

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.

1. Introduction

 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 30cm, 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. **2. 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 (Figure 1). Rainfall was 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 208 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.

3. Results

Temporal changes in burrow dynamics in ungrazed plots

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. 297 Burrows retained in the next month $(n = 646$ burrows) were significantly deeper (Fig 4b), and had significantly wider entrance diameters (Fig 4c) than burrows 299 that were lost ($n = 229$ burrows).

4. 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

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.
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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.

5. 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.

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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

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.