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

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**Abstract.** The endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) is found only in a few remaining patches of South Australian native grassland, most of which are used for live stock grazing. The lizards occupy spider burrows, they mate in October-November and females produce litters of one to four live born neonates in mid-January-mid-March. In this study we use ultrasound scans of females and observations of neonates in their maternal burrows to investigate how grazing affects the fecundity of the pygmy bluetongue lizard. We predicted that lizards in moderately grazed paddocks would have a higher reproductive output than lizards in hard grazed paddocks. Ultrasound scans indicated that this hypothesis was correct by showing a higher mean number of yolk sacs in females from moderately than from hard grazed paddocks. Females from moderately grazed paddocks also gave birth significantly earlier than females in hard grazed paddocks. The higher number of yolk sacs did not result in a significantly higher number of neonates observed in the burrows, which indicates that the weekly burrow observations used in this study may underestimate true fecundity. Understanding how grazing affects the fecundity of the pygmy bluetongue lizard is essential to the future management of this endangered species. This is not only because grazing is used to manage the habitat of all currently known lizard populations, but also because successful reproduction will be needed in those populations to supply the “surplus” individuals predicted to be essential for relocation programs to ensure the survival of the species.

**Keywords:** conservation, grassland management, reproduction, ultrasound.

**Introduction**

Intensive grazing by domestic livestock presents a challenge to many native species (James, 2003; Blevins and With, 2011; Wouters et al., 2012; Pafilis et al., 2013; Howland et al., 2014) as the grazing has the potential to change vegetation and soil structure (Fleischner, 1994; Dorrough et al., 2004; Bertiller and Ares, 2011). A reduction in the vegetation cover by grazing animals means that ground living animals such as lizards have less cover, and this could lead to a higher predation pressure (Castellano and Valone, 2006) and potentially change lizard behaviour (Wasiolka et al., 2010). Less vegetation could also reduce the density of herbivorous insects (Rambo and Faeth, 1999; Dorrough et al., 2004; van Klink et al., 2015) and thereby reduce food availability to insectivorous lizards.

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

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

For any endangered species, understanding human impact on fecundity is essential for conservation management. Almost all of the native grassland habitat of the pygmy bluetongue lizard is now used for livestock grazing. Understanding the impact this grazing has on lizard reproduction is essential for the future management of the species. Grazing could increase the fecundity of pygmy bluetongue lizards by clearing the spaces between the grass tussocks, thereby enabling the lizards to thermoregulate and hunt more efficiently translating into better body condition in preparation for breeding. Pettigrew and Bull (2012) showed that lizards living in burrows where the surrounding vegetation had been removed basked for longer times and made more predation attempts than lizards in burrows with denser surrounding vegetation due to increased visibility. Overgrazing can, however, lead to reduced local abundances of grasshoppers (Nielsen, 2017), the lizard’s most common prey (Fenner et al., 2007; Ebrahimi et al., 2015), and therefore to lizards with lower body condition. Due to higher prey abundance and a lower predation pressure, we hypothesized that lizards would have higher fecundity in paddocks with a moderate level of grazing and an intermediate vegetation density, than in paddocks with more severe grazing pressure.

In this study we utilised a direct and an indirect method of estimating lizard fecundity. We used ultrasound scans of wild caught female lizards to measure the maternal investment in the form of produced yolk sacs, and optic fiberscope assisted observations to count neonates in the lizard burrows. Ultrasound scanning can be used to confirm whether female reptiles are gravid and to provide an estimate of clutch sizes for both oviparous and viviparous species (Gartrell et al., 2002; Gilman and Wolf, 2007; Sacchi et al., 2012). Early in gestation, fluid filled foetal membranes or yolk sacs can be seen in the scans as round or elongated structures, while embryonic movement, heart beat and skeletal features are visible during later stages (Gartrell et al., 2002; Gilman and Wolf, 2007). Maternal stomach, gut and other intestinal or skeletal structures can sometimes obscure the scanning image or appear similar to yolk sacs and embryonic membranes, but can in most cases be distinguished as they are more continuous structures (Gartrell et al., 2002; Gilman and Wolf, 2007). Some viviparous lizards also retain infertile yolk sacs throughout gestation, which can lead to overestimation of the actual litter size (Gartrell et al., 2002). Ultrasound scans have, however, been successfully used on lizards as small as 40-50 mm snout-vent length (SVL) (Gilman and Wolf, 2007; Sacchi et al., 2012). Estimates of clutch size become harder, with a tendency to overestimate, as clutch size increases (Gartrell et al., 2002; Gilman and Wolf, 2007) but Sacchi et al. (2012) reported that 20 out of 22 common wall lizards (Podarcis muralis) with a mean clutch size of 3.6, laid exactly the same number of eggs as predicted by ultrasound scans. Gillman and Wolf (2007) found a highly significant correlation between the clutch size predicted by ultrasound scans and the number of eggs laid in five other
species of lizards. Although, what is measured via yolk sac numbers is in fact a measure of maternal investment and not of fecundity. For a species where the yolk is provisioned early in the offspring’s development, rather than through pregnancy, it is likely that maternal investment correlates with fecundity. Therefore, although some caveats need to be made, we consider yolk sac numbers to be a valid, although indirect, indication of fecundity. It seems unlikely that an animal in a low resource system like semi-arid grassland would evolve a system whereby it wastes a lot of energy on producing yolk sacs, without any gain in fecundity.

