30 searches, a highly significant deviation from random (Fisher's exact test, P<0.0001). In the remaining searches, the dog seemed uninterested in the task and simply sat at a random scent container without even sniffing at it. The change in behaviour could easily be used to discount these false positives.

Field training

During the field training in March and October – December 2014, the dog found and correctly indicated several known lizards in their burrows during independent searches. As these were training sessions, the general progress of the training was recorded, but detailed records were not kept of exact number of burrows found, or of false positive responses. However, our available records indicated that the dog found at least 30 already identified occupied burrows from 9 weeks of independent searches, with 5-6 searches per week. She also found three previously undiscovered burrows occupied by lizards. False positive responses, where the dog came back and sat down in front of the handler, putting her nose on the ground often without any burrows nearby were rare and easily identifiable because of the distinct behaviour .

Field Trial

In each of the three searches in the field trial in late February 2015, we confirmed a lizard occupied the target burrow after the search was completed. In the first search the dog found a lizard, but in a different burrow to the target burrow. Nevertheless, this was considered a success. No lizards were found within 10 minutes in the remaining two searches even though lizards were in the burrows and the dog passed straight over those burrows.

Lizard activity during the time when the field trial was conducted was very low. The entrance of 22 burrows was video-recorded for a combined duration of 32 hours (approximately 1.5 hours per burrow) in early March 2015. Only 5 of the 22 lizards spent any time at the entrance or outside their burrow during the recordings. The recordings showed that lizards spend an average of 5.2% of the time (range 0-83.1%) at the burrow entrance. Mean time spent at the burrow entrance was shorter than in March 2014 (mean 25.7%; range 0-94.6%), a marginally insignificant difference(Mann-Whitney test U=70.0; P=0.0509).In March 2015 lizards also spent significantly less time at the entrance than in an earlier main activity season (October - December 2013) (mean

44.8%; range 0-99.4%; Mann-Whitney test U=79.5; P<0.0001).

Discussion

Although dogs have been used previously in monitoring programs of more mobile reptiles , this study is to our knowledge the first to use a detection dog to find burrow living lizards. The laboratory trials clearly showed that our dog was capable of detecting the scent of the pygmy bluetongue lizards. It also showed that the dog could distinguish pygmy bluetongue lizard scent from the scent of other lizard species.

The combined results of the field training and field trial gave a strong indication that the dog could also find pygmy bluetongue lizards in the field. During training, the dog still correctly identified three previously unknown lizards in their burrows, as well as locating more than 30 known lizards. It also found an unknown lizard during the field trial. During training, the dog did not show any tendencies to give false positive responses, by indicating on unoccupied burrows. Her location of four previously unknown lizards by coincidence is, therefore, very unlikely.

Video recordings conducted immediately following the trial in 2015showed that most lizards had become inactive and were rarely appearing at their burrow entrances, possibly resulting in very little scent around the burrow. During the searches the dog passed straight over two occupied lizard burrows, seemingly without any reaction. This lack of response was a clear change in behaviour, compared to the previous field training when she consistently indicated occupied burrows. Due to this lack of success, the trial was discontinued after only three searches, to avoid unintentionally training the dog to ignore occupied burrows.

We suggest two possible explanations for the lack of response by the dog in the February field trial - the dog may have forgotten her training while ill, or an inactive lizard sitting in a burrow with one entrance and no airflow, spreads very little scent. To test if the dog was still able to identify and distinguish the scent of pygmy bluetongue lizards, was trained for two days in the laboratory. This training followed the protocol of the previous laboratory trials, with the exception that the handler swapped the samples around between searches. As the dog showed no reduced response in the laboratory (results not shown), we think this explanation is unlikely. It seems more likely that an inactive lizard in a burrow with one entrance and no airflow spreads very little scent. If the dog relies on recently deposited scent from the lizard at the burrow entrance, then the inactivity identified during the time of the field trial, may result in the dog ignoring occupied lizard burrows.

Although the data regarding lizard activity were recorded a short time after the field trial, we think it is likely that the lizards had similarly reduced activity during the trial. This is supported by previous studies showing a general decrease in lizard activity late in the activity season (Pettigrew & Bull, 2012)

A general concern with the field training and field trial is the possibility that the handler may influence the dog by unintentional cues (Lit et al., 2011). We did not have access to independent assistants to find occupied burrows but the handler was aware of the possible risk and, once the initial conditioning had been finalised, made every effort to avoid signalling to the dog. Part of the training was recorded and assessed by an experienced professional dog trainer, who found no indication of handler signalling to dog in the training method. The dog was on a 10m lead usually ahead of the handler, and generally did not seek contact with the handler during searches, minimising the risk of unintentional signals. During the field trial the dog did not find any of the three target burrows, but found one lizard in a burrow previously unknown to the handler. We find it unlikely that these results can be ascribed to the dog using unintentional cues from the handler to locate occupied lizard burrows.

Previous studies have shown that the distance dogs can detect scent can vary from centimetres to kilometres (Woollett et al., 2015). Results presented here suggest that pygmy bluetongue scent is at the lower end of this scale. While pygmy bluetongue lizards may signal conspecifics using olfactory cues to indicate burrow ownership (Fenner & Bull, 2011a) and mating readiness (Ebrahimi et al., 2014), their sedentary burrow occupancy (Bull et al., 2015) increases the risk of predation (Ebrahimi et al., 2015a). Reducing detectability by predators by leaving less scent around the burrow, could advantage the lizards. Less scent may be deposited by reducing the time spent at the burrow entrance.

Previous studies have also shown variability in the detection ability among individual dogs (Browne et al., 2015; Smith et al., 2003). Since our study is based on a single dog, the conclusion that dogs could be used to assist in pygmy bluetongue lizard monitoring needs to be treated with some caution.

