Optimising translocation strategies in the conservation management of reptiles; a case study of an endangered Australian skink *Tiliqua adelaidensis*

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SUMMARY

Translocation is a powerful tool in conservation management. It is relevant for species that have lost a large part of their habitat, or live in a highly fragmented habitat, or have suffered intensive land degradation such as agricultural activities or urban developments. It is also relevant for species that are faced with exotic predators or diseases, and for those with a current distributional range that may become unsuitable with future climate changes. There are a range of terms currently used to describe translocations to augment existing populations, or to introduce individuals to a currently unoccupied site that may be within or outside of the known historical range. Reintroduction, assisted colonisation, reinforcement and ecological replacement are all considered different kinds of translocation performed for different purposes. However, the ultimate goal of each is to increase the chance of survival of a threatened species, with the different approaches appropriate to use in different circumstances. The success of any translocation may be diminished by the high tendency of translocated individuals to disperse from the release site. Many successful translocations have involved releases on islands where a geographical barrier prevents dispersal. For translocations to mainland sites, conservationists have attempted to reduce dispersal with different methods such as soft release strategies (containing the released individuals for a period of time while they adjust to the novel conditions at the release site) or by adding supplementary food. An important component of investigations into translocation success is behavioural changes in the translocated individuals. Behavioural ecology may help conservationists understand how individuals react

to the stress and the novel conditions they experience in the translocation process, and how best to adjust management procedures to minimize the impact of these behavioural changes.

In this project we conducted a series of simulated translocation experiments, within large circular cages, on the endangered Australian skink, the pygmy bluetongue lizard (*Tiliqua adelaidensis*) and recorded individual behaviours under alternative release conditions to understand how change those conditions might modify behaviour and improve translocation outcomes.

The pygmy bluetongue lizard was thought to be extinct until 1992 when it was rediscovered from the stomach of a dead elapid snake, *Pseudonaja textilis,* near Burra in the mid north of South Australia. Pygmy bluetongue lizards use narrow vertical and single entrance burrows that are made by lycosid and mygalomorph spiders. They occupy vacated burrows and use them as refuges from climatic extremes and from predators, and they use the burrow entrances as ambush sites from which they detect passing invertebrate prey. They leave their burrows rarely for catching prey, defecation, finding mates, or for moving to new and better burrows. This species lives in highly fragmented native Australian grasslands, and all of the few populations now known are on privately owned land. Simulation modeling of climate change scenarios has suggested that translocation will be an essential long-term conservation management strategy for this species. Because there is a high tendency for translocated reptiles to disperse from translocation sites, there is a need to document the set of conditions that will minimize this tendency for this species. This information may be helpful for future translocation program for other reptile species too.

I did 10 simulated translocation experiments with pygmy bluetongue lizards. I changed one factor in each experiment. Experiments included adding supplementary food, changing vegetation density, providing higher burrow densities and so on. In each experiment I video-recorded all lizard behaviours during the normal daily activity time. This allowed me to compare lizard behaviour in alternative control and experimental conditions. I investigated how the factor I changed could increase or decrease risk of dispersal and at the same time how other behaviour such as basking, movement and agonistic interaction toward each other changed. A summary of the results is that dispersal was reduced when lizards had higher vegetation, more supplementary food, more burrows available, or burrows more tightly clustered, and when lizards were confined to the release area for a short time period. Dispersal was also reduced if the surrounding area was disturbed, if there were fewer conspecific cues (implying that higher release density may lead to higher dispersal, and if releases took place later in the activity season.

During the experiments I also gathered information about the natural history of this species. I found that the spiders, that are important ecological engineers for pygmy bluetongue lizards by providing burrows for them, are also important enemies of this species because they can kill lizards that try to take over burrow ownership. I also found that the traditional design of artificial burrows that has been accepted by natural populations of pygmy bluetongue lizards and that has been shown to enhance wild population density, leads to more risky behaviour of resident lizards than natural burrows. I have suggested redesign by adding a small chamber at the bottom of the burrows to allow lizards to turn around underground. I found that pygmy bluetongue lizards stay in flooded burrows even when they are underwater up to their necks. I also showed that the lizards accepted alternative natural burrows such as mouse burrows if their entrance diameters are not bigger than spider burrows (about 2 cm). These could be an additional resources of natural burrows in future translocation sites.

Finally I used the data that came from the huge data files (16544 hours of video footage) from the simulated translocation experiments to produce decision tree models and predict how the parameters I investigated can be important in real translocations. I provided two different decision trees; one informing how each of behavioural parameter I studied is likely to be affected by each of the alternative parameter states I investigated, such as vegetation density, supplementary food and soil disturbance. The second model indicated how each of behaviour might be altered by interactions with other behavioural parameters and environmental parameters that I changed. These two model can be used by conservation managers to make decisions under different circumstances for pygmy bluetongue lizards at translocated sites or to predict lizard behaviour in different translocation sites. In a broader context, these models with a few modification could be applied other translocation programs and used to predict translocated species behaviour under different situations.

Briefly, data from this thesis provide new insights into the relationships between behavioural ecology and conservation management with a focus on translocation strategy. The results show how changes in behaviour can be used to predict future translocation success or failure, and how we could manipulate the release conditions for translocated species to alter their behaviour and encourage them to stay at the release sites, thereby increasing the chance of translocation success. This thesis also shows how data from simulated translocation experiments could be useful before actual translocations are attempted. The next stage in developing strategies for the conservation management of the pygmy bluetongue lizard is to see whether the trends from simulated releases in cages are translated into real translocation situations.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Ebrahimi

Mehregan Ebrahimi

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Organisation of Thesis

This thesis contains 11 chapters, based on experiments and observations on translocation and behavioural ecology of the endangered pygmy bluetongue lizard. Chapter 1 is a general introduction that outlines the background and theory of this project and the aims of this research. The general methods of the experiments in simulated translocations are given in the second chapter and results from a series of these experiments are presented from chapter three to chapter eight, in the format of published, under review or submitted manuscripts. Chapter nine includes more published manuscripts that describe some natural history observations derived during the study, that add to our understanding of the biology and conservation management of the pygmy bluetongue lizard. Chapter ten is a conclusion in which I discuss the results of all experiments in this thesis, show how they can improve future translocation attempts in lizards in format of manuscript, and suggest future research on reptile translocations.

PUBLICATIONS ARISING FOR THIS THESIS AND STATEMENT OF CANDIDATE CONTRIBUTION

This thesis is predominantly composed of papers that have been published, accepted for publication, or have been submitted (or are near to being submitted) for review in peer reviewed journals. I did all of the field work, collection of data, analysis and most of the interpretation. I took advice from my supervisor Prof. C. M. Bull for directions of analysis and for the formatting and presentation of all of the papers. All co-authors have given me permission for this work to be used in my thesis. The paper order reflects the thesis structure rather than chronological order. Below I list the papers and estimate my contribution to each.

Chapter 3

Ebrahimi, M. and Bull, C. M. (2013). Determining the success of varying short-term confinement time during simulated translocations of the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*). Amphibia-Reptilia 34, 31-39.

ME 80%, CMB 20%

Chapter 4

Ebrahimi, M. and Bull, C. M. (2012). Food supplementation reduces post release dispersal during simulated translocation in the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*). Endangered Species Research, 18, 169-178.

ME 80%, CMB 20%

Chapter 5

Ebrahimi, M. and Bull, C. M. (2013). Resources and their distribution can influence social behaviour at translocation sites: lessons from a lizard. (Under review).

ME 80%, CMB 20%

Chapter 6

Ebrahimi, M. and Bull, C. M. (2013). Behavioural changes in an endangered grassland lizard resulting from simulated agricultural activities. (Under review).

ME 80%, CMB 20%

Chapter 7

Ebrahimi, M. and Bull, C. M. (2014). Visual conspecific cues will not help in pygmy bluetongue lizard translocations. Applied Animal Behaviour Science, 151, 102-109.

ME 80%, CMB 20%

Chapter 8

Ebrahimi, M. and Bull, C. M. (2013). Effect of time of release on behaviour and movement of translocated lizards. (Under review).

ME 80%, CMB 20%

Chapter 9

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

ME 80%, ALF 10%, CMB 10%

Ebrahimi, M. and Bull, C. M. (2012). Lycosid spiders are friends and enemies for the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*). Transactions of the Royal Society of South Australia, 136 (1): 45-49.

ME 80%, CMB 20%

Ebrahimi, M., Schofield, J. A. and Bull, C. M. (2012). Getting your feet wet. Responses of the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) to rain induced burrow flooding. Herpetology Notes, 5, 297-301.

ME 80%, JAS 5%, CMB 15%

Ebrahimi, M., Schofield, J. A. and Bull, C. M. (2012). *Tiliqua adelaidensis* (Pygmy Bluetongue Lizard). Alternative Refuge. Herpetological Review, 43 (4) 652-653.

ME 80%, JAS 5%, CMB 15%

Chapter 10

Ebrahimi, M. Ebrahimie, E. and Bull, C. M. (2013). Minimising the cost of translocation failure by using decision tree models to predict species behavioural response in translocation sites. (Prepared for Conservation Biology)

ME 80%, EE 10% CMB 10%

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1. BIODIVERSITY AND EXTINCTION

In the current biodiversity crisis, there is an urgent need to establish conservation management practices that will help conserve our more threatened species. This thesis considers one endangered lizard, the pygmy bluetongue lizard, *Tiliqua adelaidensis*. It considers one conservation strategy for that lizard, translocation, and the practical management strategies that might increase the success of translocation events. The thesis asks what strategies would reduce dispersal away from translocation release sites in the immediate period following release of animals into a new site.

The world is currently experiencing a rapid loss of biodiversity (Balmford et al., 2003; Jenkins et al., 2003). It is hard to explain and assess the relative importance of all the factors that threaten ecological communities, but there is no doubt about the role of anthropogenic effects on species extinctions. The fossil record of the earth shows faunal and floral extinctions have increased dramatically during certain periods (Fiedler and Jain, 1992). In fact, of all the extinctions that have occurred across past eras, in the current era we have recorded only a small proportion of those extinctions, including some conspicuous species such as the passenger pigeon and the dodo. However present rates of species extinction are estimated to be between 1000 and 10,000 times the rate seen through most of geological history (Purvis et al., 2000). Evidence shows that whenever humans reach a new land mass unusual bursts of extinction occur among a wide range of different taxa. For instance, extinction pulses followed human arrivals 30,000 to 50,000 years ago in Australia, 11,000 to 12,000 years ago in North and South America, 1,400 years ago in Madagascar and 1,000 years ago in New Zealand (Jeffries, 2006). Human population growth, consumption patterns and human associated habitat loss or degradation (through grazing, hunting, demand for freshwater and materials) caused initial species loss, and those losses have increased with overexploitation, pollution, introduced species, exotic diseases and urban infrastructure. All these factors have negative influences on native, endemic species and reduce their populations, threatening global biodiversity (Czech et al., 1997; Hughes et al., 1997; Wilcove et al., 1998; Dale et al., 2002; Balmford et al., 2003; Hambler, 2004).

Furthermore, many extinctions are likely to go un-noticed as they happen among the little studied species, for instance, invertebrates and microbes (Hambler, 2004). Many invertebrates have probably become extinct without our ever knowing about them. Those that have left fossils that we have discovered are likely to be a biased sample of previous biodiversity in that they probably had on average unusually large geographic ranges, dense populations, or long persistence time (Erwin and Anstey, 1995). We do not know the actual number of species that have gone extinct and are irreversibly lost from the earth, nor do we know the number of species in the world today. In spite of this, unfortunately, the number of current species at risk of extinction still increases each year (Hughes et al., 1997; Chapin Iii et al., 2000). We can only estimate a crude number of extinctions in a taxonomic group or in a geographical area based on some broad ecological generalizations (Hambler, 2004). Jeffries (2006) listed several main approaches that can be used to predict extinction rates;

A. *Estimates from recent past extinction rates*

To estimate past extinction rate fossils are the best evidence although there is no consistent fossil record across all taxa, for instance mammal and bird fossils records are patchy while marine molluscs have the best fossil records perhaps because of the better environment for the fossillisation process. From available fossils we can compare the extinction rate from past to present, and it has been suggested that mammal extinction rate was one species every two hundred years from fossil records compared with twenty species during the twentieth century (Jeffries, 2006).

B. Prediction from habitat loss

Species habitat is one the best factors to estimate extinction rates. In general, a large area of a particular natural habitat can support more species than a small area. It was shown there is a direct relationship between habitat loss and species extinction, the larger area that was destroyed the more species have gone extinct. Therefore, habitat loss is one of the major causes of species extinction. Estimates for extinction by habitat loss up until 2020, have varied between 2% to 25% of all of the world species and the estimate of forest destruction shows 1-10 per cent extinction of all the world's species (Heywood and Watson, 1995; Jeffries, 2006).

C. Prediction from changing the status of threaten species

We can also estimate extinction rates from the rate of accumulation of species, which are considered extinct according to the red list and the red data books of the International Union for the Conservation of Nature (IUCN). These estimates are based largely on vertebrate species, but current estimates from these data predict 50% of current extant species will be lost within 200 to 500 years (Jeffries, 2006).

D. Phylogenetic approach

Molecular phylogeny can indicate the evolutionary history of taxa. It defines individual lineages and the times of splits within larger taxa. Extinction rates can be estimated by comparing the expected numbers of species within lineages, assuming equal rates of speciation, with the actual numbers now recognized (Zink and Slowinski, 1995; Bennett and Owens, 1997).

E. Human energy use

Many aspects of human activity, such as agricultural activities, deforestation, mining, and urban development, have direct effects on species populations and survival. There is a correlation between human activity as measured by regional energy consumption and local extinction rate. We should then be able to predict future extinction rates based on regional human energy use in the future (Ehrlich, 1994).

These various estimation methods help us to get an idea about potential species extinction rates into the future. With improved knowledge of the current status of species (discussed briefly in the next paragraph) we can embark on proper management strategies for conserving species.

According to the red list book of IUCN (2012) 737 species (vertebrate and invertebrate) are considered recently extinct or extinct in wild, 10,820 animals species are currently threatened with extinction (vulnerable, endangered, or critical endangered), and 3,253 species are listed as near threatened or conservation dependent out of 49,826 animal species listed in IUCN. Sanderson et al. (2002) suggested that approximately 83% of the earth surface has been influenced by human activities and they usually coincides with land use invasive species. These two factors have had a great effect on biodiversity and native species (Trakhtenbrot et al., 2005). For instance, a growing body of evidence for birds and mammals suggests that over the last few thousand years, the most important agent of direct change in the environment has not been climate change but human disturbance and alteration of habitats. Most extinctions of entire species in recorded history are attributable to some aspect of human intervention (Fiedler and Jain, 1992). Habitat loss, fragmentation and degradation are considered to be the most important factors that threaten biodiversity (Primack, 2006). These processes continue to reduce species biodiversity and increase species extinction rates.

In brief, nowadays, the loss of biodiversity is one of the most important problems that threatens life on the earth. Conservation management is urgently needed to reduce extinction rates and to using natural resources in a sustainable way for the future. This thesis aims to improve conservation management for at least one endangered species.

1.2. CONSERVATION MANAGEMENT

The concept of conservation has been developed over thousands of years and has taken a variety of forms across many parts of the world. The International Union for Conservation of Nature (IUCN) defines conservation as "the management of human use of the biosphere so that it may yield the greatest sustainable benefit to the present generation, while maintaining its potential to meet the needs and aspirations of future generations" (IUCN, 1980). Thus conservation is positive, embracing preservation, maintenance, sustainable utilization, restoration and enhancement of the natural environment (Fiedler and Jain, 1992). Hambler (2004) suggests a broader definition: "conservation is the protection of wildlife (include all non-domestic species and populations of plants, micro-organisms and animals) from irreversible harm". Although conservationists have different ideas about the definition of conservation, all of them accept that protection of wildlife from extinction is the fundamental aim of conservation. How we can minimize the extinction rate of threatened species is the central problem in conservation. The lack of systematic evaluation of the effectiveness of conservation programs is another problem that has been highlighted as a key problem inhibiting advances in scientific management for conservation (Sutherland, 2000). Two different conservation paradigms suggest alternative pathways of populations towards extinction that can provide one systematic approach to conservation (Caughley, 1994). The first, the small population paradigm, focuses on problems in population genetics and dynamics that occur in small populations and increase their risk of extinction. The second, the declining population paradigm, deals with detecting and diagnosing the processes responsible for population declines (Caughley, 1994). Both paradigms try to find the best way to protect the species and reduce the rate of extinction and each of them is focused on the reasons why a species is at risk of extinction (Quinn and Hastings, 1987). These different directions in conservation management can help us to improve the outcomes of conservation management programs. An ongoing issue is that conservation management programs are regularly confronted with limited resources of personnel, time, funding, and expertise. Thus they require us to know which natural areas and ecological processes most need such programs. Habitat diversity is often important to conservationists because it is probably one of the main foundations for promoting species diversity in an area (Hambler, 2004). Fiedler and Jain (1992) catgorised natural areas and ecological processes which require active conservation management to prevent serious decrease and degradation of resources and local extinction. If the species we study is considered to belong in one of the categories below, we need an active conservation management plan to conserve it from future extinction.

A. Areas with low resilience to perturbations

There are three reasons why systems may be sensitive to perturbations. They may be areas with low net primary productivity such as deserts, mountain tops, or tundra. They may be systems with low thresholds of stability to particular types of perturbations, or they may be areas with low connectivity between habitat patches such as isolated islands or wetlands, or highly fragmented habitats. In all cases, the impacts of perturbation may overlap in time, and rates of recovery are likely to be slower with more frequent perturbations (Fiedler and Jain, 1992). Studies in the Mojave desert in California found degradation and perturbation were caused by factors that included livestock and grazing, linear corridors (road ways, rail ways, and pipelines), mining, military training operations, off-road vehicles, invasive species, air pollution and anthropogenic fires (Lovich and Bainbridge, 1999). Natural recovery from these perturbations in such sites is very slow because, extreme temperatures, intense sun, high winds, limited moisture and the low fertility of desert soils reduce the rate at which populations can respond during natural recovery after a disturbance (Lovich and Bainbridge, 1999). Many Australian natural habitats are fragmented, and although they may still have resistance to natural fires, the increased frequency of drought seasons, intentional fire, agricultural activities and development can decrease resistance threshold of such habitats.

B. Sites with vulnerable locations

Some areas are vulnerable because there is a high chance they will experience a major disturbance. These are sites with locational or situational sensitivity, for instance habitats near expanding urban populations, sites with high recreational development or with agricultural potential, and sites downwind or downstream from a major source of pollution (Fiedler and Jain, 1992). Urban development produces the greatest local extinction rates through the complete elimination of most native habitats (McKinney, 2002; McKinney, 2006). In other words, urban development is one of the human activities with the highest impact on the environment (Czech et al., 2000). Until recently, agricultural activities were considered of less concern for conservation, but that opinion changed dramatically in Britain in the 1960s and by the end of that decade most conservationists were antagonistic towards farmers and vice versa (Gall and Orians, 1992). After that many studies illustrated the adverse impact of agricultural sites on wildlife. For instance a survey in UK showed a wide range of detrimental pollution and land degradation derived from agricultural activities, such as from pesticides, nitrogen compounds in fertilisers, farm livestock wastes and soil erosion and associated nutrient losses (Skinner et al., 1997). Livestock grazing is another problem associated with agriculture that has serious ecological costs. Grazing has reduced the density and biomass of many native plant and animal species, has reduced overall biodiversity, has aided the spread of many introduced weed species, has interrupted ecological succession, has impeded the cycling of the most important limiting nutrient (nitrogen), and has changed habitat structure and disturbed community organization (Fleischner, 1994).

Finally, uncontrolled contributions of pollutants to the environment have led many species to extinction or to the verge of extinction. Environmental pollution is closely related to the survival of species because it can lead to increases in death rate and decreases in reproduction and birth rate. In fact, the deterioration of the environment due to the presence of pollutants can cause both physiological and biophysical changes in populations (Srinivasu, 2002).

Except for the national parks and conservation parks, most of the land area is privately owned in many developed countries such as Australia. These habitats may be secure for the short term, but their status may easily be changed by landowner decisions. Many of these habitats have some rare, endemic or endangered species which make them more vulnerable to change from individual decisions.

C. Rare species, habitats and resources

Rarity of a species is not necessarily a sign that it requires active conservation management. Some species may be locally rare, but are naturally widespread. These will require less attention than other species that become uncommon due to environmental change, for instance through human activity. In addition, some species with ranges that have recently shrunk to small habitat fragments need more immediate attention than a rare species with a stable habitat. For instance *Tiliqua adelaidensis* is an endangered lizard species which lives in privately owned grassland habitats that are highly fragmented. If anything changes in these grassland such as increased intensive of agricultural activities, it may increase the chance of extinction of this species. Although there may be a direct impact of agricultural activities on this lizard the more important resource that is necessary for this species, shelter, is highly affected by agricultural activities. Shelter can act as key resources for this endangered lizard. These shelters are single entrance burrows constructed by lycosid and mygalomorph spiders and studies have shown these spider strongly avoid ploughed areas (Sharp et al., 2010).

D. Keystone resources

If particular species or resources in the ecosystem have specific ecological roles, then the absence of those species or resources can have great impact on the biodiversity of the community, or cause major changes to the community structure and function (Payton et al., 2002). Such species or resources are called keystone species or resources (Fiedler and Jain, 1992). For instance, the rodent *Dipodomys spectabilis* acts as a keystone species and has a role as an indicator of the status of an ecosystem with respect to the irreversible degradation threshold (Krogh et al., 2002). The burrow-mounds which are made by this species cycle nutrients into soil patches that are important for supporting rare plant populations. Thus these rare plant population depend on the rodent, and can easily vanished if the rodent becomes extinct.

The concept of keystone resources allows conservation managers to combine some attractive features of single species management and ecosystem management approaches. This can prevent ambiguities in management directions, and can focus conservation efforts on a single species or resource, while having an impact on the whole community. This approach reduces the need to examine every resource or species, because the keystone species or resource affects many other species in the community (Simberloff, 1998). These keystone species can be more vulnerable if they live in fragmented habitat. Any small change in these habitats can easily reduce local populations and conserving these species can help other species to maintain their population. For example if lycosid and mygalomorph spiders which are affected by agricultural activity in fragmented habitat were eliminated from their habitat, the pygmy bluetongue lizard that relies on the burrows these spiders construct, will be adversely impacted as well.

E. Small and fragmented habitats

Habitat fragmentation has a negative impact on conservation and there are an increasing number of studies based on habitat fragmentation (Andren, 1994; Boswell et al., 1998; Hager, 1998; Vos and Chardon, 1998; Debinski and Holt, 2000; Keller and Carlo R., 2003; Keller et al., 2004). There is a general pattern of biological impoverishment of fragmented habitats compared with more intact ones. Many studies also find that fragmented habitats support fewer species than natural, more continuous habitat and that abundance of widespread generalist species increased in these fragmented habitats (Harrison and Bruna, 1999). Fragmentation of habitats is not as serious a problem for biodiversity if populations that are distributed among isolated habitats can migrate occasionally between fragments. Fragmentation becomes an increasing problem when populations are not able to migrate and habitat quality within the fragment is too poor or the area too small to maintain viable populations. Some other mechanisms also impact biodiversity in fragmented habitats such as the adverse role of physical and biological edge effects (Harrison and Bruna, 1999). The minimum habitat size (or the maximum tolerance of habitat degradation) can be highly variable among species within the fragments, even among closely related taxa. For example collared peccaries can survive in smaller and more fragmented habitats than white-lipped peccaries (Fiedler and Jain, 1992). But although the level of impact of habitat fragmentation differs from one species to another, population decline or even local extinction of one species can negatively impact several other dependent species. For instance the army ant *Eciton burchelli* is a keystone species which lives in fragmented tropical rainforest. Declines in ant populations could result in population decreases of some birds species which use army ant paths to locate their food (Boswell et al., 1998). One other important impact of fragmented habitat is that populations in such habitat have reduced gene flow and genetic connectivity with other populations, reducing the potential range of genetic variants, and the potential for an evolutionary response to new predators, invasive species or even changes in microclimate (Primack, 2010). Habitat fragmentation can have many other adverse impacts, including limiting dispersal and opportunities for colonization, restricting access to food and mates, dividing populations, changing microclimates, increasing the incidence of fire, increasing impacts of interspecific interactions and increasing the local impacts of disease (Laurance et al., 1998; Debinski and Holt, 2000; Trombulak and Frissell, 2000; Nepstad et al., 2001; Primack, 2010).

All of these processes can reduce species populations and potentially lead the species to extinction. There are several different conservation management strategies developed to conserve species from extinction in the face of these various threats. Each of these strategies has some advantages and some disadvantages so we need to understand them and choose between them carefully if we want to get the best management outcomes from them. The first step is to have a better comparative understanding of each strategy.

1.3. DIFFERENT CONSERVATION MANAGEMENT STRATEGIES

A number of different conservation management programs have been used to reduce the risk of extinction of different individual species or to prevent losing biodiversity in a region. There are different conservation management strategies for each of above causes of diversity loss. These include selecting, designing and managing protected areas, landscape-scale conservation, conserving the evolutionary process, ecological restoration and protecting species by using *in situ* or *ex situ* conservation (Pullin, 2002). These alternative strategies are discussed below.

1.3.1. PROTECTED AREA

Maintaining a protected area or reserve is one conservation strategy. This not only protects a targeted endangered species, but also protects the intact ecosystem and the rest of the biodiversity it contains. Thus it is a good way to reduce biodiversity loss. The size of protected area can vary from a few hundred square meters to thousands of square kilometers. MacArthur and Wilson (1967) in the theory of island biogeography inspired conservationists and ecologists to realise how the size of reserve and connectivity are important in conservation management of species. Many conservationists followed the theory of island biogeography and agreed that single large reserve could serve better species diversity than many small reserves. This idea led to the famous SLOSS debate (single large or several small reserves) between the 1970s and 1980s. However, recently, Tjørve (2010) with regards to species diversity models, suggested that the question about the size of the reserve is not an issue and we should consider when we should use the large or small reserve area to maintain maximum species diversity. So the size of reserves could be varied from big to several small areas in different circumstance. The other issue is that the area that is actually available for protection is often already fragmented, or adjacent to habitat already degraded by urban or agricultural development or other human activity, so the internal dynamics of the protected area ecosystem has already been fundamentally changed. Therefore, these protected reserves need intensive management to retain the communities and their vulnerable species.

1.3.2. LANDSCAPE-SCALE CONSERVATION

Emphasis on landscape-scale conservation is one form of protected area management. It moves the emphasis from species conservation in fragmented habitats or single small-protected areas, to enhancing connectivity and species movement between the patches. In this strategy the habitat is considered as a network of patches from the micro to the macro scale and the species within the patches are represented by both their temporal and spatial dynamics. The enhancement of movement between these patches can be achieved by wildlife corridors or stepping stone habitats (Pullin, 2002).

1.3.3. CONSERVING THE EVOLUTIONARY PROCESS

A long-term view of conservation is conserving the evolutionary process, as well as conserving a species or its habitat. This means retaining sufficient genetic diversity to allow the species to respond to the inevitable biological and physical changes that it will experience in the future. This is probably one of the major aspects of conservation strategy that will need to be developed in future because all biodiversity depends on evolutionary processes (Crandall et al., 2000; Pullin, 2002).

1.3.4. ECOLOGICAL RESTORATION

Ecological restoration refers to the repair of some of the damage already done to an ecosystem. For instance, there are many examples where degraded agricultural landscapes, or mining sites, are being restored by re-establishing the native vegetation, or by removing invasive weeds. The scale of ecological restoration can be from restoring single species to whole ecosystems. The most important challenge is to understand and recreate the complexity of the original ecosystem and determining how it can be restored to work again.

1.3.5. In situ CONSERVATION

Protecting specie as *in situ* conservation is evaluating each species in terms of its probability of extinction, which is done now in the Red book. The role of this form of conservation is to estimate the genetic diversity of rare species and small population and to construct a genetic management strategy. The information from such *in situ* conservation strategies can help us to identify species that need more intensive conservation techniques such as *ex situ* conservation.

1.3.6. EX SITU CONSERVATION

When a species population is too small, or the threats, for instance form introduced predators or exotic pathogens, are too overwhelming to allow the population to persist, one strategy may be to remove all or part of the population from the wild and keep them in captivity or at an alternative location. This is known as *ex situ* conservation, and is only appropriate if the ultimate aim is to subsequently release the population, to the same site when the threats have been reduced, or to a new site with diminished threats.

Understanding the mechanisms that caused the rarity and the historical patterns of decrease of population size is critical to assess whether this is an appropriate action and to develop conservation management policy and strategy. For example, populations that have recently fragmented into several small populations, may lose genetic diversity and become more vulnerable to extinction. In that case translocation between small populations can help to increase population heterozygosity. By way of contrast, translocation may carry the risk of outbreeding depression in recipient populations if different populations have different coadapted genomes (Templeton, 1986; Fiedler and Jain, 1992). In recent years, there has been a tremendous increase in the use of capture-release programs, captive breeding and transferring animal populations between different sites for recovering endangered species (Griffith et al., 1989; Maunder, 1992; Snyder et al., 1996). The capture and release strategy is to capture animals from the wild, keep them for a period in a controlled and protected environment and then release them. In some cases this period may simply cover a vulnerable stage of the life history, and allow higher survival thorough that period. For instance, the egg and juvenile stages are more vulnerable and they can be taken from the wild and then released back into the original population when they have achieved a size or age when they are less vulnerable. Kuehler et al. (1996) assessed the success of captive rearing and release of some endangered Hawaiian forest birds and suggested that restoration could not be successful unless the threats from predators and disease could be controlled in the natural habitat. Jiménez-Uzcátegui et al. (2006) reported that for giant tortoises (*Geochelone* spp.) and land iguana (*Conolophus subcristatus*) on the Galapogas Islands, captive rearing and release were successful and they increased the number of individual in populations. Captive breeding takes this concept further by holding individuals for longer periods, while there is some general broader threat to all life stages, such as an introduced predator or exotic pathogen. The population is allowed to breed in captivity to increase numbers and to sustain the viability of the population for as long as it takes to reduce the threat. These programs are nowadays used increasingly as tools for conservation management of endangered species. Frogs have been one of these taxa where sustainable breeding captive populations have been established, for instance to protect them from the impacts of chytrid fungus (Buley and Villavicencio, 2000; Kinne, 2005). One example is the Amphibian Ark program where populations of frogs that are likely to be wiped out by chytrid fungus have been retained in zoos, as breeding populations for eventual release when the chytrid threat has been reduced (Balmford et al., 1996; Conservation Breeding Specialist Group, 2013). Although captive breeding seems to be a powerful tool to save threatened species, it faces two important problems. First how we can manage a relatively small captive population without compromising the population viability through inbreeding depression. Second, is the cost of space, staff and money required in a single species program, justified in the overall conservation context, where there are limited resources to spread around many endangered species (Torbjörn, 1995). Captive breeding has been challenged as a viable conservation strategy because of the small size of any founder population, the removal of natural selection pressures, and the likely rapid adaptation to captive condition (Miller and Hedrick, 1993; Woodworth et al., 2002; Griffiths and Pavajeau, 2008). On the other hand, this may be a last resort solution for an iconic species, and provides a chance to establish a new population in a release site. These arguments make captive breeding and release a controversial technique in conservation management. The release site of individuals produced in a captive breeding program could be within the range of existing populations, or outside the range. In that case individuals will be translocated to new population sites. In other words, the captive breeding strategy could be used with other techniques such as translocation.

1.3.7. TRANSLOCATION

The term translocation generally refers to human-mediated movement of a living organism (or organisms) from one area to another area, which could be accidental or intentional. When individuals of a species are intentionally moved to an area to improve the conservation status of the species this is known as conservation translocation. Translocations could be to the indigenous range of the species or outside of it (IUCN, 2013b). Translocation is a useful tool in conservation management for species, which have lost their previously occupied habitat, or are faced with exotic predators or pathogens, or are living in highly fragmented habitat, or that are in habitats likely to be altered as a result of predicted climate change.

According to the IUCN position statement on translocation of living organisms, two types of translocations can be considered, population restoration and conservation introduction (IUCN, 2013b). These are explained below. As the below are definition we have used the IUCN words here.

A. *Population restoration*

When an organism is moved to and released into another part of its current or previous distribution (indigenous range) in any conservation action it is referred to as population restoration. This involves two alternative activities

- **Reinforcement** is adding individuals to an existing population. This can improve viability in the resident population by increasing population size, and genetic diversity. Augmentation, supplementation, re-stocking and enhancement (in plants) are all synonyms of reinforcement.
- **Reintroduction** refers to the intentional movement of individuals into part of the dispersal range of the species, where it is no longer

found as a result of human activity or other ecological processes. The aim is to re-establish populations at sites of local extinction.

B. *Conservation introduction*

If an organism is intentionally moved and released outside of its indigenous range to enhance its conservation status it is known as conservation introduction. Again this can involve two alternative activities.

- **Assisted colonisation** is an intentional and mediated movement of an organism by humans to outside of its indigenous range to protect the population from extinction. Assisted colonisation is used when protecting the organism in its habitat is less feasible. Examples include moving a species to a predator free off-shore island, when mainland populations are catastrophically threatened by processes (such as invasive predators) that are going to be difficult to control in the immediate future. Assisted colonisation is the foremost type of translocation.

  Other terms often used to explain the same process include introduction, assisted migration and managed relocation.
- **Ecological replacement** is releasing an organism outside of its indigenous range to perform a specific ecological function after extinction or reduction of the endemic species that previously performed that function. In this activity the ecological function is restored by releasing another species that has the same ecological functions. The introduction

may be a different sub-species or closely related species to the one that is locally extinct. In the conservation of Andean condors, exotic herbivores act as an ecological replacement of native herbivores (Lambertucci et al., 2009). Native herbivores that have vanished from condor habitat need to be replaced with another herbivore to maintain food supply for condors. Lambertucci et al., 2009 explain this in more detail, and they called it an ecological replacement.

Ecological replacement is synonymously referred to as taxon substitution, ecological substitution/proxies/surrogates, susbspecific substitution and analogue species.

Each of these methods leads to different strategies, but all can reduce the chance of species extinction by moving individuals to new or existing habitat or by increasing the number of individuals in existing population or restoring an ecological function (IUCN, 1987; Ebenhard, 1995; Hodder and Bullock, 1997; IUCN, 1998; Tenhumberg et al., 2004; Rout et al., 2005; IUCN, 2013b).

Animal translocation is an important option for conservation management these days. There have been many translocations over the last few decades. But relatively few studies have evaluated the effectiveness of these translocations (Stander, 1990; Weilenmann et al., 2010). Unfortunately, the outcomes of many animal translocations have not been documented due to inadequate resourcing after the event, but where they have been monitored after release many translocations have failed to establish viable populations at the release site. For instance, a report from 20 years ago suggested that from more than 1000 bird translocations approximately half of them have failed (Kleiman, 1989).

One of the problems with translocation is that in reinforcement programs, the translocated animals may not fit into the social system of resident populations, or in other translocations the animals suffer from stress or unfamiliarity with the site of release. Therefore they may disperse from the translocated site (Linnell et al., 1997; Miller et al., 1999; Gusset et al., 2009; Massei et al., 2010). Surveys on the effect of habitat novelty, stress, food resources, and reproductive opportunities after translocation, indicate these factors can all lead to reduced body condition, and increase the chance of mortality and of dispersal from the translocation site if they are ignored (Armstrong et al., 1999; Armstrong and Perrott, 2000; Bernardo et al., 2011; Mitchell et al., 2011; Drake et al., 2012).

One other current issue in translocation is that the translocated individuals must be derived either from a captive breeding program or they must be captured from the wild. If the target species is endangered, then it is likely that relatively low numbers of individuals will be available for the translocation. We cannot capture significant numbers from donor populations that are already in decline. However, the number of founders in a translocation plays an important role in determining the success or failure of the conservation management strategy, with more founders leading to higher success (Griffith et al., 1989; Wolf et al., 1996; Wolf et al., 1998; Fischer and Lindenmayer, 2000; Rout et al., 2007). Captive bred animals can be used to increase the number of founders, however other studies have shown that wild animal usually have more chance to establish new populations in translocation sites (Griffith et al., 1989; Lubow, 1996).

Although all of above mentioned factors influence the outcome of translocation attempts, success of a translocation depends on three main processes (Griffith et al., 1989; Seddon, 1999; Letty et al., 2000; Teixeira et al., 2007; Reynolds et al., 2008; Moorhouse et al., 2009; van Heezik et al., 2009; Chauvenet et al., 2012);

a) The settlement of enough animals in the translocation site;
b) The survival of enough of those settlers until they are ready to reproduce;
c) Successful contact for reproduction, and recruitment of new cohorts;

These can each be directly affected by the origin of the founder population and its size. Failure in any of these processes can lead to an unsuccessful translocation. When translocated animals cannot established viable populations in their new location and disperse from it, the species will disappear from the translocation site even if the process takes several years. Failures in previous translocations have resulted from high rates of dispersal away from the release sites, or low survival and low rates of reproductive recruitment at the site (Scott and Carpenter, 1987; Griffith et al., 1989; Wolf et al., 1996; Hodder and Bullock, 1997; Armstrong et al., 2007; Seddon et al., 2007; Armstrong and Seddon, 2008; Moorhouse et al., 2009; Ruffell and Parsons, 2009; van Heezik et al., 2009; Jachowski et al., 2011). The ability to reproduce also may change after translocation. For instance successful reproduction in reintroduced forest birds on New Zealand islands depends on the provision of supplementary food (Armstrong et al., 2002). So far, the majority of research effort has focused on survival rates of translocated individuals, and successful reproduction after release to increase the chance of successful translocation (Armstrong et al., 1999; Towns and Ferreira, 2001; Armstrong et al., 2002). Another important issue, dispersing away from the translocation site, particularly in the period following release, is relatively neglected. Dispersal can strongly affect population viability and successful reproduction by changing the availability of mating partners, or changing the sex ratio, among the individuals that stay. An area of research that requires more focus, is how to persuade translocated animals to stay in the novel habitat where they are released (Letty et al., 2000).

The issue of dispersal has been a controversial subject within the field of animal translocation. Studies have shown that translocated individuals tend to make longer moves at their new site, and to have poorer survival than other individuals at the source population (Hein and Whitaker, 1997; Plummer and Mills, 2000; Sullivan et al., 2004; Rittenhouse et al., 2007). More mobile species also have a stronger tendency to disperse from the translocated site, perhaps because unfamiliarity with the new site leads them to risk dispersal to find better habitat (Stenseth and Lidicker, 1992; van Heezik et al., 2009). But the tendency to disperse is affected by a number of other factors such as local population density, resource availability or resource quality, social interactions among conspecifics, interspecific competition, or the density of predators (Dodd and Seigel, 1991; Stenseth and Lidicker, 1992; Armstrong and McLean, 1995; Ims and Hjermann, 2001; Towns and Ferreira, 2001; Skjelseth et al., 2007; van Heezik et al., 2009). Essential to any translocation program is an understanding of the drivers of post release movement, why and when it happens, how many individuals are affected and how far they move from the release site. If we can neutralize the drivers, we might be able to decrease post release movement, and increase the chance of successful translocation. For instance, one method that has been used in previous translocations is called soft-release, where individuals are confined within the release site for a period of time to allow them to become familiar with it, before the confinement is finally removed. In some studies post release dispersal has been reduced after soft release compared with hard release (no confinement after release) (Bright and Morris, 1994; Linnell et al., 1997; Letty et al., 2000; Clarke et al., 2012). In those cases it appears that confinement may also decrease species stress by keeping them in one part of the translocated habitat for a certain amount of time. Adding food could also decrease the tendency of individuals to disperse, and allow them to improve body condition for reproduction after translocation (López-Bao et al., 2008; Schoech et al., 2008). Manipulating other habitat factors such as cover for refuge might also reduce dispersal and improve the translocation result. If we can find which set of habitat factors can encourage translocated individuals to stay in the translocation sites, we might be able to introduce more informed translocation protocols. In this thesis study, simulated translocation was used to investigate these questions in an endangered lizard species. Here the "translocations" were performed in an enclosed space to explore management procedures. These were thus simulated translocations before any actual translocation takes place. In the thesis I suggest that this experimental approach could be an option, at least for relatively small species that can be contained within enclosures that represent their natural home range areas, to test different habitat factors and other possible theory. This should allow us to more easily find which set of factors can help us to perform more successful translocations.

1.3.8. REPTILE TRANSLOCATION

What do we know about previous translocation in reptiles? From 3755 reptiles species which were listed in the IUCN red list for 2012, 1089 species were classified in the categories between "near threatened" and "extinct" (IUCN, 2012). Reptiles are as much in danger of extinction as any other vertebrate group. There are many papers that have addressed the threats to persistence of reptile species (Towns and Ferreira, 2001; Araújo et al., 2006; Gardner et al., 2007; Brown et al., 2008; Fordham et al., 2012). As for other taxa, habitat loss and fragmentation, habitat change and degradation, invasive species and climate change are some of more important reasons of reptile declines (Blaustein and Kiesecker, 2002; Gardner et al., 2007; Fordham et al., 2012). In general the threats can be divided into direct factors, such as habitat loss and habitat change, and indirect factors such as global climate change and pollution that affect habitat suitability (Collins and Storfer, 2003). As discussed above one option to conserve reptiles from those threats could be translocation to more suitable habitat. In previous reports of conservation management of reptiles, threatened species have been translocated to establish new populations (Dickinson and Fa, 2000; Towns and Ferreira, 2001; Nelson et al., 2002; Pernetta et al., 2005), and to rescue animals from development sites (Platenberg and Griffiths, 1999). Translocation has also been used to remove "nuisance" animals (snakes) belonging to more common species from urban areas (Shine and Koenig, 2001; Sullivan et al., 2004; Germano and Bishop, 2009). Some research has shown that reptile population can become established if they are transferred to a small island with appropriate habitat, low predator density, and few opportunities for substantial dispersal (Dickinson and Fa, 2000; Knapp, 2001; Nelson et al., 2002). However, an early review suggested that reptile translocations on mainland sites are often unsuccessful (Dodd and Seigel, 1991) although more recently Germano and Bishop (2009) reported more successful translocation in amphibians and reptiles in a review of cases that also included more island translocations.

Reptiles that have been translocated could have become stressed in the process, affecting their health and cognitive ability (Teixeira et al., 2007). In addition, translocated individuals tend to make longer moves at their new site, increasing energy costs and exposure to predators, so these individuals have poorer survival than other individuals that are at the source population or residents at the translocation site (Hein and Whitaker, 1997; Plummer and Mills, 2000; Sullivan et al., 2004; Rittenhouse et al., 2007). This "unsettled" effect can persist up to 2 years after the translocation (Reinert and Rupert, 1999), and translocated individuals that survive often have poorer body condition than residents (Platenberg and Griffiths, 1999). Release site selection to provide optimal microhabitat is most important in a successful translocation program, encouraging the best set of conditions for individuals that do not disperse, and reside near the release site (Dickinson et al., 2001; Pernetta et al., 2005).

If translocated animals are penned for a time to become familiar with the site, they may become less stressed and disperses less. For instance, gopher tortoises that were penned at a release site for 9-12 months were more likely to stay when finally released (Tuberville et al., 2005). Abundance of suitable refuges can also increase survival. Therefore, natural refuges could be augmented with artificial refuges to improve the quality of the release site. These refuges may both provide additional shelter for reptile populations, and attract individuals to use them, helping in reptile monitoring surveys (Reading et al., 1997).

Artificial refuges for reptiles come in different forms such as brick pavers (Webb and Shine, 2000), or burrows (Souter et al., 2004). Different species of reptiles prefer different kinds of artificial refuges. All artificial refuges must have two important characters, suitable thermal conditions and safety from predation (Milne and Bull, 2000; Goldsbrough et al., 2006).

This review suggests there are several ways to reduce dispersal and increase the chance of successful translocation in reptiles. A fundamentally important first step in translocation in reptiles is to understand how we can encourage or persuade the translocated population to stay close to the site of release. All features of the habitat will be important, but we need to indicate which factors the translocated populations are most sensitive to, and how can we best use those factors in the first stages of the translocation release. Understanding animal behaviour in novel habitats and according to habitat change also gives us important information about how we can choose and manage the right conservation strategy.

Behavioural ecology and conservation

An important component of the response to translocation release will be the behaviour of the released animals. Understanding how animals interact with their environment, with conspecifics and with individuals from other species is called behavioural ecology (Arora and Kanta, 2009). Behavioural ecologists investigate how individuals survive and reproduce in different ecological circumstances (Caro, 1998). Behavioral studies have wide ranging importance for conservation. For instance in captive breeding and reintroduction programs the captive raised animal must be able to interact appropriately with wild conspecifics and be able to respond to physical and biological challenges, such as predation (Shumway, 1999; Caro, 2007). Many vertebrate species come with innate behavioural responses, but can also learn survival skills from their parents, by trial and error learning, or by observation. Studies have shown that many species learn responses such as preferring some odours, from their early experience (Shumway, 1999), and behaviour can play an important role in solving conservation problems. Sutherland (1998) listed 20 areas in which behavioural studies can help to solve conservation problems (Table 1-1). Caro (2007) also discussed nine areas of behavioural study which can improve conservation attempts such as responses to human activity, to land use change and to reintroduction. A comparison between the two papers shows that both authors have the same broad ideas, but that Sutherland (1998) expanded them, into more detailed categories. He included as separate behavioural characters, species isolation, dispersal in fragmented population, predicting the consequence of environmental change, behavioural manipulation, release schemes, and habitat requirements of species of conservation concern. Sutherland (1998) pointed out that retaining species diversity depends on mating isolation among sympatric species and if something disrupts this isolation we could lose species diversity. For instance different species of cichlid fish in Lake Victoria were reproductively isolated by recognising and responding to particular species specific colours and markings of their conspecific mates, until agricultural activity and deforestation increased the turbidity of the water, reducing the effectiveness of visual signals in the water, and disrupting cichlid conspecific mate choice, which in turn allowed cross species mating, reducing species diversity, and leading to duller-colored fish with fewer colour morphs (Seehausen et al., 1997). Although we should mentioned that braking down sympatry could conceivably release genetic diversity by creating new forms.

Among the behaviours relevant to translocations, dispersal has an important role in ecology and conservation management. It impacts population dynamics, gene flow, community structure, speciation, and local adaptation, and it is influenced by, among other things, habitat fragmentation and climate change (Dieckmann et al., 1999; Heidinger et al., 2009). Dispersal influences translocation success because translocated animals can have altered dispersal and homing behaviour (Tomiyama, 1992; Armstrong and Herbert, 1997; Clarke and Schedvin, 1997; Bélangerz and Rodríguez, 2001). For instance male voles which were released to an unfamiliar site moved further than those that were held for an equivalent time and then released in familiar sites (Jacquot and Solomon, 1997). In addition social interaction of translocated and resident individuals can cause new individuals to increase their movement and dispersal behaviour (Heidinger et al., 2009). Social behaviour, and its impact on dispersal, can in turn be affected by the degree of habitat fragmentation (Ylönen et al. (1990).

Behaviour of animals can change when their environment changes, for instance as a result of climate change or habitat fragmentation. Predicting the consequence of habitat change is a powerful way to predict patterns of dispersal of a species. It could also help us to manage the species conservation strategy with more confidence. Fordham et al. (2012) showed how predicted climate change might affect existing populations of an endangered skink (*Tiliqua adelaidensis*) in South Australia, and suggested relocation as an adaptive strategy to mitigate the impacts of climate change. If we understand how behavioural decisions by individuals change we can predict how they might respond to novel habitats (Goss-Custard and Sutherland, 1997; Sutherland, 1998). Thus understanding how behaviour is affected by different environmental factors is highly important for determining conservation management strategies for instance during translocations.

Table 1-1. List of 20 areas that were suggested by Sutherland (1998) for which behavioural studies can help to solve conservation problems.

1) Small population extinctions
2) Mating systems and inbreeding depression
3) Species isolation
4) Dispersal in fragmented populations
5) Predicting the consequences of environmental change
6) Reducing predation
7) Retaining cultural skills
8) Behavioural manipulations
9) Release schemes
10) Habitat requirements of species of conservation
11) Minimum area necessary for reserves
12) Captive breeding
13) Reproductive behaviour and reproductive physiology
14) Trade-offs in habitat preferences
15) Measuring deteriorating or stressful conditions
16) Census techniques
17) Exploitation
18) Increase in human population
19) Discounting
20) Increasing conservation concern

This knowledge can allow behavioural manipulation, an increasingly important area in conservation projects. Manipulating behaviours to increase reproduction and productivity in animal, for example by removing eggs to induce second clutching, is well known in conservation programs (Rodger, 1989; Sæther et al., 1993; Mate et al., 1998; Koivula et al., 2003). Predator manipulation (specifically reduction in encounter rates with predators) also influences population dynamics and is widely used for wildlife management programs (Salo et al., 2010). Behavioural manipulations on lizard populations during translocation are not often used and need to be considered as an option to boost translocation success.

One of the major problems in a translocation program is the post release movement of released animals away from the site where they are released, and potentially out of the selected favorable habitat. This dispersal could be reduced if we had a better understanding of the behaviour of translocated species. One of the best way to decrease post release movement is by using soft release, the initial confinement of the released animals at the release site while they become familiar with the site (Kleiman, 1989; Tuberville et al., 2005; Teixeira et al., 2007). But many other factors can affect the final success of hard releases (no confinement) or of soft releases once the confinement is removed. For instance Scillitani et al. (2012) suggested that behaviour played an important role in movement and settlement of translocated male Alpine ibexes. Several translocation attempts for that species failed because of post release dispersal resulting from the social interactions among the released animals (Mihoub et al., 2011). There was then a behaviorally mediated Allee effect such that the population declined because reproductive adults could not find partners. According to their results, species that exhibit more aggregative social behaviours need to be released in larger cohesive groups, while for those that depend on habitat quality, competitive interactions need to be reduced by releasing in smaller groups (Mihoub et al., 2011).

The habitat requirement of a species is an important component in conservation management, and plays a key role in translocation. Most habitat requirements are connected to and influence behaviours like home range maintenance, social system, diet choice and breeding behaviour (Powell and Bjork, 1995; Sutherland, 1998; Moorhouse et al., 2009; Burger and Both, 2011). Therefore understanding how animals respond to different habitat factors can improve our judgment to find appropriate habitat for translocation.

In summary behavioural ecology plays an important role in conservation management, but far too little attention has been paid to this component. Perhaps more study should be focused on this aspect of conservation strategy to provide a sounder knowledge base for delivering successful translocation options. This thesis will explore the behaviour of one endangered Australian skink in a series of simulated translocations, with the aim of providing a better understanding of how to manipulate behaviour to increase translocation success.

1.4. AUSTRALIAN REPTILES: A CASE STUDY

Among 9766 reptile species in the world (Uetz, 2013), approximately 950 species are found in Australia, of which 93% are endemic and 5% (46 species) are classified as threatened species (Chapman, 2009). Although most of the families of reptiles found in Australia have species distributed in other parts of the world all have endemic Australian species (Wilson and Swan, 2010). Skinks (Scincidae) are the most diverse family of lizards in Australia with more than 370 species. They are one of the most successful families of vertebrates in Australia, with a diversity of different body forms and a distribution covering most of Australia. Although many skink species are common and widespread, others are threatened by climate change, habitat fragmentation and degradation, agricultural activity, and introduced predators (Gibbons et al., 2000; Gardner et al., 2007). Independent of their conservation status Australian skinks are a unique taxonomic lineage that deserves protection from threatening processes or extinction.

1.4.1. THE MABUYA GROUP OF SKINKS

Australian skinks belong to the Lygosominae subfamily of the family Scincidae, and are represented within this subfamily, by three lineages, the Eugongylus group, the Sphenomorphus group and the Mabuya group (Greer, 1989; Austin and Arnold, 2006; Skinner et al., 2011). The Mabuya group contains seven genera and 50 species, most of which are endemic to Australia. The genus *Egernia* has 17 species, *Liopholis* 11 species, *Bellatorias* 3 species, *Lissolepis* 2 species, *Cyclodomorphus* 9 species, *Tiliqua* 7 and *Corucia* 1 species that is endemic to the Solomon Islands (Cogger, 2000; Gardner et al., 2008). This group of species includes some of the largest (70-380 mm snout to vent length) and best known skinks in the world (Greer, 1989; Chapple, 2003; Gardner et al., 2008). The study species of this thesis comes from the genus *Tiliqua*, and the general biology and behaviour of species in that genus are given below.

1.4.2. TILIQUA

There are seven described species in the genus *Tiliqua* (Table 1-2; Figure 1-1). All are viviparous, long lived and late maturing skinks (Chapple, 2003). They vary in size, morphology and ecology. *Tiliqua scincoides intermedia* with an average snout to vent length (SVL) of 371 mm is the biggest member of this genus and *T. adelaidensis* with SVL 95 mm is substantially smaller than any other member of the genus.

