Behavioural factors affecting translocation success in the pygmy bluetongue lizard

by

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Contents

Thesis Abstract

Translocations are one method to conserve endangered species, but translocation success must be improved, especially for translocations of captive-sourced animals. Some reasons for translocation failure include selecting inappropriate age classes or individuals lacking foraging and predator avoidance skills. Selecting appropriate age classes is important, as behaviour can vary over life stages, making some life stages more likely to disperse or be preyed upon. Captive animals are more likely to lack foraging and predator avoidance skills, as the captive environment can be too simplistic and exclude predators to ensure the survival of the captive animals.

The pygmy bluetongue lizard (*Tiliqua adelaidensis*), is an endangered lizard that will require translocating to mitigate the risks of climate change and habitat loss when the current range of distribution becomes unsuitable in future. A captive breeding population can supply stocks for future translocations, but there are knowledge gaps in age specific behaviours and the effect of captivity on foraging ability and predator avoidance.

The primary aim of this thesis is to investigate factors that affect translocation success to improve the outcome of translocations, using the pygmy bluetongue lizard as a case study. I investigated behavioural differences firstly, between neonates and adults and secondly, among juvenile, immature and adult age classes to identify suitable age classes for translocation. I compared foraging ability and body condition between lizards maintained under a hand-feeding regime versus lizards that foraged on released crickets. The effect of captivity on predator recognition behaviour was investigated, comparing captive born, wild born and wild lizards to a range of reptile scents. I also investigated grass use in the pygmy bluetongue lizard, which had not been studied previously and may be an important factor in selecting appropriate translocation sites.

My results show that neonate behaviour differs significantly from adults, particularly basking and burrow movements, which makes the neonate age class less suitable for translocations. I found that pygmy bluetongue lizard behaviour changes ontogenetically, with activity levels highest in early life stages, progressively decreasing with age toward adulthood. Foraging ability and body condition were similar between the hand-fed and self-fed regimes, however hand-feeding was found to alter behaviour in a way which may increase predation risk in lizards released into the wild. Pygmy bluetongue lizards were found to innately recognise predator chemical cues, however they did not show avoidance behaviour, suggesting that other cues may be required to elicit a response. Grass tussocks were found to be an important temporary refuge for lizards when they are moving around out of burrows.

The research presented in this thesis contributes significantly to our knowledge of pygmy bluetongue lizard behavioural ecology, filling in gaps on age specific behaviours, habitat use and the effects of captivity on necessary life skills. My findings are important for future conservation management of this species and will improve translocation success. However, my research also has broader implications, and can inform captive management and translocation of other endangered species. It also highlights the importance of the captive environment in providing opportunities to study behaviour in species that can be hard to monitor in the wild and can reveal undiscovered behaviours.

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.

Signed:

Tara Daniell

Date 13/07/2020

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Animal Welfare approval

All research procedures were approved by the Flinders University Animal Welfare Committee (permit numbers: E439/16, E440/16, E453/17) and the Wildlife Ethics Committee (project number: 28/2017).

Preface

Chapter 1 and Chapter 7 are the thesis general introduction and discussion chapters respectively. As there are introduction and discussion sections to all data chapters (Chapters 2 to 6), these general chapters will cover the broad thesis topics but in less detail to avoid repetition. The data chapters are either published or in the submission process, hence there are differences in writing style between chapters to meet journal requirements and as there are co-authors, ‘we’ rather than ‘I’ is used, however I am the primary author for each chapter. In order to maintain flow, formatting and reference style have been made consistent for all data chapters.

Chapter 3 has been published in Austral Ecology, chapter 2 is in preparation and chapter 4, 5 and 6 have been submitted to journals for consideration. The following chapter – Chapter 1, provides a brief background of translocations, the factors that affect translocation success and the natural history of the pygmy bluetongue lizard.

Chapter 1.

Thesis Introduction

General introduction

Earth is currently in the midst of the sixth mass extinction event, different from previous mass extinctions in that it is caused by anthropogenic activity (Pimm et al., 2014, Ceballos et al., 2015, Ceballos et al., 2017, Shivanna, 2020). Anthropogenic factors threatening species include climate change, habitat loss, fragmentation, habitat degradation, pollution, overexploitation, exotic predators, exotic competitors and diseases (Fahrig, 2003, Frankham, 2008, Moritz and Agudo, 2013, Ceballos et al., 2017). Species can survive changing environmental conditions by adapting to the changing environment or dispersing to more suitable habitat (Nogués-Bravo et al., 2018). However, species in fragmented habitats or species with limited dispersal may require human-mediated movement such as translocations. It is now more important than ever to conserve biodiversity before it is too late. There are a broad range of actions that must be taken to conserve threatened fauna and flora species however this thesis focuses on animal translocations and the management of captive populations.

Translocations and captive breeding

The aim of many captive breeding programs is the release of offspring back into the wild, and one method to do this is by providing stocks for translocations. As defined by the IUCN Reintroduction and Invasive Species Specialist Group, a translocation is the human-mediated movement of living organisms from one area with release in another (IUCN/SSC, 2013). Translocations may involve animals from wild or captive sources. Reinforcement, augmentation, supplementation or re-stocking is defined as the intentional movement and release of an organism into an existing population of conspecifics, while a reintroduction is defined as the intentional movement and release of an organism inside its indigenous range from which it has disappeared (IUCN/SSC, 2013). Conservation introductions are defined as the intentional movement and release of an organism outside its current indigenous range (IUCN/SSC, 2013).

Captive breeding can conserve species that cannot be protected in the wild or need additional assistance. Captive breeding programs have three main purposes — safeguarding a species in captivity whilst threats in the wild remain (Connolly and Cree, 2008), establishing a source population for translocations or reintroductions (Seddon et al., 2007), and rearing vulnerable life stages before release into the wild — known as captive rearing, head-starting or supportive breeding (Heppell et al., 1996, Ford, 2002). For example, a captive insurance population of the Tasmanian devil (*Sarcophilus harrisii*) is being maintained to safeguard the species from the devil facial tumour disease (Keeley et al., 2012), Mexican wolves (*Canis lupus baileyi*) and red wolves (*C. rufus*) have been bred in captivity for reintroduction (Hedrick and Fredrickson, 2008) and many fish, reptile and insect species undergo head-starting to supplement wild populations (Philippart, 1995, Adamski and Witkowski, 2007). Reptile and amphibian species generally have low parental care, high fecundity and low juvenile survival making them suited to head-starting (Heppell et al., 1996). However, it should be noted that in some reptile species, capturing juveniles during surveys can be difficult, causing misleading estimates of survival rates (Pike et al., 2008).

Vertebrate species such as fish, amphibians, reptiles and mammals have been captive bred successfully (Mawson, 2004, Frankham, 2008, Germano and Bishop, 2009). Amphibians and reptiles are suitable candidates for captive breeding as they are highly fecund with few behavioural issues, such as stereotypic behaviours, arising due to the captive environment (Griffiths and Pavajeau, 2008, Pavajeau et al., 2008, Germano and Bishop, 2009). The number of amphibian species involved in captive breeding or reintroduction programs has increased by 57%

(77 species) over the seven years since the release of the IUCN Amphibian Conservation Action Plan, with a large focus on securing captive assurance populations (Harding et al., 2016). Many reptile species have been bred successfully in captivity (Pedrono and Sarovy, 2000, Moore et al., 2007, Mendyk, 2012). A captive breeding program has prevented the extinction of two Christmas Island species which have not been sighted in the wild since 2012 and 2010 respectively, Lister's gecko (*Lepidodactylus listeri*) and the blue-tailed skink (*Cryptoblepharus egeriae*) (Andrew et al., 2018). Unfortunately, this program was too late to save another species, the Christmas Island forest skink (*Emoia nativitatis*), as only females could be captured which have all since died (Andrew et al., 2018).

There are genetic and behavioural issues that can arise from keeping wild animals in captivity, including a loss of genetic diversity, inbreeding, genetic adaptation to captivity, abnormal behaviours and a loss of life skills. Maintaining genetic variability and minimising inbreeding are important to maintain fitness, evolutionary potential and give populations the best chance of survival when reintroduced to the wild (Frankham, 1995). It is important to avoid genetic adaptation to captivity, as genotypes that may be beneficial in the wild may be selected against in captivity, causing negative impacts when captive bred individuals are reintroduced into the wild (Frankham, 2008, Montgomery et al., 2010). Genetic adaptation to captivity can be minimised by reducing the number of generations held in captivity, fragmenting captive populations and allowing gene flow by crossing captive populations (Frankham, 2008). Abnormal behaviours can arise in captivity, which can be reduced by providing enrichment and replicating natural habitat in enclosures to improve animal welfare (McDougall et al., 2006, Mason, 2010). The captive environment can result in a loss of natural foraging and predator avoidance skills (DeGregorio et al., 2013, Jolly et al., 2018) which can reduce survival in animals released into the wild.

Captive bred animals can have reduced survival in translocation projects compared to wild sourced animals. Translocations of captive-bred individuals have higher failure rates (Fischer and Lindenmayer, 2000, Jule et al., 2008). Rummel et al., (2016) found that translocation success was significantly more affected by the origin of individuals (wild versus captive-bred) than the number of released animals or program duration. Translocations were more successful when using wild fish and mammals and to a lesser extent birds, however, in an opposite trend, translocation success was higher when using captive-bred herpetofauna compared to wild herpetofauna, possibly due to the benefits of captivity and high physiological and behavioural plasticity compared to other taxa (Rummel et al., 2016). The results suggest using wild-caught fish, mammals and birds for translocations, but captive-bred herpetofauna and when this is not possible the best alternative is long project duration of 10 to 30 years (Rummel et al., 2016). Potential reasons for reduced survival of captive released animals include a lack of fear towards humans and a lack of life skills – social, foraging and predator avoidance (Jule et al., 2008, Rummel et al., 2016).

