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Artificial burrows with basal chambers are preferred by pygmy bluetongue lizards, *Tiliqua adelaidensis*.

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Suggested Running Head: Burrows with basal chambers for pygmy bluetongue lizards

Abstract

Natural refuges are sometimes supplemented with artificial refuges to enhance populations of endangered species, or to improve the success of translocation and relocation programs. The design and structure of these artificial structures should incorporate key features of natural refuges. We aimed to improve the design of artificial burrows currently used in the conservation of the pygmy bluetongue lizard, *Tiliqua adelaidensis*, by comparing burrows with or without a basal chamber. We found that lizards chose burrows with chambers significantly more often, but that neither the size of the chamber, nor the substrate lining the chamber influenced the choice. Incorporating a basal chamber into the design of artificial burrows should provide more favourable artificial refuges for these lizards and should be incorporated into future conservation management programs.

Introduction

All animals require refuge to shelter from extremes of climate and from predators, or as secure places for the production of offspring. Artificial refuges such as nesting boxes for birds and bats, are now widely used in conservation management (Goldingay and Stevens, 2009; Lambrechts et al., 2010), particularly where natural refuges have been reduced by anthropogenic activity (Gibbons and Lindenmayer, 2002). Consideration of the relative quality of nesting boxes and natural cavities for birds (Lindenmayer et al., 2009; Robertson and Rendell, 1990), has stimulated research on how varying structure can influence the protection offered by artificial refuges and the breeding success within them (Homan, 2000; Brazill-Boast et al., 2012).

Terrestrial reptiles typically refuge in burrows, rock crevices or hollow logs (Pianka and Vitt, 2003). Artificial cover items or burrows have been used to enhance local reptile populations, or to improve the likely success of translocations (Nelson et al., 2002; Webb and Shine, 2000, Grillet et al, 2010). Designing artificial refuges for reptiles has involved documenting properties of natural refuges (Goldsborough et al., 2006), replicating essential features of those refuges in artificial structures, and recording choices that reptiles make between alternative structures in the field (Lettnick and Cree, 2007), or in laboratory conditions (Arida and Bull, 2008; Mensforth and Bull, 2008). An implication is that the design that individuals consistently choose in trials, will more likely benefit a conservation program.

The pygmy bluetongue lizard, *Tiliqua adelaidensis*, is an endangered scincid lizard (SVL 85 - 105 mm) currently restricted by extensive agricultural clearance to a few remnant fragments of native grassland in South Australia (Hutchinson et al., 1994). Modeling suggests that translocations will be essential to conserve this species into the future (Fordham et al., 2012). The lizards spend most of their time associated with single entrance vertical burrows constructed by spiders (Milne and Bull, 2000). They refuge in the burrows, bask at their entrances, ambush invertebrate prey from them (Milne et al., 2003a), and vigorously defend them from approaching conspecifics (Fenner and Bull, 2011). A shortage of suitably deep burrows can limit population expansion (Souter et al., 2007). Thus supplementary artificial burrows might augment existing populations, and enhance the success of translocations.

In previous research pygmy bluetongue lizards have used artificial vertical burrows with entrance diameters and depths reflecting the preferred dimensions of natural burrows (Milne and Bull, 2000). They have been constructed either as simple holes in the ground, or as hollowed out wooden dowling tubes, hammered into the ground (Milne et al., 2003b) and have had uniform internal diameters. These have been readily accepted by the lizards both in the laboratory (Milne and Bull 2000), and in the field where occupying lizards showed no reduction in body condition or reproductive fitness (Milne et al., 2003b). Addition of these artificial burrows to one field site led to a rapid increase in local population density and an enhanced retention of juvenile recruits (Souter et al., 2004). Overall, these results suggest that artificial burrows could be useful tools in conservation programs.

However, lizards enter and emerge differently from natural and artificial burrows (Ebrahimi et al., 2012). In natural burrows they enter head first, and later emerge head first. In artificial burrows they enter head first, quickly back out tail first, then re-enter tail-first, to be positioned to emerge head first next time. This difference probably resulted from an enlarged basal chamber, where lizards could turn around inside natural burrows (Ebrahimi et al., 2012). Artificial burrows, as currently designed, have no basal chamber. The additional time that lizards spend exposed on the surface during the reversing behaviour into artificial burrows would increase exposure to predators, already an apparently significant threat in natural populations (Fenner et al., 2008), suggesting re-appraisal of the design of artificial burrows (Ebrahimi et al., 2012).