Table 1-2. All species of genus *Tiliqua* (Meiri, 2008).

Species	**Adult SVL**
Tiliqua adelaidensis	95
Tiliqua gigas	343
Tiliqua multifasciata	300
Tiliqua nigrolutea	368
Tiliqua occipitalis	320
Tiliqua rugosa	350
Tiliqua scincoides	371

Most of the species of *Tiliqua* are commonly known as blue tongue lizards (even though *T. adelaidensis*, the pygmy bluetongue lizard, has a pink tongue!). They use their coloured tongue in an open mouth display as a defensive response to intruders (Greer, 1989). Among the seven species *T. rugosa* has been the best studied for its behaviour, ecology, physiology and morphology (De La Lande et al., 1962; Bull, 1995; Bull and Pamula, 1996; Main and Bull, 1996; Bull and Baghurst, 1998; Bull et al., 1998; Kerr et al., 2003b; Leu et al., 2011; Godfrey et al., 2012; New et al., 2012).

Figure 1-1. Six species of the genus *Tiliqua.* Photo were taken by Dr Aaron Fenner and Mehregan Ebrahimi. *Tiliqua gigas* photo: http://commons.wikimedia.org under free copy right license.

Several studies have compared feeding behaviour of *Tiliqua*. It seems the smaller members of this genus prey mainly on arthropods while the larger members are omnivorous and eat a considerable amount of plant material (Greer, 1989; Hutchinson et al., 1994; Fenner et al., 2007). They usually have a courtship in spring and summer. Mating has been described in different species to involve the male grasping the female's head, neck or shoulder, then the female raising her hind body, by straightening her hind legs to allow the mounted male to mate (Greer, 1989; Shea, 1992; Bull and Pamula, 1996; Edwards et al., 2002). Most members of genus *Tiliqua* are solitary for most of the time, with normally only one individual or two individuals found together. Many of them have territories or stable home ranges (Greer, 1989; Bull and Baghurst, 1998; Bull and Freake, 1999). They are large and relatively slow moving skinks, they are mostly diurnal, and they usually shelter under dead plants, under litter, or in burrows made by other animals (Greer, 1989; Koenig et al., 2001; Kerr et al., 2003a). In fact, they appear to be incapable of digging their own burrows (Greer, 1989). Studies has shown some members of the genus, such as *T. rugosa,* can discriminate among scent cues from different conspecific individuals, and these olfactory cues also help them to locate partners, and their own offspring. Other environmental olfactory cues may be used to recognize places that are within their home ranges (Greer, 1989; Zuri and Bull, 2000).

One of the seven *Tiliqua* species *Tiliqua adelaidensis* is listed as Endangered (IUCN, 2013a) and because of its specific habitat needs and the extreme fragmentation of its native grassland habitat, and predicted future changes to that habitat under climate change, the future for this species is unclear. Therefore, we selected *T. adelaidensis* (pygmy bluetongue lizard) as the case study for this thesis.

1.4.3. TILIQUA ADELAIDENSIS (PETERS, 1863)

Tiliqua adelaidensis or the pygmy bluetongue lizard (Figure 1-2) is the smallest member of the genus *Tiliqua* (average snout-to-vent length of 95 mm) (Fenner et al., 2007). Prior to the 1990s, fewer than 20 specimens had been collected between 1863 to1959, and it was considered to have probably become extinct. Its known distribution, from those specimens, extended about 150 km from south to north, from the Adelaide Plains to Burra in the mid north of SA (Ehmann, 1982; Shea, 1992). The last live specimen was recorded from Marion (a suburb of Adelaide), in 1959. There were vigorous attempts to find specimens of this species during the 1960s to the 1980s, but all attempts were unsuccessful, so it was believed that the pygmy bluetongue lizard was extinct, the only Australian skink to be lost after European settlement (Armstrong et al., 1993; Cogger, 2000). Then in October 1992, a *T. adelaidensis* was found dead in the stomach of a road-killed brown snake (*Pseudonaja textilis*) near Burra in South Australia (Ehmann, 1982; Armstrong and Reid, 1992; Armstrong et al., 1993), and subsequent searches have located around 30 extant populations, all in the mid-north region of South Australia.

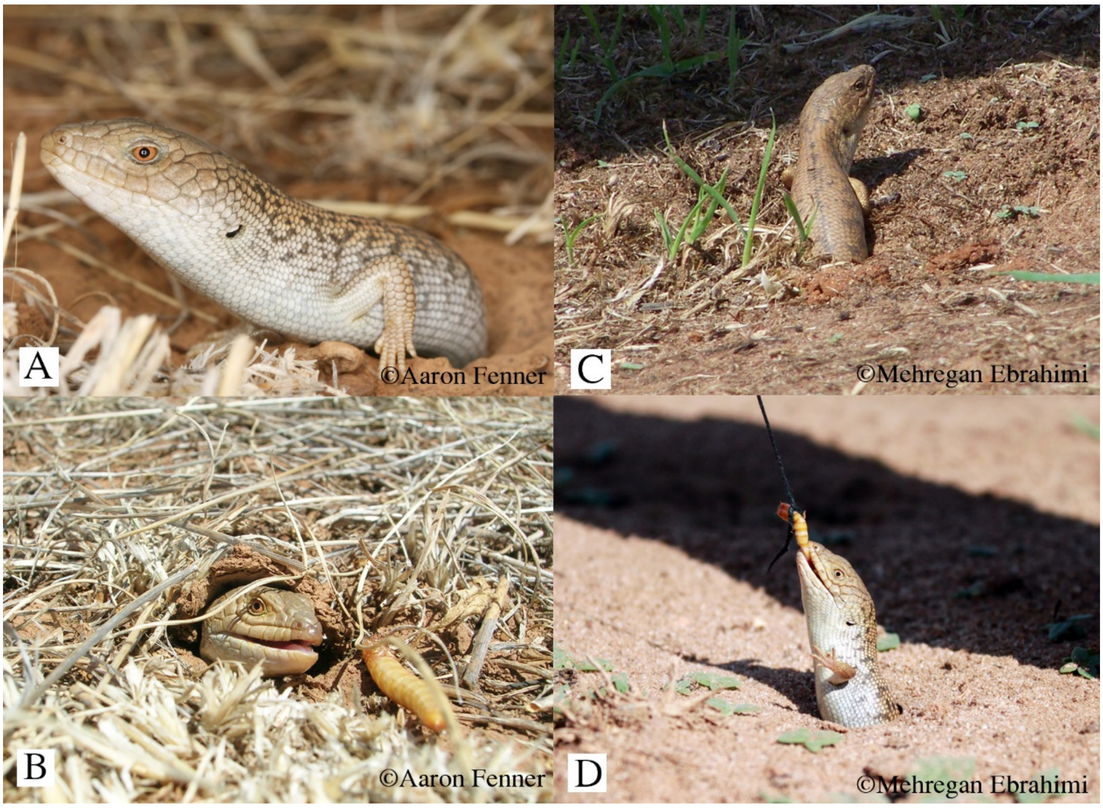

Figure 1-2. Pygmy bluetongue lizards in natural A and B and artificial burrows C and D. Photo were taken by Dr Aaron Fenner and Mehregan Ebrahimi

Most current known populations of *T. adelaidensis* and historical locations are shown in Figure 1-3. The presence of lizards is closely associated with small remnant patches of native grassland. This habitat was once much more widespread, but has now largely been degraded by extensive agricultural activity in the region.

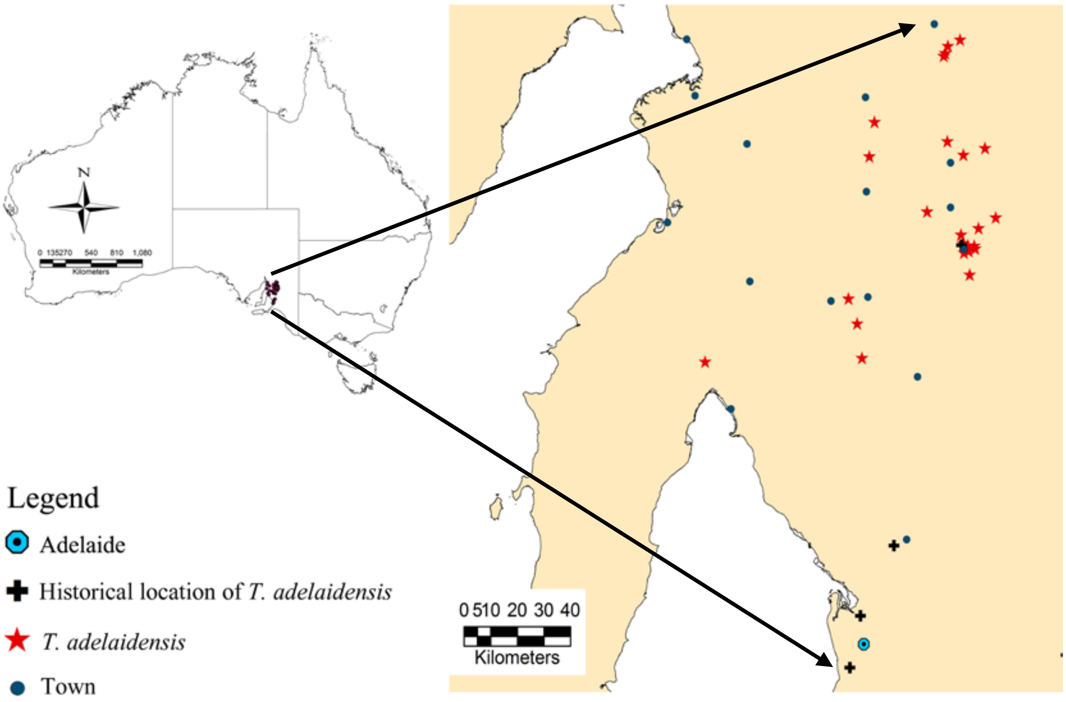

Figure 1-3. Historical and present populations of *T. adelaidensis*

These population sites are isolated remnant patches of native grasslands with dominant species of spear grass (*Stipa* spp.), wallaby grass (*Danthonia* spp.), iron grass (*Lomandra* spp.) and wire grass (*Aristida behriana*) (Hutchinson et al., 1994; Milne, 1999). *Tiliqua adelaidensis* prefers grasslands with an open area between clusters of grass. Ungrazed grasslands with a thick layer of thatch appear to be less suitable because the lizards cannot see as easily insects or other prey. The species also appears to avoid areas that have been ploughed (Souter, 2003). An essential habitat feature for the pygmy bluetongue lizard is the presence of the single entrance, narrow vertical burrows they use for shelter. In natural populations lizards spend most of their time closely associated with their individual burrows, refuging inside the burrows from predators and climatic extremes, and using the burrow entrance as a site for basking and for ambushing passing invertebrate prey. The lizards cannot dig their own burrows but rely on vacant burrows left by lycosid and mygalomorph spiders. Each lizard normally occupies a burrow alone, and they prefer deeper burrows (>30 cm in depth) and burrows with an entrance diameter that is slightly wider than their head width (average 15.1 mm). Large adult lizards prefer deeper and wider burrows while juveniles occupy burrows that are less deep, and have narrower entrances (Hutchinson et al., 1994; Milne and Bull, 2000; Milne et al., 2003b). *Tiliqua adelaidensis* spend most of their time associated with their burrows and normally leave the burrows only briefly and only in a few circumstances (Milne et al., 2003b) such as for darting out to catch prey (Hutchinson et al., 1994; Milne et al., 2003b), and for defecation (Fenner and Bull, 2010). Males make more extensive forays from their burrows while searching for females in the breeding season (Fenner and Bull, 2009; Schofield et al., 2012) and sometimes lizards seek new burrows (Milne et al., 2002b; Fellows et al., 2009; Fenner and Bull, 2011b). The species is largely solitary; tolerating neighbors in near-by burrows that can be as close as 1 m apart (Souter, 2003), but not tolerating conspecifics who approach within a few centimeters of their burrows (Fenner and Bull, 2011a). They use an aggressive burrow defense up to a short distance from the burrow that does not require full emergence (Fenner and Bull, 2011a). This observed tendency of *T. adelaidensis* to have infrequent movement was confirmed by genetic data showing restricted gene flow even within small populations. and significant genetic structure in a population occupying continuous habitat (Smith et al., 2009). Mothers and babies share maternal burrow for a few days to a few weeks after birth in Jan-Feb (Milne et al., 2002b), but the juveniles always disperse from maternal burrows at some time during late summer. The only other case of burrow sharing is when males enter female's burrows during mating (Milne et al., 2003b).

It is clear that burrows play an important role in the survival of *T. adelaidensis*. Using artificial refugia is a common approach in many conservation programs (Sullivan et al., 2000; Spring et al., 2001; Harper et al., 2005; Beyer and Goldingay, 2006). For the pygmy bluetongue lizard, some research has focused on whether lizards will accept artificial burrows in natural populations. Various forms of vertical holes in the ground, made by hammering a steel peg into the ground, or by use of an auger, or by drilling out the centre of a length of wooden dowling, have all been trialed and all have been accepted by lizards as alternative burrows, as long as they are deep enough and have appropriate entrance diameters (Milne et al., 2003a; Souter et al., 2004). In fact, adding artificial burrows to experimental quadrats caused an increase in local lizard density, presumably reflecting that local population size is limited by the availability of appropriately sized burrows (Souter et al., 2004).

Previous studies have also showed that lizard behaviour is changed by grazing. In lab and field trials, where grazing was simulated by a decrease in local vegetation cover, *T. adelaidensis* spent more time basking with simulated grazing around their burrow entrance, but they were reluctant to occupy artificial burrows in areas with simulated heavy grazing within natural populations (Pettigrew and Bull, 2011a; Pettigrew and Bull, 2012). Fenner and Bull (2007) reported that grassland fire affects the body condition and behaviour of pygmy bluetongue lizards. After fire, although there were no records of adults killed by fire, there were significant reductions in activity and foraging and lizards developed poorer body condition in burnt compared to unburnt sites. These results show how the grassland habitat plays an important role in the survival of this endangered species. Since the species has a very small geographical range and depends on undisturbed grasslands, all populations are potentially threatened by agricultural activity in this habitat (Milne, 1999).

1.5. STUDY AIMS

The study aimed to determine the set of conditions most likely to persuade translocated individuals of the pygmy bluetongue lizard to remain close to a release site (There is more information regarding the two alternative terms "release site"(= point of release), and "release area" (=wider region into which released animals are expected to disperse and establish) in chapter 4).

Nowadays many factors threaten biodiversity, and habitat loss, fragmentation and climate change are some of the important factors (Andren, 1994; Boswell et al., 1998; Keller et al., 2004; Shoo et al., 2006). Mobile species may be able to respond to local declines in habitat quality by dispersing and establishing in newly suitable habitat fragments (Parmesan and Yohe, 2003; Root et al., 2003), but other species which are less mobile, such as many reptiles, cannot exit an unsuitable habitat, and therefore may become trapped in it. Some ecologists believe that the best way to conserve these endangered species is by translocation (Hulme, 2005). However, animal translocation is not always successful (Westoby and Burgman, 2006) and many reptile translocations in past decades have failed (Dodd and Seigel, 1991; Germano and Bishop, 2009).

We simulated translocations of one endangered Australian skink (*Tiliqua adelaidensis*) as a case study to identify how environmental factors and novel habitat can affect translocation success. We recorded short-term differences in lizard behaviour at simulated release sites with different treatments applied, to identify how habitat factors influence behavioural change, and how habitat can affect the tendency of lizards either to disperse away, or to establish new populations at the release site.

The pygmy bluetongue lizard is an appropriate case study in conservation management, because, all known populations live in highly fragmented patches of native grassland, and all are on private land. Each could potentially suffer unsuitable conditions, resulting from individual land management decisions by the property owners, such as insecticide-based locust control, changed grazing regimes, or altered infrastructure of tracks and dams. Additionally, although these populations have suitable habitat at present; habitat quality is likely to change due to future climate change (Delean et al., 2013). Therefore, the present time offers a good opportunity to develop information about a future approach to a strategy for conservation. This project can also provide much important information about conservation strategies for other endangered lizards.

A major advantage of the pygmy bluetongue lizard for experiments that simulate translocations, is the relatively small space needed to sustain an individual lizard. Because each lizard rarely moves more than a metre from the burrow that it occupies, several can be contained within a relatively small area, and relatively short dispersals can be taken to indicate that the lizard wants to move. All of this activity can be confined within a manageable and observable area, so that all behaviours can be seen and no lizards actually escape by their dispersal.

CHAPTER TWO

2. GENERAL METHODS

2.1. CAPTURE AND TRANSLOCATION OF PYGMY BLUETONGUE LIZARDS

We captured 8 adult male and 8 adult female of pygmy bluetongue lizard from two populations near Burra (4 males and 4 females from each), South Australia (33° 42´S, 138° 56´E) on 30th of September 2009 (Figure 2-1). Lizards were captured by fishing rod with a meal worm attached to a short string (50 cm) at the head of the rod (Figure 2-2). Lizards were measured and weight (Table 2-1) then transferred to Animal Care Unit of Flinders University, Adelaide. Each lizard was held in an individual plastic box (52.5 x 38 x 31cm) with one artificial burrow, and kept in a 25°C room with a 12: 12 LD light regime. Crickets and meal worms were added to individual plastic boxes every third day, before the lizards were transferred to Monarto Zoo for the first experiment on 25 October 2009. Between experiments during that spring and summer lizards were kept at Monarto Zoo in another 25°C room, also with a 12: 12 LD light regime. After the final experiment in the 2009-2010 field season we moved the lizards back to the Flinders University Animal Care Unit. Temperature and light were gradually reduced to 15°C and 10:14 LD over the austral winter and then brought back to 25°C and 12:12 LD by the following spring for the next field season.

Table 2-1. Pygmy bluetongue lizard specimens that were used in this survey.

Lizard ID	**Sex**	**Weight (gm)**	**SVL (mm)**	**TBL (mm)**
2123	Female	10	89	144
2216	Female	11.5	98	155
2224	Female	13	89	149
11067	Female	11	87	140
11068	Female	13	93	144
11069	Female	9	81	135
11070	Female	12	91	153
21063	Female	10	86	141
150	Male	10	86	145
1183	Male	14	86	149
11071	Male	10	86	135
11072	Male	14	91	151
21062	Male	12	91	147
21064	Male	10	82	137
21065	Male	9	81	135
21075	Male	9	78	129

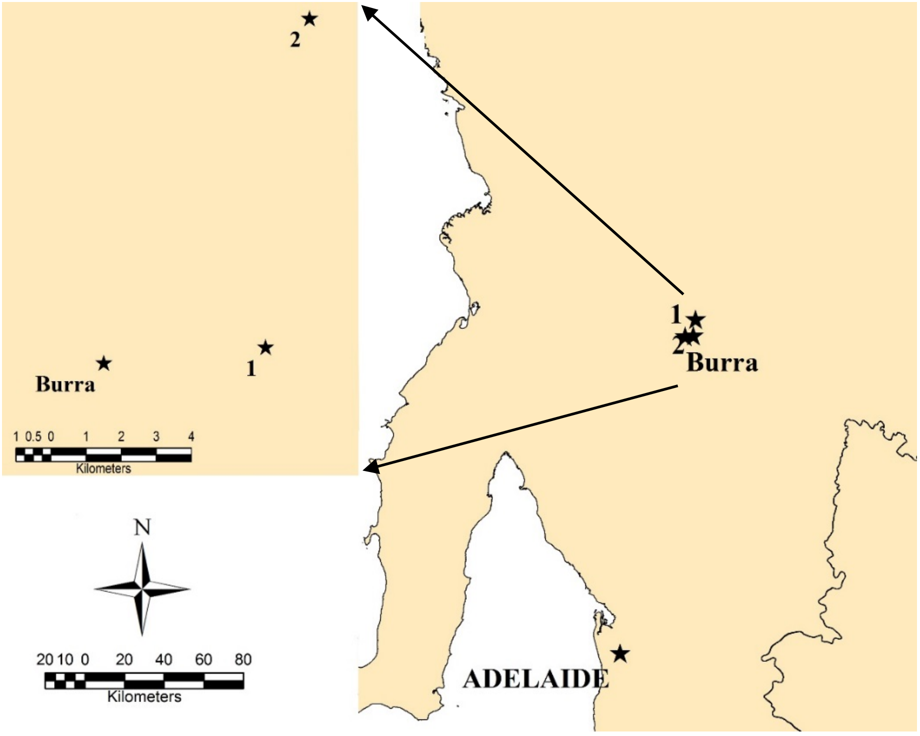

Figure 2-1. Pygmy bluetongue lizards were captured from these two populations (Black star 1 and 2).

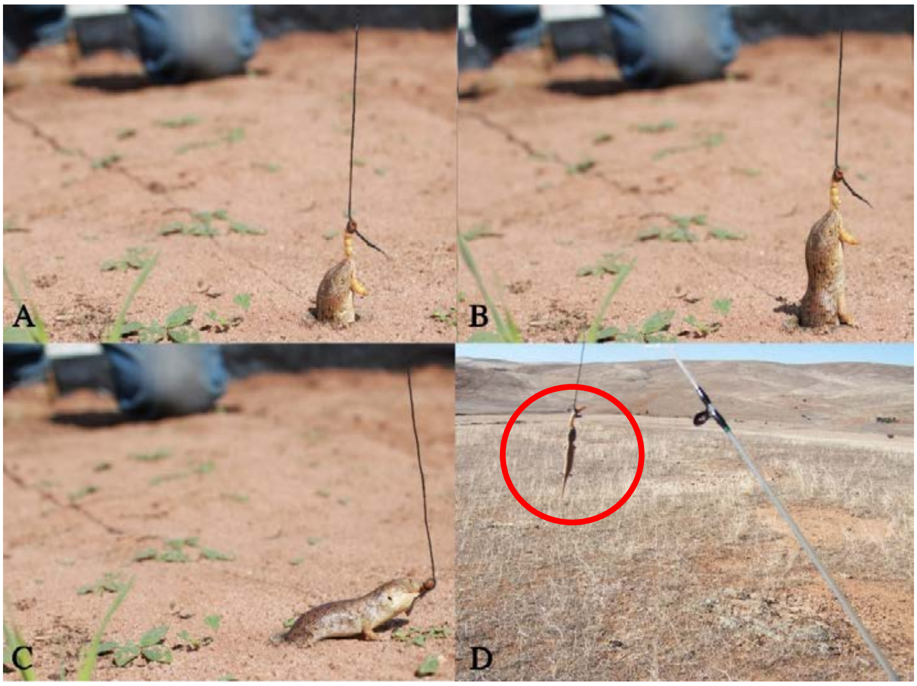

Figure 2-2. Catching a pygmy bluetongue lizard with a fishing rod and meal worm. A) Lizard bites at the meal worm from inside the burrow, B) trying to pull lizard out of the burrow, C) lizard now almost out of the burrow and D) lizard is completely out the burrow.

2.2. STUDY AREA

Four enclosures were established at Monarto Zoological Park in South Australia (Figure 2-3), to contain the simulated translocation experiments. This park is an open-range animal sanctuary and has an area of 10 square kilometers. It is located at Monarto, (35°06´08˝S 139°08´33˝E) approximately 70 km from Adelaide (Figure 2-3). This site is a suitable choice for a translocation study and for future captive breeding of *Tiliqua adelaidensis*, because it is probably within the historic range of this endangered species (Ehmann, 1982; Armstrong and Reid, 1992).

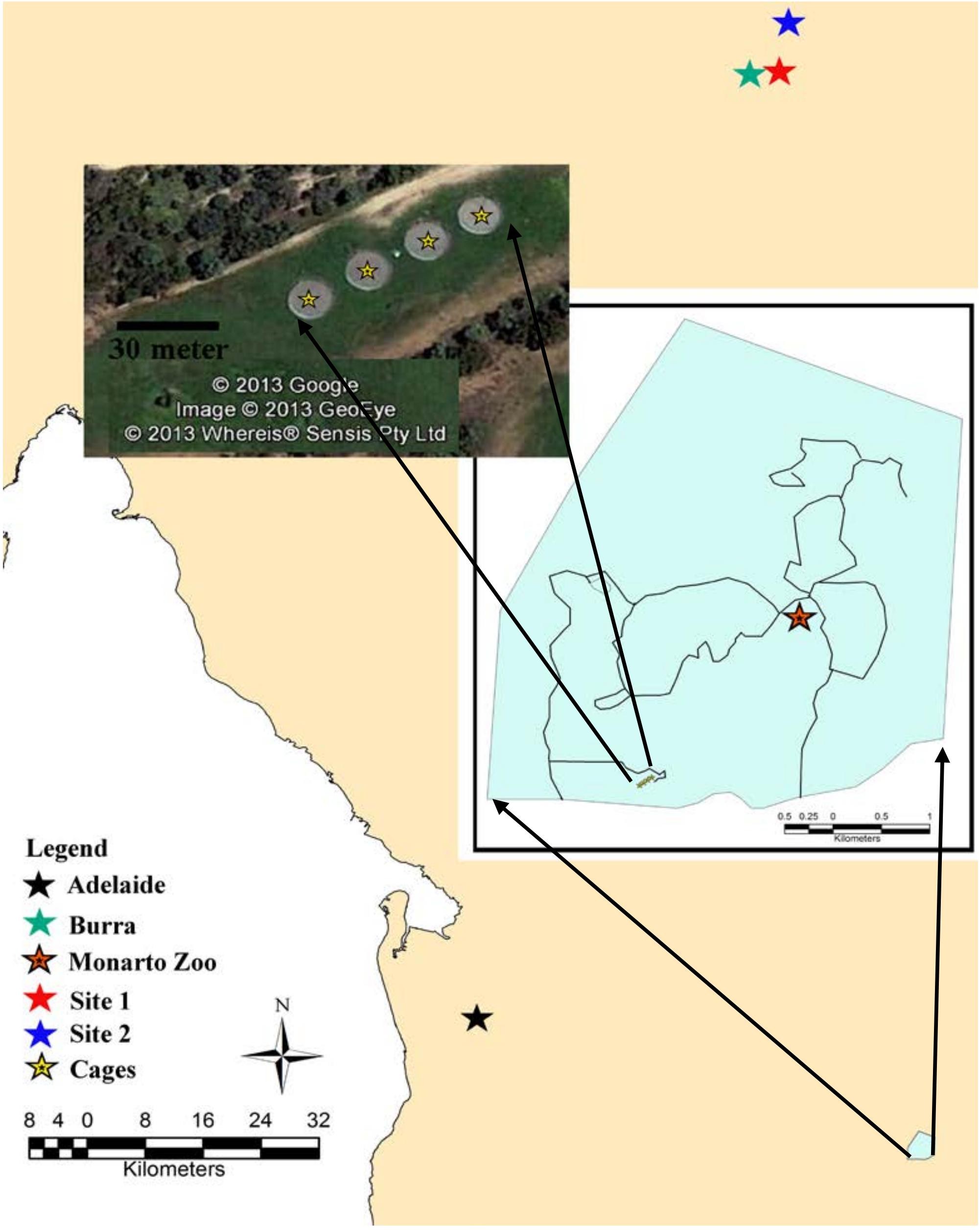

Figure 2-3. Four cages that were established at Monarto Zoo. Site 1 and 2 are were the pygmy bluetongue lizard were captured.

2.3. ENCLOSURES

We established four circular 15 m diameter enclosures in a line and 5 m apart (Figure 2-4). Each enclosure had a one metre high galvanized iron wall with a bird wire roof (Figure 2-4). We divided each enclosure into three areas. One was a central 4 m diameter (2 m radius) circular area as the main experimental lizard habitat, which was lightly vegetated with annual grass, normally cut to ground level before each experiment started. The central area was surrounded by the second area, a 5 m wide ring of lightly tilled, bare ground representing a matrix of unsuitable habitat for lizards. The third area was a 0.5 m wide marginal area with intact grasses, around the inner perimeter of each cage (Figure 2-5). Specific exceptions to this normal arrangement in individual experiments are described in the relevant chapters. Resident lizard infrequently moved from their burrows and even restricted their aggressive burrow defense to a distance that does not require them to completely emerge (Milne et al., 2003b; Fenner and Bull, 2011a). Rare movement beyond burrows are for prey capture or defecation (Milne et al., 2003b; Fenner and Bull, 2010). These reports were confirmed by restricted gene flow even among small patches of continues habitats (Smith et al., 2009). As the individual pygmy bluetongue lizards need very small spatial requirement the size of enclosures I used were appropriate for this species.

Figure 2-4. Established cages at Monarto Zoo.

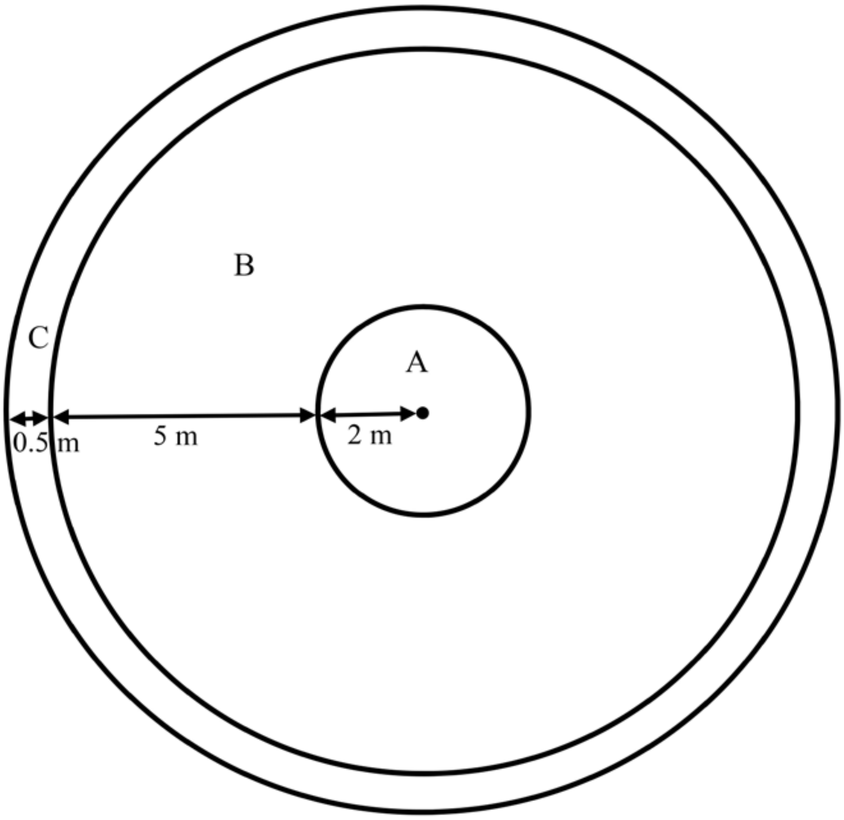

Figure 2-5. A) Central area, B) Unsuitable habitat and C) Marginal area.

2.4. ARTIFICIAL BURROWS

Previous studies have shown that *T. adelaidensis* accept and use vertical artificial burrows (Milne et al., 2003a; Souter et al., 2004). We constructed artificial burrows from 30 cm lengths of 3 cm diameter wooden dowling with drilled-out 2 cm diameter central holes (Figure 2-6). To insert each burrow into the ground, we made a 3 cm diameter hole in the ground with an auger and hammered the artificial burrows into them until the entrance was flush with the ground surface We normally placed 41 artificial burrows in the 4 m diameter central area of each cage, one burrow in the centre, and the rest arranged in three concentric rings, 8 in the first ring and 16 in second and third rings. These burrows were 65-75 cm apart. We also added 30 artificial burrows, evenly spaced around the 0.5 m wide marginal area, to provide refuges for (and allow monitoring of) any lizards that dispersed from the central area (Figure 2-7).

Figure 2-6. Wooden dowling artificial burrows. The upper two images are the two halves of a burrow that has been cut open to reveal the internal structure.

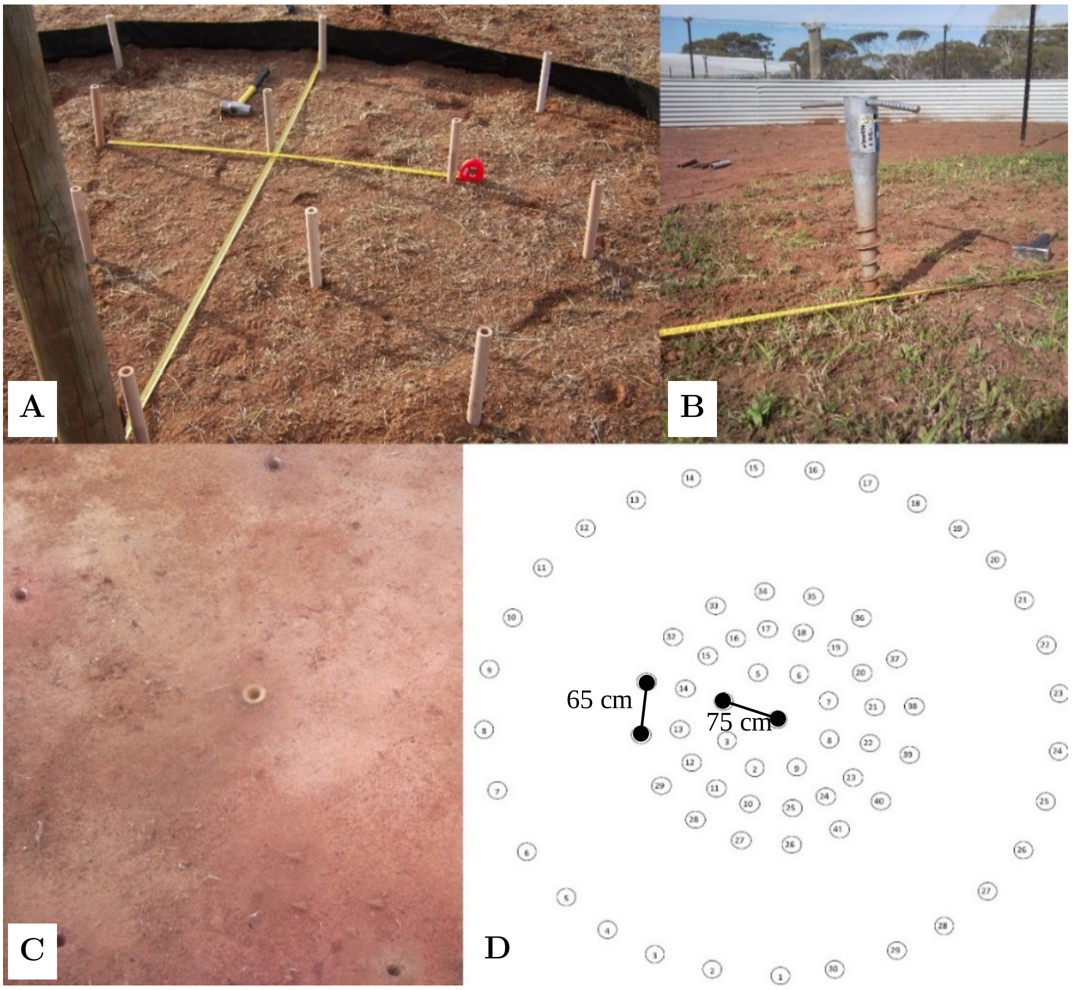

Figure 2-7. A) Artificial burrows before we hammered them into the ground, B) Auger for making holes for artificial burrows, C) Burrows hammered into the ground, D) Final layout of burrows in cages.

2.5. ENCLOSURES AND ARTIFICIAL BURROWS MAINTENANCE

Between experiments and between separate trials within experiments, we cleaned any accumulated debris from the burrows and cut the grass with a line trimmer (Figure 2-8) within the enclosures. Burrow cleaning was accomplished using a cordless drill with a 35 cm long, 1.9 cm diameter drill bit (Figure 2-8).

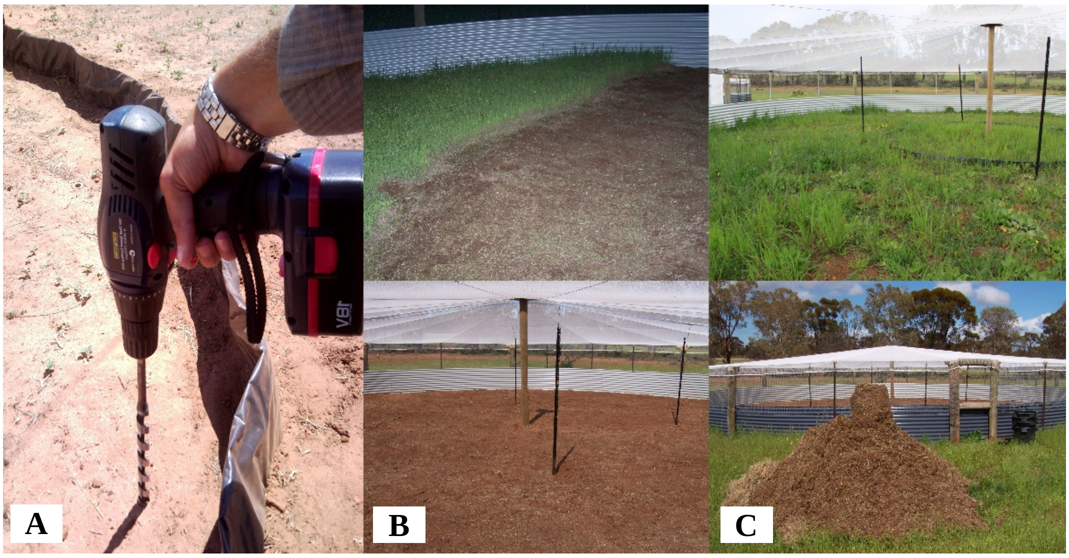

Figure 2-8. A) Cleaning burrows, B) and C) Cutting grass in cages.

We also checked each enclosure regularly to remove any resident wolf spiders and fill in any burrows they had started to construct (Figure 2-9 a-b). Mice also sometimes dug into the cages and constructed two kinds of holes, one the normal hole, which the entrance was inside a cage (Figure 2-9 d). The other was a passage way between the inside and outside the cage wall (Figure 2-9 c). Both types of holes were destroyed as soon as they were detected, and if mice were inside the cage they were removed (Figure 2-9 e). No lizards used these potential escape routes before they were detected and destroyed.

All of above maintenance activity happened between experiments while lizards were not in the enclosures, or, during experimental trials, early in the morning before lizard activity had started, so that any disturbance would not have affected normal lizard behaviour.

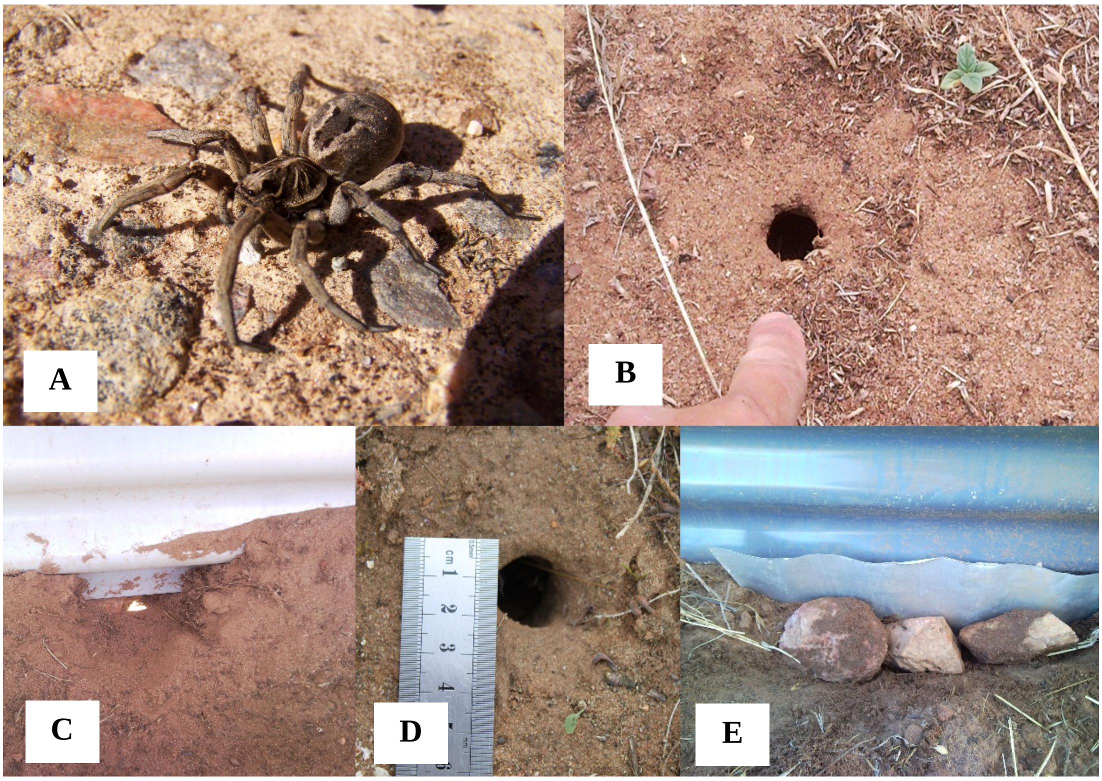

Figure 2-9. A and B) Wolf spider and its burrow, C and D) Mouse holes; E) mouse hole that was closed from outside the cage wall.

2.6. SURVEILLANCE CAMERAS

Four surveillance cameras (Longse: LICS23Hf, 3.5 mm lens) were suspended from the roof of each cage so that their combined field of view covered the entire central area of the cage. This allowed us to record all behavioural activity of each lizard within that area from 07:00 to 18:00 h on each day of each experiment, without the potential disturbance from the presence of an observer. We used a DVR h.264 with 16 channels (ESW26) to record footage from the 16 cameras. Four 12 V batteries (Power PS Sonic, Ps121000U, 100 Amp.hr) supplied power for the DVR and cameras. We used two batteries each day, while the other two were being charged for use on the next day. We also used a Samsung 15 inch LCD monitor to remotely monitor video images, and confirm the system was operating during experiments. Components of the recording system are shown in Figure 2-10. Overall, 16 cameras, each filming for 11 hours each day, over 94 days, produced image data that required ten 1.5 and 2 TB hard drives for storage.

Figure 2-10. DVR, cameras and batteries we used for filming lizard behaviour

In addition to the filming of the central area of each cage, during each experiment the status and location of each lizard was checked twice a day, once early in the morning before lizard activity had started, and the other late in the evening after lizard activity had finished. During these checks, we used a pen torch to inspect each burrow to detect where the lizards were located. In particular we confirmed the location of those lizards that had moved out of the field of view of the cameras, and into burrows in the marginal area.

We used two digital thermometers, one at each end of the line of enclosures, to provide continual records of ambient shade temperature during trials in each experiment. In addition we used climatic data from Pallamana Aerodrome (35.07° S, 139.23° E), 10 km from Monarto Zoo to confirm the site readings.

2.7. DATA COLLECTION AND EXTRACTING DATA FROM FILM FOOTAGE

Most of the analyses in this project were derived from viewing the video footage that recorded *T. adelaidensis* activity during each day of each experiment. The amount of data was very large. In total, there were 16544 hours of footage, which came from 16 cameras that recorded 11 hours a day for 94 days. This is the equivalent of 690 twenty four hour days. To watch these at normal speed for 12 hours a day would have taken about 3.7 years. To reduce this time we used Elecard AVC HD Player (version 5.7 build 24606.100529) with superfast speed in the lengthy periods when there was no lizard activity, only slowing the playback to normal speed when lizards were active. In this way every 7 hours of actual footage could be viewed in one hour. Nevertheless the entire viewing process took 296 days. We recorded all extracted data in Microsoft Excel 2010 spreadsheets and prepared them for further analysis.

2.8. DATA ANALYSIS

We used Microsoft Excel 2010, IBM SPSS Statistic 21 and RapidMiner 5.3.013 to analyse the data. For plotting the results we used SigmaPlot 5.0. The details of data analyses for individual experiments are explained in each chapter. But generally each experiment was designed so that there were two replicate cages for each of two alternative treatments in the central area of the cages. Normally four lizards were released into the central area of each cage and observed, normally, for the four following days. We tested hypotheses in each experiment, about the relative tendency for the alternative treatments to encourage lizards to establish residency and remain in burrows in the central area. Individual assays are indicated with each experiment, but among the behaviours that we analysed were basking time and number of movements that lizards made around a burrow, as indicators the lizard was settled there, and moves between burrows either within the central area, or to the marginal area. Those latter moves to the marginal area were taken as a direct indication that the lizard was likely to disperse across inhospitable substrate under the conditions provided.

We recognize that four days is not necessarily going to encompass all of the relevant period when dispersal may occur. But for this thesis, with the limited time available when lizards were active, the aim was to explore a wide range of alternative conditions, so a compromise was made to run many short experimental trials rather than a few longer ones. We believe though, that many of the significant dispersal events in translocations happen soon after release, when the animals are most stressed and most unfamiliar with the release site (Wanless et al., 2002; Rouco et al., 2010; Mitchell et al., 2011). So a primary aim of all of the experiments could be viewed as determining the set of conditions that will best encourage animals to stay where they are released in the immediate period after release. Other studies may need to follow up with longer term observations, perhaps focusing on the set of conditions derived from the work in this thesis.

CHAPTER THREE

3. SHORT-TERM CONFINEMENT TIME

3.1. HARD RELEASE OR SOFT RELEASE

For translocation to be successful the released individuals must survive predation and exposure, locate food and shelter resources, locate each other for reproduction, and produce sufficient recruits to allow the population to persist and grow. Usually translocations are to sites with optimal habitat, and an immediate problem following release is if released individuals disperse away from the release site. This dispersal will take them away from the resources and habitat of the release site, and make it difficult for reproductive contact.

There have been many records of translocations in the past decade, but their success has not always been clear. One of the most important causes of this uncertainty is that translocated animals tend to disperse from novel habitats (Dodd and Seigel, 1991; Attum et al., 2010). Unfamiliarity with the new habitat and handling stress before release can increase the chance of dispersal if animals are directly released to a translocation site (hard release). An alternative (soft release) has been to keep animals for a period of time in enclosures at the translocation site before release, allowing them to become familiar with the local conditions and reducing stress, and, it is suggested, decreasing post release dispersal. An increasing number of papers report that the soft release strategy can improve translocation success (Kleiman, 1989; Bright and Morris, 1994; Letty et al., 2000; Teixeira et al., 2007). They report that with soft release animals have less stress and are more likely to establish their home range around the release site (Bright and Morris, 1994). However, to this stage, there has been no analysis of the optimal period of soft release in most of these published papers. Most of papers report long confinement times. For instance Teixeira et al. (2007) showed gopher tortoises dispersed less from a translocation site after they had been kept in enclosures at the site for 12 months before they were actually released. But keeping animals for a long time in enclosures before release can have significant financial costs to a conservation program. It can also have some negative impacts on the animals such as dependency of the animals on the provided resources, lack of awareness of predation risks, and the risk of spreading diseases during close confinement. Short term confinement before actual release at translocation sites might reduce stress, allow some familiarisation with the site, but reduce the financial and fitness costs described above.

In chapter three we compare two short-term confinement strategies to find out how it can alter the tendency of lizards to disperse from a novel habitat.

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

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3.2. ABSTRACT

Translocation is a powerful tool in conservation management, but one of the major problems of this tool is dispersal after release. Translocated animals might disperse from prime habitat and face unsuitable habitat and possible increased exposure to predators. This might lead to decline of a translocated population and could compromise the success of translocation. 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 an Australian lizard, 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. Future research could determine the effectiveness of different durations of confinement for releasing animals at translocation sites.

Keywords: Australia, Conservation, Endangered, Lizard, Soft release, *Tiliqua adelaidensis*, Translocation.

3.3. 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; Wolf et al., 1996; White et al., 2003; Reynolds et al., 2008), 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; Letty et al., 2000; Heidinger et al., 2009). Each of these factors could cause high rates of mortality or dispersal from the translocation site very soon after the release event (Boissy, 1995; Armstrong et al., 1999; 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) (Tuberville et al., 2005; Tuberville et al., 2008; Attum et al., 2010).

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 (i) the dependency of the animals on provided resources, (ii) the risk of spreading disease among the confined animals, and (iii) the risk of stress from confinement (Reed and Stockdale, 1994; Lovegrove, 1996; Letty et al., 2000) 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, 2013a). 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., 2003b). 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., 2003b), 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., 2003b), 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 successfully 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.

3.4. METHODS

We captured sixteen pygmy bluetongue lizards (eight males and eight females) from two wild populations near Burra, South Australia (33°42´S; 138°56´ E). All currently known populations are in this area, and our permit conditions specified that collections should be spread over two sites. 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). Monarto Zoo is an open sanctuary which could be a possible translocation site and a safe place for a new population. 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).

3.4.1. Experiment design and data collection

In our experimental system we simulated the initial phases of a translocation release within the central part of the circular cages. Within those cages we monitored behaviours, such as movement, burrow changing and agonistic interactions, that might lead to dispersal. We derived the tendency to disperse by the number of times lizards moved from the 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).

Burrows are a fundamental resource requirement for this species and the lizards spend most of their time associated with their burrows (see Introduction). To provide this resource in the cages 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., 2012a). 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 from the same population 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° 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 in analyses of any of the seven parameters, because sample sizes became too small.

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.

3.4.2. 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 that might be related to dispersal tendencies, such as movement between burrows, activity time, agonistic interactions and time spent basking. 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.

3.5. 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.

3.5.1. EFFECT OF AMBIENT TEMPERATURE

Daily maximum temperatures varied by almost 20°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, $p < 0.001$); where lizards spent less time basking on hotter days (Figure 3-1). 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, $p < 0.005$); where lizards were more likely to move away from the central area after warmer nights.

3.5.2. 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 3-1. 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.

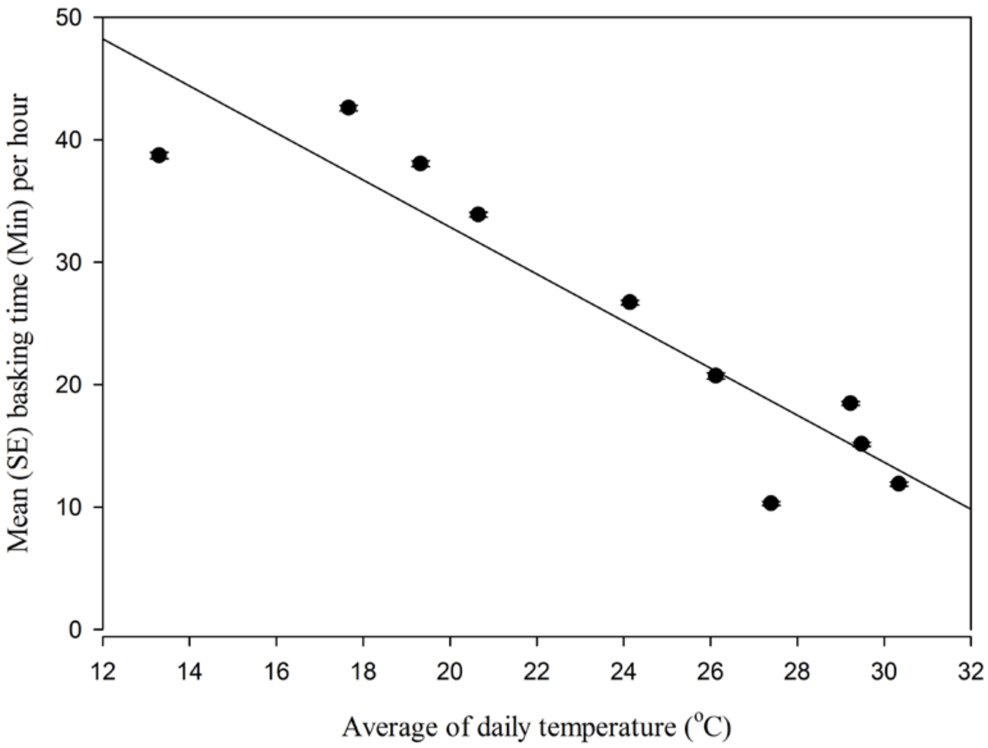

Figure 3-1. Relationship between mean basking time (min) per hour and average daily temperature (°C).

For mean basking time per hour there was a significant interaction effect of treatment and day (Table 3-1). Figure 3-2 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).