Nevertheless, surveys have so far depended on visual detection of lizard burrows by human researchers, followed by time consuming inspection of each burrow with an optic fiberscope, which is labour intensive and highly dependent on both observer experience and field conditions such as the density of vegetation on the ground. Detection dogs may be an efficient and easily added supplement to traditional survey methods, reducing time and cost. We recommend that surveys should be conducted between October and December when lizards are most active and most likely to produce detectable scent at burrow entrances. Surveys with dogs should also be conducted on cool days, or in the early morning, to minimise the need of the dog to pant as this can lead to reduced searching efficiency (Gazit & Terkel, 2003; Smith et al., 2003).

The dog in this study was trained by a non-professional trainer (TN) with some experience in other kinds of dog training and in consultancy with two experienced detection dog trainers. Detection dogs could be a valuable component of a community monitoring program using volunteers with some dog training experience

In order to assess the advantages of using detection dogs, further research should be directed into determining their efficiency of dogs, especially the area dogs can cover per hour, how long they will effectively participate in the search, and the effect of dense vegetation where human surveyors are unlikely to detect most lizard burrows.

Acknowledgements

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Chapter 7: Ecological and behavioural observations

This chapter includes three manuscripts with potential importance to the future management of the pygmy bluetongue lizards.

The first manuscript investigates how pygmy bluetongue lizards utilize random weather events such as morning dew and rainstorms, as an opportunity to drink. Other dessert dwelling animals are known to supplement the water they get from their food through water harvesting (Comanns et al., 2011; Glaudas, 2009; Sherbrooke, 2004), but this is the first study to investigate the pygmy bluetongue lizards use of freestanding water. If further investigation shows that this water source is important to the lizards, it would be valuable to include this knowledge in future management plans.

The second manuscript examines the indirect effects of red foxes (*Vulpes vulpes*) digging for the lizards. Foxes are known invasive predators of many native reptiles, mammals and birds, in some cases with catastrophic effects for local populations (Moseby et al., 2009; Moseby et al., 2011; Olsson et al., 2005; Read & Scoleri, 2015; Wayne et al., 2011). Although the pygmy bluetongue lizard has several native predators, foxes are capable of digging for the lizards which is something none of the native predators can do. The manuscript investigates the destructive effects of fox digging the lizard burrows. Even though the fox rarely gets deep enough to extract the lizard itself, the lizard burrow structure is compromised. Lizard burrows are an important resource for the lizard populations and predator control could be a necessary initiative to protect the lizards and their burrows.

The final note is based on an observation of a dead lizard near another lizard's burrow. The dead lizard showed clear signs of having fought and been killed by the resident lizard. Pygmy bluetongue lizards are known to defend their burrows (Fenner & Bull, 2011b) and possibly use their scats to mark their territory (Ebrahimi et al., 2016; Fenner & Bull, 2010), but have never been reported to kill each other. This observations indicates that caution would have to be taken, particularly when lizards are introduced to each other, and at relatively high local densities as a part of captive breeding programs.

7.1 A thirsty little lizard; drinking by the pygmy bluetongue lizard

Abstract

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is an endangered scincid lizard, endemic to the Mid North region of South Australia, where it occupies heavily fragmented patches of native grassland. Pygmy bluetongue lizards live in spider burrows and rarely venture far from their burrows. This limits their access to free standing water, and it was previously unknown whether lizards acquired all the water they needed from their food, or whether they could access an alternative source of water. This paper presents 27 observations of lizards drinking either from drops of morning dew or from rain drops accumulated on vegetation close to their burrow entrances. These observations suggest that pygmy bluetongue lizards can supplement their water requirements independent of free standing water sources, like streams or ponds, if there is vegetation that can collect dew and rain close to burrow entrances. Vegetation that can hold water droplets for a short time provides flexibility for the lizards in maintaining their water budgets. The water collecting function of vegetation should be included as a factor in short term management decisions about the level of grazing in the lizard's habitat. In the longer term the benefits of having plants that can accumulate water drops will need to be considered if suitable relocation sites are to be found.

Introduction

All animals need water, whether it is gained through consumption of food or by drinking. Getting enough water is a constant challenge to animals living in arid and semi-arid habitats. Reptiles solve this challenge in a number of ways. Some, for example arid living padloper turtles (*Homopus femoralis*), are simply most active when water is available (Loehr, 2012) while others have developed behaviours, like dorso-ventral flattening reported in desert living rattle snakes, to collect rain water, which the snakes sobsiquently drank (Comanns et al., 2011; Glaudas, 2009; Sherbrooke, 2004; Sherbrooke et al., 2007; Vesely & Modry, 2002). Some lizards have evolved specialised morphological features that help to collect and access condensed water on their surface. For instance in the arid living Australian thorny devil (*Moloch horridus*), condensed water is channeled along grooves between scales from the whole body into the mouth (Comanns et al., 2011; Sherbrooke, 2004; Sherbrooke et al., 2007). Others like the desert dwelling Gila monster (*Helodermqa suspectum*) use their bladder as an internal water reservoir, which can be filled when water is available (Davis & DeNardo, 2007). In times of water shortage, Australian sleepy lizards (*Tiliqua rugosa*), in their semi-arid chenopod scrubland habitat, can take advantage of unpredictable rainfall events to gain water, even when substrate moisture is only briefly available, and at times when the lizards are normally inactive (Kerr & Bull, 2004).