Despite translocations of captive-bred animals being less successful than wild animal translocations, there have been some successes suggesting it is a viable conservation strategy. For example, the introduction of captive-bred dibblers (*Parantechinus apicalis*) onto Escape Island in Western Australia resulted in breeding and dispersal of young in the first year of release and the following three years post-release monitoring (Moro, 2003). Successful reintroductions have occurred of captive bred marsupial and reptile species in Western Australia (Mawson, 2004) and for wolves (Hedrick and Fredrickson, 2008) and the Sonoran pronghorn (*Antilocapra americana sonoriensis*) in America (Horne et al., 2016). Survival was similar between wild-reared and captive-reared groups for the takahē, (*Porphyrio hochstetteri*) a flightless bird in New Zealand (Maxwell and Jamieson, 1997) and Mauritius kestrel (*Falco punctatus*) (Nicoll et al., 2004). Genetic monitoring of two small freshwater fish species (*Nannoperca obscura*) and (*Nannoperca australis*) found that released captive-bred fish had survived and reproduced in the wild (Attard et al., 2016). In South Australia’s Arid Recovery Reserve, one reptile and five locally extinct mammals were reintroduced with mixed success. Reintroduction of the greater stick-nest rat (*Leporillus conditor*), burrowing bettong (*Bettongia lesueur*), greater bilby (*Macrotis lagotis*) and western barred bandicoot (*Perameles bougainville*) were all successful based on short and medium-term success criteria (Moseby et al., 2011). However, reintroduction of the Woma python (*Aspidites ramsayi*) and numbat (*Myrmecobius fasciatus*) failed due to predation by the native mulga snake and avian predators respectively (Moseby et al., 2011). A lack of predator avoidance ability in captive-bred animals can result in translocation failure, but not all species have this issue, thus further research is required.

Evidence suggests that translocations using captive-bred herpetofauna can be a useful conservation method. Germano and Bishop (2009) reviewed amphibian and reptile translocations between 1991 and 2006 and found no significant difference in success rates between captive-bred and wild sourced individuals. A study that monitored wild-born versus captive-bred lacertid lizard *Psammodromus algirus* in fragmented habitat found that captive-bred lizards were larger, dispersed more frequently among nearby fragments and showed similar or higher values of survival and activity compared to the wild born lizards (Santos et al., 2009). In Suter’s skink (*Oligosoma suteri*), captive-reared lizards that were larger with lower body condition (leaner) had higher survival, suggesting that body size and condition are good indicators for selecting translocation candidates for this species (Hare et al., 2020). Additionally, captive-bred iguanas were successfully reintroduced on islands in the Galapagos (Tzika et al., 2008). A translocation of the Otago skink (*Oligosoma otagense*), to a fenced mammalian predator-free ecosanctuary was successful with lizards surviving and breeding within 15 months (Bogisch et al., 2016), although another translocation of captive sourced Otago skinks to a fenced ecosanctuary had variable results due to mice preying upon lizards (Norbury et al., 2014). Studies have indicated that translocations of captive individuals of the threatened ploughshare tortoise (*Geochelone yniphora*) and the desert tortoise (*Gopherus agassizii*) could be viable conservation strategies (Pedrono and Sarovy, 2000, Field et al., 2007). The previous successes suggest that captive breeding of reptiles for translocations and reintroductions could be a successful conservation strategy. However, there are some important knowledge gaps that need attention to improve translocation outcomes.

This thesis focuses on the effect of captivity on important life skills – foraging ability and predator avoidance, and age-specific behavioural shifts that may mean some age classes are more suitable candidates for translocation. Although the captive environment can have negative effects, it can also allow the study of behaviours that are hard to observe in the wild, such as age-specific behavioural shifts.

Age specific behavioural shifts

Behaviour can change ontogenically – thus some age classes may be more appropriate for translocating. Behavioural characteristics and age specific variations can vary from species to species (Letty et al., 2007). The behaviours that vary among age classes can be related to dispersal, migration, activity levels, foraging and predator avoidance (Morafka et al., 2000, Letty et al., 2007, Campioni et al., 2020). It is therefore important to study the behaviour throughout all life stages, especially for species subject to conservation management actions.

In many species, neonate and juvenile age classes are dispersers (Morafka et al., 2000, Bowler and Benton, 2005, Laarman et al., 2018, Delisle et al., 2019, Moss et al., 2020). Dispersal away from the release site is a major cause of translocation failure (Germano and Bishop, 2009), hence age classes that have high dispersal tendencies may be inappropriate candidates for translocation if they disperse out of suitable habitat. It has been suggested that life stages that naturally disperse are good candidates for translocation, as they can disperse within the release site and are less likely than adults to have homing tendencies (Letty et al., 2007, Germano and Bishop, 2009). Although adults are more likely to display homing behaviour, the use of soft-release techniques that allow animals to become familiar with the release site have proven successful in reducing homing tendencies (Tetzlaff et al., 2019). Furthermore, juvenile dispersal distances in a translocation situation may be greater than natural juvenile dispersal. For example, reintroduced juvenile Spanish imperial eagles (*Aquila adalberti*) were found to disperse greater distances compared to naturally dispersing wild juveniles (Muriel et al., 2015). Mortality risk has been found to increase with greater dispersal distances (Johnson et al., 2009), hence dispersal tendencies must be considered when selecting candidates for translocation.

Age-specific behavioural differences can result in differences in predator risk and avoidance behaviours among age classes. Differences in activity levels among age classes may result in certain age groups being more at risk of predation, particularly if such activity involves foraging or moving around in open spaces or away from refugia. Activity levels can be higher in neonate and juvenile age classes, possibly due to the need to acquire resources for growth (Sinervo and Adolph, 1994, Morafka et al., 2000, Watters, 2009). Foraging behaviour can also differ among age classes in terms of diet, foraging method, foraging site fidelity and frequency (Wikelski and Trillmich, 1994, Morafka et al., 2000, Schiel and Souto, 2017, Votier et al., 2017). Predator avoidance behaviour also differs among age classes. In the Iberian rock lizard (*Lacerta monticola*), juveniles have shorter approach distance than adults despite juveniles having slower sprint speeds which increases predation vulnerability (Martín and López, 2003). The shorter approach distances in juveniles could be a result of a trade-off between predator avoidance and thermal costs of refuge use or due to the smaller body size of juveniles making them less visible to predators (Martín and López, 2003). In reptiles, neonates and juveniles have been found to display distinct antipredator behaviours such as freezing, erratic escape routes, and tail vibrations (Morafka et al., 2000). Differences in predator risk and predator avoidance behaviours between age classes could affect translocation success if not taken into consideration, thus knowledge of age-specific differences is important. It is also important to research the effect of the captive environment on life skills to ensure translocation candidates will be able to forage and avoid predators successfully in the wild.

The effect of captivity on foraging ability

Captive environments provide animals with a steady food supply, reliably available refuges and a predator-free environment. This is conducive to enhanced survival, reproduction and growth rates, but it can result in individuals that lack foraging and predator avoidance skills and are ill equipped for reintroduction to the wild. Captive bred animals must have the skills to hunt or forage successfully and efficiently in order to survive when released into the wild. Inexperience with foraging was suggested as a potential reason for increased vulnerability to predation in a reintroduction of the woma python (*Aspidites ramsayi*) (Read et al., 2011) while starvation resulted in mortalities in translocated captive-born carnivores and reptiles (Jule et al., 2008, Nafus et al., 2017). In captive giant pandas, (*Ailuropoda melanleuca*) captive-born and wild-born pandas displayed the same feeding behaviours, however captive-born pandas spent more time feeding on less nutritious parts of bamboo compared to the wild-born pandas (Swaisgood et al., 2018). Time spent in captivity can also affect foraging ability, as wild-caught ratsnakes (*Elaphe obsoleta*) were found to be less successful and slower to react to prey the longer they were kept in captivity (DeGregorio et al., 2013). As foraging ability can be altered by captivity, it is important to research the foraging ability of potential translocation candidates to determine if the captive environment has reduced foraging ability and identify the best husbandry methods.

Providing captive animals with more naturalistic environments can promote natural foraging behaviour and improve survival when released into the wild for some species. Juvenile captive black-footed ferrets (*Mustela nigripes*) were more likely to make a successful kill as they matured and gained more experience, if they were raised in an enriched environment that encouraged foraging behaviour (Vargas and Anderson, 1999) and ferrets conditioned in quasi-natural outdoor pens had higher survival post-release compared to those in cages with or without prior experience with live prey (Biggins et al., 1999). Orphaned wild southern sea otter pups (*Enhydra lutris nereis*) raised by surrogate otters in captivity developed foraging skills earlier than pups raised in isolation (Nicholson et al., 2007). Survival of captive surrogate-reared juveniles was comparable to wild-reared sea otters, whereas survival of pups not reared by surrogates was significantly less than wild cohorts (Nicholson et al., 2007). However, in captive-reared Tasmanian devils (*Sarcophilus harrisii*), survival and body condition upon release did not differ between more simplistic captive facilities and naturalistic free-range enclosures (Rogers et al., 2016) and foraging ability did not differ between juvenile Atlantic salmon (*Salmo salar*), reared in standard versus enriched hatchery conditions (Hatanpaa et al., 2020). It is therefore important to determine the effects of captivity on foraging ability for animals that may be used for reintroductions, as effects vary widely among species.

The effect of captivity on predator avoidance

Captivity not only affects foraging ability, it can also alter predator avoidance behaviour in animals. Isolation from predators can result in the reduction or loss of predator avoidance behaviours (Jolly et al., 2018, Muralidhar et al., 2019). In oldfield mice (*Peromyscus polionotus subgriseus*), the more generations mice have spent in captivity, the less likely they are to seek refuge upon sighting a predator, and the more variable predator-response behaviors become (McPhee, 2003). In the Mallorcan midwife toad (*Alytes muletensis*), although anti-predator responses were retained in the absence of predator selection pressures in captivity, comparisons between short and long term captive populations showed anti-predator responses started to degenerate after nine to twelve generations (Kraaijeveld-Smit et al., 2006). It is therefore vital to determine predator avoidance ability of captive animals that are to be released into the wild to avoid high mortality rates.