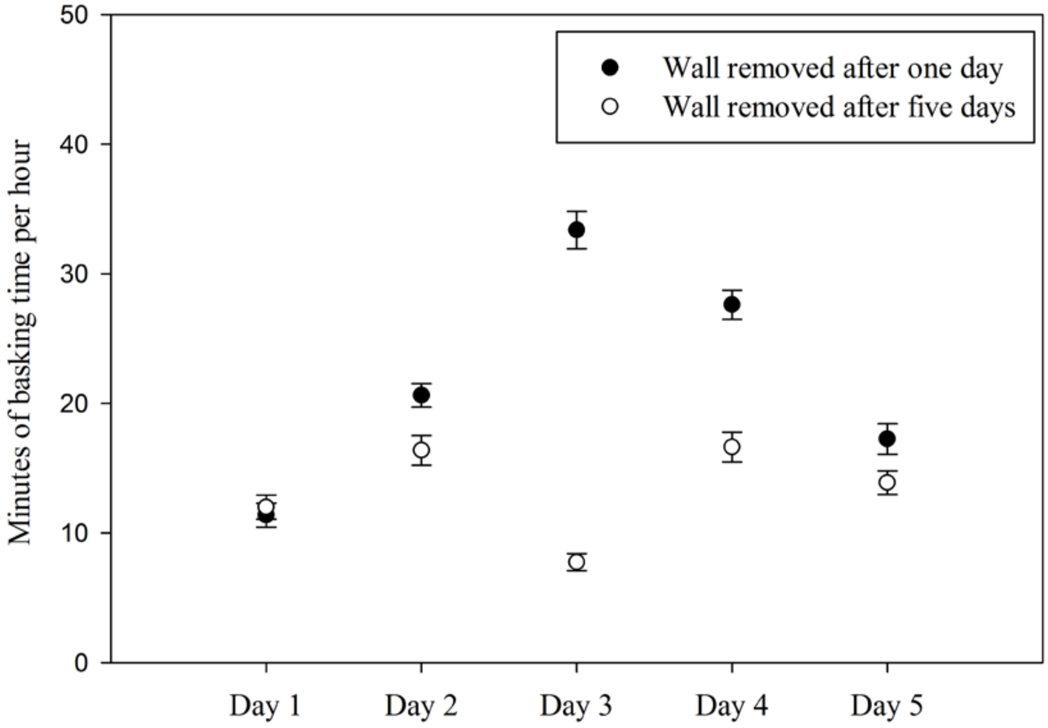

Figure 3-2. Mean (SE) basking time (mins per hour) in cages where the wall was removed after one day, and where the wall was removed after five days, in the first five days after the wall was removed.

For the number of lizards that moved to the marginal area there was also a significant interaction effect of treatment and day (Table 3-1). 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) (Figure 3-3).

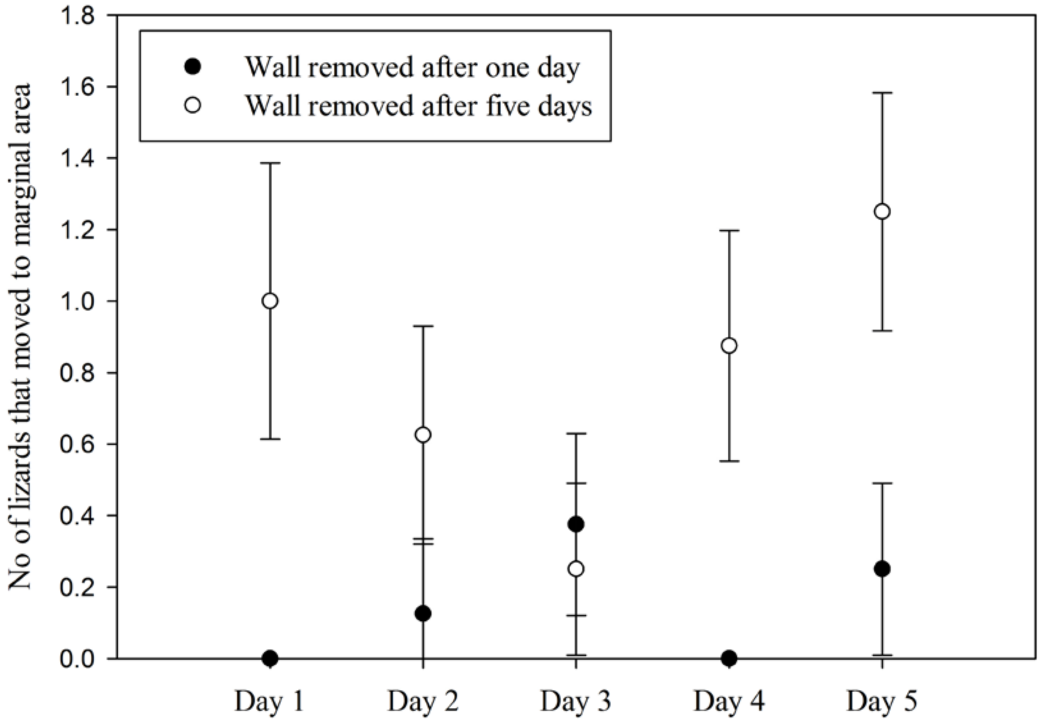

Figure 3-3. Mean (SE) number of lizards that moved to the marginal area in each cage in cages where the wall was removed after one day, and where the wall was removed after five days, in the first five days after the wall was removed.

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 3-1). Lizards moved further in one treatment than the other on some days, but that difference was reversed on other days.

Table 3-1.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 star.

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

3.6. 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 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 (Ebrahimi and Bull, 2012a), 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) as seen in translocated male rabbits, that were found to require time to explore their surroundings and their social neighborhood (Letty et al., 2000; Letty et al., 2003; Teixeira et al., 2007). 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 (Davis, 1983; Bright and Morris, 1994; Carbyn et al., 1994). 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 (Bright and Morris, 1994; Linnell et al., 1997). 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 (Bright and Morris, 1994; Hardman and Moro, 2006). 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. 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. This could be one possible project for future research.

3.7. ACKNOWLEDGEMENTS

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CHAPTER FOUR

4. SUPPLEMENTARY FOOD

4.1. RESOURCE AVAILABILITY

There are many factors that could be responsible for species extinctions (Kleiman, 1989; Maunder, 1992; Armstrong and Perrott, 2000). Reduced availability of resources such as food is one of the important factors that can force dispersal and even local population extinction. Bommarco et al. (2010) showed that reduced resources led to increased dispersal in different species of wild bees. However, it is difficult to generalise about the exact resource needs of each species or about the specific factors likely to trigger dispersal and decline (Armstrong and McLean, 1995). Similarly we also need to consider resource quality as one of the critical factors for any translocation attempts (Griffith et al., 1989; Armstrong and Perrott, 2000). Those resources that are necessary for a target species should be considered when selecting a new habitat or repairing their previous habitat (Armstrong and McLean, 1995). In translocations an initial superabundance of the resources might encourage animals to stay where they are released. One obvious resource is food availability, which can play an important role in establishing a new population by reducing dispersal, enhancing reproduction and maintaining body condition (López-Bao et al., 2008; Schoech et al., 2008). In recent years, there has been an increasing interest in how supplementary feeding can influence reproduction among individuals that have been translocated, for instance in birds (Simmons, 1993; Hoodless et al., 1999; Elliott et al., 2001; Castro et al., 2003; Robb et al., 2008). It was shown that in translocation programs supplementary feeding can improve body condition and decrease post release dispersal (Boutin, 1990; Bright and Morris, 1994), but there is little known about how it can affect dispersal in reptiles. As already mentioned one of the major reasons that reptile translocation is not always successful is a tendency to disperse after release so any attempts that decrease the probability of dispersal could be vital for reptile translocation.

Supplementary feeding can also influence other behaviours of some animals, such as the level of agonistic interactions toward conspecifics, activity times, movement and basking times (McNamara, 1987; Werner and Anholt, 1993).

Changes in these behaviours might alter the chance of dispersal from the translocation site, or increase the time translocated animals expose themselves to predators or climatic extremes outside of shelters. Reducing these behaviours in the early stages of translocation, while animals are unfamiliar with their novel habitat could be a real advantage for any translocation program. In this chapter, we investigate how short-term supplementary feeding can decrease post-released dispersal in lizards.

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Food supplementation reduces post-release dispersal during simulated translocation of the Endangered pygmy bluetongue lizard *Tiliqua adelaidensis*

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4.2. ABSTRACT

Translocation is among several tools available to conservation managers, either to augment existing populations, or to establish populations in previously occupied habitat, or in habitat identified as suitable for the future persistence of the species. Translocated reptiles do not always become established at the release area. We simulated a translocation site for an Endangered Australian skink, the pygmy bluetongue lizard *Tiliqua adelaidensis*, to investigate whether adding food would encourage released individuals to disperse less. We provided artificial burrows in a central release area within circular cages and found that lizards were more likely to remain in a burrow, spent less time exposed on the ground surface and were less likely to move out of the central area when food was provided. These modified behaviours are likely to encourage translocation success if lizards with added food expose themselves less frequently to predators, and if fewer of those lizards disperse away from the translocation site in the early days after release. We suggest that the provision of supplementary

food will be an important component of any translocation programme for this lizard.

Keywords: Supplementary food, Translocation, *Tiliqua adelaidensis*, Conservation.

4.3. INTRODUCTION

In conservation management, translocation, or assisted colonisation, is the intentional movement of individuals from one area to another (IUCN, 2013b). Translocation can be used to augment existing populations and to reintroduce a species to currently unoccupied sites within its former range, or to assist colonisation of sites outside of its historic range that are considered suitable to sustain future populations. While there is debate about the impact on other members of the ecological community where translocations are outside the historic range, translocation is still considered an important tool for conservation management (IUCN, 1987; Ebenhard, 1995; Hodder and Bullock, 1997; IUCN, 1998; Tenhumberg et al., 2004; Rout et al., 2005).

Translocation success, measured by persistence and growth of the translocated population (Seddon, 1999; van Heezik et al., 2009), is not always high (Fischer and Lindenmayer, 2000; Germano and Bishop, 2009). There are 3 major problems. One is that translocated individuals are likely to disperse from the release site as a result of locally high population density if many individuals are released in the same area, competition and predation from the resident fauna, or inadequate resource quality and availability (Dodd and Seigel, 1991;

Armstrong and McLean, 1995; van Heezik et al., 2009). A second is that translocated individuals must become familiar with local food and shelter resources, and overcome pressure from resident conspecific and other competitors for those resources. A third is that hungry individuals might increase activity levels, exposing them to higher predation risk (McNamara, 1987; Werner and Anholt, 1993). As a result of the second and third problems, even those translocated individuals that do not disperse from the release site may still lose body condition, or fail to reproduce successfully in their new habitat (Wolf et al., 1996; Towns and Ferreira, 2001; Armstrong et al., 2007). For instance, slow-worms *Anguis fragilis* that survived translocation and remained at the site often had poorer body condition than long-term residents (Platenberg and Griffiths, 1999).

Translocations may be more successful if the impact of 1 or more of these factors is reduced at the release site (Hodder and Bullock, 1997; Armstrong and Perrott, 2000), and if positive factors are enhanced to encourage individuals to stay at a new site long enough to become established. For example, as food availability influences all levels of population and community dynamics (Boutin, 1990; Anholt and Werner, 1995), supplementary feeding may be used as a conservation tool. Early in translocations this is a form of a soft release to ease the released animals into the new environment and perhaps anchor them to an area. Or this might prevent population decline caused by natural food shortages. For instance Elliott et al. (2001) found that supplementary feeding increased survival in chicks of the endangered New Zealand kakapo, and López-Bao et al. (2008) showed that endangered lynx were less likely to disperse from population sites when natural food levels were low, if supplementary food was added. However, supplementary feeding did not improve breeding success, clutch size or population density of an endangered eagle (Simmons, 1993) or reproductive success of a pheasant (Hoodless et al., 1999). Additionally, there is concern about the broader impacts of such major perturbations to natural systems both to the target species (Clout et al., 2002) and to other components of the ecological community (Robb et al., 2008).

In translocation programs, food supplementation has already been used to reduce dispersal and home range (Boutin, 1990; López-Bao et al., 2008), maintain body condition (Bright and Morris, 1994) and increase reproduction (Castro et al., 2003; Schoech et al., 2008) of translocated individuals, again with variable success (Armstrong and Perrott, 2000).

Here we investigated whether adding supplementary food would reduce short-term dispersal in simulated translocation releases of an Endangered Australian scincid lizard, the pygmy bluetongue lizard *Tiliqua adelaidensis*. This species is now restricted to a few isolated fragments of native grassland in north-central South Australia, where they occupy narrow vertical burrows, constructed by spiders. They take refuge in these burrows and use the entrances for basking and as ambush sites for passing invertebrate prey.

In natural populations, pygmy bluetongue lizards spend most of their time associated with an individual burrow (Milne et al., 2003b), rarely leaving it, and even reducing their aggressive burrow defense to a distance of less than 20 cm from the burrow entrance, which does not require them to completely emerge (Fenner and Bull, 2011a). Normal movements beyond the burrow are for prey capture (Milne et al., 2003b) or defecation. For instance, they deposit scats at distances between 25 and 70 cm from their burrows (Fenner and Bull, 2010), although males make occasional longer moves searching for female mates in spring (Fenner and Bull, 2009). Also, there is occasional dispersal within the population to seek new burrows (Fellows et al., 2009; Fenner and Bull, 2011b). Out of their burrows, lizards are more vulnerable to predation from birds and snakes (Hutchinson et al., 1994; Milne et al., 2003b; Fenner and Bull, 2011a; Fenner and Bull, 2011b), as indicated by the high incidence of tail damage (Fenner et al., 2006). For lizards seeking a new burrow, there are additional exposure risks if no suitable alternative burrow is available, or if dispersal takes them out of suitable habitat.

Although existing populations appear secure in the short term, changes in land use and climate pose longer-term threats to this species. Translocation of pygmy bluetongue lizards to additional sites within their historical range is a management option, and will have a greater chance of success if translocated individuals can be encouraged to remain where they are released. Dispersal away from the release site may remove lizards from the various management arrangements designed to promote their well-being.

Our study aimed to explore the influence of food supplementation on those behaviours of the lizard that might be related to whether it tended to stay at, or disperse from, the translocation site immediately after released. This study was not intended to determine whether adding supplementary food would enhance the overall success of a translocation in long term.

4.4. MATERIALS AND METHODS

Eight male and 8 female pygmy bluetongue lizards were captured from 2 populations near Burra (4 males and 4 females from each), South Australia (33° 42´S, 138° 56´E) in September 2009, and transported to Monarto Zoo (35° 06´S, 139° 09´E). They were individually held for 3 d in plastic boxes (52.5 × 38 × 31 cm), each fed 3 mealworms on the first day, and then left unfed for 2 d prior to the experiment.

For the experiment, 4 circular cages (15 m diameter) were built at Monarto. They had 1 m high galvanised iron walls and bird-proof wire roofs and were located in a line, about 5 m apart. Each cage included a central 4 m diameter circular area, lightly vegetated with annual grasses, but cut to ground level before the start of the experiment, representing the translocated habitat. Surrounding that was a 5 m wide ring of lightly tilled, bare ground representing unsuitable habitat, and then an untilled margin, 0.5 m wide, around the inner perimeter of the cage (Figure 4-1). We constructed artificial burrows for the lizards from 30 cm lengths of 3 cm diameter wooden dowling, with a drilled-out center (2 cm internal diameter), hammered into the ground until the entrance was flush with the ground surface. Pygmy bluetongue lizards in natural populations accept artificial burrows of similar design (Milne et al., 2003a; Souter et al., 2004; Ebrahimi et al., 2012a). We placed 41 artificial burrows in the central area of each cage, 1 in the center of the area and the others in 3 concentric rings of 8, 16 and 16 burrows, spaced 65 cm apart, with individual burrows within each ring 65 cm apart in the inner ring, and 75 cm apart in the outer ring. No burrows were placed in the area of unsuitable habitat, but 30 burrows were evenly spaced around the perimeter ring (Figure 4-1).

The combined field of view of 4 surveillance cameras (Longse: LICS23Hf, 3.5 mm lens) mounted above each cage covered the entire ground surface in the central area only. The cameras recorded all lizard activity in that area during daylight hours from 07:00 to 18:00 h on each day of the experiment, on a 16-channel h.264 DVR (ESW26), powered by 4 batteries (12 V). One digital thermometer at each end of the line of cages recorded continuous shade temperature each day, and from these we derived daily minimum, maximum and average temperatures. We also used temperature recordings from a weather station at Pallamana Aerodrome (35.07° S, 139.23° E), 10 km from Monarto Zoo to confirm the site readings.

Within each of the 4 cages, we released 2 male and 2 female lizards into separate central burrows at 07:00 h on 15 November 2009 and confined them to the central area for the rest of that day with a temporary circular wall of 20 cm high black plastic. These walls were removed before lizard activity started on the second day.

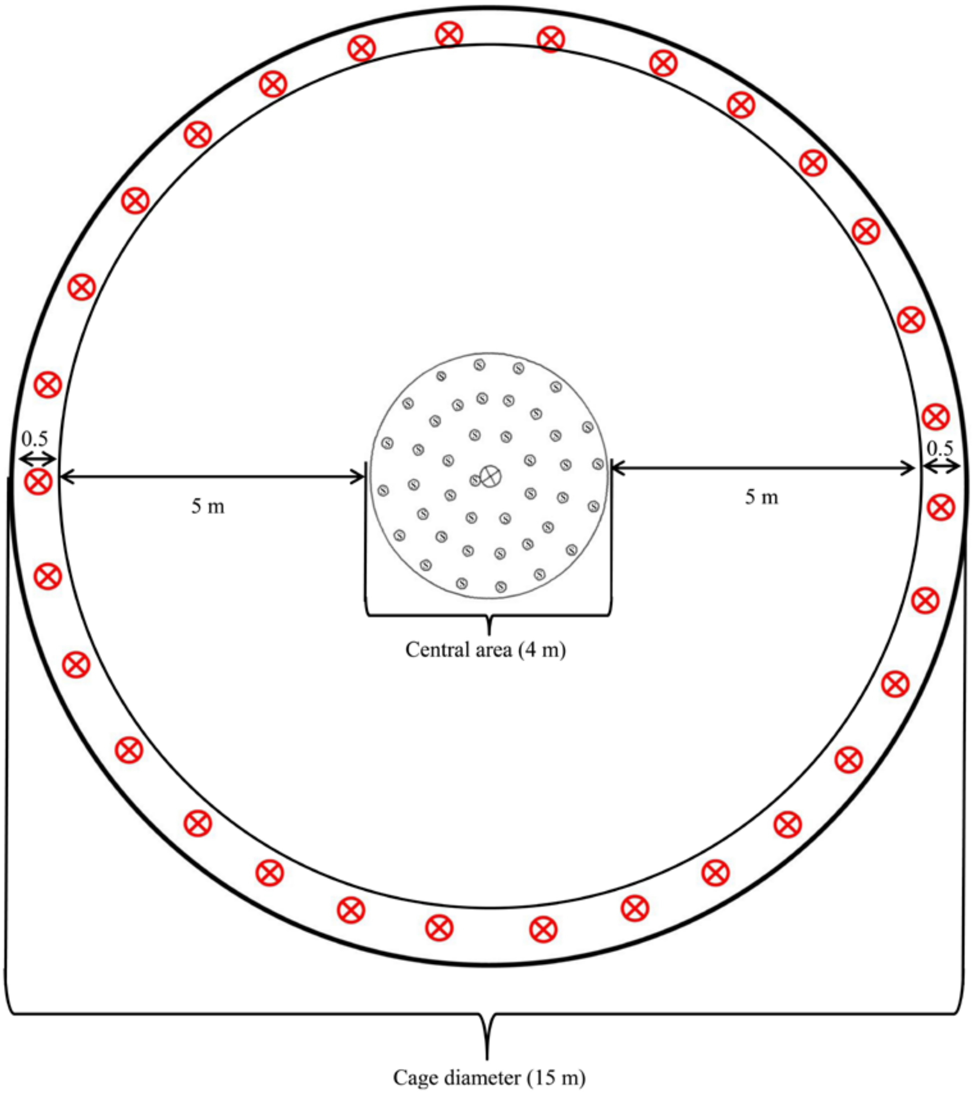

Figure 4-1. Experimental cage design, showing the locations of the artificial burrows within the central and peripheral (marginal) areas. ○ represents artificial burrows in the central area; ⊗ represents burrows in the marginal area.

To determine the influence of food availability on lizard movements after release, the experimental cages were divided into 2 treatments, fed and unfed. Lizards in 2 of the cages were fed daily for 7 d, while those in the other 2 cages were unfed. All lizards were then left unfed and undisturbed for 2 d before the experiment continued with the fed and unfed cage treatments reversed in the following 7 d. Between 12:00 and 13:00 h on the first day, and on each of the next 6 d, we fed each lizard in 2 of the cages with 3 meal worms (average mass = 7.7 g). We placed 1 mealworm at the entrance of each occupied burrow after the previous mealworm had been consumed. Lizards in the other 2 unfed control cages received no supplementary food over this period, but the same time was spent by an observer around each occupied burrow. On every feeding day, the lizards consumed all 3 offered prey items with no apparent change to the rate of consumption, suggesting that the lizards did not become habituated to the feeding regime, or vary in satiation levels during it. From the video recordings, only 3 cases of lizards feeding on incidental invertebrate prey were observed during the experiment. One was in a cage receiving supplementary food, and 2 were in unfed cages.

Lizard behavioural parameters were derived from the video recordings, and data were analysed from the last 6 d (Days 2 to 7) of each 7 d feeding period. On Day 1 of the first week, the lizards were still confined to the central area by the plastic wall, and we wanted to use a comparable period of days in the second week. We tested whether adding food altered lizard behaviour using repeated-measures analysis of variance (ANOVA) with supplementary food (added or not added), and day of feeding period (Days 2 to 7) as within-subjects factors, and the order of presentation (food then no food, or no food then food) as a between-subjects factor. We used the Greenhouse-Geisser correction where data were non-spherical. The effect of temperature on lizard behaviour was examined by Pearson correlation. The parameters that we analysed were activity time (h d^{-1}), total activity (no. d^{-1}), number of movements (no. d^{-1}), number of burrow changes (no. d^{-1}), average basking time (min h^{-1}) and number of fights (no. d^{-1}).

Daily activity time was defined as the time from when the head of a lizard first emerged from the burrow entrance to when the lizard retreated into its burrow for the last time on that day. In 12 cases (out of 192 lizard-days of filming) a lizard was in a burrow in the marginal area, at the perimeter of the cage, at the start or the end of filming. In those cases we could not determine the total activity time because the marginal area was not filmed. We defined total activity as the number of activity events for each lizard for each day. An activity event occurred when a lizard emerged from its burrow, either partially to bask at the burrow entrance or fully to bask or move around. An event started when the head of a lizard first emerged from its burrow, and finished when the lizard completely re-entered the same or another burrow. Among activity events, we defined those when the lizard fully emerged from its burrow as movements. We counted the total number of movements for each lizard for each day. One movement was from when a lizard fully emerged from a burrow to when it entered the same or a new burrow. During movements, lizards walked around the cage area, basked, foraged, defecated or sought a new burrow. Among the movements, we recorded the number of times each lizard changed burrows within the central area each day. Burrows in the marginal area were not in the field of view of the cameras. However, we recorded whether lizards had moved into a marginal burrow, by undertaking 3 visual inspections each day: before filming started, after it had finished, and in the middle of the day, when supplementary food was added to the treatment cages. This may have underestimated the actual use of marginal area burrows, but the level of error should have been comparable across cages and across experimental treatments, allowing unbiased analyses of this parameter. The average daily basking time (min h^{-1}) was calculated for each lizard from the total time it spent basking at its burrow entrance in the central area, divided by the total filming hours when we knew the lizard was in the central area. We did not include the rare (and brief) cases when a lizard basked while away from its burrow entrance.

When a lizard approached another, there was always an agonistic interaction, involving either a brief scuffle, or 1 lizard running away from the other. We defined all of these as fights, and counted the number of fights lizard^{-1} d^{-1}.

For activity time, number of activity events and number of movements, we were unable to get complete data from those lizards that left the central area during a day. In our analyses of those parameters, we used each cage as a replicate, with the mean values only from fully documented lizards in the cage on that day. For the other behavioural parameters, we used data from individual lizards as replicates.

4.5. RESULTS

We recorded 2298 activity events by the 16 lizards during 12 d of filming. Of those, 1352 cases were of lizards partially emerging from their burrow to bask at the burrow entrance before re-entering the same burrow, and 708 cases were of lizards that completely emerged, to move around before returning to the same burrow. In 238 cases, lizards completely emerged and moved to a new burrow. Of those, 182 moved to a new burrow in the central area, and 56 moved to a new burrow in the marginal area. Among the 946 cases of lizards that had completely emerged, there were 45 cases where 2 lizards displayed aggression towards each other, with 12 of the 16 lizards displaying aggression to another lizard at least once.

4.5.1. EFFECT OF AMBIENT TEMPERATURE

Temperatures recorded at the study site were within 1 to 2°C of those at the Pallamana Aerodrome weather station. Temperatures were higher and stable throughout the first period of the experiment, with some cooler days in the second period (Figure 4-2). Correlation analyses with behavioural parameters (Table 4-1) were only significant for mean basking time (min h^{-1}). On days with higher average and maximum daily temperature, lizards basked significantly less (Figure 4-3).

4.5.1. ACTIVITY TIME

The total time that lizards remained active per day varied significantly among days (Table 4-2), from <2 h (food added: Day 7 of period 1) to >7 h (no food added: Day 4 of period 2, Figure 4-4). Although day-to-day variation in ambient conditions probably influenced this variation, activity time was not significantly related (in a linear fashion) to daily temperature (Table 4-1). In relation to our specific hypothesis, the analysis showed a significant effect of food treatment on activity time (Table 4-2), with fed lizards remaining active for about 1.5 h less each day (mean ± SE daily activity time: fed lizards, 4.04 ± 0.40 h; unfed lizards, 5.51 ± 0.53 h). The non-significant food × day and food × order interactions (Table 4-2) suggest that the decreased activity time of fed lizards was consistent across days and across treatment orders. The significant food × day × order interaction (Table 4-2) probably results from ambient conditions differing on particular days within each of the feeding periods (e.g. Day 6 in period 1 was warmer than Day 6 in period 2; Figure 4-2).

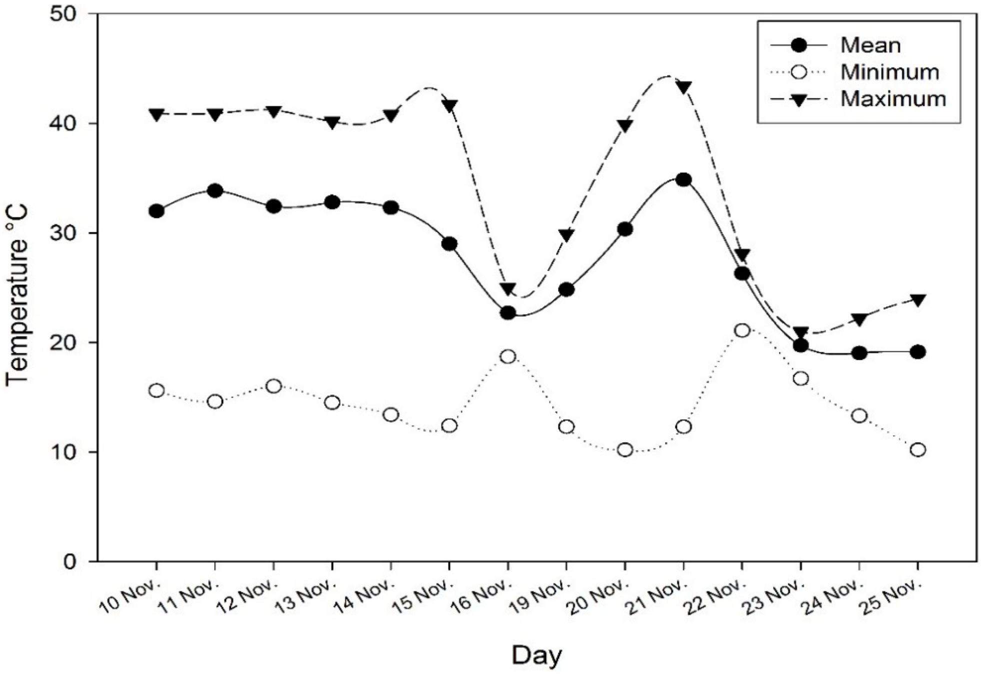

Figure 4-2. Mean, maximum and minimum temperature (°C) on each day of filming lizard activity in November 2009

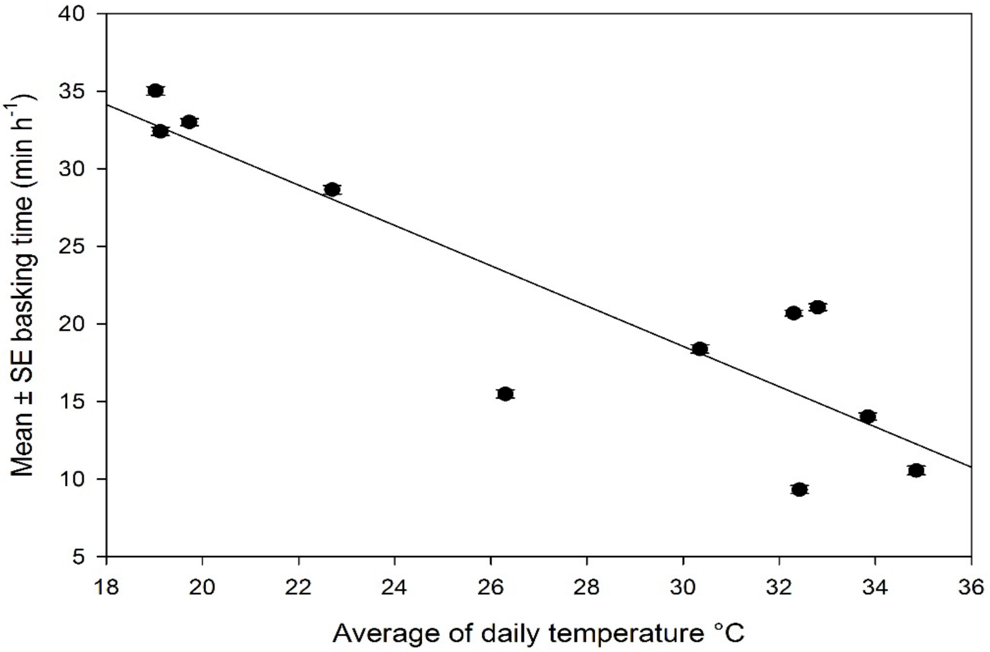

Figure 4-3. *Tiliqua adelaidensis.* Mean ± SE basking time (min h^{-1}) and average daily temperature (°C) including data from both fed and unfed treatments. n = 16 lizards

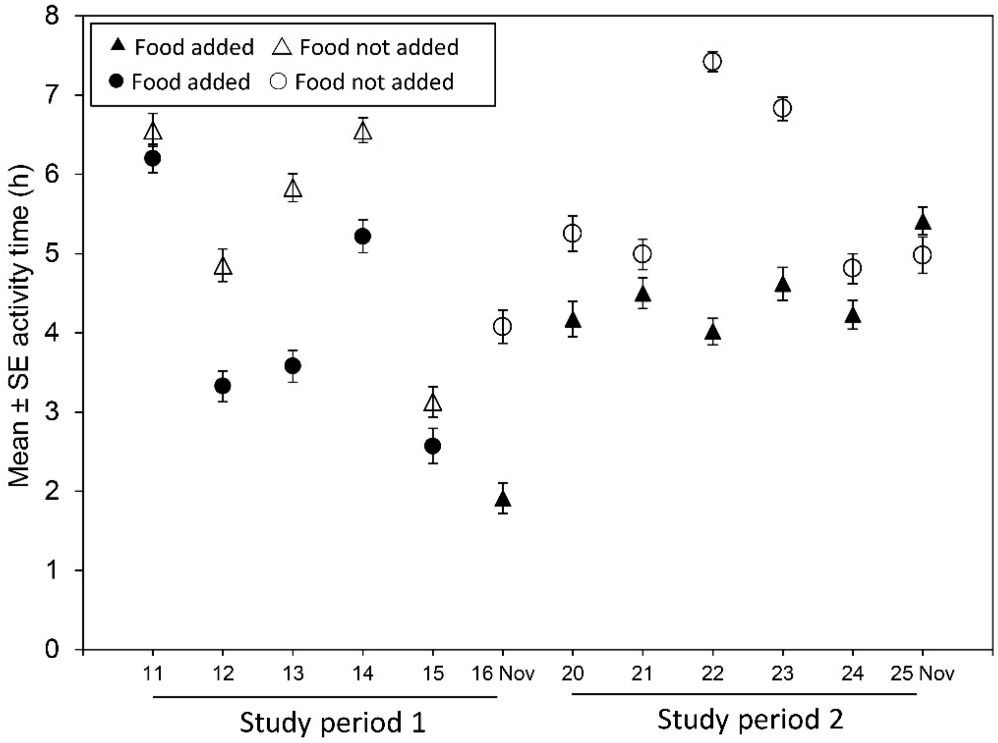

Figure 4-4. *Tiliqua adelaidensis.* Mean ± SE daily activity time (h d^{-1}) of lizards in each treatment (n = 8 lizards per treatment group). Triangles represent lizards that were unfed in the first week and fed in the second week. Circles represent lizards that were fed in the first week and unfed in the second week.

4.5.2. *TOTAL ACTIVITY AND NUMBER OF MOVEMENTS*

Food addition significantly reduced the number of activity events for lizards each day by about 40% (Table 4-2; mean number of daily activity events: fed lizards, 7.78 ± 0.12; unfed lizards, 13.94 ± 0.14). The difference was consistent across days and treatment orders (no significant food × day or food × order interactions, Table 4-2). The significant effect of day (Table 4-2) on the number of activity events again probably reflects changing ambient conditions or degrees of lizard satiation over the experimental periods, although we detected no significant linear effect of daily temperature (Table 4-1).

Table 4-1. *Tiliqua adelaidensis*. Pearson correlation between lizard behavioural parameters and daily average, minimum and maximum temperature. Values with star are significant at $p < 0.05$. In each case $n = 12$ d

Behavioural parameter		**Temperature**		
		Daily average	**Daily minimum**	**Daily maximum**
Activity time (h d^{-1})	r	−0.234	−0.008	−0.328
	p value	0.464	0.981	0.298
Total activity (no. d^{-1})	r	−0.476	−0.211	−0.515
	p value	0.118	0.511	0.086
Movements (no. d^{-1})	r	−0.131	−0.152	−0.088
	p value	0.686	0.636	0.785
Moves to marginal area (no. d^{-1})	r	−0.225	−0.152	−0.491
	p value	0.482	0.638	0.105
No. burrow changes (no. d^{-1})	r	−0.138	−0.073	−0.214
	p value	0.668	0.822	0.504
Basking time (min h^{-1})	r	−0.679	−0.132	−0.738
	p value	0.015*	0.683	0.006*
Fights (no. d^{-1})	r	0.265	0.076	0.334
	p value	0.405	0.815	0.288

There was a significant food × day effect on the mean number of movements lizard^{-1} d^{-1} outside of the burrow (Table 4-2). Fed lizards moved less often than unfed lizards earlier in the food addition period, but the difference between treatments was reduced on the last 2 d (Figure 4-5). Of the 946 total movements recorded, only 56 were to marginal area burrows, with 37 (66%) of those made by unfed lizards. The significant food × day interaction (Table 4-2) resulted from more moves lizard^{-1} d^{-1} to the marginal area by unfed lizards than fed lizards on most, but not all, days. The tendency for supplementary feeding to reduce the number of moves away from the central area became stronger later in the feeding period (Figure 4-6). The significant day × order effect probably again reflects different ambient conditions on specific days within each feeding period.

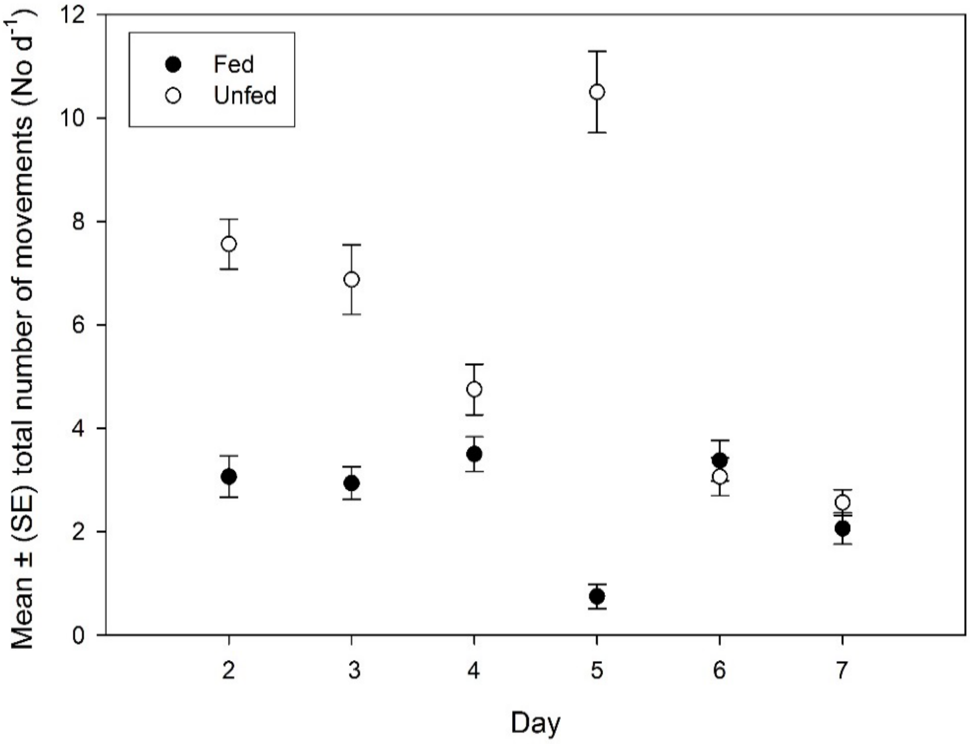

Figure 4-5. *Tiliqua adelaidensis.* Mean ± SE total number of movements lizard^{-1}d^{-1} in fed and unfed groups combining the alternative orders of treatment presentation. n = 16 lizards per treatment.

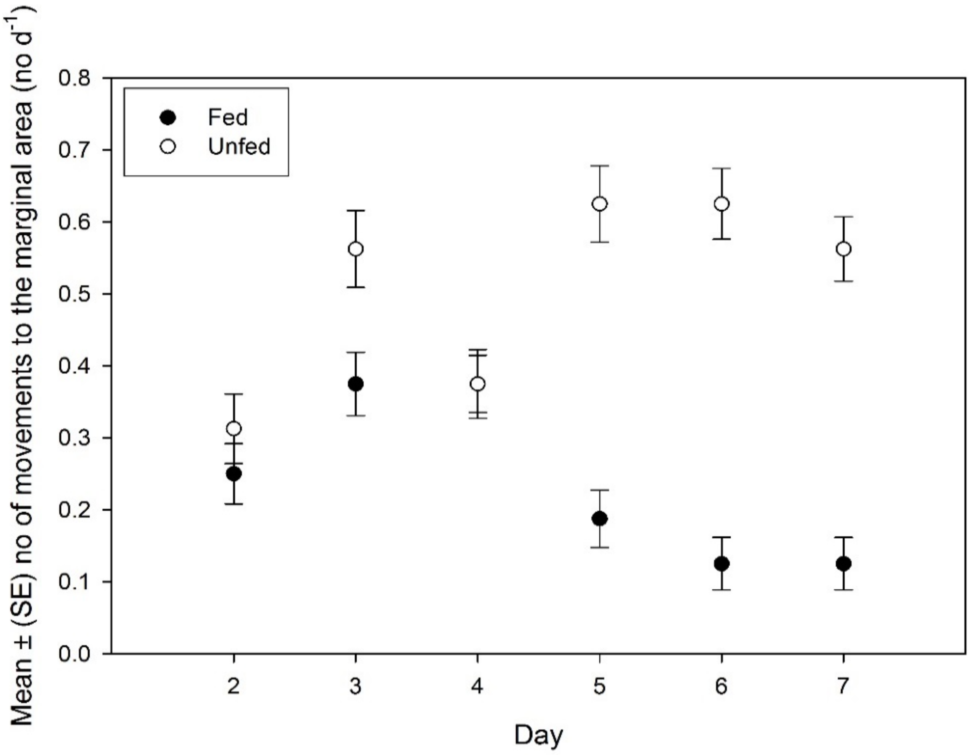

Figure 4-6. *Tiliqua adelaidensis.* Mean ± SE number of lizards observed in marginal areas per day in fed and unfed groups combining the alternative orders of treatment presentation. n = 16 lizards per treatment

4.5.3. BURROW CHANGES WITHIN THE CENTRAL REGION

Of the 182 cases where a lizard changed its burrow within the central region, 121 (66.5%) were by unfed lizards. The analysis showed a significant effect of food addition that was consistent across days and presentation order (Table 4-2). Fed lizards (mean 0.50 ± 0.010 changes d^{-1}) changed burrows significantly less often than unfed lizards (mean 1.125 ± 0.016 changes d^{-1}).

4.5.4. BASKING TIME AND NUMBER OF FIGHTS

Fed lizards basked at their burrow entrance for significantly shorter times (about 10 min less each hour than unfed lizards: 18.84 ± 1.10 min h^{-1} versus

28.58 ± 1.42 min h^{-1}; Table 4-2). The significant effects of day and the day × order interaction on basking time probably reflect the impact of daily changes in ambient conditions shown previously.

Although the mean number of fights $lizard^{-1}$ d^{-1} was small, there was a significant interaction effect of day × order (Table 4-2). Lizards that were fed and then unfed fought less frequently than lizards that were unfed and then fed, although that difference generally decreased with time (Figure 4-7). There was no significant effect involving the feeding treatment.

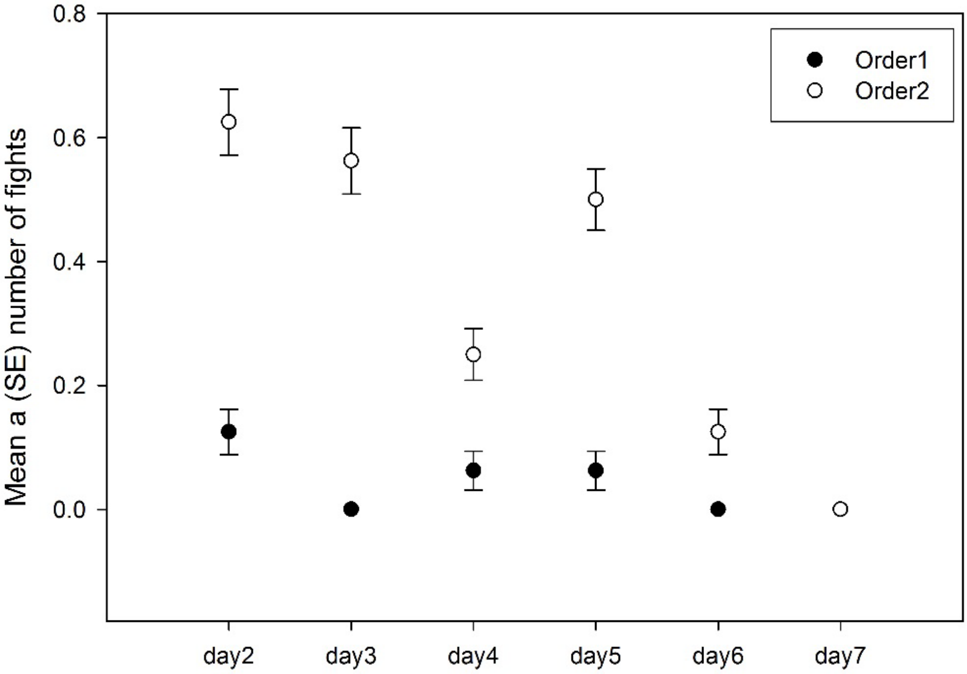

Figure 4-7. *Tiliqua adelaidensis.* Mean ± SE number of fights $lizard^{-1}d^{-1}$ in fed and unfed groups combining the alternative orders of treatment presentation. n = 16 lizards per treatment

Table 4-2. *Tiliqua adelaidensis*. Repeated-measure analyses of variance for behavioural parameters in response to the addition of supplementary food. Values with star indicate significant effects ($p < 0.05$)

Behavioural parameter		**Effect**						
		Food	**Day**	**Order**	**Food × order**	**Food × day**	**Day × order**	**Food × day × order**
Activity time (h d^{-1})	*F*	20.69	7.59	9.45	14.09	0.89	1.24	6.31
	p value	0.045*	0.003*	0.092	0.064	0.522	0.358	0.007*
	df	1, 2	5, 10	1, 2	1, 2	5, 10	5, 10	5, 10
Total activity (no. d^{-1})	*F*	18.73	3.63	1.44	12.88	1.25	0.95	2.95
	p value	0.049*	0.039*	0.352	0.070	0.355	0.488	0.068
	df	1, 2	5, 10	1, 2	1, 2	5, 10	5, 10	5, 10
Movements (no. d^{-1})	*F*	4.41	2.55	3.66	0.52	3.54	3.29	2.90
	p value	0.171	0.097	0.195	0.543	0.042*	0.052	0.071
	df	1, 2	5, 10	1, 2	1, 2	5, 10	5, 10	5, 10
Moves to marginal area (no. d^{-1})	*F*	8.86	0.66	0.04	0.21	4.47	2.64	0.81
	p value	0.010*	0.656	0.834	0.654	0.001*	0.030*	0.543
	df	1, 14	5, 10	1, 14	1, 14	5, 10	5, 10	5, 10
No. burrow changes (no. d^{-1})	*F*	5.16	1.08	0.20	0.33	1.36	0.38	0.90
	p value	0.039*	0.376	0.655	0.572	0.248	0.856	0.481
	df	1, 14	5, 10	1, 14	1, 14	5, 10	5, 10	5, 10
Basking time (min h^{-1})	*F*	8.22	7.74	0.47	4.17	1.11	2.44	0.70
	p value	0.012*	0.001*	0.503	0.060	0.359	0.042*	0.624
	df	1, 14	5, 10	1, 14	1, 14	5, 10	5, 10	5, 10
Fights (no. d^{-1})	*F*	0.19	4.52	18.99	0.19	0.87	2.83	2.17
	p value	0.664	0.009*	0.001*	0.664	0.502	0.022*	0.066
	df	1, 14	5, 10	1, 14	1, 14	5, 10	5, 10	5, 10

4.6. DISCUSSION

We detected significant differences between fed and unfed lizards for a number of behavioural parameters, which may have implications for the success of translocation programs. We also observed strong day-to-day variation in a number of behaviours, probably driven by differences in satiation levels of lizards, or by variation in ambient climatic conditions. We could only detect a linear correlation with daily temperature for 1 parameter, mean time spent basking per hour, with lizards basking for less time when temperatures were higher. This apparently anomalous result for an ectothermic reptile could be explained by the hotter days over the first week of the experiment when the lizards probably needed to shelter in their burrows more often to avoid over-heating.

Fed lizards were consistently less active than unfed lizards, as measured both by the duration of activity during a day, and the number of activity events in a day. These results conform with other studies that have reported higher activity (Abrams, 1993; Werner and Anholt, 1993; Anholt and Werner, 1995) and increased predation risk (McNamara, 1987; Anholt and Werner, 1995) when resources are scarce. In a translocation, higher activity rates would lead to longer periods of exposure out of burrows, and higher risk of predation in a new location. Additionally, in our system, more active pygmy bluetongue lizards are more likely to disperse from a release site.

In our experiments, adding supplementary food reduced the overall number of moves, the number of times lizards changed burrows in the central release area, and the number of moves out of the central area. We interpret our result to infer that lizards are more likely to seek alternative burrows, or to attempt to move to alternative sites if they perceive that there is a low chance of capturing invertebrate prey from their current burrow. Bright and Morris (1994) reported that translocated dormice showed a similar reduction in dispersal movements when supplementary food was added, and Ruffell and Parsons (2009) reported that translocated bats provided with supplementary food remained at the release site.

When we reversed the treatment conditions and stopped adding supplementary food to 1 group of lizards, they became more active, moved more, and were more likely to disperse out of the central area. Thus, at least for newly translocated lizards, the perception of adequate food supply must be sustained if movements are to be suppressed. For the gopher tortoise, it has been suggested that programs of supplementary food addition must be sustained for up to 2 yr for the successful establishment of the translocated population (Tuberville et al., 2005; Field et al., 2007).

In our study, adding food also reduced the time lizards spent basking. Basking at the burrow entrance may be both for thermoregulation and for surveillance of passing prey items (Hutchinson et al., 1994; Milne et al., 2003b). The decreased basking time of fed lizards may be related to prey searching, since fed and unfed lizards would have experienced similar thermal conditions. This result also reflects the common observation that satiated animals are less likely to expose themselves to predation risks (McNamara, 1987; Werner and Anholt, 1993).

Although agonistic interactions with conspecifics may be an additional stimulus for translocated animals to disperse, our results did not show any overall difference in the incidence of fights between fed or unfed lizards. Therefore, this is unlikely to have been a factor in the dispersal difference we observed. However, on some days we observed those fed in the second week to fight more often than those fed in the first week. This result may reflect higher activity levels and encounter rates between hungrier lizards.

Supplementary feeding is a common management technique used to increase reproductive activity and fecundity in endangered birds in their endemic or translocated sites (Armstrong et al., 1999; Castro et al., 2003; Armstrong et al., 2007; Schoech et al., 2008), and to enhance fitness of translocated mammals (Licht, 1974; Rose, 1982; Bright and Morris, 1994; Ruffell and Parsons, 2009). We did not maintain supplementary feeding for a long enough period to explore this possible advantage for translocated lizards. Indeed, our study was not designed to explore the overall impact of food addition on translocation success, but rather to determine whether behaviours related to dispersal from a translocation site were influenced by food supplementation.

However, we have shown that supplementary food can significantly alter some critical components of daily behaviour for lizards that are released into a novel environment. Notably, fed lizards were more likely to reduce their risks of predation and remain closer to their release site. Supplementary feeding may be an important component to improve establishment success for this species at other sites, and may be useful to decrease dispersal from a translocation site in other endangered reptiles.

4.7. ACKNOWLEDGEMENTS

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CHAPTER FIVE

5. BURROW DENSITY AND LAYOUT

5.1. THE IMPORTANCE OF REFUGES

We have already discussed in chapter four the importance of resource availability for translocation attempts. We suggested in that chapter that adding supplementary food can decrease early post release movement and help individuals of the translocated species establish new home ranges. One other resource that is highly important for all species, and for translocated individuals of those species is refuges. Often species use refuges to protect themselves from predators, and harsh climate, and they can use their refuges as vantage points to observe and ambush passing prey (Schwarzkopf and

Alford, 1996; Williams et al., 1999; Grillet et al., 2010). Besides those functions, refuge occupancy can effect social interactions. If the density of available shelters is low, then competition for those refuges among conspecific individuals is likely to be high. If shelters are too close to each other they may increase the degree of overlap of adjacent home ranges which again might encourage competitive agonistic interactions between neighbours. Therefore it is important to understand the details of spatial distribution of refuges as well as the optimal density of refuges in translocation sites to minimize the competitive interactions that may lead to some individuals dispersing. In this chapter we explore how burrow density, layout and the closeness of individual release locations, affect short-term behaviours in a simulated translocation.

Applied Animal Behaviour Science (Under review)

Resources and their distribution can influence social behaviour at translocation sites: lessons from a lizard.

Mehregan Ebrahimi and C. Michael Bull

5.2. ABSTRACT

In a translocation program, social interactions among released individuals can influence both the stress levels and the tendency for the individuals to remain at the site where they have been released. In hard releases, stress from social interactions may lead to early dispersal away from the release site. In soft releases, where individuals are confined together for periods of time at the release site, before ultimate release, stress levels from social interactions may become even higher as individuals are unable to move away. In this study we investigated how variation in the abundance and distribution of a fundamental habitat resource, refuge burrows, influenced behaviour and possible subsequent translocation success, of newly released individuals of the endangered Australian pygmy bluetongue lizard, Tiliqua adelaidensis, in simulations of translocation releases. Our aim was to determine if there was an optimal distribution of burrow resources that would minimize behaviours likely to lead to dispersal from the release site. Lizard movement around burrows and burrow changes differed when burrow density was changed (mean (SE) number of movements 3.73

(0.02) and mean (SE) number of burrow changes 0.06 (0.006) in low burrow density, and mean (SE) number of movements 1.88 (0.02) and mean (SE) number of burrow changes 0.50 ± 0.008 in high burrow density). When lizards were released in burrows near to each other they changed burrows more often and had more fights than when released in burrows further apart (mean (SE) number of burrow changes 0.97 (0.01) and mean (SE) number of fights 0.04 (0.004) when lizards released 50 cm from each other, mean (SE) number of burrow changes 0.22 (0.009) and mean (SE) number of fight 0.003 (0.001) when lizard released 150 cm from each other). These results suggest significant changes in behaviour can be induced by altering the way resources are distributed at a releases site. We suggest that understanding the social organization of any endangered species, and whether it can be manipulated, will be an important component of planning a translocation release program.

