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Varying short-term confinement time during simulated translocations of the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*)

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Abstract

Translocation is a powerful tool in conservation management, but one of the major problems of this tool is dispersal after release because of a tendency of animals to disperse from unfamiliar sites. We assessed whether short-term confinement within enclosures at the translocation site can significantly decrease post release movement, if confinement allowed animals to become familiar with the new habitat, and to overcome handling related stress. We simulated the translocation of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis* into the centre of a large enclosure and compared the behaviour between individuals confined to the central region for one or five days before release. We found that lizards confined for five days spent less time basking, and were more likely to disperse than lizards confined for just one day. We suggest that short-term confinement of lizards induces additional stress and that extra days of short-term confinement will not necessarily improve the success of a translocation.

Keyword: Soft release, Translocation, endangered, lizard, *Tiliqua adelaidensis*, Conservation.

Introduction

One of the more common practices in wildlife management and conservation biology is translocation and relocation (IUCN, 1998). The success of a translocation program can be measured by the survival rate and breeding success of the translocated individuals (Griffith et al, 1989; Reynolds et al, 2008; White et al, 2003; Wolf et al, 1996), but what determines whether a translocation will be successful is not yet well understood. Potential factors include the suitability and novelty of the new habitat, the degree of social disruption following the translocation, and the level of stress during the handling and release process (Curio, 1996; Heidinger et al, 2009; Letty et al, 2000). Each of these factors could cause high rates of mortality or dispersal from the translocation site very soon after the release event (Armstrong et al, 1999; Boissy, 1995; Schoech et al, 2008). Leaving the site soon after release could result in not being able to locate adequate resources; being more susceptible to predation; exposure to climatic extremes; and being away from potential mating partners (Bright and Morris, 1994; Rosatte et al, 2002; Teixeira et al, 2007).

Translocations and reintroductions have had limited success in reptiles (20-40% successful; Dodd and Seigel, 1991; Germano and Bishop, 2009), with handling stress and immediate dispersal after translocation cited as contributing factors. Translocations to small islands, where dispersal is restricted, have had greater success (Dickinson and Fa, 2000; Knapp, 2001; Nelson et al, 2002). This suggests that reducing the opportunities for dispersal following release may be an important factor for successful translocation in reptiles. In mainland translocations of reptiles such as gopher tortoises, soft release (keeping the animals in enclosures for a period of acclimatization to the release site before final release (Kleiman, 1989; Teixeira et al, 2007)) has improved the rate of retention of released individuals compared with hard release strategies (animals released directly into a new site without any pre-adaptation or human support after release) (Attum et al, 2010; Tuberville et al, 2005; Tuberville et al, 2008).

Animals may only require a short confinement of a few days to overcome the stress induced from captive handling, and to develop some preliminary familiarity with the site, but they might require longer periods to familiarise themselves with the new site more completely (Tuberville et al, 2005). Longer confinements will, however, be more expensive to maintain, and may increase the dependency of the animals on provided resources, and thus perhaps reduce longer-term success of the translocation.

We investigated the success of two different short-term durations of confinement in decreasing dispersal after release of the pygmy bluetongue lizard, *Tiliqua adelaidensis*. This species is found in only a few small fragments of native grassland in the mid-north of South Australia. The habitat in its previous range has been substantially reduced by agricultural activities, and the lizard is classified as endangered (IUCN, 2011). Fordham et al (2012) have shown that, under realistic climate change scenarios, the current population sites of lizards will decrease in quality, but that translocation of lizards into parts of their previous range will allow the species to persist. Thus the development of procedures for optimising translocation success in this species has become a management priority.