Predation has been attributed as a major cause of mortality in releases of captive animals (Jule et al., 2008, Maran et al., 2009, Hamilton et al., 2010, Rummel et al., 2016). A reintroduction using captive-bred Woma pythons (*Aspidites ramsayi*) failed as all individuals were killed by the native Mulga snake, despite efforts to rear woma pythons to a size where predation was less likely (Read et al., 2011). Predation by mountain lions was the primary cause of mortality in reintroduced bighorn sheep (*Ovis canadensis*), (Ostermann et al., 2001). Captive-born Vancouver Island marmots (*Marmota vancouverensis*) were shown to have higher mortality compared to wild-born marmots with predation being a major cause, and captive-born marmots were more vulnerable to avian predation (Aaltonen et al., 2009). While the authors suggest exposing captive marmots to avian predators before release to enhance survival, they cautioned it would prove expensive and logistically challenging to implement, and the benefits were unproven (Aaltonen et al., 2009).

Predator naive animals lack the ability to recognise predators and respond with effective predator avoidance behaviours (Banks and Dickman, 2007). Exposing naive captive animals to potential predators to enhance predator avoidance behaviours prior to release, is a possible method for reducing mortality rates in translocations using captive animals, thus improving translocation success. McLean et al., (1999) showed that conditioning to predators was possible in New Zealand robins (*Petroica australis*), where young predator-naive birds learnt to respond fearfully whether they were free-living with their parents or on their own in captivity. Juvenile black-tailed prairie dogs (*Cynomys ludovicianus*) successfully underwent training by exposure to a range of predators in conjunction with prairie dog alarm vocalisations, which enhanced anti-predator behaviour in captivity, and survival upon release in the wild (Shier and Owings, 2006). However, some species can innately recognise predators despite being captive-born and lacking previous experience with predators (Martín et al., 2015, Landová et al., 2016). It is therefore important to determine if captive animals can innately recognise predators or not, particularly for species in which predator recognition has not been studied previously or the species has been held in captivity for many generations, as naive animals may require training to improve translocation success.

Habitat use

Habitat fragmentation and degradation or loss are major threats to species worldwide (Böhm et al., 2013, Clemann, 2015). Habitat use can relate to foraging, shelter, seeking refuge from predators or temperature extremes and reproduction. Habitat requirements vary among species, sexes and age classes (Ramírez-Macías et al., 2017, Delisle et al., 2019). Understanding habitat use and resource requirements of a species is critical for conservation management and captive husbandry. In some species that are cryptic or hard to observe in the wild, captive populations can be used to learn more about behaviour and habitat requirements (Prystupczuk et al., 2019).

In grassland ecosystems, vegetation provides animals with cover from predators, foraging sites and basking sites (Olsson, 2001, Block et al., 2012). Vegetation can also be used as temporary refuges from potential predators as animals move through the habitat for foraging or other activities (Block et al., 2012). Other refugia commonly used by a range of species include burrows, rocks or logs (Michael et al., 2004, Webb and Whiting, 2005, Jarvie et al., 2016, Nafus et al., 2017). Habitat clearing or overgrazing can reduce vegetation cover, which can have adverse impacts on species diversity, abundance and dispersal for vertebrate and invertebrate species (Howland et al., 2014, Ebrahimi and Bull, 2015, Bröder et al., 2019). Invasion by non-native plant species can also have detrimental impacts. Predation risk increased for locusts (*Locusta migratoria migratoria*) in pasture in which vegetation complexity was reduced due to invasion by structurally simple grassland species, compared to locust in structurally complex native tussock grasses (Norbury and van Overmeire, 2019).

Pygmy bluetongue lizards

Distribution and habitat

The pygmy bluetongue lizard (PBT) (*Tiliqua adelaidensis*) was thought to be extinct before its rediscovery in the stomach of a road-killed eastern brown snake near Burra in mid-north South Australia in October 1992 (Armstrong and Reid, 1992). Records show the pygmy bluetongue lizard had a historical range that ran from the mid-north of the state near Burra to the Adelaide Plains (Souter et al., 2004). The current distribution is restricted to isolated populations in native grassland, mostly on farming properties, in the mid-north region of South Australia (Milne et al., 2002). Summers are hot and dry and winters are cool and moist with an average annual rainfall of 446 mm (Milne et al., 2002). The grassland species consist of endemics including spear grasses (*Austrostipa* spp.), wallaby grasses (*Danthonia* spp.), wingless bluebush (*Maireana enchylaenoides*), and brush wire-grass (*Aristida behriana*), while common exotic species include thread iris (*Gynandriris setifolia*), onion grass (*Romulearosea* sp.), wild oats (*Avena barbata*), rats tail (*Vulpia myuros* f. *myuros*) and storks bills (*Erodium* spp.) (Souter et al., 2007).

Pygmy bluetongue lizards inhabit narrow, single entrance, vertical burrows constructed by lycosid and mygalomorph spiders (Hutchinson et al., 1994). They spend most of their time in the burrows which provide a site to bask and ambush prey or shelter from predators and temperature extremes (Milne et al., 2003a). Lizards prefer burrows 30 cm or deeper and it is likely that lizards compete for this limited resource (Milne and Bull, 2000, Fellows et al., 2009). Pygmy bluetongue lizards will use artificial burrows, which can be used to supplement natural burrows and enhance population density, with lizards generally preferring vertical burrows of the narrowest size they can fit into and adults preferring deeper burrows than juveniles (Milne and Bull, 2000, Souter et al., 2004). The PBT appears to display a central-place territorial defence system, defending their home burrow from conspecific models placed near their burrow (Fenner and Bull, 2011a). Pygmy bluetongue lizards use scats as social signals to indicate burrow occupancy to conspecifics by scatting in the direction of their nearest neighbours (Fenner and Bull, 2010, Ebrahimi et al., 2016). They will take longer to inspect a burrow before entering if a scat is present and will respond more cautiously if the scat is from a male rather than a female, and are more likely to choose a vacant burrow with a nearby scat as it indicates previous occupancy and burrow quality (Fenner and Bull, 2011b). Pygmy bluetongue lizard populations are a mix of dispersers and residents — dispersers that occupy a burrow for a short time, and residents that can occupy the same burrow over a season or consecutive years (Bull et al., 2015). Females are more likely to be residents than males, and most dispersal happens in early spring when males search for mates (Bull et al., 2015). However, dispersal is restricted and populations are within fragmented habitat, preventing lizards from dispersing to other populations (Smith et al., 2009).

Morphology and diet

Pygmy bluetongue lizards (Figure 1) display sexual dimorphism, with adult males (93.4 mm mean snout to vent length — SVL) on average being shorter than females (98.3 mm mean SVL) and having a larger head size than females (Hutchinson et al., 1994). Body condition in the PBT varies substantially from year to year and the causes are uncertain (Shamiminoori et al., 2014). Body condition was generally higher earlier in the season, which could be explained by ecological factors such as prey abundance, and males generally had a higher body condition than females (Shamiminoori et al., 2014). Pygmy bluetongue lizards are omnivorous, consuming mostly invertebrates and some plant material. Consumption of invertebrates such as grasshoppers and spiders is reduced from spring to summer, while plant consumption increases (Fenner et al., 2007, Ebrahimi et al., 2015).

Figure 1: Pygmy bluetongue lizard during monthly capture to obtain body measurements.

Reproduction

Mating in PBTs occurs during October and November, when males leave their burrows and search for females (Schofield et al., 2013). Female movements away from the burrow and returning to the burrow along the same path have been observed in October but not in other months, and these specific movements are thought to be used by females to attract mates by laying an olfactory signal along the path (Ebrahimi et al., 2014). Males were observed approaching female burrows in October following the same path the female had previously taken and attempting to mate (Ebrahimi et al., 2014). Offspring are born in late January to mid-March and receive minimal parental care as neonates disperse from the natal burrow within weeks after birth (Milne et al., 2002). A comparison of female fitness in artificial burrows and natural burrows in a natural population of PBT, found females in artificial burrows had better body condition and gave birth to offspring that were heavier and in better body condition than females in natural burrows (Milne et al., 2003b).

Simulated translocation experiments

A range of simulated translocation experiments have been conducted on the PBT to investigate factors that could improve translocation success including supplementary feeding, resource distribution, short-term confinement, conspecific cues and time of release. When lizards were provided with supplementary food they were found to be more likely to remain in a burrow, basked less, moved around on the surface less, and were less likely to disperse from the central release area (Ebrahimi and Bull, 2012). Lizards that are on the surface are at greater risk of predation, therefore supplementary feeding could improve translocation success by reducing lizard movements and dispersal, hence reducing predation risk and promoting establishment at the release site (Ebrahimi and Bull, 2012). A simulated translocation testing the effect of resource distribution on dispersal found that lizards changed burrows less, but made more movements around burrows and moved further when changing burrows when burrow density was low and that lizards released closer together changed burrows more often and had more agonistic interactions than lizards released further apart (Ebrahimi and Bull, 2014a). These results suggest that lizards may be more likely to remain in a release area if burrow densities are high and lizards are released at least 2.5 m apart because any exploratory moves will be shorter and conspecific aggression and stress lower (Ebrahimi and Bull, 2014a). Short-term confinement (five days) and the presence of conspecific cues resulted in lizards basking less — an indication of stress, and an increase in movements from the burrow and out of the release area which could equate to dispersal in a real translocation (Ebrahimi and Bull, 2013, Ebrahimi and Bull, 2014c). Short-term confinement and the presence of conspecific cues are unlikely to improve translocation success in the PBT and could even be detrimental (Ebrahimi and Bull, 2013, Ebrahimi and Bull, 2014c). A simulated translocation spanning two activity seasons, found that the best time of year to translocate PBTs is late summer to autumn, when lizards are less active, reducing the chances of dispersal from the release site (Ebrahimi and Bull, 2014b). However, field translocations in the wild are required to determine if survival is improved by reduced dispersal at certain times of year.

Predators

The eastern brown snake (*Pseudonaja textilis*) is a known predator of the PBT, and potential avian predators include the nankeen kestrel (*Falco cenchroides*), brown falcon (*Falco berigora*) black shouldered kite (*Elanus axillaris*) and Australian magpie (*Gymnorhina tibicen*) (Armstrong and Reid, 1992, Fenner et al., 2008). The European fox (*Vulpes vulpes*), and cats (*Felis catus*) and dogs (*Canis familaris*) are also potential predators (Fenner et al., 2008). Field observations have revealed that foxes will dig out PBT burrows, posing a risk of predation to lizards in refugia and burrow destruction and subsequent reduction in supply of suitable burrows (Nielsen and Bull, 2016). Pygmy bluetongue lizards are attacked relatively frequently, with most survivors showing tail damage (attacks anterior to the tail are probably fatal) (Fenner et al., 2008). Observations of PBT show they suffer a substantial degree of sub-lethal injury, with adults found to have suffered injuries significantly more often than sub-adults (Fenner et al., 2008). This could mean that adults are more likely to survive an attack than sub-adults or they have had more exposure to attacks over their longer lifetime.