One option might be to increase the internal diameter uniformly along the burrow length, for instance by using a larger diameter peg to hammer holes into the ground, or larger diameter dowling. However, Milne and Bull (2000) showed that lizards prefer a burrow entrance diameter no bigger than the size of their head, presumably to block the entrance from potential rivals or predators. An alternative is to construct an artificial burrow with an enlarged chamber at its base, to allow lizards to turn around while inside the burrow.

We provided pygmy bluetongue lizards with artificial burrows with and without basal chambers to determine if they discriminated, and whether the size of the chamber and various structural features of the chamber influenced choices. Our overall aim was to improve the design of the artificial burrow for use in future conservation management programs.

Methods

Twelve wild-caught pygmy bluetongue lizards (8 males; 4 females) were individually housed in a room kept at 27^{o}C during the light phase (0700 – 1900h) and 20^{o}C during the dark phase of a 12:12 photo-cycle. Plastic cages (50 x 30 x 30 cm) were filled to 20 cm with sand, provided with a single, standard artificial burrow (see below) for shelter, and heated with a 60 W lamp during the light phase. Five two-day experiments were conducted in these cages during June and July 2011. Each experiment involved eight of the lizards (5 males: 3 females), so that each lizard was used in three or four experiments, with at least six days between experiments. Lizards were fed twice a week outside experimental times, and water was available *ad libitum*.

Experiments tested the preference of lizards among five alternative burrow structures. In burrows 1 – 4 all inside surfaces were lined with glued-on sand. (1) The standard artificial burrow was a 200 mm long, straight, cylindrical tube with an internal diameter of 20 mm, constructed from plastic tubing. Burrows were inserted at a 60^{o} angle into the substrate with the entrance was at the sand surface. (2) The tennis ball chambered burrow consisted of a 100 mm length of the same plastic tubing, inserted through a circular hole flush to the inside surface of a 100 mm diameter tennis ball. (3) The large plastic chambered burrow had a 150 mm length of plastic tubing inserted through a circular hole in the lid, and flush to the inside surface of a large cylindrical (60 mm diameter x 50 mm deep) plastic container. (4) The small plastic chambered burrow was constructed as above except that the tubing was 160 mm long, and the plastic container was 40 mm diameter and 40 mm deep. (5) The unlined large plastic chambered burrow was constructed as in (3) above except the sand lining was only applied to the plastic tubing and not to the chamber.

The first experiment was conducted in eight home cages where lizards had been refuging in their standard artificial burrows. Burrow entrances were 7.5 cm from one randomly allocated cage end. At 0930 h a tennis ball chambered burrow was buried into the sand 7.5 cm from the other end of each cage. Lizard behaviour was recorded for 6 h (1000 - 1600 h) on that day and on the next day, on video cameras mounted directly above individual cages. From the recordings we determined how long lizards spent either refuged in or basking at the entrance of each burrow on each day, and we used repeated measures analysis of variance to compare the times. Burrow type and trial day (day 1 or day 2 of filming) were within subject factors, and we interpreted a significant burrow type x day interaction to indicate a changing preference as the lizards became more familiar with the available choices. We also counted the number of emergences and entrances from each burrow type that were head first or tail first, and used chi-squared tests to determine if the proportion of head first entrances differed between the two burrow types.

The other experiments were similar in design and analysis, offering a choice of two alternative burrow types. We compared the response of lizards to the standard artificial burrow and a large plastic chambered burrow (experiment 2); to the standard artificial burrow and a small plastic chambered burrow (experiment 3); to small and large plastic chambered burrows (experiment 4); and to large plastic chambered burrows with and without sand lining in the chamber (experiment 5). In experiments 2 – 5, lizards were removed from their home cages at 0930 h while the two alternative burrow types were inserted at opposite ends, as in the first experiment. Lizards were then replaced centrally in their home cages, at 1000 h as filming began. Experiment 4 continued for an extra (third) day. Between experiments cages were cleaned with 70% ethanol, then with distilled water. Fresh sand and new burrows were provided for each experiment.

Results

During experiment 1, two lizards did not emerge from their original burrows and were omitted from analyses. For the remaining six lizards ANOVA showed a significant burrow type x day interaction (Table 1). Lizards spent significantly more time in the tennis ball burrow on the second day, but not on the first (paired t-tests; Day 1: t_5 =

2.06, P = 0.93; Day 2: t_5 = 61.30, P = <0.001) (Figure 1). In fact all six lizards spent the entire 6 h filming session of the second day in their tennis ball burrows.