The pygmy bluetongue lizard is a scincid lizard, and the smallest member of the genus *Tiliqua* with an average adult snout-to-vent length of 95 mm (Armstrong and Reid, 1992; Armstrong et al, 1993; Hutchinson et al, 1994).The lizards occupy narrow vertical burrows as refuges, and bask at the burrow entrance to ambush passing invertebrate prey (Hutchinson et al, 1994; Milne et al, 2003). Individual lizards have very small spatial requirements, and can occupy burrows as close as 1 m apart. Resident lizards infrequently move from their burrows (Milne et al, 2003), and even restrict their aggressive burrow defense to a distance that does not require them to completely emerge (Fenner and Bull, 2011a). Rare movements beyond the burrow are for prey capture (Milne et al, 2003), defecation (Fenner and Bull, 2010), males searching for females in the spring (Fenner and Bull, 2009), or for seeking new burrows (Fellows et al, 2009; Fenner and Bull, 2011b). These observations of restricted movement have been confirmed by reports of significant genetic structuring between sample sites within a single population that suggest restricted gene flow even among small patches of continuous habitat (Smith et al, 2009). These observations all suggest that realistic simulations of translocation of lizards can be conducted within relatively small enclosures.

Our aim was to explore behaviours during the short time frame immediately following a release, and to compare two alternative soft release strategies for their impact on minimising this dispersal.

Methods

In our experimental system we simulated the initial phases of a translocation release within the central part of large circular cages. Within those cages we monitored behaviours that might lead to dispersal, and we derived the tendency to disperse by the number of times lizards moved from that central area across a less hospitable matrix, to burrows around the inner circumference of the cage. We have already used this system to show that adding supplementary food within the release site reduced the tendency of lizards to disperse (Ebrahimi and Bull, 2012a).

We captured sixteen pygmy bluetongue lizards (eight males and eight females) from two populations near Burra, South Australia (33º42´S; 138º56´). Experiments were conducted in four 15 m diameter circular cages at Monarto Zoo, approximately 70 km SE of Adelaide (35°06′S; 139°09′E). Each cage had a 1 m high-galvanised iron wall and was covered with a bird-proof wire roof. The cages were in line, and adjacent cages were about 5 m apart. Each cage was divided into three areas; a) a 4 m diameter circular central area that was lightly grassed and was the area where lizards were released; b) a surrounding 5 m wide ring of bare ground that represented an unsuitable matrix, c) an 0.5 m wide ring around the perimeter of the cage (which we called the marginal area).

Experiment Design and Data Collection

We constructed artificial burrows from 30 cm lengths of 3 cm diameter wooden dowling with a 2 cm diameter hole drilled out of the centre. We used an auger to make 30 cm deep and 3 cm diameter holes in the ground and hammered the artificial burrows into these holes until they were flush with the ground surface. Lizards have accepted these type of burrows as refuges previously (Ebrahimi and Bull, 2012b; Ebrahimi et al, 2012). The central area of each cage had 41 artificial burrows. One burrow was located in the centre of the cage, and 40 were spaced evenly in three concentric rings 65-75 cm apart. There were no burrows in the matrix of unsuitable habitat, but 30 additional burrows were spaced evenly around the perimeter ring of the marginal area, to monitor lizards if they dispersed from the central release area. We cut the grass in all areas of each cage to ground level before the experiment started, to allow clear images of lizard behaviour.

We released two male and two female lizards into separate burrows in the central region of each of the four cages, at 0700 h on 25 Oct 2009. We initially prevented lizard dispersal by fencing the central area with a 20 cm high black plastic wall. In two cages we removed the wall at 0700 h (before lizard activity had started) on 26 Oct, one day after the initial release. In the two other cages we removed the wall at 0700 h on 30 Oct, five days after the initial release.

Four surveillance cameras (Longse: LICS23Hf, 3.5 mm lens) were mounted above each cage to monitor lizard activity in the central area. The cameras filmed all lizard activity in that area during daylight hours from 0700-1800h for ten days from 25 Oct – 3 Nov. The footage was recorded on a 16 channel h.264 DVR (ESW26, Economical), powered by four 12 V batteries. We checked the status and location of each lizard each morning before filming and each evening after filming by using an optical fiberscope (Olympus IF8D4X2-10L) and portable light source (Olympus KLS-131) to inspect all of the artificial burrows (Milne and Bull, 2000). Temperatures were recorded every day by two digital thermometers, placed in shade at each end of the line of cages. These temperatures were always within 1-2^{o} C of recordings from a weather station at Pallamana Aerodrome (35.07° S 139.23° E), 10 km from Monarto Zoo.

