Impacts of sheep grazing on burrow use by spiders and pygmy bluetongue lizards (*Tiliqua adelaidensis*)

by

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This thesis is dedicated to both my supervisor Mike Bull and my cat Lulu, who provided support to me throughout my PhD in different ways. Lulu in particular moved interstate with me while I pursued my dreams, and sacrificed many hours, usually allocated to pats and treats while I went away to do my fieldwork.

Lulu

~ February 2004 –July 2017 ~

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Thesis scope and objective

The chapters of this thesis examine how ecosystem engineering spiders and lizards persist together in sheep grazed native grasslands, specifically identifying the effects of grazing on burrow use and persistence of spiders, lizards and burrows. This information is essential for preservation of pygmy bluetongue lizard habitat, conservation of the spiders and lizards, and informing future translocations of lizard populations.

Specifically the aims of this study are to:

1. Identify the effects of sheep grazing on burrow use by lizards and spiders, and burrow availability
2. Identify the effects of sheep grazing on burrow depth - a vital factor for lizard use of burrows
3. Investigate how grazing influences population dynamics of spider species
4. Identify how grazing influences spider reproductive output
5. Identify how spiders and lizards co-exist together, utilising spider-dug burrows

Organization of this thesis

The thesis is organised in manuscript format with each data chapter representing a potential or actual peer-reviewed publication. The chapters are:

1. General introduction
2. Clayton and Bull (published): The impact of sheep grazing on burrows for pygmy bluetongue lizards and on burrow digging spiders
3. Clayton and Bull (published): The impact of sheep grazing on the depth of spider burrows and of burrows selected by the pygmy bluetongue lizard (Tiliqua adelaidensis)
4. Clayton et al. (in prep): Sheep grazing results in lower reproduction of burrowing spiders in native grasslands of South Australia
5. Clayton et al. (in prep): Differential response to disturbance and niche partitioning in temperate grasslands
6. General discussion and conclusion

Thesis Abstract

Habitat fragmentation and alteration as a result of anthropogenic land-use has drastically impacted ecosystems worldwide. Native grassland habitats are perhaps one of the most destroyed due to their suitability to ploughing and grazing livestock. While many species have the potential to be negatively impacted by changes to their ecosystem, the impact on certain species is likely to have wider implications for ecosystem function.

Ecosystem engineers are species that provide resources to other species through their actions. Burrowing organisms provide a wide range of ecosystem services to the environment, one of which is refuge space for other organisms. For some species burrows provide refuge from predation, while for others they provide respite from an otherwise uninhabitable climate. Impacts of habitat destruction on engineering species may therefore influence a wide range of other species within an ecosystem.

The pygmy bluetongue lizard is an Endangered species, restricted to fragmented native grasslands in the Mid North region of South Australia. It obligatorily occupies burrows dug by wolf and trapdoor spiders which act as ecosystem engineers in these grasslands. Native grasslands in this region are predominantly grazed by sheep, thus, sheep grazing has the potential to drastically impact on both lizard and spider populations.

This study aimed to determine how lizards persist with their burrow-engineers, despite the potential for spiders and lizards to cause fatality to one another. It also aimed to investigate how sheep grazing affects spider burrows, lizards and spiders. Twelve 30 x 30m plots were monitored monthly during two Austral spring-summer lizard activity seasons. Within each plot I monitored all spider burrows and their occupants to determine the dynamics of burrow use. Sheep grazing was introduced into half of the study plots in each season to investigate the effects of grazing on lizard habitat.

The results presented in this thesis show that lizards displayed spatial and temporal niche partitioning, selecting empty burrows, and showing a preference for trapdoor spider burrows. This selection of vacated burrows and of a particular subset of burrows has likely played a major role in their ongoing persistence utilising burrows engineered by these spider groups.

Sheep grazing had differential effects on pygmy bluetongue lizards and the spider groups. Grazing resulted in a decline in wolf spiders and their burrows, but did not reduce the abundance of trapdoor spiders, their burrows, or of the lizards. Grazing also resulted in a decline of reproductive output for both spider groups. Preferential selection of trapdoor spider burrows by lizards is likely to play a role in the retention of these lizards in native grasslands.

This thesis provides new information for conservation of the pygmy bluetongue lizard, identifying interactions between co-existing species and impacts of grazing. As well as direct benefits to future translocations of these lizards, I have provided insights that can benefit other grazed land, native grasslands, and burrow-occupying communities. The knowledge gained through this study has broad implications for the management of grassland habitats and provides insights into the interactions of cryptic grassland species and their response to disturbance.

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.'

Jessica Clayton

07.09.2017

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Statement of Authorship/Contribution

Chapters 1 & 6: JC (100%)

Chapter 2: (published as 'The impact of sheep grazing on burrows for pygmy bluetongue lizards and on burrow digging spider')
Data collection: JC (100%)

Statistical analyses: JC (90%) and MB (10%)

Manuscript writing: JC (80%) and MB (20%)

Chapter 3: (published as 'The impact of sheep grazing on the depth of spider burrows and of burrows selected by the pygmy bluetongue lizard (Tiliqua adelaidensis)')
Data collection: JC (100%)

Statistical analyses: JC (90%) and MB (10%)

Manuscript writing: JC (80%) and MB (20%)

Chapter 4:

Data collection: JC (100%)

Statistical analyses: JC (90%) and MB (10%)

Manuscript writing: JC (70%), MB (10%), MG (10%), MH (5%) and AF (5%)

Chapter 5:

Data collection: JC (100%)

Statistical analyses: JC (90%) and MB (10%)

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We, the undersigned have agreed to co-authored work being included in this thesis, and agree with the above stated contribution to thesis chapters:

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Michael Gardner (Primary supervisor)

Mark Hutchinson

Aaron Fenner

All research procedures reported in this thesis follow the guidelines for the use of animals in research (Flinders University) and were approved by the Animal Welfare Committee of Flinders University (permit: E368).

Preface

This thesis contains four data chapters (Chapters 2-5) that communicate research undertaken as part of a doctoral program. Statements connecting the work of previous and subsequent chapters are provided between chapters. Each chapter was written to stand alone, therefore references are provided at the end of each chapter.

Two chapters have been published (Chapters 2 and 3) while two chapters are in preparation for submission to journals (Chapters 4 and 5).

Appendix 3 consists of a publication that is associated with work in this thesis (a short communication published as a result of data collection for this thesis).

Chapters are presented in a format according to the journal in which they have been published as is the referencing within these chapters. Within text references to other chapters of this thesis are in the format e.g. Clayton et al. Chapter 3. Although I conducted the majority of the work, chapters have been written as manuscripts. As such, the plural "we" is generally used instead of the singular “I” due to the contribution of co-authors. The Statement of co-authorship details the contribution of each author to each chapter.

The following chapter (Chapter 1) presents an introduction that places the work of this thesis in a broad ecological context, providing background information on theory relating to the thesis.

Chapter 1

Introduction

General Introduction

The distribution of animals in any ecosystem is determined by a complex series of responses to the physical and biological characteristics of their environment. A major goal in many ecological studies is to understand the distribution and abundance of species based on how organisms interact (Hastings et al. 2007). These interactions between organisms include competition for abiotic and biotic resources, predation, parasitism and mutualism. Non-social behaviours such as attraction to resources are also a large influence on the distribution of organisms across landscapes.

Attraction to resources can be driven by behaviours that strengthen nutritional or physical condition of an organism, minimize the risk of predation or a trade-off between these factors (Grear & Schmitz 200). Organisms themselves can also play a role in the creation of resources. When an organism provides a resource through the modification of their environment, the organism is often referred to as an ecosystem engineer.

Many organisms modify their environments. This can be through physical changes to abiotic conditions or the production of resources. As a result of ecosystem engineering, the environment will support a different range of species, with different dynamics to what would be present if the ecosystem engineer was not. While not involving direct trophic interactions between species these interactions are nevertheless important and common (Jones et al.199).

Ecosystem engineering is an important function within many ecosystems, potentially resulting in resources being supplied or taken away from a wide range of non-engineering species. It is being recognized increasingly in conservation and invasive species studies (Buse et al.2008, Mullan Crain &

Bertness 2006, McKechnie 2006, Jouquet et al. 2006). Ecosystem engineers have the potential to have numerous positive impacts on communities and ecosystems. Consequently, ecosystem engineers may be a useful conservation target. By managing a single species an entire ecological community can be influenced. Which ecosystem engineers are of high importance for conservation is dependent upon the background environment, limiting factors and ecosystem functions of interest (Mullan Crain & Bertness 2006). An example of the importance of ecosystem engineering to the persistence of an organism is the use of spider burrows by pygmy bluetongue lizards (Tiliqua adelaidensis). This endangered lizard species is restricted to spider burrows, and thus, reliant on the spiders which engineer these burrows (Milne et al. 2003, Fellows et al. 2009).

These lizards exclusively live in native grassland habitat and most of these grasslands are grazed by sheep, thus, disturbance also has the potential to influence burrow use and availability for lizards and spiders.

This chapter will provide a review of factors which are fundamental to understanding the influence of ecological engineer species on other organisms. It will also discuss what we know to date of the ecology of pygmy bluetongue lizards and the two burrow digging spider groups associated with these lizards.

Ecosystem Engineers

The concept of an organism as an ecosystem engineer was first introduced in 1993 during the Cary Conference at the Institute of Ecosystem Studies (U.S.A) (Lawton & Jones 1993). Although it had been well established that organisms alter their environments, there was no unifying concept that examined the relationship between resource use of individuals, population and community dynamics, and the biogeochemical processes of ecosystems (Berkenbusch & Rowden 2003).

Following this conference, Jones et al. (1994) further defined the concept of an ecosystem engineer. Their definition stated that an ecosystem engineer was an organism that directly or indirectly modulates the availability of resources to other species, by causing physical state changes in biotic or abiotic materials.

They identified two major categories for ecosystem engineers (autogenic and allogenic) and provided a six-part framework for assessing the impact of an organism as an ecosystem engineer. These six factors are:

1. Life time per capita activity of individual organisms
2. Population Density
3. The spatial distribution, both locally and regionally, of the population
4. The length of time the population has been present at a site
5. The durability of constructs, artifacts and impacts in the absence of the original engineer
6. The number and types of resource flows that are modulated by the constructs and artifacts, and the number of other species dependent upon these flows.

In addition to these mechanistic classifications, ecosystem engineering can also be classified based on the presence or absence of a positive feedback loop (Jones et al. 1994). The modifications that rganisms create can influence their biology via feedback effects. By modifying their environment, and controlling resource flows, these engineering organisms are likely to modify natural selection pressures which are present in their own local environment as well as in the selective environments of other organisms (Jouquet et al. 2006). For example, beavers (Castor canadensis) build dams, which create ponds. The beavers (C. canadensis) then use these ponds as a habitat, refuge from predation and place to forage for food, thus, the ecosystem engineering conducted by the beaver results in a positive feedback for the beaver (C. canadensis). Ecosystem engineering can also result in a negative feedback for the engineering organism. For example, soil disturbance caused by gophers (Geomys bursarius) constructing gopher mounds has the potential to increase vegetation and as a consequence grasshopper numbers, which has the potential to increase competition for food resources between grasshoppers and gophers (Jones et al. 1994).

Beaver (C. canadensis) dams can however have a negative effect on other organisms, for example, they exclude many organisms by flooding out an area, which, without the presence of the beaver (C. canadensis) dam would normally support a different variety of organisms (Jones et al. 1997). That being said, habitat diversity is perhaps the most significant resource provided by an ecosystem engineer. This habitat can be created via the ecosystem engineer's actions, such as the habitat typically provided by beavers (C. canadensis), termites and earthworms for example, or, it can be provided by the structure of the organism itself, such as in the case of trees and coral reefs (Jones et al. 1994, Jouquet et al. 2006, Wright et al. 2002).

While ecosystem engineering can restrict organisms that previously inhabited an area, it can also create new habitats and provide areas for organisms that could not persist without these modifications. At a scale which encompasses unmodified habitats, engineered habitats, and degraded areas abandoned by ecosystem engineers, the overall effect of ecosystem engineering is hypothesized to result in higher species richness due to the increase in habitat diversity (Jones et al. 1997). Numerous studies provide support for this hypothesis (Lill & Marquis 2003, Castilla et al. 2004, Martinsen et al. 1990). An example of this is the presence and absence of beavers (C. canadensis). Natural sites with and without beavers (C. canadensis) have a low degree of species composition overlap. Habitat modifications made by beaver (C. canadensis) activity increased herbaceous plant species numbers by more than 33% compared with natural sites (Wright et al. 2002).

There are many other organisms, which play an important role as ecosystem engineers on a less obvious scale. The impact of ecosystem engineering is often viewed at a landscape level. However, many engineers may have an important impact on the behaviour of other organisms. By studying these impacts, insights can be gained into the demography of the non-engineering species. An example of this is the relationship between Gunnison's prairie dog (Cynomys gunnisoni hollister) and darkling beetles (Eleodes hispilabris). The activity of prairie dogs leads to microhabitat changes, which influence the movement patterns and potentially detectability of darkling beetles (Bangert & Slobodchikoff 2004).

The impact of ecosystem engineers is likely to differ across environmental stress gradients. Ecosystem engineers that ameliorate physical stress are critical to ecosystem functioning in extreme environments, while in physically benign environments ecosystem engineers are likely to support ecosystem processes by creating predator- or competitor-free spaces (Bertness 2006). The effects of ecosystem engineers across stress gradients has rarely been explicitly examined (Wright & Jones 2004). Examples of shifts in species interactions through ecosystem engineering that occurs across physical gradients can however be found in the scientific literature. The importance of ecosystem engineers in different physical environments is apparent at multiple scales, including local, regional and biogeographic. For example, studies at a small scale, such as that of an intertidal shoreline, have been extensively examined (Menge et al. 2008, Castilla et al. 2004, Bos et al. 2007, Thomas et al. 1998).

Types of Ecosystem Engineers

Ecosystem engineers are divided into two main categories, autogenic ecosystem engineers and allogenic ecosystem engineers (defined below). Most allogenic and autogenic ecosystem engineering is spatially explicit, frequently contributing to habitat heterogeneity (Dangerfield et al. 1998).

Autogenic Ecosystem Engineers

Autogenic ecosystem engineers are defined as organisms that change the environment via their own structures (i.e. their living and dead tissue) such as trees in a forest. Through the growth of leaves, trunks, roots, branches and bark, trees create a variety of habitat changes, which support many organisms. For example, a tree may impound water creating a small aquatic habitat which may support a highly specialized insect fauna (Fish 1983). Or, the leaves, which accumulate on the forest floor, may alter the microenvironment of soil, changing surface structure, affecting drainage and influencing the types of seedlings, which can grow. This in turn influences the plant composition of the forest (Facelli & Pickett 1991).

Whether or not to include the direct provision of resources by one organism to another in the form of living or dead tissues as ecosystem engineering is debatable. In earlier papers it was excluded while in more recent papers it has been included. Jones et al., (2007), argued that changes in living space via branch growth of a tree has more in common with creation of living space in soil cavities caused by root growth (which is engineering) than it does with consumption of tree tissues (which is not engineering) (Jones et al. 1994, Jones et al. 1997).

Allogenic Ecosystem Engineers

A number of species which perform allogenic ecosystem engineering are prominent throughout the literature. These largely include examples of mammals, birds and reptiles. The role of birds in providing habitat has been widely assessed with examples from across many ecosystems (Sekercioglu 2006, Casas-Crivillé & Valera 2005, Heeb et al. 2000). The European bee-eater for example, is abundant in arid and semi-arid areas where it selects sandy cliffs in wadis. It is one of few bird species capable of modifying its habitat by digging long burrows. By doing so, this species may cause bioturbation through erosion and may also enhance biodiversity by creating habitat (Casas-Crivillé & Valera 2005). This species is an example of an ecosystem engineer which is also classed as a keystone species. While many species can be considered ecosystem engineers, a species with an impact that is large relative to their abundance is considered a keystone species and as such, only a proportion of ecosystem engineers qualify as keystone species (Jones et al. 1994).

Elephants (Loxodonta africana) provide large-scale ecosystem engineering through their daily activities. Physical disturbances caused by their movements alter vegetation structure, which in turn alters the fire regime, effects food supply and the population dynamics of other animals. Ultimately, their actions result in alterations to soil formation, riparian zones and biogeochemical cycling

(Naiman 1988). These mammals are one of only a few animals, which have been documented to cause a number of critical habitat modifications above and below ground. There are many other documented examples of mammals causing habitat changes through their daily activities and a large proportion of these organisms are digging animals.

Burrowing ecosystem engineers

Burrows are an important source of habitat for many organisms. They provide shelter, refuge from predation and foraging sites (Ceballos et al. 1999, Shipley & Reading 2006). Further, the presence of animal burrows can also lead to changes in plant species composition. The extent to which these changes are influenced by the particular soil characteristics of the burrows compared with changes in herbivory taking place (due to changes in animal composition) are however, often unclear.

Burrows created by a variety of different ecosystem engineers create habitat for organisms, many of which could not persist in an area without them. Burrows of the gopher tortoise (Gopherus polyphemus) provide habitat to a wide range of other animals, including a known 302 invertebrate species and 60 vertebrate species (Lips 1991). The burrow systems created by kangaroo rats (Dipodomys ingens) have been found to influence plant and small mammal communities in the San Joaquin Valley of California. The burrowing activity of kangaroo rats (D. ingens) resulted in higher species diversity for both plants and small mammals, compared with unmodified habitat (Prugh & Brashares 2011). For another species of kangaroo rat (Dipodomys spectabilis), the dispersion pattern of mounds has a major influence on the spatial structuring of rodent communities (Schooley & Wiens 2001).

In New Zealand, fairy prions (Pachyptila turtur) share burrows in the ground with tuatara (Sphenodon punctatus). The environmental impacts of both species have been heavily researched, particularly in respect to their impact on forest vegetation. The guano deposition of fairy prions (P. turtur) in particular has led to higher levels of nutrients in these burrows, thus influencing the plant species composition significantly (Mulder & Keall 2001). Mulder and Keall (2001), suggest that high densities of burrows have a negative impact on the plant community as the soil pH is very high resulting from the higher level of nutrients.

Invertebrates also play a major role in creating and modifying environments. Termite's account for 40-65% of total soil macrofauna biomass in many tropical ecosystems (Wood & Sands 1978). Species within the genus Macrotermes in particular construct large epigeal nests and extensive underground systems. These constructions have major effects on soil properties (Dangerfield et al. 1998). Termites provide a prime example of an allogenic ecosystem engineer, and the study of them in this context could lead to valuable information on how they impact the structure and maintenance of ecosystems. They have an enormous impact on the distribution of other organisms, for example, in a woodland habitat of southern Zambia, half the woody plant species were found exclusively on termite mounds (M. falciger) (Timberlake 1995).

Earthworms provide another example of a group of significant ecosystem engineers. They are perhaps one of the most widely studied burrowing invertebrates. Their burrows create habitat for a large number of species, including a variety of other invertebrates, which use these burrows as retreats. Through their daily activities, earthworms affect soil profiles, nutrient and organic matter dynamics, soil mesofauna, and plant composition and distribution (Lavelle et al. 2006).

The importance of burrowing organisms in modulating the flow of resources is evident. Although many spiders have not been the targets of studies assessing the role of organisms as ecosystem engineers, a number of burrowing species in particular play an important role in providing habitat to other organisms. For example, the grassland earless dragon (Tympanocryptis pinguicolla) is a small agamid, restricted to patches of native grassland to the east of Canberra (Robertson & Cooper 2000). This species shelters in grass tussocks and arthropod burrows, most commonly burrows constructed by wolf spiders (Osborne et al. 1993).

Like the grassland earless dragon (T. pinguicolla), many lizards use burrows for shelter. Some will dig their own burrows, but many use burrows constructed by other organisms (Hawkins & Nicoletto 1992, Vitt & Caldwell 1993). For lizards in harsh environments, such as arid regions, burrows may provide refuge, not only from predation, but also from over-heating. Many of these arid-dwelling animals would simply not be able to persist in these environments without the burrowing species.

While habitat constructed by ecosystem engineers does provide more habitat availability to a range of organisms, it also has the potential to result in competition for this new resource. One way in which individuals minimise potential competition for resources is through niche partitioning.

Niche partitioning

Niche partitioning occurs when multiple competitive species coexist. In order for persistence of these species to occur, there is often differential use of resources by individuals to allow for coexistence (Amarasekare 2003). This can occur temporally or spatially and can occur on multiple scales. For example, temporal niche partitioning can occur within a day (e.g. use of a common resource at different times of day) or across a season (e.g. use of a common resource at different times of the month or year). Spatial niche partitioning can occur on a fine scale (e.g. within a nest or burrow), within a community (e.g. differential use of resources within a woodland) or a large scale (e.g. across a landscape). Forest bats provide an example of temporal and spatial niche partitioning, with species accessing the same food source at different times of day, and species also utilising different stratifications of the forest (Delaval et al. 2005). Other well documented examples of niche partitioning include the partitioning of space (position in water column) in plankton (Winder 2009, Tamaki et al. 2008), the temporal partitioning of visits to water holes by animals (Valeix et al. 2007, Adams & Thibault 2006, Valeix 2011) and the partitioning of nest sites by ants and birds (Albrecht & Gotelli 2001, Martin et al. 2004).

Niche partitioning is a well-documented concept. Numerous studies have investigated the partitioning of resources between organisms at multiple scales, and more recently, anthropogenic influences on niche partitioning have been incorporated into studies (Schuette et al. 2013, Maelfait & Hendrickx 1997, Williams et al. 2010). Humans have the potential to drastically alter interactions between competitive species, through restrictions and alterations to their habitat. Analysis of stable isotopes has even been used to identify diets of organisms before and after the introduction of anthropogenic disturbance. For South American fur seals (Arctocephalus australis), recent changes in stable isotopes have been identified and it is thought that these changes reflect a decline of sea lions (Otaria flavescens) resulting from the sealing industry and a shift to smaller demersal prey due to larger fish being captured by fishing industries (Drago et al. 2017). While anthropogenic activity has the potential to influence all organisms, it may potentially impact more broadly if it affects the construction and persistence of habitats engineered by organisms.

The concepts of niche partitioning and ecosystem engineers have seldom been investigated together. It stands that ecosystem engineers have the potential to drastically influence niche partitioning by other species which utilise the engineered habitat or surrounding habitat. Burrows for example may provide refuge from predation, or may provide thermal conditions tolerable to a species which otherwise could not persist in the area. Here, I discuss one such group of organisms which may produce burrows and are seldom incorporated into ecological studies – spiders.

Spiders in Ecology

There is a growing acknowledgement that invertebrate groups must be included in management of the environment as they are an important part of almost all ecosystems. They tend to be highly abundant in most ecosystems, providing an important resource to other organisms (e.g. food, habitat, refuge) (Fellows et al. 2009, Scheu & Schaefer 1998, Vickery et al. 2001). Spiders in particular are among the most specious of arthropod orders, inhabiting a wide range of habitats. They are a diverse group, and are one of the most abundant and dominant of invertebrate predators.

In recent times, spiders have started being included in assessments of environmental disturbance, particularly in agriculture. This is largely due to the fact that they contribute greatly to biodiversity in agroecosystems and also play an important role in natural pest control (Marc et al. 1999). As a group, they have been identified as potential biological indicators, largely due to the fact that they are easily impacted by their environment. Spiders are sensitive to small changes in habitat structure, vegetation complexity and a variety of microclimate characteristics. As such, they can provide an early indication of habitat degradation and for this purpose have been used in nature conservation and management projects (Skerl & Gillespie 1999, Churchill & Arthur 1999, Maelfait & Hendrickx 1997).

Most work conducted on the biology of spiders has been descriptive and population dynamics have seldom been mentioned. This general lack of work on a conspicuous and important component of terrestrial communities is likely to be related to some of the characteristics of spiders themselves. They are not commonly harmful or beneficial to humans, have poorly marked life stages, are difficult to age and sex determination is only possible in the later instars.

The Australian spider fauna consist of approximately 75 families. Ecologically, they are one of the least known of any continent. In 1988, Raven estimated that only about one fifth of the spider fauna in Australia had been described. While in general spiders have been poorly represented in studies and particularly their population dynamics, some spiders groups in Australia have been the subject of longterm studies. In recent years, several spider groups in particular have been the focus of research by a number of Australian based arachnologists. These are the trapdoor spiders (mygalomorphae), wolf spiders (Lycosidae), orb weaving spiders (Araneidae) and jumping spiders (Salticidae). Of these groups, trapdoor spiders and wolf spiders are dominated by numerous burrowing species. As the topic of this thesis relates to burrowing spiders, I will focus on the current knowledge of lycosid spiders and mygalomorph spiders.

Lycosidae – the wolf spiders

Figure 1. A wolf spider on the end of an optiscope with its burrow in the background

The lycosidae (wolf spiders) are a dominant group within the arthropods on all continents. Though currently being revised, in Australia, there are currently 168 species of wolf spider and 28 genera. This figure is however likely to be only a small proportion of the true number of lycosid spiders on this continent (Framenau 2012). They are generalist species', occupying a wide range of habitats. Even the most inhospitable environments are potential habitats for lycosid spiders, with species being recorded from above the snowline and within swamps and deserts (Main 1981).

In recent years, researchers at the Western Australian Museum have focused research on documenting the biodiversity and systematics of Australia's lycosid spiders, along with other invertebrate groups (for example, see Framenau & Yoo 2006, Framenau et al. 2006, Framenau 2002, Murphy et al. 2006). There is still however, little known about the life cycle of Australia's lycosid spiders. It is suspected that within the larger species, females may live for one to two years, while males are thought to die shortly after mating (Humphreys 1976a). Comparable species of lycosids in the northern hemisphere have a two year life cycle (Humphreys 1976b).

In the Lycosidae, male spiders generally become vagrants and leave their burrows shortly after maturation to search for females, whereas female spiders rely on burrows until their death. In many lycosid species, the female produces a pheromone that is associated with the draglines that elicits courtship behaviour in males. The courtship of lycosid spiders often involves visual elements as well as acoustic or vibratory elements (Witt & Rovner 1982).

Lycosid spiders lay their eggs in large woven egg sacs, which female wolf spiders will carry with them, attached to their spinnerets. Lycosids are thought to be one of few groups of, which show some form of maternal care or investment into spiderling survival. However, a study performed by Main (1984) demonstrated that this behavior is an instinctive act. This study demonstrated that the egg sac may be replaced by an object of similar size and weight and be given the same amount of care by the female.

Burrowing lycosids have also been documented to sun their egg sacs, rotating them periodically to maintain an even temperature and reduce the incubation time. They will carry their egg sac for approximately 5-6 weeks, before tearing them open for the spiderlings to emerge (Edgar 1971). The spiderlings will then climb onto the dorsal side of the female's abdomen, where they cling to hairs and pile up 4 or 5 deep. They will stay here for several weeks, before dispersing and seeking refuge under rocks, crevices and amongst vegetation (Rovner, Higashi & Foelix 1973).

Burrowing Behaviour in Lycosid Spiders

Spiders within this family do not rely on webs to capture their prey. Instead, they will either actively hunt for prey, or sit and wait for passing prey (Marshall 1995). This is largely related to the fact that most wolf spiders are ground dwelling spiders, which dig burrows or shelter under rocks and crevices. The burrows can vary in size, depth and shape. Some burrows are quite shallow, with palisades (a funnel-like fortress over the top of a burrow) and others are to up to 250mm deep such as those dug by Lycosa forresti, one of the larger wolf spiders (Main 1984). These palisades are usually made of dry grass and twigs, woven and bound together using silk and mud. The exact purpose of a palisade over a burrow is not clear, however, one popular hypothesis is that the grass palisade prevents water from flooding the burrow during rainfall events (McKay, 1979).

Some Lycosid species will make doors for their burrows. Unlike mygalomorph spiders, the doors are loosely attached and can open 180° to lay flat on the ground (Hawkeswood 2003). Lycosids are not equipped to dig burrows in very hard compacted soil. This could either mean that lycosid spiders are restricted in their distribution to areas of softer soil, or that their digging activities are restricted to times of higher rainfall. It is more likely that the latter is the case, as lycosid species have been reported in almost all habitats (Main 1976).

One possible purpose of digging a burrow was described by Humphreys (1978). He noted that a burrow would permit a spider to access a wide range of temperatures throughout the day and act to protect the spider from extreme cold in winter. This is likely to be of high importance to spiders in extreme environments, such as those of the arid regions in Australia. Humphreys (1975) showed that one particular lycosid spider, Geolycosa godeffroyi exhibited behavioural thermoregulation throughout the year. The spiders would bask in the sun during the day, and withdraw into the burrow at night. As a result of this, some burrowing species are thought to be diurnal predators. Contrary to this, Shook (1978) suggests that desert dwelling spiders avoid desiccation by seeking protection in their burrows during the day and become active only at night. It is most likely that species will utilize their burrows differently, dependent on the environmental conditions they are exposed to.

Mygalomorphae – The Trapdoor spiders

Figure 2. A trapdoor spider (Blakistonia aurea) in its burrow

In Australia, there are ten families of Mygalomorph spiders (Trapdoor). The family Idiopidae (formerly Ctenizidae) is by far the best known in terms of taxonomy, and the one which fits the best image model of a trapdoor spider (Raven 1988). Much like lycosids, mygalomorphs inhabit almost all terrestrial environments, and are just as prolific in terms of species numbers and abundances. There are however, some distinct differences between the two spider groups, in both life-history traits and their general biology. Recently, Rix et al. (2017) reported drastic declines of trapdoor spider populations in Southern Australia. They attribute these declines to intensive land clearing and stocking within the region. These findings show some of the effects that we as a species are having on an already understudied group of organisms, revealing the need for an increased understanding of these effects, both for spiders and for coexisting species.

While there are lycosid species, which do not dig burrows, instead seeking refuge in crevices and under rocks, all mygalomorph species dig burrows or construct a retreat in the ground, and very rarely in trees (Lindsey 1998). Mygalomorph spiders can be long lived, with specimens in captivity recorded to live for up to 20 years and longer (Hawekswood 2003). They can take up to 4 or 5 years to reach sexual maturity (Main 1976). Mygalomorph spiders found in burrows tend to be juveniles, females and immature males. Males resemble females until after the last moult, when spines on the legs and abdomen become apparent along with the full development of papal organs. Adult males will lead a roving life, especially when searching for a mate. While females are long-lived, these males will die after mating (Main 1957, Main 1976).

In many Mygalomorph species, breeding patterns are closely related to the wet and dry seasons of the year (Hutcheson 1962). Male mygalomorph spiders are probably led to a females burrow through a combination of smell and touch stimuli. Young are thought to hatch during the dry season and disperse at the onset of the next wet season (Lee & Southcott 1979, Main 1957). It may be up to a year before young spiders disperse. Much like the lycosids, mygalomorph spiders can have up to 200-300 eggs deposited in one egg sac. This egg sac will be attached to the wall at the bottom of the burrow, and after hatching, the young will stay in the burrow until they are about 5mm in length. The number of young may vary due to geographic region, or age and size of the female parent (Main 1957). After reaching this size spiderlings will then leave to construct their own burrows (Forster & Forster 1973).

Burrowing Behaviour in Mygalomorph Spiders

Mygalomorph burrows are very diverse. They may be vertical inclined, straight or winding, sometimes forked or with an escape chamber often built into the side of the main tunnel (McCullough 2000). Mygalomorph spiders plaster the walls of their burrow with a mixture of mud and saliva, which hardens in a pottery-like finish. This is often covered with a closely woven silk which clings to the burrow wall (Main 1984). Given the investment that is undertaken into the construction of such a burrow, it is not surprising that a female mygalomorph spider will spend its entire life within the same burrow, enlarging the burrow and door as it molts and grows in size (Main 1976). At the onset of summer, most mygalomorph spiders will close their doors with a collar of silk from the top of the burrow lining and some will add a plug of silk (Main 1957). It has been suggested that the sealing of the trapdoors is to prevent desiccation (Hutcheson 1962) though it may also be an anti-predator measure.

Burrowing spiders and the pygmy bluetongue lizard

Hutchinson et al. (1994) observed a number of lycosid species in association with pygmy bluetongue lizards (T. adelaidensis). They documented two large lycosid species, Lycosa gilberta and Lycosa stirlingae as well as a number of other smaller lycosids. Milne (1999) noted that two large lycosid species within pygmy bluetongue habitat breed in late summer and early autumn. He also observed cohorts of juvenile spiders, which began to dig and enter burrows in April and May the following year. The mygalomorph spiders in association with the lizards are not as well documented, however, Hutchinson et al. (1994) observed at least one species of trapdoor spider, and McCullough (2000) went on to observe a further three mygalomorph species.

While these spiders all exist within the same area as the lizards, it is not yet clear which spiders play an important role in digging the burrows needed by pygmy bluetongue lizards. It is thought that one of the lycosid species is likely to be a burrow stealer (L. gilberta), not digging its own burrow, and one is thought to not produce burrows of a suitable depth (L. pardosa) (Milne 1999).

Little is known of digging habits of Lycosa stirlingae, however experiments conducted by Milne (1999) found that transplanted Lycosa stirlingae would dig burrows and females would dig burrows more often than males. The burrows dug by L. stirlingae during the 7 weeks of the study were also significantly shallower than the burrows they were originally removed from. A study of captive spiders by McCullough (2000) also indicated that L. stirlingae would readily construct burrows whereas L. gilberta will not.

Background to the pygmy bluetongue lizard

Figure 3. A pygmy bluetongue lizard and its burrow in the background

The pygmy bluetongue lizard is the smallest member of the genus Tiliqua. It also is the only lizard within this genus which has a pink tongue rather than blue. It is classed as endangered under the Australian Environment Protection and Biodiversity Conservation Act (1999).

Pygmy bluetongue lizards were thought to be extinct until 1992, when a specimen was discovered, dead in the stomach of a brown snake, (Pseudonaja textilis), near Burra, South Australia (Armstrong & Reid 1992). After extensive surveys in the area, live individuals were located occupying vertical burrows; thought to be constructed by lycosid and mygalomorph spiders (Armstrong & Reid 1992). Prior to its rediscovery, the species had last been recorded from Marion, a suburb of Adelaide, in 1959 (Ehmann & Strahan 1982). Previous records indicated that pygmy bluetongue lizards had a wide distribution, with a range extending at least 150km from Adelaide Plains to Burra in the mid north of South Australia (Ehmann & Strahan 1982). There were, however, no recorded collections of this species, despite many search efforts after 1960. As a consequence, pygmy bluetongue lizards were thought to be extinct (Ehmann 1982, Cogger 1992). The rediscovery of a population of this species allowed for study of the basic biology and ecology of the lizard, previously known from only 20 museum specimens (Ehmann & Strahan 1982).

Since the rediscovery of the pygmy bluetongue lizard, approximately 35 known populations have been identified (Fig. 4). The distribution of these populations is heavily fragmented, however all populations exist within fragments of native grassland, on privately owned properties. These native grasslands now comprise of both native grass species, and an exotic plant component (Tremont & McIntyre 1994). The main reason for the isolation of populations and restriction in distribution of the lizard is agricultural activity, such as ploughing. This type of activity permanently alters the soil composition and plant communities as well as directly killing lizards or leaving them without shelter (Hutchinson, Milne & Croft 1994). As such, the main driving force thought to be responsible for the decline of pygmy bluetongue lizards is habitat modification through land clearing. (Ehmann & Strahan 1982).