Keywords: Behaviour, *Tiliqua adelaidensis*, Burrow density, Translocation, Burrow layout

5.3. Introduction

In translocations, a potential dilemma is that high densities among the group of individuals released, could increase the chance that at least some individuals might survive, persist at the release site, and establish a new population or contribute to the existing population. But, on the other hand, a high release density could increase competitive and social interactions among the released group, or with existing conspecific residents. Those interactions might increase levels of stress, and increase the chance of rapid dispersal away from the release site (Morris, 2003; Goymann and Wingfield, 2004; Anders, 2006; Fletcher, 2007), or reduce fecundity and juvenile survival among individuals that stay (Clutton-Brock et al., 1987). For instance, we previously reported that reducing supplementary food caused lizards, newly introduced to an area, to stay active more, to spend more time basking, and to disperse more quickly from a simulated translocation site (Ebrahimi and Bull, 2012). Here we focus on the short period immediately following a translocation release, and the social interactions in that period that might determine whether an individual will stay close to where it is released or disperse away from the release site. Our aim was to determine whether the tendency to disperse could be reduced by adjusting the way that resources were distributed at the release site.

Successful translocations aim to keep the initial group of released individuals within the release area around the release point (Mihoub et al., 2011; Rickett et al., 2013). While some limited movement around the release point is desirable, wider movement is likely to take animals to poorer habitat, to disperse individuals and make it harder for them to find mating partners, and to make monitoring the success of the management strategy more difficult. To achieve this goal of low dispersal, one factor we need to understand is how the spatial distribution of resources within a release location affects social interactions and the tendency of individuals to remain where they are released. Animal social interactions can be affected by a range of ecological factors (Alexander, 1974; Bronikowski and Altmann, 1996; Lancaster et al., 2011) such as shelter, food and vegetation density (Graves and Duvall, 1995; Johnson et al., 2002; Tanner and Jackson, 2012). Adverse social interactions, affecting translocation success, might be reduced by manipulating one or more of those factors. Understanding the influence of habitat resource distribution and availability is crucial. A low density of resources could increase the frequency of social interactions (Lancaster et al., 2011). For instance solitary scorpions, under conditions of reduced shelter and food, increased their agonistic interactions, leading to an increase in mutilations and deaths (Warburg, 2000). The level of social stability in animals can be influenced both by the level of available resource, and by the way the resource is distributed (Carr and Macdonald, 1986; Ditchkoff et al., 2006; Reynolds and Bruno, 2013). From a management perspective we need to know whether we can manipulate the distribution of resources to improve retention success.

As in many other animal translocations, reptiles tend to disperse from the site where they are released (Dodd and Seigel, 1991; Germano and Bishop, 2009). Additionally, the social system of many reptile species is primarily solitary (Visagie et al., 2005; Leu et al., 2011), meaning that aggregations following translocation release are likely to induce dispersal. Lizards also live in heterogeneous habitats, for instance requiring both shelter refuges and open areas for thermal basking (Gálvez-Bravo et al., 2009), so will need a complete range of their habitat resources at release sites.

We investigated these issues in simulated translocation releases of the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis* in South Australia. The lizards currently occupy a few isolated fragments of native grassland, with genetic evidence suggesting very little recent migration between patches (Smith et al., 2009). Most of their previously more continuous grassland habitat has been lost as a result of agricultural clearances. Fordham et al. (2012) showed that, for realistic climate change scenarios, translocations may be the best management option to retain viable populations of this endangered species into the future. An essential resource for this species is the single entrance, narrow, vertical burrows, constructed by lycosid and mygalomorph spiders, which the lizards occupy. They spend most of their time either refuged in the burrow, or using the burrow entrance to bask, and as an ambush site to catch their invertebrate prey (Hutchinson et al., 1994; Milne et al., 2003b). They rarely leave their burrows, even during aggressive burrow defence against rival conspecifics (Fenner and Bull, 2011). Normal movements beyond the burrow are for prey capture (Milne et al. 2003b) or defecation. For instance, they deposit scats at distances of between 25 and 70 cm from their burrows (Fenner & Bull 2010). Males make occasional longer moves searching for female mates in spring (Fenner & Bull 2009). The lizards cannot dig their own burrows and are reliant on a supply of suitably deep burrows (usually deeper than 25 cm) provided by the spiders (Milne, 1999; Souter, 2003). They are currently confined to grassland patches with an adequate supply of suitable burrows. A shortage of suitable burrows can limit pygmy bluetongue lizard population expansion (Souter et al., 2007), but artificial burrows added to current population sites augment existing populations (Souter et al., 2004) and could be provided at a release site in a translocation program. In that case a successful translocation would rely on the lizards remaining within an area where burrows were provided.

In our study we used artificial burrows as the resource, and investigated how the availability and distribution of burrows affected the behaviour of lizards in simulated translocation releases. We were specifically interested in the immediate responses of lizards in the first days after a release, and examined aspects of their behaviour and tendency to move. We assume that any behavioural change can directly or indirectly affect the tendency of a lizard to stay at the release site. For instance if a lizard is emerged from its burrow and active for a longer time, that may increase the chance of exposure to agonistic interactions with conspecifics which could increase the probability of dispersal. Therefore, in this study, we measured a range of behavioural changes that we considered could affect the tendency of a lizard to move or stay at a translocation site. Our aim was to develop an understanding about the design of a release site, and the location of resources within that release site, that might minimise the chance of lizards moving from the site, or experiencing stressful social interactions at the site, in the days immediately following the release.

5.4. METHODS

5.4.1. EXPERIMENTAL TRIALS

We used 16 *T. adelaidensis* (eight males (average snout-to-vent length (SVL) 85.1 ± 0.2 mm) and eight females (average SVL 89.2 ± 0.2 mm)) that had been captured from two populations near Burra, South Australia (33º42´S; 138º56´E), and held in individual plastic boxes (52.5 × 38 × 31) at room temperature (25 °C) and fed three meal worms each day.

We conducted three experiments using four circular cages (15 m diameter) that were located in the grounds of Monarto Zoo, South Australia (35°06′S; 139°09′E). Each cage had a 1 m high-galvanised iron wall and bird-proof wire roofs. The four cages were located in a line, about 5 m apart.

We simulated the first stages of the soft release translocation method. Throughout each experiment, lizards were confined to a central 2 m radius area within each cage using a 20 cm high black plastic wall (Ebrahimi and Bull, 2013). 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. In previous studies lizards have readily accepted these artificial burrows both in the field and in cages (Milne et al., 2003a; Ebrahimi et al., 2012). 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. The number and arrangement of burrows in the central part of each cage varied with the treatment in each of three experiments, as described below.

Although the confined area that we used of just over 12 m^2 was small, lizards in natural populations rarely move more than a few centimetres from their permanent burrow refuge, and agonistic interactions only occur when conspecifics approach to within 5 cm of an occupied burrow (Fenner and Bull, 2011). Our broad hypothesis was that social interactions would be most likely during the first few days after release, as the lizards establish their burrow ownership, and that the density and the arrangement of the burrows in the release site will influence the intensity of those social interactions, and the subsequent levels of normal behaviours in the lizards.

5.4.1.1. EXPERIMENT ONE: BURROW DENSITY

The first experiment tested the effect of burrow density on lizard behaviour. The alternate treatments are shown in Figure 5-1A and 5-1B. Two cages had high burrow density. We distributed 41 artificial burrows evenly around the central area, as previously described (Ebrahimi and Bull, 2012), one in the middle, and then 8, 16 and 16 burrows in three concentric rings. In this arrangement burrows were on average 63 (SE = 0.01) cm apart. The other two cages had low (10) burrow density, with 2, 4 and 4 artificial burrows in three concentric rings, and spaced between 100 and 120 cm apart. For this experiment, we ran three 4-day trials in each cage. Each trial commenced at 0700 h on the first day, when four lizards were released at the same time onto the ground in the centre of the experimental area of the cage. The three sets of trials in this first experiment started on Jan 13, Jan 19 and Jan 25, 2010. Lizards were returned to their plastic boxes, and were fed three mealworms for the 2 days between trials. For each trial there were different combinations of four lizards in each cage, selected from the 16 available lizards, although individual cages retained their treatment status across trials.

5.4.1.2. EXPERIMENT TWO: RELEASE LOCATION

In the second experiment we tested the effect of the closeness of the release locations to each other. The alternate treatments are shown in Figure 5-1C and 5-1D. Each cage had 41 burrows in the experimental area. In two cages the 41 burrows were arranged in concentric rings as in the high density treatment of experiment one (burrow density), and three lizards were released at the start of each 4 day trial into three burrows, in a triangular formation, that were 150 cm from each other. In the other two cages, 38 burrows were arranged as above, but lizards were released into three additional burrows that had been moved to a central triangular formation, within 50 cm of each other. Three sets of trials started on Feb 2, Feb 8 and Feb 14, 2010 with lizards removed from the cages for 2 days in between trials as before. For each trial, there were different combinations of three lizards for each cage, selected from the 16 lizards.

5.4.1.3. EXPERIMENT THREE: BURROW CLUSTERING

The third experiment considered the influence of burrow clustering. The alternate treatments are shown in Figure 5-1E and 5-1F. Each cage had 41 burrows. Burrows in two cages were evenly spaced as before (63 cm apart), while burrows in the two other cages were clustered. For clustering, we placed one burrow at each apex of a centrally located equilateral triangle with 2.5 m sides. Then we placed nine burrows 10.4 cm apart around the circumference of a 15 cm radius circle around each apex, creating three clusters of 10 burrows. Another 11 burrows were placed singly around the experimental area, each 75 cm from any other burrow. At the start of trials, three lizards were released in each cage 250 cm apart in the three apex burrows of the clustered arrangement, and 150 cm apart as in experiment two, in the evenly spaced burrow arrangement. Thus lizards were initially released further apart in the clustered burrow treatment than in the evenly spaced burrow treatment. Three trials started on Mar 5, Mar 11 and Mar 17, 2010. The selection of three lizards for each trial was the same as in experiment two (release location).

Note that all of the experiments were conducted several months after the spring mating period for these lizards (Oct-Nov) (Hutchinson et al., 1994;

Fenner and Bull, 2009) and we did not consider that sexual differences played an important part in the responses we observed. We consider that this period of the year would be the optimal time for translocations as stressful interactions involved with mating behaviour would be infrequent.

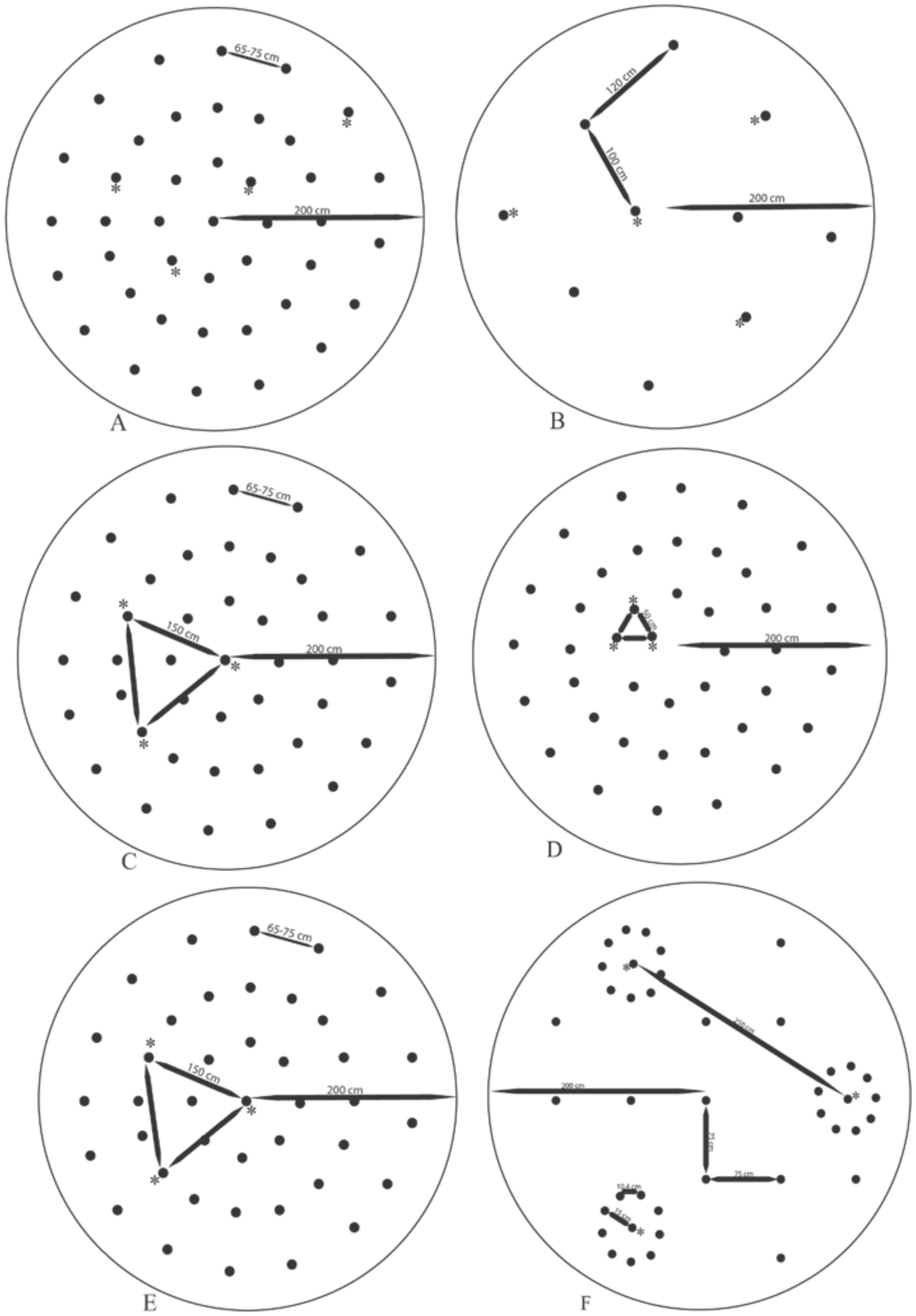

Figure 5-1. Layout of the two treatments in each experiment. Experiment 1one: Burrow density, A with 41 artificial burrows and B with 10 artificial burrows; Experiment 2two: Release location, C and D; Experiment 3three: Burrow clustering, E and F. Stars near burrows are the release points.

5.4.2. BEHAVIOURAL PARAMETERS

We mounted four surveillance cameras (CCD video camera, LICS23HF and lens 3.6 mm, Normal recording mode (continues), 30 fps, Longse, China) above the central area of each cage, with a combined field of view that covered the entire experimental area. On each day of each trial we used the cameras to record all lizard activity during daylight hours from 0700 to 1800 h. From the playback, we derived seven behavioural parameters that allowed us to compare the behaviour of the lizards in each treatment. These were total activity time (h d^{-1}), basking time (min h^{-1}), number of movements around burrow, number of burrow changes, the number of fights, the mean distance between lizards, and the distance between burrows when there was a burrow change. Below we define each of the behavioural parameters that we measured from the video recordings.

1) Activity time was defined as the period from when the lizard head first emerged from a burrow in a day to when the lizard retreated into its burrow for the last time on that day. In this definition activity time could include periods when the lizard had retreated into a burrow during the day, if it subsequently re-emerged later on the same day. In the first experiment, in which lizards were released onto the ground early on the morning of the first day, we allowed lizards to retreat to their first burrow before starting to monitor for the first emergence. 2) Basking time was defined as the period of time when the lizard was at least partially emerged (5 -98% of body outside of

the burrow) and was located at the entrance of its burrow. We calculated basking time (min h^{-1}) as the time (in minutes) that a lizard spent basking in a day, divided by 11, the number of hours filmed per day. Basking time did not include time when the lizard had retreated into its burrow. In other experiments with pygmy bluetongue lizards we have showed that lizards that spend more time basking are less likely to disperse (Ebrahimi and Bull, 2013). 3) We defined a lizard as having moved around its burrow if it fully emerged from the burrow, moved about, usually for a very short distance, no more than 10 cm from the burrow entrance, and then returned to the same burrow. During these movements, we observed some cases of lizards walking around their burrow entrance no more than 5 cm from it, some cases of lizards basking while fully emerged, and some cases of lizards that moved 10 cm away from the burrow entrance for defecation or darted out to catch prey within 10 cm of the burrow entrance. We counted the number of times that each lizard made one of these movements on each day in each trial. 4) We defined a lizard as changing burrows if it emerged from one burrow, and then located, and retreated into another burrow. 5) When two lizards approached each other on the ground surface, there was always an agonistic interaction involving the lizards scuffling, or one running from the other. We counted each agonistic interaction as a fight. 6) For distance between lizards, we located the burrow occupied by each lizard at the end of each day, in each cage, and took the average of the distances between each pair of individuals. 7). Finally we measured the straight-line distance between burrows following a burrow change, and derived two measures for a lizard if it made two or more burrow changes in a day, the sum of all of the distances moved in the day, and the average distance of each move. We used both measures in separate analyses, and found no difference in the results, so here only report results using the average distance per move.

5.4.3. *STATISTICAL ANALYSES*

We derived parameter values from each of the 4 days of video recording in each trial. We conducted preliminary analyses for each behavioural parameter using mixed effects models with repeated measure (Bolker et al., 2009), and including treatment (burrow density (experiment one), release location (experiment two) and burrow clustering (experiment three)) as fixed between subjects factors, day (1-4) and trial (1-3) as repeated within subjects factors, individual lizards as a random factor, and sex as a covariate. We found no significant effect of either individual lizards or of lizard sex on any of the behavioural parameters in any of the three experiments. We then used repeated-measures ANOVA (Hand and Taylor, 1987) for each behavioural parameter, in each experiment, with day (1-4) and trial (1-3) as within-subjects factors and treatment (burrow density (experiment one), release location (experiment two) and burrow clustering (experiment three)) as the between- subjects factors. In these analyses, we used the Greenhouse-Geisser correction where data were non- spherical.

Because the same lizards were used, although in different combinations and in different cages, in all three trials of experiment one, and because we selected 12 of the 16 lizards for each trial in the last two experiments, lizards may have become familiar with the experimental layout as the trials progressed. If that familiarity influenced their responses to the alternative experimental treatments in any of the experiments we would have expected to see significant trial x treatment interaction effects from the analyses.

Continuous temperature records were taken every day by two digital thermometers, placed in the shade at each end of the line of cages. We also used temperature recordings from a weather station at Pallamana Aerodrome (35° 04' S, 139° 13' E), 10 km from Monarto Zoo.

5.5. RESULTS

Among the lizard behaviours recorded in each experiment, basking was consistently the most commonly observed, and fighting the least commonly observed (Table 5-1).

Although the analyses (Table 5-2) showed a number of significant relationships between behavioural parameters and day or trial number, there were no correlations with ambient temperature (using either the daily mean, the daily maximum or the daily minimum temperature). Nevertheless we believe those significant effects of trial and day represented differences in ambient conditions or in the physiological condition of the lizards over different times.

Table 5-2 also shows no significant interactions between treatment and trial for any behavioural parameter in any experiment. Any increasing familiarity with the experimental arrangement over successive trials in an experiment, did not influence the responses of the lizards to the alternative treatments.

Table 1. Number of cases of each activity recorded during each experiment.

Experiment	**Activity**				**Total**
	Basking	Movement	Changing burrows	Fights	
One: Burrow density	474	308	21	4	807
Two: Release locations	381	378	65	6	830
Three: Burrow clustering	438	255	126	7	826

5.5.1. EXPERIMENT ONE: BURROW DENSITY

We tested how change in burrow density could influence lizard behaviour to suggest how the burrow density provided could affect post release movement. The number of movements, the number of burrow changes, and the distance of burrow changes all showed significant main effects of burrow density (Table 5-2). Lizards moved more (3.73 ± 0.02 moves/lizard/day) but changed burrows less (0.06 ± 0.006 changes/lizard/day) when burrows were at low density, than when burrows were at high density (1.88 ± 0.02 moves/lizard/day; 0.50 ± 0.008

changes/lizard/day). When lizards changed burrows the distance moved was further when burrows were at low density (215 ± 0.08 cm), than when burrows were at high density (101 ± 0.09 cm). Activity time and basking time were not affected by the experimental treatment, although they varied among days (as did the changing burrow distance), or on different days among trials (Table 5-2), probably as a result of differences in ambient conditions. For distance between lizards at the end of each day, there was a significant three way interaction (burrow density x trial number x day; Table 5-2). This reflected a trend at least in trial 1, for lizards to move further apart from each other between day one and day two in the high density burrow treatment, while those separations had already been achieved by the end of day one in the low burrow density treatment (Figure 5-2). Mean distance between pairs of the four lizards in each cage seemed to stabilise by day four at between 1.4 – 1.8 m apart in all treatments and trials.

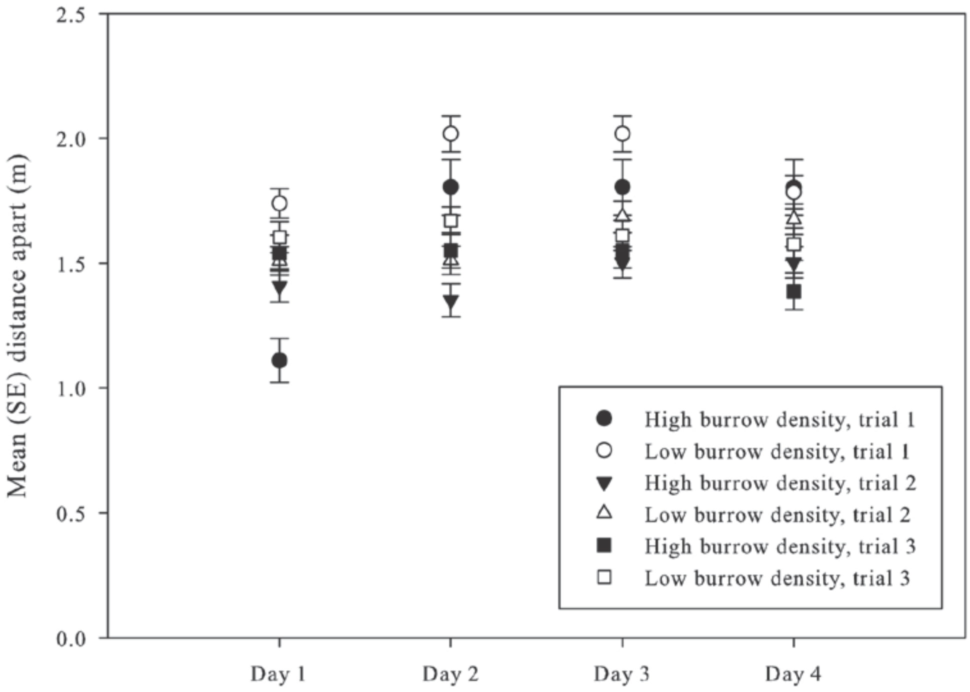

Figure 5-2. The mean distance apart of lizards at the end of each day of each trial in experiment one.

5.5.2. EXPERIMENT TWO: RELEASE LOCATION

We tested whether there were behavioural differences between lizards initially released close to each other or further apart. We hypothesised that lizards may be more stressed if closer to conspecifics, and that different stress levels may be shown by behavioural changes. The results showed there were significant main effects of treatment for two behaviours (Table 5-2). Lizards changed burrows more often (0.97 ± 0.01 changes/lizard/day) and had more fights (0.04 ± 0.004 fights/lizard/day) when they were released closer to each other, than when released further apart (0.22 ± 0.009 changes/lizard/day; 0.003 ± 0.001 fights/lizard/day). There were no significant treatment x day of trial interaction effects for either of those behaviours, indicating that the behavioural differences remained consistent even after the lizards were allowed time to adjust their spatial proximity. The number of moves had a significant treatment x day effect (Table 5-2), with lizards released closer to each other always moving more, but that difference changing with the day of the experiment (Figure 5-3a). Similarly, distance between lizards had a significant treatment x day effect (Table 5-2) with lizards released closer together increasing their distance apart over successive days, while those released far apart retained that distance over the 4 day trials (Figure 5-3b). The three lizards in each cage achieved mean separations of between 1.4 and 1.8 m by the end of day four, although those released closer, were still closer together by day four (Figure 5-3b). Activity time, basking time and distance moved when changing burrows were not significantly affected by the treatment in these trials, only varying with day and trial number, as in experiment one.

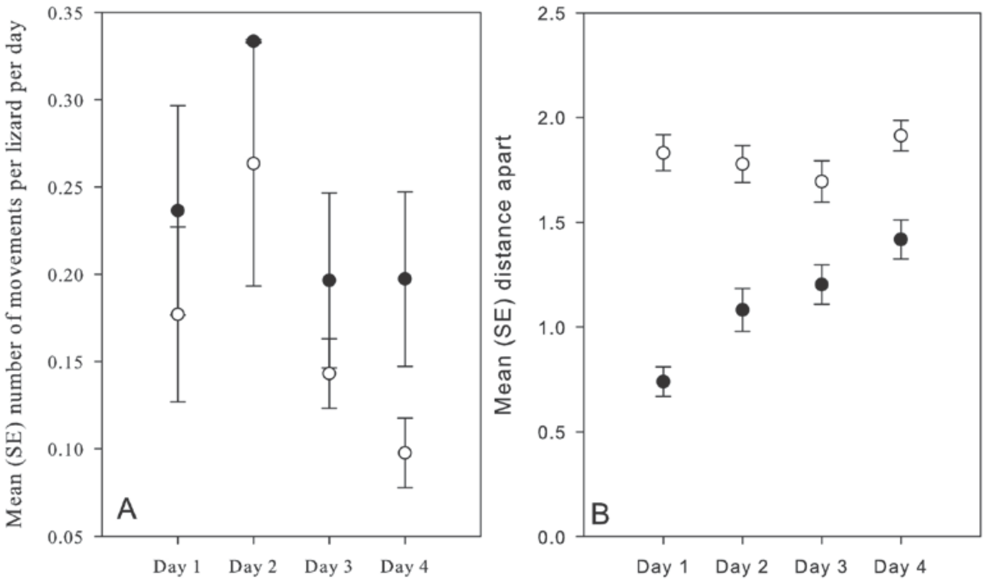

Figure 5-3. (A) Mean number of movements per lizard per day, and (B) mean distance apart at the end of each day when lizards were released close to each other (filled circles) or further apart (open circles) in experiment two.

5.5.3. EXPERIMENT THREE: BURROW CLUSTERING

In the last experiment we asked how burrow arrangement (clustered or equally spaced from each other) could impact lizard behaviour and consequently post release movement. In this experiment there were significant main effects of treatment on basking time, movement and distance moved when changing burrows (Table 5-2). Lizards spent more time basking (22.04 ± 0.06 min/h-) and made fewer movements (2.94 ± 0.04 moves/lizard/day) in the clustered arrangement (when lizards were released further apart), than in the evenly spaced arrangement (11.68 ± 0.06 min/h-; 5.66 ± 0.04 moves/lizard/day). When lizards changed burrows they moved shorter distances when burrows were clustered (41.9 ± 0.30 cm) than when burrows were evenly spaced (106.8

± 0.30 cm). There were also significant day x treatment effects for the number of burrow changes, for the number of fights and for the distance apart between lizards (Table 5-2). In each case the largest difference between treatments was on day one, with reduced differences on later days (Figure 5-4). Thus there were more burrow changes (Figure 5-4a), less fights (Figure 5-4b), and greater distance apart (Figure 5-4c) on day one when burrows were clustered. Other effects of day and trial probably reflected changes in ambient conditions.

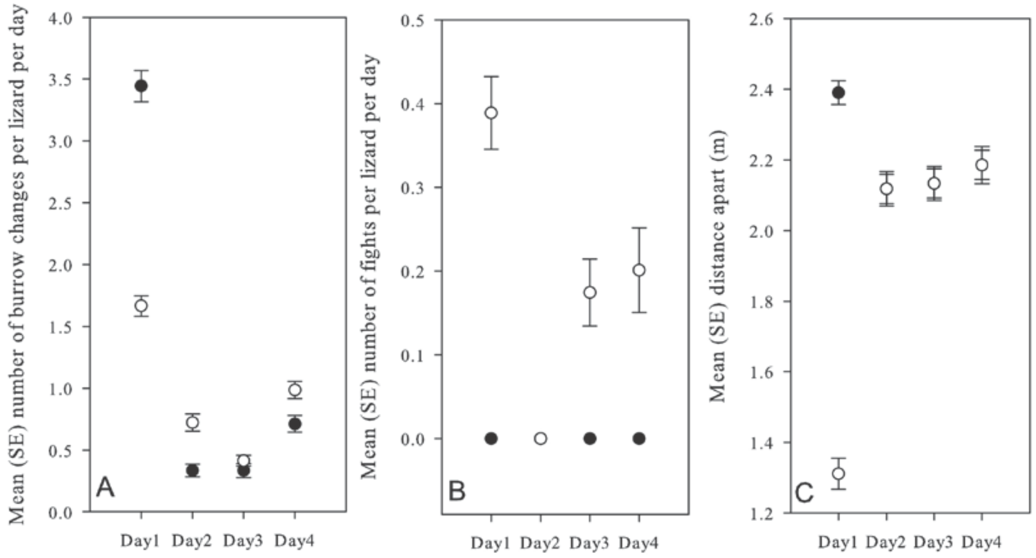

Figure 5-4. (A) Mean number of burrow changes, (B) mean number of fights, and (C) mean distance apart at the end of each day, for lizards released 150 cm apart in evenly spaced burrows (open symbols) or 250 cm apart in clustered burrows (filled circles) in experiment three. (Where mean values coincide only the open.

Table 5-2. Result of repeated-measures analyses of variance for each behavioural parameter in experiment one. Significant P value indicated with star.

	Effect		**Activity time**		**Basking time**		**Movement**		**Burrow change**		**Fights**		**Distance of movement**		**Distance from conspecific**		
		df	*F*	p value	*F*	p value	*F*	p value	*F*	p value	*F*	p value	*F*	p value	df	*F*	p value
Experiment one	Treatment	1,14	2.54	0.133	2.11	0.168	5.20	0.039*	6.85	0.020*	3.50	0.082	6.41	0.024*	1, 22	0.98	0.333
	Day	3,42	1.15	0.340	0.61	0.610	1.61	0.199	0.95	0.423	3.50	0.082	5.06	0.004*	3, 66	7.14	0.001*
	Trial	2,28	2.65	0.088	5.29	0.024*	0.16	0.847	1.34	0.276	1.40	0.263	0.19	0.826	2, 44	1.34	0.270
	Day x treatment	3,42	0.64	0.592	0.76	0.520	0.61	0.612	1.26	0.300	3.50	0.082	0.35	0.788	3, 66	0.89	0.450
	Trial x treatment	2,28	0.80	0.458	0.67	0.518	1.20	0.314	0.08	0.992	1.40	0.263	0.29	0.745	2, 44	0.12	0.885
	Day x trial	6,84	5.75	0.001*	2.69	0.078	0.43	0.852	1.46	0.245	1.40	0.224	1.95	0.082	6,132	6.73	0.001*
	Day x trial x treatment	6,84	0.67	0.667	0.71	0.639	0.02	1.000	0.70	0.644	1.40	0.224	0.90	0.497	6,132	2.85	0.012*
Experiment two	Treatment	1,10	1.86	0.203	0.80	0.390	0.14	0.708	6.30	0.033*	14.4	0.003*	3.93	0.075	1,10	30.5	0.001*
	Day	3,30	5.60	0.004*	3.62	0.024*	3.41	0.030*	0.23	0.869	1.41	0.259	1.15	0.345	3,30	5.85	0.003*
	Trial	2,20	0.17	0.838	0.16	0.847	1.48	0.251	2.31	0.128	0.15	0.861	0.48	0.626	2,20	1.69	0.209
	Day x treatment	3,30	0.29	0.830	0.27	0.846	5.62	0.004*	0.23	0.869	1.61	0.208	0.19	0.899	3,30	4.73	0.008*
	Trial x treatment	2,20	1.07	0.361	1.81	0.189	0.73	0.492	1.75	0.201	0.67	0.523	0.73	0.492	2,20	1.10	0.352
	Day x trial	6,60	4.48	0.023*	3.76	0.003*	0.67	0.668	2.36	0.120	1.61	0.208	3.97	0.002*	6,60	0.26	0.952
	Day x trial x treatment	6,60	0.21	0.971	0.72	0.635	0.75	0.609	2.07	0.081	1.37	0.238	0.45	0.824	6,60	0.38	0.889
Experiment three	Treatment	1,10	2.88	0.120	6.94	0.025*	5.58	0.040*	0.40	0.541	2.96	0.116	13.4	0.004*	1,10	19.3	0.001*
	Day	3,30	3.10	0.103	11.0	0.001*	11.7	0.001*	12.6	0.001*	2.93	0.091	9.03	0.001*	3,30	6.56	0.002*
	Trial	2,20	0.15	0.858	4.05	0.033*	0.16	0.851	0.57	0.575	1.81	0.327	1.21	0.316	2,20	1.43	0.262
	Day x treatment	3,30	0.72	0.545	0.78	0.515	0.54	0.656	3.26	0.035*	2.93	0.040*	0.48	0.695	3,30	3.95	0.017*
	Trial x treatment	2,20	1.32	0.289	0.34	0.714	0.72	0.496	0.83	0.448	1.81	0.327	1.21	0.318	2,20	0.40	0.675
	Day x trial	6,60	0.92	0.486	2.45	0.081	1.31	0.356	0.72	0.493	2.38	0.052	4.17	0.014*	3,30	0.72	0.628
	Day x trial x treatment	6,60	133	0.259	2.24	0.051	1.65	0.148	1.51	0.188	2.38	0.149	3.56	0.026*	3,30	0.89	0.506

5.6. DISCUSSION

Reptile species often select habitats based on the availability and quality of refuge shelters (Pianka, 1966; Heatwole, 1977; Beck and Jennings, 2003) and for many species, this is because the availability of permanent, secure refuges is crucial for their persistence (Langkilde et al., 2003). For a wider range of taxa, the provisioning of release sites with adequate refuge resources will be a vital component of the success of any translocation program, particularly in the period soon after release when individuals are adjusting to novel features of the releases site (Griffith et al., 1989; Gedeon et al., 2012).

Our first experiment reflected this requirement for abundant refuge resources. When lizards were presented with low burrow densities in experiment one, they made more movements out and back to the same burrow, changed burrows less often, but moved further when changing burrows than at high burrow densities. With more available burrows, lizards may have been able to more quickly assess closer unoccupied alternatives. Those burrow changes in both treatments led to a stabilisation of distance apart over the 4 days of the each trial.

One of the important problems in any translocation attempt is the stress of the released individuals soon after the release (Mihoub et al., 2009) resulting from capture, handling and release into an unfamiliar location. One specific additional cause of stress can be from agonistic interactions with conspecifics

(Letty et al., 2000; Teixeira et al., 2007; Drake et al., 2012). This stress can lead to post-release movement in the release habitat, with more exposure to climatic extremes and to predators, and more movement away from the release site. Examples of this include translocated birds (Kemink and Kesler, 2013) and snakes (Reinert and Rupert, 1999). The way that the available refuges are organised in a release site may have an important influence on the level of stress. Too few refuges, or refuges spaced too close together may lead to more frequent interactions for refuge ownership and higher stress levels.

In our second experiment, with burrow density kept stable, lizards released closer to each other had more fights, more movements out and back to the same burrow, and more burrow changes than lizards released further apart. They reacted to the proximity of conspecifics with aggressive social behaviours, and with increased movement patterns that would put them at increased risk from predation. Again the burrow changes led to them ending further apart, particularly among the lizards released close together. In the third experiment the clustered burrow treatment had lizards both with a higher local density of burrows and with a greater initial distance apart from other lizards than the evenly distributed burrow treatment. In the clustered arrangement, lizards moved in and out of their burrows less and basked more, suggesting they were less stressed, and more likely to settle where released. Confirming that interpretation, although the lizards with clustered burrows changed burrows more often on the first day of trials (as lizards did with higher burrow density in experiment one) they had fewer fights with conspecifics and retained a distance apart of just over 200 cm, a level of separation that the lizards in evenly spaced burrows also rapidly achieved in this experiment. This was presumably achieved by the evenly spaced lizards (that were initially closer together) moving further when they changed burrows.

In summary our results suggested that pygmy bluetongue lizards rapidly adjusted to the local density of burrows and to the proximity of conspecifics in those burrows. Any movements by lizards to change burrows in a real release will increase both their exposure to predation, and the likelihood that they will leave the area where burrows have been provided. They may then find themselves in habitat with fewer suitable refuges, thus reducing the chance of success of the translocation. Our experiments showed that lizards may be more likely to remain in the area where they are released if there is a high local density of refuges, so that exploratory moves can be short and secure, and if the distance apart from released conspecifics is relatively high, to reduce stress from agonistic interactions. In our study, lizards basked more, a sign of unstressed behaviour, when released at 250 cm apart, than at closer distances.

5.7. CONCLUSION

More generally the study suggested that in any translocation program, resource availability and distribution at the release site could have profound and significant influences on the behaviour of the released individuals in the critical first days after release in a new site. If translocated animals are initially confined to familiarise themselves with local conditions, as in the soft release strategy often advocated for translocations, high local density may increase the chance of adverse social interactions. If we understand, for any species, how resource distributions at the release site can affect levels of interactions, then manipulations may become possible (Gedeon et al., 2012) to reduce the impact of those interactions on the stress both within an enclosure and at the wider release site. Our study suggests there will be a benefit of understanding how resource distributions affect newly released individuals at a release site before the translocation release is initiated, for a wider range of animal species where translocation strategies are being explored.

5.8. ACKNOWLEDGMENTS

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Welfare Committee (approval no.E206) and was conducted under DENR Permit (G25011).

CHAPTER SIX

6. SIMULATED AGRICULTURAL ACTIVITIES

6.1. THE IMPORTANCE OF LIZARD BEHAVIOUR CHANGES

Understanding how animal behaviour can be altered by environmental change is crucially important for behavioural studies (Muller et al., 1997; Stoddard, 1999; Peters and Ord, 2003) and conservation management programs such as translocation (Sutherland, 1998). This information leads us to identify what set of factors are needed to avoid any change in normal behaviour. Changing behaviour can be more significant for an animal in a novel habitat because it could increase the chance of dispersal or predation. However understanding drivers of behavioural change can also help us to enhance conservation programs such as translocation by manipulating the species behaviour in the new habitat.

One of the common causes of environmental change in many endangered species habitat is agricultural activities such as over grazing and ploughing. These can change vegetation density which could cause changes in movement patterns, exposure to predation, and food availability for many species (Ivask et al., 2008; Pettigrew and Bull, 2011a; Pettigrew and Bull, 2012). Although in translocation programs we try to find the best habitat condition for the target species, identifying optimum habitat condition for species that have already lost their natural habitat, and live in highly fragmented patches of modified habitat which has already been affected by agricultural activities is difficult. Even if agricultural activities are stopped in such habitats some level of grazing and habitat management may still be required to prevent weeds over-running the space, and to avoid species diversity loss (Fuhlendorf and Engle, 2001; Smart et al., 2005). Therefore we need to understand the level of management and habitat manipulation that should be used to restore species diversity or maintain translocated population in a new habitat. In this chapter we examined lizard behavioural change in two alternative conditions, high vegetation density and barren ground. We also added variation in the surrounding matrix, with or without simulated ploughing to identify how that factor could alter lizard behaviour.

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Behavioural changes in an endangered grassland lizard resulting from simulated agricultural activities.

Mehregan Ebrahimi and C. Michael Bull

6.2. ABSTRACT

Agricultural modification of landscapes is one of human activities that profoundly affect habitat for endemic species. Most Australian native grasslands have now been taken over for agriculture activities, which dramatically changed these grassland ecosystems. Now only tiny fragments of the once more continuous native grasslands remain, and this has had a negative impact on species that occupy this habitat. One important question is how agricultural activities have altered the behaviours of endemic species in these fragmented habitats. One such species is the endangered *Tiliqua adelaidensis* which is endemic to native grasslands in South Australia. Current population sites of this species are grazed by domestic stock. We found simulated grazing increased the time that lizards spent basking at their burrow entrance, reduced the tendency of lizards to move outside of their burrow, or to move to a different burrow, but it increased the tendency to disperse away from the patch of habitat provided. Simulated ploughing of the surrounding habitat led to a reduction in dispersal rates. These results suggest that heavy grazing would have adverse impacts on existing populations of *Tiliqua adelaidensis.* They confirm that lizards avoid ploughed substrate, perhaps explaining previous observations of extremely low gene flow between adjacent populations.

Keywords: Grazing, Ploughing, Lizard, Behaviour, Pygmy bluetongue lizard

6.3. INTRODUCTION

Habitat degradation because of human activity threatens the viability of many animal species (Pimm and Raven, 2000; Primack, 2010; Read and Cunningham, 2010). Agricultural activities such as grazing, ploughing and cropping are among the most widespread forms of habitat degradation of native grasslands and woodlands (James, 2003; Pafilis et al., 2013). Continuous grazing by cattle or sheep with associated trampling effects, can reduce vegetation density, change micro habitats, and increase soil hardness, and these impacts affect the diversity of endemic invertebrates and vertebrate (Smart et al., 2005; Hoffmann and James, 2011; Dorrough et al., 2012). A major goal in the conservation of these already degraded habitats is to minimise the effect of such activities on the native species that still persist. One direct way is to stop agricultural activity in designated reserves, but further management is often required during habitat restoration. For instance endemic macro herbivores may need to be introduced to the habitat, or limited grazing from exotic grazers may be required to prevent overgrowth from exotic weeds (Rambo and Faeth, 1999; Fuhlendorf and Engle, 2001; Smart et al., 2005). Where agricultural grazing must be continued, Fuhlendorf and Engle (2001) advocated a grassland management strategy including focal point grazing to increase habitat heterogeneity and biodiversity. Cell grazing is an alternative process where, instead of continuous grazing of one large paddock, stock are regularly moved between a series of smaller paddocks (Dorrough et al., 2004). This increases the rest time and reduces the grazing pressure on individual paddocks. Sharp et al. (2010) showed that cell grazing had a smaller negative impact on burrowing spider populations than traditional continuous grazing. Thus managed grazing can be used to reduce the negative impact on native biodiversity and might even be recommended to maintain habitat heterogeneity. Other agricultural activities such as ploughing for cropping can have more serious effects on micro habitat and on invertebrate diversity (Stašiov et al., 2010). Ploughing not only changes the environmental conditions, inhibiting occupancy for many species, but it can directly kill invertebrates and reduce their populations (Thorbek and Bilde, 2004; Ivask et al., 2008).

A specific question is how lower levels of these agricultural activities might affect the behaviour of native animal species, and the levels of disturbance that can be tolerated, to allow endemic biodiversity to persist away from reserves. Grazing that alters microclimates, and decreases cover, could cause animals to reduce their levels of activity, and ploughing might produce behavioural barriers to dispersal. We must identify and recognise these changes and evaluate how the agricultural habitats need to be managed to reduce impacts on the behaviour of native species.

To address these questions we chose to study an endangered Australian grassland skink, the pygmy bluetongue lizard (*Tiliqua adelaidensis*) now restricted to a few small isolated patches of the highly fragmented native grasslands of South Australia. This lizard uses burrows constructed by mygalomorph and lycosid spiders both as refuges and as sites to ambush passing prey (Hutchinson et al., 1994). The burrows are single entrance vertical holes in the ground, normally occupied by only one lizard. In their natural populations, lizards spend almost all of their time associated with their burrow, and rarely change burrows or move away from the immediate surrounding area, except for brief excursions to capture a passing prey item (Hutchinson et al., 1994; Milne et al., 2003b). Occupied burrows are vigorously defended against approaching conspecifics (Fenner and Bull, 2011a) although neighbours are tolerated in burrows as close as 1 m apart (Milne et al., 2003b).

Although all known populations are on remnant fragments of native grassland, the sites are all on privately owned land, they all have been extensively invaded by exotic grasses and weeds, and they are mostly grazed by sheep (Souter, 2003). No populations are known from sites that have recently been ploughed or cropped. The persistence of populations in grazed grasslands suggests that the lizard can tolerate some level of agricultural grazing. A major question for conservation managers is to determine the impact of different levels of grazing, both to provide appropriate advice to conservation sympathetic landholders, and to develop specific management options for future reserved areas.

Previous studies have suggested that dispersing pygmy bluetongue lizards will accept artificial burrows inserted into the ground within native grassland patches (Milne et al., 2003a), but that they will not move into burrows in ploughed fields even if the burrows are immediately adjacent to occupied native grassland patches (Souter, 2003). Nor will they move into artificial burrows in patches of native grassland from which all of the above ground vegetation has been removed, simulating heavy grazing (Pettigrew and Bull, 2011a). Further studies have explored the behaviour of lizards in the immediate surroundings of their burrow under different levels of simulated grazing as represented by different vegetation densities around the burrow (Pettigrew and Bull, 2012; Pettigrew and Bull, 2013). Those studies, showing that lizards basked for longer with more of their body emerged from the burrow, and more frequently detected and attacked prey, when there was less grass around the burrow, suggested that a moderate level of grazing might benefit the lizards (Pettigrew and Bull, 2012; Pettigrew and Bull, 2013). Benefits might be derived from less cover allowing more efficient thermal basking and allowing wider visual fields to observe potential prey. In those previous experiments the simulated grazing was restricted to a small area immediately around the burrow entrance. Questions that remain unanswered include whether similar effects are induced by a more realistically wider area of simulated (or real) grazing, and whether other behaviours such as movement away from the resident burrow are also affected. Specifically, conservation managers will be concerned about dispersal behaviour, both because dispersal within populations will help genetic mixing, and because dispersal away from population sites might reduce population densities, particularly if there is no balancing immigration from other sites.

In the current study we predicted that, at levels of grazing that adversely affect the lizards, they would be more likely to attempt to move burrows, to find alternative sites where conditions improved. Dispersal comes with risks for pygmy bluetongue lizards, both because it increases their exposure to predators (and we know predation rate is high (Fenner et al., 2008a; Fenner et al., 2008b)), and because there is a chance they might disperse out of the small remnant fragment of native grassland habitat, and into the less suitable surrounding habitat.

To test those predictions, we manipulated simulated grazing levels at a wider scale than previous studies, and compared behavioural responses of lizards within replicated small habitat patches. We also added a second agricultural practice, with simulated ploughing of the area surrounding a habitat patch. The aim of the study, was to confirm that previously reported behavioural responses to grazing are consistent at this larger scale of habitat manipulation, to explore the effects of grazing and ploughing on dispersal behaviour, and to develop more informed management options.

6.4. METHODS

We established four 15 m diameter enclosures at Monarto Zoo (35°06´S 139°09´E), near Adelaide in South Australia. Cages were located in a line, 5 m apart. They had 1 m high galvanized iron walls and bird wire roofs to prevent avian predation. Each cage was divided into three areas, a central 4 m diameter circular area, with artificial burrows (see below), where lizards were released, surrounded by a matrix, consisting of a 5 m wide ring of bare ground that was considered unsuitable lizard habitat, and a 0.5 m wide ring around the inner perimeter of the cage. Artificial burrows for lizards were constructed from 30 cm lengths of 3 cm diameter wooden dowling with the central 2 cm drilled out. These were hammered vertically into augured holes in the ground until their entrance openings were flush with the ground surface. Pygmy bluetongue lizards readily use these in the same way as natural burrows (Milne et al., 2003a; Ebrahimi and Bull, 2012a; Ebrahimi et al., 2012a). We placed 41 artificial burrows in the central area of each cage, one in the centre and 40 in three concentric rings with 8, 16 and 16 burrows spaced 65-75 cm apart. We added another 30 burrows, spaced evenly around the perimeter ring, to act as refuges for any lizard that dispersed from the central area across the matrix, and to allow us to monitor that dispersal.

We planted tussocks of wild oats *Avena barbata* (mean height 20.8 ± 0.03 cm) at a density of 93 tussocks/ m^2 in the central area of two cages. *Avena barbata* is an exotic weed but it grows densely among the native grasses within natural population sites of pygmy bluetongue lizards (Souter et al., 2007). In the other two cages all grass was cut to ground level in the central region. Thus there were two treatment cages, grass (with *Avena*) and no grass, with two replicate cages for each treatment.

Sixteen pygmy bluetongue lizards (8 male and 8 female) were captured from two populations near Burra in South Australia (33°42´S; 138°56´E) in Sept 2009. They were kept in individual plastic boxes (52.5 x 38 x 31cm) in a room with ambient light and temperature, and fed every day with mealworms or crickets.

6.4.1. EXPERIMENT ONE (VEGETATION DENSITY EXPERIMENT)

The first experiment started at 0700 h on 1 December 2009, when we released four lizards, two males and two females, into the central region of each cage. We confined them to that region for 24 h, using a temporary, 20 cm high, black plastic wall (Ebrahimi and Bull, 2013b) to allow them to become familiar with the release environment. We then removed the wall and observed lizard behaviour in each cage over the next four days. This was the first phase of the experiment.

On the evening of Dec 5, we removed all of the lizards from their overnight refuge burrows, held and fed them for three days, and then released them in the same combinations back into the cages, but with each group of four lizards now in a different cage with a different treatment. This was the second phase of the experiment. Thus groups of lizards that had been in the grass treatment in the first phase were now released in a cage with no grass, and vice versa. Timing of the release, initial confinement and observations were the same as in the first phase of the experiment.

We observed lizard behaviour, with four surveillance cameras (Longse: LICS23Hf, 3.5 mm lens), attached at 1.5 m above ground level to four star pickets in each cage, with a combined field of view that covered the 4 m diameter central area of that cage. The cameras recorded all lizard activity in the central area of each cage during the daylight hours 0700 – 1800 h on each day of the experiment, on a 16 channel h.264 DVR (ESW26), powered by four 12 V batteries.

No supplementary food was provided in the cages, although lizards could prey on natural invertebrate fauna. Video images were not detailed enough to allow us to document feeding, particularly in the grass treatment.

We derived seven behavioural parameters for each lizard from the video recordings. 1) Activity time was defined as the total time in a day from when the head of the lizard first emerged from its burrow until the last time it retreated into a burrow on that day. 2) We considered a lizard to be basking if it was sitting partially or fully emerged at its burrow entrance, and we calculated basking time for a lizard each day, in minutes per hour, by dividing the total time it was observed to be basking by 11, the total hours of video recording for that day. 3) We defined a movement if a lizard fully emerged and walked away from its burrow entrance, and then retreated back into the same burrow. Movements could include walking around the burrow area, moving to bask away from the burrow entrance, emerging to prey on passing invertebrates, or leaving the burrow for defecation. 4) We defined a burrow change if a lizard emerged from one burrow and subsequently entered another burrow within the central region that could be seen on the video recordings. 5) We defined a movement to the perimeter if a lizard was observed on the video recording to have left the central area. We counted the number of movements, the number of burrow changes in the central area, and the number of moves to the perimeter, for each lizard on each day. 6) Where a lizard changed burrows within the central region, we measured the direct line distance between the old burrow and the new one. 7) During the experiment lizards sometimes contacted each other, and this agonistic encounter always resulted in a brief scuffle, or one lizard running away from the other. For the seventh behavioural parameter, we recorded the number of these "fights" per lizard per day.

For analyses, we examined whether lizard behavioural parameters were influenced by the grass or no grass treatment in the central region of the cages, using repeated-measures ANOVA. We used individual lizards as replicates, with a single average per day (n = 4 days) value for each lizard in each phase of the experiment. Mostly, those averages were derived from the complete four days of observations, but where a lizard left the central area and could not be observed for part of one day, the average values for activity time, basking time, number of moves, and number of fights for that lizard were calculated for the three other days when it was entirely within the central region. In the analyses, treatment (Grass or No Grass) was the repeated measure, and order of presentation (Grass first or No Grass first) was the between subjects factor. We used the Greenhouse-Geisser correction where data were non spherical.

Before and after each phase of the experiment we weighed each lizard, and then calculated the body mass gain or loss of the lizard over the five days. We used repeated-measures ANOVA as above to examine the influence of treatment and order of treatment on mass change.

After this experiment was completed, the lizards were held in individual cages in a 25^{O}C room with a 12: 12 LD light regime in the Animal Care Unit of Flinders University, Adelaide. Temperature and light were gradually reduced to 15^{O}C and 10:14 LD over the austral winter and then brought back to 25^{O}C and 12:12 LD by the following spring and in time for the next experiment.