Experiment 2 showed a significant main effect of burrow type but no significant burrow x day interaction (Table 1). Lizards spent more time in the large plastic chambered burrows and this response was consistent across the two filming days. Two lizards went directly to the large plastic chambered burrow and did not inspect the standard burrow over the filming period. The other six lizards entered the standard burrow first, but were all in the large plastic chambered burrow by the end of the second day.

Results for experiment 3 were similar to experiment 2, with lizards consistently spending significantly more time in the small plastic chambered burrow than the standard burrow (Table 1). For the first three experiments, lizards entered burrows during the filming period exclusively tail first in the standard burrows and exclusively head first in the chambered burrows. In each experiment the differences in method used to enter each burrow type were significant, experiment 1 (χ^2 = 111, d.f. = 1, *P* = <0.001), experiment 2 (χ^2 = 114, d.f. = 1, *P* = <0.001) and experiment 3 (χ^2 = 91, d.f. = 1, *P* = <0.001).

In experiment 4 there was no significant difference in the mean time that lizards spent in large and small plastic chambered burrows, and no indication of a preference developing over three days of filming (Table 1). Similarly, experiment 5 showed that lizards spent equal time in large plastic chambered burrows with or without sand lining, without developing any preference over two days of filming (Table 1).

Discussion.

The results from this captive colony of pygmy bluetongue lizards were quite clear, lizards consistently and significantly chose artificial burrows with basal chambers over those with no chambers. And lizards consistently entered chambered burrows head first and unchambered burrows tail first, confirming reports by Ebrahimi et al. (2012). The tail first option allowed lizards to subsequently emerge head first, but our results suggested lizards prefer burrow structures that allow them to enter head first, and turn around inside the burrow.

Burrows with three different sized basal chambers were all preferred over unchambered burrows, but lizards showed no preference between burrows with a large or a small chamber. Nor did they discriminate between chambered burrows with or without sand lining. Chamber presence, rather than its size or surface seemed to be the important criterion. We thought that sand might provide better grip for the lizards. That did not seem to make any difference in the perceived quality of the burrow, at least over the short time of our experiments.

Laboratory experiments on captive populations of wildlife will never replicate behaviour in natural populations, but the results from these trials were so convincing, that they encourage future development of a new design for artificial burrows that include basal chambers. In previous field studies, lizards readily adopted artificial burrows (Milne and Bull, 2000; Milne et al., 2003b, Grillet et al, 2010) and local populations increased when they were added (Souter et al., 2004, Doré et al., 2011a, 2011b), suggesting that burrows are a limiting resource, and that the more favoured burrow structure will have strong conservation benefits. A problem for pygmy bluetongue lizards is to develop a method to insert artificial burrows with wider bases than entrances, that leaves the soil structure, and the associated ecological and microclimatic processes intact. Efficient deployment of the newly designed artificial burrows, and their acceptance by lizards in natural populations, will be the subject of on-going research.

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References

Arida, E.A., Bull, C.M. (2008): Optimising the design of artificial refuges for the Australian skink, *Egernia stokesii*. Appl. Herpetol. **5**: 161-172.

Brazill-Boast, J., Pryke, S.R., Griffith, S.C. (2012): Provisioning habitat with custom-designed nest-boxes increases reproductive success in an endangered finch. Austral Ecol. (in press)

Doré, F., Grillet, P., Thirion, J-M., Besnard, A., Cheylan, M. (2011a): Implementation of a long-term monitoring program of the ocellated lizard (*Timon lepidus*) population on Oleron Island. Amphibia-Reptilia. **32**: 159-166.

Doré, F., Grillet, P., Thirion, J-M., Besnard, A., Cheylan, M. (2011b): Premiers résultats d'un suivi à long terme sur une population de Lézard ocellé *Timon lepidus* sur l'île d'Oléron. Rev. Ecol. (Terre et Vie). **66**: 205-214.

Ebrahimi, M., Fenner, A.L., Bull, C.M. (2012): Lizard behaviour suggests a new design for artificial burrows. Wildlife Res. **39**: 295-300.

Fenner, A.L., Bull, C.M.(2011): Central-place territorial defence in a burrow dwelling skink: aggressive responses to conspecific models in pygmy bluetongue lizards. J. Zool. **283**: 45-51.

Fenner, A.L., Bull, C.M., Hutchinson, M.N. (2008): Injuries to lizards: conservation implications for the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*. Wildlife Res. **35**: 158-161.