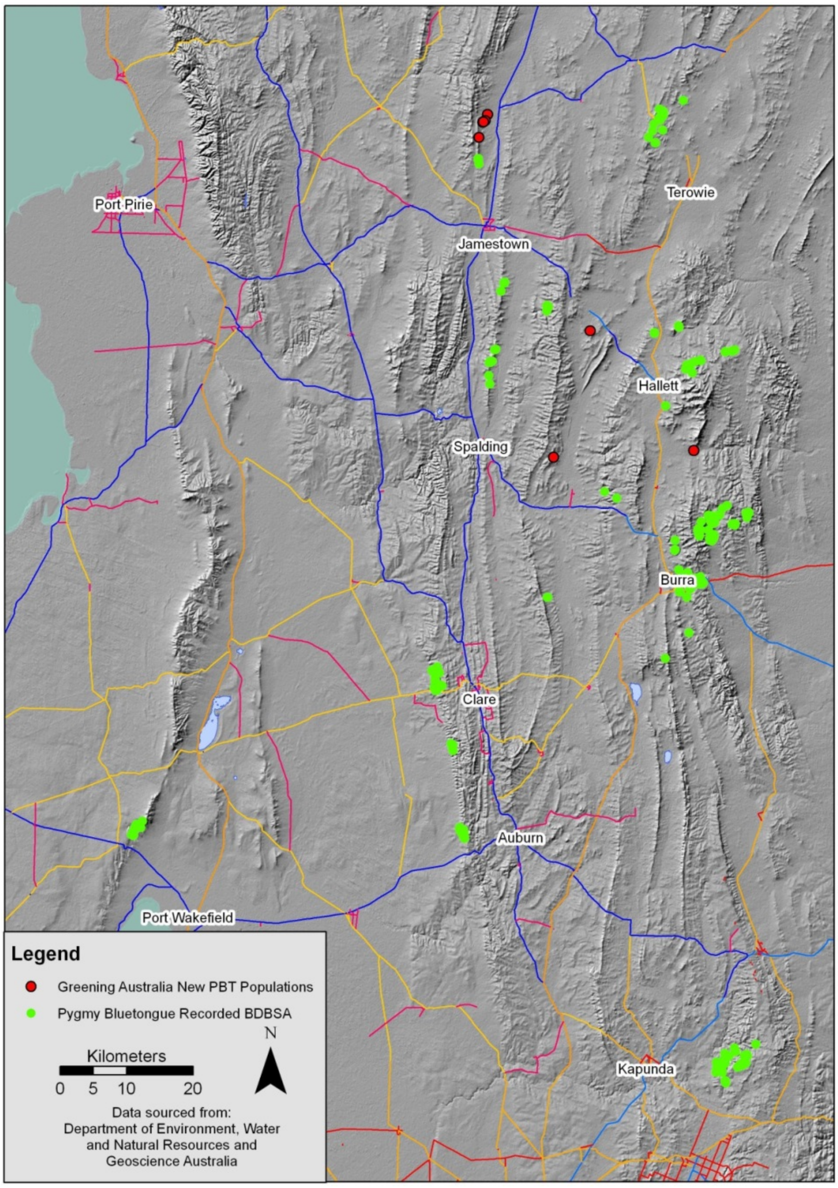

Figure 4. Known distribution of pygmy bluetongue lizards in the Mid North of South Australia (map source: Greening Australia).

Ecology of the pygmy bluetongue lizard

The pygmy bluetongue lizard has a snout to vent length (SVL) of approximately 95mm. Females and males can be distinguished by their differing body proportions, with females generally having a smaller head and more elongated body than males, which have a bulkier head and shorter body (Hutchinson et al. 1994). These lizards are restricted to spider burrows, specifically those of lycosid and mygalomorph spiders. They use these burrows as basking sites, places for refuge from predation and ambush sites for prey. They may reside in burrows which are in close proximity to other burrows occupied by pygmy bluetongue lizards, however, they will defend their burrows aggressively against rivals (Fenner & Bull 2011).

Burrows with a depth of approximately 250 mm or more are preferred, with a diameter of between 16 - 22 mm, depending on the lizard's size (burrows which are a close fit to the lizards head are selected) (Hutchinson et al. 1994, Milne & Bull 2000, Milne et al. 2003). The lizards have not been documented to modify these burrows, however, the activity of the lizard at the surface does cause beveling at the entrance (Hutchinson et al. 1994). A recent publication by Bull et al (2015) reported that pygmy bluetongue lizards use multiple strategies with burrow occupancy, with some lizards identified as 'drifters', staying in a burrow for a short period of time before moving to find a new burrow, and some lizards being long-term residents, occupying the same burrow for multiple years. These results highlight the importance of both burrow stability and burrow abundance pygmy bluetongue lizards.

Mating occurs from October to November. This involves the male lizard dragging the female lizard out of the burrow, and mating occurring at the surface (Milne et al. 2003). Research by Ebrahimi and Bull (2015) suggests that female lizards leave scent trails in order to attract mates. Between mid-January and late March, the female will have litters of live-born young in their burrow, which disperse from the maternal burrow between one and six weeks after birth (Milne et al. 2002, Hutchinson et al. 1994).

In recent years, the focus of research on conserving pygmy bluetongue lizards has shifted to translocations. Ebrahimi and Bull (2013) identified strategies for improving translocation outcomes, by conducting experimental trials on captive pygmy bluetongue lizards. They assessed one and five minute confinement times to test if a longer (five minute) time would improve the likelihood of lizards remaining in their translocation burrows. There results suggest that short-term confinement time of five days did not provide any extra benefits to lizards compared to a one day confinement, and in fact resulted in higher dispersal of lizards from the translocation burrow(Ebrahimi & Bull 2013). They also determined that food supplementation would enhance the success of translocations in this species by reducing post-release dispersal (Ebrahimi & Bull 2012a), and that burrow density influenced how frequently lizards changed burrows (Ebrahimi & Bull 2014). Another factor which is an essential component to investigations of translocations for this species is the effect of sheep grazing on lizards and spiders. It is highly probable that like the habitat lizards currently occupy, translocation sites will be sheep-grazed to some capacity. As such, incorporating grazing in studies of lizard populations will improve the chances of conservation of the species, both within current populations and within future populations if translocations are to occur.

Grazing

Sheep grazing has the potential to be beneficial and/or detrimental to persistence of the species and to the persistence of spider burrows. Grazing has the potential to influence soil and vegetation structure, species diversity and composition (Dennis et al. 2008, Bilotta et al. 2007, Dorrough et al. 2004). Previous studies investigating the effects of sheep grazing on pygmy bluetongue lizards have shown that it can result in alterations to lizard basking behaviour (Pettigrew & Bull 2012, Pettigrew & Bull 2011, Ebrahimi & Bull 2015). These studies have had contrasting findings. Pettigrew and Bull (2014) suggest that lizards exposed to simulated grazing may benefit from the reduction of vegetation associated with grazing by increasing their ability to detect prey, and increasing their opportunity for basking. Increased basking time in grazed treatments was also reported in Pettigrew and Bull (2012). Ebrahimi and Bull (2014) found that simulated grazing led to lower body condition of lizards, decreased the tendency of lizards to move from their burrows, but increased their tendency to disperse away from the patch of habitat provided during simulated translocations. Pettigrew and Bull (2011) reported that lizards showed preference for burrows in vegetated habitat and basked for longer in these burrows compared to burrows in simulated grazed habitat.

Grazing also has the potential to influence burrow stability and availability.

There is currently limited knowledge of how grazing influences spider burrow stability. A study by Sharp et al. (2010) investigated how set stocking and rotational grazing influenced the persistence of spider burrows (artificial), and determined that there was no significant difference in the effect of these grazing regimes on burrow stability, however, this study did not test the overall effect of grazing on burrow stability. Nielsen and Bull (2017) reported that burrows containing over-wintering lizards were less likely to be destroyed by sheep grazing activity, and that heavy grazing increased the chances of burrow destruction. Due to the reliance of lizards on spider burrows and on burrowing spider populations, it is critical that an understanding of how sheep grazing affects burrowing spiders be established.

Within populations of pygmy bluetongue lizards, burrows housing spiders and lizards are interspersed. To date, there has been no record of burrows being synchronously occupied by both lizards and spiders (Fellows et al. 2009). Milne (1999) reported that in laboratory trials, nine out of ten lizards displaced spiders from artificial burrows, and probably consumed them. There have also been cases of lizard fatalities due to spider bites. Two lizard specimens from enclosures at the Monarto Zoo were recently discovered with puncture wounds, inflicted by spiders (Ebrahimi & Bull 2012b). In 2016, two neonate lizards at Monarto Zoo were found dead and the cause of death was found to be redback spider bite (Latrodectus hasselti). These interactions between lizards and spiders provide an extra complexity to their relationship, as both can potentially impact negatively on one another. Due to the exclusive reliance of both animal groups on burrows, competition for burrow resources has the potential to result in population declines of spiders and/or lizards when burrow numbers are low.

According to McCullough (2000) lycosid spiders may potentially occupy many burrows throughout their life span. He observed most burrow construction occurring between July and August and suggested that this may be due to increased malleability of soil. His study did not continue into the summer months. McCullough's research (2000) is unpublished, and is to date almost all that has been discovered about the distribution of the spiders associated with the lizards. There are still major gaps in our understanding of the population dynamics of these spiders, and the relationship between these lizards and spiders.

Most research conducted to date has been centralized around pygmy bluetongue lizard populations, without focusing in any great detail on the spider populations. This is largely due to the need for understanding the basic ecology and population dynamics of these lizards; however, in order to provide adequate conservation management to this species, an understanding of the population dynamics of associated spider species is also required.

The importance of lycosid and mygalomorph spiders in the conservation of pygmy bluetongue lizards has been identified by the scientific literature. These lizards rely extensively on spider burrows for survival, thus preservation of the burrows is essential for the long-term sustainability of this species (Milne et al. 2003). A critical resource for the establishment of juvenile lizards is the availability of burrows of a suitable size (Milne and Bull 2002), and an abundance of lizard-suitable burrows is critical to dispersing lizards.

This thesis

While well recognized as an important kind of ecological interaction, physical ecosystem engineering by organisms is diverse with varied consequences, presenting challenges for developing and using general understanding. Although there is an increasing awareness of the importance of these interactions, these relationships between species (whereby species-specific abiotic interactions occur), are not commonly included in models of natural systems. Further, incorporating the partitioning of these resources by organisms has rarely been investigated.

While the influence of invasive ecosystem engineers has been fairly well documented, there has been little effort to determine what factors will influence which species are lost from a habitat if an ecosystem engineer was to be removed. A greater understanding of the significance of ecosystem engineering is important, especially when considering conservation of endangered species and the impact that the presence or absence of an ecosystem engineer may have. Further, a number of plant and animal groups have been the target of studies assessing the impact of ecosystem engineering. This is largely owing to the fact that these species provide good models of ecosystem engineers, and/or perform this role on a large and obvious scale. There are however a lot of organisms which perform important ecosystem engineering which have been largely ignored in the literature. Spiders, for example, have largely been overlooked.

This thesis aims to address gaps in our understanding of the relationship between pygmy bluetongue lizard populations and burrow digging spiders. While it is known that spiders are potential predators of juvenile lizards, as discussed above, they are also essential engineers that build the burrows required by the lizards (Fellows et al. 2009, Ebrahimi & Bull 2012b). As far as we know, the lizards cannot construct the burrows themselves. As such, any longer term conservation plan must include sustaining spider populations to keep digging burrows. Further, any information gathered on the spatial and temporal distribution of burrow-digging spiders within pygmy bluetongue habitat has been anecdotal. An understanding of the distribution of these spiders in relation to pygmy bluetongue lizards is crucial. By advancing our knowledge of the habitat requirements and population dynamics of hole-digging spiders, the chances of successful translocation of lizard populations can be improved.

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Chapter 2

The impact of sheep grazing on burrows for pygmy bluetongue lizards and on burrow digging spiders

Pygmy bluetongue lizard habitat being grazed by sheep

Chapter Preface

While the effects of grazing on pygmy bluetongue lizard behaviour have been investigated, there has been little research into the effects of grazing on spiders or spider burrows – both essential to conservation of the lizard. Spiders not only provide an ecosystem engineering service to lizards by producing burrows, which lizards exclusively utilise (Milne, Bull & Hutchinson, 2003), but they also maintain existing burrows, potentially improving persistence of the burrows and enhancing burrow numbers. Any impact of grazing on spiders or their burrows has the potential to both directly and indirectly impact lizard survival.

This chapter investigates the impact of grazing on the persistence and construction of spider burrows (wolf spider burrows and abandoned trapdoor spider burrows) as well as the impacts of grazing on the occupancy of these burrows by wolf spiders and pygmy bluetongue lizards.

The impact of sheep grazing on burrows for pygmy bluetongue lizards and on burrow digging spiders

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Abstract

Grazing by domestic stock has altered and degraded natural grassland ecosystems worldwide, directly and indirectly impacting the endemic plant and animal species occupying those grasslands. The pygmy bluetongue lizard (Tiliqua adelaidensis) is an endangered species, restricted now to fragments of native grassland habitat in South Australia, which are predominantly grazed by sheep. These lizards exclusively occupy burrows dug by spiders, and use them as refuges, basking sites and ambush points. They do not dig their own burrows and rely on co-existing spiders for this essential resource. We asked how sheep grazing influences construction and persistence of spider burrows, by comparing burrow dynamics in adjacent grazed and ungrazed grassland habitat. In ungrazed plots spider burrows increased over one spring and summer period, particularly after a summer rain event that softened the soil. In grazed plots more existing burrows were destroyed, presumably by sheep trampling, and fewer new burrows were constructed, leading to a net loss in burrow numbers over the same period. However, in this short study, grazing did not affect the number of pygmy bluetongue lizards or the number of lycosid spiders. Burrows that were lost tended to be shallower and to have smaller diameter entrances than those that were retained, suggesting that the best burrows for lizard refuges were more likely to persist despite sheep activity. However, heavy grazing may have negative impacts on both lizards and spiders, resulting from a reduction in available burrows and in spider digging behaviour.

Introduction

Anthropogenic activity has caused major changes to ecosystems through habitat alteration or deterioration, and has reduced biodiversity on a global scale. In Australia, about 60% of the land area has been affected by grazing of introduced domestic ungulates and much native habitat has been cleared for this agricultural practice (Jansen & Robertson, 2001; Fleischner, 1994). This paper explores an indirect impact of grazing on an Endangered Australian scincid lizard that occupies remnant patches of native grassland in South Australia, through changes in the burrowing behaviour of the lycosid spiders that provide lizard refuges.

Livestock grazing reduces plant diversity and the structural complexity of native vegetation (Dorrough, Ash & McIntyre, 2004; Adler, Raff & Lauenroth, 2001; Yates, Norton & Hobbs, 2000). Of specific relevance to ectothermic arthropods and lizards, grazing can alter microhabitats, and the ranges of associated available microclimates and thermal opportunities (Vitt et al., 1998), potentially leading to decreases in their population densities (Woodcock et al., 2005). Moderate grazing may also benefit some species if a reduced cover of vegetation provides better opportunities for behavioural thermoregulation, foraging and detecting potential predators (Ebrahimi & Bull, 2013; Pettigrew & Bull, 2012; Schofield et al., 2012; Schofield et al., 2014). Thus the impact of grazing on a particular habitat can be complex, potentially benefiting some species while disadvantaging others.

Grasslands are among the most utilized and least protected terrestrial habitats in the world (Tarboton, 1997). In South Australia, clearing of native grasslands for cultivation over the past 150 years has left extant less than 5% of the previous area of grassland ecological communities (Hyde, 1995). Remnant grassland patches are highly fragmented and often exposed to inappropriate stocking (Hyde, 1995) . While grasslands require some disturbance to maintain plant diversity, community structure and composition, European settlement has drastically altered the way these grasslands are disturbed (Lewis et al., 2008). Prior grazing disturbance came from native herbivores, predominantly large marsupials such as kangaroos and wombats. Grazing reduces competitive exclusion allowing the persistence of annual forbs, and grazing also prevents the accumulation of a dense thatch of dead dry grass, allowing the establishment of native grasses and forbs (Lodge & Murphy, 2002; Dorrough et al., 2004).

More recently, in South Australia, remnant patches of native grassland, consisting of both native and exotic plant species, have been predominantly grazed by sheep. Sheep grazing may be required to control introduced species and maintain what remains of the native plant biodiversity. Grazing may also influence the endemic animal species that inhabit those grasslands.

The pygmy bluetongue lizard, Tiliqua adelaidensis, is an endangered grassland species, endemic to South Australia, and now restricted to a few isolated remnants of native grassland in the mid north region of the state. It is a medium sized skink, measuring up to 107mm snout-vent length (SVL) (Hutchinson, Milne & Croft, 1994), which refuges in vacated mygalomorph and lycosid spider burrows, using the burrow entrance to bask and as an ambush point for passing invertebrate prey (Milne & Bull, 2000; Souter et al., 2007; Fellows, Fenner & Bull, 2009). An important question to consider when managing the conservation of this species is how the grazing regime influences the fitness of the species.

Pettigrew and Bull (2011, 2012, 2014), simulated heavy grazing by removing all vegetation to ground level from immediately around burrows. They reported that lizards in the field avoided occupying new burrows with simulated grazing, and that lizards in new burrows in the laboratory were more active above ground when burrows had more surrounding vegetation (Pettigrew & Bull, 2011). Similar reduced above-ground activity, and a subsequent decline in body condition was reported for pygmy bluetongue lizards following vegetation clearance by grassland fire (Fenner & Bull, 2007). Reduced vegetation around the burrow may result in a higher perceived risk of predation, thus, less above-ground activity, particularly in a new unfamiliar burrow. Alternatively, reduced shade may mean lizards need less time basking to reach optimum temperatures (Pettigrew & Bull, 2011). In contrast, three other studies showed that pygmy bluetongue lizards in established burrows spent more time emerged and searching for prey at the burrow entrance after simulated grazing (Pettigrew & Bull, 2012; Ebrahimi & Bull, 2013; Pettigrew & Bull, 2014). This may result from local reductions in prey abundance requiring longer to encounter prey (Ebrahimi & Bull, 2012), or from an increased ability to detect prey or approaching predators (Pettigrew & Bull, 2012).

Grazing might affect not only these lizard behavioural responses, but also the supply of spider burrows. Populations of pygmy bluetongue lizards occupy most available burrows that are deeper than 300mm, and may be limited by the number of suitable deep burrows (Fellows et al., 2009). Lizards rely on the resident spider population to supply the burrows, so an impact of grazing on spider burrowing will indirectly affect the lizards. Spiders are sensitive to changes in habitat structure (Duffey, 1993), and the diversity of spiders within a grazed habitat may be largely influenced by stocking rate and grazing regime (Bell et al., 2001). For example, after extreme grazing, a spider assemblage may consist mostly of 'pioneer' species, typically species that are active aeronauts, able to disperse into disturbed (grazed) habitats (Bell, Wheater & Cullen, 2001), rather than burrowers. However, few studies have focused specifically on how grazing pressure and sheep trampling influence burrowing spiders and burrow persistence. Sharp, Schofield and Fenner (2010) compared the relative impact of cell grazing and set stocking, two alternative sheep grazing regimes, and reported no significant differences in lizard or spider population abundance, or on burrow longevity. They suggested that burrow destruction by grazing sheep may play a relatively minor role in spider and lizard population dynamics.

In the current study, we compared plots with no grazing to those with heavy grazing to further explore the role of sheep grazing on the abundance of natural spider burrows and their occupation by lizards and spiders. While lizards readily use artificial burrows (Souter, Bull & Hutchinson, 2004), their installation and maintenance as a conservation tool is labour intensive, and maintenance of natural burrow digging spiders would be a better management option. Understanding how the numbers of natural burrows are influenced by grazing will be an important key to the conservation of this endangered lizard species.

Materials and Methods

Site Description

The study was conducted over seven months during one austral spring and summer period, from September 2012 to March 2013, within a 70 ha site, the “Tiliqua” property of the Nature Foundation of South Australia, near Burra, South Australia (33°42′S, 138°56′E). The site has been described previously (Milne, 1999; Souter, 2003), as Site 2 and consists of semi-arid native grassland partially invaded with exotic weeds. The area has hot, dry summers and cool, moist winters. From 1961 to 2012 the average annual rainfall at Burra, approximately 8km from the study site, was 431.1mm (Bureau of Meteorology, 2012). Rainfall over the study period was 86.8 mm, below the average (139.1 mm) for those seven months (Bureau of Meteorology, 2012). The highest monthly rainfall (February 2013: 32mm), consisted of three downpours, including 24.6mm on one day (Fig 1). Grass density was low at the site, with much bare ground reflecting the relatively low rainfall over the study period.

The Tiliqua property has six experimental paddocks ranging in size from 3.49 – 6.86 ha, arranged in a north-south line along its eastern edge. In May 2012, we established twelve 30 m x 30 m plots, one in each paddock, and spaced 100-200 m apart from each other, and one outside of each paddock and about 50 m west of the fence line. In September 2012, we searched along 30, 1 m wide transects in each plot, locating as many vertical burrows as possible. We measured the depth and entrance diameter of each burrow, and used an optic fiberscope (Medit Inc 2 way articulating FI Fiberscope) to inspect for lizards or spiders occupying burrows, as in Milne and Bull (2000). The only lizards detected in the burrows were pygmy bluetongue lizards, and from over 400 lizard records, only nine were juveniles. We defined a spider burrow as any burrow with a depth of 14mm or more, and with an entrance diameter range 6– 35mm. We marked the location of each spider burrow with a 300mm plastic tent peg and noted whether it was constructed by a lycosid or mygalomorph spider. Mygalomorphs generally constructed deeper burrows, with thicker silk lining, and with a trapdoor lid. Among the spider burrows we defined those suitable for pygmy bluetongue lizards as deeper than 120 mm, and with an entrance diameter between 10 - 22mm (Milne et al. 1999). We repeated this survey of each plot in five of the next 6 months (Oct – March) omitting December. In each monthly survey we noted new burrows that were detected, and previously detected burrows that could no longer be found close to their marker peg.

Local farmers routinely rotate sheep around different paddocks, leaving paddocks ungrazed for some of each year. We used stocking rates consistent with local practice. The whole study site, and all 12 plots, remained ungrazed throughout 2012, including the times of the first three surveys (Sept – Nov 2012). Then, for three months from January 2013, 200 sheep were introduced into the western part of the Tiliqua property, outside of the experimental paddocks (and their six survey plots), at a density of about 4 sheep per hectare. Thus six survey plots (inside the paddocks) were excluded from sheep grazing over the entire study period, while the other six (outside the paddocks) had no grazing for the first three surveys, but were grazed for the last three surveys. We used our surveys to test the impact of sheep grazing on the numbers of burrows, and on the numbers of spiders and pygmy bluetongue lizards in those burrows.

Analysis

We conducted two analyses. First we considered only control ungrazed plots, to determine temporal patterns of burrow dynamics in undisturbed grassland across the six surveys. We used seven parameters in separate repeated-measures ANOVAs, and investigated the impact of month on each. Parameters were the number detected per plot in each survey of (i) spider burrows (ii) pygmy bluetongue lizard suitable burrows, (iii) empty burrows, (iv) lycosid spiders found in burrows, (v) pygmy bluetongue lizards found in burrows, (vi) newly constructed burrows, and (vii) previously detected burrows lost since the last survey. Since mygalomorph spiders conceal occupied burrow entrances with well-disguised trapdoors, we were not confident that we had detected and counted all these burrows. We considered this relatively unimportant for the dynamics of the lizards and the burrows they use, because mygalomorphs normally remain in the same burrow for several years (Main, 1976), and lizards only occupy abandoned mygalomorph burrows (Fellows et al., 2009). After abandonment, the trapdoor lids detach from the burrow entrances, and the burrows are easier to detect.

In our analyses we pooled lycosid burrows and abandoned mygalomorph burrows, but excluded any occupied mygalomorph burrows that were found, with trapdoors still in place. For the first five parameters we had data from each of the six surveys. However, for changes in number of burrows between successive surveys (number of new burrows and number of burrows lost) we had no data from the first survey and we omitted data from the two month interval between surveys in November and January, leaving only four sets of data (changes from Sept to Oct, from Oct to Nov, from Jan to Feb, and from Feb to March).

Second, to assess the impact of sheep grazing, we compared the same seven parameters between the grazed and ungrazed plots, before and after the grazing was imposed. For the first five parameters we derived a mean value for each plot from the three months before grazing, and from the three months after grazing. For the parameters of burrow change, we included two measurements before (Sept to Oct; Oct to Nov), and two measures after sheep were introduced (Jan to Feb; Feb to March). For these two parameters we then calculated the total number of burrows either lost or gained rather than a mean per month. We used repeated-measures ANOVAs, with time (before and after the introduction of sheep) as the within-subjects factor, and treatment (sheep or no sheep) as the between-subjects factor. An impact of sheep grazing on any parameter should have been detected by a significant time x treatment interaction effect.

We then included two additional parameters, burrow depth and entrance diameter, and assessed whether burrows that were retained between months were different in depth and diameter from burrows that were lost. We selected all burrows within each plot, which were present between two consecutive months (October – November or February – March) and compared their depth and entrance diameter (mm) in the first month to those which were present in the first month but lost in the second month. In analyses the response variables were the mean values per plot of burrow depth or diameter for burrows of each alternative status (retained or lost). We used repeated-measures ANOVA's with time (before and after grazing) and burrow status (retained or lost) as within-subjects factors and treatment (sheep or no sheep) as the between-subjects factor.

We used natural log transformations where necessary to ensure data were normally distributed. In all repeated-measures analyses, we used Mauchly's test to determine whether data were spherical, and applied the Greenhouse-Geisser correction when they were not.

Results

Temporal changes in burrow dynamics in ungrazed plots

For five of the seven parameters measured in ungrazed control plots there were significant differences among months (Table 1). There were increases from Sept to March in the mean number of spider burrows (Fig 2a), in mean number of pygmy bluetongue suitable burrows (Fig 2b), and in mean number of empty burrows (Fig 2c). The mean number of lycosid spiders in burrows decreased over the seven months (Fig 2d), while the mean number of new burrows detected in successive months remained relatively stable until Feb, but then more than doubled from Feb to March (Fig 2e). The mean number of pygmy bluetongue lizards per plot (overall mean= 7.63; SE=0.05; range= 1 – 16) did not change significantly among months. The overall increase in the number of spider burrows over the study period resulted from an excess of newly detected burrows each month, particularly between February and March (Fig 2e), over the number that were lost (overall mean burrows lost per month = 4.83; SE=0.09; range= 0 - 8).

Grazing impact on burrow dynamics

Including both grazed and ungrazed plots resulted in significant time x treatment interaction effects for four of the measured parameters (Table 2). After grazing commenced, the mean number of spider burrows per plot (Fig 3a), the mean number of new burrows per plot (Fig 3b) and the mean number of unoccupied burrows per plot (Fig 3c) all increased in ungrazed plots, but decreased in grazed plots. The mean number of burrows lost between surveys decreased in ungrazed plots but increased in grazed plots (Fig 3d). Although there was a highly significant main effect of time for the mean number of lycosid spiders in ungrazed plots, reflecting the decline in numbers over the study period detected in the previous analysis, neither the number of spiders, nor the number of pygmy blue tongue lizards showed a significant effect of the grazing treatment (Table 2).

There were no significant main effects of treatment, nor any significant interaction effects on either burrow depth or burrow entrance diameter (Table 3). However, there was a highly significant main effect of time on burrow entrance diameter (Table 3), with burrows measured in October having smaller entrances on average than those measured in February (Fig 4a). There was also a significant main effect of burrow status for both depth and entrance diameter. Burrows retained in the next month (n = 646 burrows) were significantly deeper (Fig 4b), and had significantly wider entrance diameters (Fig 4c) than burrows that were lost (n = 229 burrows).

Discussion

Our results indicate that spider burrow dynamics are influenced by both seasonal changes and sheep grazing. Grazing may have an indirect adverse impact on pygmy bluetongue lizards through a reduction in available spider burrows, an essential resource for the lizards.

Temporal changes in burrow dynamics

The decline of lycosid spiders in ungrazed plots across the study (Fig 2d) was consistent with a trend reported in a previous year (Fellows et al., 2009). This probably happens each year because many lycosid spiders have annual or biannual life cycles (Framenau, 1997; Schaefer, 1987) , and adult lycosids probably die after reproduction (Humphreys, 1976) .

Despite the decline in lycosids the number of spider burrows (Fig 2a), and the number of lizard suitable burrows (Fig 2b) increased over the study, leading, with fewer spiders, to an increase in the number of unoccupied burrows in ungrazed plots (Fig 2c). Burrows accumulated because more new burrows were detected each month than were lost. Possibly increased experience and reduced grass density meant some burrows that were always there were more readily detected later in the season. However, the rapid increase in new burrows late in the season was more likely related to the substantial rainfall event in late February (Fig 1). This would have softened the soil, making burrow construction easier than in the dry, hard soil conditions present earlier in the season.

The low rate of burrow loss in the ungrazed plots reflected both the generally dry weather conditions and the lack of trampling by sheep. Burrows can be destroyed if they fill with debris from water run-off (Ebrahimi, Schofield & Bull, 2012), but in the dry conditions, over most of the study this was not a problem. Additionally, undisturbed burrows in hard, compact soil were unlikely to collapse. Although we detected no significant change in lizard numbers in ungrazed plots, the increase in burrow numbers, and in lizard suitable burrows, would probably provide opportunities for increased recruitment to the lizard population in subsequent seasons (Souter et al., 2004).

Grazing impact on burrow dynamics

After sheep grazing was introduced there were significant declines in the number of burrows (Fig 3a), in the number of new burrows detected (Fig 3b) and in the number of empty burrows (Fig 3c), and a significant increase in the number of burrows lost (Fig 3d) relative to ungrazed plots. Sheep grazing at the level imposed in this study had a negative impact on spider burrows.

Sheep may impact spider burrows directly through trampling. If they tread on or near a burrow entrance they are likely to destroy it. Additionally, trampling can reduce structural quality by compaction and soil homogenization (Betteridge et al., 1999) . As sheep break up surface soil crusts, the loose fine dust can blow into and fill up empty spider burrows. Grazing may also reduce vegetative structure and allow a wider spread of water born debris to fill burrows during rain (Ebrahimi et al., 2012). An additional impact of sheep may be that their presence disturbs the digging activity of spiders, explaining the lower numbers of new burrows constructed in grazed plots.

Although grazing significantly reduced the number of spider burrows, the number of lizard suitable burrows was not significantly affected (Table 2). This can be explained because the burrows lost were significantly shallower (Fig 4b) and had smaller entrances (Fig 4c) and were less suitable for lizards than burrows which remained intact. Either larger burrows required more trampling to destroy, or they were more likely to be maintained by occupants after minor damage.

Grazing impact on pygmy bluetongue lizard population dynamics

Pygmy bluetongue lizard populations are limited by the number of suitable spider burrows (Souter et al., 2004; Fellows et al., 2009). Lizard numbers did not decline significantly in grazed plots in this study, but the number of spider burrows, and amount of spider burrowing activity did decline. In particular there was a decline, in grazed plots, in the number of smaller burrows that might have grown with further excavation to become future replacements for the larger burrows.

This suggests two major concerns about sheep grazing and pygmy bluetongue lizards. First, in the short term, there will be fewer small burrows, of the size preferred by juveniles (Milne & Bull, 2000) available to shelter dispersing neonates after litters are produced in summer (Milne, Bull & Hutchinson, 2002). Second, in the longer term, there will be fewer deep burrows preferred by adults

(Fellows et al., 2009) as replacements for burrows destroyed by natural processes, or to replace the accelerated destruction from sheep grazing.

However there are three additional points. First, pygmy bluetongue lizards occupy grassland habitats that have almost certainly been grazed by mammals long before European settlement and the introduction of sheep (although sheep hooves are more likely to break up soil surfaces). Second, grazing impacts are probably complex. In addition to effects on burrow dynamics, in grazed sites lizards bask more, disperse less and capture prey more frequently than in ungrazed sites (Pettigrew & Bull, 2012; Ebrahimi & Bull, 2013). Thus, sheep grazing can have positive and negative impacts on the lizards. Finally our experimental grazing trials were conducted at one relatively high sheep density in a period of the summer, and in a year when natural vegetation was relatively sparse. More moderate grazing may have had less impact on burrow numbers.

Conclusion

In this short study grazing did not significantly affect the abundance of spiders, but it did result in a decline of spider burrows. In other studies, grazing has resulted in a decline of both abundance and species richness of invertebrate species, including arachnids, and this negative influence becomes greater with increased grazing intensity (Boschi & Baur, 2007; Dennis, Young & Bentley, 2001). The lack of difference in lizard abundance between grazed and ungrazed habitat in the current study was not unexpected, as previous studies assessing how grazing influences lizard behaviour have returned mixed results. These multiple studies on pygmy bluetongue lizards have shown that grazing can be both beneficial and detrimental for lizards, perhaps depending on grazing intensity and regime, and conditions of the season. An implication for other endemic species that live in native grasslands, is that the impact of agricultural grazing is unlikely to be simple to understand, and is likely to require detailed investigation.

Grazing has become an important management tool for maintaining native grassland habitats. Some level of grazing will probably be needed to conserve the grasslands themselves. Our study reveals that the relationship between sheep grazing, spider burrows, spiders and lizards is complex. The impact of grazing is also likely to be influenced by other factors, such as rainfall. Even rainfall is likely to have complex components, with lag effects from rainfall in previous seasons as well as direct impacts from heavy summer storms all potentially influencing the number, persistence and construction of new spider burrows. Further research is needed to determine an appropriate level of grazing, and an appropriate grazing regime to improve retention of burrows suitable to lizards. An encouraging sign is that lizards have persisted in some sheep grazed remnants of native grassland for over 100 years.

Acknowledgements

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Table 1. Results of repeated-measures ANOVAs for the effect of month on mean values of each of seven burrow parameters in ungrazed plots.

Bold denotes significant effects (P<0.05).

Table 2. Results of repeated-measures ANOVA for effects of time (before and after sheep grazing) and treatment (grazed and ungrazed) on seven burrow parameters. Results show F values with P in brackets; df = 1,10 for all F values.

Bold denotes significant effects (P<0.05)

Table 3. Results of repeated-measures ANOVA for effects of time (before and after sheep grazing), status (burrow remained intact or burrow was lost) and treatment (grazed and ungrazed) on depth and diameter of burrows between months (October and November or February and March) Results show F values with P in brackets (df = 1,10 for all F values).

Bold denotes significant effects (P<0.05)

Figure 1. Monthly rainfall (mm) at Burra between September 2012 and March 2013

Figure 2 Mean values each month for the five burrow parameters that were significantly affected by month in ungrazed plots: a) mean number of burrows; b) mean number of pygmy bluetongue suitable burrows; c) mean number of empty spider burrows; d) mean number of lycosid spiders and e) mean number of new spider burrows.

Figure 3 Comparisons of burrow parameters in treatments (grazed and ungrazed), before and after grazing a) mean number of burrows; b) mean number of new spider burrows; c) mean number of empty spider burrows and d) mean number of spider burrows lost during the 2012/2013 field season.

Figure 4 (a) mean burrow entrance diameter of all burrows in October and in February; and for burrows that remained intact in a subsequent month or were lost in a subsequent month, (b) mean burrow depth; and (c) mean burrow entrance diameter.

Tables

Table 1. Results of repeated-measures ANOVAs for the effect of month on mean values of each of seven burrow parameters in ungrazed plots. Bold denotes significant effects (P<0.05).

	Total Burrows	Lizard Suitable Burrows	Empty Burrows	Lycosid Spiders	Pygmy Bluetongue Lizards	New Burrows	Burrows Lost
F	9.146	3.420	6.131	3.378	2.44	6.927	0.524
df	5,25	5,25	5,25	5,25	5,25	3,15	3,15
P	**<0.001**	**0.017**	**0.033**	**0.018**	0.122	**0.004**	0.672

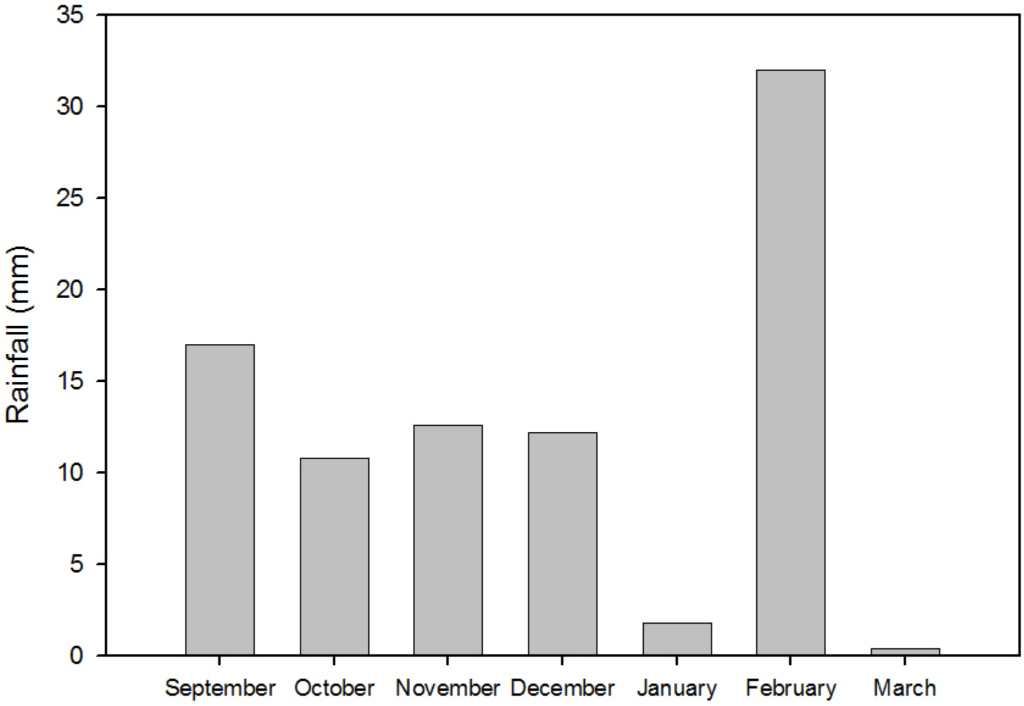
Table 2. Results of repeated-measures ANOVA for effects of time (before and after sheep grazing) and treatment (grazed and ungrazed) on seven burrow parameters. Results show F values with P in brackets; df = 1, 10 for all F values. Bold denotes significant effects (P<0.05).