For the pygmy bluetongue lizard, previous estimates of fecundity have been derived from observations of neonate lizards in the maternal burrows (Milne et al., 2002; Shamiminoori et al., 2015). Two previous studies using that method have reported different mean numbers of neonates per litter [Milne et al. (2002): 3.23 neonates per burrow; Shamiminoori et al. (2015): 1.58 neonates per burrow]. This difference may have been due to two sources of possible bias. First, female lizards occasionally block the burrow, remaining above their offspring and preventing reliable fiberscope observations. Second, some neonate lizards can disperse within their first week (Milne et al., 2002), so that a survey that first detects a litter a week after it is produced, may detect only a portion of the original litter members. Both errors can be minimised by doing frequent burrow checks throughout the breeding season, and should apply equally to all observations in a study. The observed differences could however also have reflected real fecundity differences resulting from different ecological conditions during the two studies.

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

Methods

The study was conducted during two lizard activity seasons (October to March 2013-2014 and 2014-2015) at the ‘Tiliqua’ property of the Nature Foundation of SA, near Burra, South Australia (33.67°S; 138.93°E) that has been previously described (Fenner et al., 2007; Pettigrew and Bull, 2011; Clayton and Bull, 2015). The study site is in an isolated patch of native grassland that has not been ploughed for at least four decades, but has been used for sheep grazing for over a century. The region has hot dry summers and cool wet winters with an average annual rainfall at Burra of between 400-500 mm. The study site was divided into six adjacent experimental paddocks (size range 3.5-6.9 ha), arranged in a north to south line along the eastern edge of the property, each with similar elevation and slope. During the study alternative sheep grazing regimes were applied to these paddocks in an alternate pattern. We took advice on stocking rates from the property manager, but the number of sheep applied to a particular grazing regime varied between the two seasons as different rainfall patterns led to variation in vegetation growth. All results are shown as means ± SE unless otherwise indicated. During the first season (2013-2014) half of the six paddocks were left ungrazed, while moderate grazing (2.70 ± 0.11 sheep/day/ha) was applied to the other half. In the second season (2014-2015), moderate grazing pressure (1.54 ± 0.15 sheep/ day/ ha) was maintained on the same three paddocks, while the three previously ungrazed paddocks were changed to a hard grazing pressure (2.69 ± 0.26 sheep/day/ha). We previously reported a significantly greater reduction in vegetation levels with increased grazing pressure (Nielsen et al., 2017). Very rarely we saw kangaroos in the paddocks, and their grazing impact was considered to be limited and spread evenly across all paddocks.

Season 1, 2013-2014

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

Between January 15 and February 13 2014, we inspected 342 burrows and recorded clutches of neonates in 39 burrows. Using an optic fiberscope, we inspected the natal burrows to count the number of neonates that could be seen
associated with each female. The mean litter size was compared between moderately grazed and ungrazed paddocks. However, we did not extract adult lizards from their burrows, and could not determine sex from the fiberscope images. Thus, females without litters could not be differentiated from males and therefore the proportion of females producing litters could not be determined in the first season.

**Season 2, 2014-2015**

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

From mid-January 2015, we checked all 38 burrows of the ultrasound scanned lizards for neonates weekly. When these surveys started three of the female lizards were no longer in their original burrows and they were excluded from any future surveys and analysis. We detected the first neonates on January 21, 2015, and weekly inspections were made over the next five weeks, with a final inspection in the second week. For each burrow in each survey we recorded if the adult female was still present and the number of neonates we could see. Adult mothers sometimes blocked the burrow so that the fiberscope probe could not pass, in which case only neonates that were above the adult could be recorded. Thus, the number counted may have been lower than the actual litter size. The maximum number of neonates recorded over consecutive inspections was used as an estimate of the litter size. Neonates typically leave their natal burrow at some time over the six weeks after birth (Milne et al. 2002), so subsequent surveys are also likely to underestimate actual litter size. Day of birth was calculated from January 21, the first observation of neonates in the second season. We assumed either a neonate or an adult female had dispersed from the burrow if it was not detected there on two consecutive surveys. A separation time was estimated for each burrow, from the birth date to the first time when only one lizard (either the female or a single neonate) was left in the burrow, or the burrow had been completely abandoned. Neonates are never found with adult lizards in the following spring and so adults and neonates must all separate sometime before they become inactive in winter. In this study any mothers and neonates that had not yet separated by the last survey in week seven (March 9), were assumed to have separated in the following week.