Threats

Threats to the PBT include climate change, habitat loss and agricultural activities. Climate change has been predicted to have a negative impact on population abundance (Delean et al., 2013) and management actions including translocations will be required for the species to persist (Fordham et al., 2012). Habitat loss due to changing land use is a major threat (Smith et al., 2009). Historically, the PBT's distribution range extended to the Adelaide Plains, but currently the species only exists in fragmented patches in mid-north South Australia due to urbanisation and agricultural practices (Smith et al., 2009). Plowing is also a threat as it destroys the burrows lizards rely upon and reduces dispersal, which could explain the low gene flow between adjacent populations (Souter et al., 2007, Ebrahimi and Bull, 2015). Heavy grazing may result in the loss of burrows, reduce spider digging activity and cause burrow destruction after heavy rainfall washes debris into burrows or causes collapse (Ebrahimi et al., 2012, Clayton and Bull, 2015). In areas that are heavily grazed, lizards have been shown to avoid artificial burrows and are more likely to disperse which could negatively impact recruitment and population sustainability (Pettigrew and Bull, 2011, Ebrahimi and Bull, 2015). This suggests that heavy grazing could negatively affect the PBT, as dispersal from native grassland habitat increases the risks of predation and dispersing into unsuitable habitat.

Conservation management

As the PBT is an endangered species, the establishment of a captive breeding program is one of the recovery actions detailed in the Species Recovery Plan (Duffy et al., 2012). Projections using bioclimatic envelope modelling and plant-habitat modelling have shown PBT range contractions of 60% and 42% by 2100 for the respective models under a worst-case scenario of no climate mitigation (Delean et al., 2013). Translocations will be an important management strategy for the species to persist (Fordham et al., 2012). Lizard stocks will be required for translocations and a captive bred population could supply some of these stocks.

In spring 2014, sixteen adult PBT (eight males and eight females) were captured and brought to Monarto Zoological Park (35°06′S; 139°09′E) to establish a captive breeding colony. The PBTs were captured from two sites near Burra, South Australia (33°42′S; 138°56′E) between 22 and 29 September 2014. The two sites, Tiliqua and Main Site, are approximately 8 km apart and were treated as two populations. Capture was by the fishing rod method (Hutchinson et al., 1994). A mealworm was tied to the rod with cotton string and used to pull the lizard from the burrow while it gripped the bait, or to lure the lizard from its burrow to be captured. Eight lizards (four of each sex) were obtained from each site. Sex was identified in the field based on snout-vent length and head size, although this proved to be difficult and not entirely accurate. Lizards were grouped in enclosures by population, with a maximum of three adults per enclosure. Enclosures were situated within a larger dome enclosure to exclude predators (Figure 2).

Figure 2: Lizard enclosures situated within a larger predator-free enclosure at Monarto Zoo.

Thesis scope and aims

Although translocations are needed to conserve some species, especially in the face of a changing climate, the success rate of translocations needs to be improved. We know that animal behaviour can be affected by the captive environment, and that this can negatively affect translocation success when captive animals are used. It is therefore important to conduct research into the effects of captivity, particularly on endangered species subject to translocation projects. Furthermore, behavioural studies in captive environments that mimic a natural or semi-natural setting can provide an opportunity to study behaviours that are difficult to study in the wild, allowing researchers to fill in knowledge gaps on species ecology and behaviour.

This thesis aims to investigate key questions and fill knowledge gaps regarding lizard behaviour to improve the success of translocations and the husbandry of captive animals that may be used in future translocations. The primary aims were to;

1. Determine how behaviours change ontogenetically in the pygmy bluetongue lizard
2. Determine how behaviour differs between neonates and adults
3. Determine the effect of captivity on lizard foraging ability and behaviour
4. Determine if predator recognition is innate in the pygmy bluetongue lizard, how captivity affects predator avoidance behaviour and if lizards respond more strongly to a known predator compared to other predatory reptile species
5. Investigate how pygmy bluetongue lizards use grass tussocks

Thesis outline

Chapter 1 is the general thesis introduction and Chapter 7 is the general discussion. These two chapters are kept brief to avoid repetition of the data chapters.

Chapter 2 investigates how lizard behaviour changes over time for juvenile (two years old), immature (three years old) and adult age classes, which has previously not been studied and reviews literature concerning ontogenetic behavioural changes in lizards. My two main aims were to 1) determine the behavioural differences among the three age classes – adult, immature and juvenile, 2) compare ontogenetic changes in behaviour in the pygmy bluetongue lizard to other lizard species as reviewed from the literature.

Chapter 3 aims to fill a significant knowledge gap in neonate behaviour – we know that neonate pygmy bluetongues are actively dispersing shortly after birth in late summer, but no other behavioural information has been obtained. Therefore, this chapter aims to 1) compare the behaviour of neonate and adult lizards to determine if behaviour differs between the age classes and 2) observe how adult behaviour changes over the activity season. Information on neonate behaviour is vital to determine appropriate age classes for translocation of lizards.

Chapter 4 focuses on the effect of the captive environment on foraging ability. This is significant as many captive animals may lack the skills to successfully forage once released into the wild. The aims were to 1) determine if hand-feeding, a husbandry method used by keepers to ensure lizards are getting enough sustenance, alters foraging ability in pygmy bluetongue lizards, 2) identify if lizards have an innate foraging ability and 3) compare other behaviours between hand-fed and self-fed lizards which may negatively affect lizard survival in the wild.

Chapter 5 investigates the effect of captivity on predator recognition and whether the pygmy bluetongue lizard can innately recognise predators. The specific aims are to determine; 1) if captive-born pygmy bluetongue lizards recognise predators innately; 2) if captive-born lizards have reduced predator recognition compared to wild lizards and if time spent in captivity reduces responses to predators; 3) if captive pygmy bluetongue lizards respond more strongly to a known predator, the eastern brown snake (*Pseudonaja textilis*), than other predatory reptiles; and 4) if the avoidance response to predator detection differ between naive and experienced lizards. This research is significant as predation is a major cause of mortality in translocations of captive animals, thus a better understanding of predator recognition and the effect of captivity can improve translocation success.

In chapter 6, I investigate the level of grass use in captive pygmy bluetongue lizards. Previous research has investigated the impact of grazing intensity and subsequent vegetation cover on burrow stability, basking and foraging but there has been a lack of research on how lizards use the grass tussocks when out of their burrows. This is a significant knowledge gap, as tussock grasses may be important as temporary refuges when lizards are dispersing, foraging or seeking mates and may therefore require consideration in wild populations and when determining translocation sites. The research aims were to 1) investigate how often lizards spend in grass tussocks when out of burrows, 2) determine if there is an age difference in grass use between adults and juveniles and 3) determine if there is seasonal variation in grass use over the activity season.

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Chapter 2

How activity and movements decrease with age in the pygmy bluetongue lizard and comparisons of ontogenetic behavioural changes in other lizards.

Adult and neonate pygmy bluetongue lizards (photo: Aaron Fenner)

Chapter Preface

Age-specific behavioural differences can relate to dispersal tendencies, activity levels and habitat preferences which can result in some age classes being potentially more vulnerable to predation. Understanding age-specific behaviours is an important aspect of species ecology. It is also vital to understand how behaviour varies over animal life stages to better inform captive husbandry management and can potentially help identify suitable candidates for translocation. In this chapter, I investigate how behaviour changes with age in the pygmy bluetongue lizard and review ontogenetic behavioural differences in other lizards for the same behaviours that I used for pygmy bluetongues. As the pygmy bluetongue lizard is a well-studied species, it could possibly be used to inform about age-specific behaviours in other similar lizard species.

How activity and movements decrease with age in the pygmy bluetongue lizard and comparisons of ontogenetic behavioural changes in other lizards.

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Abstract

Behaviour can vary over the life cycle of species, which can result in differences in dispersal, activity and habitat use. A captive population of the endangered pygmy bluetongue lizard which consisted of three age classes allowed for investigation of ontogenetic changes in behaviour. This study compares juvenile, immature and adult pygmy bluetongue lizard age classes to further elucidate how behaviour changes over the lizard life cycle and reviews literature regarding ontogenetic changes in behaviour in lizard species to understand how the pygmy bluetongue lizard fits and if it can be used to inform on ontogenetic changes in other lizard species. We filmed lizards from October to March and recorded basking time, burrow movements, time sitting on the surface, time walking the enclosure wall, and number of burrows occupied. We found that behaviour changes ontogenetically in the pygmy bluetongue lizard, with activity levels highest at an early life stage, and progressively decreasing with age. Our review showed that ontogenetic changes are also common in other lizard species, with dispersal and activity being high in neonate and juvenile classes, and differences occurring in foraging, predator avoidance and habitat use. As activity levels

and movements decrease with age in the pygmy bluetongue lizard, immature and adult age classes may be less likely to be preyed upon than younger age classes which may have implications for future translocations.

Introduction

Ontogenetic changes refer to changes that occur over the growth and development of an animal's life cycle. Such changes can relate to embryonic development, morphology, diet and various behaviours (Morafka et al., 2000, Nasri et al., 2018). The dietary components consumed can change with age, with juveniles of semi-herbivorous lizard species often consuming mostly prey items and increasing consumption of plant matter as they mature (Beuttner and Koch, 2019) or insectivorous lizards increasing the size of prey items with age (Suarez et al., 2000, Whitfield and Donnelly, 2006, Lahti and Beck, 2008). Common behaviours that vary ontogenetically across a range of taxa including birds, mammals, invertebrates, fish and herpetofauna include dispersal, habitat use and diet or foraging techniques (Andrews et al., 2010, Stevens et al., 2014, Campioni et al., 2020). For example, there are dietary, parental care, habitat and movement differences in bearded seal (*Erignathus barbatus*) pups, in which young receive maternal care before transitioning to aquatic feeding after weaning, when pups show a peak in exploratory movements prior to settling along the coast (Hamilton et al., 2019).