From video recordings, and inspections, we calculated seven parameters that described lizard behaviour on each day as follows:

1) Activity time for each lizard on each day was defined as the total time from when the lizard head first emerged from its burrow entrance in the morning to when the lizard retreated into its burrow for the last time for that day. This activity time could include periods when the lizard had temporarily retreated into its burrow during the day (for periods ranging from several seconds to several hours).

2) The mean basking time per hour, was the total time on each day that each lizard spent basking at the entrance of its burrow, divided by the total filming hours of that day when we knew the lizard was in the central area. A lizard was defined as basking if at least a portion of its head was emerged. Lizards never basked when they were away from their burrows

3) Movement (two parameters): A lizard was defined to have moved when it had completely emerged from its burrow to move around the cage area, forage, or defecate. Some movements ended when the lizard returned to its previous burrow. Other movements resulted in the lizard entering a new burrow. Thus we recorded two movement parameters, the total number of movements by each lizard each day, and the number of movements that led to a burrow change.

4) We also recorded the number of lizards in a cage that moved to the marginal area of that cage each day. This was determined by two visual inspections of the marginal burrows, one early in the morning and one late in the afternoon.

5) In cases where a lizard changed its burrow, we estimated the minimum distance of movement (cm), as the straight line distance between consecutively occupied burrows. When a lizard moved within the central area, movements between burrows were observed directly on the video recording. When a lizard moved from the centre to the marginal area, the marginal burrow it was located in that evening was assumed to be its first destination.

6) The number of fights per lizard per day included any incident of agonistic interaction between two lizards. Lizard gender was not included as a factor.

We were unable to get a complete data set for all lizard behaviours because some of the lizards moved to the marginal area and out of the field of view of the cameras during some days. In analyses of activity time and number of moves, we used each cage as the replicate with the mean values per cage for the fully documented lizards in that cage on that day. For other behavioural parameters we used average data per hour from each lizard from the period when the lizard was in the central area.

Analysis

Our analyses were designed to compare the behaviour of lizards that had been confined to a simulated release site for a short (one day) or for a longer (five day) period. We asked whether variation in the confinement time affected the tendency of lizards to disperse from the release site in the period immediately after the confining conditions were removed (or whether it affected behaviours, such as movement between burrows, activity time, agonistic interactions and time spent basking, that might be related to dispersal tendencies). We compared the first five days of filming after the wall was removed in each cage, namely days 2-6 in the two cages where the wall was removed after one day, and days 6-10 in the two cages where the wall was removed after five days. We also analysed data from the last five days of filming (days 6-10) in each treatment, but have not presented those results here. The trends in each analysis were identical. We compared lizard behaviour between the two treatments using the seven behavioural parameters described above. We used repeated measures ANOVA with day as the within subjects factor, and treatment (wall removed on day 1 or day 5) as the between subjects factor. Lizard gender was not included, because we were exploring generalised trends, and because our relatively low sample size restricted the number of variables that could be considered in the analyses. We used the Greenhouse-Geisser correction where data were non-spherical. The effect of temperature on lizard behavioural parameters was examined by Pearson correlation.

Results

We recorded 3535 activity events from the 16 lizards during 10 days of filming. Of those events, 2989 (84.6%) were observations of basking at the burrow entrance, and 504 (14.3%) were of lizards moving out of their burrows. There were 21 (1.2%) observations of lizards fighting each other. Among the 504 moves, there were 314 cases (62.3%) where lizards returned to the same burrow, 144 cases (28.6%) where lizards moved to a new burrow in the central region, and 46 cases (9.1%) where lizards moved to marginal burrows.