Fordham, D.A., Watts, M.J., Delean, S., Brook, B.W., Heard, L.M.B., Bull, C.M. (2012): Managed relocation as an adaptation strategy for mitigating climate change threats to the persistence of an endangered lizard. Glob. Change Biol. (in press) doi: 10.1111/j.1365-2486.2012.02742.x

Gibbons, P., Lindenmayer, D. B. (2002): *Tree hollows and wildlife conservation in Australia*. CSIRO, Collingwood.

Goldingay, R. L., Stevens J. R. (2009): Use of artificial tree hollows by Australian birds and bats. Wildlife Res. **36**: 81-97.

Goldsbrough, C.L., Shine, R., Hochuli, D.F. (2006): Factors affecting retreat-site selection by coppertail skinks (*Ctenotus taeniolatus*) from sandstone outcrops in eastern Australia. Austral Ecol. **31**: 326-336.

Grillet, P., Cheylan, M., Thirion, J.M., Doré, F., Bonnet, X., Dauge, C., Chollet, S. Marchand, M.A. (2010): Rabbit burrows or artificial refuges are a critical habitat component for the threatened lizard, *Timon lepidus* (Sauria, Lacertidae). Biodivers Conserv. **19**: 2039-2051.

Homan, P. (2000): Excluding the common myna *Acridotheres tristis* from artificial nest boxes using a baffle. Vic. Nat. **117**: 75.

Hutchinson, M. N., Milne, T., Croft, T. (1994): Redescription and ecological notes on the pygmy bluetongue lizard *Tiliqua adelaidensis* (Squamata: Scincidae) Trans. Roy. Soc. S. Aust. **118**: 217-226

Lambrechts, M. M., Adriaensen, F., Ardia, D. R., et al. (2010): The design of artificial nestboxes for the study of secondary hole-nesting birds: a review of methodological inconsistencies and potential biases. Acta Ornithol. **45**: 1- 26

Lindenmayer, D. B., Welsh, A., Donnelly, C., Crane, M., Michael, D., Macgregor, C., McBurney, L., Montague-Drake, R., Gibbons, P. (2009): Are nest boxes a viable alternative source of cavities for hollow-dependent animals? Long-term monitoring of nest box occupancy, pest use and attrition. Biol. Conserv. **142**: 33- 42

Lettink, M., Cree, A. (2007): Relative use of three types of artificial retreats by terrestrial lizards in grazed coastal shrubland, New Zealand. Appl. Herpetol. **4**: 227-243.

Mensforth, C.L., Bull, C.M. (2008): Selection of artificial refuge structure in the Australian gidgee skink. Pac. Conserv. Biol. **14**: 63-68.

Milne, T., Bull, C.M., (2000): Burrow choice by individuals of different sizes in the endangered pygmy blue tongue lizard *Tiliqua adelaidensis*. Biol. Conserv. **95**: 295-301.

Milne, T., Bull, C.M., Hutchinson, M.N. (2003a): Use of burrows by the endangered pygmy blue-tongue lizard *Tiliqua adelaidensis* (Scincidae). Wildlife Res. **30**: 523-528.

Milne, T., Bull, C.M., Hutchinson, M.N. (2003b): Fitness of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis* in artificial burrows. J. Herpetol. **37**: 762-765.

Nelson, N.J., Keall, S.N., Brown, D., Daugherty, C.H. (2002): Establishing a new wild population of tuatara (*Sphenodon guntheri*). Conserv. Biol. **16**: 887-894.

Pianka, E.R., Vitt., L.J. (2003): Lizards: Windows to the Evolution of Diversity. University of California Press, Berkeley.

Robertson, R. J., Rendell, W. B. (1990): A comparison of the breeding ecology of a secondary cavity nesting bird, the tree swallow (*Tachycineta bicolor*), in nest boxes and natural cavities. Can. J. Zool. **68**: 1046-52.

Souter, N.J., Bull, C.M., Hutchinson, M.N. (2004): Adding burrows to enhance a population of the endangered pygmy blue tongue lizard, *Tiliqua adelaidensis*. Biol. Conserv. **116**: 403-408.

Souter, N.J., Bull, C.M., Lethbridge, M.R., Hutchinson, M.N. (2007): Habitat requirements of the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*. Biol. Conserv. **135**: 33-45.

Webb, J. K., Shine, R. (2000): Paving the way for habitat restoration: can artificial rocks restore degraded habitats of endangered reptiles? Biol. Conserv. **92**: 93-99.

Table 1. Results from repeated measures ANOVAs for each of the five experiments. P values in bold are significant at P < 0.05.