Many reptile taxa display differences in behaviours among age classes. These differences can include dispersal, habitat use, foraging and predator avoidance tactics and exploratory behaviour (Morafka et al., 2000, Graeb et al., 2006, Takeuchi, 2009, Ramírez-Macías et al., 2017, Votier et al., 2017). Whilst predator avoidance has been found to be innate in some lizard species and antipredator tactics were the same between adults and juveniles (Van Damme et al., 1995, Stapley, 2004, Landová et al., 2016), in other species tactics differ among age classes. Juvenile southern water skinks, (*Eulamprus heatwolei*), were found to have a stronger avoidance of predator chemical cues than adults due to their small size making them vulnerable to predation (Head et al., 2002).

The pygmy bluetongue lizard, *Tiliqua adelaidensis,* is an endangered species found in native grasslands in South Australia, which will require conservation translocations to combat the effects of climate change on their distribution range (Fordham et al., 2012, Delean et al., 2013). This lizard species spends the majority of their time associated with natural burrows constructed by spiders, which provide a site to bask, ambush prey and take refuge from predators and temperature extremes (Hutchinson et al., 1994, Milne et al., 2003). They are known to leave their burrows to forage, defecate and seek mates in spring (Milne et al., 2003, Schofield et al., 2013, Ebrahimi et al., 2014, Ebrahimi et al., 2016). Females give birth to offspring in late summer and the neonates then disperse within weeks of birth from the natal burrow (Milne et al., 2002). We know that dispersal predominately occurs during the spring when males leave burrows to search for mates and in late summer or autumn when neonates disperse from the natal burrow (Schofield et al., 2013). Lizards are at increased risk of predation when out of their burrows from native predatory birds and eastern brown snakes, *Pseudonaja textilis* (Hutchinson et al., 1994, Fenner et al., 2008).

The pygmy bluetongue lizard has distinct ecological and habitat preferences but is readily studied, thus could provide insight into less easily studied lizard species. This study aims to compare juvenile (one-year-old), immature (two-year-old) and adult pygmy bluetongue lizard age classes to further elucidate how behaviour changes over the lizard life cycle. We investigated time spent basking, number of burrow exits, time sitting on the surface, time walking the enclosure wall and number of burrows occupied. As we know that neonates are more active than adults, we predicted that juvenile and immature lizards would display higher levels of activity compared to adults, but that immature lizards would be less active than juvenile lizards. We then conduct a review to answer two questions. Firstly, given that the pygmy bluetongue lizard has such distinctive ecology, we compare ontogenetic age shifts in behaviour between the pygmy bluetongue lizard and other lizard species to identify generalities. To do this, we review the literature on ontogenetic behavioural differences among lizard species to compare ontogenetic behaviour in the unusual pygmy bluetongue lizard to other lizard species. Secondly, if there are generalities among lizard species, what can we learn about broader lizard behavioural ontogeny from our findings from the pygmy bluetongue lizard.

Methods

Study population

The study was conducted at Monarto Zoo, South Australia (35°06′S; 139°09′E) from October 2017 to March 2018. There were 45 pygmy bluetongue lizards in the breeding collection at Monarto Zoo, consisting of 14 wild caught adults captured from two wild populations, and 31 captive born juveniles of which 13 were born in January / February 2016 and 18 were born in February 2017. At the time of the study, the 2016 cohort were in their second year of life and are henceforth referred to as immatures, whilst the 2017 cohort were in their first year and referred to as juveniles. Adults had been held in captivity for four years at the time of this study.

Housing

Ten enclosures 0.65 m high (2.4 m x 1.2 m) were filled with sandy loam to a depth of 400 mm. Artificial burrows were formed from 300 mm long wooden dowels, 30 mm in diameter with an 18 mm diameter circular hole drilled through the centre, placed vertically into the sand, with the entrances flush with the sand surface. Each enclosure was divided into three sections, and each section had six burrows approximately 30cm apart (Figure 1). Adults were housed singly to prevent breeding, while juveniles were housed in pairs. There were four native grass tussocks – two each of *Austrostipa* sp. and *Austrodanthonia* sp. in each section that provided shade and surface shelter. The enclosures were situated within a larger 15 m diameter circular caged area netted on the sides and the roof to prevent predators entering.

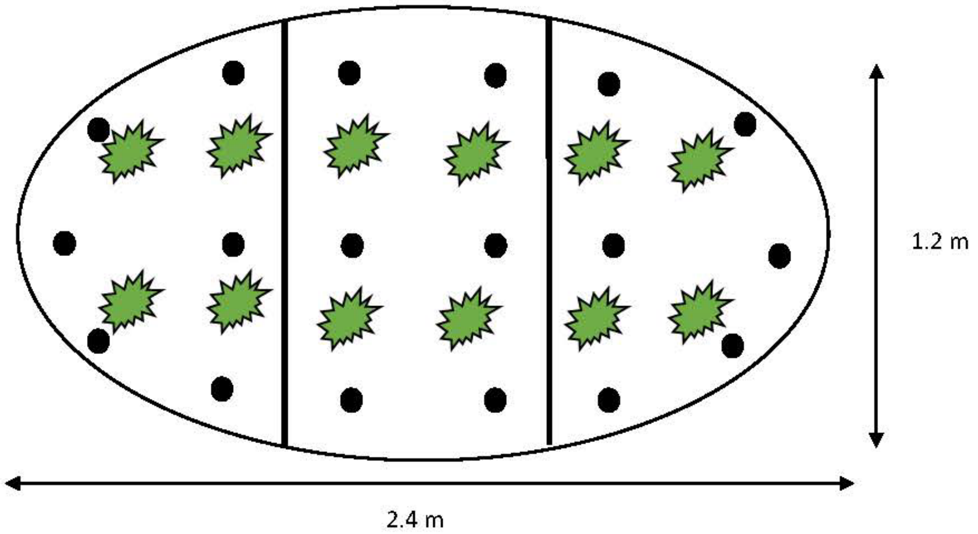

Figure 1: Diagram of enclosure divided into three sections, large black circles represent burrows and green clumps represent grass tussocks.

Age class behavioural comparisons

All 45 lizards were filmed with 15 Movii Neo cameras for four hours per day between 10 am and 2 pm as lizards are known to be active during these hours throughout the activity season (Milne et al., 2003). Filming occurred for three days per lizard in October, due to a delay in commencing the experiment, and for four days per lizard per month for the rest of the season spanning November to March to record behaviour. Pygmy bluetongue lizards are active from spring to autumn, but we chose to begin behavioural observations in October when lizards are more active (PBTs mate in October) and finish in March when activity declines. As cameras were limited, the adults and two juveniles were filmed on the same day, and the remaining juveniles filmed on a separate day. From video playback, the following behaviours were recorded; basking time, burrow exits, time sitting on the surface, time walking the enclosure wall and number of burrows occupied. These behaviours were chosen as they can be used to compare activity and movement levels between age classes. For more information, see Chapter 3, p. 77.

Statistical analysis

Primer-e v7/PERMANOVA+ was used to conduct univariate, multifactorial, repeated measures PERMANOVA tests based on Euclidean distance matrices. For the age class comparisons, age (adult, immature, juvenile) was a between subjects factor. Month and day (nested within month) were within subjects factors. Average daily maximum temperature (°C) was analysed as a covariate.

Results

Age class behavioural comparisons

The total footage collected over the 46 days over the activity season from October to March was 496,800 minutes; by age class this was 198,720 minutes for juveniles, 143,520 minutes for immatures and 154,560 minutes for adults. Lizards basked for a total of 65,043 minutes, exited burrows 1110 times, spent 703 minutes sitting on the surface and spent 320 minutes walking the enclosure wall.

Basking time

There was a significant difference among age classes in mean basking time (Table 1). Time spent basking was higher in juveniles (67.36 minutes ± 2.63 SE) and immatures (65.32 minutes ± 3.44 SE) compared to adults (54.74 minutes ± 3.66 SE). There were also significant differences in basking time among months and days (Table 1). Mean basking time was highest early in the activity season before decreasing toward the end – October 124.20 minutes ± 5.77 SE, November 89.14 minutes ± 3.52 SE, December 83.01 minutes ± 3.78 SE, January 42.76 minutes ± 3.35 SE, February 37.20 minutes ± 3.48 SE and March 16.09 minutes ± 2.14 SE. There was not a significant interaction effect between month and age in basking time (Table 1).

Burrow exits

The number of burrow exits made by pygmy bluetongue lizards differed significantly among age classes (Table 1). Juveniles exited burrows more often (1.83 exits ± 0.15 SE) compared to immatures (0.65 exits ± 0.06 SE) and adults. The number of times lizards exited their burrows also differed significantly among months and among days (Table 1). The mean number of exits for each month were: October 1.85 exits ± 0.24 SE, November 2.45 exits ± 0.27 SE, December 1.10 exits ± 0.13 SE, January 0.64 exits ± 0.10 SE, February 0.38 exits ± 0.06 SE, and March 0.21 exits ± 0.04 SE. There was a significant interaction effect between month and age for number of exits from burrows (Table 1). Juveniles made more burrow exits than immature and adult age classes in October and November and movements from burrows declined in later months for all age classes (Figure 2).