	Total Burrows	**Lizard Suitable Burrows**	**Empty Burrows**	**Lycosid Spiders**	**Pygmy Bluetongue Lizard**	**New Burrows**	**Burrows Lost**
Time	3.47 (0.092)	8.97 **(0.013)**	35.46 **(<0.001)**	28.95 **(<0.001)**	0.667 (0.433)	3.289 (0.100)	7.964 **(<0.001)**
Treatment	1.043(0.331)	1.422 (0.261)	1.052 (0.329)	1.796 (0.210)	4.784 (0.054)	0.494 (0.498)	9.36 **(0.012)**
Time x Treatment	11.547 **(0.007)**	2.004 (0.187)	14.778 **(0.003)**	2.880 (0.121)	1.437 (0.258)	9.400 **(0.012)**	12.936 **(0.005)**

Table 3. Results of repeated-measures ANOVA for effects of time (before and after sheep grazing), status (burrow remained intact or burrow was lost) and treatment (grazed and ungrazed) on depth and diameter of burrows between months (October and November or February and March) Results show F values with P in brackets (df = 1,10 for all F values). Bold denotes significant effects (P<0.05).

	Depth of burrows	**Diameter of burrows**
Time	2.413 (0.151)	49.296 **(<0.001)**
Status	62.419 **(<0.001)**	7.690 **(0.020)**
Treatment	0.045 (0.836)	0.001(0.977)
Time x Status	1.466 (0.254)	1.744 (0.216)
Time x Treatment	2.361 (0.155)	2.292 (0.161)
Status x Treatment	1.990 (0.189)	0.360 (0.562)
Time x Status x Treatment	0.101 (0.757)	1.730 (0.218)

Figures



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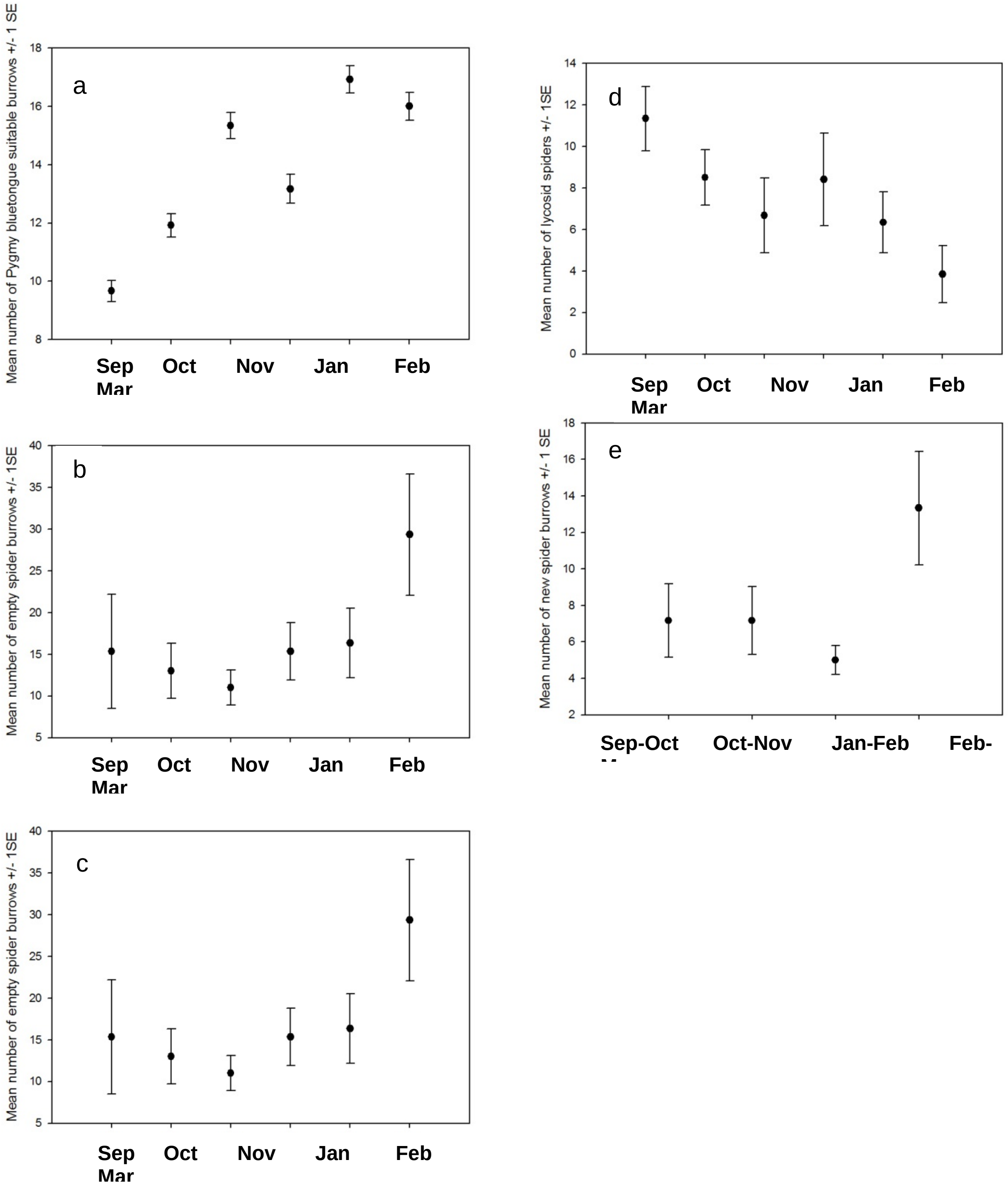

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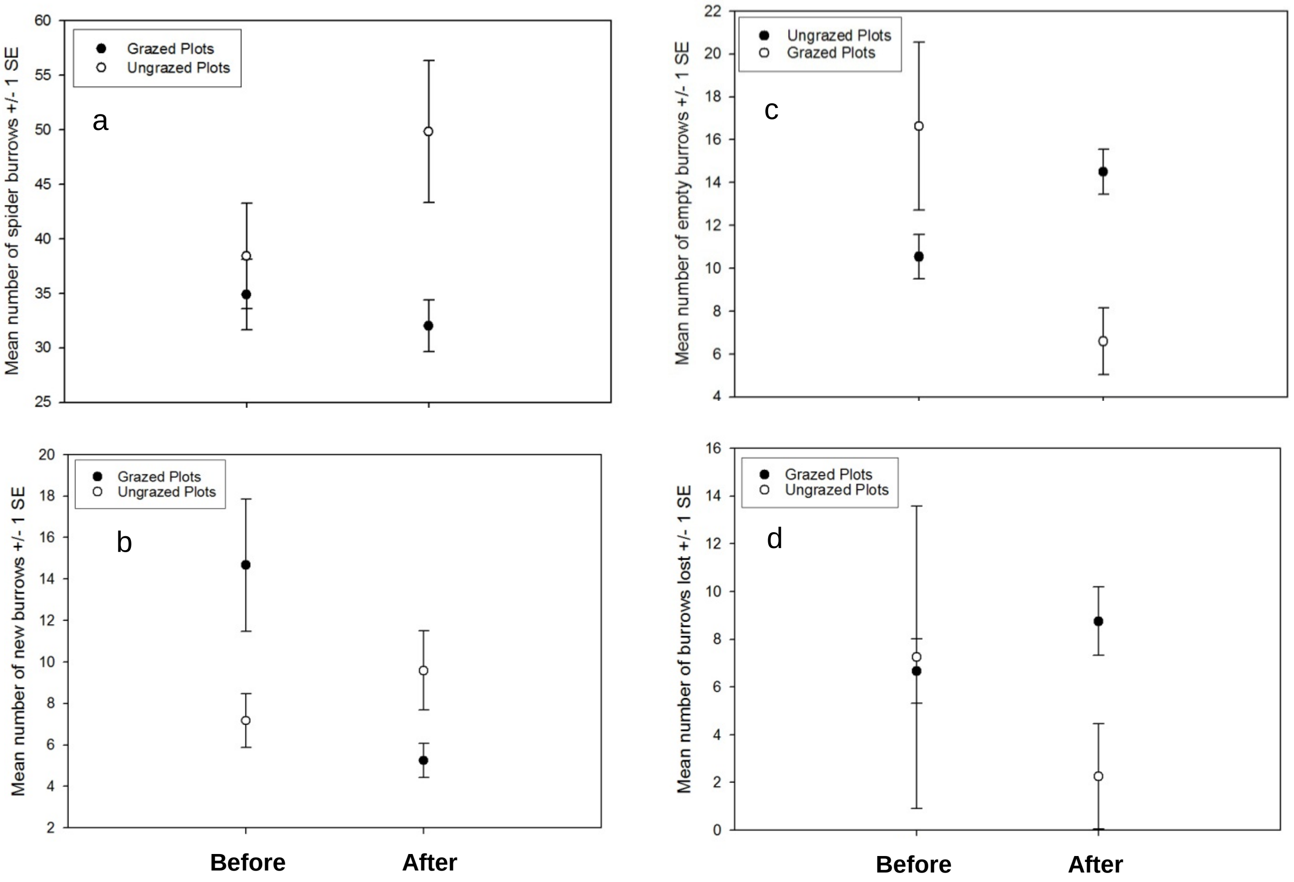

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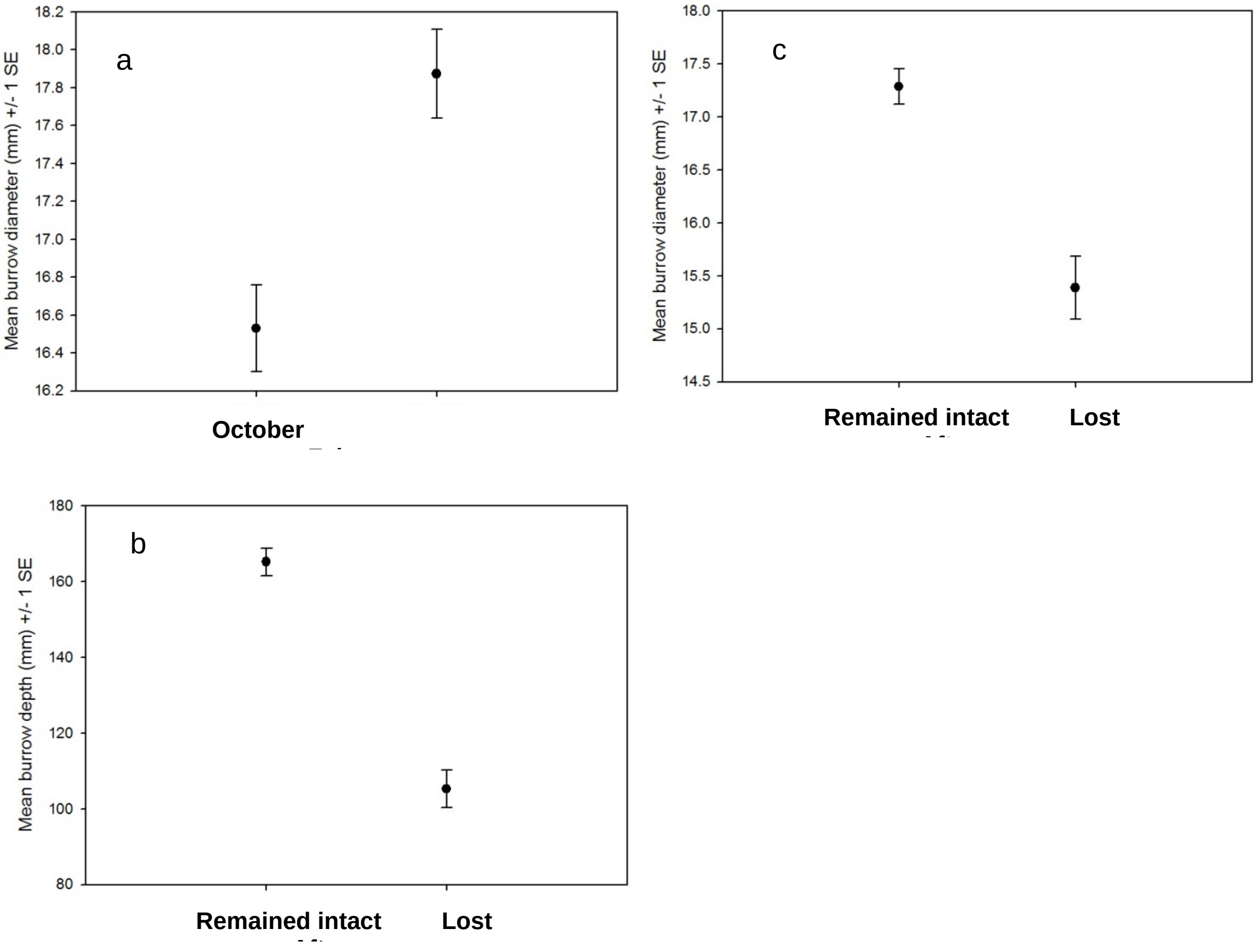

Figure 4. (a) Mean burrow entrance diameter of all burrows in October and in February; and for burrows that remained intact in a subsequent month or were lost in a subsequent month, (b) mean burrow depth; and (c) mean burrow entrance diameter.

Chapter 3

The impact of sheep grazing on the depth of spider burrows and of burrows selected by the pygmy bluetongue lizard (Tiliqua adelaidensis)

Measuring of a wolf spider burrow observed at the Tiliqua Reserve, and its occupant patiently waiting at the entrance

Chapter Preface

Grazing can have a range of impacts on the organisms within an ecosystem. In my previous chapter, I reported that sheep grazing in pygmy bluetongue lizard occupied grasslands resulted in a reduction of spider burrows, and I also reported that grazing resulted in a higher mean burrow depth when compared to ungrazed habitat (Clayton and Bull, 2015). There are a range of potential influences on this differential burrow depth. One commonly reported purpose of burrow use is to access suitable thermal conditions, escaping potentially lethal conditions aboveground (Humphreys 1975; May 1979; Sunday et al. 2014). Grazing may lead to changes to the microclimate (e.g. temperature and/or humidity) of burrows, changes in behaviour of burrow engineers to compensate for altered microclimatic conditions, or increased destruction of a cohort of burrows with similar dimensions. Changes in spider behaviour may have huge implications for future inhabitability of grasslands under climate change. If spiders alter their behaviour in order to maintain suitable burrow temperature, then it may increase their potential to persist within a particular habitat. Further, it may also extend the ability of lizards to persist in these habitats. Alternatively, if particular cohorts of burrows are more at risk of destruction under sheep grazing pressure, there may be implications for some burrow occupants. This chapter explores the effects of grazing on spider burrow depth and implications for the pygmy bluetongue lizard.

The impact of sheep grazing on the depth of spider burrows and of burrows selected by the pygmy bluetongue lizard (Tiliqua adelaidensis)

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Abstract

Context

Grazing by domestic stock can potentially influence ecosystems positively or negatively, depending on the grazing regime and intensity. The pygmy bluetongue lizard (Tiliqua adelaidensis) is an endangered species, restricted to fragmented grasslands in the Mid North region of South Australia, predominantly grazed by sheep. These lizards refuge in vertical burrows. They do not dig their own burrows but rely on co-existing spiders for this essential resource.

Aims

We investigated how sheep grazing influenced spider burrows. We predicted that grazing sheep might trample and destroy shallow burrows; that burrows in grazed habitat with less vegetative cover would have higher temperatures in summer, and that, in compensation, spiders would dig deeper burrows in grazed habitat.

Methods

We monitored 12 30m X 30m plots monthly, over two austral spring/summer periods (Sep-Mar, 2012-2014). We recorded the number, depth and occupant of all spider burrows in those plots in each survey. We also measured temperatures inside artificial burrows in grazed and ungrazed habitat over a three day period in September 2014.

Key results

Mean burrow depth increased with grazing. This was not because individual burrows became deeper, but because the shallowest burrows were lost where there were sheep. Burrows were significantly hotter in grazed habitat, but lizards consistently chose deeper, cooler burrows, and there was no difference in depth of the subset of burrows that were occupied by lizards, between grazed and ungrazed treatments. We detected no difference in spider digging activity between grazed and ungrazed plots.

Conclusions

Sheep grazing can destroy shallow burrows, probably through trampling, and because sheep produce burrow filling debris. However, grazing did not affect deeper burrows occupied by pygmy bluetongue lizards.

Implications

This short study indicated that moderate levels of grazing have low impact on deeper burrows suitable for pygmy bluetongue lizards and for lizard persistence. It explains how sheep and lizards have co-occurred for so long, but leaves open the question of how the loss of shallow burrows affects populations of the burrow-digging spiders that are an essential component of the lizard environment.

Key words: grazing, pygmy bluetongue lizard, spider, burrow, grassland

Introduction

A global shift towards intensive land use for agriculture and urbanisation has increased fragmentation of native ecosystems. Among the changes, grazing by livestock, particularly in the last century, has intensified the impact on biodiversity (Foley, DeFries et al. 2005) and has usually resulted in negative changes in species diversity, community structure and changes in a range of soil characteristics (Fleischner 1994), although these impacts may differ among habitats, grazing regimes and grazer species. This paper considers the impact of grazing livestock on the burrows of an endangered Australian grassland lizard.

Native grasslands are considered to be among Australia's most threatened ecosystems (Williams and Cary 2001). They have a long history of grazing by native and exotic species, and have been exposed to many potential biological and physical disturbances. In South Australia, a shift towards human land use has resulted in the destruction of more than 95% of pre-European native grasslands (Hyde 1995). Most of the remaining native grasslands have been extensively grazed by sheep, and invaded by exotic annual plant species. While some level of grazing may be beneficial to control weeds, sheep grazing probably disturbs this habitat in a different way than the pre-European grazing by endemic macropods and other mammalian herbivores (Lewis, Clarke et al. 2008). Correct management of these new grazing regimes is critical to maintain at least some elements of native grassland ecosystems.

In grasslands, grazers can substantially reduce vegetation cover. Grazer driven ecosystem change can then result through associated changes in the microclimate, and therefore the suitability of the habitat for certain animal and plant species, with ultimate impacts on the overall community structure. Grazed habitats, with reduced vegetative cover, become locally hotter and drier, with the impact increasing as the grazing pressure become greater (Gardiner and Hassall 2008; Whitman 1974). Grazed habitats also lose the structural diversity of the vegetation needed to support complex communities (Dennis, Young et al. 1998).

Grazing can then have varying flow-on impacts on animal species. Studies on bird, small mammal, spider and insect groups have described both negative and positive impacts of grazing, often measured as changes in species richness and species abundance (Jones 1981; Loe, Mysterud et al. 2007; Read and Cunningham 2010; Vulliamy, G. Potts et al. 2006). In arid and semi-arid systems, the grazing impacts are often greater at sites closer to watering points, often man made water sources, where domestic stock graze more intensively (Brits, Van Rooyen et al. 2002; Churchill and Ludwig 2004; Hendricks, Bond et al. 2005; Kovac and Mackay 2007; Macchi and Grau 2012).

In addition to the impact through reduced vegetation cover, livestock grazing can affect endemic diversity by changing the physical attributes and soil characteristics of the ecosystem. These changes can result directly from trampling, or indirectly, by rain and wind shifting grazer disturbed soil. Ungulate grazers can alter a range of soil characteristics (Augustine and McNaughton 2007; Teague, Dowhower et al. 2011) including the distribution of nitrogen and organic carbon (Augustine and Frank 2001), the rate of nitrogen cycling, and the composition and abundance of both plants (Singer and Schoenecker 2003; Tracy and Frank) and soil biota (Mohr, Cohnstaedt et al. 2005; Tracy and Frank). Animal trampling can compact soil, with adverse effects on porosity, water infiltration rates, and soil strength (Lobry de Bruyn and Kingston 1997; Mulholland and Fullen 1991; Singleton and Addison 1999). Of particular concern for burrow dwelling animal species is the impact that trampling grazers might have on any burrows in the soil.

Among the many animal species inhabiting native grasslands, fossorial species in particular are likely to be at high risk from burrow-threatening, grazing disturbances. While there are many studies on the impacts of grazers on overall ecosystem quality and above ground biodiversity, the impact of grazing on burrows and burrow inhabiting species is surprisingly less well documented. Grazing is reported to result in trampling and destruction of the burrows of burrowing owls (Speotyto cunicularia) in grasslands of North America (Dechant, Sondreal et al. 2002), and of burrowing small mammals in

Mediterranean montane grasslands (Torre, Díaz et al. 2007). Grazing can also alter the microhabitat around intact burrows, largely through reduced vegetation cover and exposure to higher above-ground temperatures. This can alter the microhabitat in and around the burrow to become either more or less suitable for a burrow dwelling species, depending on its requirements. For desert tortoises (Gopherus agassizii) grazing reduces habitat suitability because they prefer a high percentage of vegetation cover and low disturbance (Grandmaison, Ingraldi et al. 2010). Alternatively, some grazing may increase habitat suitability for burrowing owls (S. c. hypugaea) and blacktail prairie dogs (Cynomys ludovicianus) which select sites with less vegetation and more bare ground (Dechant, Sondreal et al. 2002; Uresk, MacCracken et al. 1981). For some other burrow dwelling species, there may be a trade-off between beneficial and detrimental effects of grazing. Yellow mongoose (**Cynictis penicillata**) benefit from the presence of small shrubs for burrow protection, but, prey availability declines with more shrubs; they select burrow sites with an intermediate shrub density, resulting from moderate grazing (Blaum, Rossmanith et al. 2007). The relationship between grazing and microhabitat suitability is therefore complex and variable, depending on individual species requirements.

Burrows in grassland are critical to the survival of a wide range of animals. Burrows provide a buffer from the above-ground climate, protection from above-ground predators and ambush points for capturing prey (McGinnis and Voigt 1971; Pavey, Burwell et al. 2010; Pike and Mitchell 2013; Rothermel and Luhring 2005). These benefits of burrows are available both to the animals that dig them, and also to the many species that exploit and occupy burrows dug by other animals (Jones and Franz 1990; Read, Carter et al. 2008; Smith and Foggin 1999). In Australia, many reptile species gain refuge and thermoregulatory benefits from burrows, including grassland inhabitants such as the grassland earless dragon (Tympanocryptis pinguicolla) and Bynoe's gecko (Heteronotia binoei) (Pianka 2013; Stevens, Evans et al. 2010).
Many of the burrows used by these grassland reptiles are constructed by arthropods (Cocroft and Hambler 1989; Fellows, Fenner et al. 2009; Stevens, Evans et al. 2010; Turner 2014), and their continual availability may be critical for species persistence. Artificial burrows have been successfully trialed as additional refuge resources for a number of grassland lizard species (Souter et al. 2004). However, maintaining the

continued supply of the natural burrows that they utilise would be a more efficient management practice. The impact of grazing on grassland spider burrows in particular has been rarely studied. As these burrow resources are key to the survival of at least one endangered lizard species, a greater understanding of the consequences of grazing pressure for this resource is essential.

The pygmy bluetongue lizard (Tiliqua adelaidensis) is an endangered lizard, restricted to remnant fragments of native grassland in the Mid North region of South Australia. It has a snout-vent length of 85-105mm, and exclusively occupies burrows built by mygalomorph or lycosid spiders, using them as refuges, and using their entrances as basking sites and ambush points (Milne, Bull et al. 2003). Pygmy bluetongue lizards rarely completely leave their burrows (Pettigrew and Bull 2012) and can occupy the same burrow for multiple activity seasons (Bull, Godfrey et al. 2015). Almost all known population sites are used for livestock grazing. This has probably been important in controlling the abundance of introduced weeds (Souter and Milne 2009), but for more informed conservation management we need to know how the lizards and their resources are impacted by grazing. Although pygmy bluetongue lizards prefer to occupy burrows with more surrounding vegetation, they basked for longer at the burrow entrance and were more successful in prey capture in burrows with simulated grazing (Pettigrew and Bull 2011; Pettigrew and Bull 2012; 2014). Enhanced prey capture rates with less vegetation could result either from higher prey visibility, or from there being longer periods in the day when conditions are thermally suitable for lizards to remain at the burrow entrance (Ebrahimi and Bull 2012; Pettigrew and Bull 2012). Thus moderate grazing may have some direct benefits for lizards.

However, grazing may have a negative impact on the persistence of the spider burrows that the lizards occupy. High quality burrows (more than 300 mm deep) are in short supply (Fellows, Fenner et al. 2009), and adding artificial deep burrows led to local increases in lizard density (Souter, Bull et al. 2004). These lizards do not dig their own burrows, but rely on burrows dug by spiders (Milne, Bull et al. 2003).   We have previously reported that sheep grazing resulted in a decline in the number of new burrows constructed, and an increase in the number of existing burrows lost, relative to ungrazed areas (Clayton and Bull, 2015). Our current study explores in more detail the normal range of burrow depths in a pygmy bluetongue lizard population site, and the differential effects of grazing on burrows of different depths within the site.

Material and methods

Study site

The study was conducted within a 70 ha site, the Tiliqua property of the Nature Foundation of South Australia, approximately 8 km from Burra, South Australia (33°42′S, 138°56′E). The site is a semi-arid native grassland partially invaded with exotic weeds (Clayton and Bull 2015; Milne 1999; Souter, Bull et al. 2007). Surveys occurred over two field seasons, during the austral spring and summer period from Sept – March, of 2012/2013 (season 1) and 2013/2014 (season 2). The area has hot, dry summers (average maximum temp in January is 31^{0}C) and cool, moist winters (average maximum temp in July is 12.8^{0}C), and lizards are normally only active during our designated spring and summer field season. Over the period 1961 – 2014 the average annual rainfall at Burra was 431 mm, with most rain in the winter months (Bureau of Meteorology, 2014). The two seven-month periods Sept – March in the current study, were much drier (89.0 mm) in season 1 than in season 2 (217.8 mm).

Grazing treatments

The Tiliqua property contained six experimental paddocks that ranged in size from 3.49 – 6.86 ha, arranged in a north-south line along the eastern edge of the property, and a larger paddock to the west. We established six 30 m x 30 m plots, one in each experimental paddock, and spaced 100-200 m apart from each other, and an additional six plots in the larger paddock, arranged in a straight line 50 m west of the experimental paddock fence line. From September 2012 we surveyed the plots each month (Sept – March) during the two successive field seasons. In the first season, we surveyed all twelve plots, while in the second season we surveyed only the six plots within the individually fenced experimental paddocks.

There were no sheep on the Tiliqua property over 2012, including the first four months, Sept – December, in the first field season. Then, from Jan – March 2013 (second half of season one), sheep were introduced at a rate of four sheep per hectare to the larger western paddock, and to the six survey plots in that larger paddock, but sheep were still

excluded from the six experimental paddocks. Thus six plots were exposed to grazing and six plots remained ungrazed in the second half of the first field season.
From April 2013, and throughout the second field season (Sept 2013 – March 2014), sheep grazed in three of the experimental paddocks at a rate of five sheep per hectare, while the other three paddocks remained ungrazed. Grazed and ungrazed treatments were alternated across adjacent paddocks. Thus, three survey plots in those paddocks were exposed to grazing and three plots remained ungrazed throughout the entire second season. We varied the number of sheep per hectare between seasons to maintain the same level of moderate grazing in response to higher rainfall and greater vegetative growth in the second season, and with advice from local graziers'. Stocking rates reflected normal practice among local graziers' and we assume they reflected historical rates of grazing.

We previously reported for field season one (Clayton and Bull 2015) a significantly greater increase in the mean number of spider burrows per plot in ungrazed (46.44 (SE 0.25) to 59.06 (SE 0.29)) than in grazed paddocks (47.22(SE 0.22) to 42.22 (SE 0.21)) when comparing months before and after sheep were added. The mean number of burrows per plot that were occupied by lizards (mean 6.99; SE 0.073) was not significantly different across time or grazing regimes in season one.

In the second field season, previously unreported, there was no significant impact of either time or grazing treatment on the mean number of spider burrows (mean 48.78; SE 0.45) or of burrows with lizards (mean 8.59; SE 0.26) per plot (Appendix 1).
The focus of the current paper was on any impact of grazing on the depth and microclimatic conditions of burrows.

Vegetation Biomass

In each of six months (Oct 2013 – Mar 2014) during the second field season, we collected with scissors all above-ground vegetation from five replicate 300mm X 300mm squares just outside the edge of each plot within the experimental paddocks (five samples x six plots x six months). Five different sample squares were selected around each plot in each month. Samples consisted of both live plants and dead vegetation that had accumulated on the ground surface. Samples were dried at 70^{O}C for

24 h, then weighed. An index of dry vegetation biomass was derived for each plot in each month, from the average of the five replicate sample weights.

We compared mean vegetation biomass per plot between grazed and ungrazed treatments, in the second field season, using repeated-measures ANOVA, with month (October – March) as a within-subjects factor, since the same plots were repeatedly sampled, and treatment (grazed, ungrazed) as a between-subjects factor.

Burrow surveys and burrow dimensions

Following Clayton and Bull (2015), we defined a spider burrow, independent of any occupants, as a burrow that was deeper than 14 mm and had an entrance diameter of between 6 – 35 mm. In each season, we thoroughly searched each survey plot for all spider burrows we could find, once each month from Sept – March (except Dec 2012). We searched each plot along 30, 1 m wide transects to locate as many burrows as possible. We then used an optic fiberscope (Medit Inc 2 way articulating FI Fiberscope) to inspect each spider burrow for occupants and to measure burrow depth, as in Milne and Bull (2000). Most burrows were between 50mm and 250mm deep (Fig 1). We marked the location and identity of each spider burrow with a 300 mm polypropylene tent peg. In subsequent monthly surveys we noted whether previous spider burrows were still present, and marked any newly detected spider burrows. We defined a spider burrow as established if it was detected in three consecutive surveys. We defined a lizard occupied burrow as a spider burrow that had a lizard occupant on at least one survey. We used the observational data to compare burrow depths of spider burrows and of lizard burrows in grazed and ungrazed conditions.

Spider and lizard burrows

We asked three specific questions.

1. Does grazing influence the depth of established spider burrows or of lizard burrows?

We first analysed the influence of grazing on the depth of all established spider burrows (those present on three or more consecutive monthly surveys) regardless of the occupant. In season 1, we calculated their mean depth in each plot, first in Nov 2012 (before grazing) and then in March 2013 (three months after grazing was introduced).

We used our 24 measurements of mean burrow depth (12 plots x 2 times) in a repeated-measures ANOVA with time (Nov, March) as a within-subjects factor and treatment (grazed, ungrazed) as a between-subjects factor. Because no plots had been grazed in November, the interaction effect of time x treatment was used to indicate any impact of grazing.

In season 2, we measured established burrow depths in January, February and March. We expected grazing to have an impact in each month. We used the 18 measures of mean burrow depth per plot (6 plots x 3 months) in a repeated-measures ANOVA using time (Jan, Feb and Mar) as the within-subjects factor and treatment (grazed, ungrazed) as the between-subjects factor. Here we were exploring temporal changes in burrow depth as well as an effect of grazing treatment.

We repeated these analyses in each season considering only lizard occupied burrows. Additionally, we compared the impact of grazing on lizard occupied burrows (lizard burrows), with those never found to be occupied by lizards (non-lizard burrows). We calculated for each plot the mean depth of all lizard burrows and of all non-lizard burrows, and then conducted analyses as above, but including burrow status (lizard burrow, non-lizard burrow) as an additional within-subjects factor.

2. Do burrows change depth?

Next we asked whether depth changed over time in individual burrows, and whether grazing impacted those changes. Preliminary analyses (not shown) found little change in depth from month to month of already established burrows. Instead, we focused on newly detected burrows that we considered more likely to undergo additional excavation. We acknowledge that some of these may have been present but undetected in previous months. We measured the difference in burrow depth from the month of first detection to the next month. In season 1, we calculated the mean change in new burrow depth per plot, first from October to November, before grazing commenced, and then from February to March, for all new burrows first found in February, after the introduction of grazing. To determine whether grazing influenced change of burrow depth we used similar repeated-measures analyses to those described above.

In season 2, we used similar analyses on mean burrow depth change of new burrows from one month to the next, with time of season (early: combined data from burrow depth changes from Sep-Oct, Oct-Nov and Nov-Dec and late: combined data from Dec-Jan, Jan-Feb and Feb-Mar) as a within-subjects factor and treatment (grazed, ungrazed) as a between-subjects factor.

3. Does the depth of a burrow influence whether it is likely to remain intact?

In season 1, we compared mean spider burrow depth in each plot in September (before grazing) between those that remained (intact) and those no longer detected (lost) three months later (November). Similarly we compared burrow depths in January (after grazing) between those still intact and those lost during the March survey. We used repeated-measures ANOVA on the mean burrow depth per plot with time (before and after grazing), and burrow status (intact or lost) as the within-subjects factors and treatment (ungrazed or grazed) as the between-subjects factor. As before we used the interaction effect of time x treatment as an indicator of the impact of grazing.

Similarly in season 2, we compared the mean depths of those burrows that remained intact and those that were lost over the next three months early in the season (September) and later in the season (January). Again we used repeated-measures ANOVA with time (early, later) and burrow status (intact or lost) as within-subjects factors and treatment (ungrazed or grazed) as a between-subjects factor. Here the main effect of treatment was used to indicate an impact of grazing.

To further explore the influence of burrow depth on burrow persistence, we recorded the proportion of shallow (less than 150mm) and deep (150mm or more) burrows in each plot that remained intact 3 months later.

In Season 1, we made this comparison from September to November (before grazing) and then from Jan – March (after grazing). We used repeated-measures ANOVA on the arcsin transformed proportions of intact burrows per plot, with time (before and after grazing) and depth class (shallow and deep) as the within-subjects factors, and treatment (ungrazed or grazed) as the between-subjects factor.

Similarly, in Season 2, we compared the proportion of shallow and deep burrows that persisted for three months, using early (Sep-Nov) and late (Jan-Mar) season as the time intervals.

Microclimatic conditions in burrows

We also asked if sheep grazing influenced thermal conditions inside the burrows. In Sept 2014 we constructed 16 vertical artificial burrows (250mm deep; 19mm entrance diameter) at the Tiliqua study site. We followed the method of Souter et al. (2004) by hammering a steel rod 250 mm into the ground, and then removing it. Eight artificial burrows, constructed in this way, were spaced 1 m apart in a heavily grazed paddock (11 sheep per hectare) and eight in an adjacent ungrazed site in the Tiliqua reserve. We suspended, on a cotton thread, one temperature measuring Ibutton data logger (hydrochron DS1923-F5#) in four burrows in each grazing treatment at a depth of 100mm, and in the other four at 200mm below the ground surface. Additional data loggers were placed at the entrance (00mm depth) of two burrows in each treatment (total 20 data loggers). The data loggers recorded temperature every 30 min for three days from 1000 h on 30 September 2014, in the early part of the lizard activity season.

We compared temperature at each depths within burrows (100mm and 200mm) in grazed and ungrazed areas of the study site. We calculated a mean daily midday temperature and a mean temperature range for the three days for each logger. We used two way ANOVAs with temperature (midday or daily range) as the dependent variable, and depth (100mm and 200mm) and treatment (grazed and ungrazed) as fixed factors.

General statistical methods

In all analyses we compared measured parameters between grazed and ungrazed treatments. We used natural log transformation of data where necessary to ensure they were normally distributed. In repeated-measures ANOVAs, we used Mauchly's test to determine whether data were spherical, and applied the Greenhouse-Geisser correction where they were not.

Results

Vegetation biomass

In the analysis of dry vegetation biomass in season 2 there was a significant main effect of grazing treatment but no significant effect of month and no significant interaction between month and treatment (Table 1). Grazed plots had consistently lower vegetation biomass over all samples.

Burrow dynamics

Does grazing influence the depth of established spider burrows or lizard burrows? Analysis of the mean depth of established burrows, in season 1 showed a highly significant interaction effect of time x treatment (Table 2). Mean burrow depth became shallower from November to March in ungrazed plots, but deeper in grazed plots (Fig 2a). The interaction effect time x treatment was also significant in season 2 (Table 2). Grazed plots always had deeper burrows but the difference became greater in February and March, later in the summer (Fig 2b).

For lizard occupied burrows, there was no significant effect of either time or grazing treatment, nor any significant interaction effect (time x grazing treatment), on mean depth per plot in either season (results not shown).

Burrow status (occupied or not occupied by lizards) had an additional significant main effect on burrow depth in each season, with a significant time x status interaction in season 1 (Table 3). Lizard burrows were always deeper than non-lizard burrows although that difference was greater in November than in March of season 1.

Do burrows change depth?

Newly detected burrows had usually changed in depth by the next month, although the direction and amount of change varied considerably, and there was no consistent pattern across time (Appendix 2). Analyses (Table 4) detected no significant effects of either time or grazing treatment on the mean depth change of new burrows per plot, in either season.