6.4.2. EXPERIMENT TWO (SURROUNDING MATRIX PLOUGHED AND VEGETATION DENSITY EXPERIMENT)

In the second experiment, the central area of each cage at Monarto was the same as in first experiment, two with and two without grass. Then in two cages, one with and one without grass, we ploughed a 2 m wide ring in the matrix around the central area, leaving a 3 m wide unploughed ring of the matrix before the perimeter region of the cage. We used a hand-held shovel, and broke up the matrix soil surface to a depth of about 30 cm. We ran three trials starting at 0700 h on Oct 20, Nov 16 and Nov 26, 2010. In each trial lizards were confined to the central area for one day, and then the central barrier was removed, and their behaviour was recorded for the next four days as in the first experiment. In this experiment the 16 lizards were randomly assigned to new groupings of four lizards in between trials, and each trial was considered to provide independent replicates of the four combinations of treatments. Between trial 1 and trial 2 we replanted the central regions of the "grass" treatment cages, and re-ploughed the 2 m wide circle for the "ploughed" treatment cages.

For analyses we derived one overall mean per cage for each behavioural parameter, from all four lizards over all four days (or fewer if the lizard left the central area) in each trial. These mean values were used as the dependent variable in two way ANOVAs testing the impact of central treatment (grass/ no grass) and matrix treatment (plough/no plough).

6.5. RESULTS

6.5.1. EXPERIMENT ONE

Basking was the main activity of lizards during the filming sessions, with an overall average of 11.47 ± 0.08 min spent basking each hour. Lizards spent most of their time associated with their burrows, and movements around the burrows (0.50 ± 0.01 moves per lizard per day), or away from their burrows (0.63 ± 0.1 moves per lizard per day: = number of burrow changes + no of moves to perimeter) were infrequent.

For five of the seven behavioural parameters we measured there was a significant main effect of the experimental treatment, but with no significant effect of order or of the interaction between order and treatment (Table 6-1). That is the treatment had a similar significant impact independent of the order in which the two alternatives were presented to the lizards. When there was no grass, lizards were active for longer in each day, and basked for longer in each hour of the day (total activity time 3.42 ± 0.07 hour day^{-1}; basking time 15.65 ± 0.16 min h^{-1}) than when there was grass (2.17 ± 0.07 hour day^{-1}; 7.30 ± 0.17 min h^{-1}). In cages with no grass lizards moved around their burrows less often, changed burrows less often, but moved to the perimeter more often

(0.33 ± 0.02 moves per lizard per day; 0.28 ± 0.02 burrow changes per lizard per day; 0.36 ± 0.02 moves to the perimeter per lizard per day) than lizards in cages with grass (0.66 ± 0.03 moves per lizard per day; 0.46 ± 0.03 burrow changes per lizard per day; 0.16 ± 0.02 dispersals per lizard per day).

There was no difference between treatments in the distance moved during burrow changes within the central area (overall mean 114.72 ± 0.35 cm), or the number of fights between lizards (0.06 ± 0.01 per lizard per day)

For body mass change there was significant interaction of treatment x order (Table 6-1). Lizards always gained body mass in the grass treatment, but either gained less mass or actually lost mass in the no-grass treatment depending on whether that treatment came first or second in the sequence. (Figure 6-1).

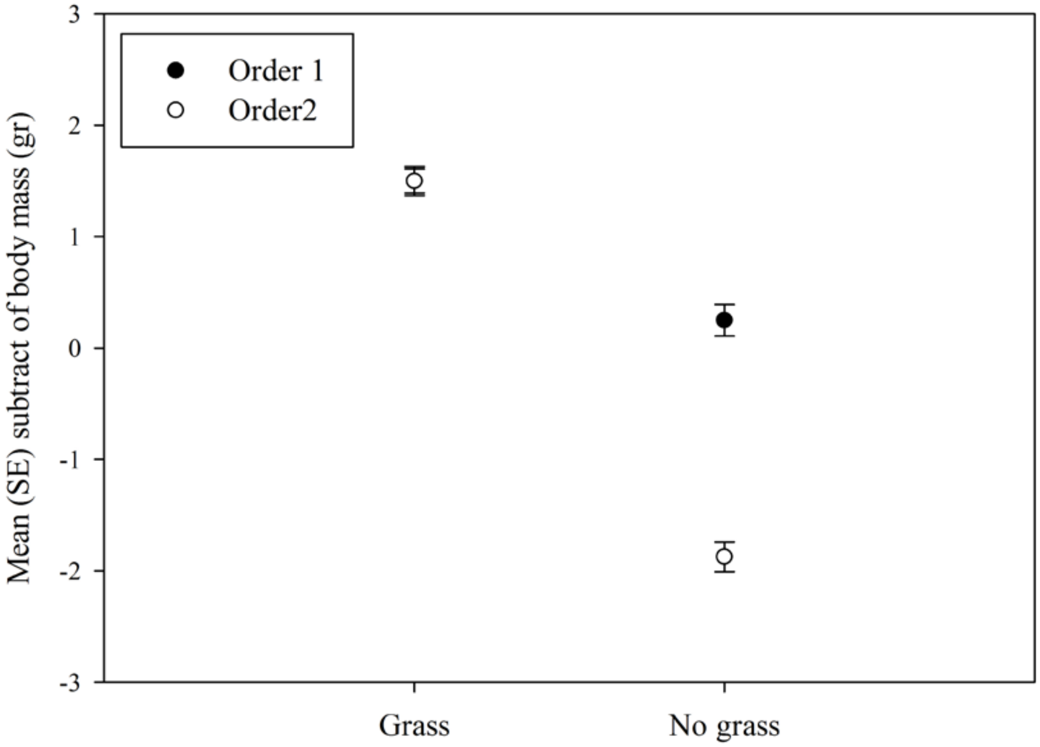

Figure 6-1. Mean (SE) change in body mass over the five days in each treatment phase of experiment 1, for lizards presented with grass first and then no grass (Order 1) or no grass and then grass (Order 2) N = 8 lizards in each category.

6.5.2. *EXPERIMENT TWO*

In the second experiment there were no significant interactions between the central treatment (grass/ no grass) and the matrix treatment (plough/ no plough) for any behavioural parameter, allowing us to examine the main effects separately. Five of the seven behavioural parameters showed a significant effect of the central grass/ no grass treatment (Table 6-2). Lizard responses were similar to those from the first experiment. In cages with no grass, lizards were active for longer in each day and basked for more minutes each hour (total activity time 3.56 ± 0.04 hour day^{-1}; basking time 20.03 ± 0.14 min h^{-1}) than in cages with grass (2.0 ± 0.05 min day^{-1}; 14.0 ± 0.16 min h^{-1}). In cages with no grass lizards moved around their burrows less often than in cages with grass (No grass: 0.66 ± 0.03 moves per lizard per day; grass: 1.35 ± 0.04 moves per lizard per day), but they moved to the perimeter significantly more often in cages with no grass (Figure 6-2). Although there was no effect of either treatment on the number of moves to a new burrow within the central area (overall mean 1.42 ± 0.03 moves per lizard per day), the distance moved between burrows in those moves was significantly further when there was no grass (127.4 ± 0.46 cm) than when grass was present (23.9 ± 0.20 cm). Lizard body mass increased significantly more in cages with grass (+1.73 ± 0.18 g) than in cages with no grass (-0.80 ± 0.20 g) (Table 6-1).

There was only one significant main effect of ploughing. Lizards moved significantly less often through the matrix to the perimeter when the matrix had been ploughed (Figure 6-2). There were no fights recorded during this experiment.

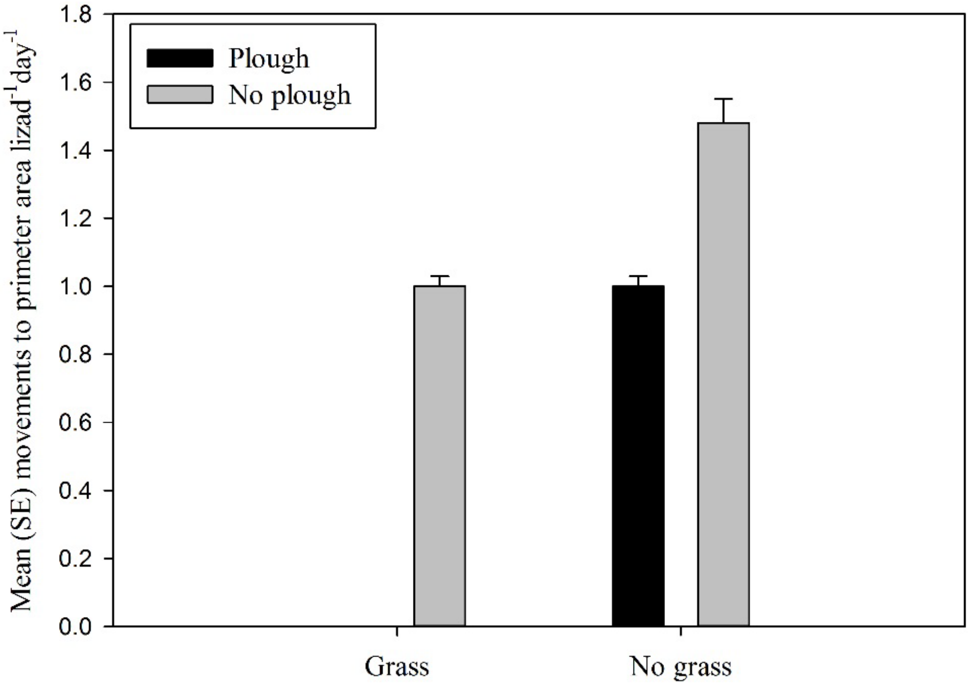

Figure 6-2. Mean number of lizards per day that moved to the perimeter area in each treatment combination in experiment 2.

Table 6-1. Repeated-measure analyses of variance for behavioural parameters in experiment 1 considering responses of lizards to Grass or No Grass treatments. The number of fights is omitted as there were no significant effects. Values with star indicate significant effect ($p < 0.05$). df = 1, 14 for all effects.

		Treatment	**Order**	**Treatment x Order**
Activity time	*F*	29.52	2.52	1.93
	p value	0.001*	0.135	0.186
Basking time	*F*	10.46	4.01	0.21
	p value	0.006*	0.065	0.649
No. moves	*F*	9.10	0.07	0.94
	p value	0.009*	0.788	0.347
No. burrow changes	*F*	7.90	0.01	0.12
	p value	0.014*	0.925	0.731
No. moves to perimeter	*F*	8.22	0.06	0.61
	p value	0.012*	0.812	0.447
Distance moved	*F*	0.97	2.50	0.71
	p value	0.339	0.136	0.413
Body weight change	*F*	27.61	11.83	5.83
	p value	0.001*	0.004*	0.03*

Table 6-2. Two way analyses of variance for behavioural parameters in response to centre treatment (grass or no grass) and matrix treatment (ploughed or not ploughed). Values with star indicate significant effects ($p < 0.05$). df = 1, 8 for all effects.

		Plough	**Grass**	**Plough x Grass**
Activity time	*F*	0.69	6.60	0.01
	p value	0.428	0.033*	0.904
Basking time	*F*	0.15	24.91	0.01
	p value	0.701	0.001*	0.926
No. moves	*F*	2.24	8.39	0.90
	p value	0.172	0.020*	0.370
No. moves to perimeter	*F*	5.88	5.88	2.14
	p value	0.041*	0.041*	0.181
Distance moved	*F*	0.20	23.21	3.54
	p value	0.661	0.001*	0.097
Body weight change	*F*	0.82	8.69	0.02
	p value	0.391	0.018*	0.875

6.6. DISCUSSION

Our results from two experiments over two years showed a consistent influence of vegetation density on the behaviour of the pygmy bluetongue lizard. When there was no grass within the central region of the cages, lizards were active over a longer period of each day, and they basked for a higher proportion of time, than in cages with grass. When there was no grass, they also moved about on the surface close to their burrows less often, and changed burrows within the central area less often, but were more likely to disperse away from the central area than when there was grass. If this behaviour is replicated in natural populations, then reducing grass levels, for instance by heavy grazing, could lead to increased dispersal and reduction of population size.

Although we introduced lizards to entirely novel habitats in these trials, the results are completely consistent with patterns shown by Pettigrew and Bull (2012) who described lizard behaviour when grass was clipped away from a small area around natural pygmy bluetongue lizard burrows. That study reported that when there was less grass, lizards spent longer periods basking, and spent more time fully emerged than when there was more grass. Similarly, they reported in another study (Pettigrew and Bull, 2011a) that lizards were less likely to move into artificial burrows if there was simulated grazing around those burrows, consistent with the current result that lizards were more likely to disperse away from treatments with no grass. In the current study, however, we were comparing simulations of no grazing with simulations of very heavy grazing pressure, involving the complete removal of grass from the no grass treatment cages. We cannot use these results to comment on any potential advantages or disadvantages to lizards with a less severe level of grazing. Some grazing might be beneficial to the lizards if it were to open up the habitat without completely removing grass cover.

The only inconsistency from previous results concerns the greater weight gain in cages with grass than in cages with no grass for the lizards in the current study. Weight gain presumably reflects the ability of lizards to find and capture invertebrate prey. Previous research had showed lizards captured prey more frequently in treatments with less grass (Pettigrew and Bull, 2012). The current results may simply reflect that there was more natural prey available in the cages when there was grass, rather than any effect on the efficiency of prey capture. Other studies have also shown that grazing can decrease the available prey for lizards (Fair and Henke, 1997; Pafilis et al., 2013). Reduced numbers of available prey in our experimental cages with no grass, could also explain why lizards bask for longer when grass density is lower, a consistent observation across this and previous studies. In another experiment, Ebrahimi and Bull (2012a) showed that lizards basked less when they were fed supplementary food. Although we used the term "basking" for the behaviour of sitting partially exposed at the burrow entrance, Ebrahimi and Bull (2012a) suggested that basking lizards are also keeping watch for passing prey items, and that lizards that have fed less remain exposed for longer at their burrow entrance to increase their chance of encountering and capturing some prey items. This increased basking time, while allowing the potential for more prey captures, might come with the increased risk of longer exposure to predation.

Pygmy bluetongue lizards are preyed upon by both snakes and avian predators (Fenner et al., 2008b). The conflicting demands of detecting prey and avoiding predators might influence a range of other behaviours. Thus when grass was present, and there was more cover from predation, lizards completely emerged, and moved on the surface around their burrows more often. They also changed burrows within the central area more often, perhaps reflecting a lower chance of their movements being detected. On the other hand, when there was no grass, lizards were more likely to attempt to leave the central area of the cage, perhaps because of a perception of higher predation risk, or of lower prey availability in the habitat. Ebrahimi and Bull (2012a) similarly reported that lizards without supplementary food were more likely to disperse. In this study it is difficult to differentiate between whether the dispersal response was a direct consequence of the altered vegetation structure or an indirect consequence of the altered vegetation changing the food levels.

Our study is not unique in exploring the impacts of grazing on endemic lizards. Several other studies have shown how changes in vegetation density, induced by agricultural grazing, influence the population density of individual lizard species, and the broader lizard community structure (Jones, 1981; Read, 2002; James, 2003; Castellano and Valone, 2006).

The disruption of the soil surface, by simulated ploughing in the matrix around the central areas of the cages in our experiments had no significant influence on either the behaviour of lizards within the central area, or their weight gain, but it did significantly reduce the tendency for lizards to disperse across the matrix. This is consistent with previous unsuccessful attempts to use artificial burrows to encourage lizards to colonise previously ploughed fields, immediately adjacent to existing population sites (Souter, 2003).

The experiments confirmed two established recommendations for sustainable management of pygmy bluetongue lizard populations in agriculturally modified habitats. These are that population sites should not be subjected either to heavy grazing or to ploughing. They also provided new insights into the behavioural responses of this lizard to reduced vegetation density and specifically into the balance between the advantages (increased opportunities to see passing prey) and disadvantages (increased risk of being detected by a predator) of exposure at their burrow entrances, and of moving around on the surface.

Finally, because we were placing our lizards into a novel habitat, our results provided some new insights into possible short-term responses of lizards to translocation. Modelling has suggested that some form of translocation will be essential for the ultimate persistence of viable populations of this species (Fordham et al., 2012). For any future translocation program we need to understand what will encourage lizards to remain close to where they are released. This is to ensure that they do not move away from areas of preferred habitat, but also to ensure that translocated individuals can locate conspecifics for mating. In our trials, at least over a few days, lizards were less likely to disperse from the release area if there was more vegetation. Irrespective of whether that was a response to more cover or more food, the management implications are the same. Release sites should not be heavily grazed.

The fact that "ploughing" inhibited dispersal may be less useful as a translocation management tool. Although this disruption of the habitat may keep released lizards in one place, it will also reduce the overall habitat quality for later population expansion into the surrounding landscape. Nevertheless it suggests that some form of matrix manipulation might provide a short term reduction of dispersal from releases.

An implication for conservation management on land that is primarily agricultural could be to recommend that the land should not be heavily grazed or left ungrassed because that could decrease prey and increase dispersal for native species like the pygmy bluetongue lizard. Similar recommendations have emerged from parallel studies of other lizards (Vitt et al., 1998; Stašiov et al., 2010; Pafilis et al., 2013).

6.7. ACKNOWLEDGMENTS

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CHAPTER SEVEN

7. CONSPECIFIC CUES

7.1. IMPORTANCE OF CONSPECIFICS

Conspecifics have an important role to maintain and establish a new population, and it has been shown that the more conspecific individuals that are released at a translocation site the greater the chance of successful translocation (Griffith et al., 1989; Veltman et al., 1996). Individuals are also influenced by the behaviour of conspecifics and understanding these behavioural changes could be vital for conservation management programs such reintroduction and translocation. So by understanding how species response to a range of cues from conspecifics we can potentially use this information to manipulate behaviours in a future conservation management program. For instance if the target species is attracted to conspecifics we may can reintroduce more individuals to small local population, or add conspecific cues such as odours to the release site. If they are highly competitive we may need to release individuals further from each other, reduce possible conspecific signals, or increase other resources to reduce agonistic interaction. These agonistic interaction can increase dispersal rates, especially for individuals that are not familiar with a new habitat, or they might increase the risk of predation. In this chapter we investigate how lizards respond when visual cues from conspecific models are presented in simulated translocation site for pygmy bluetongue lizards, which are essentially solitary in their social organisation. In this chapter we show how conspecifics can increase early dispersal after release, and we derived some new information about alternative personality among individuals which could be important for individual selection from the donor population for future translocation.

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Visual conspecific cues will not help in pygmy bluetongue lizard translocations

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7.2. ABSTRACT

Where a translocation program is used to reinforce an existing population of an endangered species, the response of the introduced individuals to cues from conspecific residents will have an important impact on the success of the translocation. If those cues induce the translocated individuals to stay at the release site the translocation is more likely to succeed than if the cues cause individuals to move away. We used conspecific models of the endangered Australian pygmy bluetongue lizard to identify behavioural parameters relevant to translocation success, which change when the visual conspecific cues are presented. Pygmy bluetongue lizards typically remain in or at the entrance of their refuge burrows. In the presence of conspecific models, introduced lizards significantly increased, and nearly doubled, the number of movements out of their burrows (mean (SE) number of movements with models = 0.44 (0.03); without models = 0.25 (0.03); $P = 0.012$) and more than doubled the number of movements away from the release area (mean (SE) number of movements with models = 0.28 (0.03); without models = 0.08

(0.02); P = 0.003), suggesting they would be less likely to remain within a resident population where they were released. We found that, by the end of the first day of experimental trials 11 of 16 lizards in treatments with models present had occupied burrows that did not have a model nearby, and that number increased to 14 of 16 lizards by the fourth day. The results suggest that cues from conspecifics will not encourage translocated lizards to stay at a release site.

Keywords: Conspecific models, Behaviour, Lizards, Dispersal

7.3. INTRODUCTION

A range of behavioural responses to conspecific individuals, particularly responses associated with agonistic or mating behaviours, are mediated by unique cues, and models that contain features of those cues can be used to manipulate animal behaviour in practical ways (Craven, 1984), including their use in conservation related translocations.

For many endangered species, one potential management strategy is conservation translocation, the intentional movement and release of individuals primarily for conservation benefit (IUCN, 2013). Two important problems in any translocation attempt are the initial stress on release, and the tendency to disperse from unfamiliar habitat (Mihoub et al., 2009). Examples of post-release movement in release habitats include translocated birds (Kemink and Kesler, 2013) and snakes (Reinert and

Rupert, 1999). The novel location and resource competition from conspecific residents may increase the stress level of translocated animals (Letty et al., 2000; Teixeira et al., 2007; Drake et al., 2012), but stress may be reduced if individuals recognise conspecific cues that allow them to rapidly identify refuge shelters or feeding locations (Lorenzo and Lazzari, 1996; Göth and Evans, 2004; Gautier et al., 2006; Kullmann et al., 2008). In those cases the provision of conspecific cues may reduce both stress and the tendency to disperse. For instance Ahlering et al. (2010) reported that, in 20 of 24 reviewed studies, songbirds were encouraged to settle in habitat where conspecific songs were played. Alberts (2007) suggested that captive reared individuals of the endangered Caribbean rock iguana, when released back into the wild, may be more likely to preferentially settle where there are familiar cues such as known conspecifics or their odours. On the other hand, in species that are aggressively territorial, the use of conspecific cues may have the opposite effect, and increase stress. The potential to use conspecific cues to promote translocation success needs to be examined carefully on a species by species basis.

The endangered pygmy bluetongue lizard (*T. adelaidensis*) is now restricted to a few isolated fragments of its native grassland habitat in the mid-north region of the state of South Australia, Australia. Its current distribution is a small part of its previous range, most of which has been taken over by cereal cropping and grazing farmland. Its endangered status has resulted from the now restricted geographical range, and from the isolated nature of the few remaining small populations. Models that explore likely future climate change scenarios within the range of this lizard, show that reinforcement or reintroduction translocation will be a certain requirement for the future preservation of this species (Fordham et al., 2012). If we adopt that strategy, we need to know how best to prevent translocated lizards from dispersing away from release sites. Can we use cues from conspecifics, to encourage them to preferentially settle close to where they are released?

The pygmy bluetongue lizard is normally solitary and lizards spend most of their time associated with single entrance burrows constructed by lycosid and mygalomorph spiders (Hutchinson et al., 1994; Fenner and Bull, 2011b). Individuals usually occupy a single burrow for extended periods of time and most suitable burrows are taken by lizards (Hutchinson et al., 1994; Milne et al., 2003; Souter et al., 2004; Fellows et al., 2009). This suggests there is competition between lizards for limited high quality burrows, and although occupied burrows can be as close as 1 m apart (Fenner and Bull, 2009) lizards actively defend a very small area with a radius of less than 15 cm around their burrow entrance from approaching conspecifics (and from conspecific models) (Fenner and Bull, 2011a). This would suggest that conspecific cues might increase stress in newly introduced lizards. On the other hand, when in a novel environment, lizards recognise conspecific olfactory signals and prefer to choose unoccupied burrows that have previously held a conspecific (Fenner and Bull, 2011b); that is they select refuges where other lizards have been. In that case, conspecific cues that are not directly challenging might help lizards adjust to a novel environment. In the current study we asked whether the provision of conspecific models near some, but not all burrows in a novel habitat, might reduce or increase movements and dispersal among newly introduced pygmy bluetongue lizards.

7.4. METHODS

7.4.1. EXPERIMENTAL TRIALS

We used eight male (average snout-to-vent length (SVL) 85.1 ± 0.2 mm) and eight female (average SVL 89.2 ± 0.2 mm) pygmy bluetongue lizards that had been captured from two natural populations near Burra, South Australia (33°42´S; 138°56´E). These lizards had been used in several other short behavioural experiments during the austral spring and summer of 2009/10 and 2010/11 (Ebrahimi and Bull, 2012; 2013a; b; c), and so had briefly experienced the experimental habitat of the current experiment (total of 60 days in the cages over a two year period). Before the current experiment the lizards were held in individual cages (52.5 x 38 x 31cm) in ambient conditions and fed every day with crickets and mealworms.

The experimental cages have been described previously (Ebrahimi and Bull, 2013b) as four, 15 m diameter cages at Monarto Zoo, 70 km SE of Adelaide, South Australia (35°06′S; 139°09′E). Each cage had a 1 m high galvanized wall and a bird-proof wire roof. Each cage was divided into three areas; a 2 m radius central area where lizards were released, which was lightly vegetated with annual grass cut to ground level before the experiment started, and where burrows were provided, a 5 m wide ring of marginal habitat, similarly vegetated but with no burrows, and a 0.5 m wide perimeter area around the inside cage wall, again similarly vegetated but with burrows. We considered the no-burrow habitat marginal because we assumed that lizards would perceive they were exposed and at risk where there were no burrows. We placed 41 artificial burrows into the central area, one in the centre and 40 in three concentric rings, so that burrows were 65-75 cm apart. We also spaced 30 burrows evenly around the inside cage perimeter (Figure 7-1). Burrows were made from 30 cm lengths of 3 cm diameter wooden dowling with the central 2 cm diameter drilled out. These were hammered into 30 cm deep, 3 cm diameter holes drilled into the soil surface. The burrows in the perimeter area allowed us to detect lizards that had dispersed from the central area.

We made 40 polyurethane models from a previously produced mould of a male pygmy bluetongue lizard. To produce a model colour that resembled that of the lizards, we added 1 mg of oxide brown colour (Diggers oxide colouring, Recochem, Australia) to 500 mL polyurethane (Easy Flo 60 Casting Polyurethane, Polytek Development Corp., Solid Solutions, Australia). Fenner and Bull (2011a) have previously reported that pygmy bluetongue lizards attacked these models if they were placed 5 cm from their burrow entrances, and that they differentiated between these models (that they attack), and models of a similar sized sympatric skink species, or a similar sized inanimate stick (that they do not attack).

Two replicate trials were conducted to test whether the behaviours of lizards differed in the presence or absence of the model lizards. At the start of each trial we placed 20 models in the central area of each of two cages. Models were located 5 cm from the entrance of alternate burrows. We considered the models were located at a position relative to the burrow entrance that a basking lizard might adopt. There were four models spaced around the inner concentric ring of eight burrows, and eight models spaced around the two outer rings, each of 16 burrows (Figure 7-1). The other two cages were left with no models. In the first trial, we released four lizards (two males and two females) into the central region of each cage at 07:00 h on 17 January 2011 and confined them for 24 h in the release area with a temporary, 20 cm high, black plastic wall. This allowed the lizards to become familiar with the release environment (Ebrahimi and Bull, 2013b) and the models. Then we removed the wall and recorded lizard behaviour for the next 4 days. Thus day 1 of the trial was the day after the wall had been removed. Because filming was continuous while lizards were active we could follow the behaviours of each individual lizard over the four days of each trial. At the completion of the first trial, on the evening of 21 January 2011, we removed lizards from their burrows and kept them individually in their holding cages, with ambient temperature and light, and fed them for 3 days. Then, for the second replicate trial, we randomly chose new combinations of two male and two female lizards, and released them into the same four cages (two with and two without models).

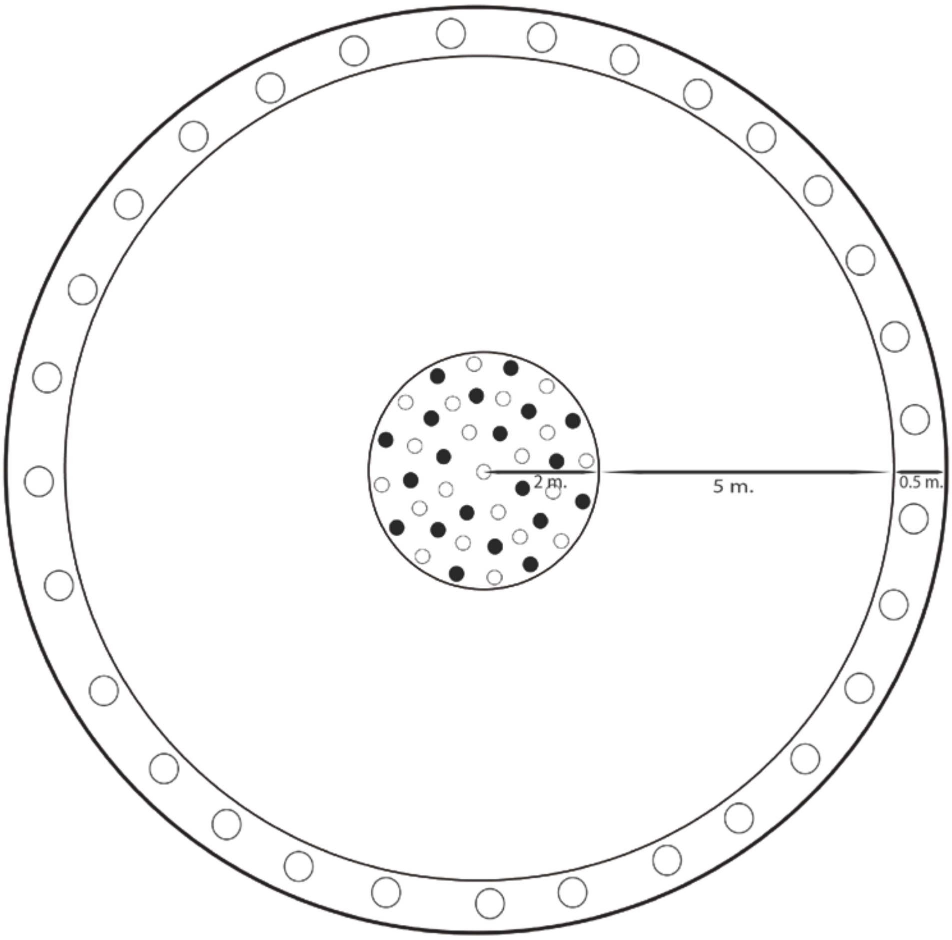

Figure 7-1. The layout of each cage used in the experimental trials, showing burrows with models placed 5 cm from the burrow entrance (in the model addition treatment; filled circles) and the burrows with no models (open circles)

7.4.2. BEHAVIOURAL PARAMETERS

In each trial, we observed lizard behaviour using four surveillance cameras suspended above each cage (CCD video camera, LICS23HF and lens 3.6 mm, Normal recording mode (continues), 30 fps, Longse, China). The cameras had a combined field of view covering the complete central area, and we used them to record continuously lizard behaviour from 07:00h to 17:00h during each day of each trial. We also confirmed the location of each lizard every day in the early morning and late afternoon by inspecting each burrow with a small torch. In the cages with models, we recorded, at the end of each day, how many lizards were in the 20 burrows with adjacent models, and how many were in the 21 burrows without models. We did not add supplementary food during the experiment, but lizards could prey on naturally occurring invertebrates.

We derived six behavioural parameters from the video recordings in each cage during each replicate trial; 1) Total activity time (h d^{-1}) which was defined as the period from the first time the head of a lizard emerged from its burrow to the last time that lizard retreated completely into its burrow on that day; 2) Basking time (min h^{-1}) which was defined as the period of time when the lizard was at least partially emerged (5 -98% of body outside of the burrow) and was located at the entrance of its burrow. We divided the total min spent basking each day by 11 (the total h of filming in a day) to calculate the basking time as min h^{-1}; 3) Number of movements around burrow. In some cases lizards fully emerged from their burrow, moved about, usually for a very short distance, no more than 10 cm from the burrow entrance, and then returned to the same burrow. These movements included lizards that just walked around the burrow entrance no more than 5 cm from it, lizards that basked while fully emerged, and lizards that moved 10 cm away from the burrow entrance for defecation or darted out to catch prey within 10 cm of the burrow entrance. We recorded the number of movements by each lizard on each day; 4) Number of burrow changes. In some cases, lizards fully emerged from their burrow, moved more than 10 cm from that burrow (distance of actual moves are given in the results), and entered another burrow in the central area. We recorded the number of burrow changes for each lizard on each day; 5) Distance moved. If a lizard had moved to one or more different burrows within the central area during a day we measured the distance moved as the direct line distance between the burrow the lizard was in at the start of the day to the burrow it was in at the end of the day; 6) Number of movements to the perimeter area, which was defined as the number of times a lizard left the central area, moved across the habitat matrix, and was subsequently discovered occupying a burrow in the perimeter region. In terms of the translocation simulation, we considered that these represented dispersal events away from the release site, because lizards normally move less than 1 m from their occupied burrows (Milne et al., 2003; Fenner and Bull, 2011a) and because lizards that ended in perimeter burrows had to leave the central area where burrow refuges were available and cross the burrow-less matrix.

For each cage, on each day, we calculated a mean value per lizard of each parameter, and used that mean value for the cage in subsequent analyses. Normally this was a mean from four lizards, although in some cases, when a lizard had moved to the perimeter ring, and out of the field of view of the cameras, the mean for that day was derived from three or fewer lizards. On the first, second and fourth day, data from one cage had to be calculated from just three lizards and on the second and fourth day from one other cage data had to be calculated from just two lizards. We observed no agonistic interactions among live lizards in any cages, but recorded the number of attacks that lizards made on the model lizards.

We also recorded the overall number of behavioural activities by the lizards, including emerging to bask, movement around the burrow, burrow changes, movements to the perimeter area, and attacks on models. Although we used the same lizards, they were in different combinations in the second trial and we considered that we had four independent replicates, two from each trial, of each treatment (with or without models). Thus data from the two sets of replicate trials were combined in the analyses to ask two questions. We first asked whether lizards in cages with models (four cages total; two cages in each of the two trials) showed any specific responses to those models, and if this response changed with experience over the 4-day trials. We then asked if lizards differed in their behaviours if they were in cages with (four cages) or without (four cages) conspecific models.

7.4.3. STATISTICAL ANALYSES

In the cages with models we used contingency chi-squared analysis to determine if burrow choice was random or was influenced by the presence of the models. Then, after the first day of each trial, we compared by t-test the behavioural parameters of those lizards that were in burrows with models and those in burrows without models. We did not repeat these analyses for days 2 to 4 because of the low numbers of lizard that remained in burrows with models on those days. In the cages with models, we used repeated measures ANOVA (Hand and Taylor, 1987) to determine whether attack rate on models changed with day of the trial. To compare other behavioural parameters between those lizards that did or did not attack models at least once, we used a repeated measures ANOVA for each of the six behavioural parameters, with day of trial as a within subjects factor and lizard response to the model (attacked model/did not attack model) as a between subjects factor.

We again used repeated measure ANOVA to examine whether the treatment of adding conspecific models to cages altered lizard behaviour, for each of the six behavioural parameters. We used the average behavioural parameter value per cage (eight cages in total, four cages in each of the two trials) per day as the dependent variable, day (1-4) as a within subjects factor and cage treatment (model/no model) as the between subjects factor. For all repeated measures ANOVA's we applied the Greenhouse-Geisser correction when data were non- spherical.

7.5. RESULTS

7.5.1. DO LIZARDS RESPOND TO THE MODELS?

From 16 lizards (in four replicate cages with models present), five occupied burrows with models 5 cm from the burrow entrance at the end of the first day (two in the first and three in the second trial). This was reduced to three lizards on the second day, and to two lizards on the third and fourth days (one in each trial) of the experiment. While chi squared tests showed no significant deviation from random choice on the first day ($X^2 = 2.25$, d.f. = 1, P = 0.13) lizards had a significant preference for burrows without models by the second day ($X^2 = 6.25$, d.f. = 1, P= 0.012), and the third and fourth days ($X^2 = 9.0$, d.f. = 1, P= 0.002). In the cages with models, the mean number of lizards that stayed in burrows near models significantly reduced from the first to the fourth day of the trials ($F_{3,9} = 9.00$, P<0.005; Figure 7-2a).

On the first day of each trial, in cages with conspecific models, lizards that had chosen burrows with models had significantly shorter overall activity time and spent significantly less time basking than lizards that had chosen burrows without models (Table 7-1). Additionally those lizards that ended the day in burrows with models had changed burrows significantly more often on that day, than lizards that ended the day in burrows without models (Table 7-1). Two different lizards (two females) that occupied burrows with a conspecific model 5cm from the burrow entrance, attacked the models during days 1, 2 and 4 (a total of five times for both females and all days) and appeared to be trying to push the models further away. There was no significant effect of day on the mean number of attacks on models ($F_{3,9} = 0.60$, P = 0.63).

Repeated measure analyses showed significant effects of the response of lizards to models (whether they did or did not attack the models) on three behavioural parameters (Table 7-2). The lizards that attacked the models stayed active for longer (mean 3.71 ± SE 0.03 h d^{-1}) than those that did not (2.98 ± 0.11 h d^{-1}), and changed burrows more often (Figure 7-2b; Table 7-2). The lizards that attacked the models were also significantly more likely to stay in the central release area (0.0 movements to the perimeter area per day) than those that did not (0.56 ± 0.11 movements to the perimeter area $lizard^{-1}$ day^{-1}) (Table 7-2). There was also a significant effect of day on total activity time, and a significant interaction effect between day and response to models, for the number of burrow changes (Table 7-2).

Note that these results are derived from a small sample size (only two lizards attacked models), and an uneven distribution of lizards between the two categories attacked model (two lizards) or did not attack model (14 lizards).

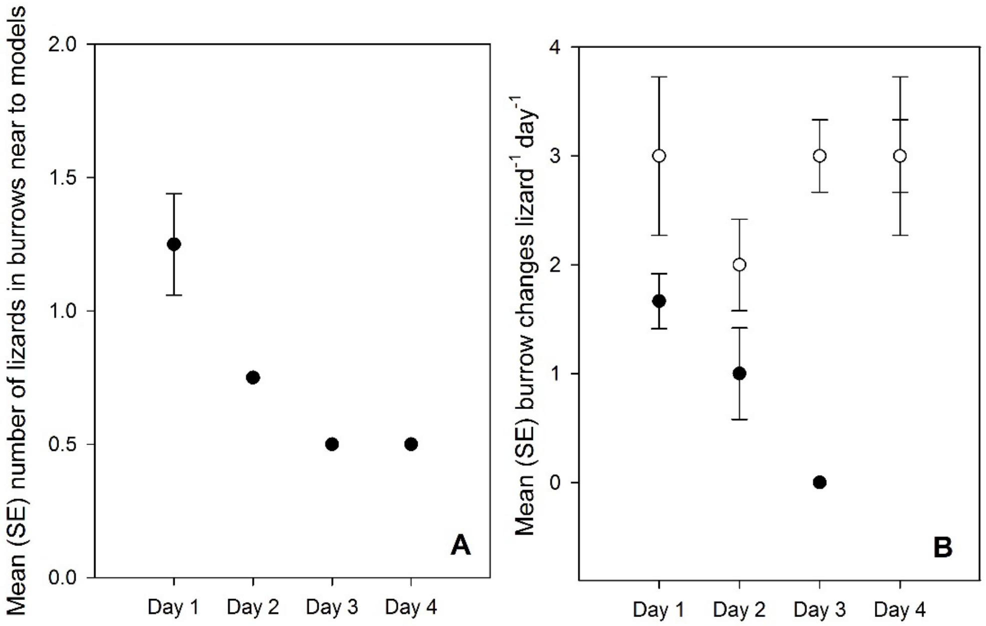

Figure 7-2. For cages with models: A) Mean number of lizards in burrows near to models on each day. B) Mean number of burrow changes of two lizards that attacked the model (open circles) and 14 lizards that did not attack the model (closed circles).

Table 7-1. Two sample t-tests, for lizards in cages with models, comparing, for each of six behavioural parameters, the five lizards, that , by the end of the first day of the trials, occupied burrows with models located 5 cm from the burrow entrance, with the 11 lizards that occupied burrows without adjacent models. Values in bold indicate significant effects ($P < 0.05$).

Behavioural parameter	**Burrow occupied**	**Mean**	**SE**	**df**	**t**	**p value**
Total activity time (h d^{-1})	No Model	4.19	0.16	14	2.402	0.031*
	Model	0.76	0.20			
Basking time (min h^{-1})	No Model	18.13	0.27	14	2.628	0.020*
	Model	7.63	0.18			
No. movements around burrow	No Model	1.82	0.15	14	0.745	0.469
	Model	0.88	0.19			
No. burrow changes	No Model	0.75	0.09	14	-2.650	0.019*
	Model	2.00	0.01			
Distance moved (cm)	No Model	12.46	0.47	14	0.173	0.865
	Model	10.00	0.94			
No. movements to perimeter area	No Model	0.28	0.05	14	-0.959	0.354
	Model	0.48	0.13			

Table 7-2. Repeated-measure analyses of variance for behavioural parameters comparing the two lizards that attacked models and the 14lizards that did not attack models, in the cages with model lizards. Day (1-4) was the repeated measure, and response to model (did or did not attack) was the between subjects factor. Values of P in bold indicate significant effects ($P < 0.05$).

		Total activity time (h d^{-1})		**Basking time (min h^{-1})**		**No. movements around burrow**		**No. burrow changes**		**Distance moved (cm)**		**No. movements to perimeter area**	
	df	*F*	p value	*F*	p value	*F*	p value	*F*	p value	*F*	p value	*F*	p value
Response to model	1, 14	6.57	0.022*	0.05	0.824	1.30	0.272	21.06	0.001*	0.06	0.809	6.95	0.020*
Day	3, 42	5.20	0.004*	1.19	0.324	1.23	0.311	3.66	0.020*	0.05	0.904	0.13	0.938
Response to model x Day	3, 42	1.85	0.153	1.34	0.272	0.29	0.827	4.17	0.006*	1.29	0.287	0.29	0.826

7.5.2. DO LIZARDS BEHAVE DIFFERENTLY WITH AND WITHOUT MODELS PRESENT?

We recorded 429 separate behavioural activities from 1280 hours of recordings. Emerging to bask was the most common lizard activity (234 basking events) followed by movement around the burrow (135 movement events). The experimental treatment (with or without models) did not affect total activity time, basking time, or distance moved when changing burrows (Table 7-3). There was a significant interaction of treatment x day for the number of lizard movements around the burrow (Table 7-3); lizards consistently made more movements around the burrow in the cages with models, although the difference between treatments was much smaller on the first day of the trials (Figure 7-3). Also lizards changed burrows significantly more (Figure 7-4a), and made significantly more movements to the perimeter area (Figure 7-4b) in cages with models than in cages with no models (Table 7-3).

Table 7-3. Repeated-measure analyses of variance for behavioural parameters comparing mean daily values for lizards in four cages with models and four cages without models. Day (1-4) was the repeated measure, and treatment (models present or models absent) was the between subjects factor. Values in bold indicate significant effects ($P < 0.05$).

		Total activity time (h d^{-1})		**Basking time (min h^{-1})**		**No. movements around burrow**		**No. burrow changes**		**Distance moved (cm)**		**No. movements to perimeter area**	
	df	F	p value	F	p value	F	p value	F	p value	F	p value	F	p value
Treatment	1, 6	2.103	0.197	0.001	0.988	7.199	0.036*	51.76	0.012*	0.150	0.710	22.04	0.003*
Day	3, 18	0.788	0.516	0.893	0.464	1.881	0.169	3.120	0.052	0.147	0.930	1.387	0.279
Treatment x Day	3, 18	1.126	0.365	0.269	0.847	27.18	0.005*	0.724	0.529	1.826	0.179	2.677	0.078

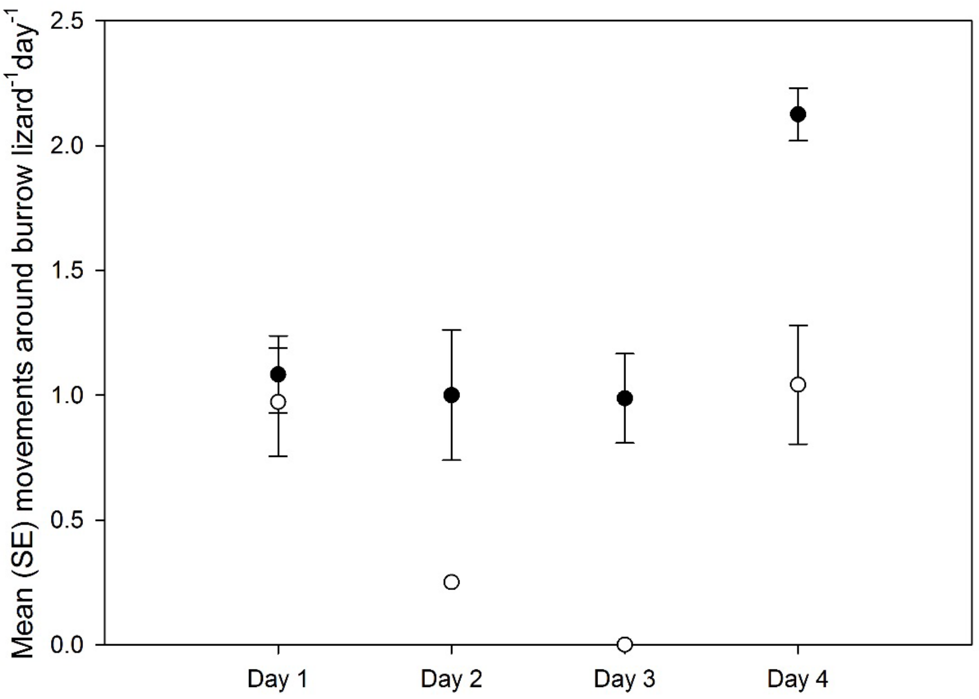

Figure 7-3. Mean number of movements around burrows per lizard on each trial day in cages with models (filled circles) and without models (open circles).

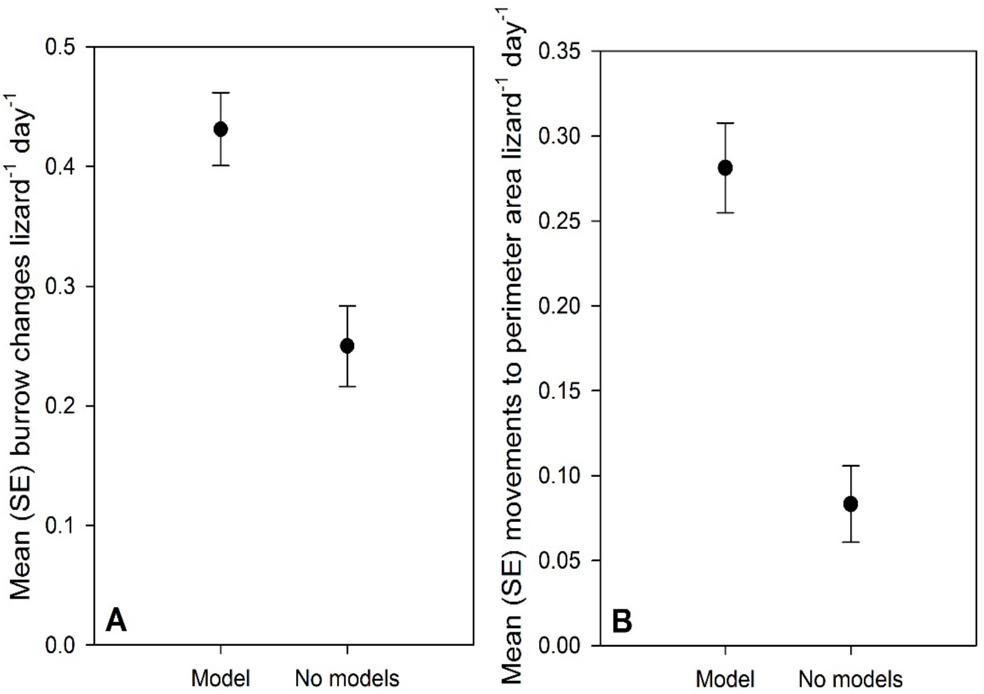

Figure 7-4. In cages with and without burrows: A) the mean number burrow changes per day; and B) the mean number of movements to the perimeter area per day.

7.6. DISCUSSIONS

First considering the lizards in the cages with models present, our results showed that lizards responded to the conspecific models. They tended to avoid burrows that were close to the models, and, rarely, they attacked the models. This is consistent with the report of Fenner and Bull (2011a) who suggested that pygmy bluetongue lizards defend a small area immediately around their burrow entrance (radius less than 15 cm) and that they aggressively attacked conspecific models placed 5 cm from their burrows. We deduced from the current study, that lizards in a new habitat will reduce agonistic interactions with conspecifics by avoiding burrows they perceive to be occupied. Those lizards that occupied burrows close to models at the end of the first day, showed behaviours consistent with being negatively affected by the presence of a conspecific; they were active over a shorter time, and basked for shorter periods than lizards in the same cages but in burrows without models close by.

Comparing lizards in cages with models and in cages without models, we found that the presence of models did not affect total time active or basking time. Perhaps this was because, after the first day, most of the lizards in cages with models had selected burrows that did not have a model 5 cm from the burrow entrance, and there was no immediate perceived threat from closely adjacent conspecifics. However the presence of models significantly altered movement behaviours. Lizards in cages with models moved around the burrow more, changed burrows more, and moved to the perimeter area more. Thus, even with apparently unoccupied burrows available, the presence of an apparent conspecific in another burrow within 65 – 75 cm induced this extra activity. We have previously shown a similar result (Ebrahimi and Bull, 2013c), that lizards changed burrows more, and made more moves to the perimeter area if they released into burrows that were close together than if they were released into burrows that were further apart.

We can draw three broad conclusions from our results about the use of conspecific models for the translocation of pygmy bluetongues, and the translocation of other species that have a similar solitary social organisation, and with defence of a central refuge. First, the lizards responded to visual cues from models as if they were real conspecifics. This confirms our earlier studies on this species (Fenner and Bull, 2011a), and the reports of many other behavioural ecologists since Tinbergen (1948). In any conservation program where increased conspecific presence is a management option to enhance retention of translocated individuals at the release site, models are adequate substitutes even if they contain less than the complete sensory signal range of real conspecifics.

Second, in the case of pygmy bluetongue lizards, models induced more movements among lizards that had been introduced to a release site. There were more movements away from the burrow entrances when models were present than when models were absent, both for lizards that returned to the same burrow (movements around the burrow) and for lizards that changed burrows. And there were more movements to the perimeter area when models were present than when models were absent, out of the central release area and across a burrow free area. In terms of translocations, these movements would increase the exposure of the lizards to visual predators such as birds (Fenner et al., 2008), and increase their probability of leaving the release site. Although our experiments were conducted over a relatively brief period, we suggest that behaviours immediately after release are likely to be most significant in determining the success of a translocation. The likely drivers of this behavioural change (increased movement by lizards when models are present than when models are absent) are either a perceived increase in the threat to burrow security, a perceived increase in the likelihood of aggressive encounters, or a perceived increase in competition for invertebrate prey. Whatever the mechanism, the message for conservation management is that, for this species, a high density, or a perceived high density at the release site is likely to lead to behaviours that will reduce the chance of a translocated individual staying and surviving. The broader implication for any translocation program is that it will be important to understand the local density that can be tolerated by a target species, and whether conspecific cues will negatively impact translocation success at those densities. This might be particularly important in reinforcement translocation programs designed to augment existing populations, where resident individuals may inhibit the settlement of introduced individuals. A novel, but as yet unexplored suggestion from the current study, is that the placement of conspecific models in locations surrounding a release site might inhibit dispersal away from that site if an initial aim is to retain released animals near to the release site.

A third conclusion is that the presence of conspecific cues at the release site would be detrimental to any translocation program for pygmy bluetongue lizards. This contrasts with studies of other species such as Caribbean rock iguanas (Alberts, 2007; Ahlering et al., 2010) where conspecific cues have reduced the stress levels and allowed translocated animals to adjust more rapidly to their new habitat at the translocation site. Our third conclusion emphasises the need for detailed understanding of the social structure of the species before embarking on any translocation attempt.

7.7. CONCLUSION

In cages with conspecific models we found lizards responded to the models by avoiding occupancy of burrows with a model near the entrance. The visual cue from the model appeared to induce behavioural avoidance. In the presence of conspecific models, compared to the absence of models, introduced lizards significantly increased the number of movements around burrows, the number of burrow changes and the number of movements to the perimeter area of the experimental cages. These behavioural changes could decrease the success of a translocation by increasing exposure to predation and dispersal from the release site. The results suggest that cues from conspecifics will not encourage translocated lizards to stay at a release site. This contrasts with other studies where conspecific cues have increased translocation success, and our results suggest that a careful assessment of reactions to conspecific cues will be required before they are considered in any translocation program.

7.8. ACKNOWLEDGEMENTS

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CHAPTER EIGHT

8. EFFECT OF SEASON OF RELEASE

8.1. TIME OF TRANSLOCATION

We have already shown a number of different sets of conditions at the release site that could help us to improve the result of actual translocation in lizards. These include availability of food and burrows, short term confinement at the site, conspecific cues, and so on. We have also shown how these factors can alter lizard behaviour, but one other important factor for animal translocation is the time of release. Bright and Morris (1994) suggested that animal release should not be in the season of food shortage which could reduce the chance of success, but is this the only effect or there are more seasonal impacts on the release time? It is necessary to find the best season for translocating animals, and the best way to find it is to monitor behaviour relevant to release success in different seasons in a new habitat. For instance animals may move about more, and thus be more likely to quickly disperse from a release site, if they are let go in the breeding season. In this chapter we will show how *Tiliqua adelaidensis* responds to being released in our experimental translocation arenas, in different seasons.