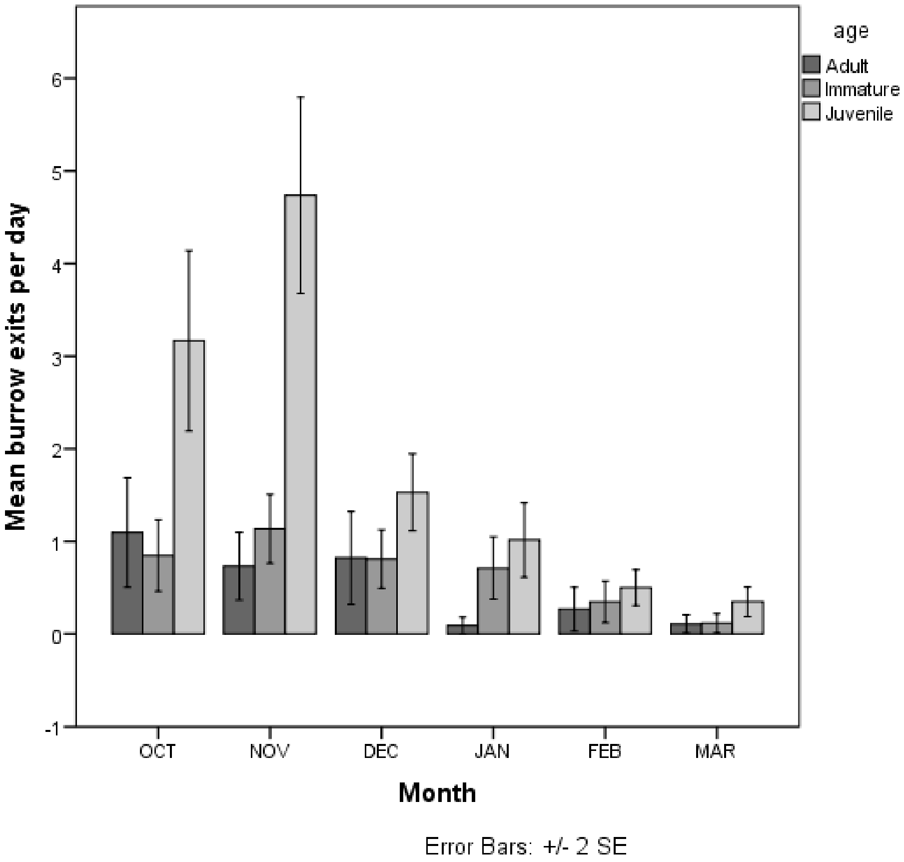

Figure 2: Mean number of exits from burrow per day ± SE over each month of the activity season made by lizards of the three age classes.

Burrows occupied

The number of burrows occupied by pygmy bluetongue lizards differed significantly among age classes (Table 1). Juveniles occupied more burrows (2.01 burrows ± 0.11 SE) than immatures (1.32 burrows ± 0.05 SE) or adults (1.30 burrows ± 0.06 SE). The number of burrows occupied also differed significantly among months (Table 1). The mean number of burrows occupied each month were: October 1.9 burrows ± 0.16 SE, November 2.58 burrows ± 0.22 SE, December 1.59 burrows ± 0.09 SE, January 1.32 burrows ± 0.08 SE, February 1.14 burrows ± 0.04 SE and March 1.08 burrows ± 0.03 SE. There was a significant interaction between month and age (Table 1), as juveniles occupied a greater number of burrows per day in October and November than other age classes (Figure 3).

Walking the enclosure wall

The mean time lizards walked the enclosure wall by age class was as follows; juveniles 0.37 minutes ± 0.08 SE, immatures 0.11 minutes ± 0.03 SE, and adults 0.42 minutes ± 0.10 SE. There was a significant difference among age classes in time spent walking the enclosure wall (Table 1). The time lizards spent walking the enclosure wall differed significantly among months and among days (Table 1). The mean time pygmy bluetongue lizards spent walking the enclosure wall each month was as follows; October 0.79 minutes ± 0.22 SE, November 0.75 minutes ± 0.16 SE, December 0.33 minutes ± 0.11 SE, January 0.03 minutes ± 0.02 SE, February 0.05 minutes ± 0.02 SE and March 0.02 minutes ± 0.01 SE. There was a significant interaction between month and age class (Table 1, Figure 4).

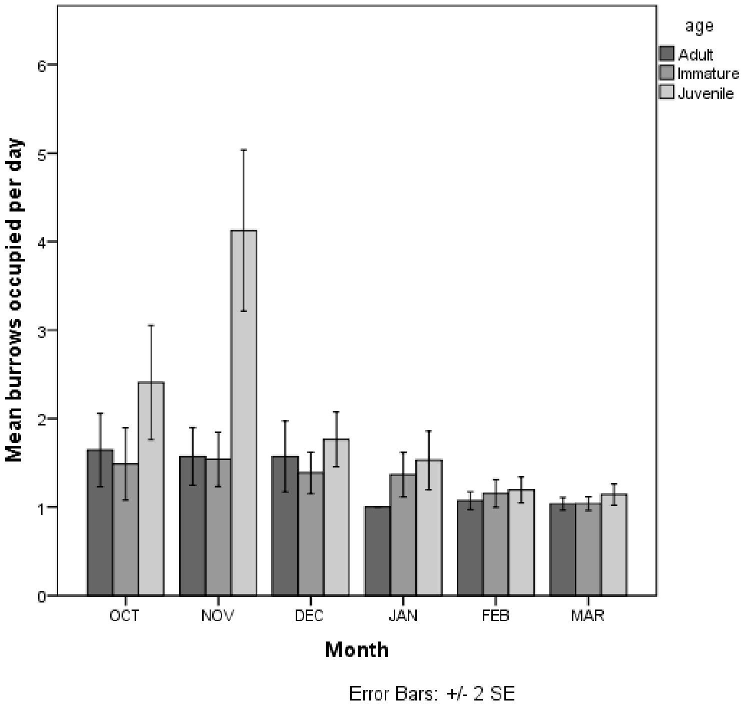

Figure 3: Mean number of burrows occupied per day ± SE over months by the three age classes.

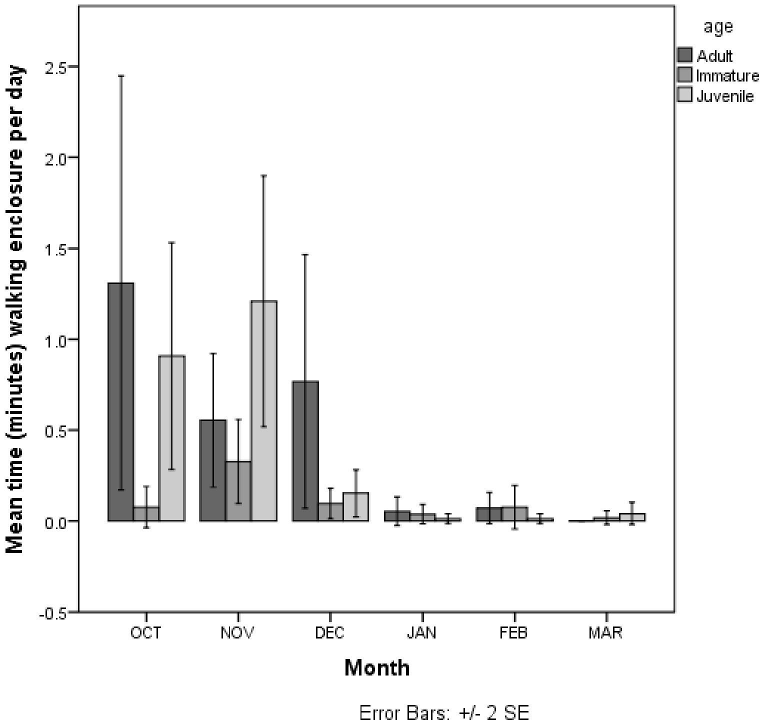

Figure 4: Mean time (minutes) ± SE spent walking the enclosure wall over months by the age classes.

Sitting on the surface

The mean time spent sitting on the surface by juveniles was 0.67 minutes ± 0.13 SE, for immatures 0.72 minutes ± 0.21 SE and for adults 0.66 minutes ± 0.20 SE. There was no significant difference among pygmy bluetongue lizard age classes in time spent sitting on the surface or for interactions between month and age (Table 1). There was a significant difference among months and among days in time pygmy bluetongue lizards spent sitting on the surface (Table 1). The mean time lizards spent sitting on the surface was: October 2.20 minutes ± 0.56 SE, November 0.77 minutes ± 0.15 SE, December 0.33 minutes ± 0.09 SE, January 0.48 minutes ± 0.27 SE, February 0.45 minutes ± 0.21 SE and March 0.22 minutes ± 0.08 SE.

Table 1: PERMANOVA results for the behavioural variables; basking time, burrow exits, time spent sitting on the surface, time spent walking the enclosure wall and number of burrows occupied for the three age classes – juveniles, immatures and adults. Month = filming month, Day = filming day, Age = age class. df = degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F = F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations. Bold indicates significant P value.

Basking time						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	7669.80	1534.00	16.75	**<0.001**	9936
Day	40	3189.60	79.74	8.80	**<0.001**	9885
Age	2	467.45	233.73	4.76	**0.001**	9924
Month x age	9	199.28	22.14	1.77	0.138	9931
Day x age	27	346.44	12.83	1.42	0.08	9896
Res	951	8612.70	9.06			
Total	1034	20485.00				
Burrow exits						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	119.91	23.98	8.07	**<0.001**	9947
Day	40	103.73	2.59	5.91	**<0.001**	9848
Age	2	73.60	36.80	21.03	**<0.001**	9942
Month x age	9	22.98	2.55	5.11	**0.001**	9951
Day x age	27	13.62	0.50	1.15	0.278	9919
Res	951	416.87	0.44			
Total	1034	750.71				
Sitting on the surface						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	31.65	6.33	4.81	**0.002**	9951
Day	40	46.40	1.16	2.11	**<0.001**	9877
Age	2	0.64	0.32	0.45	0.577	9935
Month x age	9	7.31	0.81	0.97	0.509	9950
Day x age	27	23.35	0.86	1.57	0.078	9899
Res	951	522.74	0.55			
Total	1034	632.09				
Walking enclosure wall						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	21.06	4.21	7.93	**<0.001**	9945
Day	40	18.66	0.47	1.81	**0.004**	9887
Age	2	1.87	0.94	2.57	**0.036**	9928
Month x age	9	4.66	0.52	2.76	**0.028**	9949
Day x age	27	4.91	0.18	0.71	0.776	9920
Res	951	245.02	0.26			
Total	1034	296.17				
Burrows occupied						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	22.64	4.53	9.13	**<0.001**	9937
Day	40	17.34	0.43	3.03	**<0.001**	9878
Age	2	9.26	4.63	11.03	**<0.001**	9934
Month x age	9	9.21	1.02	5.71	**0.001**	9955
Day x age	27	4.92	0.18	1.27	0.185	9912
Res	951	136.13	0.14			
Total	1034	199.49				

Discussion

Differences in basking time, and burrow movements in pygmy bluetongue lizard age classes

We found a significant difference among age classes in time lizards spent basking, and the number of burrow exits, burrows occupied and time walking the enclosure wall with activity levels generally decreasing with age (with the exception of adults walking the wall in October and December) and as the activity season progressed. Juveniles (one year old) basked longer and made more burrow movements than adults, with immature lizards (two years old) basking a similar amount to juveniles but being more similar to adults in the number of burrow exits and burrows occupied. Although an infrequent behaviour, adults walked the enclosure wall more than juveniles and immature lizards in October and December, whilst juveniles displayed this behaviour more than adults and immature lizards in November. The higher levels of basking and burrow movements in juveniles could be due to this age class being in active growth, whilst immature lizards have almost matured into adults. In our study, juveniles occupied more burrows than older age classes. Whilst I couldn't measure dispersal as lizards were housed in enclosures, the changing of burrows and walking the enclosure wall could represent dispersal. In a wild population, the juvenile lizards that change burrows may be more likely to disperse from the area as this activity represents an exploration of the surrounding area. A potential explanation for adults walking the enclosure wall in spring could be that adult lizards were attempting to disperse in search of mates.