Does the depth of a burrow influence whether it is likely to remain intact?

In both seasons, analysis of burrow depth showed a significant main effect of burrow status (remained intact or lost), with intact burrows deeper than those that were lost (Table 5, Fig 3). In season 1 there was also a significant time x burrow status interaction. Intact burrows had similar mean depths (around 180 mm) in both early and late season samples, but after grazing started, the “lost” group included some deeper burrows, resulting in an increase in the mean depth of that group (although this happened in plots that were both grazed and ungrazed) (Fig 3a). Although this might suggest a greater risk for deep burrows after grazing started we found no significant treatment x burrow status interaction in season 1 and no main effect of grazing treatment in season 2.

However, when we divided all of the burrows into shallow and deep categories, in season 1, there was a significant time X depth X treatment interaction effect for the proportion of burrows that persisted (Table 6). Before grazing was imposed, there was a consistent pattern in both grazed and ungrazed treatments, for a higher proportion of deep than of shallow burrows to persist for three months. After the grazing treatment was added, deep and shallow burrows had equal and high persistence over three months in the still ungrazed treatment plots, but shallow burrows had much lower proportional persistence than deep burrows in the plots with grazing. That is, grazing significantly reduced the proportion of shallow burrows that remained intact (Table 6, Fig 4a). In season 2, there was a significant depth X treatment interaction on the proportion of burrows remaining intact. While comparable proportions of shallow and deep burrows persisted in the ungrazed plots, fewer shallow than deep burrows persisted in the grazed plots (Fig 4b). Thus results from both seasons consistently suggested that shallow burrows were at greater risk than deep burrows when sheep were grazing.

Microclimatic conditions in burrows

Mean surface temperatures were higher than inside burrows in both grazed and ungrazed treatments (Appendix 3). Within burrows, there were significant main effects of depth and of treatment, and no significant depth x treatment interaction, for both mean midday temperature and mean daily temperature range. Midday temperatures were always cooler, and daily temperature ranges were smaller, deeper into the burrow, and in the ungrazed paddock (Fig 5).

Discussion

Vegetation Biomass

Grazing had a significant impact on vegetative biomass as expected, with grazed paddocks having less vegetation biomass. This presumably arose from the combined effects of sheep both consuming and trampling the plants. Reduction of vegetative biomass from livestock grazing has been previously well documented (Mwendera, Saleem et al. 1997; Pucheta, Cabido et al. 1998; Reeder and Schuman 2002). Previous studies have discussed how a reduction in vegetation may have positive (more basking opportunities, easier to detect insect prey) and negative (lower prey density, more exposure to avian predators) impacts on burrow occupants such as pygmy bluetongue lizards (Ebrahimi 2015; Pettigrew and Bull 2011; Pettigrew and Bull 2012). Our focus in the current study was on how grazing affected the burrows that the lizards rely on for refuges.

Does grazing influence the depth of established spider burrows or lizard burrows?

The mean depth of established burrows in both season 1 and season 2, was significantly deeper in grazed than ungrazed treatment plots, and became deeper after grazing was applied in season 1. Two explanations, explored in later analyses, were (i) that spiders dug deeper burrows in the presence of grazing sheep, or (ii) that sheep were more likely to damage shallow burrows than deep burrows.

The burrows found occupied by lizards were significantly deeper than those with no lizard occupancy observed. Milne and Bull (2000) reported that adult pygmy bluetongue lizards, given a choice, preferred deeper burrows, both in field and laboratory experiments. Their explanation was that deep burrows were better refuges against predators that can enter the burrows, such as brown snakes (Pseudonaja textilis) (Fenner and Bull 2014; Fenner, Schofield et al. 2008; Hutchinson et al. 1994). Also, Fellows et al. (2009) found lizards occupied deeper burrows than spiders at the same study site as ours. Later analysis in this current study allowed us to consider two other hypotheses for this pattern of preference for deeper burrows. One, based on observations that lizards can occupy the same burrow for extended periods over consecutive seasons (Bull et al. 2015), is that deeper burrows are more stable for long term lizard occupancy. The other is that deeper burrows better protect lizards from extreme surface temperatures.

We found no effect of grazing on the depth of lizard burrows. Lizards were able to locate and occupy burrows of similar depth whether or not sheep were grazing. This suggests that there was no immediate direct impact of grazing on the short-term availability of suitable burrows for lizards. The grazing related loss of shallower burrows did not affect lizard occupancy in deeper burrows. However, we could not determine, from this study, any longer term impacts. For instance, if the spiders that dig the burrows have fewer shallow burrows in which to start deeper excavations, then the longer-term replacement of deep burrows may be threatened.

Do burrows change depth?

One hypothesis to explain why average burrow depth was greater in grazed than ungrazed treatments was that spiders dug deeper burrows in places where sheep were grazing. We have previously reported that spiders regularly dig new burrows, and more of those new burrows appear in ungrazed than in grazed plots (Clayton and Bull 2015). Our question now is whether they continued to excavate the burrows after they have reached a depth sufficient to be called spider burrows in our classification. We detected regular changes in individual burrow depth from one survey to the next. Some burrows became shallower from the accumulation of debris and collapsing burrow walls. Others became deeper as spiders and lizards cleared out the debris or as spiders dug the burrows. It was difficult to differentiate or separate these alternative processes and we recorded both increases and decreases in individual burrow depth over each time period. Importantly, we detected no consistent pattern either considering all burrows or considering only newly established burrows, for individual burrows to become deeper in grazed than in ungrazed plots. We could not separate the impact of spider digging from other factors likely to influence individual burrow depth, nor could we find evidence to support any difference in digging activity of spiders between grazing treatments. Our hypothesis of a differential level of spider excavations with or without sheep was not supported by our data.

Does the depth of a burrow influence whether it is likely to remain intact?
We have previously shown that the net number of burrows in a plot results from the loss of some old burrows and the formation of some new ones (Clayton and Bull 2015). Our alternative hypothesis for the change in mean burrow depth between grazing treatments, was that more shallow burrows were destroyed, leaving a greater proportion of deep burrows in grazed than in ungrazed plots. Our analyses confirmed that the mean depth of lost burrows was shallower than of those that persisted, and that a higher proportion of shallow burrows was lost when sheep grazed than in ungrazed plots.

This probably resulted from a higher level of sheep disturbance in grazed habitat. Sheep are likely to directly impact burrows by trampling, and to affect them indirectly by breaking up surface lichen crusts, creating dust, and displacing vegetation and surface debris. Debris and dust may then drift into burrows, transported either by wind or water (after rain). A previous study (Ebrahimi, Schofield et al. 2012) reported that removal of surface vegetation led to more pygmy bluetongue lizard burrows being destroyed following rain. Deeper burrows may be more resistant to this degradation, either because they have a greater depth to fill with debris before they are lost, or because they are more likely to have resident lizards or spiders that may clear out the debris to maintain them.

Other studies have reported similar impacts of grazing on soil and on burrows in the soil. Greene et al. (1994) reported that high grazer stocking densities, with greater hoof activity and lower organic matter, resulted in increased susceptibility to erosion of the surface soil. They have reported trampling of Agassiz's desert tortoise (Gopherus agassizii) burrows by bighorn sheep (Ovis canadensis nelsoni) (Agha, Delaney et al. 2015), trampling of burrowing owl (Athene cunicularia) burrows by livestock (Holmes et al., 2003), and more general trampling disturbance to soil (Torre, Díaz et al. 2007). However, less attention has been given to the differential stability of burrows with alternative characteristics.

One explanation for why lizards preferred to occupy deeper burrows may be that those burrows are inherently more stable. Perhaps spiders can only construct very deep burrows where the soil structure is firmest. Alternatively, deep burrows may be more stable, and persist for longer because they are more likely to be occupied by lizards and the movement of the lizard in the burrow continuously clears away debris, and maintains the burrow structure.

Microclimatic conditions in burrows

Another explanation for why lizards prefer deep burrows was that deeper burrows allow better protection of lizards from thermal extremes. Our results confirm the many other studies that have shown less extreme temperatures underground in burrows than on the surface during the heat of the day, and smaller temperature ranges (Ke and Lu 2009; Roper, Bennett et al. 2001; Zimmerman, O'Connor et al. 1994). Additionally our results show that, for a given burrow depth, burrows in grazed treatments had less protection from those extremes, so burrow inhabitants would need to go deeper in the burrow for the same protection. The vegetation around a burrow probably provides some additional insulation against temperature extremes. While we found no evidence that grazing and the reduction of vegetation cover led spiders to dig deeper burrows, we suggest that lizards in deep burrows can adjust their depth underground to accommodate changes in surface temperature. Thus the impact of grazing on their thermal environment will be minimal, if deep burrows remain available.

Broader impacts of grazing and burrow depth

The long term impacts of the decrease in numbers of shallow burrows in grazed treatments for both spider and lizard populations, are not known. However both pygmy bluetongue lizards and spiders have persisted in native grassland habitats that have probably been grazed by introduced sheep for over a century (Pettigrew and Bull 2014). While burrows are probably a limiting resource for populations of this lizard (Fellows, Fenner et al. 2009; Souter, Bull et al. 2004), the demonstration in the current study, that deeper burrows persist despite the presence of grazing, probably explains how lizards can co-exist with sheep. Indeed grazing may be important to remove vegetation, to prevent the build-up of a thatch of dead grass on the surface, and to allow lizards basking opportunities around their burrows, and enhanced prey capture (Pettigrew and

Bull 2012). It is important to further investigate how this loss of shallow burrows affects the population sizes of lizards and the spiders that construct lizard burrows, and the level of grazing management that will provide the necessary benefits but also minimize any potential negative effects of burrow damage by sheep.

Nielsen and Bull (unpublished data, 2016) reported that pygmy bluetongue lizards from the same study area had lower body condition and later dates of parturition in grazed than ungrazed habitat. Thus, while grazing may not impact the short-term availability of suitable refuge burrows for these lizards, it probably has flow on effects on a range of other processes that themselves influence lizard fitness in their native grassland habitat.

Sheep grazing is likely to be an important management tool for conservation of the pygmy bluetongue lizard. It has the potential to be both beneficial and detrimental to lizard populations, depending on its use. This study indicates that grazing at this level may pose minimal short term impacts on burrows suitable to pygmy bluetongue lizards and lizard persistence, however further investigation into the ongoing effects of losing a large proportion of shallow burrows due to grazing is required. Further research into the most appropriate grazing density and regime is critical in the conservation of this species and its burrow resources.

More broadly, burrows, and other refuges, provide an opportunity for ectothermic reptiles to behaviourally adjust their body temperatures and to respond to any moderate changes in ambient temperature that are likely to result from predicted climatic changes (Kearney et al. 2009). Understanding the dynamics of the lizard – burrow interaction will be critical in future management of this and of other endangered lizard species.

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Tables

Table 1. Results of repeated-measures ANOVA for mean above-ground vegetation biomass (g) in Season 2, between month (October – March) and treatment (grazed and ungrazed). P values in bold indicate significant effects (P<0.05).

Vegetation Biomass (g)	df	F	P
Month	5,20	1.996	0.196
Treatment	1,4	7.914	**0.048**
Month X Treatment	5,20	0.386	0.697

Table 2. Results of repeated-measures ANOVAs for mean depth per plot of all established spider burrows, assessed independently in Season 1 and Season 2, in grazed and ungrazed treatments measured in Nov and Mar (season 1) and in Jan, Feb and Mar (season 2). P values in bold indicate significant effects (P<0.05).

	df	F	P
Season 1			
Time	1,10	0.542	0.479
Treatment	1,10	0.112	0.744
Time X Treatment	1,10	11.490	**0.007**
Season 2			
Time	2,8	36.468	0.616
Treatment	1,4	1.496	0.288
Time X Treatment	2,8	4.713	**0.044**

Table 3. Results of repeated-measures ANOVAs for mean depth per plot of all established spider burrows (burrows present for 3 months or more) and differences in status (lizard occupied and lizard unoccupied burrows) assessed independently in Season 1 and Season 2, in grazed and ungrazed treatments measured in Nov and Mar (season 1) and in Jan, Feb and Mar (season 2). P values in bold indicate significant effects (P<0.05).

	df	F	P
Season 1			
Time	1,10	1.023	0.336
Status	1,10	240.635	**<0.001**
Treatment	1,10	0.081	0.782
Time X Status	1,10	28.526	**<0.001**
Time X Treatment	1,10	4.132	0.069
Status X Treatment	1,10	0.012	0.915
Time X Status X Treatment	1,10	0.053	0.822
Season 2			
Time	2,8	4.207	0.056
Status	1,8	38.352	**0.003**
Treatment	1,8	0.212	0.669
Time X Status	2,8	0.117	0.891
Time X Treatment	1,8	0.471	0.640
Status X Treatment	2,8	0.273	0.629
Time X Status X Treatment	2,8	0.825	0.445

Table 4. Results of repeated-measures ANOVAs for differences in depth of new burrows measured from one month to the next assessed independently in Season 1 and Season 2 in grazed and ungrazed treatments, measured before (Oct-Nov) and during (Feb-Mar) grazing (Season 1) and early (combined data from Sep-Oct, Oct-Nov and Nov-Dec) and late (combined data from Dec-Jan, Jan-Feb, Feb-Mar) (Season 2). P values in bold indicate significant effects (P<0.05).

	df	F	P
Season 1			
Time	1,6	2.101	0.197
Treatment	1,6	0.900	0.379
Time X Treatment	1,6	1.474	0.270
Season 2			
Time	1,4	2.004	0.230
Treatment	1,4	0.043	0.846
Time X Treatment	1,4	4.374	0.105

Table 5. Results of repeated-measures ANOVAs for differences in burrow depth of burrows which remained intact between months and burrows which were lost, assessed independently in Season 1 and Season 2 in grazed and ungrazed treatments, measured before (combined data from Sep-Nov) and during (combined data from Jan-Mar) grazing (Season 1) and early (combined data from Sep-Nov) and late (combined data from Jan-Mar) (Season 2). P values in bold indicate significant effects (P<0.05).

	df	F	P
Season 1			
Month	1,10	3.273	0.101
Status	1,10	50.402	**<0.001**
Treatment	1,10	0.024	0.881
Time X status	1,10	6.918	**0.025**
Time Xtreatment	1,10	0.012	0.915
Status Xtreatment	1,10	0.105	0.753
Time X Status X Treatment	1,10	0.309	0.590
Season 2			
Time	1,4	3.733	0.126
Status	1,4	40.728	**0.003**
Treatment	1,4	0.245	0.647
Time X Status	1,4	0.311	0.607
Time X Treatment	1,4	1.035	0.367
Status X Treatment	1,4	0.173	0.699
Month X Statust X Treatment	1,4	0.684	0.455

Table 6. Results of repeated-measures ANOVAs for differences in proportion of shallow and deep burrows remaining intact between times assessed independently in Season 1 and Season 2, in grazed and ungrazed treatments. Measurements made before (proportion of burrows in Sep that remained in Nov) and during (proportion of burrows in Jan that remained in Mar) grazing (Season 1) and early (proportion of burrows in Sep that remained in Nov) and late (proportion of burrows in Jan that remained in Mar) (Season 2). P values in bold indicate significant effects (P<0.05).

	df	F	P
Season 1			
Time	1,10	2.235	0.166
Depth	1,10	11.080	**0.008**
Treatment	1,10	13.33	**0.004**
Interaction Time X Depth	1,10	3.724	0.082
Interaction Time Xtreatment	1,10	3.198	0.104
Interaction Depth Xtreatment	1,10	0.112	0.744
Interaction Time X Depth X Treatment	1,10	12.607	**0.005**
Season 2			
Time	1,4	0.387	0.567
Depth	1,4	20.752	**0.010**
Treatment	1,4	0.909	0.394
Interaction Time X Depth	1,4	1.025	0.369
Interaction Time X Treatment	1,4	3.643	0.129
Interaction Depth X Treatment	1,4	7.683	**0.050**
Interaction Time X Depth X Treatment	1,4	0.085	0.785

Figures

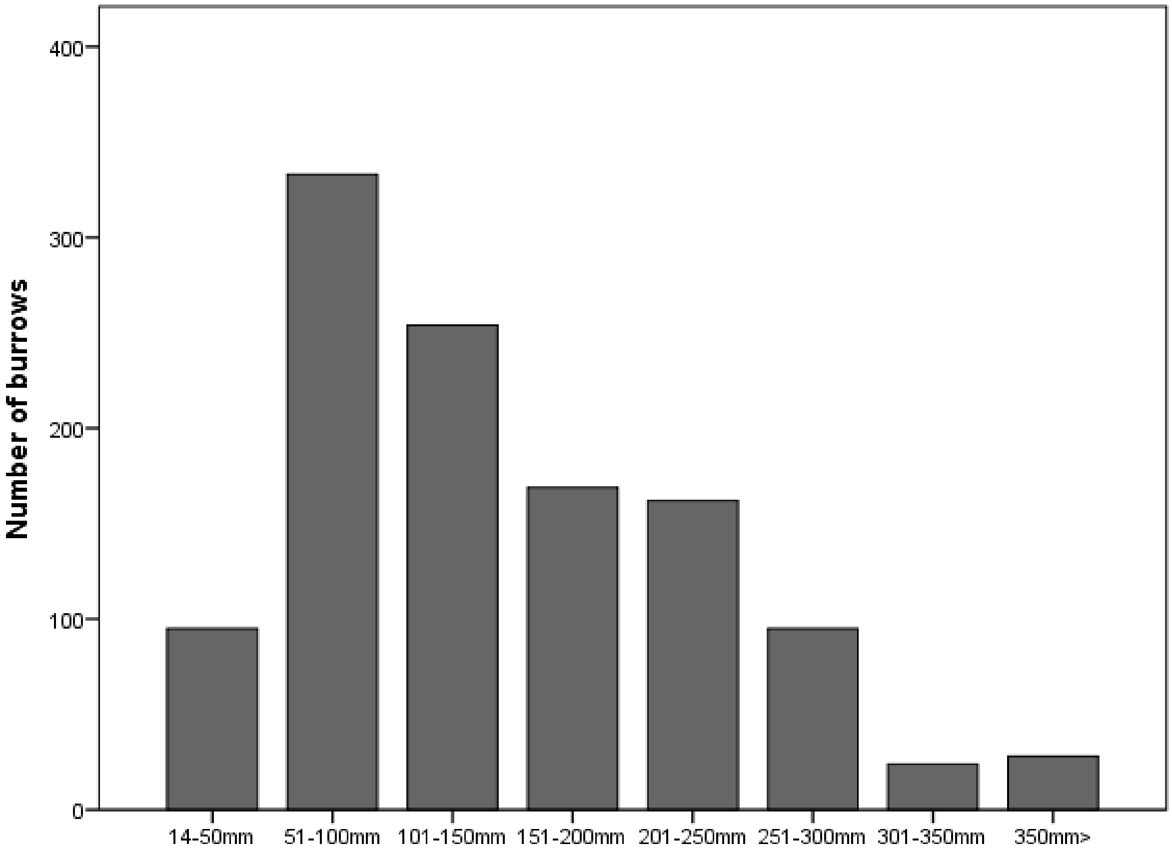
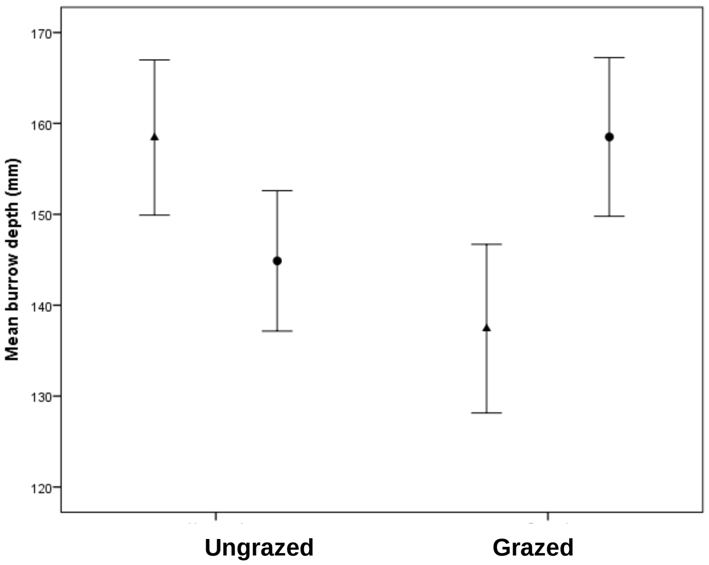
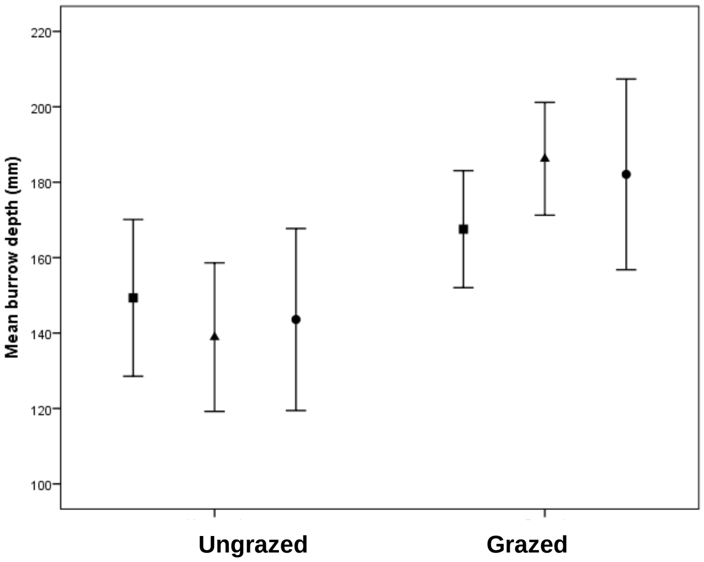


Figure 1. The frequency distribution (with 50 mm increments) of depths of all established spider burrows recorded in September and January of both field seasons.

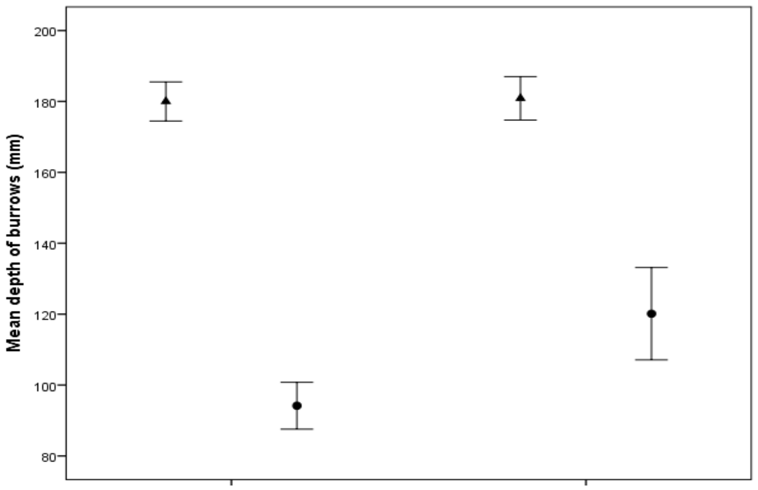


b) Season 1

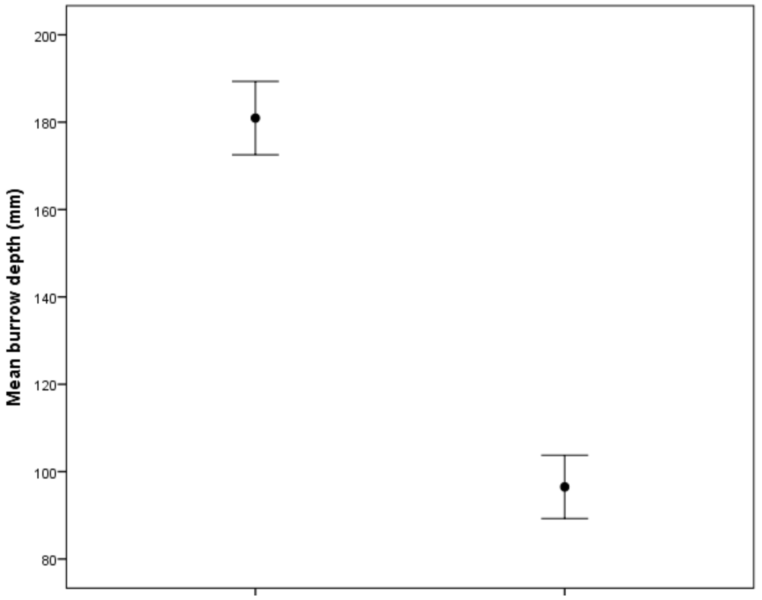


a) Season 2

Figure 2. Mean (+/- 1 SE) depth of established burrows (mm) in grazed and ungrazed treatment plots (a) before (Nov =▲) and after (March = ●) grazing was applied in season 1; and (b) in the last three months of season 2 (Jan = ■; Feb = ▲; and Mar = ●).

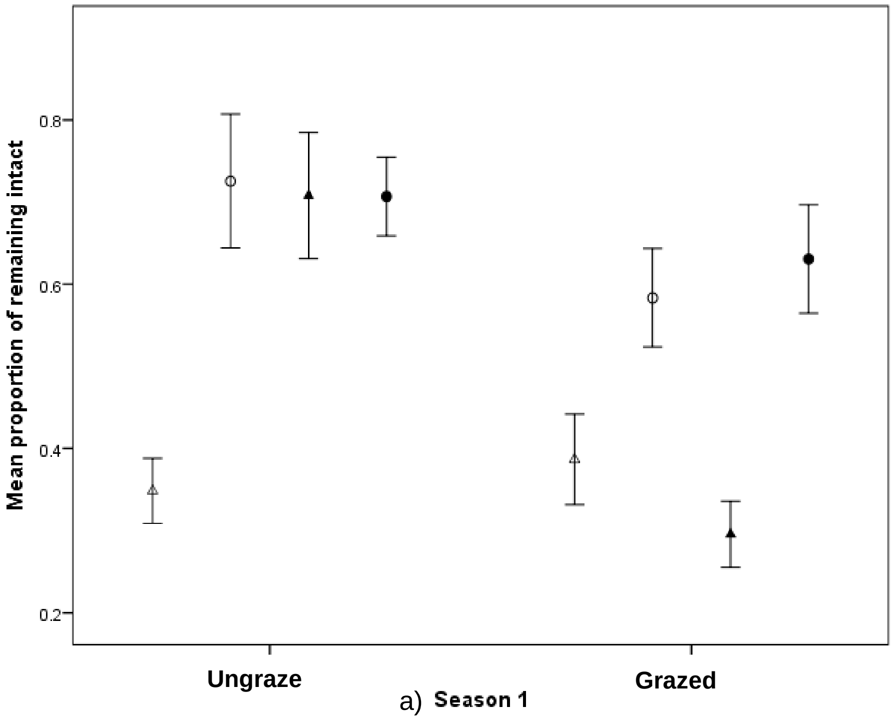


a) Season 1 **Before grazing** **After grazing**

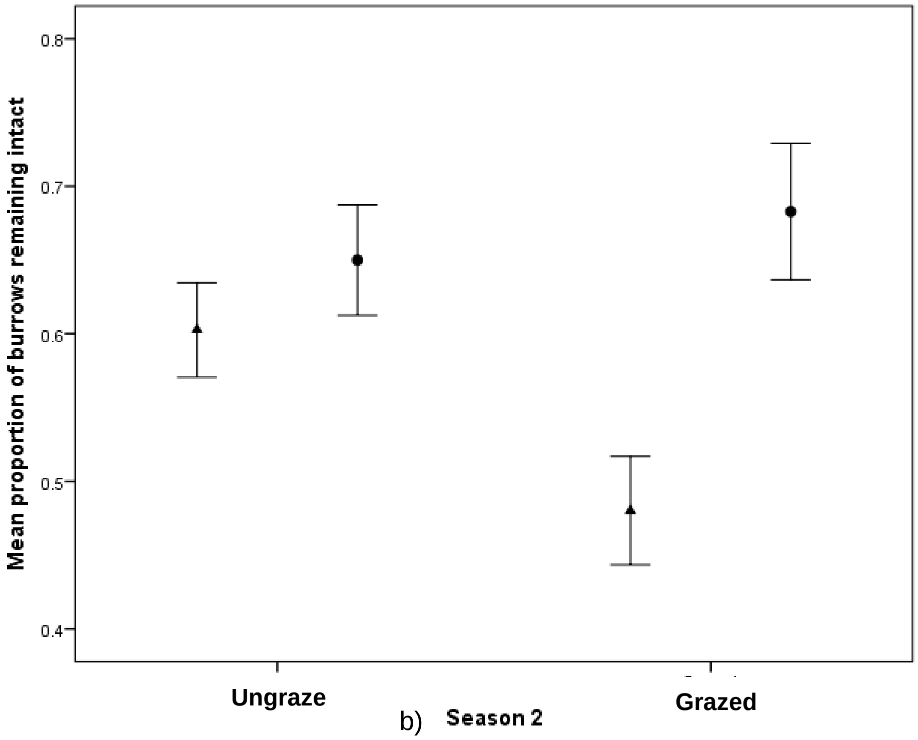


b) Season 2 **Remained intact** **Lost**

Figure 3. Mean (+/- 1 SE) burrow depth (mm) of intact (▲) and lost (●) burrows in grazed and ungrazed treatment plots (a) before (Sep-Nov) and after (Jan-Mar) grazing was applied in season 1; and (b) and between status (intact and lost) in the last three months of season 2(Jan-Mar).



a) Season 1



b) Season 2

Fig 4. Mean (+/- 1 SE) proportion of shallow (less than 150mm) and deep (150mm or more) burrows remaining intact in ungrazed and grazed treatment plots (a) before (shallow = △, deep = ○) and after (shallow = ▲, deep = ●) grazing was applied in season 1 (Sep-Nov = before, Jan-Mar = after); and (b) early and late in the lizard season in season 2 (Sep-Nov = early, Jan-Mar = late) (shallow = ▲, deep = ●).

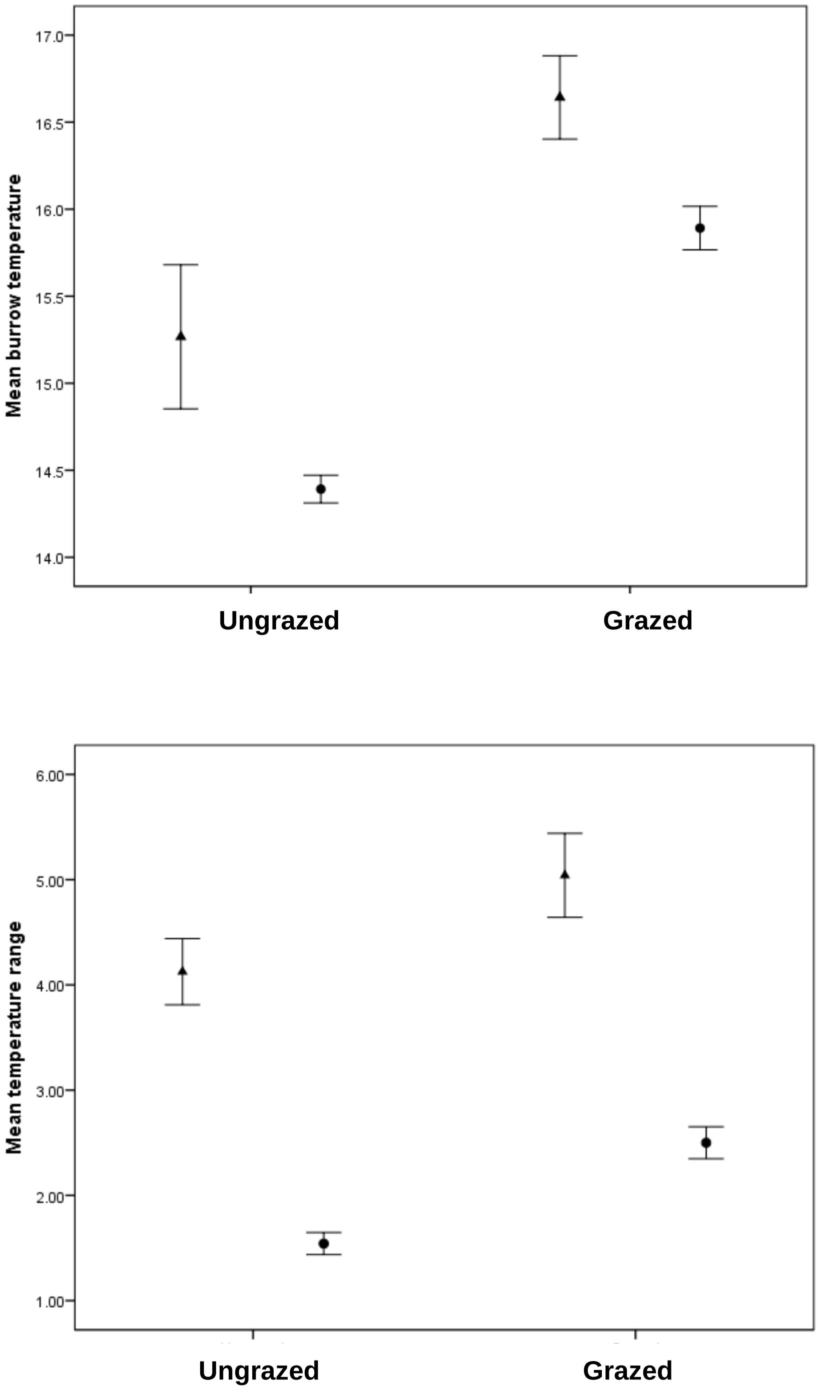


Figure 5. Mean (+/- 1 SE) (a) temperature (degrees Celsius) of burrows, and (b) temperature range (degrees Celsius) of burrows at 100mm (▲) and 200mm (●) depths (recorded at 12pm on three consecutive days).

Appendix 1. Results of repeated-measures ANOVA for the number of spider burrows and the number of lizard burrows per plot between months in season 2 (Sep – Mar).

The P value in bold indicates a significant effect (P<0.05)

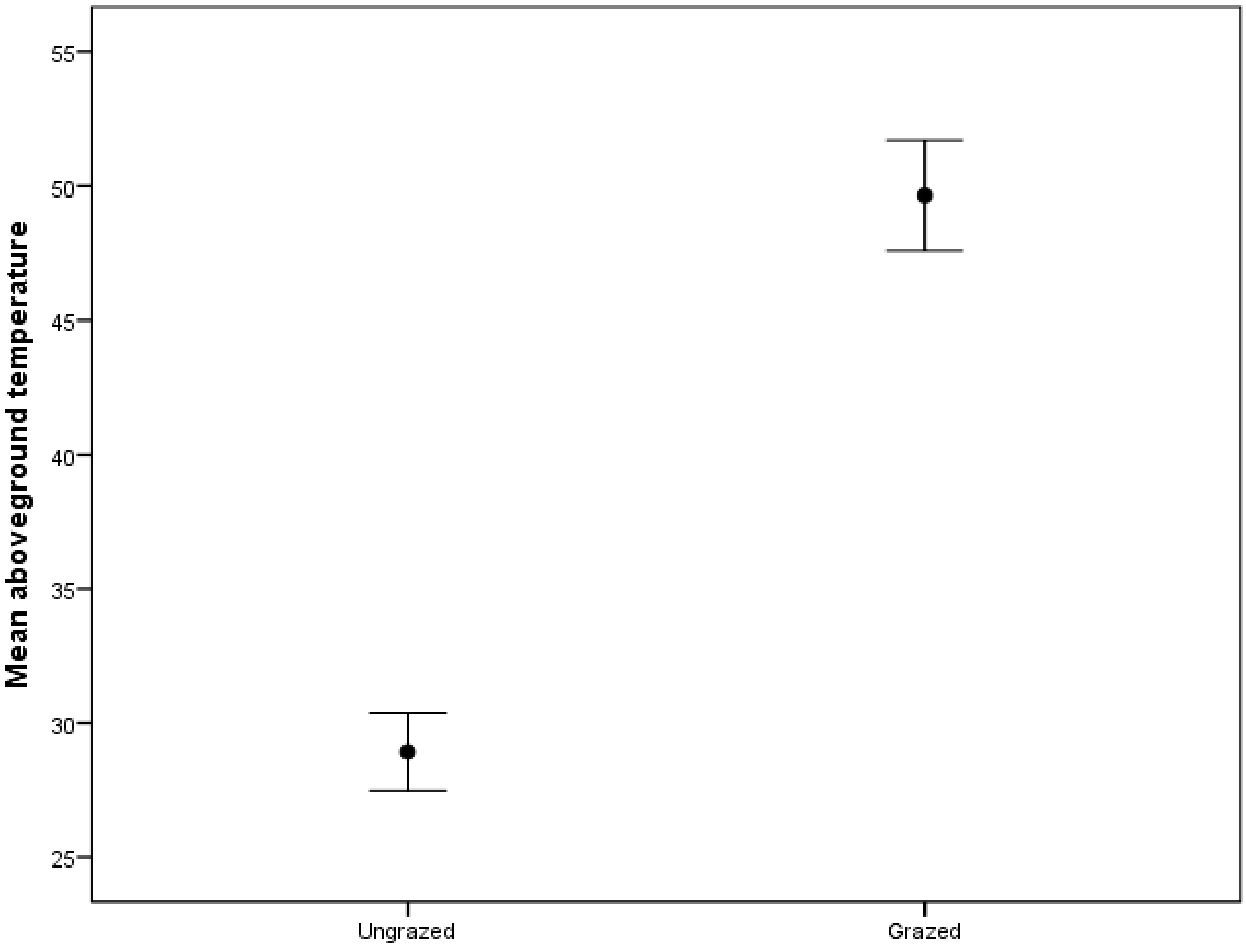
	F (P) ; df = 6,24	
	Total Burrows	Pygmy Bluetongue Lizard
Time	0.486 (0.562)	1.191 (0.354)
Treatment	0.134 (0.733)	0.438 (0.544)
Time x Treatment	0.469 (0.570)	1.631 (0.248)

Appendix 2. Mean (of mean per plot) and range of the change in new burrow depth between months for season 1 and season 2.