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is an endangered skink (IUCN 2014). It has a mean adult snout-vent length of 95mm, and is the smallest member of the genus *Tiliqua*.(Milne, 1999)

The species is endemic to a small region in the Mid North region of South Australia, where it occupies a few remaining patches of native grassland, a formerly common habitat now largely converted into crop land. This region has an annual rainfall of 400-500 millimeters, but most of the rain falls in the cool winters when the lizards are inactive, while less rain falls during their summer activity period.

Pygmy bluetongue lizards are apparently unable to dig their own burrows, but instead occupy single entrance, vertical burrows originally built by lycosid and mygalomorph spiders (Milne & Bull, 2000; Milne et al., 2003b; Souter et al., 2007). The lizards are solitary and spend most of their lives inside their burrows or at it's entrance. The burrows function as shelters from extreme weather, refuges from predators and bushfires, and ambush sites to capture passing insects (Fenner & Bull, 2007; Fenner et al., 2007; Milne et al., 2003b). Lizards may occasionally stalk prey for short distances, but apart from this they only leave their burrow to defecate, or if they have to find a new burrow, or during the spring mating season, when predominantly males move about outside their burrows looking for mates (Fenner & Bull, 2011a; Milne et al., 2003b; Schofield et al., 2012).

They will gain some water from their insect prey, but little is known about whether lizards need additional water, particularly in summer, when prey may become scarce, and when free standing water is hard to find. We describe how pygmy bluetongue lizards can gain water during summer, without leaving their burrow, by drinking from dew and raindrops that have accumulated on nearby vegetation.

Methods

Observations were conducted within the "Tiliqua" property of the Nature Foundation of SA, near Burra, South Australia (33.67^{O}S; 138.93^{O}E), over two austral spring and summer seasons 2011/2012 and 2013/2014. Spring and summer is when the lizards are active and appear at their burrow entrances. They remain inactive inside their burrows over the cooler winter months. The results developed from serendipitous observations derived during two separate and different studies. Both studies used video surveillance to investigate lizard behaviour with as little disturbance as possible.

In the 2011/2012 season up to three CCTV cameras (SONY Effio 2.8mm-10mm, 30 fps) were positioned 100 cm above the entrance and the immediate surrounding area of each of 23 occupied burrows. The cameras were set to record from sunrise to sunset on 10 consecutive days each month for five months (October - February). The field of view from the cameras centred on the burrow entrance and included a surrounding area of 60 x 60 cm.

In 2013/2014 we used two higher resolution digital video cameras (Panasonic HX-WA30) mounted on tripods (Velbon videomate 438) 100 cm from the burrow entrances and 140 cm above them. On each of 44 days between October 2013 and March 2014, we filmed a different pair of occupied burrows for 75 – 100 min. On most days filming took place between 1200 - 1600 h, although when the maximum temperature for a day was expected to exceeded 35^{O}C , conditions that inhibit pygmy bluetongue lizard activity(Ebrahimi & Bull, 2014) we filmed before 1200 h or after 1600 h. The field of view of the cameras included an area with a radius of approximately 20 centimetres around the burrow entrance.

We observed playback of the video recordings and identified lizard behaviours that appeared to represent drinking from droplets of water close to the burrow entrance formed as dew or from rain.

Results

The observations described here, are the first descriptions of pygmy bluetongue lizards imbibing water from dew and raindrops. Typical behaviours that we describe as drinking were seen from the close up footage during a rain shower on 13 Feb 2014, with both of the filmed lizards on that day extending their tongues repeatedly towards raindrops caught on vegetation. This drinking behaviour differed in several ways from the more normal, and regularly observed sensory tongue flick (Figure 7. 1). Although both involve tongue movements, the sensory tongue flick is a short quick movement (0.1-0.3 sec), where the tongue is kept slim. Often the lizard's nose is pointing upwards from the burrow entrance during sensory tongue flicking. Drinking involves slower tongue movements (0.4-0.5 sec), during which the tongue is kept wide probably in order to extract as much water as possible. The lizard keeps its nose close to the substrate while licking accumulated water. Drinking can happen as single licks or in series of up to 30 repeated licks following immediately after each other. One drinking session can consist of multiple repeated series of licks. On 13 Feb 2014 rain started falling soon after filming had commenced, and water droplets were available to the lizards for almost the entire 92 min filming session. During this session one lizard licked 825 times.

Over the two seasons, similar drinking behaviour was observed from lizards in 18 burrows. In some burrows lizards were observed drinking on more than one day, and we recorded 27 separate 'drinking days', cases when a lizard was observed drinking on a day. In 15 cases lizard drank from morning dew that had accumulated in droplets on plants, while the remaining 12 were from rain. Drinking was recorded throughout the activity season, with the earliest record on 19 Oct and the latest on 13 Feb. While drinking from droplets of dew was recorded only early in the morning (0440 - 0758 h), drinking from rain could occur throughout the day (0508 – 1903 h). In 2011/12, when lizards were filmed for the whole day, the mean time spent drinking per day for 25 drinking days was 51 min (SE = 13.52: range = 3-260 min).

Discussion

Getting enough water is a common problem for many animals and the locations of water sources have been shown to directly impact the local distribution of a reptile population (Lillywhite et al., 2008).

In the case of the pygmy bluetongue lizard, the problem of getting enough water seems, to some degree, to have been resolved by licking morning dew or raindrops off the surrounding substrate or vegetation, taking advantage of unpredictable periods of high atmospheric moisture. Although sensory tongue flicking is commonly observed in the pygmy bluetongue lizard (Fenner and Bull 2011), the behaviour described here was only recorded when either rain or dew were present, and differed substantially from the sensory tongue flick. We considered this to provide strong circumstantial evidence that the lizards were drinking. Burrow living has a number of advantages for these lizards (Fenner & Bull, 2007; Fenner et al., 2007; Milne et al., 2003b), but since they rarely venture far from their burrows, the need for water could become a disadvantage, particularly if their invertebrate prey are scarce. Our observations showed that they can overcome this problem by taking advantage of droplets of water from rain and dew that appear close to the burrow entrance at irregular intervals.