Effect of Ambient Temperature

Daily maximum temperatures varied by almost 20^{o}C over the ten day filming period , although we found no difference between treatments in the mean values of temperature parameters on each day for days one to five after wall removal (removed after one day: 26-30 Oct; removed after five days: 30 Oct – 3 Nov) (paired t-tests: average temperature: t_4 =1.27, p = 0.27; maximum temperature: t_4 =1.13, p = 0.32; minimum temperature: t_4 =1.14, p = 0.32). Only two of the behavioural parameters we examined were significantly correlated with daily temperature measures. Basking time per hour was significantly negatively correlated with daily maximum ($r = -0.923$, $p < 0.001$) and with daily average temperature ($r = -0.925$, $p < 0.001$); lizards spent less time basking on hotter days (Fig 1). There was also a significant positive correlation between minimum daily temperature and the number of lizards that moved to the marginal areas ($r = 0.810$, $p < 0.005$); lizards were more likely to move away from the central area after warmer nights.

Effect of Treatment: Wall Removed After One Day or Five Days

Comparisons of lizard behaviour between treatments in the five days after wall removal are shown in Table 1. There were no significant differences between treatments for total activity time, total movements, the number of times lizards changed their burrows, the distance of movement, or for the numbers of agonistic interactions. However, there were significant differences between treatments for mean basking time per hour, and for the number of lizards per cage that moved to the marginal area.

For mean basking time per hour there was a significant interaction effect of treatment and day (Table 1). Fig 2 shows that the difference between treatments in mean basking time varied from day to day, although there was a consistent trend for lizards to bask longer when the wall was removed after one day (22.05 ± 0.56 mins/hr), than when the wall was removed after five days (13.25 ± 0.45 mins/hr).

For the number of lizards that moved to the marginal area there was also a significant interaction effect of treatment and day (Table 1). The amount of difference varied from day to day, but there was a consistent trend for more lizards to move to the marginal area when the wall was removed after five days (mean 0.8 ± 0.14 lizards per cage per day), than when the wall was removed after one day (mean 0.15 ± 0.09 lizards per cage per day) (Fig 3).

We also observed in the video recordings, 24 attempts to get past the plastic wall during days 3 – 5, by six of the eight lizards in the cages where the wall was in place for five days. These lizards moved up to the wall, were deflected from their path, and then moved along the wall edge for up to 1 m. This behaviour was not observed in any lizards in the one day when the plastic wall was present in the other treatment group.

For the minimum distance moved when a lizard changed burrows, there was a significant interaction effect of treatment and day but no consistent main effect of treatment (Table 1). Lizards moved further in one treatment than the other on some days, but that difference was reversed on other days.

Discussion

On the question of short term confinement benefit for translocation release of the pygmy bluetongue lizard, this study found that one day confinement was better than five days. After the plastic wall was removed, two behavioural parameters mean basking time per hour and the number of lizards that moved to the marginal area, showed consistent differences between the two treatments. When the lizards had been confined for five days, they spent less time for basking, and they moved from the central area to the marginal area more often than when they had been confined for one day. Our analysis indicated this was not an effect of differences in ambient temperature. In the context of translocated lizards, a shorter basking time suggests that they were less settled in their burrow occupancy, perhaps as a result of higher stress. A higher rate of movement out of the central area suggests they were less likely to remain at the translocation release site.

Short term confinement might actually add to the stress of the translocation process (Adams et al, 2011). Translocated male rabbits were found to require time to explore their surroundings and their social neighbourhood (Letty et al, 2000), and restrictions that prevent that exploration in both rabbits and lizards may cause stress. We noted that lizards confined for five days made repeated attempts to cross the wall, and this may have led to an accumulating increase in their stress levels over those five days. In contrast those lizards confined for a single day may have suffered little stress beyond the initial handling and release into an unfamiliar site, and may have quickly recognised the absence of suitable burrows in the matrix beyond the central region. The lower stress levels in lizards confined for just one day may then explain why those lizards basked for longer and why they were less inclined to disperse away from the release site (Teixeira et al, 2007).