Experiment	**Burrow**			**Day**			**Burrow x Day**		
	d.f	***F***	***P***	***d.f***	***F***	***P***	***d.f***	***F***	***P***
1.Standard vs Tennis Ball	1, 5	88.97	**<0.001**	1, 5	1.51	0.273	1, 5	26.01	**0.004**
2. Standard vs Large chamber	1, 7	28.5	**0.001**	1, 7	0.304	0.598	1, 7	0.704	0.429
3.Standard vs Small chamber	1, 7	8.216	**0.024**	1, 7	0.364	0.565	1, 7	1.567	0.251
4. Large vs Small chamber	1, 7	0.050	0.830	2, 6	0.382	0.562	2, 6	0.318	0.623
5. With vs Without sand lining	1, 7	0.326	0.586	1, 7	0.125	0.170	1, 7	1.223	0.305

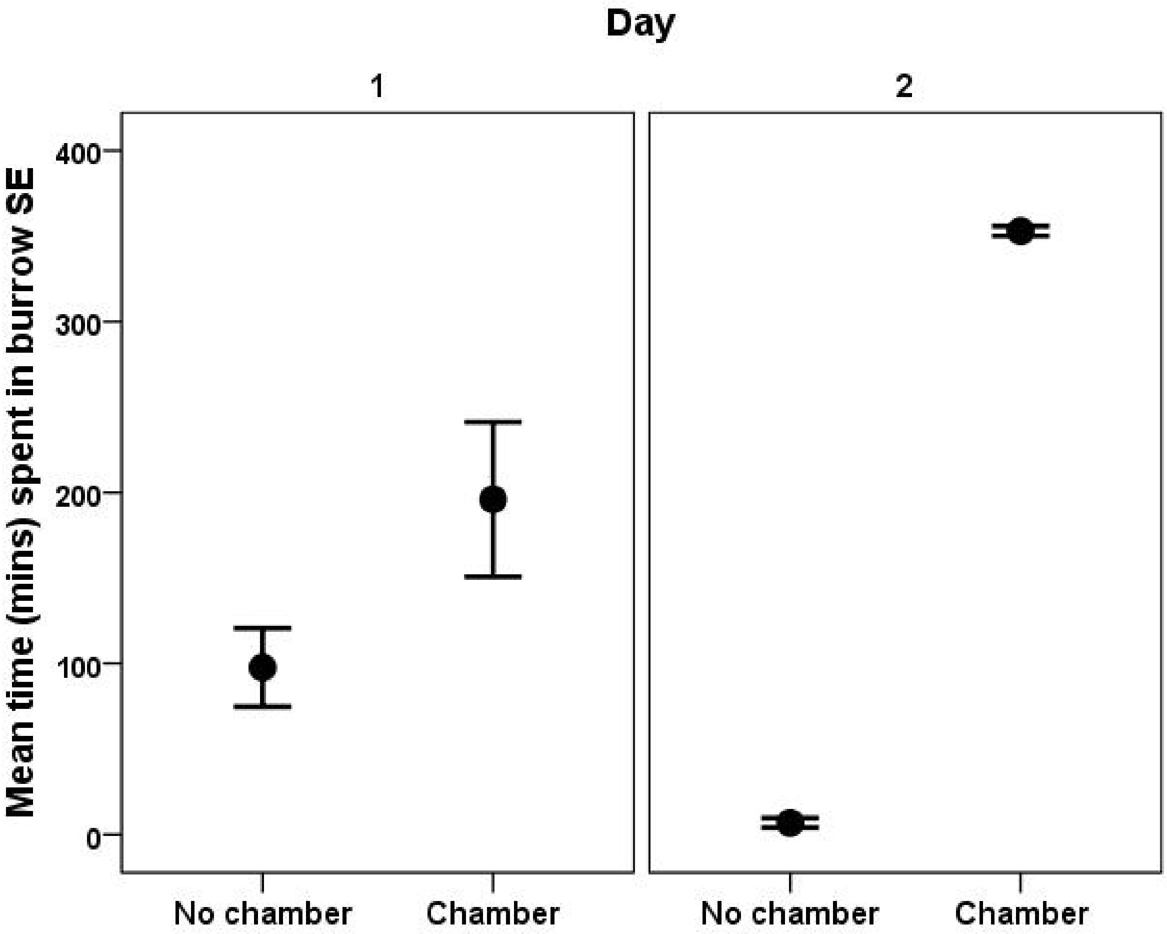

Figure 1. Mean (SE) time in minutes lizards spent in the standard artificial burrow (No chamber) and the tennis ball burrow (Chamber) on each of the two filming days of experiment 1.