Knowing when and how the pygmy bluetongue lizard can find supplementary water is an important component to the management of this endangered species. Apart from the water gained from their food, rain and dew must be the only other source of water normally available. None of the known populations are close to a free standing water body, and the lizards rarely move from their burrows to allow contact with temporary pools or puddles.

In the short term, the amount of grazing by sheep and cattle in an area occupied by a lizard population could impact the availability of water from this source. Dew normally forms as water droplets on the low grassland vegetation, and although raindrops might hit bare ground, summer rainfall will rarely be heavy or persistent enough to create free standing water close to burrow entrances. Most rain will be almost instantly absorbed by the dry soil. Lizards are therefore mostly dependent on water drops suspended from vegetation close to the burrow for their prolonged summer drinking sessions.

Overgrazing that has removed much of the vegetation will make it more difficult for lizards to find water to drink, especially to find dew in the morning when temperatures are low, and when lizards are even more unlikely to venture away from their burrows.

In the long term, climate change is likely to result in warmer and drier conditions in South Australia, fewer cool, dew forming mornings in the spring and summer, and fewer rainfall events during the period when these lizards are active. Models predict that relocations will have to be a part of future management, if this species is to persist (Fordham et al., 2012). In that case it will be essential to take water sources, and vegetation that can accumulate water droplets, into account when searching for suitable relocation sites.

This is the first reported observation of drinking behaviour in the pygmy bluetongue lizard, and the data presented here are serendipitous. Future research could investigate the physiological and ecological effects of drinking, and what advantage this behaviour gives the lizards.

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Figure 7. 1 Photo A shows a lizard drinking by licking raindrops of vegetation Photo B shows the same lizard sensory tongue flicking

7.2 Impact of foxes digging for the pygmy bluetongue lizard (*Tiliqua adelaidensis*).

Abstract

The endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) is endemic to heavily fragmented patches of native grassland in the Mid-North region of South Australia. The lizards live in spider burrows, and suitable burrows can be a limiting factor to local populations. The invasive red fox (*Vulpes vulpes*) is a likely predator of the lizards. No previous research has focussed on the effects of foxes on pygmy bluetongue lizards. This paper presents observations from three sampling rounds in a single lizard activity season. During each round, burrows were located and lizard presence or absence, fox digging and burrow condition were recorded. Out of 256 monitored lizard burrows, 12 were dug out by foxes. The data show that foxes actively seek to prey on pygmy bluetongue lizards. In several cases, the fox gave up before reaching the lizard. Dug out burrows were quickly abandoned by the surviving lizards and became filled in, reducing the supply of suitable burrows for the population. These data highlight the need for fox management around pygmy bluetongue lizard populations, especially if future management of the species involves relocation, as small newly established populations could be vulnerable to fox predation and secondary effects of burrow destruction.

Introduction

Since its introduction in Australia in about 1871 the red fox (*Vulpes vulpes*) has become a threatening invasive predator. The fox is adaptable, and with a diet varying in size from insects to large mammals (Davis et al., 2015; Spencer et al., 2014) it is affecting a long list of native fauna (Moseby et al., 2009; Moseby et al., 2011; Olsson et al., 2005; Read & Cunningham, 2010; Read & Scoleri, 2015; Wayne et al., 2011). Apart from a direct impact of predation (Moseby et al., 2011), foxes can also have an indirect effect on the food web, by preying on native predators and thereby releasing predation pressure on some prey species (Moseby et al., 2009; Read & Scoleri, 2015). For some native reptiles, removal of foxes can either increase (Wayne et al., 2011) or reduce (Moseby et al., 2009; Olsson et al., 2005) their abundance, depending on the trophic interactions between native and introduced predators.

The pygmy bluetongue lizard (*Tiliqua adelaidensis*), with a mean adult snout-vent length of 95mm, is the smallest member of the genus *Tiliqua* (Shamiminoori et al., 2014). With most of its former habitat now converted into crop lands, this scincid lizard is currently considered endangered both in South Australia and internationally (IUCN), and is restricted to a few remaining patches of native grassland in the Mid-North region of South Australia.

Pygmy bluetongue lizards inhabit single entrance, burrows originally constructed by lycosid or mygalomorph spiders (Milne & Bull, 2000; Milne et al., 2003b; Souter et al., 2007). The lizards are solitary and rarely leave their burrows, which function as ambush sites, from where they can prey on passing insects, as well as acting as refuges from extreme weather conditions, bushfires and predators (Fenner & Bull, 2007; Fenner et al., 2007; Milne et al., 2003b).

Being apparently unable to dig their own burrows, pygmy bluetongue lizards defend their burrows vigorously against potential rival conspecifics (Fenner & Bull, 2011b) and suitable burrows are thought to be a limiting resource in some populations (Souter et al., 2007). Lizards occasionally leave their burrows temporarily to defecate or stalk close-by prey, but burrows only seem to be permanently vacated if they get filled in, get too small (for growing juveniles and sub-adults) or during the spring mating season when predominantly males move around to locate females (Ebrahimi et al., 2014; Fenner & Bull, 2011a; Milne et al., 2003b; Schofield et al., 2012).

Reported native predators of the pygmy bluetongue lizard are eastern brown snakes (*Pseudonaja textilis*) and various species of birds (Fenner et al., 2008). As none of these have the capacity to dig, lizards will in most cases be safe once inside their burrows. The introduced fox can probably locate lizard burrows by scent, and may be able to dig the lizards out. This paper reports incidents of fox digging for pygmy bluetongue lizards, and investigates what happens to the lizards and their burrows after fox digging.