Month	Season 1	Season 2
Oct-Nov	-7.14 (range= -19.69 to +21.33)	
Nov-Dec	n/a	-4.00 (range = -17.75 to +20.67)
Dec-Jan	n/a	1.81(range= -48 to -33.33)
Jan-Feb	-12.87 (range=-22.95 to -2.8)	-0.042(range= -12 to +13.5)
Feb-Mar	-16.10 (range= -43.8 to -4.61)	2.46 (range= -5.5 to +7.33)

*** A negative value indicates that a burrow has become shallower from the first month to the second*

Appendix 3. Mean (+/- 1SE) ground surface temperature (degrees Celsius) recorded at 12pm over 3 days in ungrazed and grazed treatments.



Chapter 4

Sheep grazing results in lower reproduction of burrowing spiders in native grasslands of South Australia

A female wolf spider carrying her spiderlings

Chapter Preface

In Chapter 2 and 3, I identified the effects of grazing on spider burrows. I showed that grazing had a negative impact on burrow persistence and that shallow burrows were being destroyed under these grazing pressures. While the retention of existing burrows is crucial to survival of the current populations of spiders and lizards, recruitment of future spider populations will also be critical. In this chapter, I investigate one aspect of sustaining spider populations – reproductive output. I aimed to identify the effects of sheep grazing on reproductive output of wolf and trapdoor spiders at the Tiliqua Reserve, thus increasing our knowledge of indirect impacts on pygmy bluetongue lizards.

Sheep grazing results in lower reproduction of burrowing spiders in native grasslands of South Australia

Jessica Clayton, Prof Michael C. Bull, Dr Mark Hutchinson, Dr Aaron Fenner, Assoc Professor Michael Gardner

In preparation for submission

Abstract

Burrowing spiders are cryptic, yet important species within an ecosystem. Through their burrow engineering, they have the potential to provide habitat to other species. In native grasslands of South Australia, wolf and trapdoor spiders dig burrows which the endangered pygmy bluetongue lizard obligatorily occupies. Lizards are reliant on this burrow resource, therefore retention and recruitment of suitable burrows is critical to conservation of the lizard species. We aimed to investigate the effects of sheep grazing on reproductive output of wolf and trapdoor spiders in these grasslands. We monitored 12 30 x 30m plots monthly, over two austral spring/summer periods (Sep-Mar, 2012-2014), recording the abundance of wolf and trapdoor spiders and the number of these with egg sacs. Our results indicate that sheep grazing may have a negative impact on spiders, through a decline in their reproductive output. This effect of grazing appears to be dependent on timing of grazing. Future research into grazing regimes which reduce this impact of grazing on reproductive output of spiders will be critical for maintenance of spider populations in these grasslands.

Introduction

Grassland habitats are perhaps one of the most exploited and destroyed habitat types in the world. Less than 5% of native grassland habitat remains extant in South Australia, and much of this habitat is heavily fragmented and inappropriately grazed (Hyde, 1995). This combination of factors has had drastic impacts on the fauna and flora that are native to these habitats. The impacts of grazing on native grassland habitats have been well documented (Jansen et al. 2013; Naeth et al. 1990; Pucheta et al. 1998; Souter and Milne 2009; Tracy and Frank 1998). Numerous studies have reported a decline of species diversity and/or altered species richness associated with heavy grazing for both flora and fauna species (Bell et al. 2001; Leonard and Kirkpatrick 2004; Teague et al. 2011; Torre et al. 2007). Alternatively, grazing can result in increased abundance or diversity of species (Collins et al. 1998; Loe et al. 2007). Grazing therefore has the potential to be beneficial or detrimental to maintenance of a habitat dependent on the type of grazing (organism), regime (frequency and length), and environmental factors (e.g. rainfall, temperature, vegetation types) (Bilotta et al. 2007; Fuhlendorf et al. 2006; Milchunas and Lauenroth 1993; Pykälä 2003).

Native grassland habitats consist of a mosaic of vegetation with open patches of bare ground and lichen crusts. Invasive plants alter this structure by filling gaps of bare ground and increasing biomass. Grazing has the potential to maintain this mosaic structure to some extent through a reduction in biomass (Austrheim and Eriksson 2001; Belsky 1992; Cipriotti and Aguiar 2005; van der Maarel and Titlyanova 1989). On the contrary, if intensive, grazing may also lead to the break-up of this lichen crust, thus destruction of the grassland habitat (Belnap and Eldridge 2001; Eldridge and Greene 1994). These impacts have flow-on effects to fauna and flora within the ecosystem (Bardgett et al. 1998; Den Herder et al. 2003; Socher et al. 2012). It is therefore critical for grazing within grassland habitats to be managed in an appropriate manner for maintenance of the grassland structure and species diversity.

One particular group of organisms which have been understudied in grassland ecosystems are spiders. Burrowing spiders in grasslands provide an ecosystem engineering service to other species through their creation of burrows (Fellows et al. 2009; Jones et al. 1994; Milne et al. 2003). Previous studies have had mixed findings in regards to the impact of grazing on spiders. Many studies of grassland spiders have reported reduced abundance in intensively grazed habitats, and a change of spider diversity associated with changes to land use (Bell et al. 2001; Churchill and Ludwig 2004; Szinetár and Samu 2012). Alternatively, some studies have reported no effect of grazing on spider assemblages (Batáry et al. 2008; Jansen et al. 2013; Samu et al. 2010). While some spider species appear to be sensitive to grazing disturbance, some species appear to colonise grazed sites. Spiders capable of large dispersal distances, such as spiders with aeronautical capabilities may be less impacted by grazing practices as they are capable of dispersing in and out of the habitat with each cohort of spiderlings, thus can reinhabit habitats which are heavily disturbed rapidly (Duffey 1998; Meijer 1977; Weyman et al. 2002). Most studies discussing grazing and spiders examine web building and vegetation dwelling spiders. To date there have been very few studies on the effects of grazing on burrowing spider fauna, a group likely to be vulnerable to grazing pressure due to many species having low dispersal capabilities and their reliance on soil stability for burrow maintenance.

Recently, Rix et al. (2017) documented the widespread decline of trapdoor spiders in Southern Australia. The decline was largely attributed to changed and inappropriate land use practices since European settlement, and they also suggested that remaining populations may be experiencing contemporary population declines. Not only do spider declines impact on the spider species itself, but on other species which compete with spiders for resources, provide resources to spiders or are predated on by spiders (Dennis 2003).

In grasslands of South Australia, wolf and trapdoor spiders provide an ecosystem engineering service to other organisms within the grassland. Pygmy bluetongue lizards are endangered lizards which exclusively occupy burrows dug by these spiders (Fellows et al. 2009; Hutchinson et al. 1994; Milne et al. 2003; Milne 1999). For these lizards to persist in the wild there must be ongoing recruitment of wolf and trapdoor spiders in order to provide a continual supply of burrows.

One activity which threatens to impact survival of both spiders and lizards in these grasslands is sheep grazing. Native grasslands of South Australia are almost all grazed by sheep, and to date there have been no studies investigating how sheep grazing affects reproductive success in these burrowing spiders. Previous research has shown that sheep grazing can result in the destruction of shallow spider burrows, likely to be dug by wolf spiders (Clayton and Bull, 2015), and can also result in a decline of wolf spiders (Clayton et. al., Thesis Chapter 5). We also know that adult trapdoor spider abundance and adult burrow abundance do not appear to be altered by sheep grazing at this level (Clayton et. al., Thesis Chapter 5).

In this paper, we aimed to investigate the effect of sheep grazing on burrowing spiders in native grasslands of South Australia. We know from previous research that there is a huge variation in reproductive traits and strategies by spiders (Eberhard 2004; Herberstein et al. 2011; Huber 2005; Uhl 2000; Uhl et al. 2010). In many spiders, including some wolf and trapdoor spiders, females are able to store sperm to use at a later time (Michalik et al. 2005; Useta et al. 2007). We also know that many temperate spiders appear to mate in the cooler, wetter months, and produce young in the warmer months (Costa and Pérez-Miles 2002; Main 2010; Main 1978). Many spiders, including wolf and trapdoor spiders, will seal their burrows when they have offspring (Aisenberg et al. 2010; Aisenberg et al. 2007; Main 2010; Main 1978) providing a stable microclimate and reducing the risk of disturbance and predation (Main 1978). Wolf spiders may seal their burrow shut when they have offspring, while trapdoor spiders will seal their burrow shut after mating and unseal it when juvenile spiders are ready to disperse (Main 1957; Main 1978).

Wolf spiders and trapdoor spiders have vastly different life histories. Wolf spiders are short-lived compared to trapdoor spiders, with many wolf spiders typically having annual or biannual life cycles, compared to lifespans of over 20 years for many trapdoor spider species (Framenau 1997; Humphreys 1976; Main 1976). Most wolf spiders, like trapdoors, are sit-and-wait predators, but they are also more likely to move from their burrow. Some species have been observed to leave their burrow to hunt (Cady 1983; Kovac and Mackay 2007; Ward and Humphreys 1981). They are also more likely to rebuild a burrow if disturbed at their burrow or if unable to capture sufficient prey, and some species potentially inhabit vacated burrows dug by other spiders (McCullough 2000). Trapdoor spiders on the other hand will occupy the same burrow for their lifetime, enlarging this burrow as they grow in size (Ward and Humphreys 1981; Main 1957; Main 1976; Main 1978). A commonality between the two groups is that mating appears to correlate with a particular set of environmental conditions. Male spiders within a species will leave their burrow in search of a mate at about the same time, resulting in a mass movement of male spiders (Main 1976; Main 2001). This potentially means that when other detrimental factors are at play during this time, reproductive success of that cohort of spiders may be drastically impacted.

Many spiders, including wolf and trapdoor spiders produce a high number of offspring per capita. There are a wide range of potential factors which are likely to influence recruitment of spiderlings, including the environmental conditions at the time of dispersal (temperature, rainfall, wind etc), availability of resources in the area they disperse to (prey, habitat etc) and abundance of predators. Human-induced impacts from agricultural practices, such as the administering of pesticides, pollutants and the manual disturbance of habtitat through activities such as ploughing and grazing also have the potential to drastically influence the recruitment of spiderlings in a particular area. Although high numbers of spiderlings may be produced by an individual spider, only a fraction of them are likely to reach adulthood due largely to the numerous threats detailed above.

We hypothesise that sheep grazing will result in a decline of reproduction in both spider groups. We predicted that both spider groups would be negatively impacted by sheep grazing but the effects will be greater for wolf spiders. We have previously reported that shallow burrows, likely dug by wolf spiders are more likely to be destroyed in grazed habitat (Clayton and Bull 2015; Clayton and Bull 2017), thus we expected a greater impact of grazing on wolf spider reproductive output compared to trapdoor spiders We also predicted that time of grazing would have an effect on spider reproductive output. Where grazing had been implemented for a short time, in late summer, we expected to see less impact on reproduction compared to a grazing regime implemented over a long period of time, encompassing all seasons. This is because spiders are likely to mate earlier than they produce egg sacs, therefore, a grazing regime which is introduced closer to the time of egg sac production is likely to impact on fewer stages of spider reproduction.

Methods

The study was conducted at the Tiliqua Reserve in the Mid North of South Australia. It is a 70 ha property of the Nature Foundation of South Australia (33°42′S, 138°56′E). The site is a semi-arid native grassland invaded by exotic weeds (Clayton and Bull 2015; Milne 1999a; Souter et al. 2007). Surveys occurred over two survey seasons, during the austral spring and summer period from Sept – March, of 2012/2013 (season 1) and 2013/2014 (season 2) (Clayton and Bull, 2017). The area has hot, dry summers (average max January temp of 31^{O}C) and cool winters (average max July temp of 12.8^{O}C). During the period 1961 – 2014 the average annual rainfall at Burra, approximately 8km from the study site, was 431 mm, with the majority of rain falling in the winter months (Bureau of Meteorology, 2014). Rainfall during the survey season differed between years, with 89.0mm in season 1 and 217.8mm in season 2 (Clayton and Bull, 2017).

At the study site, two major groups of spiders were found; wolf spiders (Lycosidae) and trapdoor spiders (Mygalomorphae) each with two main species. The main wolf spider species were Tasmanicosa ramosa and T. gilberta, while the main trapdoor spiders were Blakistonia aurea and Aganippe substritis (family: Idiopidae). For this paper we did not differentiate between spider species within each of these spider groups.

In each of the two seasons, we systematically searched each survey plot for spider burrows once each month from Sept – March (except Dec 2012). We searched each plot along 30, 1 m wide transects to locate burrows and used an optic fiberscope (Medit Inc 2 way articulating FI Fiberscope) to inspect each unsealed spider burrow for occupants as in Milne and Bull (2000). We also recorded when burrows contained spiders with egg sacs (for wolf spiders only as trapdoor spider burrows were firmly sealed shut when they had egg sacs) or spiderlings (for both wolf and trapdoor spiders). We also recorded when trapdoor spider burrows were sealed shut (and were not inspected).

Sheep grazing was conducted in six of twelve plots, from January to March of season 1 (4 sheep per hectare), and in three of six plots from April prior to field surveys through to the end of season 2 (March) (5 sheep per hectare) (Table 1).

Analysis

We defined spider reproductive output by the presence of an egg sac (for wolf spiders) or spiderlings (for wolf spiders and trapdoor spiders) in a spider burrow. We inspected wolf spider burrows, regardless of whether they were sealed or not as it was easy to replace the plug produced by wolf spiders. For trapdoor spiders, we only inspected burrows which were unsealed, thus, did not observe trapdoor spiders with egg sacs, but with spiderlings only.

To confirm that the data we were using were representative of patterns of reproductive output at the study site, we first wanted to determine whether there was an effect of time of season (early, late) or of grazing treatment (ungrazed, grazed) on the abundance of shut trapdoor spider burrows. This analysis allowed us to determine whether there was a difference in the number of these burrows between treatments, and so allowed us to determine if the subset of burrows that we were able to access was the same between treatments.

To compare reproductive output between the two groups of spiders, we calculated the number of each type of spider per plot for each month of the study, and the number of spiders that showed evidence of reproduction (i.e. had an egg sac or spiderlings in the burrow). From this we calculated the proportion of spiders in each plot/month which were reproductive. We then calculated the mean proportion of reproductive spiders early (Sep-Nov) and late (Jan-Mar) in each plot in each season. We used arcsin transformation, then repeated-measures ANOVA on the proportion of spiders exhibiting reproductive output with time (early, late), treatment (grazed, ungrazed) and spider type (wolf, trapdoor) as within-subjects factors.

Results

There was a significant effect of time on when trapdoor spiders sealed their burrows shut but there were no effects of treatment indicating that trapdoor spiders will seal their burrows at a certain time of year, irrespective of grazing treatment (Table 2) (mean sealed trapdoor burrows per month = 38.58, with a range from 0-108). There were significantly more trapdoor spider burrows sealed in plots at the end of the field season compared to early in the season (Fig 1).

There was a significant difference in the time of season that spiders have offspring, with more spiders having offspring late in both seasons (Table 2, Fig 2). In season 1, where grazing was not introduced until late in the season, there was no effect of treatment on reproductive behaviour. Contrastingly, in season 2 there was a significant effect of treatment, and an interaction of time and treatment on spider reproductive behaviour (Table 3). A higher proportion of spiders showed reproductive behaviour in ungrazed plots compared to grazed plots late in the season (Fig 3).

Discussion

Sheep grazing has the potential to be beneficial or detrimental to burrow digging spiders and lizards which inhabit their burrows. Our study resulted in a reduction in reproductive output of spiders exposed to sheep grazing, indicating that grazing may therefore pose threats to spider populations and other burrow occupants in the long-term. Well managed timing of grazing is paramount to the minimisation of these potential negative impacts on burrowing spiders.

We predicted that wolf spider and trapdoor spider reproductive output would be differentially impacted by grazing, and that the effects would also differ dependent on the timing and duration of sheep grazing. Our results do not entirely support our hypotheses. We expected to see more effect of grazing on wolf spider reproductive output, however we found that grazing had a significant impact on both wolf and trapdoor spider reproductive output and there was no difference between the spider groups. Sheep grazing resulted in a decline of reproductive output in both spider groups.

We found no significant effect of grazing on reproductive output in the first year of the study which had a short grazing regime implemented in late summer. Our second season of grazing, which was carried out from autumn prior to the season and right through the season resulted in a significantly lower proportion of spiders showing reproductive output in grazed compared to ungrazed plots.

Our results supported our hypothesis that sheep grazing would have a greater impact in our second season, when sheep grazing commenced during autumn prior to our field surveys, and continued throughout all surveys. We expected to see less impact in the first season compared to our second. Our reasoning was that mating would have already taken place prior to the introduction of sheep in the first season; however in the second season grazing impact would be consistent throughout mating and production of egg sacs.

A likely explanation for why we did not see a differential impact of grazing on each spider group is that the main effect of grazing on reproductive output occurs out of the burrow. Male wolf and trapdoor spiders leave their burrows in search of mates, and this occurs over a short time period, meaning that males from a particular cohort of spiders will be searching for mates at similar times. Grazing has the potential to disrupt this behaviour as there will likely be increased disturbance and decreased vegetation/refuge in grazed habitat compared to ungrazed habitat (Austrheim and Eriksson 2001; Belsky 1992; Cipriotti and Aguiar 2005; van der Maarel and Titlyanova 1989). We would expect to see a greater decline of reproductive output observed in wolf spiders compared to trapdoor spiders if, as we predicted, our observed decline of reproductive output was related to burrow stability. This however was not the case. Other factors likely to be influenced by grazing impact which may result in a decline in reproductive output include increased predation risk due to decreased vegetation, increased soil compaction, thus, increased water run-off, and a reduction of prey items leading to reduced ability for spiders to invest in reproduction (Batáry et al. 2008; Dennis et al. 1998; Jansen et al. 2013; Maelfait and Hendrickx 1997). Further research needs to be conducted to identify which key factors have influenced these effects on spider reproductive output.

While our results show that spider reproductive output may be reduced by sheep grazing if grazing is implemented during times when male spiders are searching for mates, we have not assessed whether this observed decline in reproductive output has resulted in a decline of spider recruitment. Further research into the longer term impacts of this observed decline in reproductive output on recruitment will be essential to inform future conservation efforts of both the pygmy bluetongue lizard and spider groups.

Although in the current study it appears that grazing has likely had an effect on spider reproductive output through disturbing their activity outside of burrows, the potential influence of burrow destruction on spider persistence within a particular habitat cannot be completely dismissed and should be further explored. For example, Long-term, if wolf spiders are at greater risk of having their burrows destroyed, and this pressure is repeated regularly, it may lead to a decline in spider populations great enough to then impact survival of those populations within the ecosystem. Declines of trapdoor spiders reported by Rix et al. (2017) reveal a strong connection between decline and agricultural land use. The persistence of burrow dwelling invertebrates in the future will be dependent upon suitable grazing regimes as this study, and previous research highlight the direct and indirect effects of grazing on spider persistence within these grasslands (Clayton and Bull, 2015, Clayton and Bull, 2017).

The observed impacts of grazing on spider reproductive output in our study may have large implications for wolf and trapdoor spiders, as well as other inhabitants of spider engineered burrows and of native grasslands. A negative effect of grazing on reproductive output may in time result in an overall decline of spiders. Depleted spider populations will lead in a decline of burrows, impacting a wide range of burrow-utilising species. Spiders act as both a predator for some species and a prey to others, thus spider declines also have the potential to drastically alter food webs for many organisms (Barton and Schmitz 2009; Carter and Rypstra 1995; Cronin et al. 2004).

The individual life-histories of each spider group means that they are likely to be differentially impacted by declines of reproductive output over time. The long-lived trapdoor spiders may have more opportunity to recruit new cohorts if grazing pressure varies from year to year. On the other hand, if grazing pressure persists for an extended period of time, the limited ability of trapdoor spiders to disperse means that it will be a very long time before this spider group are able to recolonise areas of localised extinction if they are unable to produce sufficient offspring to maintain population growth and stability. On the other hand, wolf spiders have shorter lives and are more mobile and thus more likely to be impacted over a short time period. However, wolf spiders have a greater potential of recolonising areas of localised extinction so have greater potential to recolonise an area than trapdoor spiders (Duffey 1998; Öberg and Ekbom 2006; Main 1957; Main 2001).

In these grassland ecosystems, endangered pygmy bluetongue lizards exclusively utilise spider burrows as refuges, basking sites and ambush points (Milne et al. 2003). As they cannot dig their own burrow, a decline in spider reproductive output may impact habitat availability to lizards if it results in a decline in spiderlings reaching adulthood. Grassland habitats need to be managed in a way that supports maintenance of the mosaic structure of the grassland, without resulting in reduced reproductive output and burrow destruction (Clayton and Bull, 2015). By reducing grazing at times of year when spiders are likely to be mating, the impact of grazing on reproductive behaviour in spiders may potentially be reduced. Future research identifying key influences on spider mating, and using modelling software to form predictions about potential influences on spider mating would be beneficial for informing farmers ahead of time about times of year to reduce grazing pressure in grassland ecosystems. This study also adds to the growing evidence of the negative impacts we as a species are having on a group of understudied and undervalued cryptic invertebrate species. The importance of these ecosystem engineers in increasingly disturbed habitats is crucial to persistence of numerous species. Not only will organisms which utilise these engineered habitats be impacted by declining spider populations, but the broader ecological community as well.

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Tables

Table 1. Grazing treatments at the Tiliqua Reserve over two field seasons from September 2012 – March 2014.

	Season 1						Season 2						
Plot	Sep	Oct	Nov	Jan	Feb	Mar	Sep	Oct	Nov	Dec	Jan	Feb	Mar
1				**Grazed at a rate of 4 sheep per hectare**									
2													
3													
4													
5													
6													
7				**Ungrazed**			**Grazed at a rate of 5 sheep per hectare**						
8							**Ungrazed**						
9							**Grazed at a rate of 5 sheep per hectare**						
10							**Ungrazed**						
11													
12													
13							**Grazed at a rate of 5 sheep per hectare**						

Table 2. The mean number of sealed trapdoor spider burrows analysed using a repeated-measures ANOVA recorded in grazing treatments (early (Sep-Nov) and late (Jan-Mar) in season 1 and season 2, analysed separately (2012-2014)). P values in bold indicate significant effects (0.05).

	df	F	P		df	F	P
Season 1				Season 2			
Time	1,10	11.408	**0.007**	Time	1,4	11.394	**0.028**
Treatment	1,10	0.072	0.794	Treatment	1,4	7.444	0.053
Time X Treatment	1,10	0.102	0.756	Time X Treatment	1,4	4.044	0.115

Table 3. The proportion of each spider type with offspring analysed using a repeated-measures ANOVA, temporally (Sep-Mar over both field seasons (2012-2014), and in grazing treatments (early (Sep-Nov) and late (Jan-Mar) in season 1 and season 2, analysed separately (2012-2014)). P values in bold indicate significant effects (0.05).

	df	F	P		df	F	P
Season 1				Season 2			
Time	1,19	5.192	**0.034**	Time	1,7	32.93	**0.001**
Treatment	1,19	0.130	0.722	Treatment	1,7	7.356	**0.030**
Spider type	1,19	0.035	0.853	Spider type	1,7	0.643	0.449
Time X Treatment	1,19	0.104	0.751	Time X Treatment	1,7	12.42	**0.010**
Time X Spider type	1,19	0.333	0.570	Time X Spider type	1,7	0.005	0.944
Treatment X Spider type	1,19	0.488	0.493	Treatment X Spider type	1,7	0.405	0.545
Time X Treatment X Spider type	1,19	0.331	0.572	Time X Treatment X Spider type	1,7	1.729	0.230

Figures

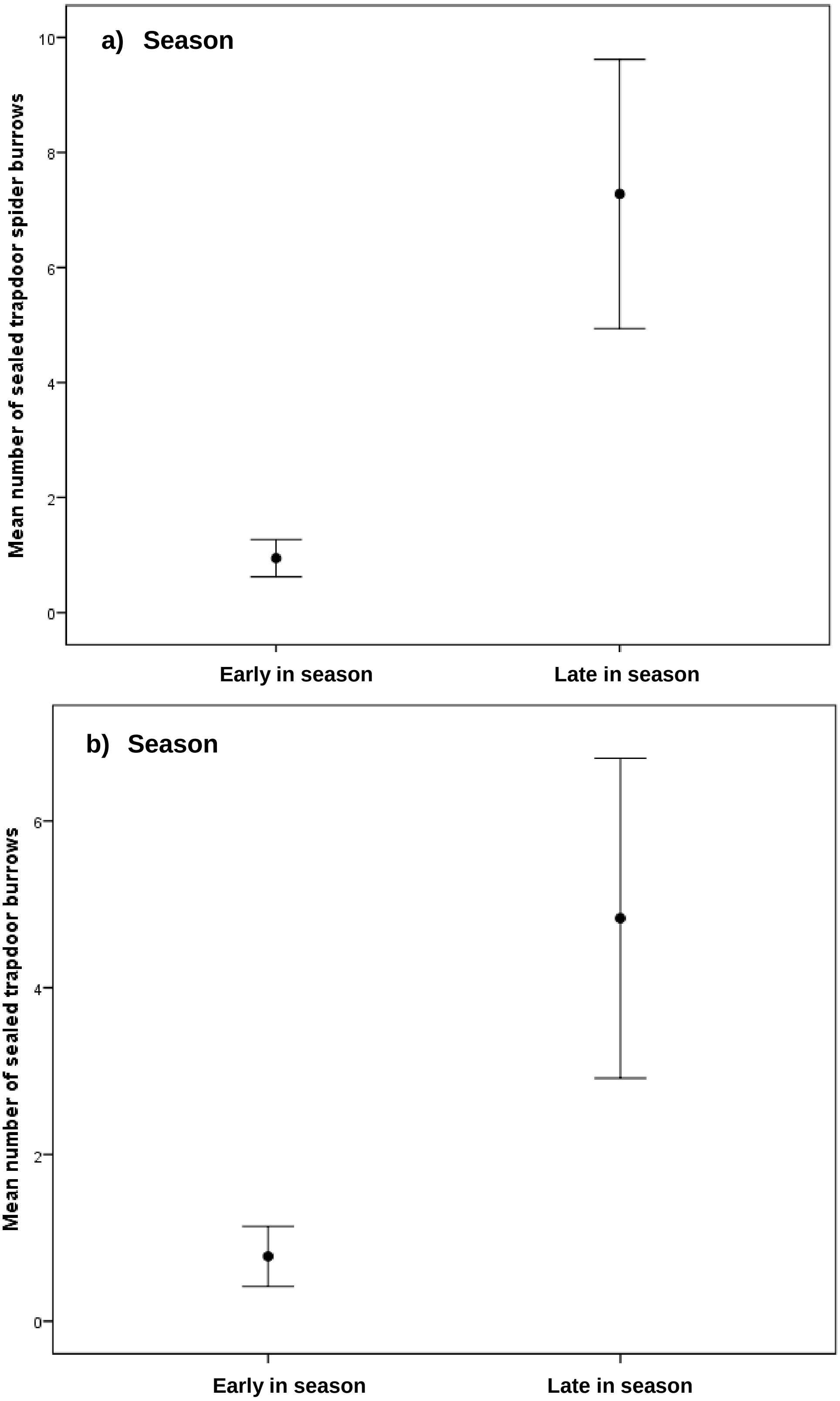
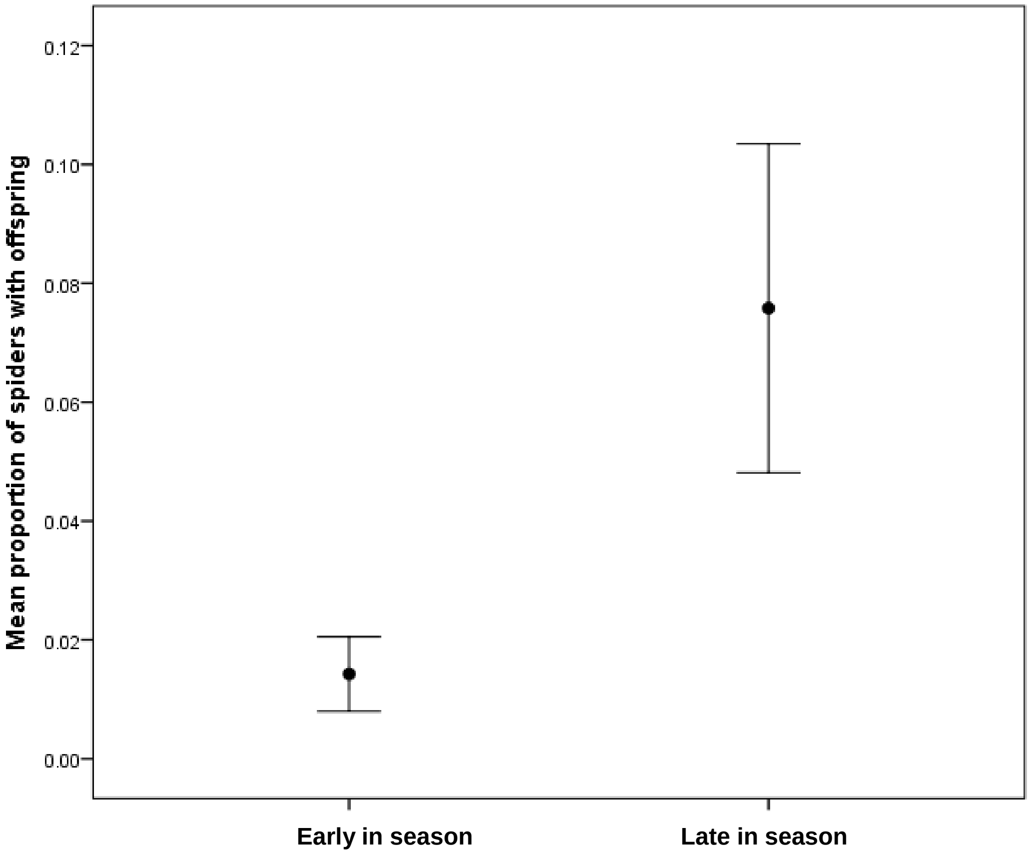


Figure 1. Mean (+/- 1 SE) number of trapdoor spider burrows that were sealed shut, early (Sep-Nov) and late (Jan-Mar) in (a) season 1 and (b) season 2.



Season 1

Figure 2. Mean (+/- 1SE) proportion of wolf and trapdoor spiders with offspring early (Sep-Nov) and late (Jan-Mar) in season 1.

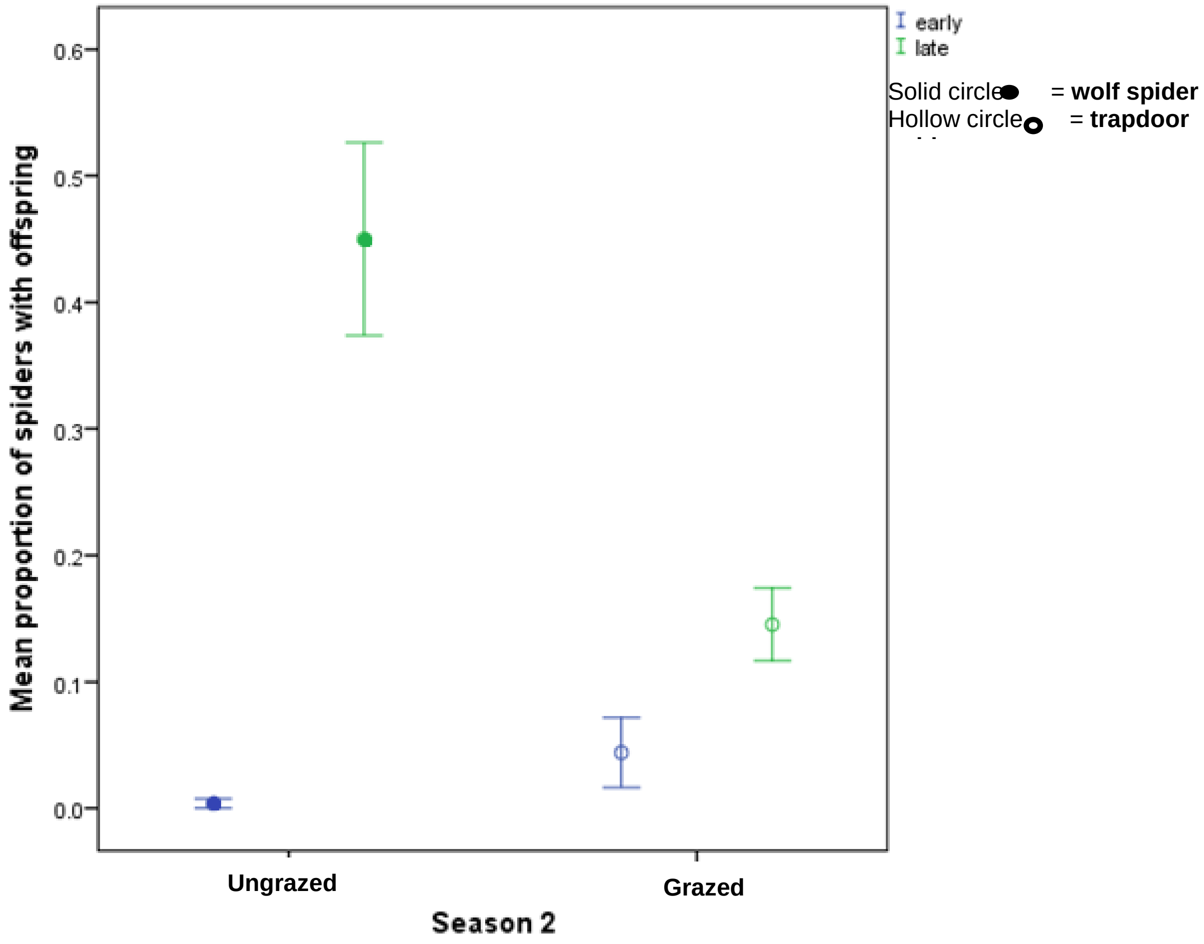


Figure 3. Mean (+/- 1SE) proportion of wolf and trapdoor spiders with offspring in grazed and ungrazed plots early (Sep-Nov) and late (Jan-Mar) in season 2.

Chapter 5

Differential response to disturbance and niche partitioning in temperate grasslands

A pygmy bluetongue lizard in its burrow

Chapter Preface

In my previous chapters, I have established that grazing has multiple impacts on spiders and their burrows. Grazing results in the destruction of shallow burrows, and I also found that lizards appear to be occupying the deeper burrows within their habitat. As lizards and spiders both require a burrow refuge for survival, a decline in burrow abundance has the potential to increase competition for burrows. Previous research has shown that spiders and lizards may cause fatality to one another. Thus, an understanding of how lizards coexist with ecosystem engineering spiders is crucial to interpreting how burrow abundances are likely to impact lizards. This chapter investigates whether lizards exhibit niche partitioning in order to coexist with their burrow engineers. I identify how burrow dynamics and spider dynamics change throughout a season in order to determine how spider populations may interact with and impact pygmy bluetongue lizard populations. As grazing is an inevitable factor in native grasslands of South Australia, I also incorporate grazing into this chapter, identifying whether grazing influences persistence of wolf or trapdoor spiders. Long-term conservation of pygmy bluetongue lizards requires long-term conservation of burrowing spider species if it is to be successful.