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Effect of time of release on behaviour and movement of translocated lizards

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8.2. ABSTRACT

Translocations are an increasingly used management option to conserve endangered animal species against a number of threatening processes. A problem is that some translocations are unsuccessful if released animals disperse away from the release site. Dispersal may be induced by stress, both from the process of handling and release, and from social interactions at the release site. A partial solution may be to modify the time when animals are released to coincide with a period of year when stress levels are low. For amphibians and reptiles that often have time in the year when they are largely inactive, release close to that time may reduce the chance that released animals will disperse. We test that hypothesis for an endangered Australian lizard, the pygmy bluetongue lizard, *Tiliqua adelaidensis.* We simulated translocation releases in a central area within large enclosures, and monitored behaviour and movements of the released lizards over a four day period. Releases were conducted in each month of the austral spring and summer, the period when this lizard is normally active, over two field seasons, and we tested our hypothesis by comparing behaviours among months. Movements, activity and rates of dispersal by lizards from the central release area all declined from early spring to late summer. Previous modeling has suggested that translocations will be an essential component in the conservation of this endangered species. The current results suggest that translocations of this species are more likely to be successful if they are carried out late in its activity season. We suggest that consideration should be given in other translocation programs involving reptiles and amphibians, to similarly releasing animals at their new sites late in their activity season.

Keywords: Behaviour, Dispersal, Translocation, Time of release.

8.3. INTRODUCTION

Conservation managers are increasingly considering translocation of endangered species to sites either within or outside of their know historic range (Seddon, 2010) to overcome problems from habitat loss or fragmentation, from climate change, or from other threats such as introduced predators (Collins and Storfer, 2003). However, translocation attempts are not always successful (Kleiman, 1989; Dodd and Seigel, 1991; Fischer and Lindenmayer, 2000; Germano and Bishop, 2009), with post-release dispersal often reducing establishment success (Rittenhouse et al., 2007; van Heezik et al., 2009; Whisson et al., 2012).

Several studies have suggested that the time of year when translocations occur can influence the subsequent success of establishment at the release site. Bright and Morris (1994) found doormice that were translocated and released in a season of relative food shortage achieved poorer body condition than those released at a later time when food was more abundant, and Coates and Delehanty (2006) found female prairie grouse translocated after their lekking period were more likely to nest and raise offspring at the release site than those translocated earlier in the season. Translocation release time might also influence the tendency for released individual to disperse. Movements are usually greater soon after release (Bodinof et al., 2012), partly as a result of the stress from the translocation process (Dickens et al., 2010). One solution may be to perform translocations at a time of reduced stress, for instance after mating activity has finished. Attum et al. (2011) recorded low dispersal of translocated tortoises that were released just before their period of inactive aestivation. For ectothermic reptiles and amphibians living in temperate habitats there is a potential conservation conflict resulting from the restricted season when individuals are normally active. On the one hand individuals in the source populations are more easily located and captured for translocation when they are most active, and released animals are more likely to be able to adjust to the novel release habitat, and to avoid predation, in that period. On the other hand, animals released while they are inactive, or approaching a period when they will become inactive in refuges, will be more likely to settle at a release site. They may subsequently emerge from their refuges less stressed and less likely to disperse. A hypothesis, derived from the above assumptions, is that translocations of reptiles and amphibians may be more likely to succeed if they are conducted towards the end of their activity period than if conducted earlier. Pragmatically, if translocations of reptiles and amphibians are to involve wild-caught animals, and if holding time is to be minimised, then they must take place within the normal activity period when individuals can be located and captured. The hypothesis is that later in the activity period will be better. In this paper we use experimentally simulated translocation releases across the activity season of an endangered Australian scincid lizard to examine the impact of the time of release. We test the hypothesis that lizards released in translocations later in the activity season are more likley to remain at the release site.

The endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*, is now restricted to a few isolated fragments of native grassland in a small geographic region in the mid-north of South Australia. This region has a mediterranean climate, with cool wet winters and hot dry summers, and pygmy bluetongue lizard activity is restricted to the austral spring and summer (September – March) (Milne et al., 2003b). The species is normally solitary and individual lizards spend most of their time associated with their single entrance, vertical burrows, which have been made by mygalomorph and lycosid spiders (Hutchinson et al., 1994; Milne et al., 2003b). They use the burrows as refuges from predators and from climatic extremes, and they partially emerge to sit at the burrow entrance to bask and to ambush passing invertebrate prey (Hutchinson et al., 1994; Milne et al., 2003b). Although they aggressively defend their burrows from conspecifics, that defence is restricted to a region of less than 15 cm from the burrow entrance (Fenner and Bull, 2011a). In natural populations they rarely emerge fully from their burrows, and their movements away from the burrow entrance are normally limited to defecation and catching prey, although there are occasional moves of adults seeking new burrows, of males seeking for female partners in spring, and of neonates and juveniles dispersing to establish their own burrows in late summer (Milne et al., 2002b; Schofield et al., 2012). When lizards move from their burrows they become vulnerable to predation from birds and snakes (Fenner et al., 2008a). Fordham et al. (2012) modeled climate induced changes in future habitat suitability for this species and suggested that translocation will be an important management option to maintain the viability of pygmy bluetongue lizard populations.

In this study our broad aim was to derive the set of conditions that would maximize the chance of successful translocations for this species. Specifically, we tested the hypothesis that translocated pygmy bluetongue lizards would be more likely to remain at the release site if the release happened later in their activity season. More generally we hoped to provide insights into appropriate times for management interventions for a range of other reptiles and amphibians where translocations are considered as an option.

8.4. METHODS

The data that we use in this paper have already been reported as the control treatments of a series of experimental studies over the austral spring and summer of 2009 – 2010 and 2010 -2011. Those studies investigated how variable conditions influence the tendency of lizards to disperse in a simulated translocation release. The methods have been previously reported in papers describing this series of studies (Ebrahimi and Bull, 2012a; Ebrahimi and Bull, 2013a; Ebrahimi and Bull, 2013b; Ebrahimi and Bull, 2013c). Briefly, we captured eight male and eight female pygmy bluetongue lizards from two populations near Burra, South Australia (33° 42´S, 138° 56´E) in September 2009. Lizards were held in individual plastic boxes (52.5 × 38 × 31) before and between trials in a room with ambient conditions, and they were fed crickets and meal worms every third day. After the last trials in the 2009-2010 spring and summer field season, lizards were kept in the Animal Care Unit of Flinders University, Adelaide in a 25°C room with a 12: 12 LD light regime. We reduced temperature and light gradually to 15°C and 10:14 LD over the austral winter, and then brought light and temperature back to 25°C and 12:12 LD by the following spring for the second field season of our trials.

For the trials, we established four circular cages (15 m diameter) in a line, about 5 m apart in the grounds of Monarto Zoo, South Australia (35° 06´S, 139° 09´E). Cages had 1 m high-galvanised iron walls and bird-proof wire roofs. We divided each cage into three areas; a central 4 m diameter circular area where lizards were released, a 5 m wide matrix, considered to be unsuitable habitat around the central area, and a 0.5 m ring around the inside cage perimeter that trapped any lizards that dispersed from the central area. 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. Artificial burrows were hammered into the ground until they were flush with the ground surface. Pygmy bluetongue lizards readily accepted these artificial burrows both in field populations (Milne et al., 2003a) and in our cages (Ebrahimi et al., 2012a). We distributed 41 artificial burrows in the central area of each cage as previously described (Ebrahimi and Bull, 2012a), one in the middle, and then 8, 16 and 16 burrows in three concentric rings. In this arrangement, each burrow was spaced an average 63 cm (SE= 0.01) from the next nearest burrow. We also distributed 30 artificial burrows evenly around the perimeter ring of each cage as refuges for lizards that dispersed from the central area. We ran a series of experimental trials during two spring and summer periods, from October 2009 to March 2010 and from October 2010 to January 2011. Each trial lasted four days, and each tested the impact of some experimental manipulation within the central cage area, for instance of food level (Ebrahimi and Bull, 2012a), of vegetation density (Ebrahimi and Bull, 2013a), or of the arrangement of the burrows (Ebrahimi and Bull, 2013c). In most trials two cages were used to apply the experimental treatment, and two were used as controls. The comparisons between experimental and control treatments have been documented in previous publications. In this paper we consider only the control treatment cages which were identical in all trials, with burrow conformation as described above, with no additional food supplementation, and with all vegetation in the central area cut to ground level. Normally there were two of these control treatments in each trial but sometimes only one. Thus the only differences among the different trials were the month and field season when the trials were run, and the increasing experience with the trial conditions over successive trials using the same group of lizards. In total we ran 17 trials across the two field seasons, with 28 control treatment cages considered in this analysis (Table 8-1).

In each trial we released four lizards in each cage (two male and two female) and confined them to the central area for one day using a 20 cm high black plastic wall (Ebrahimi and Bull, 2013b). We then removed the wall and observed lizard behaviour and movements for four consecutive days.

To observe behaviour, we suspended four surveillance cameras (Longse: LICS23Hf, 3.5 mm lens) above each cage with a field of view that covered the central 4 m diameter area. Cameras recorded lizard behaviour during each day of trials from 0700 to 1800 h onto a 16 channel h.264 DVR (ESW26), powered by four 12 V batteries.

Table 8-1. The number of trials and the number of cages with the control treatment in each trial in each month of each field season.

	Field seasons			
	2009/2010		2010/2011	
	No. trials	Cages/trial	No. trials	Cages/trial
October	1	2	1	1
November	2	2	1	2
December	1	2	1	2
January	2	1	2	2
February	3	1	0	0
March	3	2	0	0

From our filmed records we derived seven behavioural parameters in each trial that allowed us to compare lizard behaviour among different months. In natural populations, pygmy bluetongue lizards spend most of their time associated with a single refuge burrow, retreating down the burrow to escape predators and climatic extremes, or sitting at the burrow entrance to bask and to ambush passing invertebrate prey (Hutchinson et al., 1994). These lizards rarely move further than a few centimetres from the burrow entrance, although they occasionally emerge fully to bask, to capture prey, or to defecate. The behaviours described below relate to this burrow centred focus of activity.

1) Total activity time (h d^{-1}) was defined as the period from the first time the lizard head emerged from its burrow to the last time that lizard retreated completely into its burrow on that day. 2) Basking time (min h^{-1}) was defined

as the period of time when the lizard had partially emerged and remained at the entrance of its burrow. We called this basking because the lizard was exposed to solar radiation, but an additional function of this behaviour may have been to ambush passing invertebrate prey. We divided the total minutes spent basking each day by 11 (total hours of filming) to calculate the basking time as minutes per hour. 3) Number of movements around burrow. In some cases lizards fully emerged from their burrow, moved about, usually for a very short distance, and then retreated to the same burrow. These movements included lizards that just walked around the burrow entrance, lizards that basked while fully emerged, and lizards that moved away from the burrow entrance for defecation or foraging for prey. We recorded the number of these movements by each lizard on each day. 4) Number of burrow changes. In some other cases lizards fully emerged from their burrow and moved around to choose another burrow in the central area. We recorded the number of burrow changes for each lizard on each day. 5) Distance moved. If a lizard had moved to a different burrow within the central area during a day we measured the distance moved as the direct line distance between the burrow the lizard was in at the start of the day to the burrow it was in at the end of the day. 6) Number of dispersals. This was defined by the number of times a lizard left the central area, moved across the habitat matrix, and was subsequently discovered occupying a burrow in the perimeter region. In terms of the translocation simulation, these represented dispersal events away from the release site. This behavioural parameter was not recorded in trials in February and March of the first season. 7) Number of fights. When two lizards approached each other on the ground surface, they always showed some agonistic interaction, either with the lizards scuffling together, or with one running away from the other. We defined all of these interactions as fights, and recorded the number of fights per lizard on each day.

In the trials, when a lizard moved into the perimeter area, it left the field of view of the cameras, and we had incomplete information about its behaviour on that day. Therefore, for analyses, we derived one value of each behavioural parameter from each cage in each trial, using the average over all four days, from all lizards with complete data on each day.

Although the same 16 lizards were used in all 17 sets of experimental trials, in each trial we selected different combinations of four lizards for the control cages, and we treated each of the 28 sets of control cage results as independent replicates. We analysed the data in two ways. First we took the results from October to January in each season, and conducted two-way analysis of variance (ANOVA) for each behavioural parameter, with factors month (Oct-Jan) and field season (2009-2010 and 2010 -2011). A significant month x season interaction would indicate that any behavioural change across months differed between the two seasons. One interpretation of that might be that they were becoming familiar with the trial arenas and adjusting behaviours with experience. Our results (see below) did not show any significant interaction effects, so for the second analysis we pooled all trials across the two field seasons to derive mean values per month and used nested one-way ANOVAs to investigate the effect of month of release for each behavioural parameter. We used the month of the trial as a fixed factor with replicate control cages nested within months. We used the Bonferroni test for post hoc comparisons between pairs of months.

We also explored whether thermal conditions during the trials influenced any of the behavioural parameters. We derived average, minimum and maximum temperatures over each four day trial from temperature records at Pallamana Aerodrome weather station (35° 04' S, 139° 13' E) 10 km from Monarto Zoo. We then used one way ANOVAs to determine if the mean temperatures experienced differed among months, and Pearson correlations to examine the relationships between those temperatures and the average parameter values for each lizard behaviour during each trial.

The study was conducted according to the guidelines of the Flinders University Animal Welfare Committee (approval no.E206) and was conducted under DENR Permit (G25011).

8.5. RESULTS

Although there was a wide range of ambient temperatures experienced over the 17 trials (e.g. Figure 8-1), the one way ANOVA analyses did not show any significant differences among months for the mean average (F_5= 0.32, p =

0.87), minimum (F_5= 3.39, p = 0.13), or maximum temperatures (F_5= 0.44, p = 0.80). The only behavioural parameter that was significantly correlated with temperature was basking time (average temperature: r = -0.434, p = 0.021; minimum temperature: r = -0.710, p = 0.001; maximum temperature: r = -0.518, p = 0.005). When the temperature increased lizard basking time decreased (Figure 8-1).

There were no significant interactions between month and field season for any of the behavioural parameters (Table 8-2). The significant effect of month for six of those behaviours over the period October to January remained consistent between the two field seasons.

When the data were pooled across field seasons, and data from February and March 2010 were included in the analyses, five behavioural parameters retained significant differences among months (Table 8-3). For those five parameters there was a consistent trend for decreasing values as the field season progressed (Figure 8-2). Lizards spent a shorter period of the day active (Figure 8-2a), they basked for less time (Figure 8-2b), moved out of their burrows less often (Figure 8-2c), dispersed from the central area less often (Figure 8-2d), and were involved in fewer fights (Figure 8-2e) as the season progressed from spring (October) to late summer (March).

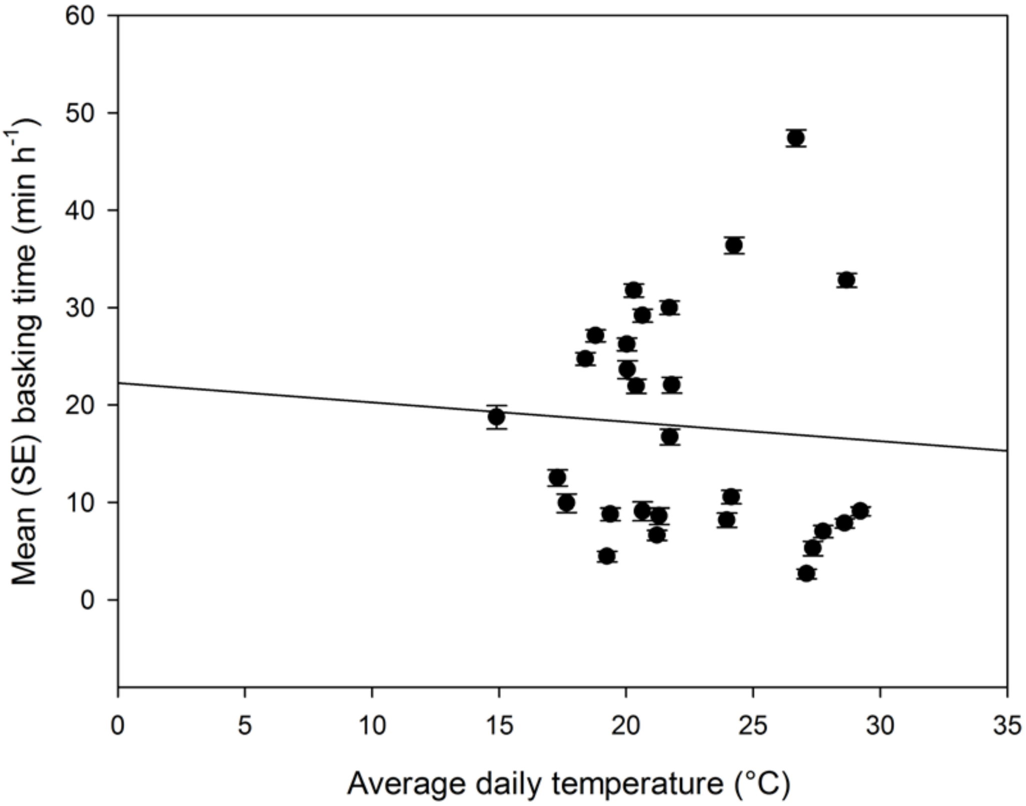

Figure 8-1.Relationship between the mean basking time of all lizards in a cage over a four day trial and the mean of the average daily temperature over the four day trial. The solid line indicates the significant negative correlation.

Table 8-2.Analyses of variance (ANOVA) considering the effect of month (Oct – Jan) and field season (2009/2010 and 2010/2011) on each of seven behavioural parameters, using months when trials were run in both field seasons. Values with star indicate significant effects (p < 0.05).

	Field season			**Month**			**Field season x month**		
	df	*F*	p value	df	*F*	p value	df	*F*	p value
Total activity time	1, 12	1.68	0.210	3, 12	4.73	0.021*	2, 12	0.70	0.510
Basking time	1, 12	0.23	0.640	3, 12	4.33	0.027*	2, 12	3.16	0.080
No. Movements around burrows	1, 12	1.38	0.260	3, 12	16.99	0.001*	2, 12	0.36	0.700
No. burrow changes	1, 12	1.02	0.330	3, 12	8.09	0.003*	2, 12	0.77	0.480
Distance moved	1, 12	0.03	0.850	3, 12	2.03	0.163	2, 12	0.91	0.420
No. dispersal	1, 12	0.01	0.910	3, 12	7.49	0.005*	2, 12	0.44	0.520
No. fights	1, 12	0.63	0.440	3, 12	4.28	0.028*	2, 12	0.45	0.640

Table 8-3. Analyses of variance (ANOVA) considering the effect of month on each behavioural parameter over all trials. Values with star indicate significant effects (p < 0.05).

Behavioural parameter	**Month**			**Nested factor (Cage(Month))**		
	df	*F*	p value	df	*F*	p value
Total activity time	5, 16	47.23	0.001*	6, 16	0.43	0.673
Basking time	5, 16	33.02	0.001*	6, 16	1.26	0.333
No. Movements around burrows	5, 16	5.03	0.036*	6, 16	2.55	0.062
No. burrow changes	5, 16	1.42	0.336	6, 16	5.51	0.003*
Distance moved	5, 16	0.63	0.682	6, 16	2.67	0.054
No. dispersal	3, 9	17.81	0.007*	6, 16	0.79	0.561
No. fights	5, 16	591.3	0.001*	6, 16	0.08	0.966

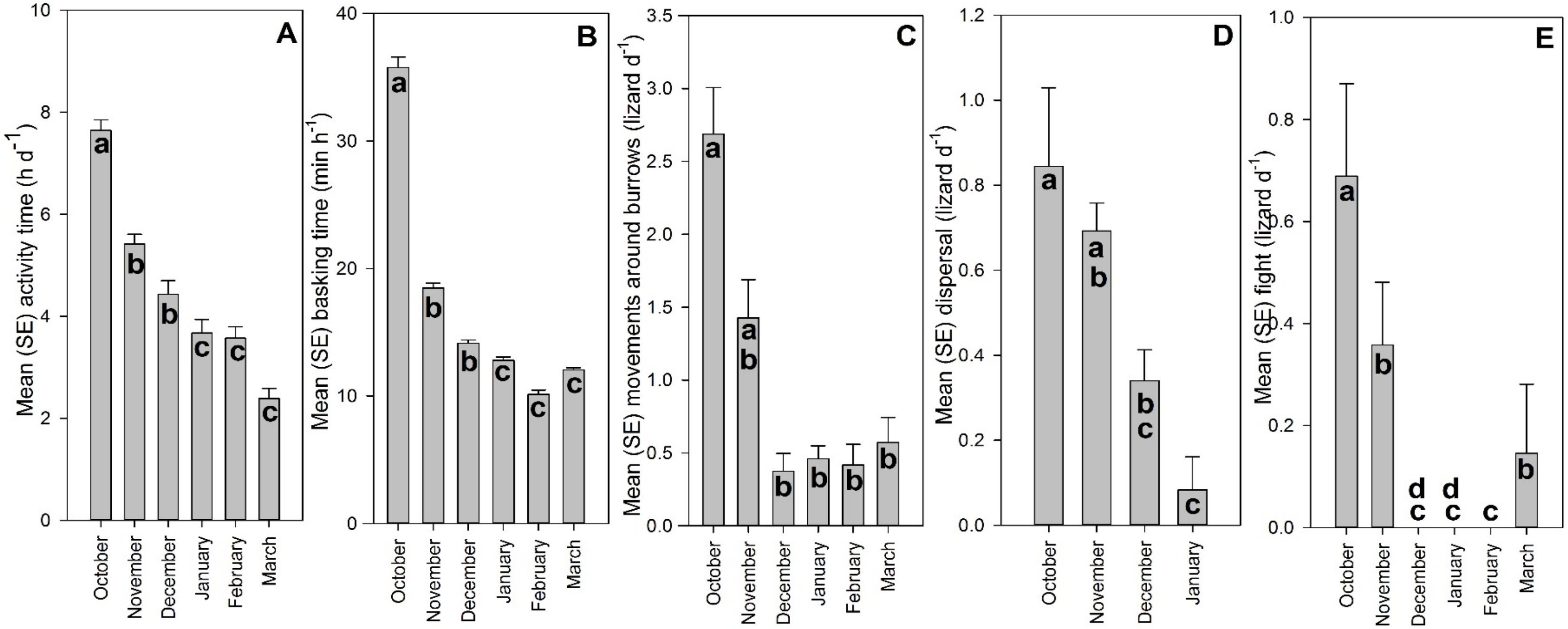

Figure 8-2.Mean and one standard error for five behavioural variables from trials in each month. Bars with different lower case letters were found to be significantly different in posthoc bonferroni pairwise comparisons. A) Mean total activity time , B) Mean basking time , C) Mean number of movements around the burrow, D) Mean number of dispersals (no data available for February and March), E) Mean number of fights.

8.6. DISCUSSIONS

The data from our series of trials showed a consistent pattern across the two field seasons of monthly differences in the behaviours of pygmy bluetongue lizards. The lack of any significant month x season interaction effects suggests that those differences represent a real monthly change in behaviour, rather than an accumulating familiarity by our captive lizards for the experimental conditions. In that case we would have expected different levels of activity or behaviour in the spring of the second field season than in the first season.

We considered whether the behavioural changes may have been temperature related. Temperatures in southern Australia generally increase from spring to summer, and one behavioural parameter, mean basking time, showed a significant negative correlation with the mean ambient temperature measured over the four day trials. This was consistent with the reduction in basking time with month within each field season. Perhaps lizards spent shorter periods emerged when it was warmer, to avoid overheating. However, other behaviours that also significantly declined over successive months, were not significantly related to temperature during the trials. And, while the temperatures experienced over the different trial periods varied, analysis showed no significant difference in temperature among months in the periods when individual trials were run, for any of the three temperature parameters that we considered. We concluded that, while some behaviours may be affected by the ambient temperature conditions, consistent behavioural changes in the lizards occurred from month to month independent of ambient temperatures.

The trends of generally reduced levels of activity and movement that we observed in the experimental arenas were consistent with reported observations from field populations. Schofield et al. (2012) found that the number of adult pygmy bluetongue lizards captured in pitfall traps was highest in the spring, coinciding with the time when mating behaviour had previously been observed (Fenner and Bull, 2009) They suggested that lizards are most likely to leave their burrows and move around (to be trapped by pitfalls) at the time when they are seeking mating partners (Schofield et al., 2012). This may be reflected by the higher rates of movements and burrow changes in spring in our trials. Changes in some of our other behavioural parameters may be correlated with these movements. For instance there may have been fewer fights in summer than spring because the lizards spent less time active, and moved less around their burrow entrances, so there were fewer opportunities for two individuals to encounter each other.

8.6.1. MANAGEMENT IMPLICATIONS

Whatever the causal explanation of the trends we observed, the results have significant implications for a conservation management program that involves translocations. Specifically, three aspects of the results suggest that lizards translocated later in the summer may have more chance of successfully establishing at the translocation site. First, lizards were less frequently active, moved about around their burrow entrance less, and spent less time basking at the burrow entrance later in the season. All of these behavioural changes would reduce the exposure of lizards to potential predators at the new site. Second, lizards interacted agonistically less often later in the season, reducing the levels of stress from intraspecific interactions. And third, the lizards dispersed away from the central release area less often later in the season.

The first period after release, while individuals become adjusted to and familiar with the novel release environment, is probably critical for the success of any translocation (Bodinof et al., 2012; Scillitani et al., 2013). Animals may be stressed from the handling, holding and transportation (Dickens et al., 2010), and unfamiliar with refuge and foraging resources (Bright and Morris, 1994; Rosatte et al., 2002; Teixeira et al., 2007), but if they can be persuaded to remain close to where they are released until they have settled into the new conditions, then there is a chance they will stay (Kleiman, 1989; Teixeira et al., 2007; Ebrahimi and Bull, 2013b). This study has suggested that, for pygmy bluetongue lizards, the time when they are released could play a significant role in determining whether or not individuals are likely to remain where they are released, and later in the activity season is a better time.

There are two broader conclusions from this study. One is that behavioural changes across different seasons might be critical for translocation success. We can speculate on how other species might be affected. For our study system the remarkable seasonal differences in adult activity behaviour that have already been reported (Schofield et al., 2012), suggested the time of release might be important. Other reptiles and amphibians with narrow mating seasons when they are more active and subject to more stress, might have similar windows of non-mating time for best translocation. Counter to this, the capture of animals from source populations for translocation, might be easiest at the times when they are most active. Among mammals and birds, that have more stable social structures across longer periods of time, and where dispersal is often among subadults and juveniles, an optimal translocation period may be less obvious.

The second conclusion is that understanding the basic behaviour of the target species is essential for optimizing translocation success (Wallace, 2000; Shier, 2006).

8.7. ACKNOWLEDGMENT

The Australian Research Council, Holsworth Wildlife Research Endowment, Sir Mark Mitchell Research Foundation, Zoos SA, the SA Department of the Environment and Natural Resources, the Field Naturalists Society of SA, the SA Museum, the Northern and Yorke NRM Board and the SA Murray-Darling NRM Board are founded this research. The Ministry of Sciences, Research and Technology of Iran sponsored the PhD studies of Mehregan Ebrahimi. Thanks go to the Mina Ansari for help during the field, and Monarto Zoo staff Jeff Lugg, Ruth Reuter, Ian Smith and David McLelland for logistic support.

CHAPTER NINE

9. NATURAL HISTORY

9.1. IMPORTANCE OF NATURAL HISTORY

Understanding the natural biology of a species, and the interactions with the habitat and resources, is vital for any conservation management program. Information about biology and ecology of target species can help us to find out the best way to conserve animals from possible extinction. For instance if we can find what types of shelters are acceptable we may able to find alternative natural refuges, or design artificial refuges for the target species. Although the research in this thesis focused on simulated translocations of pygmy bluetongue lizard, we also recorded some interesting information about the natural history and ecology of this endangered skink during the many hours of video footage. These data can increase our chance to find the best available options for conservation management of pygmy bluetongue lizard.

This chapter contains four short "natural history" papers that each describe a previously unknown aspect of the ecological needs and possible threats to the pygmy bluetongue lizard. The first paper developed from observations of how lizards entered and left the artificial burrows we provided. Those observations led to a suggestion of how to improve the design of artificial burrows. In a follow up Honours project, Staugas et al. (2013) used this design suggestion and showed that when offered a choice in the lab, lizards more often used artificial burrows with the new design. The second paper showed how pygmy bluetongue lizards respond to flooding of their burrows, and the third paper gave information about alternative natural burrows in a possible translocation site instead of artificial burrows. Finally, the last paper of this chapter showed a new insight into how pygmy bluetongue lizards interact with lycosid spiders, the ecological engineers that the lizards rely on to dig their burrows. All of these papers help us to increase our basic knowledge about pygmy bluetongue lizards. Although they were really incidental observations, they will contribute to improving the quality of any actual translocations of pygmy bluetongue lizards in the future.

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Lizard behaviour suggests a new design for artificial burrows

Mehregan Ebrahimi, Aaron L. Fenner and C. Michael Bull

9.2. NEW ARTIFICIAL BURROW DESIGN

9.2.1. *ABSTRACT*

Context. The use of artificial refuges is a common strategy for the conservation management of endangered species. However, artificial refuges may alter an animal's natural behaviour that in turn may be detrimental to the species. The endangered pygmy bluetongue lizard from Australia is one species that will accept artificial burrows.

Aims. The aim of the present research was to determine whether the normal behaviour of the pygmy bluetongue lizards differed between artificial and natural burrows, so as to determine whether the existing artificial burrow is an optimal design for this species.

Methods. In the present study we filmed the behaviour of lizards as they entered artificial and natural burrows. We compared the number of times a lizard entered a burrow, the time that lizards spent inspecting burrows, and the behaviours that lizards used when entering artificial and natural burrows.

Key result. We found that in natural burrows, lizards always entered head first, and then usually reversed direction inside, using an enlarged basal chamber, to sit with their head uppermost in the entrance. In artificial burrows, however, lizards had to enter head first, then reverse tail-first back out, and then reverse tail-first back into the burrow (so as to have their head facing upwards) We called this behaviour reversing from outside.

Key conclusion. The stereotyped reversing from outside behaviour when entering artificial burrows, and its occasional occurrence in natural burrows, suggest that it has evolved to allow lizards to use narrow burrows as well as those with a chamber, even though it can increase lizard's surface activity and exposure to predation.

Implication. The reversing behaviour from outside the artificial burrow increases exposure to potential predators, and our observations suggest that a re-design of artificial burrows to incorporate internal space for turning around may improve their effectiveness in conservation management interventions.

Keywords: Artificial burrow, Refuge behaviour, Pygmy bluetongue lizard, *Tiliqua*

9.2.2. INTRODUCTION

Many animal species require some form of refuge or shelter to protect them from predators and harsh climatic conditions (Schwarzkopf and Alford, 1996; Williams et al., 1999; Bulova, 2002; Millidine et al., 2006; Grillet et al., 2010).

Ectothermic lizards are particularly sensitive to habitat heterogeneity and benefit from the stable environment provided by shelters such as crevices or burrows. These shelters enable lizards to avoid excessive water loss and thermal extremes, and to reduce their energy expenditure (Pianka, 1966; Bennett and Nagy, 1977; Cooper et al., 2000; Blamires, 2001; Kretzer and Cully, 2001; Gálvez-Bravo et al., 2009). Among burrow dwelling lizards, some species can dig their own refuges, whereas others rely on burrows constructed by other animals (Hawkins and Nicoletto, 1992; Vitt and Caldwell, 1993; Williams et al., 1999; Cooper, 2000; Kerr et al., 2003a; Milne et al., 2003b; Goldsbrough et al., 2004; Kotler et al., 2004; Read et al., 2008; Grillet et al., 2010). In the latter case the distribution and abundance of these lizards will be influenced by the activity of the ecological engineer species that build the burrows. Factors of importance will include burrow availability, and potential conflicts between the burrow builders and the lizards (Armstrong and Griffiths, 2001; Beck and Jennings, 2003; Souter et al., 2004; Grillet et al., 2010).

For endangered species, one commonly adopted management strategy is to increase the availability of refuges or nesting sites with artificial structures (Harper et al., 2005; Beyer and Goldingay, 2006). For endangered reptiles, artificial burrows or refuges such as brick pavers have been used to supplement the supply of natural refuges (Webb and Shine, 2000; Souter et al., 2004), or to encourage occupancy to facilitate population surveys (Reading et al., 1997). In many reptile species, individuals have been shown to prefer one structure over alternatives (Goldsbrough et al., 2006), and experimental studies have been used to determine optimal refuge structures (Lettink, 2007; Arida and Bull, 2008; Mensforth and Bull, 2008). Lizard preference for a particular artificial refuge structure could be influenced by the microclimatic conditions, by the physical properties and dimensions of the refuge, and by the security it offers the occupant from predation. In conservation programs that use artificial refuges all of these aspects of artificial refuge structure need to be considered. A comparison of the ecology and behaviour of individuals using artificial and natural refuges should provide important clues about the suitability of the artificial refuge structure.

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is an endangered scincid found only in small fragments of native grassland in the mid-north of South Australia. It is 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). With no records since 1959, it was thought to be extinct (Ehmann, 1982; Cogger, 1997) until 1992, when it was rediscovered in stomach of an eastern brown snake (*Pseudonaja textilis*) that was killed on the road (Armstrong and Reid, 1992; Armstrong et al., 1993; Hutchinson et al., 1994). The lizards occupy narrow vertical burrows with a single entrance that are constructed by lycosid and mygalomorph spiders. They use these burrows as refuges, and bask at the burrow entrance to ambush passing invertebrate prey (Hutchinson et al., 1994; Milne et al., 2003b). Lizards prefer deeper burrows (>30cm in depth) with an entrance diameter slightly wider than their head width (average 15.1 mm) (Milne and Bull, 2000). Pygmy bluetongue lizards position themselves with their head uppermost inside the burrow, tilting the head slightly forward to completely fill and block the burrow space, reducing the chance of being gripped and extracted from the burrow by predators (e.g. snakes) or conspecific rivals (Hutchinson et al., 1994; Milne et al., 2003b). Pygmy bluetongue lizards have a cryptic lifestyle and spend most of their time associated with their burrow. Most juveniles have dispersed from their natal burrow after 5 weeks (Milne et al., 2002a), and occupy smaller burrows than do adults (Milne and Bull, 2000).

However, both adult and juvenile pygmy bluetongue lizards are vulnerable to predation from snakes and birds, particularly when exposed on the ground surface, for instance, while basking or searching for mates (Hutchinson et al., 1994; Fenner et al., 2008a; Fenner et al., 2008b). The longer they are exposed the higher their risk from predation (Fenner et al., 2008b).

Population surveys have shown that the deep burrows that the lizards prefer are in short supply, and that most available deep burrows at a site are occupied (Souter et al., 2004; Fellows et al., 2009). Ploughing and other agricultural activities have reduced the number of suitable burrows, and of the spiders that construct them, across much of the previous lizard distribution (Milne, 1999). One of the factors apparently preventing local population expansion appears to be a lack of suitably deep burrows beyond the local distribution of a population (Souter et al., 2007). These observations have encouraged the development of a variety of ways of constructing artificial burrows for pygmy bluetongue lizards, including hammering a steel peg into the soil to create a hole, drilling a hole with a hand auger, and using a hollowed out length of wooden dowling inserted into the ground (Milne and Bull, 2000; Milne et al., 2003a; Souter et al., 2004). These methods enable artificial burrows to be constructed to the depths, and with the internal entrance diameters that correspond to the natural burrows preferred by lizards in the field (Milne and Bull, 2000), although they lack the enlarged basal chamber that is often found in natural burrows (Milne, 1999). Lizards readily use these artificial burrows both in the field (Souter et al., 2004; Pettigrew and Bull, 2011b), and in captivity (Milne and Bull, 2000; Fenner and Bull, 2010; Fenner and Bull, 2011a). Lizards occupying these burrows show no apparent decline in survival relative to those in natural burrows, and female lizards produce litters in artificial burrows (Milne et al., 2003a). Adding burrows to a natural population led to a significant local increase in lizard density because of a decrease in the dispersal of both adults and young (Souter et al., 2004). Thus the use of artificial burrows of uniform internal diameter seemed to be a useful for the management of this endangered species.

However, the artificial burrows lack one feature that is present in the natural burrows that are constructed by spiders, namely, an expanded chamber at the base of the burrow (Milne, 1999). Lizards can probably turn around, underground, inside this chamber, so that they can enter a natural burrow head first and then subsequently emerge head first. In the present paper we compare burrow entering behaviours of pygmy bluetongue lizards in natural and artificial burrows, and reflect on some previously unexpected hazards from the use of artificial burrows as they are currently designed.

For much of the time, pygmy bluetongue lizards either shelter in their burrow, or bask at the burrow entrance with at least some of the body or tail still inside the burrow (Milne et al., 2003b; Fenner and Bull, 2011a). In that case, if it is disturbed, the lizard rapidly retreats backwards into its burrow. Our observations in the present study concerned the less frequent behaviour when a lizard completely emerged from its burrow, and then either returned to the same burrow or moved to a different burrow. We compared video recorded behaviours of lizards entering artificial and natural burrows to ask whether there were any behavioural differences that might have an impact on lizard fitness. In particular we focussed on the time that lizards were exposed to above ground predation.

9.2.3. MATERIALS AND METHODS

9.2.3.1. ARTIFICIAL BURROWS

We tested artificial burrows in four circular cages, each 15 m in diameter and covered with a bird-wire roof. The four cages were located, at Monarto Zoo, approximately 70 km SE of Adelaide (35°06´S; 139°09´E). Each cage contained 71 artificial burrows that were made from 30 cm lengths of wooden dowling with a drilled out centre of 20 mm internal diameter. The burrows were hammered vertically into the ground, so that their entrances were flush with the ground surface. We captured eight adult males (average snout to vent length (SVL) 85.1 ± 0.2 mm) and eight adult females (average SVL 89.2 ± 0.2 mm) of *T. adelaidensis* from two populations near Burra, South Australia (33°42´S; 138°56´E), and placed two males and two females in each cage on 25 October 2009. Four cameras (CCD video camera, LICS23HF and lens 3.6 mm, Longse, China) were suspended from the roof of each cage, so that their combined field of view covered a central 2 m diameter circle, which contained 41 of the artificial burrows. The other 30 burrows in each cage were spaced around the inside cage perimeter. The cameras recorded all lizard activity in the central cage area for 11 daylight hours from 0700 hours to 1800 hours for 10 days from when the lizards were first introduced to the cages. We used a 16-channel, 12 volt digital video recorder (16 channels. H264 DVR, Economical DVR, Taiwan) to record all data from cameras. The location of each lizard was confirmed each morning before filming and each evening after filming, by inspecting each burrow with a small 16 LED torch.

We viewed the video recordings to determine any movements of lizards among different burrows, to document the time that lizards spent inspecting burrow entrances, and to observe the behaviours that lizards used when entering their burrows. Because we saw every time a lizard entered a burrow in the central cage area over the 10 days of filming, we could differentiate between two cases of burrow use: 1) entry into a completely new burrow that had never been sampled before; and 2) entry into the same burrow that the lizard had used at least once before in the 10-day filming period. In the latter case, we were also able to number the consecutive entrances a lizard made to a familiar burrow.

9.2.3.2. NATURAL BURROWS

Observations were conducted over the period 2006-09, during separate studies of lizard behaviour in a field population near Burra (Fenner and Bull, 2007; Fenner et al., 2007; Fenner et al., 2008a; Fenner and Bull, 2009). A 1-ha plot in the study area contained ~500 burrows that were 10 cm or deeper, and ~50 pygmy bluetongue lizards (Fellows et al., 2009). We located 47 adult lizards by looking into lycosid and mygalomorph spider burrows using an Olympus IF8D4X2-10L optic fiber scope with an Olympus KLS-131 portable light source (Olympus, Japan). We positioned camera stands over occupied burrows 1 day before filming and then recorded lizard behaviour on two 12 volt, 4 channel, Digital Video Recorders (DVR) (Triplex DVR046, Triplex, China) each taking signals from four Sharp ¼ inch colour CCTV cameras (EY-3102) placed each day on the camera stands above the burrows. Each of the 47 burrows was filmed for 3 h in the morning of each of three consecutive sunny days. At the end of the filming we confirmed that lizards were still present in the burrows. Lizards were individually marked by toe clip to ensure that 47 different lizards were filmed.

From the video playback we selected episodes where a completely emerged lizard entered a filmed burrow, and recorded the same behavioural parameters associated with burrow entering as for the artificial burrows.

We defined inspection time as the number of seconds from when the tip of the lizard snout first came to within 5 cm of the entrance, to when it first started the final reverse into the burrow.

We calculated mean and standard error for representing the parameters in the present paper.

9.2.4. RESULTS

Recordings of both artificial and natural burrows confirmed that pygmy bluetongue lizards spent most of their activity time basking during the filming sessions. In the cages, lizards were active on average for 9.0 ± 0.04 min each hour of filming, and an average of 7.8 ± 0.03 min per hour was spent basking. In the field, lizards were active for an average of 5.5 ± 0.014 min per hour of filming, of which an average of 5.3 ± 0.013 min per hour was spent basking. In all recordings, the lizards basked at their burrow entrance and used that entrance as an ambush site to catch passing invertebrate prey in their activity times (25 attempts to catch passing prey). They were normally partially emerged, such that the amount of the body exposed above the surface varied from just the head, to all of the body except the last half of the tail. In all of those cases, whether in a natural or an artificial burrow, a lizard that was disturbed always reversed, tail first, back into its burrow. However, for lizards that had completely emerged (0.11 cases per lizard per hour in the natural habitat and 1.38 cases per lizard per hour in cages), either to attempt to catch a prey item (0.04 cases per lizard per hour in the natural habitat, and 0.14 cases per lizard per hour in cages) and return to the same burrow (0.075 cases per lizard per hour in the natural habitat, and 0.38 cases per lizard per hour in cages), or to move between burrows (0.035 cases per lizard per hour in the natural habitat and 0.43 cases per lizard per hour in cages), we observed two very different behavioural patterns in the video recordings, namely, reversing from outside the burrow and reversing inside the burrow.

In the cages, we recorded 397 cases emerged lizards entering artificial burrows. All 16 lizards exposed to artificial burrows were consistent in a behaviour that we described as reversing from outside. In each case, the lizard first inspected the entrance and the immediate surrounding surface with tongue flicks. It then inserted the front half of the head inside the burrow and stayed like that briefly, apparently inspecting inside the entrance. Next it inserted the whole of the head into the entrance and stopped again, perhaps for further inspection, before entering further into the burrow until the backlegs, and then the tail disappeared beneath the surface. Note that the 30 cm deep artificial burrows were longer than the total body length of the adult lizards (range 12.9 – 15.5 cm). After a brief time (average of 34 ± 0.04 s) the tip of the tail appeared at the entrance, vibrating against the ground surface outside, and then the lizard reversed quickly out.

In five cases the lizard appeared to detect some disturbance while reversing out, and rapidly retreated head first back into the burrow, before starting to back out again. When it was fully emerged, each lizard then curled its body into a U shape around the entrance, so that the head was adjacent to the tip of the tail, and the middle of the body (the bottom of U) was closest to the entrance. Next, the lizard slowly walked forwards away from the entrance, but uncoiling its body until the tail came to rest near the entrance. The lizard then vibrated the tip of its tail again, until it slotted into the entrance, before reversing into the burrow, tail first, until the entire body was again underground. Thus, the reversing from outside behaviour involved entering head first, re-emerging tail first and then re-entering tail-first. Following this reversal, the head of the lizard normally re-appeared at the entrance, after an average of 5.06 ± 0.02 min.

In the field population, during 423 burrow-hours of recording natural burrows of 47 lizards, we saw only 15 cases of emerged lizards entering burrows. All of them initially entered head first. In two (13.3%) cases the lizard (two different lizards) backed out and reversed direction from outside, as described above. In the other 13 cases (86.7%), the lizards remained in the burrow for an average of 6.21±1.38 min, and then emerged head first to bask at the entrance, indicating that they had reversed direction inside.

Because the video recordings were examined for burrow entrance behaviour retrospectively, we were not able either to relocate the filmed burrows, or to determine if burrows where the lizards reversed direction inside or outside were structurally different from each other.

A chi-squared contingency test, with Yate's correction for small cell size, showed a highly significant difference between lizards in artificial and natural burrows in the proportion of cases where reversing occurred from inside or outside ($X^2 = 93.5$; d.f. = 1; $P < 0.001$). More lizards reversed direction outside in the artificial burrows (100%) than in natural burrows (13.3%).

Lizards that entered artificial burrows multiple times over the 10 days of filming appeared to reduce the time that they spent in burrow-entering activities.

Among 397 cases where emerged lizards entered artificial burrows, 75 involved a completely new burrow for that lizard. In the other 322 cases, a lizard entered a burrow it had used previously, up to 10 times. Each lizard used a mean of 4.75 (SE 0.74; range 1-11) different burrows over the 10 days. For each lizard, we used the inspection time for the last time it entered a burrow in a Spearman rank correlation analysis that showed that lizards spent progressively shorter time inspecting entrances with the number of times the burrow was used increased ($r = -0.915$; $p < 0.001$) (Figure 9-1). We also calculated the mean reversal time outside of the burrow. This was the time that a lizard remained potentially exposed on the ground surface while reversing from outside. This time was not needed by lizards that reversed from inside and so represented the additional risk. We defined reversal time as the time from when the lizard emerged tail first from the burrow until when the last part of the head had gone below the surface. Lizards reversed significantly more quickly into burrows as they became more familiar with them ($r = -0.976$; $p < 0.001$) (Figure 9-2).

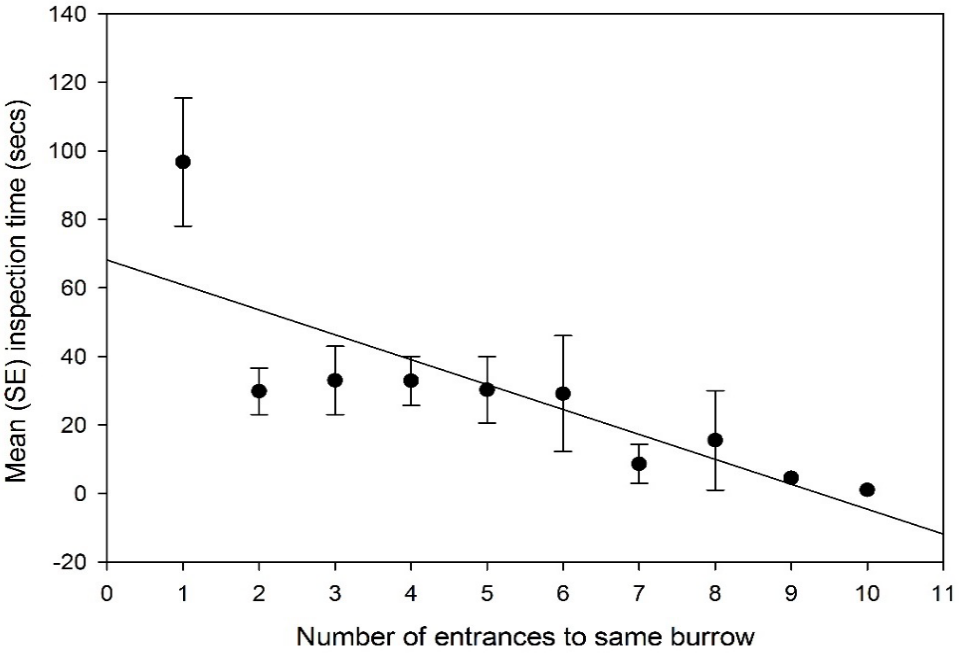

Figure 9-1. The mean inspection time (see text) for the 16 lizards during successive entries into the same artificial burrow.

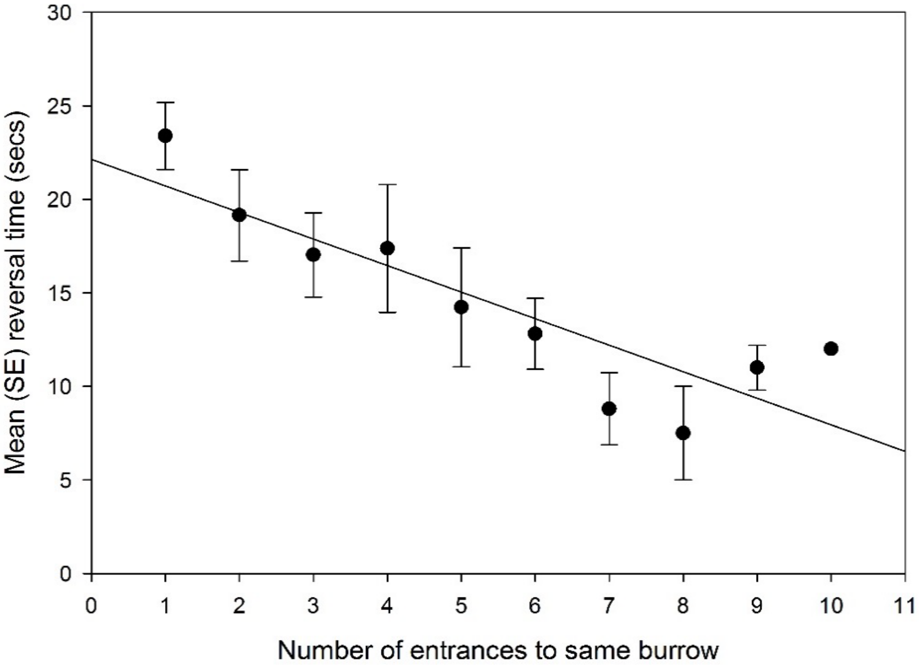

Figure 9-2. The mean reversal time (see text) for the 16 lizards during successive entries into the same artificial burrow.

9.2.5. DISCUSSION

There were two major differences between the lizards using artificial and natural burrows. One was that lizards completely emerged from burrows on many more occasions in the enclosure, so that even with fewer lizards, there were many more observations of lizards subsequently entering burrows. This may be because the lizards found the artificial burrows suboptimal, and were searching for a better quality burrow. This seems unlikely because previous studies have shown that lizards in field populations will occupy similarly designed artificial burrows, will remain in them for extended durations and will even produce litters of young within them (Milne et al., 2003a; Souter et al., 2004). A more likely explanation is that the translocated lizards were unfamiliar with the burrows and the new habitat in the cages, and were adjusting to the new conditions.

The second major difference was the incidence of reversing from outside. This behaviour was always used with the artificial burrows, and rarely used in natural burrows. One explanation for that difference is that natural spider burrows normally have an expanded chamber at their base (Milne, 1999), which would provide space for the lizard occupant to turn around without having to emerge. The complex, but stereotyped behaviour that we observed as lizards reversed from outside, coupled with the occasional observation of this behaviour in natural burrows, suggests that lizards in the field sometimes encounter natural burrows without internal turning space. An implication is that they have evolved to include reversing from outside in their normal behavioural repertoire.

The lizards probably need to reverse so that they are facing head up while refuging in their burrow. This allows them to use the large thickened scales on the dorsal surface of the head as a barrier to burrow invasion by conspecifics or predators (Hutchinson et al., 1994). It also allows them to emerge cautiously, head first, for subsequent basking episodes.

Lizards entering a burrow will have two potential threats. The first will come from resident occupants. Previous studies have shown that resident lizards will attack potential rival conspecifics that might threaten burrow ownership (Fenner and Bull, 2011a) and that lizards approach a new burrow more cautiously when there are conspecific cues, such as scats, close to the entrance (Fenner and Bull, 2011b). Alternatively, lizards may be threatened by predatory invertebrates. The spiders that build the burrows retain residency of some burrows in the field (Fellows et al., 2009) and can impose fatal bites on the lizards (Ebrahimi and Bull, 2012b). Predatory centipedes can also occupy burrows (Souter et al., 2004). Thus, lizards will need to show a level of caution, and spend time inspecting the entrance before entering.

The second threat comes from exposure to above-ground predation. Snakes and birds are likely predators that probably attack when the lizards are emerged from their burrows, and over 5% of individual lizards have tail damage, indicating near-miss predation events (Hutchinson et al., 1994; Fenner et al., 2008a; Fenner et al., 2008b). This suggests that the shorter the amount of time that lizards spend out of the burrow, the lower their predation risk. Lizards using reversing from outside the burrow have a double risk from the increased time out of the burrow, and from the time that they spend emerging tail first, and thus being unable to use visual detection of approaching danger. Anecdotal support for this risk is that, although these lizards are normally very difficult to catch by hand, in the 10 day cage trial, two lizards were hand captured while reversing out of their artificial burrows.