Methods

Observations were conducted from late October 2012 to early April 2013, within the “Tiliqua” property of the Nature Foundation of SA, near Burra, South Australia (33.67^{O}S; 138.93^{O}E). They were part of a larger experiment, following the dynamics of lizard burrows in paddocks with varying levels of sheep grazing. From late October to early December 2012, up to 50 burrows considered suitable for lizards were located in each of six paddocks, and their GPS coordinates recorded. Lizard occupancy was confirmed using an optic fiberscope to inspect the inside of the burrow, following Milne & Bull (Milne & Bull, 2000). In each of two subsequent sampling rounds round 2: January-March 2013 and round 3: March-April 2013), known burrows were checked for inhabitants and burrow condition. Those burrows that had no lizard in them and had filled in, to less than 150mm of depth or had entrances with a diameter of less than 12mm, were considered destroyed and excluded from the following round. In each survey round, the aim was to replace destroyed burrows by exactly the same number of newly located lizard-suitable burrows (32 burrows in round 2 and 18 burrows in round 3), so that there was still the same number of burrows in each survey round. Fox digging was visually identified by obvious scratch marks, piles of loose dirt and funnel shaped holes encasing the entrance. Only burrows that were checked in at least two rounds, and where a lizard was found at least once, were included in the analysis. During visits to the site, at least two visually different foxes (one was larger and with thicker fur) inhabited the area and were seen usually at least once every two days during the study. No other predators that could dig, such as dogs or goannas were ever observed in the area.

We compared the frequency of abandonment by lizards, and the frequency of burrow destruction, between burrows with and without traces of foxes digging, using Fisher’s exact test in GraphPad InStat ver. 3.06.

Results

During the season, 256 burrows with lizards were observed, with 12 of these showing signs of fox digging (Table 7. 1). By the end of the season, significantly more lizards (10 (83%) of 12) had disappeared from burrows where foxes had been digging, compared to 126 (52%) of 244 in other monitored lizard burrows (Fisher's exact test P= 0.039). Six of the lizards in fox dug burrows were still detected in the burrow on the first observation after the digging was detected, but had disappeared from the burrow by the next observation. There was a significantly higher rate of deterioration by the end of the season among burrows that had been dug by foxes (7 of 12; 58%) than among the other burrows (34 of 244; 14%) (Fisher's exact test P< 0.001). This resulted partly from the loose dirt the fox left behind filling in the burrow, but also from the crater created, with the burrow entrance in the middle, channelling loose dirt and other debris into the burrow.

Discussion

The results presented here are the first to report foxes finding and digging for the pygmy bluetongue lizard. This study clearly shows that foxes have the potential to be a threat to the endangered pygmy bluetongue lizard. The data summarise only burrows where lizard occupancy was identified either before or after the digging. These data will therefore be an underestimation of the true damage of the foxes, as burrows where the lizards had been eaten or moved, before the burrow was surveyed, are not recorded.

Other research has shown that a trained sniffer dog can detect and find pygmy bluetongue lizard burrows (T. Nielsen unpublished data 2014) and it is likely that other canids such as foxes have similar abilities. The two traits of foxes, scent detection and digging capability(Dawson et al., 2014) are not combined in any of the known native predators of this lizard (Fenner et al., 2008). Because the main defence of the lizard is retreating to their burrow (Milne et al., 2003b), and because the average depth of lizard burrows is only 23.4cm (Milne & Bull, 2000), pygmy bluetongue lizards could be vulnerable to digging predators. Lizards might be especially at risk in their spring activity season, when the ground can be soft for digging and when females are spreading scent around the entrance to their burrows (Ebrahimi et al., 2014).

However, in at least 50% of our observations (6 of 12 cases) the fox abandoned digging before it had reached the lizard. But even with the lizard unharmed, an indirect adverse effect was a rapid deterioration of burrow quality following the digging. This effect might be just as bad for the lizards as if they had been caught by the fox, since it forces them to move to another burrow, thereby both exposing them to other predators, and leaving fewer burrows for lizards. Intact burrows are in short supply (Souter et al., 2007), so this fox activity has wider implications for the overall population of pygmy bluetongue lizards.

Other studies have found that foxes, through trophic cascade effects, increase the abundance of some small reptiles (Moseby et al., 2009; Olsson et al., 2005). This is unlikely to be the case with the pygmy bluetongue lizard, as the native predators of the lizards, mainly eastern brown snakes and various raptors are unlikely to be preyed upon by foxes, to any significant degree (Davis et al., 2015; Spencer et al., 2014). Only in areas where feral cats are abundant is it likely that the presence of foxes could have a positive effect on small lizards through competition with or predation on cats (Glen & Dickman, 2005).

Models that incorporate predicted climate changes have shown that relocation is likely to be part of the future management of the pygmy bluetongue lizard, if this species is to be conserved (Fordham et al., 2012). The observations presented here highlight a possible management issue to current populations of lizards as well as to future plans to relocate the lizards. Foxes probably affect the lizards both directly, through predation, and indirectly, by destroying the burrows. This can be a serious threat to a species that only persists in small isolated populations, and has limited numbers of suitable burrows.

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	No fox digging	With fox digging
Burrows with lizards	244	12
Burrows destroyed	34	7
Lizard moves	126	10
Lizard remains	118	2

Table 7. 1 total number of burrows with lizards, number of burrows destroyed, number of lizards that had moved and lizards that had stayed during the season. Only burrows that has had a lizard in them during the season was included.