Differential response to disturbance and niche partitioning in temperate grasslands

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In preparation for submission to Ecology

Abstract

Sheep grazed native grasslands of South Australia are home to burrow engineering spiders which play an important role in the persistence of the endangered pygmy bluetongue lizard (Tiliqua adelaidensis). These spiders dig burrows which provide refuges, basking sites and ambush points for the lizards. These lizards obligatorily occupy spider burrows, however, both spiders and lizards pose a potential threat to one another. In order for coexistence of the spider groups and lizards in these grasslands, there is likely differential use, or partitioning of the burrow niche. We aimed to investigate how spiders and lizards utilize burrows and to identify if niche partitioning could provide an explanation for coexistence of spiders and these burrow-dependent lizards. Due to the potential for sheep grazing to also influence this relationship, we also aimed to identify how grazing affects burrows and burrow use. Our results show that lizards display temporal and fine-scale spatial niche partitioning in order to utilize spider burrows. Lizards move into already vacated burrows and thus reduce the risk of direct interaction with the spiders. Lizards also show preference for burrows dug by trapdoor spiders, which are deeper and less impacted by sheep grazing. Both spider groups were impacted by grazing, with wolf spiders showing a greater decline in grazed habitat, and both spider groups having a lower reproductive output. An understanding of lizard and spider dynamics, and the effects of grazing on burrow use and availability is essential to conservation of the pygmy bluetongue lizard.

Introduction

All organisms have a specific set of requirements for survival, and a large contributor to this is resource availability. Specific resources required by an organism consist of a combination of space (including refuges), food and availability of mates (Johnson 1980). Often, these same resources are required by other individuals and other species, which may result in competition. A species' niche consists of the range of conditions and resources it requires for survival in a particular habitat. Competition for a niche can be inter- or intra-species specific but ultimately results in organisms either persisting together or one out-competing the other (Kuenzler 1958; Mason et al. 2011).

In order for coexistence of species to occur, there often needs to be a difference in how each species utilises resources, or a difference in the competition for resources.

Coexistence of competing species may occur on local and regional scales, within homogenous or heterogeneous environments. Different mechanisms will influence coexistence under each of these scales and conditions. For example, coexistence in a spatially heterogeneous, competitive environment is likely to occur via mechanisms dependent on spatial variation. These mechanisms are most likely to result in local exclusion and regional coexistence rather than local coexistence. This is because one species is inevitably likely to be more competitive than the other, within the constraints of particular biotic and abiotic conditions, and vice versa (Amarasekare 2003).

Niche partitioning is a concept which helps to explain the coexistence of species which utilise a common resource, suggesting that resources may be differentially used at various spatial and temporal scales (Amarasekare 2003; Schuette et al. 2013). Therefore, spatial niche partitioning may occur when multiple species are competing for a limiting resource such as space itself (e.g. sessile organisms, plants, species requiring breeding territories etc.), or, when multiple species compete for resources that are patchily distributed in space, such as food, nutrients etc. (Amarasekare 2003). Spatial niche partitioning has been documented for a range of organisms such as ants, earthworms and ungulates (Albrecht and Gotelli 2001; Jiménez et al. 2006; Stewart et al. 2002). The partitioning of foraging areas by bats via differences in stratification of species within a forest (i.e. understorey and canopy foraging) is one example of how species utilise partitioning of space to coexist (Delaval et al. 2005). There is a huge diversity in the scale that spatial niche partitioning can occur, and this is often dependent on resource availability, requirements and other species which exploit these resources.

Similarly, temporal niche partitioning occurs when a resource is exploited by multiple species but at different times. This may occur on multiple scales, including times of day, as with the use of watering holes by large herbivores at different times of the day (Valeix et al. 2007); and large carnivorous mammals being active at different times within the night (Schuette et al. 2013). It may also occur across a season, for example differential use of food resources by ants at different times of year (Albrecht and Gotelli 2001). Ultimately, niche partitioning results in increased diversity of species within an ecosystem.

A special case of resource availability is ecosystem engineering which occurs when the organisms themselves produce the resources they require. These ecosystem engineered resources can be exploited by other species. There are numerous well documented cases of ecosystem engineering, including dam construction by beavers (Wright et al. 2002), burrow digging by gopher tortoises and burrowing owls (Kinlaw 1999; Machicote et al. 2004; Pike and Mitchell 2013) and mounds built by termites (Dangerfield et al. 1998). Each of these organisms creates habitat through physical changes to the habitat and this in turn provides a new resource to other organisms which would not persist without the presence of the ecosystem engineer. Ecosystem engineered resources are utilised by a wide range of organisms. They can provide shelter and thermal refugia, as seen in the use of gopher tortoise (Gopherus Polyphemus) burrows by over 50 vertebrate and 300 invertebrate species, including gopher frogs (**Rana capito**) and burrowing owls (**Athene cunicularia floridana**) ***(Pike and Mitchell 2013). They can also provide suitable habitat for plant species, for example, the increased plant species richness observed in alligator (***Alligator mississippiensis Daudin) ***holes compared to surrounding marsh habitat (Palmer and Mazzotti 2004).***

Adding further complexity to these interactions is disturbance. Niche partitioning and habitat use may be altered as a result of disturbance. Disturbance may be natural, for example fires, floods and volcano eruptions, or it may be human induced such as mechanical disturbance from agricultural activity (e.g. ploughing), land clearing, or chemical disturbances, for example from pesticides and fertilisers. An increasingly common source of disturbance is anthropogenic activity. Anthropogenic disturbances such as agriculture and urbanization have been reported to alter species niche selection and use in numerous studies (Hadar et al. 1999; Mason et al. 2011).

Disturbance may have positive and negative effects on species, and it may differentially impact species within the same habitat. An example of the complex effects of disturbance on species is the differential response of plants, reptiles and birds to grazing, fertiliser and tree clearing identified in South-Eastern Australia by Dorrough et al. (2012). Here, plant richness was negatively correlated with stocking rates of livestock, while there was no correlation observed for bird abundance or species richness. Reptile abundance was positively correlated with livestock densities and negatively correlated with nutrient enrichment. Similarly, differential responses of species to disturbances have been documented in numerous other studies (McIntyre and Lavorel 1994; Powell 2006; Williams et al. 2010).

It has also been suggested that response to disturbance may differ within a species (Stillman and Goss-Custard 2002). Stillman and Goss-Custard (2002) found that oystercatchers (Haematopus ostralegus) responded less to disturbance when they had a higher risk of starvation, thus, a trade-off between tolerance to disturbance and starvation risk may take place for this species. The complex nature of the responses of different taxa to disturbance means that disturbance may also have different influences on how organisms utilise resources. This may in turn have implications for niche partitioning by different species under disturbance pressure. Further, it may influence the availability of habitat produced by ecosystem engineers if these species are negatively impacted by disturbance. For example, Lenihan and Peterson (1998), reported altered fish and invertebrate abundance in response to a decline in oyster-produced reef.

In this study, we investigated niche partitioning in South Australian native grasslands which are grazed by sheep. This grassland is inhabited by the endangered pygmy bluetongue lizard. The lizard is endemic to, and restricted to, highly fragmented native grasslands in the Mid North region of the state. It obligatorily occupies spider burrows, constructed by wolf and trapdoor spiders. Adult lizards will utilise the same burrow for multiple months and even multiple seasons (Bull et al. 2015). Previously, Schofield et al. (2012) reported that adult lizards were most likely to leave burrows early in the season, and this movement was attributed to male lizards. Neonate lizards were mostly found moving from burrows in late summer, which coincides with dispersal from the natal burrow (Milne et al. 2002; Schofield et al. 2012). Adult and juvenile lizards have also been found to utilise a different range of burrow dimensions, with adults selecting burrows typically deeper than 200-300mm and juveniles typically utilising shallower burrows with dimensions of 100-200mm (Milne and Bull 2000).

Lizards and spiders can each cause fatality to one another, thus coexistence is likely to involve some form of niche partitioning (Ebrahimi and Bull 2012; Milne 1999). This system therefore gives us the opportunity to investigate how coexisting spiders and lizards exploit the same resource and whether disturbance by sheep grazing impacts on resource engineering spiders. Previous research has established that grazing can be both beneficial and detrimental to the lizards and spiders. On one hand, lizards have been reported to benefit from some grazing as it reduces vegetation density, allowing for more basking and prey capture (Pettigrew and Bull 2014). On the other hand, grazing which is too heavy may be detrimental, due to destroying spider burrows (Clayton and Bull 2015; Clayton and Bull 2017). Previous research has shown that shallow spider burrows are most vulnerable to sheep trampling, and that lizards prefer deep spider burrows, but the effect of this loss of shallow burrows has not been further examined (Clayton and Bull 2015; Clayton and Bull 2017). We previously reported (Clayton and Bull, 2015) a significant within season decrease in the mean abundance of wolf spiders per plot (Sep mean = 11.33 (SE 1.54), March mean = 3.83 (SE 1.37)), but no data from this study have been published on the abundance of trapdoor spiders at the study site.

We hypothesise that there will be differential burrow use by ecosystem engineering spiders and lizards, with lizards selecting burrows that are not utilised by the spiders. In using burrows which have been vacated by spiders, lizards will avoid interaction with the spiders, thus avoiding potential harm. We also expect that lizards may show preference for trapdoor spider burrows, which from previous research we expect are deeper than wolf spider burrows (Clayton and Bull 2017; Milne and Bull 2000). Finally, we anticipate that grazing may have differential impacts on each spider group, as spiders may engineer burrows of different stabilities. We expect that wolf spiders will be more impacted by grazing pressure, potentially being in lower abundance in plots grazed by sheep, but similar to our previous findings with lizards (Clayton and Bull, 2015), we expect to see little impact on the abundance of trapdoor spiders.

Methods

Study site characteristics

The study was conducted within a 70 ha site inhabited by pygmy bluetongue lizards, the Tiliqua property of the Nature Foundation of South Australia, near Burra, South Australia (33°42′S, 138°56′E). The site is a semi-arid native grassland partially invaded with exotic weeds (Clayton and Bull 2015; Milne 1999a; Souter et al. 2007). Surveys occurred over two field seasons, during the austral spring and summer period from Sept – March of 2012/2013 (season 1) and 2013/2014 (season 2). The area has hot, dry summers (average maximum temperature in January is 31^{O}C) and cool, moist winters (average maximum temp in July is 12.8^{O}C), and lizards are normally only active during the spring and summer. Over the period 1961 – 2014 the average annual rainfall at Burra, approximately 8km from the study site, was 431 mm, with most rain in the winter months (Bureau of Meteorology, 2014). The seven months Sept – March of the lizard activity season in the current study, were much drier (89.0 mm) in season 1 than in season 2 (217.8 mm).

Grazing treatments

The experimental set up and the different grazing treatments that were applied have been previously described (Clayton and Bull 2015; Clayton and Bull, 2017). The Tiliqua property contained six experimental paddocks that ranged in size from 3.49 – 6.86 ha, arranged in a north-south line along the eastern edge of the property, and a larger paddock to the west. In each individual paddock, a 30m X 30m plot was established. These six plots were spaced 100-200 m apart from each other, and an additional six plots were established in the larger paddock, arranged in a straight line 50 m west of the experimental paddock fence line. From September 2012 we surveyed the plots each month (Sept – March) during the two successive field seasons. In the first season, we surveyed all twelve plots, while in the second season we surveyed only nine plots, six within the individually fenced experimental paddocks and three in the larger paddock. Sheep grazing regimes were applied in each season with guidance from local farmers (See Appendix 1 for grazing regimes applied).

Spiders present and their burrows

Following Clayton and Bull (2015), we defined a spider burrow, independent of any occupants, as a burrow that was deeper than 14 mm and had an entrance diameter of between 6 – 35 mm.

At the site there were two taxonomic groupings of burrow digging spiders, wolf spiders (Lycosidae) and trapdoor spiders (Idiopidae, a family within the suborder Mygalomorphae). Each group has two abundant species that use the burrows of a size that can be used by the lizards, the wolf spiders Tasmanicosa ramosa and Tasmanicosa gilberta, and the trapdoor spiders Blakistonia aurea and Aganippe subtristis. McCullough (2000) suggested that T. gilberta was a burrow stealer rather than a burrow constructer, thus three of these abundant spider species construct and occupy burrows while one is likely to occupy burrows constructed by other spiders. In our analyses we combined data from all species in each group (wolf spider and trapdoor spider).

Trapdoor spiders remain, sometimes for years, in the same burrow (Main 1985). In contrast, wolf spiders are much more mobile, regularly adopting new vacant burrows (Marshall 1995; McCullough 2000; Ward and Humphreys 1981), and in our study were even found on some occasions in abandoned trapdoor spider burrows. To differentiate between trapdoor and wolf spider burrows we used either the presence of a trapdoor spider or the compaction of burrow walls to identify trapdoor spider burrows. All other spider burrows that were intact and clearly identifiable as spider burrows were recorded as wolf spider burrows (Trapdoor spider burrows: mean depth = 218mm, n=1418 records, range = 32mm-680mm; Wolf spider burrows: mean depth = 108mm, n=2383 records, range = 14mm-394mm). Where it was not possible to confidently identify a spider burrow as being constructed by either a wolf spider or a trapdoor spider, it was recorded as an unknown spider burrow and was not used in analysis within this paper (19.24% of 1533 different individual burrows were recorded as unknown) as in Clayton and Bull (2017).

We limited our interpretation of trapdoor spider burrow abundances to only consider declines of burrows rather than declines and increases of burrows. We used these data to compare declines in this burrow group to wolf spider burrows over time and under different experimental treatments. Our reasoning for this was that trapdoor spider burrows were more difficult to detect than wolf spider burrows and therefore we could not be confident that an observed increase in trapdoor spider burrows reflected an actual increase of this burrow type. Trapdoor spider burrows observed in this study all belonged to adult spiders as burrows of small/juvenile spiders were too difficult to detect. Thus reported increases in trapdoor spider burrows during the current study are most likely to be an artefact of changing detectability and also do not reflect recruitment of spiderlings to the area.

Burrow surveys

In each season, we thoroughly searched 12 30 x 30m survey plots (Season 1) and 9 survey plots (Season 2) for all spider burrows we could find, once each month from Sept – March (except Dec 2012). Plots were spaced 80 – 200 m apart from each other within an 800 x 100 m area of the study site. We searched each plot along 30, 1 m wide transects over a period of 7 -10 h to locate as many burrows as possible (range 16 – 102 (SE1.44) burrows per plot in each survey). We then used an optic fiberscope (Medit Inc 2 way articulating FI Fiberscope) to inspect each spider burrow for occupants, burrow type and to measure burrow depth, as in Milne and Bull (2000). In the first survey of each season, we marked the location and identity of each spider burrow with a 300 mm polypropylene tent peg. In subsequent monthly surveys we noted if previous spider burrows were still present, and marked any new spider burrows that had not been detected in the previous month.

Analysis

General statistical methods

In all analyses we compared measured parameters between grazed and ungrazed treatments. We used natural log transformation of data where necessary to ensure they were normally distributed. When using proportions, we used the arcsin transformation of data. In repeated-measures ANOVAs, we used Mauchly's test to determine whether data were spherical, and applied the Greenhouse-Geisser correction where they were not.

Spider burrows

Here, we examined the abundances of wolf spider and trapdoor spider burrows, and how these numbers changed temporally. We calculated the total number of each known burrow type (trapdoor or wolf spider) per plot (8 plots in common in both seasons and excluding Dec in both seasons) for each month of the study period. We then used repeated-measures ANOVA on the mean number of burrows per plot, with season (1, 2) burrow type (wolf, trapdoor) and month (Sep-Mar) as within-subjects factors.

Grazing as a factor in analyses

We previously reported that grazing did not have an effect on lizard abundance in Season 1 (Clayton and Bull, 2015) and observed the same pattern in Season 2 (Appendix 3). We also reported that lizards consistently occupied deep burrows and this did not differ between grazed and ungrazed treatments (Clayton and Bull, 2017). As such, we have included grazing as a factor for spider burrows and burrow use, however we have not included grazing as a factor in our analysis of lizard burrow use.

Burrow use by lizards

We first examined the previous occupancy of burrows and determined whether lizards selected empty burrows or spider occupied burrows. For this analysis we used all of the records from successive pairs of months when a lizard was recorded as a new occupant in the second month. Among burrows occupied by lizards in October we identified those that had no lizard occupant in September. Then we recorded the occupancy status of the burrow in September, as either empty, occupied by a wolf spider, or occupied by a trapdoor spider. We repeated this for each adjacent pair of months in each lizard season. We then combined data for all cases of new lizard occupancy from early (Sep-Nov) or late (Jan – Mar) in each field season. We used repeated-measures ANOVA on the mean (per plot) number of lizards newly occupying a burrow, with previous occupancy (empty, wolf spider, trapdoor spider), time (early, late) and season (1, 2) as within-subjects factors.

We then investigated whether lizards showed a preference for either wolf spider or trapdoor spider burrows. We divided the total number of each burrow type, per plot in each month, into those occupied and those not occupied by a lizard. We then calculated the proportion of each burrow type used by lizards in each plot in each survey, and, after an arcsin transformation of data, we used repeated-measures ANOVA, with season (1, 2) burrow type (wolf, trapdoor) and month (Sep-Mar excluding Dec) as within-subjects factors.

We then omitted any burrow that had a spider resident from these data. We calculated the proportion of each burrow type that were occupied by lizards, and analysed as above. We then analysed each season separately, including only burrow type (wolf, trapdoor) and month (Sep-Mar excluding Dec) as within-subjects factors.

Burrow use by spiders

Here, we examined how wolf and trapdoor spiders use burrows in grazed and ungrazed habitat temporally**.** To compare the overall population dynamics of the two groups of spiders, we first calculated the total number of spiders in each group per plot (12 plots in season 1, 9 plots in season 2) for each month of the study, then we used repeated-measures ANOVA (using 8 plots common in both seasons) with month (Sep-Mar), season (1,2) and spider type (wolf, trapdoor) as within-subjects factors. We then used repeated-measures ANOVA with month (Sep-Mar) and spider type (wolf, trapdoor) as within-subject's factors for each season separately, to include all plots in each season and Dec in season 2.

Next, to determine whether there was an effect of grazing treatment on spider dynamics in season 1, we calculated the mean total number of spiders three months before grazing (Sep-Nov) and during grazing (Jan-Mar) in the season. We used repeated-measures ANOVA with time (before grazing, during grazing), and spider type (wolf, trapdoor) as within-subjects factors, and treatment (ungrazed, grazed) as between-subjects factors. We used these results to test whether there was an effect of treatment on either or both spider group abundances. A significant interaction effect of time X treatment would indicate an overall effect of grazing.

In season 2, we used the last three months of the field season to test the effect of grazing and used repeated-measures ANOVA with time (Jan-Feb, Feb-Mar) and spider type (wolf, trapdoor) as within-subjects factors and treatment (ungrazed, grazed) as a between-subjects factor. We used these results to test whether there were differences in spider abundance between treatments, times or spider types. A significant treatment effect would indicate an effect of grazing.

For any three way interaction effects, we then analysed each time/month separately using a two way ANOVA, with number of spiders as a dependent variable and treatment (ungrazed, grazed) and spider type (wolf, trapdoor) as fixed factors.

Finally, we investigated how spiders utilise available burrows, by determining whether wolf and trapdoor spiders will inhabit burrows previously defined as empty. We divided each season into two three month periods, early (September to November) and late (January to March). We then defined an established burrow in each of those periods as one that remained intact for those 3 months. In each early period, we considered the subset of established burrows that were empty in the September or the October survey, and calculated the proportion of those burrows that had a spider occupant in the next survey (October or November). We used the average of those two proportion values to indicate the proportion of established burrows in each plot that that gained a spider occupant for each spider group for the period September to November. We then repeated this procedure for the period Jan – March, and compared the proportion of spiders that move into existing burrows in each plot for each spider group, and between early and late in the spring- summer season.

With these data we then used an arcsin transformation to test for an effect of grazing, used identical analyses as for spider abundance tests for season one, with burrows with spiders gained as the dependant variable. We used identical analyses for season 2.

Results

Spider burrows

No significant main effects or interaction effects were found when comparing the number of detected wolf and trapdoor spider burrows per plot (Table 1). Specifically the mean abundance of wolf spider (22.36; SE 1.14; range 4-62) and trapdoor spider (22.67; SE 1.40; range 4-56) burrows did not differ, and nor were there monthly changes, or year to year differences in the overall numbers of burrows in each plot.

Burrow use by lizards

We recorded a total of 41 cases of lizards moving into new burrows. The overwhelming majority (35 = 85.36%) of moves were into burrows that had been recorded as empty in the previous month. There were only five cases where a lizard moved into a burrow that a wolf spider had occupied and there was one case of a lizard moving into a burrow that a trapdoor spider had occupied. The analysis confirmed that significantly more lizards were detected moving into empty burrows than into burrows which were previously occupied by either wolf spider or trapdoor spiders (Table 2, Fig 1). This pattern was consistent in both seasons and times (early, late) of the study.

A single significant effect of burrow type was found when comparing the mean proportion of lizards occupying burrows (Table 3). Lizards occupied a significantly higher mean proportion of trapdoor burrows than wolf spider burrows. When including all wolf and trapdoor spider burrows, this was consistent between seasons and across all months of the study (Table 3, Fig 2). While lizards occupied about 30% of the detected trapdoor spider burrows, they used less than 10% of available wolf spider burrows (Fig 2). When excluding all burrows with a spider occupant from the analysis, there were significant interaction effects of season and month (Table 4). In season 1, a decrease in the proportion of trapdoor spider burrows being used by lizards was observed across the season, while an increase in wolf spider burrow use was observed in November, followed by a decrease across the season (Fig 2). In season 2, there was no significant effect of month on the proportion of lizards using each burrow type (Table 4).

Burrow use by spiders

A significant effect of month and a significant interaction effect of month X spider type in both season 1 and season 2 were found when comparing the abundance of spiders in plots (Table 5). Trapdoor spiders maintained a similar abundance over the duration of each field season, while wolf spiders steadily declined in numbers over the field season. In season 2, there were higher abundances of wolf spiders in November and March compared with season 1, and a higher abundance of trapdoor spiders (Fig 3). As previously mentioned, detectability of trapdoor spider burrows was difficult, thus the increased abundance observed in season 2 is likely an artefact of increased detector skill or changes in vegetation cover.

The grazing treatment had an effect in both season 1 and season 2 (Table 5). In season 1, there was an effect of time, as well as an interaction effect of time and treatment, and time andspider type. There was a greater magnitude of decline in spider abundance in grazed vs ungrazed plots (Fig 4a). This decline was due to a large decline in wolf spiders (Fig 4b). Trapdoor spider abundance did not differ significantly between times (Fig 4b). In season 2, there was an effect of time, as well as interaction effects of time and treatment, time and spider type and time, treatment and spider type. In grazed plots, a trend towards higher decline of wolf spiders was observed compared to ungrazed plots. Consistent with season 1, no significant difference in the abundance of trapdoor spiders was observed in grazed and ungrazed plots during season 2. (Fig 4c). There was no significant difference in the abundance of spiders between treatments or spider groups when months were analysed individually in season 2 (Table 6).

Burrow selection by wolf and trapdoor spiders was significantly different, regardless of time, treatment or season (Table 7). A significantly higher proportion of wolf spiders moved into existing burrows from one month to the next, while no trapdoor spiders were recorded to have moved into existing burrows (Table 7, Fig 5).

Discussion

We predicted that 1) there would be differential burrow use by spiders and lizards, thus, niche partitioning in order for coexistence to occur 2) wolf and trapdoor spiders would have differential responses to grazing impacts. Our results support these predictions. Empty spider burrows appear to be the key to promoting coexistence of pygmy bluetongue lizards and burrow digging spiders in the grasslands of South Australia. Fine scale spatial niche partitioning, and temporal niche partitioning have enabled the coexistence of pygmy bluetongue lizards and burrow digging spiders, despite the potential threats that they pose to each other.

The results of our study show that there was no significant difference in the abundance of wolf and trapdoor spider burrows present at the study site (Table 1). Lizards moved into previously empty burrows (Table 2, Fig 1), and despite no difference in the observed abundance of wolf and trapdoor spider burrows, lizards selected trapdoor spider burrows most often (Table 3, Fig 2). As spiders are potential predators of lizards, a likely explanation for this is that lizards avoid conflict with spiders by occupying vacated burrows. Trapdoor spider burrows were deeper compared to wolf spider burrows (Appendix 3), providing further support for the selection of trapdoor spider burrows. Clayton and Bull (2017) have previously reported that lizards occupy the deeper burrows within this site. Trapdoor spider burrows are likely to be a more suitable burrow choice for lizards, potentially providing a more stable burrow. This would require the lizard to search for a new burrow less frequently, thus reduce the lizard's risk of predation from snakes and birds of prey whilst outside of a burrow (Fenner et al. 2008). Alternatively, survival of lizards which occupy deeper burrows could be higher, due to better microclimatic conditions or better burrow stability. While these are possible influences on our results, they were not investigated in this study.

We propose that our study provides a potential explanation for how these lizards and spiders persist and coexist together. Our findings that wolf spiders decline in abundance in late summer (Fig 5) reveal that a cohort of shallow burrows become available at this time. We have also shown that lizards select empty spider burrows (Fig 1), and previous research shows that neonate lizards disperse in late summer (Milne et al. 2002; Schofield et al. 2012). This dispersal coincides with our reported increase in empty/available burrows, and while these burrows are unlikely to be suitable to an adult lizard, Milne et al. (2000) found that neonate lizards will occupy shallow burrows. Grazing pressure has the potential to reduce the availability of these burrows to neonate lizards, thus has potential to negatively impact neonate lizard survival.

Although not included in this study, it has been previously documented that many trapdoor spiders mate during the wet, autumn months (Main 1976; Main 1978). Male spiders disperse from their burrows in search of females to mate with, and die shortly after mating with numerous females (Main 1976; Main 1978). This potentially leaves a suite of burrows empty and available for lizards at the beginning of spring when they are most likely to move into new burrows (Milne et al. 2002). Due to the presence of lids on trapdoor burrows, and the compacted burrow walls, they are likely to remain intact even with the absence of a burrow occupant. We therefore predict that grazing will have less impact on vacated burrows which have an intact trapdoor spider lid (suitable for adult lizards) compared to vacated burrows which are smaller and do not have an intact trapdoor spider lid (suitable for neonate lizards).

Wolf spiders appear to utilize a wide range of burrows compared to trapdoor spiders which appear to stay in the same burrow for the duration of their life. For example, wolf spiders were recorded regularly moving into empty burrows while no records of trapdoor spiders moving into empty burrows were observed (Table 7, Fig 5). The importance of trapdoor spiders and wolf spiders to lizards may therefore differ, with wolf spiders providing maintenance of a larger number of burrows (which may include trapdoor spider burrows), and trapdoor spiders providing the most suitable burrows for lizard use.

In a captive setting, pygmy bluetongue lizard fatalities have been reported as a result of wolf spider bite (Ebrahimi and Bull, 2012). More recently, neonate pygmy bluetongue lizard fatalities by redback spiders (Latrodectus hasseltii) have also been reported (Clive, 2017, pers. communications). No documentation of fatality from trapdoor spider bites have been reported, however it is highly likely that they too may cause serious harm to a lizard. Lizards have been found to cause fatality to wolf spiders both anecdotally and in video footage, revealing the potentially tenuous nature of their coexistence (Milne et al. 1999; Ebrahimi et al., 2012). As burrows appear to be a limiting resource for the lizards, it is crucial that suitable burrows be maintained within lizard habitat. In years where fewer burrows are constructed, or more burrows are destroyed, lizards and spiders may be forced to interact with one another, or alternatively, the less competitive individual may need to seek a different refuge. This ultimately has the potential of reducing survivability of the less-competitive species in the short-term, and may alter species interactions in the long-term.

The preference towards trapdoor spider burrows by lizards is likely to be a consequence of higher stability of trapdoor spider burrows compared with wolf spider burrows. Milne and Bull (2000) noted that pygmy bluetongue lizards have specific preferences for burrow dimensions. It is also possible that trapdoor spider burrows may provide dimensions which are more suitable for pygmy bluetongue lizards than wolf spider burrows. Further, if trapdoor spider burrows are typically deeper than wolf spider burrows they may provide better protection from predators such as brown snakes (Psuedonaja textilis). Previously, we reported that grazing resulted in destruction of shallow burrows, but did not impact on the deeper burrows selected by lizards (Clayton and Bull, 2017). Lizards can utilise the same burrow for multiple seasons, therefore are likely to seek out burrows which provide stability and resilience to disturbance (Bull et al., 2016). Lizards that do not select stable burrows are at risk of needing to vacate the burrow if it is destroyed or becomes unstable, thus, are at greater risk of predation of exposure.

Previous research has highlighted the importance of grazing practices to maintenance of grassland habitats (Dorrough et al. 2004). For pygmy bluetongue lizards, appropriate grazing pressure can result in increased basking opportunity, and retention of spider burrows. Conversely, grazing pressure which is too high, or no grazing at all will likely result in either a reduction of prey capture ability for lizards or increased destruction of spider burrows (Pettigrew and Bull 2011; Pettigrew and Bull 2012; Pettigrew and Bull 2014). Our study adds to our understanding of sheep grazing impacts that wolf spiders and trapdoor spiders respond differently to this disturbance. Wolf spiders appear to be more impacted by sheep grazing compared to trapdoor spiders. This is likely related to the ecology of the species, with trapdoor spiders constructing deep, stable burrows and rarely moving from these burrows. Wolf spiders appear to be more inclined to move from their burrow and potentially as a result may be more at risk of trampling and displacement by grazers. The lizards appear to be impacted similarly to trapdoor spiders, with our previous results showing no effect of grazing on lizard abundance and burrow selection (Clayton and Bull, 2015, Clayton and Bull, 2017).

Differential responses of organisms to disturbance are well documented (La Rosa et al. 2001; Samu et al. 2010; Williams et al. 2010). The response of an organism to disturbance is dependent on a range of factors which may include life history traits, the habitat itself, the history of the habitat, duration and type of disturbance (Barrett and Guyer 2008; Belsky 1992; Smith et al. 2016). For this study system, sheep grazing disturbance had a direct effect on a refuge resource for wolf spiders and potentially neonate lizards. There is the potential that this pressure may increase competition for trapdoor spider burrows between lizards and wolf spiders, or result in a decline of spiders and thus wolf spider burrows. This may in turn impact neonate lizard survival.

Conservation of the pygmy bluetongue lizard will depend upon appropriate management of their grassland habitat. Through investigating spatial and temporal niche partitioning we have shown that retention of empty spider burrows will be crucial to the persistence of lizards. Although previous research has shown that grazing has the potential to have positive impacts on lizard behaviour, our study is the first to highlight the importance of empty burrows to lizards and to give an insight into how grazing at these stocking rates may impact lizard survivability in the long-term. While we have touched on how grazing may impact lizards and spiders, the question of how a reduction in spider abundance, or burrow abundance will impact neonate and adult lizards in the long-term remains unanswered. Further, investigations into how niche partitioning differs between adult and neonate lizards, and also if burrow use by lizards influences the partitioning of resources by spiders would significantly improve our understanding of these lizard and spider dynamics in their grassland habitat.

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Tables

Table 1. Repeated-measures ANOVA of the mean number of all wolf and trapdoor spider burrows in each month of two field seasons (Sep-Mar 2012-2014). P values in bold indicate significant effects (P<0.05).

	Df	F	P value
Burrow type	1,7	0.182	0.682
Season	1,7	0.11	0.749
Month	5,35	0.289	0.792
Burrow type X Season	1,7	2.904	0.132
Burrow type X Month	5,35	0.388	0.701
Season X Month	5,35	2.076	0.177
Burrow type X Season X Month	5,35	1.893	0.201

Table 2. Repeated-measures ANOVA of the proportion of lizards occupying burrows which were previously empty, wolf or trapdoor spider occupied. P values in bold indicate significant effects (P<0.05).

	Df	F	P value
Previous occupant	2,14	14.878	**0.004**
Time	1,7	2.280	0.175
Season	1,7	0.448	0.525
Previous occupant X Time	2,14	0.461	0.603
Previous occupant X Season	2,14	0.277	0.645
Time X Season	1,7	0.538	0.487
Previous occupant X Time X Season	1,14	0.089	0.810

Table 3. Repeated-measures ANOVA of the proportion of wolf spider and trapdoor spider burrows occupied by lizards, in each month of two field seasons (Sep-Mar 2012-2014). P values in bold indicate significant effects (P<0.05).

	Df	F	P value
Spider type	1,7	13.997	**0.007**
Year	1,7	2.765	0.140
Month	5,35	0.797	0.461
Spider type X Year	1,7	5.050	0.059
Spider type X Month	5,35	0.520	0.557
Year X Month	5,35	0.701	0.626
Spider type X Year X Month	5,35	1.189	0.335

Table 4. Repeated-measures ANOVA of the proportion of wolf spider and trapdoor spider burrows (excluding all spider occupied burrows) occupied by lizards, in each month of two field seasons (Sep-Mar 2012-2014). P values in bold indicate significant effects (P<0.05).

	Df	F	P value
Spider type	1,7	51.006	**<0.001**
Year	1,7	0.032	0.862
Month	5,35	2.310	0.065
Spider type X Year	1,7	0.170	0.693
Spider type X Month	5,35	1.012	0.425
Year X Month	5,35	2.770	**0.033**
Spider type X Year X Month	5,35	2.769	**0.033**

Season 1			
Spider type	1,11	96.738	**<0.001**
Month	5,55	5.657	**<0.001**
Spider type X Month	5,55	5.919	**<0.001**

Season 2			
Spider type	1,8	40.264	**<0.001**
Month	6,48	1.305	0.298
Spider type X Month	6,48	1.473	0.261

Table 5. Repeated-measures ANOVA for the mean number of wolf and trapdoor spiders temporally (Sep-Nov) over both field seasons, and in grazing treatments (ungrazed, grazed) early (Sep-Nov) and late (Jan-Mar) in season 1, and in the last three months of season 2 (Jan-Feb, Feb-Mar). P values in bold indicate significant effects (0.05).

	Df	F	P
Temporal			
Month	5,100	6.115	**0.001**
Spider type	1,20	0.839	0.371
Season	1,20	1.947	0.178
Month X Spider type	5,100	4.865	**0.003**
Month X Season	5,100	2.298	0.080
Spider type X Season	1,20	1.182	0.290
Month X Spider type X Season	5,100	3.376	**0.020**
Season 1			
Month	5,55	6.110	**0.003**
Spider type	1,11	0.493	0.497
Month X Spider type	5,55	7.862	**0.001**
Season 2			
Month	6,48	5.223	**<0.001**
Spider type	1,8	0.358	0.566
Month X Spider type	6,48	2.404	**0.041**
Grazing treatment			
Season 1			
Time	1,10	17.543	**0.002**
Treatment	1,10	0.144	0.712
Spider type	1,10	0.448	0.518
Time X Treatment	1,10	6.762	**0.026**
Time X Spider type	1,10	12.712	**0.005**
Treatment X Spider type	1,10	0.008	0.930
Time X Treatment X Spider type	1,10	2.540	0.142

*Table 5 continued.	Df	F	P
Season 2			
Time	1,4	120.125	**<0.001**
Treatment	1,4	1.039	0.366
Spider type	1,4	0.797	0.423
Time X Treatment	1,4	10.125	**0.033**
Time X Spider type	1,4	156.250	**<0.001**
Treatment X Spider type	1,4	3.575	0.132
Time X Treatment X Spider type	1,4	12.250	**0.025**

Table 6. Two way ANOVA for spider type and treatment in Jan-Feb and Feb-Mar of season 2 independently.

	Df	F	P
Jan-Feb 2014			
Spider type	1,8	0.484	0.506
Treatment	1,8	0.738	0.415
Spider type X Treatment	1,8	4.353	0.070
Feb-Mar 2014			
Spider type	1,8	1.445	0.264
Treatment	1,8	1.080	0.329
Spider type X Treatment	1,8	3.746	0.089

Table 7. Repeated-measures ANOVA of the proportion of each spider type which move into existing burrows in two grazing treatments (ungrazed, grazed), early (Sep-Nov) and late (Jan-Mar) in season 1 and season 2, analysed separately (2012-2014)). P values in bold indicate significant effects (0.05).