Implications for the translocation procedures for pygmy bluetongue lizards, are that extended short-term duration of confinement does not appear to have benefits over shorter confinement (at least comparing five days to one day). If anything, the results suggest translocated individuals will be more stressed and more likely to disperse if confined for the longer period. So should we consider removing the confinement step all together? Although we have not directly tested this, we believe that pygmy bluetongue lizards should be confined for at least a day to allow them to recognise and accept the resources provided at the release site, such as supplementary food, which decreases post release movement (Ebrahimi and Bull, 2012a), and artificial or natural burrows. Other research on other species has suggested that hard release translocation with no confinement at all might be less successful (Bright and Morris, 1994; Carbyn et al, 1994; Davis, 1983). Our research has not tackled the alternative strategy of much longer term confinement to allow lizards to adjust to the release site conditions over a longer period. Gopher tortoises showed increased site fidelity and a decreased activity area at the translocation release site after a long-term confinement, probably resulting from more complete site familiarisation, for instance after a period of hibernation while still confined (Tuberville et al 2005).   However, that strategy comes with additional costs of maintenance and infrastructure that may stretch limited conservation management budgets.

It is important to emphasise that we only investigated one early component of the simulated translocation process, and with a relatively small sample size of lizards. But our view is that a full understanding of the translocation process requires detailed exploration of the individual processes that take place. A critical requirement for translocation success is that individuals remain in the area where they are released in the period immediately after the release. This is because the release site has often been chosen, or manipulated, to provide optimal conditions for subsequent survival, and any dispersal will normally be to less optimal conditions. Additionally, dispersal will reduce the chance of successful mating, and increase the time that individuals are exposed to predators and climate extremes. Thus management strategies that reduce the tendency to disperse in the period immediately after release will be important. We hope that this, and other simulation experiments, such as the trials where supplementary food was added (Ebrahimi and Bull, 2012a) will provide firm indications of appropriate procedures to ensure that lizards are likely to remain where they are released, and that these results provide a strong foundation for more realistic translocation trials in the future.

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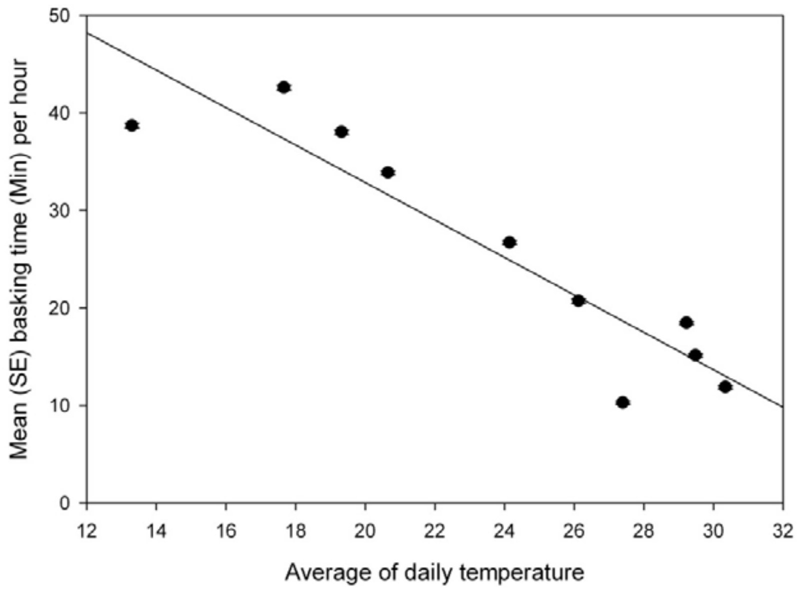
Table **1**. Repeated-measure analyses of variance for *T. adelaidensis* behaviours in response to removing temporary plastic wall after one or five days comparing the first five days after wall removal. Significant P values are indicated with *.

Behavioural parameter		Effect Treatment	Day	Treatment x Day
Activity time	***F***	5.042	1.963	1.131
	P value	0.267	0.265	0.454
	df	1, 2	4, 8	4, 8
Total movement	***F***	0.943	1.960	0.856
	P value	0.509	0.265	0.55
	df	1, 2	4, 8	4, 8
Changing burrows	***F***	0.869	0.976	2.618
	P value	0.029	0.437	0.056
	df	1, 14	4, 8	4, 8
Fights	***F***	1.129	0.747	1.050
	P value	0.363	0.416	0.095
	df	1, 14	4, 8	4, 8
Basking time	***F***	33.346	3.893	13.662
	P value	0.001*	0.012*	0.001*
	df	1, 14	4, 8	4, 8
Move to marginal area	***F***	6.443	1.370	3.657
	P value	0.039*	0.270	0.016*
	df	1, 14	4, 8	4, 8
Distance of movement	***F***	1.644	2.277	11.495
	P value	0.241	0.086	0.001*
	df	1, 14	4, 8	4, 8

FIG 1. Relationship between mean basking time (min) per hour and average daily temperature (ºC).