There is a high rate of juvenile mortality in the pygmy bluetongue lizard (Milne, 1999), but previous research has found that adults have a significantly higher number of injuries compared to subadults – subadults were classified as lizards smaller than 82 mm snout-vent length (juvenile and immature classes in this study) (Fenner et al., 2008). Two possible reasons for difference were suggested, first either subadults are less likely to survive a predator attack, or second, subadults are attacked less frequently than adults as they leave their burrows less or are less visible due to their smaller size (Fenner et al., 2008). If we assume wild pygmy bluetongue lizards behave similarly to captive lizards, we can rule out reduced burrow movements by subadults as a reason for less observed injuries in this age class. We suggest that the reason fewer injuries were found on subadults is that they are less likely to survive an attack or are less visible, as we found juveniles exit their burrow significantly more than immature or adult lizards and as such should be more exposed to predation compared to adults or immatures that behave similarly to adults.

Comparing ontogenetic changes in behaviour between pygmy bluetongue lizards and other lizards

Studies of other lizard species have examined differences in dispersal, habitat use, and basking activity which we now review. The literature was searched from the Web of Science database, with the search terms ontogenetic AND behaviour AND reptiles. From the resulting list, articles that that focused on lizards and compared age classes were included in this mini review. Table 2 provides a summary of ontogenetic behavioural studies in lizards. It is important to study behavioural variation among age classes for species in which this knowledge is lacking, as it can affect predation risk and ecological factors such as growth rates and habitat use.

Table 2: Comparative summary of studies investigating age-related differences in behaviour and any similarities with the pygmy bluetongue lizard (PBT).

Behaviour	**Species**	**Results**	**Comparison to PBT**	**Reference**
Natal dispersal	*Niveoscincus microlepidotus*	Neonate females moved further than males although movements in home range small overall.	Neonates also disperse in first year like the PBT, however sex-based differences in neonate dispersal not studied in PBT.	Olsson and Shine, 2003
	Lacerta vivipara	Rates of neonate female dispersal related to age of mother, rates of male dispersal not related.	Juveniles disperse and are independent from birth like in the PBT.	Ronce et al., 1998
	Cyclura nubila caymanensis	Neonates disperse soon after hatching. High site fidelity in adults. Females make reproductive migrations to nest.	Neonates disperse like in the PBT. Females make reproductive movements (males in PBT).	Moss et al., 2020
	Anolis limifrons	Individual home ranges overlapped as juveniles and adults. Low juvenile dispersal.	Limited juvenile dispersal associated with high mortality of adults unlike in the PBT.	Andrews and Rand, 1983
Habitat use	*Chamaeleo chamaeleon*	Juveniles found in grass, adults in shrubs / low trees.	Dissimilar to the PBT, in which all age classes spend most of the time in individual, single entrance burrows. PBT juveniles exit burrows more than immature and adult lizards, but no significant differences in time spent on the surface in the open.	Keren-Rotem et al., 2006
	Chondrodactylus turneri	Juveniles found in the open more, adults mostly in crevices.		Eifler et al., 2017
	Callisaurus draconoides	Juveniles found in areas with less vegetative cover compared to adults.		Frost and Bergmann 2012
	Microlophus atacamensis	Juveniles use lower perches than adults and subadults.		Vidal et al., 2002
	Anolis sagrei	Juveniles use lower perches and observed on the ground more than adults.		Delaney and Warner, 2016
	Meroles cuneirostris	Juveniles spent less time under vegetation cover than adults.		Childers and Eifler, 2015
Basking activity	*Sceloporus jarrovii*	Juveniles active throughout the day, adults primarily active in morning.	Similarly, we found PBT juveniles and immatures basked more than adults and juveniles moved more.	Middendorf and Simon, 1988
	Physignathus lesueurii	Sub-adults had higher levels of activity and feeding frequency compared to adults.		Avery and Meek, 2008
	Meroles cuneirostris	Juveniles moved more and for longer periods than adults.	Similar to PBT juveniles, which made more movements from burrows	Childers and Eifler, 2015
	Lacerta viridis	Juveniles were more exploratory than adults.	We did not measure exploratory behaviour, but juvenile PBT moved from burrows more, which could equate to explorative behaviour.	Bajer et al., 2015
	Podarcis hispanica atrata	Juveniles basked less frequently than adults.	Opposite finding to our PBT study.	Castilla and Bauwens, 1991

	Gallotia galloti	Adult males basked for longer durations, followed by subadults and female adults and lastly juveniles.	We did not measure basking duration, but have observed juveniles basking for short bouts, retreating and then reappearing repetitively (pers. obs).	Diaz, 1994
	Liopholis slateri	Juveniles foraged more frequently and further from burrows compared to adults.	We did not measure foraging behaviour, but found that PBT juveniles leave burrows more often than adults.	McKinney et al., 2014
	Amblyrhynchus cristatus	Younger (smaller) iguanas fed more frequently and needed to shuttle between basking sites (to warm up) and foraging sites more than adults.		Wikelski and Trillmich, 1994

Dispersal

There are often high levels of dispersal in neonate age classes, including in pygmy bluetongue lizards (Ronce et al., 1998, Morafka et al., 2000, Schofield et al., 2013, Moss et al., 2020). Conversely, there is limited juvenile dispersal in *Anolis limifrons,* likely due to the high mortality rate of adults (Andrews and Rand, 1983). Neonate dispersal distances can differ between sexes, with female neonates dispersing further than males in the southern snow skink, *Niveoscincus microlepidotus* (Olsson and Shine, 2003). Dispersal of reproductive adults occurs during the breeding season for mate-seeking behaviour or nesting, and can be biased toward males or females depending on the species (Olsson and Shine, 2003, Schofield et al., 2013, Moss et al., 2020). Juvenile dispersal can be a result of seeking available resources or establishing territories.

Habitat use

Habitat requirements and space use differences among age classes are also common. Reasons for these differences include territoriality or cannibalism of adults toward juveniles, competition or differences in foraging or thermal requirements. In the common chameleon (*Chamaeleo chamaeleon*), juveniles were found in low grass and adults in shrubs and trees with minimal overlap of habitat use and juveniles displayed active avoidance of adults by concealment or flight as adults would attack and consume juveniles (Keren-Rotem et al., 2006). Juvenile Turner's thick-toed gecko (*Chondrodactylus turneri*), were found in open areas more often than adults which were mostly found in crevices, possibly due to differences in foraging strategy or displacement by adults (Eifler et al., 2017). Juvenile zebra-tailed lizards (*Callisaurus draconoides*) were also found in areas with less vegetation cover than adults (Frost and Bergmann, 2012). Spatial segregation was found in *Microlophus atacamensis*, in which adults and subadults use higher perches and foraging areas compared to juveniles which use lower perches, possibly to avoid aggression from adults (Vidal et al., 2002). Similarly, in the brown anole lizard (*Anolis sagrei*) juveniles used lower perches which were suggested to be safer from predators and from potentially aggressive adults and were found on the ground more than adults (Delaney and Warner, 2016). In our study species, the pygmy bluetongue lizard, juveniles prefer burrows smaller in diameter than adults as such burrows provide better protection from predators (Milne and Bull, 2000). The pygmy bluetongue lizard will actively defend its burrow from conspecifics (Fenner and Bull, 2011), but there are no known observations of cannibalism of juveniles by adults.

Basking activity and movement

There is a general pattern of higher levels of activity in juvenile age classes compared to adults in lizard species. Daily activity levels vary between adult and juvenile *Sceloporus jarrovii,* with adults being active primarily in the morning and juveniles remaining active throughout the day; when adults were active in the afternoon they spent the majority of the time in shade (Middendorf and Simon, 1988). This variation between age classes is possibly a result of adults having trouble cooling down after exposure to sunlight (Middendorf and Simon, 1988). In Australian water dragons, (*Physignathus lesueurii*), sub-adults were found to be more active and fed more compared to adults (Avery and Meek, 2008) and similarly, juvenile wedge-snouted desert lizards (*Meroles cuneirostris*) made movements of greater duration and frequency and spent less time under vegetation cover compared to adults (Childers and Eifler, 2015). Exploratory behaviour was found to be higher in juvenile European green lizards (*Lacerta viridis*), compared to adults (Bajer et al., 2015). There can also be individual variation in activity levels, running speeds and morphology among neonates, likely a result of phenotypically plastic responses to the thermal environment during embryonic development (Shine and Harlow, 1993). However not all lizard species show higher activity and basking in juvenile age classes. Juvenile *Podarcis hispanica atrata* were found to bask less frequently than adult lizards (Castilla and Bauwens, 1991). A study of *Gallotia galloti*, which measured duration of basking bouts (not total daily basking time) found that adult males basked for longer periods, followed by subadults and adult females and lastly juveniles (Díaz, 1994). This could be due to juveniles heating up faster due to their smaller size. Neonate and juvenile reptiles generally have higher energy and nutrient requirements compared to adults to enable growth (Morafka et al., 2000). For this reason, higher levels of activity and foraging in juvenile age classes are expected. Juvenile Slater’s skink, (*Liopholis slateri*), were found to forage more frequently and further from their burrows compared to adult lizards (McKinney et al. 2014). In the Galapagos marine iguana (*Amblyrhynchus cristatus*), younger smaller iguanas fed more frequently and shuttled between foraging and basking sites faster than older larger animals (Wikelski and Trillmich, 1994). Thermal requirements may also play a role in predator avoidance activity, with the thermal cost of seeking refuge outweighing the benefits in some juvenile lizard species. In Iberian rock lizards (*Lacerta monticola)* approach distances of simulated predators were shorter for juveniles compared to adults (Martín and López, 2003). Additionally, a review of escape behaviour in five lizard species found that juvenile lizards had shorter flight initiation distances compared to adults (Cooper Jr, 2011). The smaller size of juvenile lizards may make them less detectable to predators, or the cost of fleeing may be greater for juveniles as they have higher energetic requirements than adults (Martín and López, 2003, Cooper Jr, 2011, Cooper, 2015, Mikula et al., 2019).