7.3 *Tiliqua adelaidensis* (pygmy bluetongue lizard). Territorial defence and fighting.

T. adelaidensis is an endangered scincid lizard endemic to a few remnant fragments of native grassland in the Mid-North region of South Australia. On 2 December 2013, an adult individual was discovered dead on the surface, twisted into an ”S” shape, apparently the result of a snapped vertebral column (Figure 7. 2). The dead lizard was lying immediately adjacent to a burrow occupied by another conspecific. *T. adelaidensis* are unable to dig burrows themselves, but spend most of their lives in burrows dug by lycosid or mygalomorph spiders. Burrows deeper than 20 cm are preferred and can be a limited resource in their grassland habitat (Souter et al., 2007). Occupied burrows are aggressively defended by both sexes, against model lizards (Fenner & Bull, 2011b). Male lizards encountering each other out of their burrows, during the mating season, fight by biting on to each other and wrestling on the ground (pers. observation), but no actual fights over burrow ownership have previously been observed. During mating, males bite on to females by the neck, resulting in scale damage. Similar damages are occationally found on the head, neck and body of males, presumably from fighting with other lizards. We could not deduce the sex of either the dead lizard (too dry) or the resident of the burrow (could not be lured out). Because the dead lizard was whole, without bite marks or parts being eaten, we consider predation or spider attack (Ebrahimi & Bull, 2012) as unlikely causes of death. The contorted body suggested that a natural death was unlikely. Although we found no scale damage to confirm it, we instead propose that the immediate proximity to an occupied burrow strongly suggests death resulting from intra-specific aggression, with the resident conspecific attacking a potential rival for burrow ownership.

Figure 7. 2 A: dead *Tiliqua adelaidensis* found next to the entrance of an inhabited burrow. B: lizard back, showing the snapped and separated vertebrae (Green arrows)

Chapter 8: Conclusion

8.1 General overview

The primary aim of this study was to determine the effects of varying grazing pressure from no grazing, to moderate grazing and to hard/intensive grazing on the pygmy bluetongue lizard and the grassland habitat it lives in. It follows on from previous studies, mainly by Pettigrew (2011), who used small scale simulated grazing in the form of clipping of the grass, to ask similar questions. This study went one step further towards understanding the real impact of grazing, by using sheep to create a real grazing scenario in six experimental paddocks containing pygmy bluetongue lizard populations.

It was hypothesized that lizards would prosper the most in moderately grazed paddocks, as the vegetation cover derived from this grazing regime would ensure sufficient prey insects and cover from predators, while still be open enough to allow efficient hunting and thermoregulation. It was expected that, in paddocks entirely without grazing, vegetation density would become so dense that it would interfere with the lizards hunting and basking behaviours, while paddocks with hard grazing would have insufficient abundance of prey insects, particularly grasshoppers. It was also anticipated that the better ecological conditions in the moderately grazed paddocks would result in lizards with better body condition and fecundity.

8.1.1 Habitat requirements.

As expected, vegetation cover decreased with increasing grazing intensity. Increasing grazing intensity was also associated with greater deterioration of burrows over winter. This is likely to be a result of trampling and increased erosion, although the analyses showed no direct relationship between the vegetation cover surrounding the burrow entrance and the persistence of the burrow. Burrows inhabited by lizards were less likely to deteriorate over winter, possibly because lizards to some degree are capable of maintaining and stabilizing the burrows.

Grasshopper numbers were also affected by grazing intensity, with an increase in grazing pressure generally causing a decrease in grasshopper numbers. This is a trend that might be predicted by the grasshopper requirement for vegetation to feed on. Less vegetation would suggest fewer grasshoppers. However, there were substantial variations in grasshopper numbers between surveys and seasons and the effect of grazing on grasshopper numbers was largest during the late summer, but limited during spring when food were plentiful. Additionally, the relatively small distance between adjacent paddocks with different grazing treatments, may have led to some blurring of any differential grazing impact on grasshopper numbers.

8.1.2 Effects on the pygmy bluetongue lizards.

The effects of grazing on the lizard choices of burrows was unclear based on the results presented in this thesis. Seen over an entire lizard activity season, burrows occupied by lizards had less surrounding vegetation cover than the average for the paddock. However, in spring, when lizards were most likely to be moving between burrows and choosing where to establish residency, there was a tendency for burrows occupied by lizards to have more surrounding vegetation than the average for the paddock. This indicates that lizards may have preferences for more densely vegetated locations when they initially choose burrows in spring. But later in the season as vegetation cover changes, they may not be willing to move burrow based on these preferences alone. Thus the actual vegetation cover around their burrows may not be due to their choice, but more due to burrow availability and changed vegetation density patterns over the season. Alternatively, lizards may choose burrow locations in spring based on other characteristics than vegetation density, but our observational data cannot allow us to resolve among these various interpretations.

The mean body condition of the lizards in the paddocks was shown to decrease with increasing grazing intensity, but although this mirrored the trend for lower numbers of grasshoppers, the connection between predator (lizard condition) and prey (grasshopper abundance) was complex. While grasshopper numbers were not affected by grazing until late summer, lizards in more intensely grazed paddocks already had lower body condition than lizards in less grazed paddocks by early summer. The results indicated that lizards were more dependent on grasshopper size than abundance, as the lizards managed to build up their body condition in a short period in spring, which aligned with the time of year where grasshoppers were largest but less abundant. A possibility that we did not explore, is that more sheep led to higher levels of disturbance of the lizards, and thus less time available for waiting for prey.