Logistically, reversing from outside is the only option available to lizards when there is inadequate space to reverse from inside. Our results showed that as lizards became familiar with an artificial burrow, they substantially reduced both the time taken to inspect the burrow, presumably with increasing confidence that there were no internal threats, and the time taken to reverse back, presumably with experience of the micro-topography around the burrow entrance. Thus, although a lack of internal turning space presents a potential increased predation risk for the lizards, they can quickly adapt to reduce that risk. This supports previous field results showing no obvious decrease in fitness of lizards using artificial burrows (Milne et al., 2003a). However, although they may reverse from outside on some occasion in natural burrows, lizards reduced the time of reversing after several attempts. This behaviour illustrates that pygmy bluetongue lizards need practice reversing from outside to reduce predation risk.

Nevertheless in terms of conservation, two priorities should be to design new artificial burrows that allow lizards to reverse directions underground, and to assess the relative effort required to insert these newly designed burrows into field locations. An artificial burrow that ends with an enlarged chamber will be harder to insert, without disturbing the surrounding soil, and it may be that the original design is a better option if fewer improved artificial burrows can be installed for an equivalent cost.

Whatever the outcome of those future investigations, the current study has shown that there is value in maintaining careful behavioural observations of the impact of interventions designed to help an endangered species.

9.2.6. ACKNOWLEDGEMENTS

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the field work. The study was conducted according to the guidelines of Flinders University Animal Welfare Committee (approval no.E206) and was conducted under DENR Permit (G25011).

9.3. FLOODED BURROWS

Burrows play a vital role in pygmy bluetongue lizards survival. The burrows are vertical holes in the ground, and during rain these can become filled with water, until they drain. We know little about how resident lizards respond to this. Heavy rain can also destroy burrows by washing clay and soil into the burrows. This paper report the effect of heavy rain on artificial burrows and show how lizards response to flooded artificial and natural burrows. The importance of this information for the conservation management of this species and probable future translocation is that we need to find translocation sites with an appropriate soil that can drain fast so burrows remain flooded for a relatively short time.

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Getting your feet wet. Responses of the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) to rain induced burrow flooding.

Mehregan Ebrahimi, Julie A. Schofield and C. Michael Bull

9.3.1. ABSTRACT

The pygmy bluetongue lizard, *Tiliqua adelaidensis*, is an endangered Australian skink. It refuges in narrow vertical burrows with single entrances, constructed by lycosid and mygalomorph spiders. Lizards spend most of their time associated with their burrows. Following heavy rainfall events we observed that some burrows failed to drain rapidly, but that lizards remained in those burrows immersed in water. The two impacts most likely to have negative effects on lizard populations were that at least one lizard was seen to become trapped in wet clay, and that some burrows, usually unoccupied ones, were degraded as debris and soil were washed into them. Burrow destruction was more prevalent in an area without grass cover, implying a detrimental impact of heavy grazing.

Keywords: Pygmy bluetongue lizard, Flooding, Burrow, Rain, Vegetation

9.3.2. INTRODUCTION

Most species live in environments that are subjected to unpredictable catastrophic events, such as fires and storms. The immediate consequences of those events are often local population decline, although species with distributions that extend beyond the affected area can rapidly recover (Driscoll and Henderson, 2008; Freeman et al., 2008; Lugo, 2008). In many cases species have developed specific adaptations to resist some of the detrimental impacts of extreme weather events (Kanowski et al., 2008) or fires (Williams et al., 2012). However, fragmentation of the ranges of many species has increased the risk of local catastrophe induced declines, because normal dispersal based recovery of those populations is now blocked (Root, 1998).

One such species is the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*, whose once widespread native grassland habitat in South Australia is now fragmented into a few isolated patches where small lizard populations persist. For this species, and others like it, a local catastrophic event may have more severe implications than for species with broader and more connected distributions. This lizard occupies narrow vertical burrows with a single entrance, and in this paper we report observations that some of those burrow fill with water after heavy rain events. We were interested in how catastrophic that may be for a local population.

The pygmy bluetongue lizard is the smallest member of genus *Tiliqua* with an average adult snout-to-vent length of 95 mm (Armstrong and Reid, 1992). All known populations are found on small fragments of native grassland in the mid north of South Australia (Hutchinson et al., 1994). The lizards select narrow vertical burrows with single entrances that have been constructed by lycosid and mygalomorph spiders. They use these burrows as refuges, basking at the entrance, ambushing passing prey from them, and producing litters in them (Hutchinson et al., 1994; Milne et al., 2003b). For *T. adelaidensis* the burrow is a central resource where they spend most of their time, and they even restrict their aggressive response to conspecific individuals to within a body length distance, so they do not need to be fully emerged (Fenner and Bull, 2011a). Adult lizards prefer burrows with an entrance slightly wider than their head width (average 15.1 mm) and deeper than 30 cm. Juveniles usually leave the natal burrows after five weeks and initially select smaller burrows (Milne and Bull, 2000).

Pygmy bluetongue lizards are vulnerable to predation from several bird species and from the brown snake, *Pseudonaja textilis*, and are more vulnerable to those predators when exposed on the surface (Hutchinson et al., 1994; Fenner et al., 2008a; Fenner et al., 2008b). Thus any event that reduces the suitability of a burrow, forcing a resident lizard to seek new burrow opportunities, will be potentially detrimental to the fitness of the lizard. Heavy rain might be one such event if burrows become flooded or destroyed by water flow. Here we report some observations of the behaviour of lizards after rain, and their responses to burrow flooding.

9.3.3. METHODS AND RESULTS

We made two sets of observations of the response of pygmy bluetongue lizards to significant rainfall events. One was from a captive population held at Monarto Zoological Park (33°36´S 138°59´E), approximately 70 km SE of Adelaide, the other was from a natural population near Burra (33°68′ S 138°94′ E), both in South Australia.

9.3.3.1. CAPTIVE POPULATION

Eight male and eight female *T. adelaidensis* from two populations near Burra, South Australia (site one: 33°36´S 138°59´E; site two: 33°37´S 138°59´E) were moved to four 15 m diameter circular cages at Monarto Zoo (35°06´S 139°09´E) in October 2009. Each cage had a galvanized iron wall, 1 m high and a bird wire roof. We released two male and two female lizards into each cage.

The cages had no natural spider burrows for the lizards to use, and 71 artificial burrows, 30 cm lengths of wooden dowling, drilled out with a central tube of 2 cm internal diameter, were hammered vertically into the ground in each cage. The burrows were open at each end, and the ground substrate was a sandy soil that we expected would allow water to drain rapidly. Pygmy bluetongue lizards readily accepted these artificial burrows (Ebrahimi et al., 2012a) (Figure 9-3A) and other artificial burrows of similar design (Milne et al., 2003b; Souter et al., 2004).

Lizards in these cages were used in a series of experiments and observations of lizard behaviour and responses to environmental variation (Ebrahimi and Bull, 2012b; Ebrahimi and Bull, 2012a; Ebrahimi et al., 2012b) during spring and summer (October-March) of two seasons 2009/2010 and 2010/2011. During these studies we recorded lizard behaviour in the daylight hours 0700 – 1800h on each day of the experiment, with four surveillance cameras (Longse: LICS23Hf, 3.5 mm lens) mounted above each cage, and a 16 channel h.264 Digital Video Recorder (ESW26), powered by four 12 V batteries. Lizard location and status were checked by inspecting each burrow with a 14 LED torch twice a day, once in the early morning and once late in the afternoon. We also checked the status of each lizard after extreme weather events such as heavy rain.

On 21-22 Nov 2009, a total of 21.7 mm of rain fell at Monarto with the rain stopping in the morning of 22 Nov. During inspection at 1000 h on that day, 10 mins after the rain had stopped, 19 of the 284 burrows were found to contain free standing water, with an average water depth of 124.5 ± 0.4 mm (range 80 – 190 mm). Three male lizards and one female were found occupying these water filled burrows, submerged in the water up to their necks during burrow inspection. In one additional burrow, although not waterlogged at the time of inspection, the rain had washed soil and debris into the burrow entrance, so that the entrance was blocked. The resident lizard had become trapped inside the burrow, as its feet were stuck in wet clay that had washed into the burrow.

This lizard was rescued and transferred to a dry burrow in the same cage. The 19 waterlogged burrows took 7-10 days to dry out.

At the time of the rainfall we were conducting an experiment on the impact of the density of grass tussocks on lizard behaviour. In two cages we had a density of 38 tussocks/ m^2 around the artificial burrows, in the other two cages there was no grass, just a bare soil substrate. A number of the unoccupied burrows in each cage were destroyed as the heavy rain washed surface clay and debris into them. Significantly more of the burrows in the bare soil cages were destroyed than in the cages with grass tussocks ($X^2 = 17.2$, d.f. = 1, $P < 0.001$) (Figure 9-4).

We left the four lizards in their water filled burrows and continued to film their behaviour over the next few days. The following day, Nov 23, was warm and sunny with a maximum temperature at the site of 23^{O}C. One lizard moved from its water filled burrow to another dry burrow on that day. The other three lizards continued to act normally in their water filled burrows, and did not vacate those burrows even though there were many dry burrows available close by. They partially emerged to bask at the burrow entrance, but when disturbed retreated to the bottom of the burrow so that they were fully immersed, and then rested in the burrow with only their nose out of the water.

9.3.3.2. NATURAL POPULATION

Further observations were derived from regular monitoring, over the same period of three 1 ha square quadrats in a natural population of *T. adelaidensis* located 9 kms from Burra (Schofield et al., 2012). We used an Olympus IF8D4X2-10L optic fiberscope to inspect for resident lizards inside over 80 natural burrows that were located within each quadrat, on monthly surveys between September and March 2009/2010 and between January and March 2011 (Figure 9-3B).

On two occasions thunderstorms resulted in heavy rain over the study site, with 24 mm of rainfall on13 Jan 2010, and 39.4 mm of rainfall on 5 Feb 2011. Inspection of all previously occupied burrows on the day after the rain found one water filled burrow on each occasion, with the resident lizard still present in each, up to its neck in water in the burrow. At the time of inspection, most of the water had drained from all of the other occupied burrows.

Figure 9-3.A. pygmy bluetongue lizard basking at entrance of artificial burrow; B. pygmy bluetongue lizard basking at entrance of natural burrow (photographer: Dr Aaron Fenner).

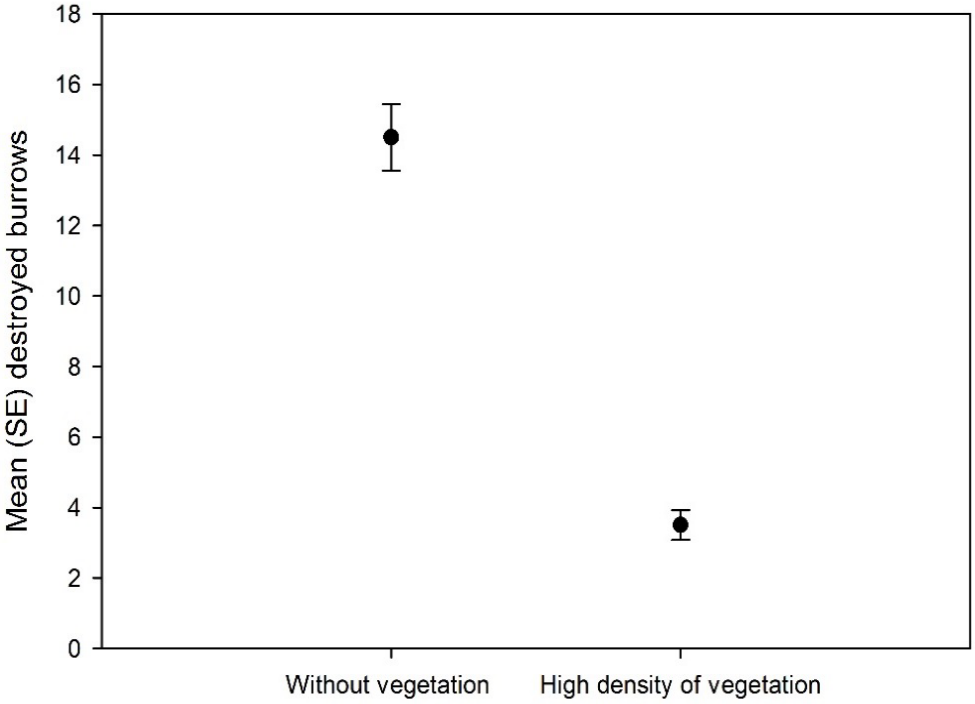

Figure 9-4. Mean number of burrows were destroyed by rain in cages with high density of vegetation and without vegetation.

9.3.4. DISCUSSION

These anecdotal observations show two impacts of heavy rain on lizards living in vertical burrows. One is that the burrows that have been selected by lizards can sometimes fill with water and take several days to drain. This included both natural burrows in field populations, and artificial burrows in our experimental enclosures. For artificial burrows the open ended tube inserted in sandy soil allowed water to drain rapidly from most burrows, but water persisted in a few. Perhaps some previously accumulated debris at the burrow base reduced drainage rates of the water in those cases. A similar explanation might account for the relatively rare cases of water filling burrows in the field population. In this study, lizards appeared to tolerate immersion in water within their burrows, at least for a few days while the burrows gradually drained, although one lizard became physically stuck in the wet clay. It is unlikely that a lizard would survive in similar circumstances in the natural population.

The study period was during the warmer spring and summer months when lizards had opportunities to emerge, bask, and dry out, but in the enclosures, only one of four lizards responded to these temporary aquatic conditions by changing to a drier burrow. Because of the increased exposure risks to potential predators from leaving an established burrow, lizards may prefer to tolerate wet conditions for short periods of time. All four lizards from the water filled burrows retained body condition and continued to participate in behavioural trials for the next 18 months without any apparent adverse impact from their brief aquatic experience.

Similarly, our less detailed observations of the field population indicated that lizards will remain immersed in water when their burrows become flooded after rain, although the duration of that tolerance was not determined. This conforms with previous observations of long term tenure of burrows by lizards. Suitable burrows are in relative short supply (Fellows et al., 2009) and resident lizards move infrequently from their burrows (Milne et al., 2003b).The area that holds natural pygmy bluetongue lizard populations has a Mediterranean climate with normally warm dry summers and cold wet winters. Our observations were during the summer, but the consequences of immersion in water may be more detrimental in winter, when colder temperatures might limit the ability of this ectothermic species to respond appropriately, and to extract themselves if they become stuck. Thus poor burrow drainage may be more of a disadvantage in the winter season when burrows are more likely to become water filled.

The second impact was on the burrows themselves. We gathered no specific information about the impact on burrows of the two rain events in the natural population, but in the Monarto enclosures burrow structures were compromised by flooding events that washed soils and debris into their entrances. This suggests that there will be a continual loss of burrows, perhaps more pronounced in winter when the rainfall is normally heavier. Souter (2003) showed that unattended burrows deteriorated and collapsed over time, and Fellows et al. (2009) reported changes in the numbers of burrows during monthly surveys within a 1 ha survey plot close to the study site of this paper". Fellows et al. (2009) also reported limited availability of suitable burrows during the activity seasons in a natural population. Presumably, rain damage to burrows as documented in the current study is one factor leading to burrow loss. There have been no observations of lizards digging new burrows, and it has been assumed that lycosid and mygalomorph spiders maintain the supply by digging new burrows over winter when the soils are softer (Milne, 1999). This study has demonstrated that rainfall events can destroy burrows, and indicates the vital role these hole digging spiders play in maintaining a supply of refuge sites for these endangered lizards.

The study also found, although without any replication, that rain damage was more severe when grass was absent, and this may be a previously unconsidered impact of grazing on pygmy bluetongue populations (Pettigrew and Bull, 2011b).

9.3.5. ACKNOWLEDGMENTS

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Tiliqua adelaidensis (Pygmy Bluetongue Lizard).
Alternative refuge

Mehregan Ebrahimi and C. Michael Bull

9.4. ALTERNATIVE REFUGE

Suitable refuges can be critical for most animal species because they protect them from predators and climate extremes (Grillet et al., 2010). Some species can construct their own shelters while others rely on burrows or structures built by other animals (Kotler et al., 2004). The distribution of an animal is thus highly dependent on the availability of refugia and distribution of the refuge building species (Souter et al., 2004). One example is an endangered scincid lizard from South Australia, the Pygmy Bluetongue Lizard (*Tiliqua adelaidensis*). This lizard normally uses burrows built by lycosid and mygalomorph spiders. The lizard uses these burrows as refuges, and basks at the burrow entrances, ambushing passing invertebrate prey (Figure 9-5) (Milne et al., 2003b). Lizards of this species prefer deeper burrows (>30cm in depth) (Figure 9-6) with entrance diameters slightly wider than their head width (average 15.1 mm) (Milne and Bull, 2000). In this note we describe observations of an alternative natural burrow system that can be used by *T. adelaidensis* and that may increase the range of options for the optimal management of existing populations, and for the possible selection of translocation sites.

Figure 9-5.Pygmy bluetongue lizard basking at the entrance of a natural burrow

Figure 9-6.A natural burrow built by a lycosid spider

On 25 October 2009, we moved eight male and eight female *T. adelaidensis* into four 15 m diameter circular cages in Monarto Zoo (35.10 S 139.15 E) approximately 70 km SE of Adelaide, South Australia. The cages had galvanized iron walls, 1 m high, that were buried 15 cm under the ground surface. Two male and two female lizards were released into each cage. There were no natural burrows in the cages initially. Instead we constructed artificial burrows from 30 cm lengths of wooden dowling with a drilled out center of 2 cm internal diameter (Figure 9-7 A and B).

Figure 9-7.A) pygmy bluetongue lizard feeding at the entrance of an artificial burrow; B) an artificial burrow

Previous studies have shown *T. adelaidensis* accept and use these artificial burrows (Milne et al., 2003a). Seventy-one artificial burrows were hammered vertically into the ground in each cage. Experiments, set up to measure the responses of the lizards to various environmental conditions in the cages, are reported elsewhere (Ebrahimi and Bull, 2012b; Ebrahimi et al., 2012a). We inspected each cage daily and removed any mice (*Mus musculus*) that had burrowed under the cage wall, and filled in any burrows they had made (Figure 9-8).

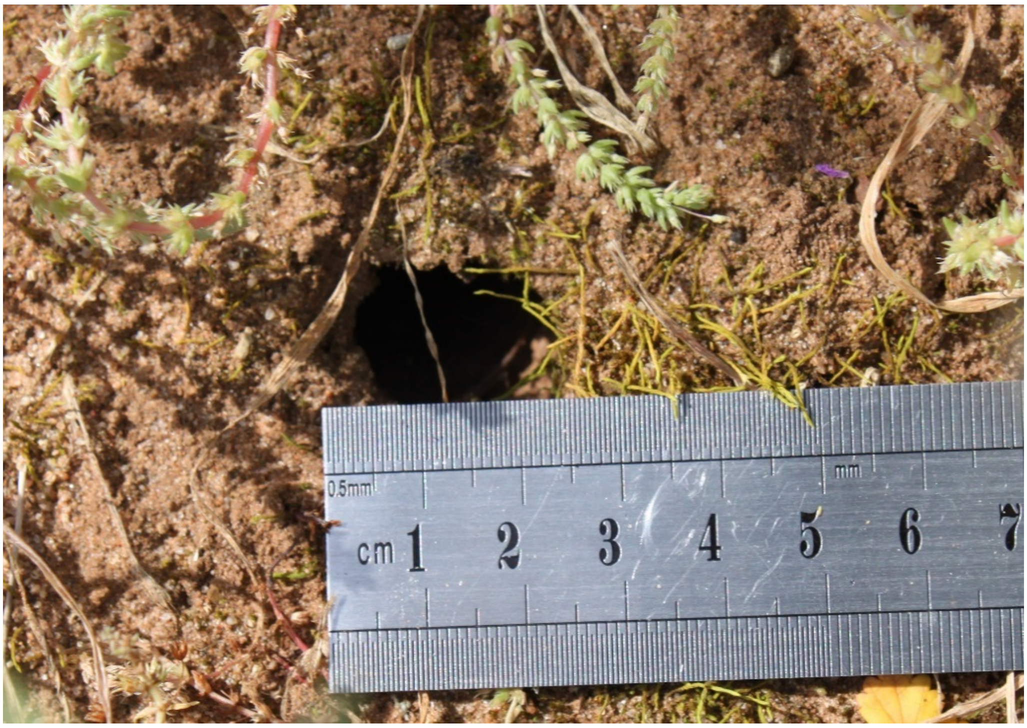

Figure 9-8. A mouse hole

During two lizard seasons at Monarto Zoo we caught 57 mice (35 in 2009-2010; 22 in 2010-2011) inside the cages. Ninety mouse holes were composed of short tunnels under the cage walls with one entrance inside and the other outside the cage. Fourteen mouse nests were found within the cages (10 in the first season; 4 in the second season) with one or two entrances inside the cages. Two of those nests were unoccupied when discovered, but 10 nests were found with a single mouse, and two had two mice. In the 2010-2011 lizard season the average diameter of 40 mouse hole entrances was 2.14 ± 0.016 cm. We found two male lizards, in the second season at Monarto, one on 22 December 2010 and the other on 27 January 2011, basking at the entrance of mouse holes inside a cage while other lizards basked at the entrance of artificial burrows. Both lizards were caught and released back into the central area of the cage, and the entrance diameters of the mouse holes were measured (each was 2.0 cm) as they were destroyed. We did not find any mice inside these burrows. One of the mouse burrows was 85 cm long and 25.5 cm below the ground at its deepest point. It had an enlarged underground chamber and another entrance outside the cage. The other was 95 cm long, 35 cm deep, had two enlarged chambers, and had a second entrance (2.2 cm diameter) inside the cage. The two entrances of this second mouse hole were about 80 cm apart. We could not prolong our observations of the lizards in these alternative refuges because we needed to prevent them escaping from their enclosures.

Other observations were conducted over the lizard season of 2009-2010, in three 1 ha square areas within a natural population of *T. adelaidensis* located 9 km from Burra (33.683 S 138.933 E), South Australia. Fences and pitfall traps were established around each area (15 cm high black plastic drift fencing;

16 pitfalls, each 30 cm diameter and 40 cm deep placed along each side of each 1 hectare quadrat), as part of a study of lizard population dynamics. We used an Olympus IF8D4X2-10L optic fiberscope to inspect each natural burrow within each area for burrow residents. Among the lizard captures we also trapped 90 mice in the pitfalls and we located and measured the entrance diameter (3.15 ± 0.14 cm) of 19 mouse holes within the study quadrats. Two mouse holes had 2.2 cm diameter entrances, within the range of entrance diameters preferred by adult lizards (Milne and Bull, 2000). The fiberscope had a functional length of 35 cm, and each of the mouse holes extended beyond that length. Thus we could not determine if any mouse holes were occupied by lizards. Nevertheless, mice provided burrows that may have provided alternative refuges for *T. adelaidensis* in a natural field population. Many studies that have noted the importance of rocks, logs, rodent burrows, cervices, dead leaves and thick bushes as a potential shelter sites for squamate reptiles (Webb and Shine, 2000; Grillet et al., 2010), but some species are more specific in their choice of refugia. Previous reports have suggested that *T. adelaidensis* uses only spider burrows (Hutchinson et al., 1994). Our observations in this report indicate this lizard will use another kind of shelter, in the form of mouse tunnels.

9.5. SPIDER AND PYGMY BLUETONGUE LIZARD INTERACTION

Lycosid and mygalomorph spiders play an important role in the ecology of pygmy bluetongue lizards by constructing the major available shelters for this Australian skink (Fellows et al., 2009). It has been shown already that the lizard spider interaction is complex. For instance Milne (1999) and Fenner et al. (2007) reported that lizards displaced lycosid spiders in laboratory terraria. An analysis of field collected lizard scats indicated that lizards ate spiders (Milne, 1999; Fenner et al., 2007). However, Milne (1999) found a dead juvenile pygmy bluetongue lizard in a burrow occupied by lycosid spider. So although spiders provide important burrow resources and possibly also a food source for lizards, their interaction could also have negative side, successfully killing some lizards that are trying to take over their burrows. The paper below reports two incidents where lycosid spiders killed adult pygmy bluetongue lizards. The importance of this paper for future translocation program is that we need to investigate and understand the lizard spider interaction more carefully before any attempts to translocate lizards into new habitat with native lycosid spiders.

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Lycosid spiders are friends and enemies of the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*)

Mehregan Ebrahimi and C. Michael Bull

9.5.1. ABSTRACT

The pygmy bluetongue lizard is an endangered species and the smallest member (average snout-to-vent length 95 mm) of the genus *Tiliqua*. It uses spider burrows with a single entrance for shelter and as sites to ambush passing prey. There is a little information about interactions between this lizard and the lycosid and mygalomorph spiders which construct the burrows they use. Surveys of the diet of pygmy bluetongue lizards show they eat lycosid spiders, and one record of a partly consumed juvenile pygmy bluetongue lizard found in a lycosid burrow suggests spiders eat lizards. This paper describes the first record of adult pygmy bluetongue lizards being killed by lycosid spiders. It suggests a complex relationship of lizards and spiders in that the lizards rely on potentially lethal co-inhabitants of their grassland habitat to construct the burrow refuges that they require. Conservation management of this endangered lizard will need to consider both the advantages and disadvantages of maintaining spiders in lizard population sites.

Keywords: Lycosid spiders, *Tiliqua adelaidensis*, Spider bite

9.5.2. INTRODUCTION

Many lizard species use burrows for shelter. Some dig their own burrows and some use burrows already constructed by other animals (Hawkins and Nicoletto, 1992; Vitt and Caldwell, 1993; Cooper et al., 2000; Milne et al., 2003b). Burrow residents are protected from extremes of ambient thermal conditions, and from other external hazards such as fires (Vitt and Caldwell, 1993; Costanzo et al., 1995; Milne et al., 2003b) and predators (Thompson, 1992; Cooper, 2000; Milne et al., 2003b). Those species that do not construct their own burrows must rely on other "burrow engineer" species. While depending on other species to provide their refuges, they may come into conflict with them during disputes over burrow ownership. The Australian pygmy bluetongue lizard, *Tiliqua adelaidensis* (Peters, 1863), is an endangered species and the smallest member (average snout-to-vent length 95 mm) of the genus *Tiliqua*. It uses spider burrows with a single entrance for shelter as sites to ambush passing prey (Milne et al., 2003b; Souter et al., 2007; Fellows et al., 2009), and has never been observed to dig its own burrows (Milne et al., 2003b; Souter et al., 2007; Fellows et al., 2009). There is a relatively little information about interactions between this lizard and the lycosid and mygalomorph spiders which construct the burrows that it relies on. Here we provide evidence for an adverse impact on lizards from interactions with those spiders.

Tiliqua adelaidensis is a scincid lizard distributed in small patches of remnant native grassland in the mid-north of South Australia. It was considered extinct until it was re-discovered in 1992, and is still classified as Endangered (Hutchinson et al., 1994). Within pygmy bluetongue lizard populations, burrows occupied by spiders and by lizards are interspersed, but no burrows have been found synchronously occupied by both (Fellows et al., 2009). An unanswered question is whether lizards actively evict resident spiders, or whether they wait until the burrow is vacated before taking over. In surveys every two weeks of burrows within a 1 ha study area, Fellows et al. (2009) reported that direct transitions from spider occupancy to lizard occupancy between successive surveys were rare, and that new lizard occupancy records were usually into burrows with no occupants in the previous survey.

Three previous observations have suggested negative interactions between spiders and lizards. Milne (1999) reported that, in laboratory terraria, nine out of ten lizards displaced lycosid spiders from artificial burrows, and probably consumed those spiders in six cases. In the field, a study of the diet of the pygmy bluetongue lizard reported lycosid spider remains in 14% of the scats examined (Fenner and Bull, 2007), indicating that the lizards naturally prey on the spiders. Conversely, Milne (1999) found a dead juvenile lizard in a burrow with a spider and suggested that resident lycosid spiders might kill, and perhaps eat juvenile pygmy bluetongue lizards that attempt to enter spider occupied burrows. The present paper reports two incidents of spiders attacking adult pygmy bluetongue lizards, and provides further evidence that spiders may have a negative impact on lizards.

9.5.3. METHODS

On 20 Oct 2009 we captured 16 pygmy bluetongue lizards (8 males and 8 females) from two populations near Burra, South Australia (population one: 33°36´S 138°59´E, population two: 33°37´S 138°59´E), and on 25 Oct 2009, we transferred four lizards to each of four 15 m diameter circular cages, with bird-wire roofs, at Monarto Zoo, approximately 70 km SE of Adelaide (35°06´S 139°09´E).

In each cage we provided lizards with 71 artificial burrows made from 30 cm lengths of wooden dowling with a drilled out centre of 2 cm internal diameter. These burrows were hammered vertically into the ground to replicate the angle of most of the natural burrows. As part of the design of a larger experiment that simulated a translocation release, we placed 41burrows within a 4 m diameter circle in the cage centre and 30 burrows spaced evenly around the internal perimeter and 1 m from the cage wall. Previous studies have shown pygmy bluetongue lizards readily accept these artificial burrows (Milne et al., 2003b; Souter et al., 2004). We defined two areas in each cage, the central (4 m diameter) area, and the marginal area outside of the centre. We used four cameras (CCD video camera, LICS23HF with 3.6 mm lens) to record all lizard activity within the central part of each cage for 12 daylight hours every day until late January. Cameras only covered the central part of each cage where simulated translocation releases occurred. We checked the status and location of each lizard early in the morning and late in the evening of each day using an optical fiberscope (Olympus IF8D4X2-10L) and portable light source (Olympus KLS-131) to inspect the artificial burrows, as described by Milne and Bull (2000).

Each day we also inspected the cage and removed any large predatory invertebrates such as spiders and centipedes. These were located either in the artificial burrows, or in their own recently constructed natural burrows. Those new burrows were destroyed. In total, we removed three centipedes and five spiders during the three month study. However, the video recordings showed two cases where a lizard was attacked by a spider before the spider was removed, and these cases are reported in this paper.

9.5.4. RESULTS

Two lizards (both females) were found dead during routine evening surveys, both in the same cage, one on 7 Dec 2009, and the second on 19 Jan 2010. Both showed bite punch markings and visible swelling around the neck (Figure 9-9). Monarto Zoo veterinarian reports concluded that the deaths were consistent with having been bitten by a poisonous arthropod. Inspection of the cage after each incident revealed a new lycosid spider (*Lycosa* spp) and its burrow in the cage in each case. Video records from the central area of the cage allowed us to see the behaviour of each lizard before it died, although critical moments in one case happened in the marginal area, outside of the camera field of view.

The first lizard was discovered dead on the ground surface near to an artificial burrow in the marginal area at 1840 h during the evening survey on 7 Dec. Video records for that lizard showed that it moved out of the central area at 1040 h on 6 Dec but returned to a burrow in the central area by 1900 h on the same day. On 7 Dec the lizard left the central area at 1000 h, and was located in a marginal burrow during the morning survey. It returned to the central area at 1030 h then changed burrows within the central area four times between 1030 – 1151 h, before moving out to the marginal area again. That evening the lizard was found dead near the entrance of the marginal burrow that it had occupied at 1000 h. We found a newly constructed burrow 1.5 m from the dead lizard, with a lycosid spider occupant. The spider measured 20.2 mm from head to thorax and was identified as a species from the genus *Lycosa*.

No evidence of the spider or its burrow was detected during the 1000 h survey of that day.

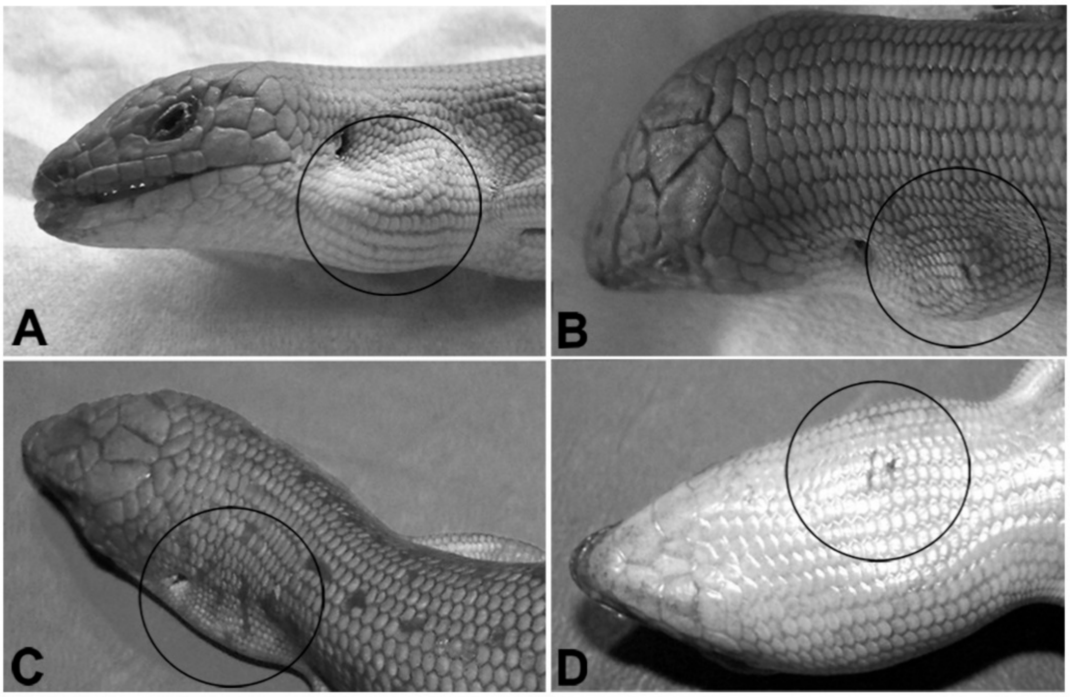

Figure 9-9. A and B are photos of adult female killed by spider on 7.12.2009, C and D are photos of adult female killed by spider on 19.1.2010. A and C show swelling around neck, whereas B and D show bite area.

The second lizard was found dead in the central area during the evening survey at 2040 h on 19 Jan. Video records showed that this lizard emerged from its burrow at 1302 h, and had an aggressive interaction (11 sec duration) with another female lizard at 1305 h. That second lizard returned to its own burrow after the interaction, but the focal lizard continued to search the surface of the central area. On this day we were conducting an experiment where lizards were confined to the central area by a temporary black plastic wall, and the lizard walked along the wall in one direction for one second before changing to move in the other direction along the wall. At 1307 h the video record showed a spider sitting on the wall and biting the lizard. Later that evening, when the dead lizard was discovered, we found a spider in a freshly dug burrow 10 cm outside of the removable wall, and adjacent to where the lizard had been bitten. The head to thorax length was 18.1 mm and it was again identified as a member of the genus *Lycosa*. No spiders or burrows had been detected in the 0900 h morning survey of that cage. The video recording showed that the lizard began unusual movement almost immediately after the bite. This movement appeared to be writhing or thrashing, with a distinct sinusoidal pattern and continued for 62 seconds, during which the lizard moved 2 m around the wall. The lizard then stopped and for the next 3.8 min its head kept slumping to the ground as it attempted repeatedly to lift its head back up to its normal position. Finally, just less than 5 min from the time of the spider bite, all movement ended, and we found the lizard in that position that evening. Meanwhile the spider had rapidly departed.

9.5.5. DISCUSSION

Most studies of lizard and spider interaction have focused on the impact of lizards as predators of spiders and as competitors for the same invertebrate prey (Spiller and Schoener, 1988; Spiller and Schoener, 1990; Taylor, 1991; Spiller and Schoener, 1998; Chase et al., 2002). Previous studies of pygmy bluetongue lizards have also identified them as predators of spiders, and other invertebrates that may also be prey to certain spiders (Fenner et al., 2007). Thus those previous studies suggested lizards may be both predators and competitors of spiders. The present study has revealed an extra complexity to this interaction.

In both of the dead lizards, signs of puncture wounds and local swelling were discovered around the neck. Both lizards were behaving normally prior to their deaths. They also were familiar with their habitat because they had been in the enclosures for more than two months. The role of lycosid spiders in causing the deaths of these two lizards can be deduced from direct observation in the second case, and from strong circumstantial evidence in the first case. Although the black plastic wall may have artificially increased the opportunity for contact between lizard and spider, in the second case, the outcome of that contact is likely to reflect what could happen in more natural surroundings. These are the first records of adult pygmy bluetongue lizards being killed by spiders, and they indicate the complexity of the relationship where lizards rely on potentially lethal co-existing species to construct their refuge shelters.

Milne (1999) observed a dead juvenile lizard present in a spider burrow that was partly consumed and he suggested lycosid spiders may be predators of juvenile pygmy bluetongue lizards. Adult lizards with an average snout to vent length of 95 mm are probably too large to be considered spider prey, and likely move too far from the spider burrow between spider bite and death, for the spider to be able to safely feed on its victim. In addition both lizards which were bitten by wolf spiders were not consumed by the spider. It is more likely, given the frequent occurrence of lycosid spider remains in lizard scats (Fenner et al., 2007), and laboratory observations of probable predation on spiders by lizards (Milne, 1999), that spiders bites adult lizards to deter potential predation by the lizard. The very rapid onset of thrashing and then death in the case that was fully recorded in the present study suggests this is an effective anti-predator strategy. In the second case the spider was out of its burrow and might have been surprised by the approaching lizard, which it bit before running away, suggesting a defensive motive for the bite. However the common occurrence of lycosid spider remains in lizard scats suggests that lizards frequently risk the consequences of spider bites. The taxonomy of Australian lycosid spiders is not entirely resolved (Hawkeswood, 2003), the diagnostic features are subtle, and we were not able to identify exactly which spider species was involved. It is possible that different species of lycosid spiders vary in the toxicity of their bites (Isbister and Framenau, 2004; Isbister and White, 2004), and that the suspected biters in this study were larger than many lycosid that co-existing with natural pygmy bluetongue populations. Furthermore, the Monarto Zoo site is outside the current restricted range of the pygmy bluetongue lizard, and we may have exposed the lizards to spider species they would not have encountered before. However, the range of the lizard is believed to have been more extensive in the past (Hutchinson et al., 1994), and it is probable that over their range they have co-existed with a wider range of spider species of variable bite toxicity.

A significant problem in translating this result to the few remaining natural populations of pygmy bluetongue lizards is the difficulty in identifying similar cases of spider induced mortality in natural populations, in the absence of intensive video surveillance. In cases of natural spider induced mortality, it is unlikely that killed adult lizards would be taken into spider holes where they could be deduced to be victims of spider bites. Lizards that have been bitten by spiders are likely to die very quickly, outside of burrows, and then be removed by scavengers before detection by researchers. We have previously suggested that the sustained conservation of this endangered lizard requires the presence of viable, high density populations of burrow digging spiders (Fellows et al., 2009). Our finding in the present study suggests that some caution is needed in considering that recommendation, and that more needs to be known about the interaction of spiders and lizards to allow a fully informed conservation strategy. More broadly this study provides another example where species interactions can have both positive and negative influences (Pruitt and Ferrari, 2011).

9.5.6. ACKNOWLEDGMENTS

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CHAPTER TEN

10. CONCLUSION

10.1. OVERVIEW

This thesis examined the impact of changes in environmental parameters, resource availabilities and social interactions in simulated translocations, using pygmy bluetongue lizards as a model. The results suggest that behavioural changes in translocated species are a key factor that can help us to reduce risk of dispersal and failure of translocation programs. The experiments described in the thesis focused on those behavioural changes in the pygmy bluetongue lizard with simulated translocation experiments.

As individuals of a translocated species may disperse immediately after release because of stress from handling and unfamiliarity with the novel habitat, I investigated the possibility of reducing this dispersal by confining individuals at the release area for a short time. I also kept in mind that pygmy bluetongue lizards are not active foragers and that they spend most of their time associated with their burrows (Milne et al., 2003b; Fenner and Bull, 2011a). Therefore, I only investigated short-term confinement time and showed that a one day confinement time reduced dispersal more than confinement for five days perhaps because of increased confinement stress. This experiment was described in chapter three. Changes in other behavioural parameters also proved that one day is as good as or better than five days confinement to reduce behaviours that may reduce translocation success.

I tested whether supplementary food may encourage newly translocated lizards to stay at their release site and to reduce early post release movement. Other studies have indicated that supplementary food does not directly improve the success of reptile translocations (Licht, 1974; Rose, 1982), but, in this experiment, supplementary food reduced activity time and basking time in pygmy bluetongue lizards, which could decrease the risk of predation, and reduced the rate of lizard dispersal from the central release site. The details about this experiment were discussed in chapter four.

In chapter five I explored behavioural difference of translocated pygmy bluetongue lizards with differences in burrow resource availability or spatial organisation. Several relevant lizard behaviours were significantly altered by changes in burrow density, burrow distribution and release distance from each other. These experiments illustrated that burrow refuges, and their distribution are critical for lizards, and, in actual translocations, we need to have enough burrows and we need to release lizards sufficiently far apart (at least more 1.4 m from each other) to reduced social interactions in this solitary lizard.

Next, in chapter six, I studied the effect of intensive grazing and ploughing on the behaviour of released pygmy bluetongue lizards. First, I cut grass to the ground surface to simulate intensive grazing and compared lizards behaviour between low and high density of vegetation. The results showed lizards spent more time basking, moved less around their burrows, but dispersed more with reduced vegetation density. This result conformed with other reports of lizard behaviour with different vegetation density in natural populations of pygmy bluetongue lizards (Pettigrew and Bull, 2011a; Pettigrew and Bull, 2012). Second I showed that soil disturbance, making the matrix around release sites unsuitable, inhibited lizard dispersal from the release site. In a previous study, Souter (2003) showed that lizards avoided artificial burrows in ploughed areas immediately adjacent to suitable habitat. Combining my experiments with the previous results suggests that pygmy bluetongue lizards may be effectively confined in a release area when there is a ploughed ring around them.

In chapter seven I investigated the influence of visual conspecific cues on the early stages after release for translocated lizards, testing whether signs of conspecifics may reduce the stress of a new habitat. In contrast, I found lizards dispersed more and changed their burrows more often when there were conspecific cues around them. Bolder individuals that attacked conspecific models and tried to push them further from their burrows, may be more suitable candidates for future translocation purposes.

In chapter eight, I showed how the time of release also could be important in lizard translocation. I released pygmy bluetongue lizards in different months of two consecutive austral spring/summer seasons. Lizards were more active and dispersed at a higher rate in early spring, than later in summer. Additionally, in the course of running this series of experiments, I also gathered several anecdotal records about some previously unrecorded aspects of the natural history of pygmy bluetongue lizards. These observations are presented as a series of separate papers in the final chapter of results of this thesis. This information is highly important for future translocation management. From the observations of lizard behaviour I found that artificial burrows can be optimized by adding a chamber at the bottom of them, which could allow more efficient lizard movements inside the burrows, particularly when they first go head first into the burrows. Second we found that lizards stay in flooded burrows after heavy rains, and they do not like to change their burrows even after a storm. They usually remain in flooded burrows until they have dried out. In addition, because at the time of a storm I was conducting an experiment on the effect of heavy grazing. I found that a higher vegetation density protects burrows from washed in debris during heavy rain, and fewer burrows were destroyed in high vegetation density areas. I also for the first time reported that pygmy bluetongue lizards accept mice burrows if their entrance diameter were not bigger than 2 cm, and mouse burrows might be exploited when considering future suitable translocation sites for this endangered species. I also reported that lycosid spiders which constructed the burrows that were used by pygmy bluetongue lizards, can kill adult pygmy bluetongue lizards that try to take over burrow ownership.

Finally, I used the data that I had collected from this series of experiments to produce decision tree models for future translocation management of this species. This modeling approach can be used for pygmy bluetongue lizards, or for ecologically similar species, or it can be easily altered to suit other reptile species. I used data on dispersal rates, derived from the result chapters. The result of decision trees models are outlined below as an overall conclusion of this thesis.

Decision tree models

Decision tree algorithms are widely used across many disciplines of science (Vlahou et al., 2003; Ebrahimie et al., 2011; Omiotek et al., 2013), including environmental science (Friedl and Brodley, 1997; Pal and Mather, 2003; Bogaert et al., 2004). Decision trees help us to identify which factors affect our target species more and how our target species respond to the changes of those factors.

One of the main problems for many conservationists is they are faced with many uncertainties in the environment where they work (Regan et al., 2005) and they need to make correct decisions as fast as possible to protect the threatened species or habitat. To help decision making, there are frame works and decision tables available from some organisation such as IUCN, but there is still a need of more detailed understanding of each species, and their responses to the decision that we make are still very important. Translocation, as one of the powerful tools available for conservation management, is also faced with decision making uncertainty. Producing a decision making chart may help conservationists to perform translocation with more confidence and to predict translocated species responses at the translocation release sites. Decision trees could help conservationists to reduce the rate of dispersal and to reduce the risk of translocation failure. In the following paper, I discuss how data from five experimental simulations of a translocation of an endangered lizard can help us to make a better decision in different circumstances. For instance, if there is low vegetation density following translocation at the release site, how we can reduce dispersal. The paper below discusses this decision making for future translocation of pygmy bluetongue lizards.

Prepared for Conservation Biology

Minimising the cost of translocation failure by using decision tree models to predict species behavioural response in translocation sites

Mehregan Ebrahimi, Esmaeil Ebrahimie and C. Michael Bull

10.2. ABSTRACT

Translocation is a powerful tool in conservation management, but the high number of failures of many translocation attempts is one reason why translocation is not recommended as a first solution. In many conservation management issues more attention is now paid to animal behaviour. Considering how behavioural parameters change may be a key to translocation success. In the present paper we used data from five simulated translocation experiments on an endangered Australian skink to derive decision tree models. We used four different decision tree algorithms (decision tree, decision tree parallel, decision stump and random forest) with four different criteria (gain ratio, information gain, gini index and accuracy) to investigate how environmental and behavioural parameters that were studied in the five experiments, and their changes, might affect the success of a translocation. The trees became more complex when we included all behavioural parameters as attributes, but these trees gave us more detailed understanding about why and how dispersal occurred. Decision tree models based only on parameters related to the release conditions were easier to follow and

might be used by conservation managers to make decisions about the translocation process in different circumstances.

Keywords: Decision tree, Translocation, Behaviour, Conservation management

10.3. INTRODUCTION

Decision tree algorithms have been used widely in health science (Qu et al., 2002; Vlahou et al., 2003; Ebrahimie et al., 2011; Omiotek et al., 2013), engineering (Kim et al., 2001; Cho and Kurup, 2011; Evans et al., 2013) and environmental sciences (Friedl and Brodley, 1997; Pal and Mather, 2003; Bogaert et al., 2004). The results from these algorithms help to quickly identify which factor or factors most strongly affect a target end-point, and provide a basis for decision making to most efficiently reach that end-point. One of the main problems for many conservation managers is that they are faced with many uncertainties in the environment where they work (Regan et al., 2005), and they need to make appropriate decisions as soon as possible to protect a threatened species or habitat. Some organisations such as the IUCN provide a general frame work and decision guideline for specific management processes, such as translocations (IUCN, 2013b), but a more detailed understanding of each species response to the decisions taken are still very important.

Assisted colonisation or translocation is a potentially powerful tool in conservation management, but is accompanied by some controversy. Relatively few previous translocations have been confirmed to be successful (Kleiman, 1989; Dodd and

Seigel, 1991; Fischer and Lindenmayer, 2000) with one probable cause of failure being the tendency of translocated individuals to disperse from release sites (Stenseth and Lidicker, 1992; Rittenhouse et al., 2007). In more recent times translocation success may have improved, particularly with releases onto islands where dispersal is limited by a geographical barrier (Germano and Bishop, 2009). Some of the reasons for dispersal after translocation have been discussed in detail by Stenseth and Lidicker, 1992; Ims and Hjermann, 2001; van Heezik et al., 2009 and include unfamiliarity with a new habitat (Tuberville et al., 2005; Ebrahimi and Bull, 2013b), handling and release stress (Dickens et al., 2010), disrupted social structures and social interactions among conspecifics (Towns and Ferreira, 2001; Skjelseth et al., 2007), and reduced resource availability or quality (Bright and Morris, 1994; Elliott et al., 2001). Each of these factors can affect individual behaviours directly or indirectly, and can also increase the chance of dispersal. Behavioral ecologists have advocated for a long time, the need to include behaviour in considerations of conservation management, if we want to reduce the risk of failure of specific conservation management decisions (Gosling and Sutherland, 2000; Festa-Bianchet and Apollonio, 2003).

Although decision trees in natural systems can be made with relatively few available data, restrictions on time, budget and labour to collect such data decrease the chance of an accurate evaluation (Goethals et al., 2006). In the case of translocations, the lack of data from experimental or simulated translocations, and a tendency not to do such research before the actual translocation takes place, decrease the precision of any model predictions about the responses of translocated species. When such data are available, decision making models can help to boost our understanding of how different habitat factors, environmental conditions and species behaviours at the translocation release site can change the outcome of the translocation. Decision trees are important algorithms for management approaches in many situations, and should be helpful in conservation management programs.

In this paper we derived four different decision tree algorithms from the data of five simulated translocation experiments on an endangered Australian skink, the pygmy bluetongue lizard (*Tiliqua adelaidensis*) (Ebrahimi and Bull, 2012a; Ebrahimi and Bull, 2013a; Ebrahimi and Bull, 2013b; Ebrahimi and Bull, 2013d). We had two aims. First, we anticipated these models would provide a better understanding of how, when and why dispersal happens under different sets of conditions at the release site. So we could use the models to plan specific procedures and sets of conditions at the release site to reduce the risk of early post release dispersal. Second, we used the models to provide broader support for the view that behavioural parameters are important for conservation management issues such as translocation (Caro, 1998; Caro, 1999; Wallace, 2000; Shier, 2006; Caro, 2007).

10.4. METHODS

The data we used have already been reported from a series of five experimental studies over the austral spring and summer of 2009 – 2010 and 2010 -2011 (Ebrahimi and Bull, 2012a; Ebrahimi and Bull, 2013a; Ebrahimi and Bull, 2013b; Ebrahimi and Bull, 2013d). Those experiments were conducted to identify how different variable conditions influence the tendency of lizards to disperse from simulated translocation sites. Details of the methods have already been reported.

Briefly we used four 15 m diameter circular cages in a line, about 5 m apart in the grounds of Monarto Zoo, South Australia (35° 06´S, 139° 09´E) with 1 m high galvanised iron walls and bird wire roofs. Each cage was divided into three areas, a 4 m diameter central area as the experimental release site, a 5 m wide matrix of unsuitable habitat, and a ring, 0.5 m wide, around the inside cage perimeter that trapped any lizards that dispersed from the central area. We hammered 41 artificial burrows for lizards (Milne et al., 2003a) into the central area and 30 around the perimeter area as previously described (Ebrahimi and Bull, 2012a). Four surveillance cameras were used to record lizard activity in the central area over, usually, four days during each experiment (Ebrahimi and Bull, 2012a). Eight male and eight female pygmy bluetongue lizards were captured from two populations near Burra, South Australia (33° 42´S, 138° 56´E) in September 2009 and four were released into the central area of each cage for these experiments. Details of the lizard biology and husbandry have been provided previously (Ebrahimi and Bull, 2012a; Ebrahimi and Bull, 2013b).

We used data from the first four days of each trial in the five experiments to make our data set. In the experiments we manipulated environmental conditions within the central release area. The experimental treatments that we changed in each experiment became the independent variables that, in the decision tree, were called regular attributes. The parameters defining these treatments are listed below. Each experiment involved several replicate trials with manipulation of a single factor. 1) Confinement time: in one experiment we initially confined lizards to the central area of the cage, in two cages for one day and two other cages for five days, then observed behaviour after the confining walls were removed (Ebrahimi and Bull, 2013b). 2) Supplementary food: three mealworms were fed to each lizard every day in two cages while we did not feed lizards in two other cages (Ebrahimi and Bull, 2012a). 3) Vegetation density: two cages had high vegetation density and to other cages had all vegetation removed to ground level (Ebrahimi and Bull, 2013a). 4) Soil disturbance: in two cages we ploughed the soil in a 2 m wide area of the matrix immediately around the central area, and we left two cages with no soil disturbance (Ebrahimi and Bull, 2013a). 5) Conspecific models: we added 18 conspecific models close to burrow entrances in two cages and left two cages without models (Ebrahimi and Bull, 2013d). In addition, because each of these experiments was conducted at a different time of year we included the month when we released lizards as the sixth attribute. For these analyses we included experiments conducted in October (two trials), November (three trails), December (two trails), and January (three trails).