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

**Results**

**Season 1, 2013-2014**

Between 14 and 26 inhabited lizard burrows were located in each paddock. In burrows where neonates were detected in season one, there were no significant differences (Mann-Whitney

\[ U = 149.0; P = 0.29 \]

between the mean number of neonates counted in the burrows from ungrazed

\[ (2.29 \pm 0.29; n = 17) \]

and moderately grazed paddocks

\[ (1.86 \pm 0.22; n = 22) \]

**Season 2, 2014-2015**

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

The mean number of yolk sacs detected by ultrasound scans of the 35 gravid females differed significantly between the two grazing treatments (Mann-Whitney $U = 89.5; P < 0.05$) with more detected in females from moderately grazed paddocks than from hard grazed paddocks (fig. 1A).

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

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

![Figure 1](https://example.com/figure1.png)
We never found any infertile yolk sacs or dead neonates during any of the burrow checks in neither season one nor season two.

Females gave birth significantly earlier (Mann-Whitney $U = 7.5; P < 0.01$) (fig. 1B) and there was a non-significant tendency for females and neonates to stay together for a shorter time (Mann-Whitney $U = 18; P = 0.092$) (fig. 1C) in moderately grazed than in hard grazed paddocks.

Among the 13 females with live embryos observed through ultrasound scans, there were significantly more live embryos than detected neonates from the same females (Wilcoxon signed-rank $Z = −2.791; P < 0.01$) (fig. 2). We also found a non-significant tendency among the 17 females with yolk sacs detected during the ultrasound scans and with subsequent observations of neonates in the burrows, for a higher mean number of yolk sacs (2.29 ± 0.56) than observed neonates (1.82 ± 0.65) per female (Wilcoxon signed-rank $Z = −1.554; P = 0.12$).

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

**Discussion**

We found no significant effect of grazing treatment on the mean number of neonates observed in litters inside female burrows in either of the two seasons. In season two, when we could identify burrows with resident females, litters were detected in 46% of those burrows. In contrast, ultrasound scans of the same females from earlier in that season showed that 92% were gravid and that females from moderately grazed paddocks carried significantly more yolk sacs than females from hard grazed paddocks. The larger number of yolk sacs, as well as the significantly earlier litter production in moderately grazed paddocks indicate a higher reproductive fitness in these paddocks compared to hard grazed paddocks. This increased reproductive fitness is likely to be related to the previously reported higher body condition of lizards in moderately than in hard grazed paddocks.

We suggest two different explanations for why the ultrasound scans produced higher estimates of both the proportion of gravid females, and the mean litter sizes of those females, than the field observations of females with their litters. The first is that not all of the observed yolk sacs were fertile, or resulted in live neonates, so the count of yolk sacs overestimates the actual number of live births. Live embryos were detected by observing movement of embryos in only 13 females and it is possible that some of the yolk sacs were fertile, or resulted in live neonates, so the count of yolk sacs overestimates the actual number of live births. Live embryos were detected by observing movement of embryos in only 13 females and it is possible that some of the yolk sacs without movement did not support a live embryo. But it is also possible that the scanned yolk sacs may have all been alive but at different stages of development, so that only a proportion of them were sufficiently advanced to show movement. The optic fiberscope inspections of burrows never found any infertile yolk sacs or dead neonates in the burrows. However, other reptile species have previously been reported to ingest dead neonates and other embryonic material soon after parturition (Lanham and Bull, 2000; Rivas, 2000), and it is therefore possible that infertile yolk sacs were present at birth but not detected. Future studies should focus on developing reliable methods to estimate...
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fecundity of these endangered lizards, if possible by doing both ultrasound scans and burrow observations in captive caged populations where the number of live neonates produced at parturition can be more reliably determined. Then the relationship between ultrasound scan counts and actual litter sizes could be more rigorously established.

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

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

Although the ultrasound scans may overestimate the number of neonates born, the significantly larger number of yolk sacs produced by females in moderately grazed paddocks, suggests that these females could produce more neonates than females in hard grazed paddocks. We also showed that parturition happens earlier in moderate than hard grazed paddocks and since the neonates need to establish their own burrow refuges, and develop energy reserves during the late summer to allow them to survive the coming winter, an earlier birth is likely to give them an advantage. Our conclusion is that hard grazing leads to a reduced yolk sac production and delays the time of birth of litters of the pygmy bluetongue lizard, which could indicate a reduction in fecundity. This probably arises because excessive grazing reduces levels of vegetation to a degree that either significantly impacts the abundance of grasshoppers and other insects that the lizards feed on, and/or significantly increases the level of predation related stress because of lowered cover. Both of these factors can lead to decreased pre-mating body condition which have been shown to delay the time of birth in garter snakes (Thamnophis sirtalis) (Gregory, 2006). The outcomes of this study are entirely consistent with a parallel set of analyses that showed lizard body condition was relatively lower in the hard-grazed paddocks (Nielsen, 2017).

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

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References


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