As a likely consequence of the lower body condition, grazing also affected the fecundity and gestation period of female lizards. Ultrasound scans of lizards showed fewer yolk sacs in gravid females from hard grazed paddocks than lizards from moderately grazed paddocks. This same trend was not found in the numbers of neonates observed in the burrows, but this could be because neonates often disperse shortly after birth and might have done so before the burrows were inspected for counts of litters. The results further showed that females in hard grazed paddocks gave birth later than females in moderately grazed paddocks.

8.1.3 Survey method

A secondary aim of the project was to investigate the possibility of using trained detection dogs to find pygmy bluetongue lizards in their burrows. Although detection dogs have been used successfully in surveys of several other animal species, including reptiles. The amount of time that pygmy bluetongue lizards spend inside their burrow in a habitat that is often hot and dry, could pose problems to a detection dog. The results showed that the detection dog trained for this study could detect pygmy bluetongue lizard scent, and discriminate between this scent and the scent from two other lizard species. It was also shown that during field training, the dog was capable of detecting inhabited lizard burrows, while ignoring other empty burrows or burrows inhabited by spiders and other invertebrates. Unfortunately the training was only completed towards the end of the study, so these capabilities could not be exploited in this study.

8.2 Implications for conservation management

Based on the results presented in this thesis, It is clear that grazing affects the pygmy bluetongue lizards and their grassland habitat in a number of ways. It was however only possible to partly confirm the hypothesis that moderate grazing is the best management practice to conserve this endangered lizard. Moderate grazing was better than hard grazing, as hard grazing caused increased burrow deterioration and decreased body condition and fecundity. The results, however, also indicated that under the circumstances applying to this study, paddocks without grazing for one season were better for lizards than moderately grazed paddocks. The lack of any negative effects found in ungrazed paddocks could however be due to the time limitations in the study. Ungrazed paddocks in this study, had been grazed before the study was started, and were only left without grazing for a total of just under two years. This would only allow for limited build up of dead vegetation. If left without grazing for a greater number of years, the amount of dead vegetation would be much larger, producing a thatch that might displace much of the current bare ground, and reducing suitable locations for lizard burrows. This study can therefore not disprove a possible negative effect of prolonged periods without grazing.

Another important point is that this study only indicates what is the best management option out of a limited range of grazing regimes. Not only are there many alternative grazing densities to be considered, but these regimes can all be adjusted in numerous ways for instance in cycling the sheep on and off the paddocks at varying time intervals. There might be better grazing options than the ones used in this study. It is also worth mentioning, that while this study searched for "ideal" lizard conditions, this is not the same as the conditions the lizards need to survive. In a semi-arid habitat, many factors may influence pygmy bluetongue lizards, and ideal conditions can be rare. Furthermore, an optimal grazing regime in one year may not be as preferable in another. Lizards are likely also to be able to survive and populations to persist under less optimal conditions. Indeed a number of pygmy bluetongue populations have persisted, probably for over a hundred years on sheep grazed paddocks with different farmers exploiting different grazing practices.

However, superimposed on this, perhaps preliminary exploration of the impact of grazing pressures, this study has introduced two new methods that, although used in studies of other lizards, have never before been used to study pygmy bluetongue lizards. Detection dogs and ultrasound scans both showed promising results, and can potentially improve efficiency and rigour of future surveys.

In conclusion, while pygmy bluetongue lizards are able to withstand some level of grazing, this thesis has shown that intensive grazing can have a detrimental impact on the lizards and their habitat. Although direct negative effects of the vegetation cover in ungrazed paddocks were not found, it is however still likely that prolonged periods without grazing can adversely affect lizard populations. Lizard habitat should therefore be grazed cautiously and with consideration to vegetation cover and environmental factors.

8.3 Future research

Although the results of this thesis improves our knowledge about grazing effects on the pygmy bluetongue lizard and although they clearly demonstrate a negative impact of intensive grazing, they also point to several unknown aspects that future research could address.

Part of the original hypothesis was that the vegetation cover in the ungrazed paddocks would impede the lizards ability to hunt and thermoregulate. The results did not show this, which is likely to be due to the short amount of time that the experimental paddocks were without grazing, but future research could focus on the effects of long term no-grazing, and particularly if, or at which threshold the vegetation cover becomes detrimental to the lizards. This information could help in the recovery of struggling lizard populations and in the establishment of newly relocated lizard populations. It may be possible to get partial answers to this question in the short term, by artificially increasing vegetation around lizard burrows.

All currently known lizard populations are on privately owned land, and a large network of connected protected habitat, suitable for pygmy bluetongue lizards, is unlikely to be part of the future. Knowledge about the amount of grazing the lizards can tolerate, while still maintaining a sustainable population, is essential to policy makers and local landowners who wish to protect the lizards but depend on the grassland as grazing for their livestock. This research should not be focussing on ideal conditions, but on the range of conditions that allow survival and sustainability and positive recruitment for the lizards.

This and previous studies have shown that the major part of lizard activity is concentrated primarily in a few month in spring. This is not only the mating season, but also the time of year when lizards build up their body condition and energy reserves to be able to reproduce and survive through the autumn and winter. Future research should investigate if lizards are particularly sensitive to grazing during this specific time of the year. This can be through direct effects of grazing on the lizards or through indirect effects, affecting their prey insects.

A central issue in this and previous studies of this species has been that lizards and their burrows are cryptic and hard to find. The results that were achieved with the detection dog trained for this study were promising and showed that a dog can find pygmy bluetongue lizards. Unfortunately it was not possible to conduct field trials that could have shown the efficiency of the dog in real lizard surveys. Future research into the efficiency of pygmy bluetongue lizard detection dogs under different conditions and vegetation densities and in comparison with visual surveys should be conducted. If this technique were implemented it could greatly improve the efficiency and time use for future research projects, it could also help generating reliable population estimates.