We then used five behavioural parameters that we recorded in each experiment, as dependent variables that we called target (label) attributes. In these analyses each behavioural parameter had one of two possible states. Each lizard was recorded either as showing the behaviour at least once on a day, or not showing the behaviour on that day. The recorded behaviours were; 1) Basking, if the lizard had partially emerged and was basking at the entrance of its burrow, we recorded it as basking. 2) Movements around burrows, in some cases lizards fully emerged from their burrow and moved about, usually for a very short distance, and then retreated to the same burrow. Movements also included lizards walking around the burrow entrance, lizards that basked while fully emerged, and lizards that moved away from the burrow entrance to defecate or forage for prey. We recorded whether a lizard did or did not move on each day. 3) Burrow changes, in some cases lizards fully emerged from their burrows and moved around to choose another burrow. We recorded if lizards did or did not change burrows on a day. 4) Dispersal was defined when a lizard left the central experimental area and moved across the habitat matrix, and was subsequently discovered occupying a burrow in the perimeter region. In terms of the translocation simulation, they represented dispersal events away from the release site. We recorded if a lizard did or did not disperse on a day. Note that with the larger cage, lizards could not move beyond the perimeter area, and often moved back to the central area. Thus a lizard could disperse on more than one day. 5) Fights, when two lizards approached each other on the ground surface, they always showed some agonistic interaction, either with the lizards scuffling together, or with one running away from the other. We defined all of these interactions as fights, and recorded whether a lizard did or did not fight on each day.

Decision tree algorithms, using machine learning techniques, have already been described and used in many other research areas (Pal and Mather, 2003; Geurts et al., 2005; Rokach and Maimon, 2005; Doğan et al., 2008). To develop decision trees for our analysis we imported the data set into RapidMiner software (RapidMiner 5.0.001, Rapid-I GmbH, Stochumer Str. 475, 44227 Dortmund, Germany). We had five target attributes (the five behavioural parameters) and produced two different types of final data sets for each target attribute. For the first type, we selected one of the behavioural parameters as a target attribute and excluded the other behavioural parameters to produce five-data sets, one data set for each behavioural parameter. Those five data sets each included six regular attributes (confinement time through to time of release) and one target attribute (one of the behavioural parameters). We considered that models produced from these first five data sets would be useful for developing management strategies for the conditions of release in future translocations. For the second type of data set, we chose again one behavioural parameter as the target attribute, but we included the other four behavioural parameters as additional regular attributes. Therefore we had another five data sets (one for each behavioural parameter) that had one target attribute (the chosen behavioural parameter) and 10 regular attributes (six representing the experimental conditions, confinement time through to time of release, plus the four remaining behavioural parameters). Data sets of this second type allowed interpretation of how other behavioural parameters can also influence the target behavioural attribute. The steps described below were then applied to all ten data sets to produce the decision tree models.

10.4.1. DATA CLEANING

Data cleaning algorithms have previously been developed to remove duplicate and correlated attributes from a data base (Pyle, 1999; Zhang et al., 2003). In our data sets we found no duplications or highly correlated attributes (with Pearson correlation greater than 0.9).

10.4.2. ATTRIBUTE WEIGHTING

In building decision trees weighting attributes can help to get more accurate models (Quinlan, 1986; Quinlan, 1990; Doğan et al., 2008). There are many different weighting algorithms that can be used for this purpose and usually one, two or a few of these algorithms have been used (Blum and Langley, 1997; Wettschereck et al., 1997; Geurts et al., 2005; Hall, 2007). In this study we applied all 10 different algorithms of attribute weightings that were available in RapidMiner software to the ten data sets, to identify the most important attributes. The algorithms are described in (Rapid-I, 2013) they were: 1) Weight by PCA (principal component analysis), this algorithm used the factors of the first of the principal components as attribute weights. 2) Weight by SVM (support vector machine), this algorithm used the coefficients of the normal vector of a linear SVM as attribute weights. 3) Weight by relief, this algorithm measured the relevance of attributes by sampling examples and comparing the value of the current attribute with the nearest example of the same and of different class. 4) Weight by uncertainty, this algorithm calculated the relevance of an attribute by measuring the symmetrical uncertainty with respect to the class. 5) Weight by gini index, this algorithm calculated the relevance of an attribute by computing the gini index of the class distribution, if the given data set would have been split according to the attribute. 6) Weight by chi squared statistic, the chi squared algorithm calculated the relevance of an attribute by computing, for each attribute of the input data set, the value of the chi-squared statistic with respect to the class attribute. 7) Weight by deviation, this algorithm created weights from the standard deviations of all attributes. The values were normalised by the average, the minimum, or the maximum of the attribute. 8) Weight by rule, this algorithm calculated the relevance of an attribute by computing the error rate of a OneR Model on the example set without this feature. 9) Weight by information Gain ratio, this algorithm was similar to the first one but it used an Information Gain ratio to calculate the attribute weight. 10) Weight by information gain, this algorithms computed the relevance of an attribute by calculating the information gain in the class distribution. The resulting weights were normalised into intervals between 0 and 1.

10.4.3. ATTRIBUTE SELECTION

Attribute weighting algorithms give us the opportunity to select attributes that have high influence on our target attributes. In this process redundant and less relevant data in the data set, which might decrease the model performance and make the training phase more difficult are eliminated (Blum and Langley, 1997; Witten and Frank, 2005). In our analysis we had ten different weighting outputs for each of the ten data sets (= 100 new data sets each with a set of weighted attributes). From each of those data sets we then eliminated those attributes with a weighting of less than 0.5, defining them as less important (Geurts et al., 2005). We also included the ten original unweighted data sets to produce 110 data sets that were then used to produce separate decision tree models.

10.4.4. MACHINE LEARNING MODEL: DECISION TREE

To extract information from a data set, machine learning offers various paradigms including unsupervised and supervised learning models. Unsupervised models used the data set without a label (a target attribute) and supervised models used the data set with a label (Geurts et al., 2005; Kotsiantis, 2007). For our data sets, that were composed of samples described by input variables (regular attributes or treatment state in each experiment) and by specific output information (target attributes or behaviour parameters), the supervised learning models were the best choice. There are several algorithms for supervised learning including neural networks, genetic algorithms and decision trees. When the number of variables is large relative to the number of samples in a data set, we need an algorithm that can identify informative attributes under those conditions, and decision tree algorithms are the best choice (Geurts et al., 2005). Additionally, decision tree models are often most appropriate for management decisions (Quinlan, 1990). There are several different algorithms for constructing decision tree models (Breiman et al., 1984; Freund and Schapire, 1995; Breiman, 1996; Breiman, 2001; Geurts et al., 2005). Typically the goal of all of them is to construct a tree with minimum generalization error, but other goals such minimising nodes and branches to make the tree less complicated also may be important (Rokach and Maimon, 2005). We did not know which algorithms would work best for our data set, so we ran four decision tree algorithms (decision tree, decision tree parallel, decision stump and random forest) to allow, by comparison, models with the smallest error, and models with the least complexity. Decision tree algorithms are based on splitting functions. The splits are based on single attributes at nodal points, with the first node representing the most important attribute, and with successive nodes representing progressively less important attributes. Thus a tree is constructed that reflects the relative importance of the regular attributes in influencing the target attribute. Among the different criteria used to determine the splitting functions are impurity based criteria, normalized impurity based criteria and binary criteria (Drummond and Holte, 2000; Rokach and Maimon, 2005). From among these, we used four different criteria (gain ratio (normalized impurity based criteria), information gain (impurity based criteria), gini index (impurity based criteria) and accuracy (impurity based criteria)) for each of the four decision tree algorithms to produce 16 decision tree models for each of the 110 data sets.

Each decision tree model has the most informative attribute forming the first node as the root of tree (the attribute that the tree starts with). Then it is divided into two branches with each branch showing alternative states of that attribute (Quinlan, 1990; Safavian and Landgrebe, 1991; D'Heygere et al., 2003). For instance in Figure 10-1 the target attribute is basking behaviour (lizard basked/lizard did not bask). The most important attribute is vegetation density which is the first node or root of the tree. One branch from that node is high vegetation density and the other is low vegetation density. If the algorithm identifies a second important attribute (soil disturbance in Figure 10-1), another node appears on the tree which again divides into two branches. This process continues until the last important attribute has been selected (time of release in Figure 10-1). At the terminal end of each sequence of branches there are leaves. The leaves show the results for the target attribute.

One leaf will show Yes (lizard basked) the other No (lizard did not bask) and the colour of the leaf, or the percentage indicates the percentage of lizards in each basking category (Yes or No) as predicted by the model.

We used 10-fold cross validation as used by (Loh and Shih, 1997; D'Heygere et al., 2003; Habashy et al., 2011) to train and test the models on all patterns, to calculate the accuracy, as defined below, of each model, and to prevent model over-fitting. To evaluate each tree by 10-fold cross validation all records in each data set were randomly divided into10 parts, with 90% of the data set used for training and 10% used for testing to perform cross validation. This process was repeated 10 times and the final accuracy was reported as the percentage of correctly classified instances (CCI). For each target attribute, we selected the single tree with the highest CCI. The algorithms and criteria that led to each selected tree differed among the different target attributes. The lowest CCI we used was 61% for movements around burrows and the highest was 93% for fights.

10.5. RESULTS

10.5.1. ATTRIBUTE WEIGHTING

The number of regular attributes with weight higher than 0.5 varied depending on the target behavioural attribute. The value for each regular attribute weight when other behaviours were excluded and included in data sets, for each of the five target behaviours are shown in table 10-1 and table 10-2 respectively.

10.5.2. DECISION TREES

We produced 1760 trees in total, which is 176 trees for each target attribute. Most trees (1600) did not have roots or leaves, and were excluded as they had no results we could use. From the remaining 160 trees with roots and leaves eight with the highest accuracy (highest CCI score) (one for each attribute, and four from each of the two types of data sets) were selected as described above (Table 10-3). Some other trees are presented in the supplementary material where outputs replicate other models, where the trees are too complex, or where the results are not relevant to the purpose of this paper. Even though one of the trees for dispersal was very complex, with 17 branches, it was retained because of the management importance of this attribute.

Table 10-1. Attribute weight and the number of weighting algorithms that gave each attribute a weight > 0.5, when one behavioural parameter was the target attribute and other behaviours were excluded.

		Weighting algorithms										
	Attribute	PCA	SVM	Relief	Uncertainty	Gini Index	Chi Squared	Deviation	Rule	Info Gain Ratio	Info Gain	No. weight > 0.5
Basking	Confinement time	0.00	0.00	1.00	0.57	0.11	0.11	0.15	1.00	1.00	0.29	4
	Supplementary	0.00	0.03	0.31	0.71	0.58	0.58	0.00	1.00	0.84	0.45	5
	Soil disturbance	0.01	0.12	0.74	0.00	0.00	0.00	0.01	1.00	0.00	0.00	2
	Vegetation density	0.02	1.00	0.61	1.00	1.00	1.00	0.03	1.00	0.85	0.86	8
	Conspecific models	0.04	0.27	0.00	0.12	0.03	0.03	0.00	1.00	0.16	0.06	1
	Month of release	1.00	0.42	0.23	0.99	0.84	0.87	1.00	1.00	0.89	1.00	8
Movements around burrows	Confinement time	0.00	1.00	0.00	0.22	0.19	0.18	0.15	1.00	0.39	0.17	2
	Supplementary	0.00	0.05	0.53	0.15	0.14	0.14	0.00	0.00	0.19	0.14	1
	Soil disturbance	0.01	0.00	1.00	1.00	1.00	1.00	0.01	0.00	1.00	1.00	6
	Vegetation density	0.02	0.07	0.94	0.31	0.36	0.36	0.03	0.00	0.27	0.35	1
	Conspecific models	0.04	0.05	0.00	0.15	0.14	0.14	0.00	0.00	0.19	0.14	0
	Month of release	1.00	0.01	0.13	0.00	0.00	0.00	1.00	0.00	0.00	0.00	2
Burrow changes	Confinement time	0.00	0.80	0.00	0.61	0.49	0.35	0.15	1.00	1.00	0.45	5
	Supplementary	0.00	0.21	0.78	0.00	0.00	0.00	0.00	1.00	0.00	0.00	2
	Soil disturbance	0.01	0.01	0.42	0.92	0.84	0.61	0.01	1.00	0.90	0.86	6
	Vegetation density	0.02	0.00	1.00	0.95	1.00	0.72	0.03	1.00	0.80	1.00	7
	Conspecific models	0.04	1.00	0.00	0.14	0.13	0.09	0.00	1.00	0.16	0.12	2
	Month of release	1.00	0.02	0.06	1.00	0.27	1.00	1.00	1.00	0.23	0.26	5
Dispersal	Confinement time	0.00	0.77	0.10	1.00	1.00	1.00	0.15	1.00	1.00	0.93	7
	Supplementary	0.00	1.00	0.04	0.00	0.00	0.00	0.00	1.00	0.00	0.00	2
	Soil disturbance	0.01	0.65	1.00	0.65	0.41	0.41	0.01	1.00	0.42	0.84	5
	Vegetation density	0.02	0.29	0.28	0.66	0.56	0.56	0.03	1.00	0.38	1.00	5
	Conspecific models	0.04	0.44	0.00	0.33	0.34	0.34	0.00	1.00	0.25	0.38	1
	Month of release	1.00	0.00	0.14	0.38	0.57	0.64	1.00	1.00	0.33	0.95	6
Fight	Confinement time	0.00	0.19	0.34	0.00	0.00	0.00	0.15	1.00	0.00	0.00	1
	Supplementary	0.00	0.61	0.00	1.00	1.00	1.00	0.00	1.00	1.00	0.69	7
	Soil disturbance	0.01	1.00	1.00	0.90	0.42	0.42	0.01	1.00	0.80	0.72	6
	Vegetation density	0.02	0.48	0.64	0.67	0.50	0.50	0.03	1.00	0.54	0.65	7
	Conspecific models	0.04	0.00	0.68	0.67	0.26	0.26	0.00	1.00	0.67	0.46	4
	Month of release	1.00	0.07	0.94	0.79	0.60	0.60	1.00	1.00	0.94	1.00	9

Table 10-2. Attribute weight and the number of weighting algorithms that gave each attribute a weight > 0.5 when one behavioural parameter was the target attribute and other behaviours were included.

		Weighting algorithms										
	Attribute	PC	SV	Reli	Uncertainty	Gini	Chi	Deviatio	Rule	Info Gain Ratio	Info	No. weight > 0.5
Basking	Movements around burrows	0.00	0.32	0.02	0.00	0.00	0.00	0.06	1.00	0.00	0.00	1
	Burrow changes	0.00	0.13	0.00	0.04	0.04	0.04	0.05	1.00	0.04	0.04	1
	Dispersal	0.01	0.60	0.53	0.19	0.10	0.10	0.02	1.00	0.22	0.12	3
	Fight	0.01	0.13	0.30	0.29	0.10	0.10	0.00	1.00	0.43	0.15	1
	Confinement time	0.01	0.00	0.42	0.60	0.15	0.15	0.16	1.00	1.00	0.32	3
	Supplementary Food	0.01	0.65	0.24	0.73	0.61	0.61	0.01	1.00	0.86	0.48	6
	Soil disturbance	0.02	0.43	0.00	0.07	0.05	0.05	0.03	1.00	0.09	0.04	1
	Vegetation density	0.03	0.58	0.57	1.00	1.00	1.00	0.05	1.00	0.87	0.87	8
	Conspecific models	0.05	0.00	0.51	0.18	0.08	0.08	0.01	1.00	0.23	0.10	2
	Time of release	1.00	1.00	1.00	0.99	0.85	0.87	1.00	1.00	0.90	1.00	10
Movements around burrows	Basking	0.01	0.00	0.00	0.02	0.01	0.01	0.03	0.00	0.02	0.01	0
	Burrow changes	0.00	1.00	1.00	1.00	1.00	1.00	0.05	1.00	1.00	1.00	8
	Dispersal	0.00	0.00	0.00	0.04	0.03	0.03	0.02	0.04	0.05	0.03	0
	Fight	0.00	0.00	0.06	0.12	0.09	0.09	0.00	0.15	0.22	0.09	0
	Confinement time	0.01	0.00	0.03	0.02	0.01	0.01	0.16	0.02	0.04	0.01	0
	Supplementary Food	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.02	0.01	0
	Soil disturbance	0.01	0.00	0.10	0.08	0.06	0.06	0.03	0.00	0.10	0.07	0
	Vegetation density	0.03	0.00	0.07	0.02	0.02	0.02	0.05	0.00	0.03	0.02	0
	Conspecific models	0.04	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.02	0.01	0
	Time of release	1.00	0.00	0.04	0.00	0.00	0.00	1.00	0.00	0.00	0.00	2
Burrow changes	Basking	0.01	0.00	0.05	0.03	0.02	0.02	0.03	0.00	0.03	0.02	0
	Movements around burrows	0.00	1.00	1.00	1.00	1.00	1.00	0.06	1.00	1.00	1.00	8
	Dispersal	0.01	0.46	0.77	0.63	0.54	0.54	0.02	0.81	0.86	0.49	7
	Fight	0.01	0.02	0.14	0.26	0.20	0.20	0.00	0.33	0.48	0.18	0
	Confinement time	0.01	0.22	0.02	0.03	0.02	0.02	0.16	0.00	0.06	0.02	0
	Supplementary Food	0.01	0.00	0.09	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0

Table 10-2. (Continued)

		Weighting algorithms										
	Attribute	PC	SV	Reli	Uncertaint	Gini	Chi	Deviati	Rul	Info Gain	Info Gain	No. weight > 0.5
Burrow change	Soil disturbance	0.02	0.00	0.13	0.04	0.04	0.04	0.03	0.00	0.06	0.04	0
	Vegetation density	0.03	0.00	0.08	0.05	0.04	0.04	0.05	0.00	0.05	0.04	0
	Conspecific models	0.05	0.08	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0
	Time of release	1.00	0.36	0.06	0.05	0.01	0.06	1.00	0.00	0.01	0.01	2
Dispersal	Basking	0.01	0.01	0.12	0.09	0.06	0.06	0.03	1.00	0.10	0.08	1
	Movements around	0.00	1.00	0.00	0.06	0.06	0.06	0.06	1.00	0.05	0.06	2
	Burrow changes	0.00	0.66	1.00	1.00	1.00	1.00	0.05	1.00	1.00	1.00	8
	Fight	0.01	0.16	0.46	0.54	0.45	0.45	0.00	1.00	0.85	0.34	3
	Confinement time	0.01	0.16	0.32	0.51	0.40	0.40	0.16	1.00	0.93	0.29	3
	Supplementary Food	0.01	0.36	0.09	0.00	0.00	0.00	0.01	1.00	0.00	0.00	1
	Soil disturbance	0.02	0.44	0.41	0.33	0.16	0.16	0.03	1.00	0.39	0.26	1
	Vegetation density	0.03	0.35	0.07	0.34	0.23	0.23	0.05	1.00	0.36	0.32	1
	Conspecific models	0.05	0.06	0.08	0.17	0.14	0.14	0.01	1.00	0.23	0.12	1
	Time of release	1.00	0.00	0.19	0.20	0.23	0.26	1.00	1.00	0.31	0.30	3
Fight	Basking	0.01	0.46	0.99	0.23	0.14	0.14	0.02	1.00	0.21	0.25	2
	Movements around	0.00	0.77	0.16	0.35	0.37	0.37	0.05	1.00	0.28	0.49	2
	Burrow changes	0.00	0.86	0.60	0.76	0.82	0.82	0.04	1.00	0.64	1.00	8
	Dispersal	0.01	1.00	0.63	1.00	1.00	1.00	0.01	1.00	1.00	0.94	8
	Confinement time	0.01	0.54	0.53	0.00	0.00	0.00	0.15	1.00	0.00	0.00	3
	Supplementary Food	0.01	0.53	0.26	0.57	0.51	0.51	0.00	1.00	0.61	0.49	6
	Soil disturbance	0.02	0.63	0.50	0.51	0.21	0.21	0.01	1.00	0.49	0.51	5
	Vegetation density	0.03	0.36	0.24	0.38	0.26	0.26	0.03	1.00	0.33	0.46	1
	Conspecific models	0.05	0.00	0.00	0.38	0.13	0.13	0.00	1.00	0.41	0.33	1
	Time of release	1.00	0.25	1.00	0.45	0.30	0.31	1.00	1.00	0.57	0.71	6

10.5.3. SINGLE BEHAVIOUR DATA SETS AND DECISION TREES

There were no trees with root and leaves for the target attribute behaviour of fights when other behaviours were excluded. Basking behaviour produced a decision tree with three branches (Table 10-3, Figure 10-1). Vegetation density was the first node, with more lizards basking in low vegetation density. In the high vegetation density the next branching node was soil disturbance in the matrix area. More lizards basked with undisturbed soil in the matrix. The final node was represented by time of release. With high vegetation density and disturbed soil in the matrix, more lizards basked when they were released in October, November and January but less lizards basked when released in December (Figure 10-1).

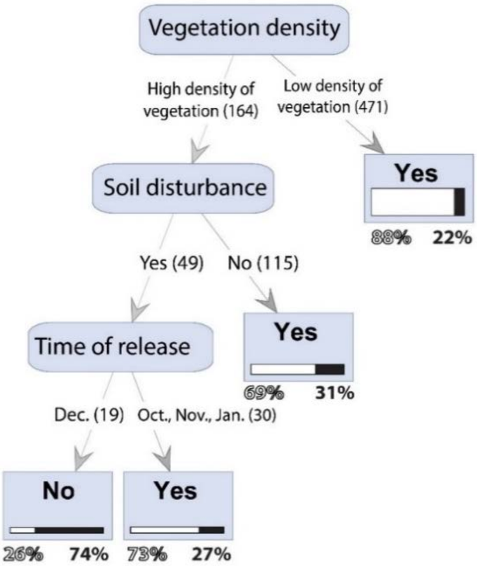

Figure 10-1. The random forest based decision tree for basking behaviour (bold Yes/No in gray box) when other behavioural parameters were excluded. The black and white bars with percentages represent the proportion of cases when lizards did bask (white) or did not bask (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

When movements around burrows was the target attribute a six branch tree with three of the nodes representing time of release was produced (Table 10-3, Figure 10-2). Soil disturbance in the matrix was the most important attribute, and the first node of the tree, with density of vegetation forming the next node, time of release the next three nodes, and confinement time, the least important of the branching nodes (Figure 10-2). The tree showed that soil disturbance in the matrix reduced the number of cases of lizards moving, and that, where soil was undisturbed, high vegetation density decreased the number of cases of lizards moving. There were fewer cases of movement in areas with low vegetation density in January than the other months, and in those other months more cases of movement in October. That October movement could be reduced more by one day than by five days of preliminary confinement to the release site (Figure 10-2).

When burrow changes was the target attribute a three branch tree was produced (Table 10-3, Figure 10-3). Supplementary food was the first node with less lizards changing their burrows when supplementary food was presented. Then time of release formed the next two nodes. Without supplementary food, there were fewer cases of lizards changing their burrows in January than other months, and in those other months more lizards changed burrows in October (Figure 10-3).

Dispersal produced a decision tree with four branches (Table 10-3, Figure 10-4). Soil disturbance in the matrix was the most important attribute, and the first node of the tree, with density of vegetation forming the second node, time of release the next node, and confinement time, the least important of the branching nodes (Figure 10-4). The tree showed that soil disturbance in the matrix reduced the number of cases of lizard dispersal (to 2%), and that, where soil was undisturbed, high vegetation density decreased number of case where lizards dispersed (to 5%). In areas with low vegetation density there were fewer cases of dispersal in November and December (4% of cases) than the other months, and in those other months (January and October) the number of cases of lizards dispersing could be reduced more by confining the lizards for one day than by for five days before removing the confinement to the release site (Figure 10-4).

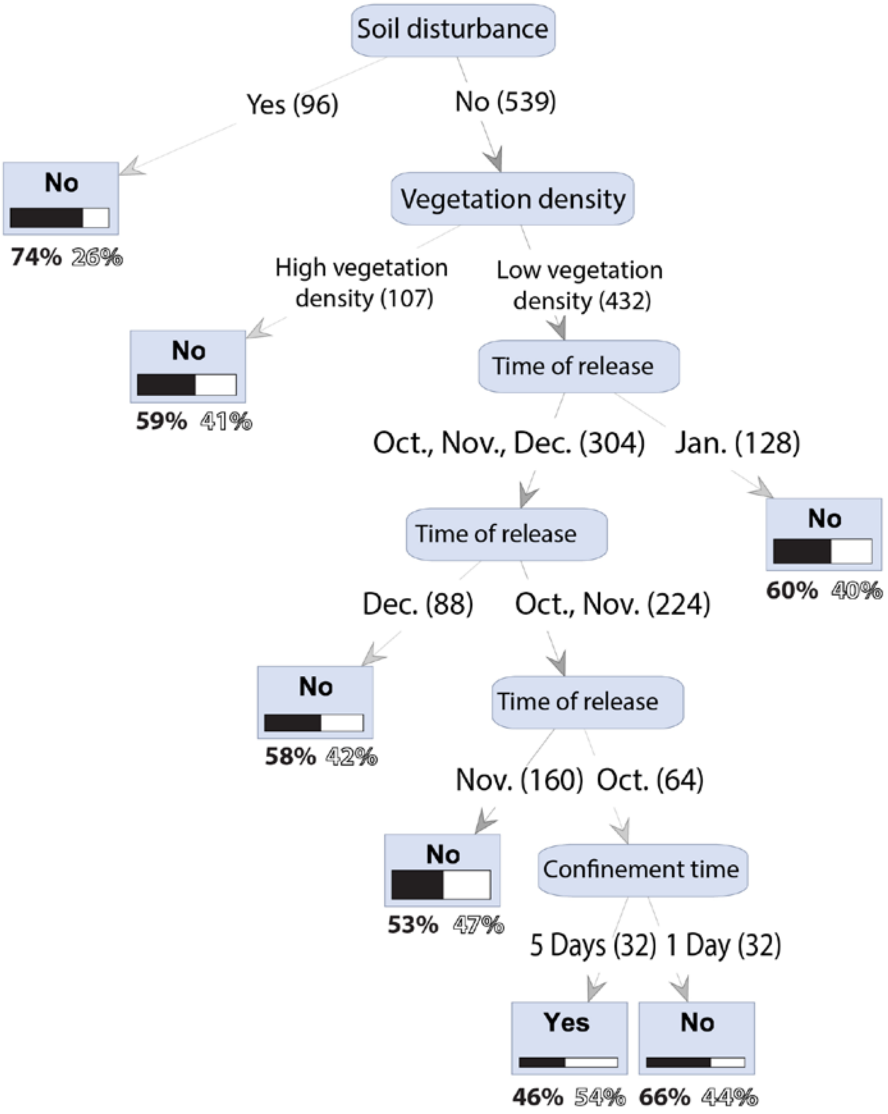

Figure 10-2. The random forest based decision tree for movements around burrows (bold Yes/No in gray box) when other behavioural parameters were excluded. The black and white bars with percentages represent the proportion of cases when lizards moved around burrows (white) or did not move (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

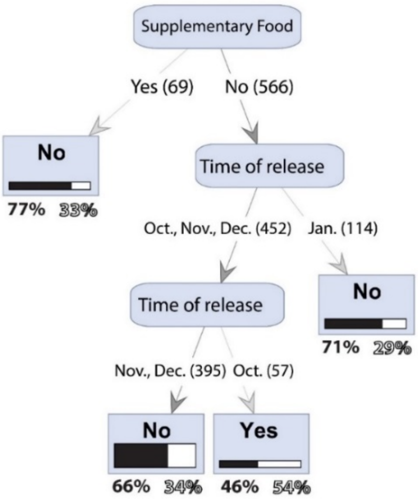

Figure 10-3. The random forest based decision tree for burrow changes behaviour (bold Yes/No in gray box) when other behavioural parameters were excluded. The black and white bars with percentages represent the proportion of cases when lizards did change burrows (white) or did not change burrows (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

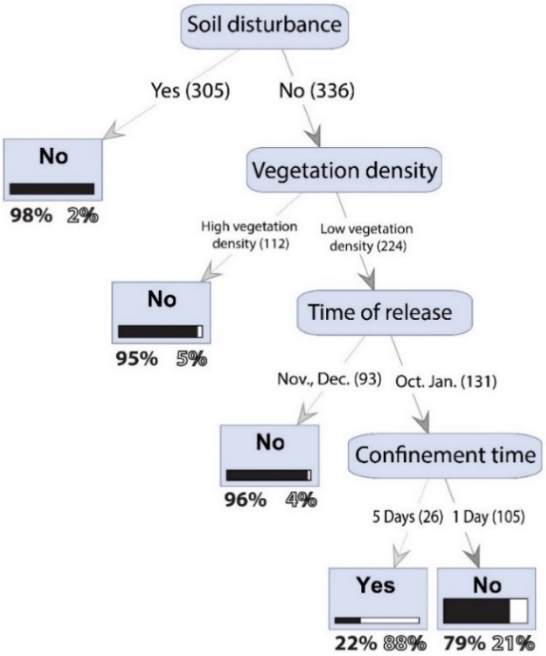

Figure 10-4. The random forest based decision tree for dispersal (bold Yes/No in gray box) when other behavioural parameters were excluded. The black and white bars with percentages represent the proportion of cases when lizards did disperse (white) or did not disperse (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

10.5.4. ALL BEHAVIOURS DATA SETS AND DECISION TREES

When basking behaviour was the target attribute the decision tree algorithm with gini index criteria produced a 14 branch tree. Because of this complexity, and because basking was not an important behaviour likely to affect translocation success we only include this tree as supplementary material.

Movements around burrows produced a decision tree with four branches (Table 10-3, Figure 10-5). Burrow change was the first node, with time of release forming the second node, fighting the next node and vegetation density the least important of the branching nodes. The tree illustrated that there were more cases of lizard moving around burrows among lizards that also changed their burrows. For those lizards that did not change their burrows there were fewer cases of movement around the burrow in January than other months (Figure 10-5), and in those other months, lizards that were not involved in fights showed fewer cases of movement around burrows (20%) than those that did fight. Among the fighters, there were no cases of lizards moving around their burrows in high vegetation density, but movement in 50% of cases in low vegetation density (Figure 10-5).

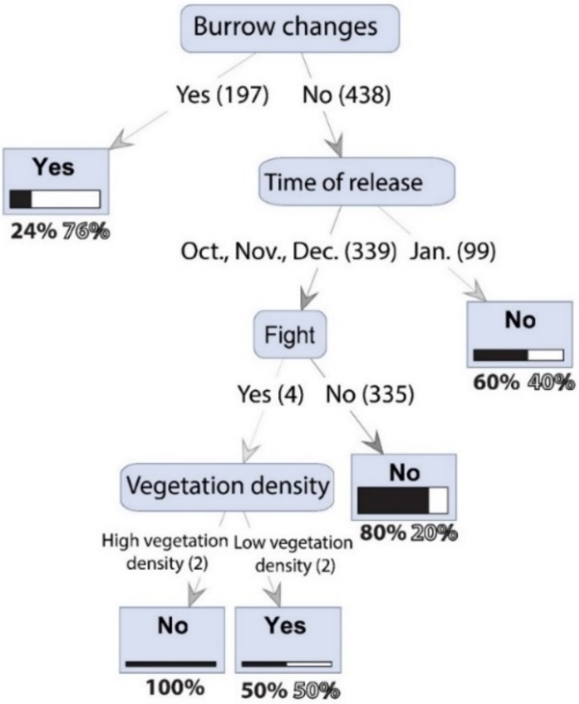

Figure 10-5. The random forest based decision tree for movements around burrows (bold Yes/No in gray box) when other behavioural parameters were included. The black and white bars with percentages represent the proportion of cases when lizards moved (white) or did not move around burrows (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

Changing burrows produced a decision tree with four branches (Table 10-3, Figure 10-6). As in Fig 5, the strongest relationship was between burrow changes and movements around burrows, but each of the branches from that first node had different secondary nodes. In cases where lizards did not move around burrows and did not have fights there were more cases of lizards not changing their burrow, although in 64% of cases where they did fight, they did change burrows. On the other branch, in cases where the lizards moved around the burrow, and did bask, they were more likely to change burrows, and among the non-baskers lizards were more likely to change burrows if not provided with supplementary food. Although this tree was quite complicated, and indicated the degree of complexity that these trees can generate, the major determining factor was whether or not a lizard moved around its burrow. The majority of leaves at the end of the branch for cases of no movement around the burrow, were for no change of burrow. Most leaves at the end of the branch for cases of movement around the burrow, were for a change of burrow (Figure 10-6).

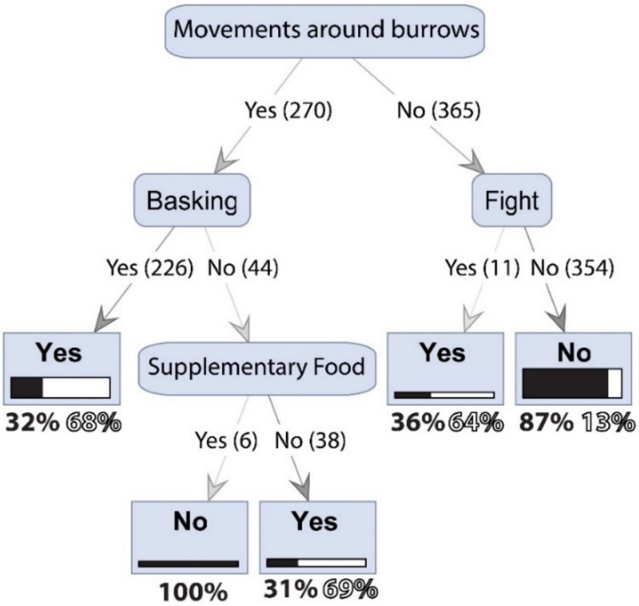

Figure 10-6. The random forest based decision tree for burrow changes behaviour (bold Yes/No in gray box) when other behavioural parameters were included. The black and white bars with percentages represent the proportion of cases when lizards changed burrows (white) or did not change burrows (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

When dispersal was the target attribute four decision tree models were produced with almost same CCI. A 17 branch tree was the main decision tree model for dispersal (Table 10-3, Figure 10-7) that cover three other small trees (three other models were added in supplementary materials). In successive nodes, there were more cases of dispersal with no matrix soil disturbance, then with low vegetation density, then in the months October, November and January (less dispersal in December), then with no supplementary food provided. Beyond the fifth node, although attributes were still important, branching became more complex and there were fewer cases at each node. There was still a tendency at multiple nodes in these higher branches for cases with lizard moving around their burrows to be less likely to disperse, and for cases where lizards changed burrows to be more likely to disperse.

The behavioural attribute of fighting produced a decision tree with four branches (Table 10-3, Figure 10-8). Dispersal was the first node, with time of release forming the second and last nodes and supplementary food an intermediate third node. The tree showed that cases of lizards fighting were less common among lizards that did not disperse. Among those that dispersed, there were fewer cases of fighting in October and January than other months. In those other months lizards that had supplementary food provided showed fewer cases of fighting (Figure 10-8), and in those did not have food there were more cases of fighting in November than December (Figure 10-8).

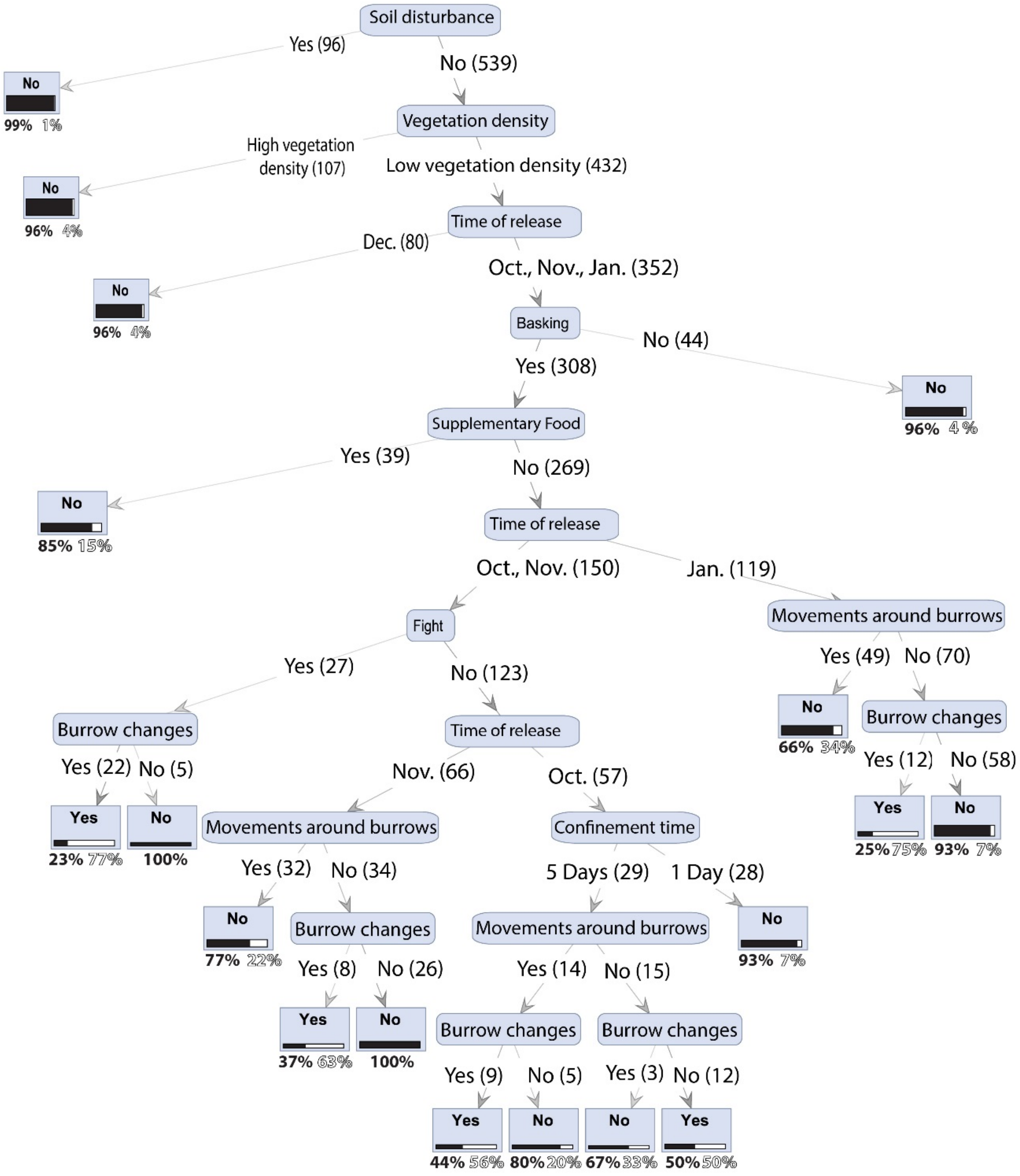

Figure 10-7. The decision tree for dispersal (bold Yes/No in gray box) when other behavioural parameters were included. The black and white bars with percentages represent the proportion of cases when lizards dispersed (white) or did not disprese (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

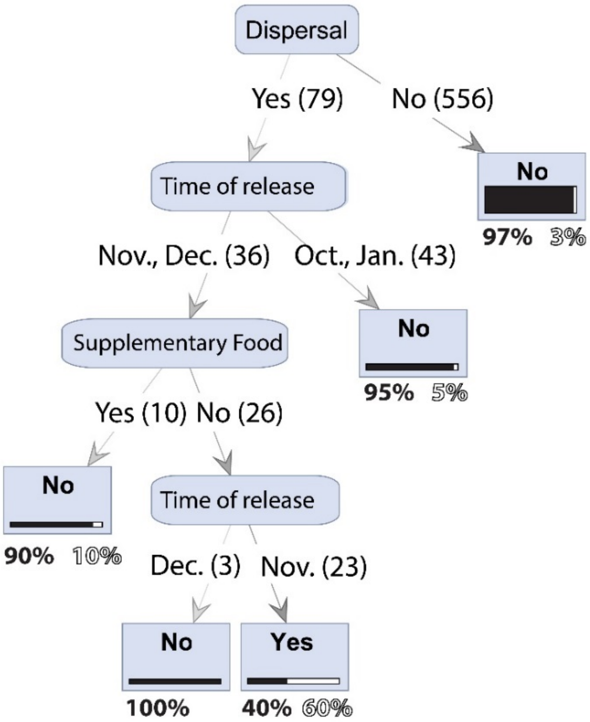

Figure 10-8. The random forest based decision tree for fight (bold Yes/No in gray box) when other behavioural parameters were included. The black and white bars with percentages represent the proportion of cases when lizards had fight (white) or did not have fight (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

Table 10-3. The properties of the eight decision tree models that were selected by the analysis. Name of data set is according their attribute weighting algorithms.

	Target attribute	Figure No.	Data set	Decision tree algorithm	Criteria	No. branches	No. leaves	CCI (%)
Single behaviour	Basking	1	Rule	Random forest	Gini index	3	4	82.2
	Movements around burrows	2	unweighted data	Random forest	Accuracy	6	7	61.0
	Burrow changes	3	SVM	Random forest	Gini index	3	4	67.0
	Dispersal	4	Info Gain	Random forest	Accuracy	4	5	87.0
All behavioural	Movements around burrows	5	Rule	Random forest	Gini index	4	5	64.0
	Burrow changes	6	unweighted data	Random forest	Gini index	4	5	73.0
	Dispersal	7	unweighted data	Decision tree	Accuracy	17	18	80.0
	Fight	8	Rule	Random forest	Info gain	4	5	93.0

10.6. DISCUSSION

10.6.1. MANAGEMENT IMPLICATIONS

For successful translocations managers need to provide a set of conditions that will encourage released individuals to stay close to the release site. For pygmy bluetongue lizards, behaviours that should indicate a likelihood to stay include basking at the burrow entrance, allowing thermoregulation and prey capture, reduced movement around the burrow (reducing exposure to predation), reduced burrow changes (again reducing exposure to predation and reducing the chance of not finding a new burrow) and reduced dispersal away from the release area. Our decision tree models with single behavioural attributes included gave indications of the sets of conditions that might promote all of those directions. We would also want to reduce the incidences of fighting among the released individuals, although no specific decision tree models provided advice on that when other behaviours were excluded from the data set. The most consistent factor influencing these behaviours in our trials was soil disturbance in the matrix around the release site. Essentially this is equivalent to a soft release in that soil disturbance made the matrix more inhospitable, making it more likely that lizards will stay in translocation sites. Milne (1999) showed that pygmy bluetongue lizards in natural habitats avoid burrows in ploughed areas and Souter (2003) showed lizard will not occupy artificial burrows in ploughed areas immediately next to population sites.

Vegetation density had an opposite effect on different behaviours in our decision tree models. Low vegetation density encouraged basking (positive for translocations), supporting observations of Pettigrew and Bull (2012). But low vegetation density also encouraged movement and dispersal (negative for translocations), as previously reported (Ebrahimi and Bull, 2013a). The effect of time of release in the decision tree models was consistent, with early release in October leading to more movement, more burrow changes, and more dispersal than other months. Perhaps times closer to the spring breeding season led more lizards to move about, confirming previous reports from field pitfall trapping (Schofield et al., 2012). Reducing dispersal from the release site is one of the main goals for translocations. For pygmy bluetongue lizards figure 4 shows the most important parameters that managers could use to decrease risk of dispersal in the early stage of translocation. Time of release, vegetation density, food supplementation and surrounding soil disturbance could all be manipulated to limit dispersal. According to our decision tree models, the presence or absence of conspecific models played a lesser role in influencing lizard behaviour. Although soil disturbance around the release site may have a short term benefit in reducing dispersal, in the long term, this may have adverse impacts in preventing the spread of an established translocation site. Our trees, based on short term behavioural changes, need to be balanced against longer term considerations.

10.6.2. BEHAVIOUR AND CONSERVATION

The decision tree models that included all behavioural attributes provide clues about relevant combinations of behaviour that may influence translocation success. The trees showed clear positive associations between movement around burrows and changing burrows. Lizards that emerged to move around their burrows more often were also more likely to move away and change their burrows. Lizards that were involved in fights were more likely to disperse. These and other relationships from the decision trees reflect the connections and interactions among the different types of behaviour that are related to successful settlement of released lizards. On the other hand there were few connections between basking behaviour and movements or dispersal, indicating that not all behaviours that we thought may be important are interconnected in influencing establishment success.

10.6.3. OVERVIEW

The main result of this study showed how decision trees with regards to animal behaviour open a new door for the study of conservation management and they give conservationists the opportunity to predict the behaviours of translocated species immediately after release. Some ethologists and behavioural ecologists believe that conservation biology lacks a theoretical backbone (Caro, 1999). Caro (2007) suggested that the interdisciplinary interface between behavioural ecology and conservation biology is the answer to many problems in conservation. For example, feeding condor chicks with condor-head-shaped puppets helped those chicks to be less attracted to the humans after release (Wallace, 2000), and translocations of black-tailed prairie dogs were more successful when the whole family, a behaviourally integrated unit, was translocated (Shier, 2006). The problem of identifying how species behaviour changes after release at translocation sites is difficult and conservation managers need to be able to identify which sorts of behaviours have negative impacts on the translocation success. Decision tree models can help us to predict which combined set of conditions can alter behaviour, which have the most influence, and which combinations work synergistically. This should give managers the opportunity to identify those behaviours that have negative or positive impacts on success, according to the models with all behaviour included. Then they could use the models to suggest interventions that could alter the negative behaviour. In addition models decrease the cost and time we need to spend to find out how and why species dispersed and directly led us to the parameters that caused specific behaviour. Developing those models before the actual translocation release might allow an improved success. As Regan et al. (2005) commented, conservationists must make decisions under severe uncertainty and decision models give the possible answer to respond to some, although not all of those uncertainties.

One problem is that not all endangered species will be as easy to work with as the pygmy bluetongue lizard. This is a small species (95 mm) with a very small normal activity range that can be easily confined within relatively small enclosures, and can be observed almost continuously around their burrows, to derive the behavioural parameters we used in this analysis. Nevertheless the benefits that are derived from the decision tree models suggest it is worth exploring ways of quantifying critical behaviours in a range of alternative conditions as background for translocation projects across a wide range of animal species.

10.7. ACKNOWLEDGEMENT

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10.8. SUPPLEMENTARY MATERIALS

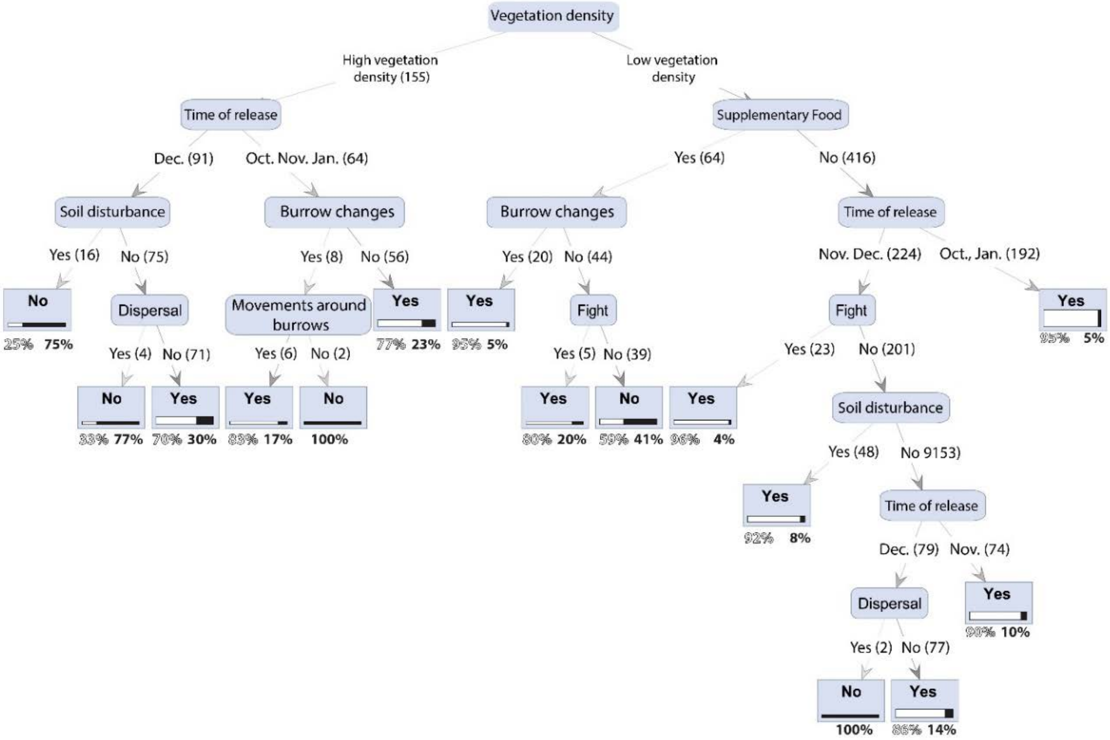

Figure 1 (supplementary material). The decision tree for basking behaviour (bold Yes/No in gray box) when other behavioural parameters were included. The black and white bars with percentages represent the proportion of cases when lizards did bask (white) or did not bask (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

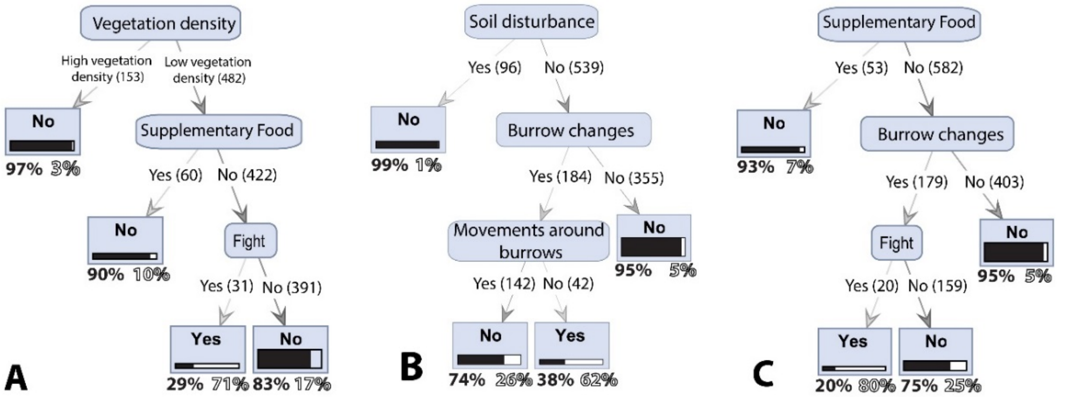

Figure 2 (supplementary material). A) The random forest based decision tree for dispersal (unweighted data set) bold Yes/No in gray box when other behavioural parameters were included, B) The decision tree paralle based decision tree for dispersal (SVM data set) and C) The random forest based decision tree algorithm for dispersal (rule data set). The black and white bars with percentages represent the proportion of cases when lizards did bask (white) or did not bask (black) in the specified set of experimental conditions. Number in bracets shows number of cases in each branch.

10.9. FUTURE RESEARCH SUGGESTIONS

This thesis has provided a basis for future research on conservation management and behavioural ecology. We have shown how manipulating habitat factors and resources at the release site can change the behavioural response of translocated lizards in the early stages after simulated release into translocation sites.

Further research with more individuals, with behavioural observations over longer time periods after release, and in the bigger areas could help us to have more data to compare with our results. The project may also be expanded to explore responses to release sites by different species, although the sedentary behaviour and small spatial requirements of the pygmy bluetongue lizards made them ideal for the relatively small experimental arenas we used in this series of trials. The results emphasized the importance of including behaviour as well as numerical dynamics to understand the success or failure of a translocation event. Some preliminary results also suggested that different behavioural types within the population may be differentially suitable as the source of translocated individuals. That aspect of behavioural ecology of the lizard needs further investigation. We used alternative states of individual environmental parameters to compare lizard behaviours, and future research may consider exploring combinations of these environmental factors to give us more information about how lizards react to changes in translocation sites.

Also the relationship between spiders and lizards needs to be better studied because spiders both benefit lizards by producing important burrow resources, but they also have the ability to kill them. Finding alternative natural burrows, or designing better artificial burrows for pygmy bluetongue lizards is highly important because of the shortage of suitable burrows in natural populations. Strategies for identifying translocation sites with adequate burrow densities, or for using available management teams to construct and maintain banks of artificial burrows will be important.

If translocation is to become one of the long-term strategies to conserve pygmy bluetongue lizards, then genetic diversity and gene flow of wild population should be studied before any translocation, so that appropriate source populations and suitable individuals can be identified for translocation.

Briefly, this thesis has provided new details of behavioural changes in simulated translocations of an endangered lizard. We produced decision trees from these detailed data which could enable us to predict lizard behavioural response at the translocation site under different circumstances. This information will help us to prevent dispersal from translocation sites and reduce the risk of predation, by controlling behaviours that lead to dispersal or to predation exposure.

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