	Df	F	P
Season 1			
Time	1,8	0.277	0.613
Treatment	1,8	2.613	0.145
Spider type	1,8	22.294	**0.001**
Time X Treatment	1,8	2.746	0.136
Time X Spider type	1,8	0.277	0.613
Treatment X Spider type	1,8	2.613	0.145
Time X Treatment X Spider type	1,8	2.746	0.136
Season 2			
Time	1,4	0.094	0.775
Treatment	1,4	4.088	0.113
Spider type	1,4	19.885	**0.011**
Time X Treatment	1,4	2.711	0.175
Time X Spider type	1,4	0.094	0.775
Treatment X Spider type	1,4	4.088	0.113
Time X Treatment X Spider type	1,4	2.711	0.175

Figures:

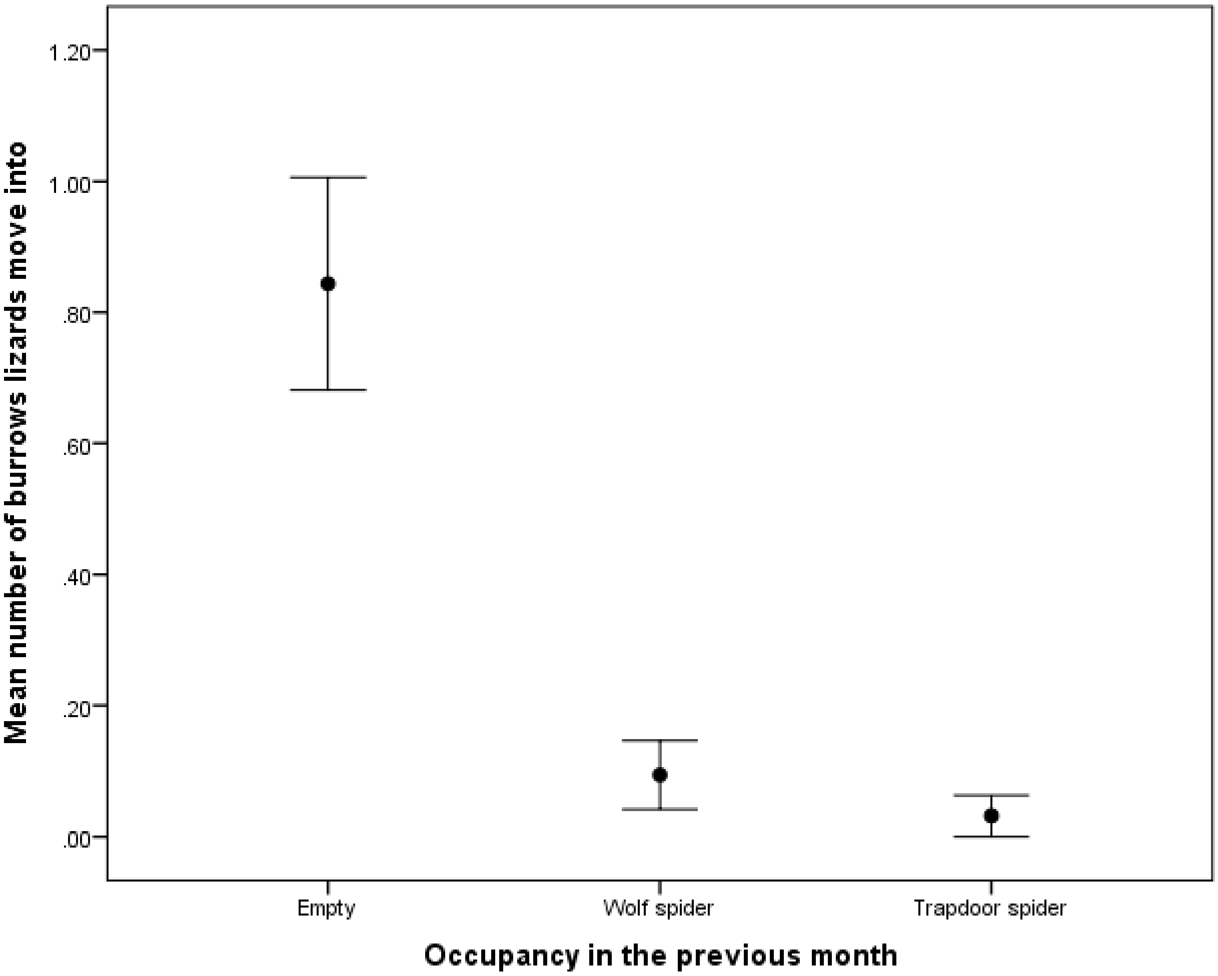

Fig 1. Mean (+/- 1 SE) number of burrows per month, which were previously empty, occupied by wolf spiders or trapdoor spiders, that were newly occupied by a lizard in both seasons combined (Sep2012-Mar2014).

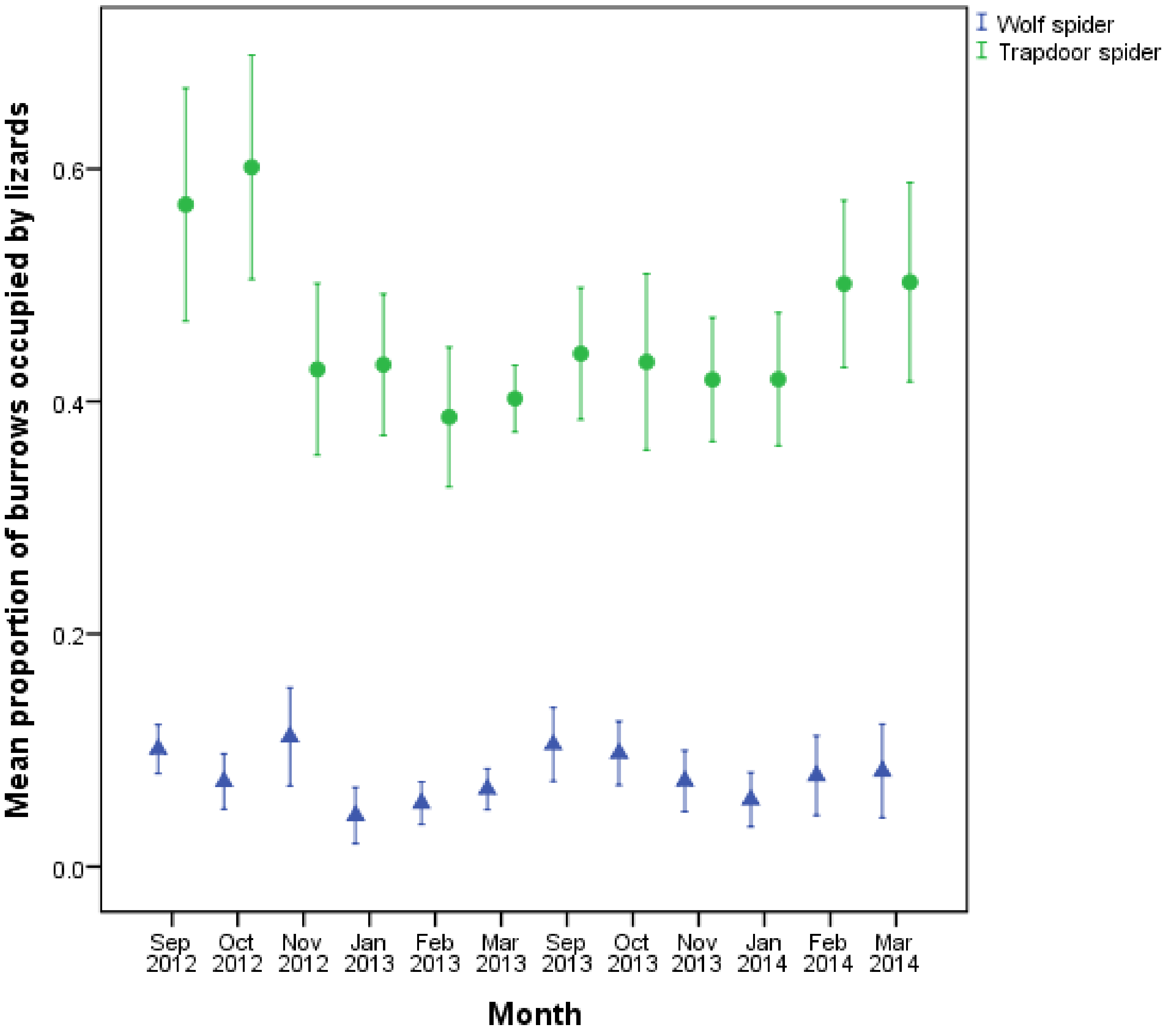

Fig 2. Mean (+/- 1 SE) proportion of lizards observed in either wolf (triangle) or trapdoor (circle) spider burrows in each month of season 1 and 2 (Sep 2012 – Mar 2014), including only burrows that were not spider occupied.

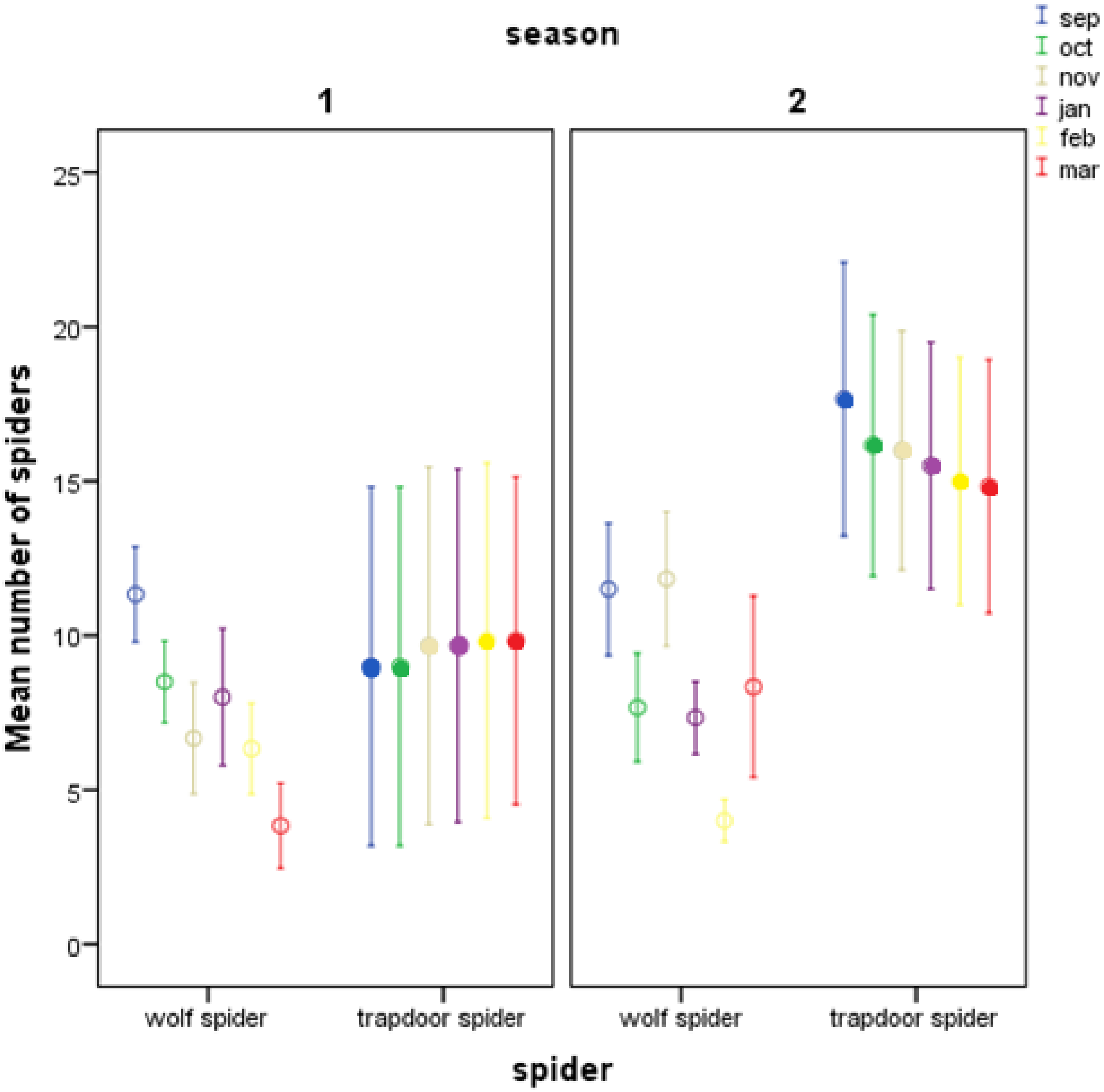

Figure 3. Mean (+/- 1SE) number of wolf and trapdoor spiders in plots, observed monthly, in season 1 (Sep-Mar 2012-201) and season 2 (Sep-Mar 2013-2014).

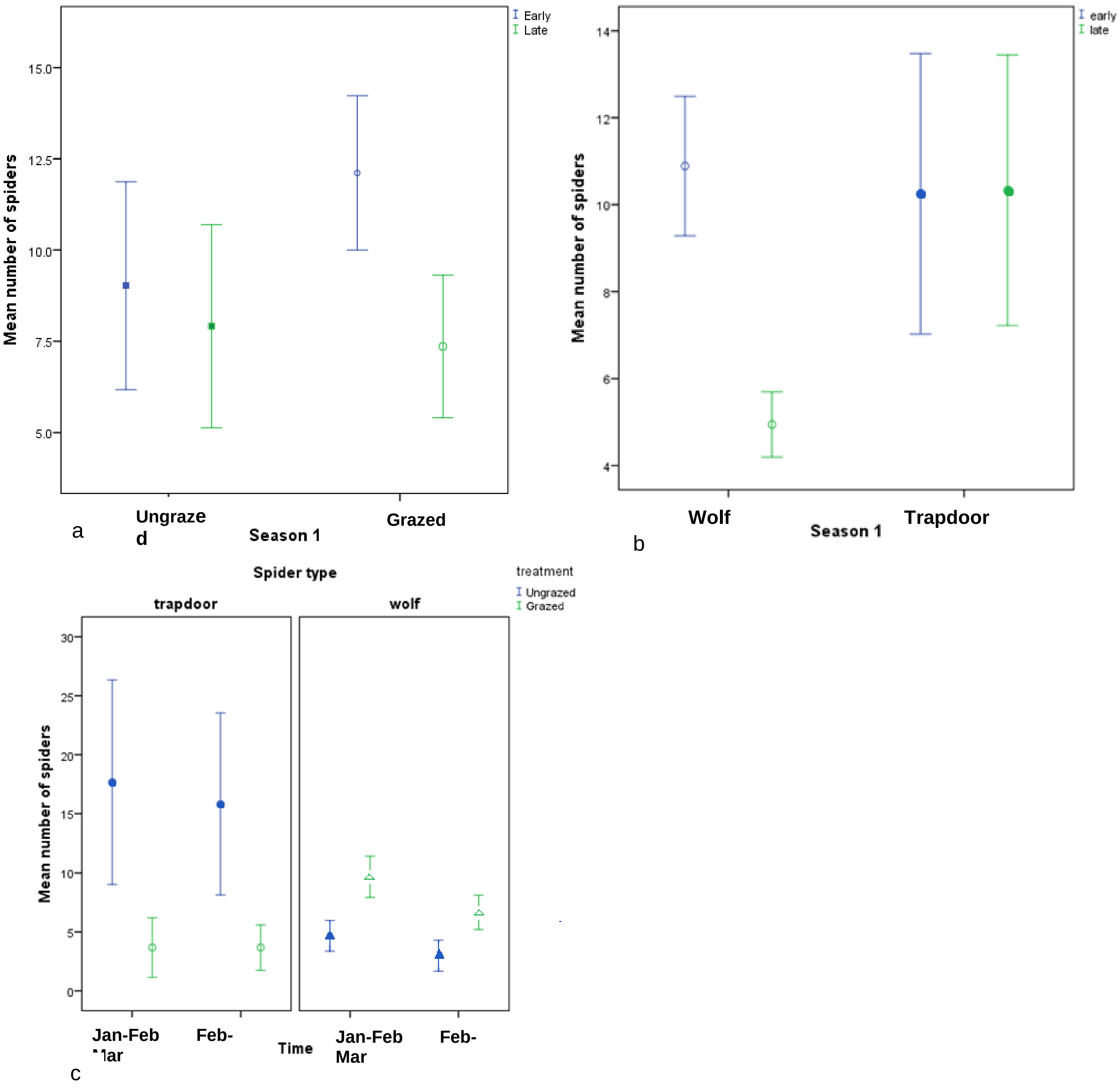

Fig 4. Mean (+/- 1SE) number of wolf and trapdoor spiders (a) combined, early (Sep-Nov) and late (Jan-Mar) in Season 1 in grazed and ungrazed plots, (b) wolf and trapdoor spiders early (Sep-Nov) and late (Jan-Mar) in season 1 and (c) wolf and trapdoor spiders late in season 2 (Jan-Feb, Feb-Mar) in grazed and ungrazed plots.

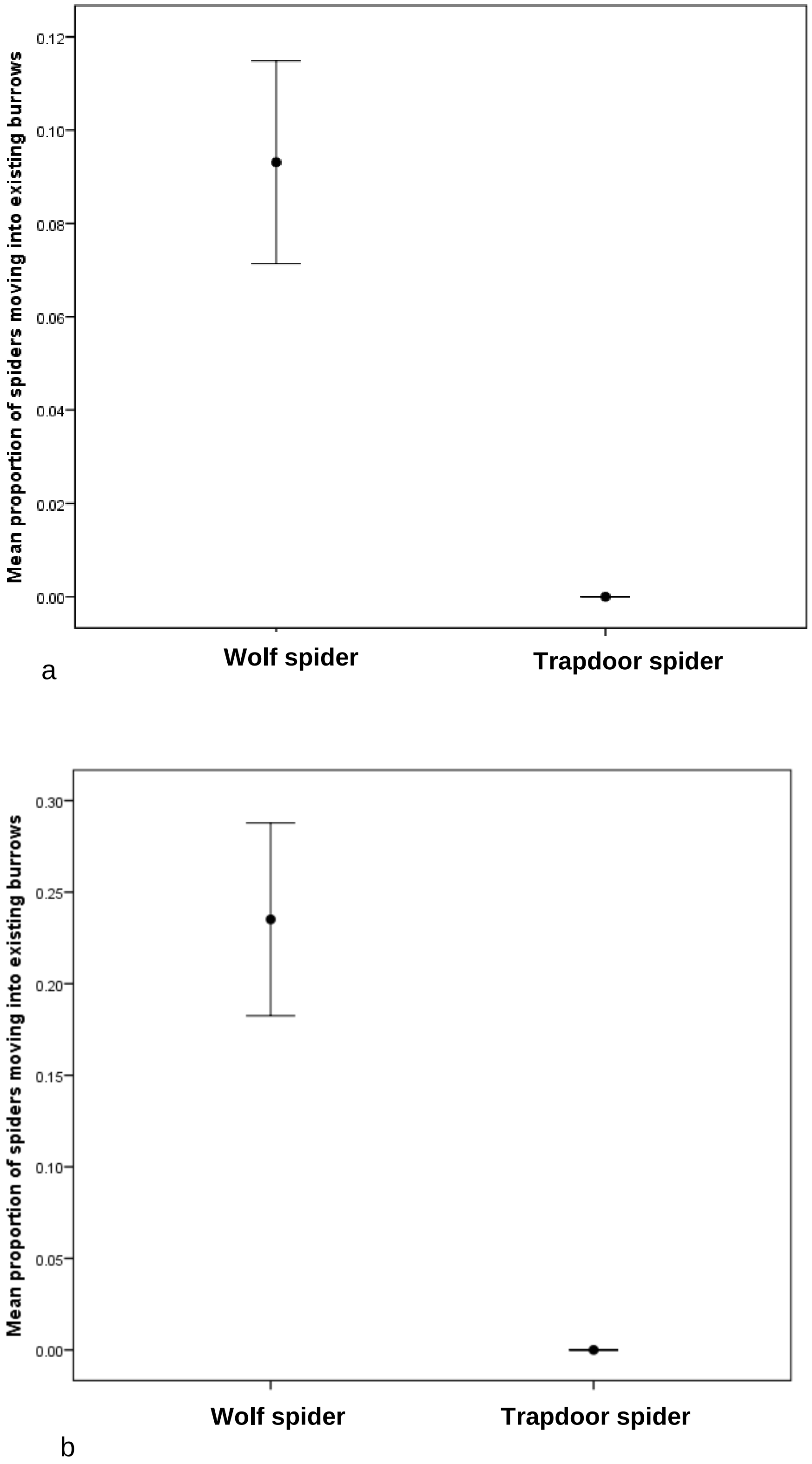

Figure 5. Mean (+/- 1 SE) proportion of wolf and trapdoor spiders which moved into burrows from one month to the next from Sep-Mar (excluding Dec) in (a) season 1 and (b) season 2.

Appendix 1. Grazing treatments at the Tiliqua Reserve over two field seasons from September 2012 – March 2014.

<table>
<tr><th></th><th colspan="6">Season 1</th><th colspan="7">Season 2</th></tr>
<tr><th>Plot</th><th>Sep</th><th>Oct</th><th>Nov</th><th>Jan</th><th>Feb</th><th>Mar</th><th>Sep</th><th>Oct</th><th>Nov</th><th>Dec</th><th>Jan</th><th>Feb</th><th>Mar</th></tr>
<tr><td>1</td><td></td><td></td><td></td><td rowspan="6" colspan="3">Grazed at a rate of 4 sheep per hectare</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>2</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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<tr><td>8</td><td></td><td></td><td></td><td colspan="7">Ungrazed</td></tr>
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<tr><td>13</td><td colspan="6"></td><td colspan="7">Grazed at a rate of 5 sheep per hectare</td></tr>
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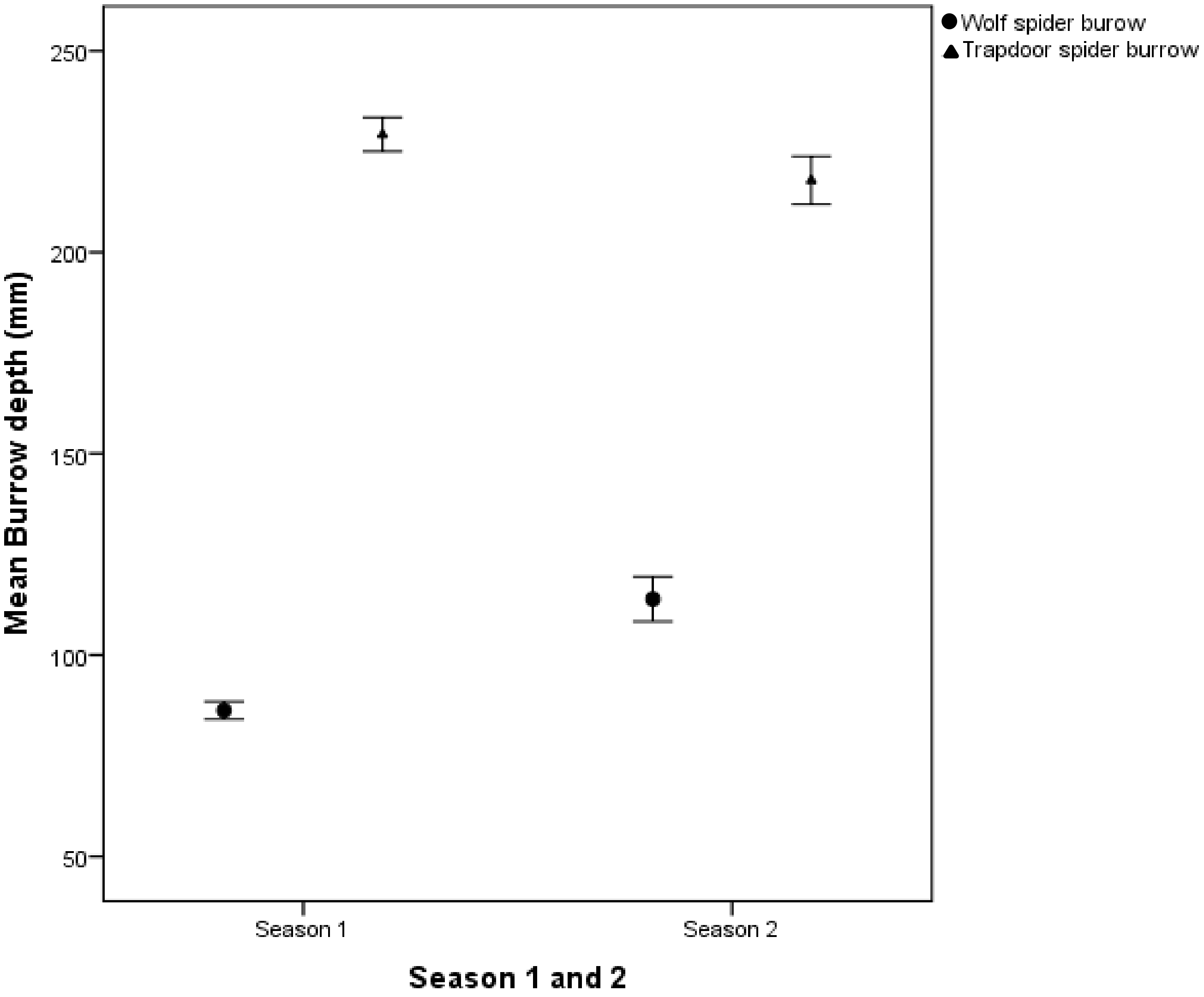
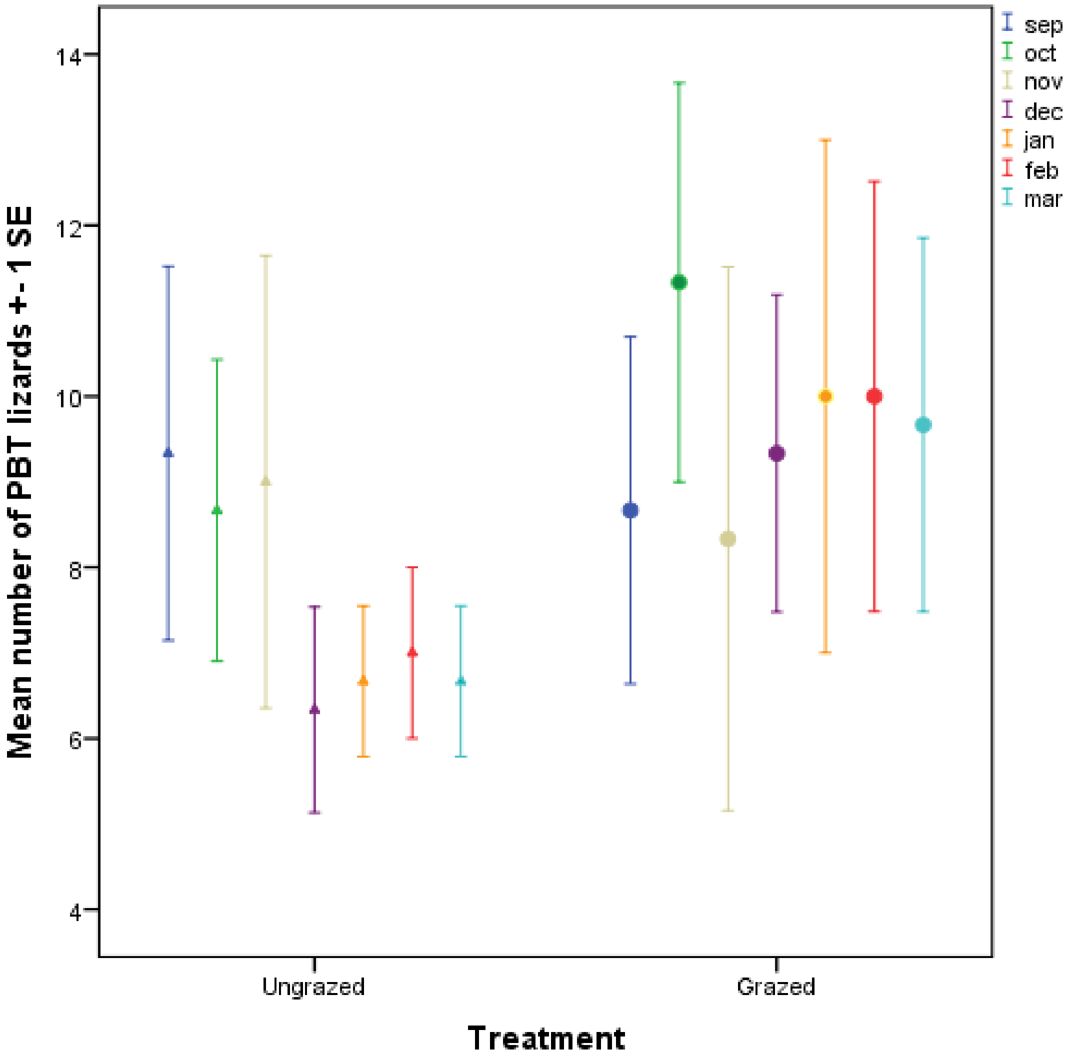
Appendix 2. The depth of trapdoor and wolf spider burrows observed in all plots during season 1 and season 2.



Appendix 3. Mean number of pygmy bluetongue lizards observed monthly (Sep-Mar) in ungrazed and grazed treatments during season 2.



Chapter 6

Thesis Discussion

Ecosystem engineering spiders and the burrows they construct are vital to the persistence of pygmy bluetongue lizards in native grasslands. In this thesis, I investigated how sheep grazing influences a group of native ecosystem engineers, and the endangered lizards which exploit their burrows. I addressed gaps in the literature, providing critical knowledge for conservation of both the lizards and spiders. Five key aims were addressed in this thesis, which all contribute to improving our understanding of burrow availability to pygmy bluetongue lizards. These aims were:

1. To identify the effects of sheep grazing on burrow use by lizards and spiders, and burrow availability
2. To identify the effects of sheep grazing on burrow depth - a vital factor for lizard use of burrows
3. To investigate how grazing influences population dynamics of spider species
4. To identify how grazing influences spider reproductive output
5. To identify how spiders and lizards coexist together, utilising spider-dug burrows

Here, I discuss the major findings of my work and how it contributes to conservation of pygmy bluetongue lizards and burrow digging spiders.

A focus of my thesis was to address a large gap in our understanding of pygmy bluetongue lizards and their relationship to sheep grazing - the impact of grazing on spider burrows. Spiders and lizards continue to inhabit native grasslands, despite the widespread introduction of sheep grazing since European settlement. My results show that sheep grazing at a moderate level (4-5 sheep per hectare) results in the destruction of spider burrows, but does not significantly affect the abundance of pygmy bluetongue lizards (Chapter 2). Previous research of grazing effects on pygmy bluetongue lizards focus on lizard behaviour with no assessment of burrow persistence or availability.

These findings reveal a potential indirect effect of grazing on lizard persistence via reduced burrow availability.

In chapter 3, I identified the cohort of spider burrows which were most impacted by grazing pressure. I found that shallow spider burrows were being destroyed by sheep grazing, but deeper burrows largely persisted. This result leads me to conclude that deep burrows are more stable under grazing pressure than are shallow burrows. I found that lizards were preferentially occupying the deeper burrows available to them and that there was no effect of sheep grazing on lizard abundance, despite a reduction of shallow burrows. Similarly Milne et al. (2000) found that pygmy bluetongue lizard occupy the deeper burrows within their habitat consistent with this theory. My results provide new insight into mechanisms the spiders and lizards use to co-exist with sheep in these habitats. My results also highlight the need to manage sheep grazing intensity as even at these moderate levels a cohort of spiders are likely being negatively impacted and a cohort of spider burrows are being destroyed.

In order for burrow recruitment and maintenance to occur, spider populations need to be present within pygmy bluetongue populations. In Chapter 4 I aimed to identify how sheep grazing affects the reproductive output of burrow digging spiders.

The production of egg sacs and spiderlings by trapdoor and wolf spiders appears to be negatively impacted by sheep grazing. I reported a significantly lower number of both spider groups producing egg sacs and spiderlings in grazed habitat compared to ungrazed; Further highlighting the importance of appropriate grazing regimes for these species (Chapter 4). For long-term persistence of spiders and lizards in native grassland habitat, maintenance of spider burrows and conditions conducive to recruitment of new burrows and spiders is essential. Similar findings of negative effects of grazing on reproductive output have been reported in other studies (Ammon and Stacey 1997; Cooch et al. 1991; Newton 2004). Grazing may be impacting spider reproductive output in multiple ways. Disturbance via trampling by sheep, altered soil and vegetative characteristics resulting in reduced success of spiders finding mates or surviving to mate and destruction of burrows may all be factors which have affected reproductive output. While I have identified the result of grazing – a decline of reproductive output, the question of what mechanism/s sheep grazing explicitly impacts remains unanswered. Further, whether this reduction of reproductive output also results in less recruitment of overall spiderlings has not been examined in this study.

Finally, a major focus of my research was to identify how lizards and spiders utilise burrows. Pygmy bluetongue lizards and burrowing spiders persist together in sheep-grazed grasslands, despite numerous potential obstacles. While lizards and spiders are both capable of causing harm and are potentially fatal to one another (Ebrahimi and Bull 2012), they persist together, utilising the same resource – burrows constructed by spiders (Milne et al. 2003).

In Chapter 5 I aimed to determine whether lizards display niche partitioning in order to exploit these spider burrows, thus allowing for coexistence. My results show that lizards display spatial and temporal niche partitioning. They select a subset of spider burrows, and will occupy these burrows at different times to spiders. Adult lizards select deep burrows, most often dug by trapdoor spiders (Chapter 3, 5). My results suggest that typically, they do not steal these burrows from spiders but utilise already vacated burrows, reducing the risk of altercations with burrow occupants (Chapter 5). Their selection of vacated trapdoor spider burrows also means that the burrows they are using appear to be the most resilient to sheep grazing.

While adult trapdoor spiders and adult pygmy bluetongue lizards appear to have stable refuges under this level of grazing pressure, there may be implication of sheep grazing for long-term lizard and spider survival. Wolf spiders appear to be impacted at multiple life stages (Chapter 3, 5). Wolf spiders were found to occupy a wide range of burrows, including shallow burrows and also vacated trapdoor spider burrows (Chapter 1, 5). They appear to be more mobile and more likely to move into existing burrows than trapdoor spiders. It is this combination of factors that make them most vulnerable to the pressures imposed by sheep grazing. A limitation of my study was that I was unable to monitor plots more than once per month. This meant that movements between these times could not be detected and therefore I could not rule out the chance that there were some interactions between lizards and spiders, particularly wolf spiders.

While not directly investigated in my study, I have identified potential mechanisms for lizard and spider populations to utilise burrows without competing for burrows. From our research, I know that lizards utilise vacated spider burrows. I also identified the population dynamics of spider cohorts within our two year study. Wolf spider abundances were highest in spring, declining by late summer. I propose that this decline leaves a cohort of empty spider burrows, of suitable dimensions for neonate lizards at their time of dispersal. Previous research has identified dispersal times for neonate lizards and this coincides with our documented decline in wolf spiders (Milne et al. 2002; Schofield et al. 2012). Due to the design of my study I was unable to monitor a burrow more than once in a month which reduced my opportunity for finding females with neonate lizards and also observations of neonate lizards in their early life. This limited me from being able to explore questions about direct effects of grazing on neonate lizards and of burrow use by neonate lizards. Future research into questions about whether these wolf spider burrows provide a refuge for neonate lizards and reduce competition for burrows with other lizards would help to improve our understanding of niche partitioning between different age classes of lizards which was not considered in this study.

I also suggest a mechanism for adult lizards finding suitable empty burrows. Trapdoor spiders are thought to mate during the cooler, wet months (Main 1957; Main 1978). While not directly investigated in my study, previous research shows that adult pygmy bluetongue lizards are most likely to move from their burrows in spring, and I suggest that at this time of year, a cohort of vacated male trapdoor spider burrows would likely be available (Fellows et al. 2009; Schofield et al. 2012). Identifying whether timing of lizard dispersal does in fact align with an increase in empty trapdoor spiders would assist in future investigations of burrow availability to lizards.

General conclusion

While I have identified a number of potential negative impacts of grazing on spiders and lizards, I have also shown the potential for long-term survival of lizards and spiders in these grasslands. Pygmy bluetongue lizards appear to utilise trapdoor spider burrows more frequently than wolf spider burrows, and these burrows appear to be less impacted by grazing pressure, at least at the levels applied during my study. Long-term, grazing at the levels imposed in my study may potentially result in a decline of spider recruitment however we need to understand more about the recruitment required by each spider group for persistence to comment further on this. Realistically, with the absence of other out of the usual events (e.g. pesticide use, extreme weather etc), provided land is grazed appropriately, both lizards and spiders can persist. What is considered 'appropriate' grazing will differ, dependent on environmental conditions at the time of grazing and the land itself. Trapdoor spiders and pygmy bluetongue lizards occupy burrows which are most resilient to grazing pressure. While I identified a negative impact of grazing on shallow burrows and wolf spiders, I suggest that grazing management, appropriate for lizard persistence will depend on a number of factors. In my study, grazing at a rate of 4-5 sheep per hectare did not directly impact lizards but did impact spider reproduction and wolf spider burrow abundance. Therefore, I suggest that even at these rates there may be some indirect detrimental effects on lizards. In order for grazing to be considered 'appropriate' for lizard persistence it should result in a reduction of vegetation cover in order to maintain the grassland mosaic structure but retention of burrows. This means that grazing intensity would need to be low enough so as not to disturb the soil crust and burrow structure. It should also be conducted at a time of year where lizards and spiders are not moving outside of burrows. I suggest that minimising grazing pressure at times of year when spiders mate (autumn and winter) will improve reproductive output of spiders.Due to the complexity of factors which will likely influence the outcome of grazing it is unfortunately difficult to specify a particular grazing regime or intensity. Further investigation into the effects of specific regimes (e.g. set stocking, rotational grazing etc) would greatly improve our ability to provide clear management plans for graziers.