Appendix A

Satellite image date	Date since last image	Mean air temperature	Rain (mm/day)	Grazing pressure Treatment group 1	Grazing pressure Treatment group 2
Season 1					
15-12-2012	N/A	N/A	N/A	N/A	N/A
05-01-2013	21	21.9	0.2	*0.0*	*0.0*
03-03-2013	57	21.3	0.9	*0.0*	*0.0*
Season 2					
10-06-2013	99	15.5	1.3	2.4	*0.0*
02-09-2013	84	9.4	2.1	2.3	*0.0*
31-10-2013	59	14.3	1.1	3.4	*0.0*
03-12-2013	33	17.2	0.1	2.0	*0.0*
09-01-2014	37	19.4	0.8	0.0	*0.0*
02-03-2014	52	23.2	1.5	0.0	*0.0*
Season 3					
26-06-2014	116	14.2	1.9	3.2	**0.9**
07-09-2014	73	8.4	2.2	3.0	**3.6**
04-10-2014	27	13.1	0.5	6.0	**4.4**
01-12-2014	58	18.5	0.5	0.3	**3.9**
02-01-2015	32	19.5	1.1	0.0	**0.0**
05-02-2015	34	19.0	2.1	0.0	**0.0**
16-03-2015	39	21.5	0.0	1.0	**1.4**

Appendix A. For each satellite image acquisition data is shown mean air temperature, rain (measured by an onsite weatherstation) and grazing pressure shown as sheep per ha per day since the previous satellite image. Note that the September and September images are outside the pygmy bluetongue lizard's activity season. Grazing pressure is shown for each of the two treatment groups, the first group consisting of paddocks 1, 3 and 5 and the second group of paddocks 2, 4 and 6. Treatment is indicated by style: *Ungrazed*; Moderate; **Hard**

Appendix B

	Survey start	Survey finish	Number of burrows per paddock	Burrows with lizards per paddock	closest satellite image date
Season 1					
Survey 1	23-10-2012	11-12-2012	50-53	32-40	15-12-2012
Survey 2	17-01-2013	13-03-2013	49-51	21-32	03-03-2013
Survey 3	25-03-2013	04-04-2013	48-50	18-28	03-03-2013
Season 2					
Survey 4	08-10-2013	24-10-2013	28-41	13-25	31-10-2013
Survey 5	28-11-2013	08-12-2013	50-52	15-31	03-12-2013
Survey 6	29-01-2014	13-02-2014	50-50	15-36	09-01-2014
Survey 7	10-03-2014	18-03-2014	42-50	13-35	02-03-2014
Season 3					
Survey 8	14-10-2014	05-11-2014	50-52	23-39	04-10-2014
Survey 9	24-11-2014	05-12-2014	50-50	20-41	01-12-2014
Survey 10	14-01-2015	28-01-2015	38-47	18-37	02-01-2015
Survey 11	16-02-2015	25-02-2015	36-42	16-32	05-02-2015

Appendix B. Survey dates, range of burrows, range of burrows with lizards and date of the satellite image used to calculate vegetation cover index for each survey.

Appendix C

	Factor	D.f.	F	P
Season 2	Survey	3, 12	20.618	**5.006E-5**
	Category	1, 4	0.026	0.879
	Treatment	1, 4	54.809	**0.002**
	Survey*Category	3, 12	4.439	**0.026**
	Survey*Treatment	3, 12	4.310	**0.028**
	Category*Treatment	1, 4	2.425	0.194
	Survey*Category*Treatment	3, 12	0.292	0.830
Season 3	Survey	1.278, 5.111	5.560^{GG}	0.060
	Category	1, 4	6.817	0.059
	Treatment	1, 4	19.128	**0.012**
	Survey*Category	3, 12	4.416	**0.026**
	Survey*Treatment	1.278, 5.111	0.700^{GG}	0.476
	Category*Treatment	1, 4	0.317	0.604
	Survey*Category*Treatment	3, 12	2.383	0.120

Appendix C. Repeated measures ANOVA (Split plot design) comparing vegetation cover index between random points and burrows occupied by lizards for each survey of season two and season three separately. d.f (used, error), F-value and P-value are shown for the within subjects effects, survey and category (lizard burrow or random point) and the between subjects effect treatment, as well as any cross effects. ^{GG} indicates that sphericity could not be assumed and the Greenhouse-Geisser correction has been used.

Appendix D

	Factor	D.f.	F	P
Season 2	Survey	3, 12	18.355	**8.855E-5**
	Category	1, 4	3.695	0.127
	Treatment	1, 4	103.054	**0.001**
	Survey*Category	3, 12	1.318	0.314
	Survey*Treatment	3, 12	3.379	0.054
	Category*Treatment	1, 4	1.508	0.287
	Survey*Category*Treatment	3, 12	0.731	0.553
Season 3	Survey	1.287, 5.148	5.440 ^{GG}	0.061
	Category	1, 4	0.741	0.438
	Treatment	1, 4	26.266	**0.007**
	Survey*Category	3, 12	1.104	0.386
	Survey*Treatment	1.287, 5.148	0.955 ^{GG}	0.445
	Category*Treatment	1, 4	0.003	0.961
	Survey*Category*Treatment	3, 12	0.753	0.541

Appendix D. Repeated measures ANOVA (Split plot design) comparing vegetation cover index between occupied and unoccupied lizard burrows for each survey of season two and season three separately. d.f (used, error), F-value and P-value are shown for the within subjects effects, survey and category (occupied or unoccupied) and the between subjects effect treatment, as well as any cross effects. ^{GG} indicates that sphericity could not be assumed and the Greenhouse-Geisser correction has been used.

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