FIG 2. Mean (SE) basking time (mins per hour) in cages where the wall was removed after one day, and where the wall was removed after five days, in the first five days after the wall was removed.

FIG 3. Mean (SE) number of lizards that moved to the marginal area in each cage in cages where the wall was removed after one day, and where the wall was removed after five days, in the first five days after the wall was removed.



Mean (SE) basking time (Min) per hour
0
10
20
30
40
50
12
14
16
18
20
22
24
26
28
30
32
Average of daily temperature

FIG 1.

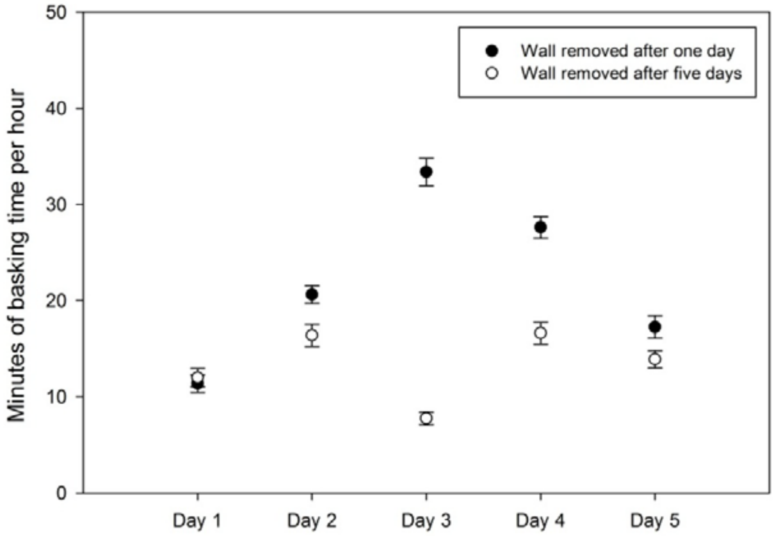

Wall removed after one day
Wall removed after five days
Minutes of basking time per hour
0
10
20
30
40
50
Day 1
Day 2
Day 3
Day 4
Day 5

FIG 2.

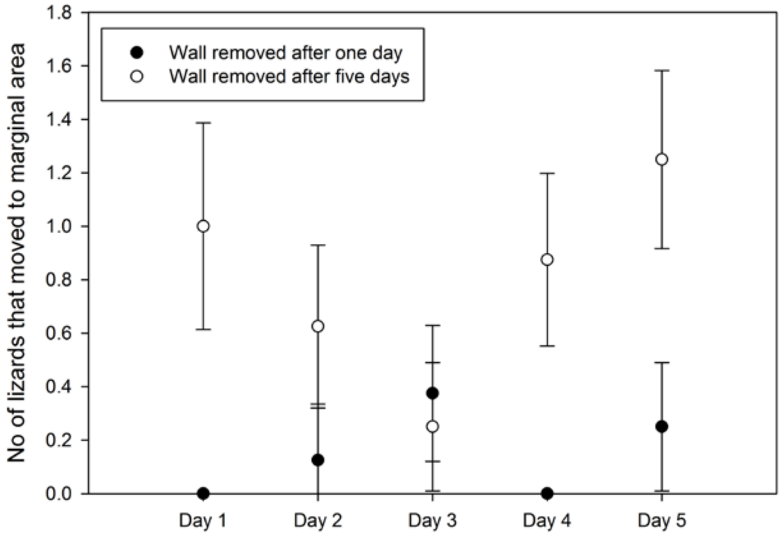

Wall removed after one day
Wall removed after five days
No of lizards that moved to marginal area
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Day 1
Day 2
Day 3
Day 4
Day 5

FIG 3.