Predictions of potential outcomes under poor grazing management can be made from the knowledge that I have developed throughout this thesis. Wolf spiders within the habitat are likely to be most directly and most quickly impacted by inappropriate grazing. Declines of wolf spiders will likely lead to a decline in maintenance of empty spider burrows. In turn, this will lead to a decline of burrows and increased competition for this vital resource. Recruitment of juvenile spiders (both wolf and trapdoor) may potentially be impacted. This may result in long-term decline of spider burrows and potential local extinctions of spider populations, however as previously mentioned further research on spider recruitment in grazed and ungrazed habitat is needed. Alternatively, as wolf spiders have greater dispersal abilities, they may be able to persist and maintain burrows while trapdoor spider numbers decline. This may still be detrimental to lizards as trapdoor spiders are responsible for digging burrows most utilized by lizards, thus the best quality habitat may not be available for lizards. If hypothetically, spider recruitment is negatively impacted and does result in a decline of spider burrows, lizards may persist for the short-term but will also suffer population declines and extinctions if these pressures persist. Ultimately, pygmy bluetongue lizards are reliant on ecosystem engineering spiders to supply them with sufficient burrows.

I provided the first investigation into the effects of sheep grazing on spider burrows and lizard and spider interactions in these habitats. While I have answered many questions and improved our understanding of these relationships, I have also exposed unanswered questions and areas which require investigation. I suggest the following next steps to improve management of these grasslands and improve the potential for long-term conservation of wild pygmy bluetongue populations.

1. Identifying the range of grazing regimes utilised by farmers in pygmy bluetongue habitat
2. Investigate how each of these regimes influences burrow construction, persistence, spider reproduction and abundances of spiders and lizards
3. Investigate the effects of time of year that grazing is implemented on the above factors
4. Provide guidance to farmers on habitat qualities that are suitable for lizards and how grazing practices can affect burrow recruitment and maintenance
5. Use this information to inform future translocations of pygmy bluetongue lizards of suitable translocation sites, and suitable management of these sites

By implementing these steps we can assist farmers to adopt or continue farming practices which will improve conservation outcomes for lizards and spiders in these grasslands. Transferring the knowledge gained from this thesis to translocation projects, and building on this knowledge has the potential to improve the outcome of future translocations. In particular, determining the effects of various grazing regimes and intensities on burrow availability, and niche partitioning by spiders and lizards will provide critical background information for future translocations of the lizards.

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Appendix 1

Spider species in the range of the pygmy bluetongue lizard

Methods

Collection sites

Spider specimens were collected from 11 sites across the range of the pygmy bluetongue lizard (Fig 1,2) (pygmy bluetongue sites 1,2, 6, 9, 10, 20, 24, 25, Hornsdale wind farm (in Jamestown), Baldina Station (in Burra) and Mokota Conservation Park (near Hallett) which is a site known to note have pygmy bluetongue lizards).

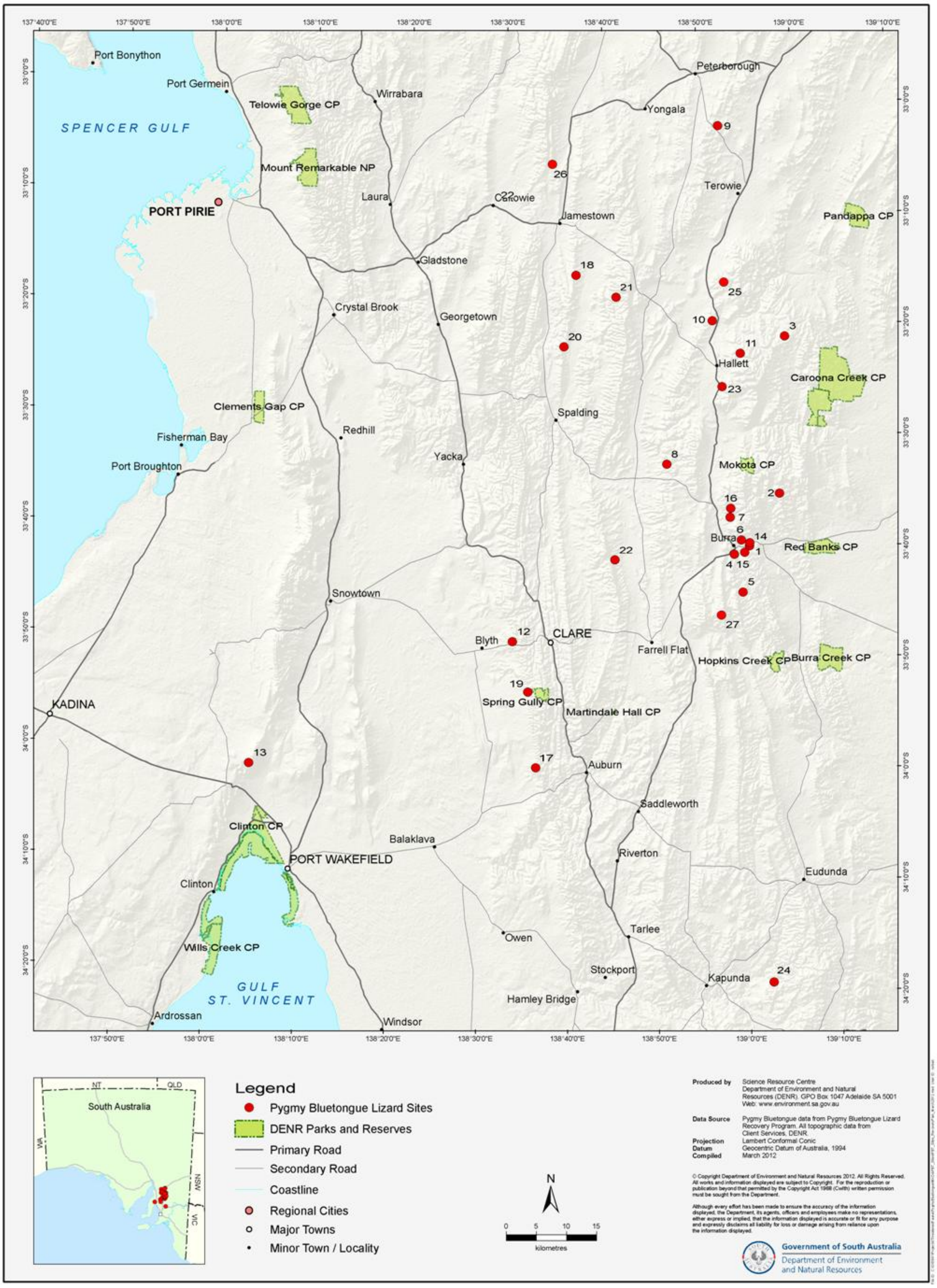

Figure 1. Numbered sites of known populations of pygmy bluetongue lizards (Tiliqua adelaidensis) (Map provided by DEWNR)

Collection methods

Spiders were collected from both burrows and the ground surface. Burrows were first inspected with an optiscope to determine the occupant. When collected from burrows, spiders were either lured out with a mealworm on string, or with the optiscope. Spotlighting was used at night in an attempt to collect mature male spiders from the surface of the ground, while spider extraction from burrows was used during the day in an attempt to collect mature females.

Spotlighting

Head torches were used at approximately the same time at night (1hr after dark) for one hour at each site. The eye-shine of spiders outside of burrows was detected by head torches and spiders were collected. This method was adopted in an attempt to collect mature males looking for mates.

Spider Identification

Spiders were preserved in 70% ethanol. Preserved wolf spider specimens were formally identified by Dr Volker Framenau at Phoenix Environmental and assistance in identification of preserved trapdoor spider specimens was received by Dr Robert Raven at the Queensland Museum, Nick Birks at the South Australia Museum and Sophie Harrison at the University of Adelaide. Spider specimens are housed at the Western Australian Museum and the South Australian Museum.

Table 1 Identified spider species across the range of the pygmy bluetongue lizard

Species	Number identified	Sites identified at
Wolf spiders		
Hogna burti	3M, 1F, 1J	6, 9, 10
Hogna crispipes	1M	10
Hogna lacertosa	2F	10
Lycosa gibsoni	7M, 6F	1,20
Tasmanicosa gilberta	10M, 11F	1, 6, 9, 24, 25, Hornsdale (Jamestown, SA)
Tasmanicosa leuckartii	1M	10
Tasmanicosa ramosa	1M, 5F	1, 2, 9
Venatrix esposica	1F	Mokota Conservation Park** not PBT habitat
Trapdoor spiders		
Blakistonia aurea	11F	1,2, 10, 20, 24, 25, Baldina Station
Aganippe subtristis	2F	2, 20
Aname tepperi	1F	10
Aname sp.	1F	2

Wolf spiders

There were 8 different species of wolf spiders identified across the range of the pygmy bluetongue lizard (Table 1). Of these species, four were observed within burrows and four were not. Lycosa gibsoni, Tasmanicosa gilberta, T. leuckartii and T. ramosa were observed within burrows while Hogna burti, H. crispipes, H. lacertosa and Venatrix esposica were captured by spotlighting and were never observed within a burrow during the two year study. Previously, McCullough (2000) reported

Lycosa stirlingae as a common species at Main Site (site 1), however recent revision of wolf spiders now identifies this species as Tasmanicosa ramosa.

Common burrow dwelling spiders in the range of the pygmy bluetongue lizard

Tasmanicosa gilberta (Female)

Female T. gilberta dorsal view

Female T. gilberta ventral view

Tasmanicosa gilberta (male)

Male T. gilberta dorsal view

Male T. gilberta ventral view

Male T. gilberta reproductive organ (pedipalp)

Tasmanicosa ramosa (Female)

Female T. ramosa dorsal view

Female T. ramosa ventral view

Other species identified at pygmy bluetongue lizards sites

Hogna lacertosa (Female)

Female H. Lacertosa dorsal view

Female H. lacertosa ventral view

Hogna burti (male)

Male H. burti dorsal view

Male H. burti reproductive organ (pedipalp)

Hogna crispipes (male)

Male H. crispipes dorsal view

Male H. crispipes reproductive organ (pedipalp)

Lycosa gibsoni (juvenile)

Juvenile L. gibsoni dorsal view

Juvenile L. gibsoni ventral view

Species found only at Mokota Conservation Park (Grasslands which do not occupy pygmy bluetongue lizards)

Venatrix esposica (Female)

Female V. esposica dorsal view

Female V. esposica ventral view

Trapdoor spiders

There were four species of trapdoor spider formally identified from spider specimens in the range of the pygmy bluetongue lizard. Of these, two built trapdoors (Blakistonia aurea and Aganippe subtristis), while two built burrows without lids (Aname tepperi and Aname sp.). While not included in spider collections, burrows thought to be dug by Anidiops sp. were also observed at the study site. Previously, McCullough (2000) anecdotally reported mouse spiders (misulena sp.) also being present within pygmy bluetongue lizard populations. These were not observed in the current study. Work carried out by McCullough (2000) was conducted at Main site (site 1) rather than Tiliqua Reserve (site 2). It appears that if these spiders are present at the Tiliqua Reserve they may be in low abundance.

Blakistonia aurea (Female)

Female B. aurea dorsal view

Female B. aurea ventral view

Female B. aurea eye group

Female B. aurea burrow. *Note: In the range of the pygmy bluetongue lizard, B. aurea burrows had a distinctive d-shaped lid. The lid was thick and plug-like. Photographed here is an example of an adult burrow, with a smaller burrow next to it from a younger spider. It is likely the offspring of the spider in the larger burrow.

Aganippe subtristis (Female)

Female A. subtristis dorsal view

Female A. subtristis ventral view

Female A. subtristis eye group

Female A. subtristis burrow. *Note: Burrows constructed by A. subtristis were similar in appearance to those of B. aurea, however, had a characteristically thin lid. The lid was more circular and had a diameter often larger than the burrow

Aname tepperi (Female)

Female A. tepperi dorsal view

Female A. tepperi ventral view

Female A. tepperi eye group

Aname sp.(Female)

Female A. sp. Dorsal view

Female A. sp. Ventral view

Female A. sp. Eye group *Note: Both Aname species constructed burrows without lids.

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Appendix 2

Co-occupancy of spider engineered burrows within a grassland community changes temporally

Jessica Clayton, Prof C. Michael Bull, Dr Mark Hutchinson, Dr Aaron Fenner, Assoc Prof Mike Gardner

This short communication manuscript is in preparation for a special edition of Austral Ecology

Abstract

Burrow-digging organisms act as ecosystem engineers, providing potential habitat to other organisms. In the Mid North region of South Australia, wolf and trapdoor spiders in fragmented grassland communities provide this service. Pygmy bluetongue lizards are an endangered skink, endemic to these grasslands. The lizards obligatorily use burrows dug by these spider groups as refuges, basking sites and ambush points. We investigated the occupancy of these spider burrows by lizards and other organisms within the grassland community, identifying the occasions that burrows were shared by multiple taxa. We found that the lizards and trapdoor spiders are predominantly solitary, while wolf spiders co-shared burrows more frequently either lizards or trapdoor spiders. There were numerous taxa that were found to regularly co-share with other taxa, particularly snails, centipedes and weevils. There was a strong temporal influence on burrow sharing, with most co-sharing occurring late in summer. This study provides an insight into the use of burrows by wolf spiders and co-existing taxa within these grassland communities. The dynamics of burrow-use by other taxa have the potential to influence long-term conservation of these lizards as burrow availability is crucial to their survival in these grasslands.

Introduction

Burrows are a valuable resource, not only for the animals that construct them, but also for other animals in an ecosystem. They provide a buffer from aboveground climatic conditions (Clayton and Bull, 2016) and are often vital to the persistence of a species within areas which may have unsuitable surface temperatures and humidity for survival (Gálvez Bravo et al. 2009). As well as these physical alterations, burrow construction can result in changes to vegetation and changes to food availability (Eisenberg and Kinlaw 1999; Wesche et al.

2007). Animals that construct these burrows are often referred to as ecosystem engineers, as they create resources for other species, through physical and biological alterations to the ecosystem (Davidson et al. 2012; Jones et al. 1994; Wright et al. 2002).

The pygmy bluetongue lizard is an endangered skink, endemic to native grasslands in the Mid North region of South Australia. The species exclusively uses wolf (Lycosidae) and trapdoor (Mygalomorphae) spider burrows as refuges, basking sites and ambush points, often utilising the same burrow for extended periods of time (Bull et al. 2015). Each adult lizard will inhabit a burrow and will defend this burrow from conspecifics (Fenner and Bull 2011). The only time that adult lizards have been observed sharing their burrow with other lizards is when mother's first give birth to young and the young remain in the burrow for a short time before leaving to find their own burrow (Milne et al. 2002). While we know about pygmy bluetongue lizard interactions with conspecifics, there have been no records of whether, and if so when, lizards will share their burrows with other species within their range. As lizards rely on burrows for long-term survival, the availability of burrows may potentially be a limiting resource for them. Lizards and other burrow dwelling species may rely heavily on burrows for immediate survival in the instance of attempted predation (Roznik and Johnson 2009), and long-term survival in maintaining adequate thermoregulation and refuge (Gálvez Bravo et al. 2009; Schwarzkopf and Alford 1996). As such, the ability to share these resources with other taxa may improve a species chance of survival. This paper reports on the frequency of observations of shared burrow use by lizards and by other burrow occupants. It also explores which taxa within burrows will co-share burrows together and how this changes temporally.

Methods

The study was conducted in the Tiliqua Reserve, near Burra in the Mid North region of South Australia (33°42′S, 138°56′E). Nine plots of 30m X 30m were established at the study site in June 2012 (Clayton and Bull 2015), and all spider burrows (17 – 119 per plot) were monitored for occupancy each month from Sep 2013 – Mar 2014, over one complete spring and summer lizard activity season. An optic fiberscope (Medit Inc 2 way articulating FI Fiberscope) was inserted into each spider burrow to view for and record burrow occupants, as in Milne and Bull (2000). Burrows included in the surveys varied from 39 – 446mm deep. Burrow inhabitants were recorded as occupants if they were within the burrow chamber. The smallest occupant we could confidently record was an ant of approximately 3mm. All smaller occupants were not considered in this study.

Analysis

We calculated the total number of burrows per plot that had multiple occupants and used repeated-measures ANOVA with month (Sep-Mar) as a within-subjects factor. We counted the burrows per plot that had multiple occupants and divided these into pairs of organisms co-sharing. We used repeated-measures ANOVA with month (Sep-Mar) and taxa-taxa grouping (pbt-wolf spider, pbt-weevil, pbt-centipede, pbt-snail, wolf spider – weevil, wolf spider – centipede, wolf spider – snail, centipede – weevil, centipede – snail) as within-subjects factors.

Results

There were a total of 12 different taxa observed within burrows throughout the duration of the study. Of these species groups, seven were found to share burrows with other species at some point during the lizard activity season, with weevils and snails the most frequent taxa to be found in shared burrows (Table 1).

Weevils: There were 305 observations of weevils in burrows. Weevils were frequently observed co-occupying burrows with other weevils and often appeared at the base of the burrow or climbing on the burrow sides. All weevils observed appeared to be the same species. The majority of weevils were observed in late summer (only 24.2% observed in the first three months of the study). It is likely that burrows provide refuge from extreme surface temperatures to weevils in late summer when they would be at risk of desiccation or exceeding critical temperature limits at the surface (Rothermel and Luhring 2005).

Snails: There were 126 observations of snails in burrows. They were usually observed on the burrow sides. In all cases the snails were identified as the introduced European snail Theba pisana that is a cereal crop pest, widespread through agricultural regions of South Australia. Snails were found in burrows in all months of the study, but predominantly in December, January and February, coinciding with hotter, drier conditions. We suggest that the snails observed using burrows were likely resultant from either incidental use through chance of encountering a burrow (likely early in the season when conditions were cooler and wetter), and intentional burrow use to avoid exposure to aboveground temperatures.

Centipedes: There were 83 observations of centipedes in burrows. They were observed in the bottom half of the burrow chamber, usually on the wall of the burrow. Centipedes were observed in burrows in all months of the study, but were in highest abundance in October and November.

Wolf spiders: There were 628 observations of wolf spiders in burrows. Due to difficulty in identification of wolf spiders they were not identified to species in the field. Wolf spiders were observed in all months of the study, but were in greatest abundance early in the season during spring and early summer (Clayton and Bull, 2015). Wolf spiders were observed at all positions within the burrow., There were numerous observations of wolf spiders sitting just within the entrance of a burrow (in the very top section of the burrow chamber) while its co-sharing occupant was at the bottom of the burrow. This was the case when wolf spiders were sharing with pygmy bluetongue lizards and centipedes.

Redback spiders: There were two observations of redback spiders. When redback spiders occupied burrows they built extensive webbing.

There was a significant effect of month and co-sharing taxa on the number of shared burrows observed, and a significant interaction effect of month X co-sharing taxa (Table 2). Weevil-centipede shared burrows were significantly higher in September than any other time of the season and compared to other co-shared burrow taxa, while weevil-snail co-shared burrows were more commonly observed than other co-shared options, observed from Nov-Mar, peaking in Jan (Fig 2).

Discussion

Though predominantly solitary, pygmy bluetongue lizards were observed in shared burrows with other taxa during this field study (Table 1). These burrow-sharing observations were predominantly with snails and weevils (Fig 1). It is likely that these observations were the result of incidental burrow sharing (snails falling into burrows by chance) or, potentially a result of a need for other taxa to escape extreme surface temperatures.

Pygmy bluetongue lizards have an omnivorous diet, including other potential burrow occupants such as beetles, cockroaches and ants (Fenner et al. 2007). Anecdotally, snail remains and weevil remains have been reported in their scats (personal communication Derne, 2016, personal observation Clayton, 2016). There is, therefore, the potential that pygmy bluetongue lizards prey on these taxa when they co-share, making co-sharing temporary. We previously reported that pygmy bluetongue lizards select vacated burrows more frequently than they select spider occupied burrows (J. Clayton, thesis Chapter 5, 2017). If lizards are able to predate on certain taxa within burrows, it is plausible that they may potentially also be moving into occupied burrows, where the occupant causes no threat, and where the burrow is of sufficient dimensions for a lizard.

Weevils and snails were most commonly observed in sharing burrows with other taxa, and most of these shared burrow observations were recorded in the summer months of the study (Table 2, Fig 2). This may be due to an increased abundance of individuals later in the season, or, it may be due to an increase in burrow use later in the season. Burrows provide a buffer from surface temperatures, thus, may reduce the risk of desiccation and reaching lethal temperature limits (Pike and Mitchell 2013; Powell et al. 2015). Previous research has shown that burrow temperatures remain cooler during the day compared with surface temperatures at the study site, providing support for the function of burrows as a potential buffer from temperature extremes (Clayton and Bull, 2016).

In most cases, taxa sharing burrows were likely to not be a threat to one another, however, where there was the potential for one occupant to predate the other, the latter occupant would be observed close to the entrance of the burrow. This may indicate that the occupant had only moved into the burrow out of necessity, and/or it may have stayed close to the entrance in case it needed to escape the other potentially predatory occupant.

These results indicate that shared burrow use may serve multiple purposes. Dependent on the requirements of the occupant, the time of year, and the other taxa occupying the burrow, it is clear that some organisms within these grasslands do co-share under certain circumstances. This research provides an initial insight into the taxa that are likely to co-share burrows within these grasslands. The question of how this influences burrow-digging spiders and the endangered pygmy bluetongue lizard remains unanswered.

Co-sharing of taxa has the potential to benefit lizards (if they are prey items), but also has the potential to be detrimental. It is clear that co-sharing of burrows is very influenced by time of year, with most co-sharing occurring late in the lizard activity season, when external conditions are likely to be extremely hot. If co-sharing of burrows increases at this time of year due to increased demand for burrow space, it is plausible that there may be increased competition for burrows and the potential for less burrows to be available to lizards. The mechanisms leading to co-sharing of burrows, and consequences of this increase in co-sharing of burrows observed in late summer require further investigation in order to improve our understanding of how burrow-dwelling organisms in these grassland communities persist together and how this impacts pygmy bluetongue lizard populations.

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Tables

Table 1. The total number of observations of burrow occupants and instances where they were observed sharing burrows with other species.

	Total observations	Shared observations
Reptiles		
PBT lizard (T. adelaidensis)	535	6
Skink (Menetia greyii)	9	0
Spiders		
Wolf spiders (Lycosidae)	628	47
Trapdoor spiders (Mygalomorphae)	881	0
Redback spider (L. hasseltii)	2	1
other invertebrates		
Centipede (Chilopoda)	83	19
Weevil (Coleoptera)	328	77
Snail (Theba pisana)	126	58
Cockroach (Blattodea)	49	26
Beetle (Coleoptera)	15	6
Ant (Hymenoptera)	13	1
Unknown larva	1	0

Table 2. Repeated-measures ANOVA of the total number of burrows with co-sharing taxa, grouped by taxa (pbt-wolf spider, pbt-weevil, pbt-centipede, pbt-snail, wolf spider – weevil, wolf spider – centipede, wolf spider – snail, centipede – weevil, centipede – snail), in each month (Sep-Mar). P values in bold indicate significant effects (P<0.05).

	Df	F	P
Month	6,48	4.033	0.002
Co-sharing taxa	8,64	9.898	<0.001
Month X Co-sharing taxa	48,384	6.236	<0.001

Figures

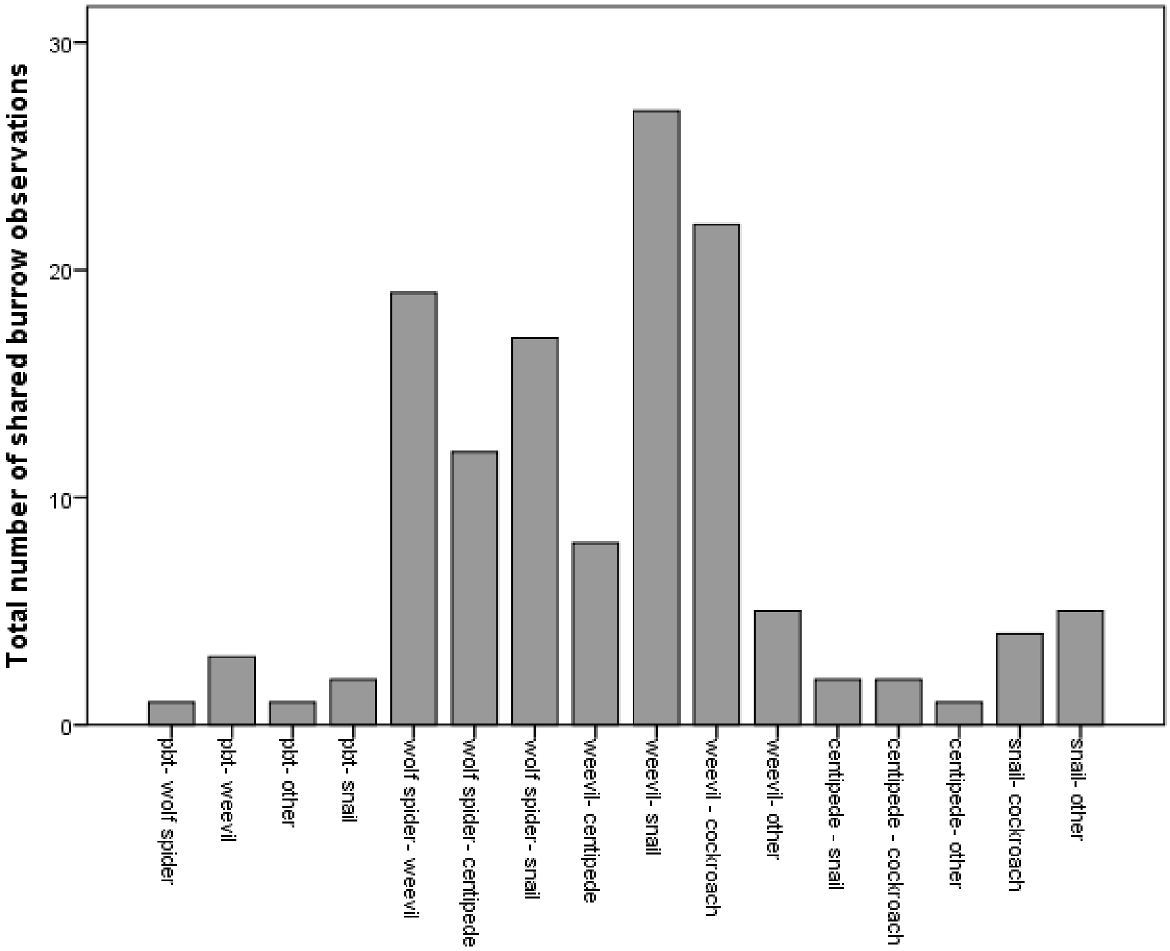

Figure 1. The total number of shared burrows for each taxa-taxa co-sharing option from Sep 2013-Mar 2014.

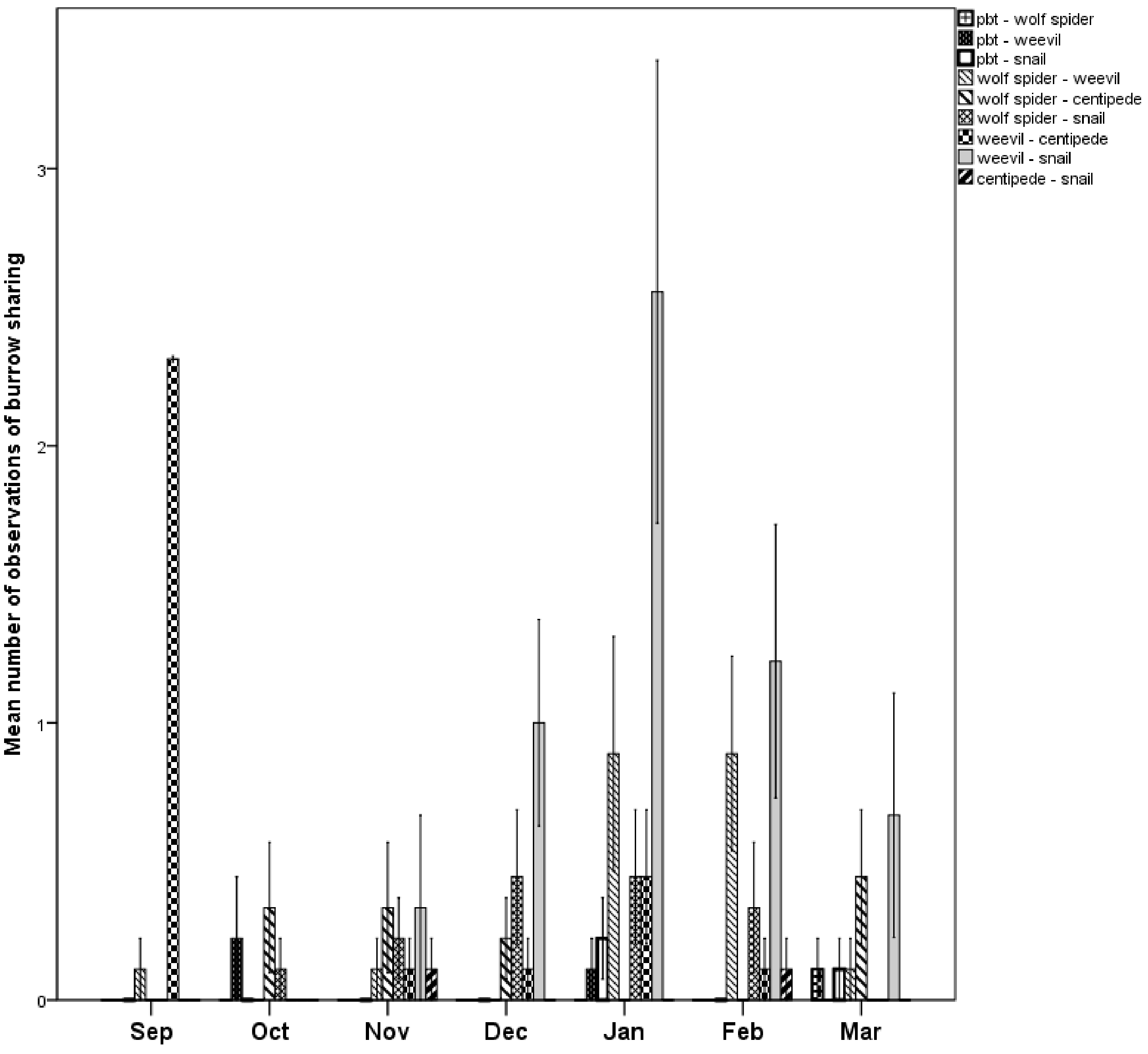

Figure 2. Mean (+/- 1 SE) number of observations with shared burrow use for each taxa-taxa combination (pbt-wolf spider, pbt-weevil, pbt-centipede, pbt-snail, wolf spider – weevil, wolf spider – centipede, wolf spider – snail, centipede – weevil, centipede – snail) in each month of the season (Sep 2013 – Mar 2014).

Appendix 3

A NON-DESTRUCTIVE METHOD FOR COLLECTION OF ADELAIDE PLAINS TRAPDOOR SPIDERLINGS (BLAKISTONIA AUREA) IN BURROWS

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INTRODUCTION:

The Adelaide Plains trapdoor spider (Blakistonia aurea) is a burrowing spider which is widespread in South Australia (Rainbow & Pulleine, 1918). It is a long-lived species, taking approximately 5 years to reach maturity. It uses its burrow as a refuge from predation and extreme climatic changes and as a point to catch passing invertebrate prey. Females also use their burrow to protect their young for the first few months of life, before the spiderlings move out to build their own (Main, 1957). These burrows provide a vital resource for the spiders themselves, but after the burrow has been vacated, it may also be a valuable resource for a wide-range of other species, including other spiders, insects and reptiles (Hutchinson et al., 1994; Clayton & Bull, 2016). The pygmy bluetongue lizard (Tiliqua adelaidensis) is an endangered species, endemic to the Mid North of South Australia and its range falls within that of B. aurea. Pygmy bluetongue lizards shelter exclusively within spider burrows, as they are not capable of digging their own burrows and are one such species which are reliant upon these burrows. As spider burrows are potentially a limiting resource for these lizards and for other burrow occupants, preservation of these burrows, rather than destructive sampling by burrow excavation, should be a considered when undertaking studies of the spiders. We developed a method of sampling spiderling trapdoor spiders (B. aurea) without destroying the adult burrow.

Previously, a common technique to collect spiders from their burrows involved excavation and destruction of the burrow. We describe a modified pooter apparatus to collect spiderlings non-destructively from trapdoor spider burrows. As far as we are aware, this is the first publication of this method to sample trapdoor spiderlings.

METHOD FOR MAKING POOTER:

The pooter was constructed by cutting two 1.5 cm diameter holes in the lid of a 500 ml plastic jar. Two lengths of 10 mm diameter flexible plastic tubing of approximately 40 cm and 50 cm respectively were cut and inserted into the 1.5 cm diameter holes. The tubes were then sealed into place using silicone glue, creating an air tight container when the lid was screwed back on. A piece of fine gauze mesh was attached over the end of the 40 cm tubing which was on the underside of the lid (facing inside the container). This was to ensure that debris is not brought up into the mouth of the user. Finally, the inside of the container was lined with cotton pads on the bottom surface, to reduce injury to any spiderlings captured.

METHOD FOR CAPTURING SPIDERLINGS:

A trapdoor spider burrow was located and inspected for spiderlings using an optic fiberscope (Medit Inc 2 way articulating FI Fiberscope). Once spiderlings were observed, the shape and orientation of the burrow, and position of the spiderlings was observed to assist in sampling the correct area and guiding the plastic tubing into the burrow. The lid was then opened enough to place the end of the longer (50 cm), uncovered tubing into the burrow (**Fig. 1**). Once inserted the tubing was guided down the burrow and the shorter, 40 cm tubing, which was covered by gauze mesh on the container end was used to suck in air and in turn, pull spiderlings from the burrow and into the pooter chamber where they would land on the cotton pad lining the bottom of the pooter. Care was taken to reduce any injury to spiderlings, by ensuring that the cotton lining was placed beneath the tubing and that air was not sucked too quickly.

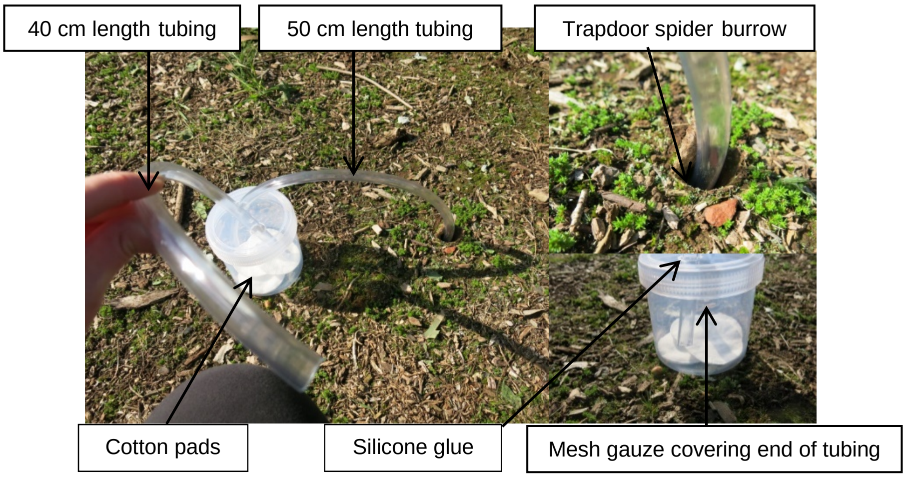

Fig. 1: Design of the modified pooter for collection of trapdoor spiderlings.

CONCLUSION:

Applying this method allowed us to sample a subset of spiderlings from a burrow, without destroying the burrow, and without killing the mature spider or killing the entire generation of spiderlings. This method was found to be suitable for spiderlings but unsuitable for adult spiders due to their ability to strongly hold onto the silk-lined burrow.

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Professor Mike Bull and David Attenborough during filming of 'Life in Cold Blood' (Photograph taken by Dale Burzacott)

To me, there seem to be two main approaches to conservation. You can spread resources widely, investing a little in a lot of different conservation interests, or you can focus deeply on one, gaining a deep understanding of a particular species over time, involving multiple stakeholders and ultimately aiming to improve the conservation outcome of that species. This is the approach that Professor Mike Bull took. The future of the pygmy bluetongue lizard is considerably brighter thanks to the attention and dedication that Mike invested in this species, and that he instilled in the people around him. Once again, thank you Mike for your time, patience and the wisdom you imparted over the years! You will be sorely missed by many people and lizards alike…