Conclusions and implications

We found that like in other lizard species, behaviour changes ontogenetically in the pygmy bluetongue lizard, with activity levels highest in earlier life stages, and progressively decreasing with age. However, not all lizard species show increased basking and activity in juveniles, thus caution in predicting behaviours in other lizard species is suggested. Our results support an ontogenetic shift in behaviours with age in the pygmy bluetongue lizard. We suggest that immature lizards are more like adults in their activity levels, and thus are less likely to be preyed upon compared to juvenile or neonate pygmy bluetongue lizards, assuming that predators can locate adult and juvenile lizards equally. Our study highlights the benefits provided by the captive environment for studying behaviours in species that are otherwise difficult to observe in the wild, such as in this case where we have been able to compare three distinct age classes over a whole activity season.

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Chapter 3

Translocation for conservation: neonates are less suitable than adults


Neonate pygmy bluetongue lizard basking at burrow entrance at Monarto Zoo.

Chapter Preface

The previous chapter investigated behavioural differences in juvenile, immature and adult pygmy bluetongue lizards and found that activity levels decrease with age. This chapter explicitly compares the behaviour of neonate and adult pygmy bluetongue lizards, with the aim of providing a better understanding of the neonate age class. I also compared behaviour over the activity season – October to March – between male and female lizards. I used this information to identify if behaviour differs between neonate and adult lizards and how behaviour changes seasonally. This information is important for the conservation of the pygmy bluetongue lizard, as to date there has been a lack of research focused on the neonate age class. Translocations will be an important conservation method for the pygmy bluetongue lizard in the future; for translocations to be successful, an important factor is selecting appropriate candidates to release. Translocations of young age classes can be a beneficial method for some species, particularly if they are reared in captivity until they reach a less vulnerable size, a practice common in fish and some egg-laying reptile species. For this reason, a good understanding of the ecology and behaviour of the species is required, as in some species neonate behaviour may increase vulnerability to predation or dispersal from the release area. This chapter has been published in Austral Ecology and has been reformatted for this thesis.

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Translocation for conservation: neonates are less suitable than adults

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Abstract:

Animal behaviour can affect the outcome of conservation translocations. It is important to understand the behaviour of the species being considered for translocation and how its behaviour varies over life stages. There may be uncertainty about what life stages are best as founders for release back into wild populations. A technique called head-starting whereby juvenile life stages are raised in captivity and then released is one potential pre-release strategy. However, juveniles of many species have a dispersive role in the life cycle, potentially raising difficulties for establishing new populations due to dispersal from the intended habitat following release. For this study, we compared aspects of the behaviour of captive adult and neonate pygmy bluetongue lizards (*Tiliqua adelaidensis*) — an endangered species for which translocation is likely to be an important management strategy — to determine if neonate behavioural characteristics are appropriate for their translocation. We filmed adult and neonate pygmy bluetongue lizards and compared their behaviour. We also filmed adults over an activity season to compare seasonal behaviour. Behavioural parameters measured included basking time, burrow exits, burrows occupied, and walking the perimeter wall. Neonates basked significantly more than adults in summer and autumn. Neonates are likely to be basking more than adults because they are in a stage of rapid growth and need to gain body mass before the winter inactivity period. Neonates

exited burrows more often than adults and used a greater number of burrows. These results indicate neonate lizards are actively exploring their habitat. Neonates are unlikely to be as suitable for translocation as they are actively moving about and more likely to be preyed upon or disperse from the translocation site. Our finding can be applied to other species that have active juvenile life stages and are at particular risk of predation due to their small size.

Introduction

Translocations, the intentional movement of individuals, are one conservation strategy for threatened species. They can be used to augment existing populations, reintroduce species to areas occupied prior to local extinction, or introduce a species outside their historical range (IUCN/SSC, 2013). Translocated individuals can be sourced from wild populations or from captivity. However, translocation success rates can be poor, and some possible explanations for failure include the number and source of individuals (captive or wild), lack of survival skills (e.g. foraging, social skills, and predator avoidance), release method and age (Fischer and Lindenmayer, 2000, Letty et al., 2007, Jule et al., 2008, Rummel et al., 2016). In order to improve translocation success, a variety of measures can be implemented. These measures can be in situ at the release site, such as temporary food provision (Ebrahimi and Bull, 2012) and temporary confinement (Ebrahimi and Bull, 2013, Sasmal et al., 2015). Measures implemented prior to release include predator avoidance training of captive animals (Griffin et al., 2000), selecting suitable age classes (Sarrazin and Legendre, 2000), and a technique known as head-starting whereby vulnerable life stages are raised in captivity (Heppell et al., 1996). In this article we concentrate on the suitability of different age classes for translocation.

In the context of translocations, it is important to understand any behavioural variation between age classes as this may impact translocation success. For example, if certain age classes are more active dispersers, they may disperse from the translocation site to unfavourable habitat — a particular concern in fragmented landscapes. Conversely, dispersal tendencies may be associated with good colonising ability, such is the case with sub-adult swift foxes (*Vulpes velox*) which colonise vacant habitat (Sasmal et al., 2015). Certain age classes may be less suitable translocators if they have a higher mortality rate due to predation, as the number of individuals needed for release would be higher to compensate. This may be less of an issue in species with high reproductive output, for example, a fish endemic to Texas, *Gambusia nobilis*, was able to produce in excess of 1,000 young per year from a founder stock of 30-40 individuals (Philippart, 1995). In comparison, the Whitaker's skink (*Cyclodina whitakeri*) can only give birth to 2-4 young annually (Towns, 1994), and would require 250 to 500 gravid females to produce 1,000 offspring. In species that have lower reproductive outputs, it may not be viable to release juveniles if that age class is known to have lower survival rates compared to sub-adults or adults, particularly if there is not a large enough source population available. Head-starting or captive-rearing is one method to overcome high mortality in juvenile life stages — by raising animals in captivity until they have reached a less vulnerable age and then releasing the animals to supplement wild populations. Species with low parental care, high fecundity and low juvenile survival are particularly suitable for head-starting projects (Heppell et al., 1996).

Studies comparing the efficacy of translocating juvenile and adult age classes have varied results and generally measure factors such as survival, site fidelity and habitat use (Nelson et al., 2002, Sasmal et al., 2015, Kraus et al., 2017, McCallen et al., 2018). The selection of the appropriate age class for translocation will largely depend on the particular species' characteristics (Letty et al., 2007). Most studies compare juveniles and adults during translocation. However, investigating behavioural differences prior to translocation — particularly those behaviours relevant to dispersal — may assist in selecting the most suitable age class.

The pygmy bluetongue lizard (PBT) (*Tiliqua adelaidensis*) is an endangered skink species found in fragmented native grasslands in mid-north South Australia. They occupy vertical burrows constructed by lycosid and mygalomorph spiders that provide protection from predators and temperature extremes (Hutchinson et al., 1994). Natural predators of PBTs include raptors, magpies and eastern brown snakes (Fenner et al., 2008). As the burrow is such a valuable resource, PBTs maintain a central-place territorial defence system, defending their burrow from conspecifics that approach too close to the burrow entrance (Fenner and Bull, 2011). PBTs are ambush predators of invertebrates that pass their burrow entrance and also consume small quantities of plant matter (Fenner et al., 2007). Males leave their burrows in search of females to mate with during spring months (Schofield et al., 2012). Females give birth to two to four offspring in late summer, and offspring disperse from the natal burrow soon thereafter (Milne et al., 2002). Neonates are small, with a mean snout-vent length of 44.5 mm and a mean mass of 1.56 g (Milne et al., 2002). As there is a high rate of mortality in juvenile PBT (Milne, 1999), there is the potential to make use of this surplus of offspring in translocations.

Translocations are a possible conservation strategy for the PBT to mitigate against a number of threatening processes that will affect the survival of this species in the future (Clive et al., 2020). Climate change will likely result in range contractions to suitable habitat and area of occupancy as predicted by bioclimatic envelope and plant-habitat models (Delean et al., 2013). Although models predict a potential southward range expansion, this is unrealistic, as the southern range is an area of urban and agricultural development where PBTs have been extirpated (Delean et al., 2013). Habitat fragmentation and low dispersal ability has resulted in isolated populations of PBTs (Smith et al., 2009). Changing land use is also an ongoing threat as agricultural activities such as ploughing or overgrazing can destroy the spider burrows that lizards require (Souter et al., 2007). Additionally, dispersal is too limited to rely on natural colonisation (Smith *et al.* 2009). Thus, translocations will be necessary to ensure lizard persistence in future as other strategies such as burrow augmentation alone will not effectively mitigate extinction risk (Fordham et al., 2012).

In order to better conserve the PBT, we need to know more about neonate behaviour following dispersal from the natal burrow. Previous field work has shown that neonates disperse soon after birth, some within the first week, and the majority having dispersed by five weeks (Milne et al., 2002). Neonates are the second most captured age class in population surveys from pit fall traps (following adult males which are highly mobile during spring) during the period of dispersal from the natal burrow (Schofield et al., 2012). A previous field study found variable but low survival rates from year to year in juvenile PBTs, with survival from birth to the following spring ranging from 6.7% for the 1993/94 season to 36% for the 1995/96 season (Milne, 1999).

There were two aims of our study using the PBT as the focal species; (1) to compare neonate and adult lizard behaviour in captivity to identify behavioural differences between age classes and (2) to observe changes in adult behaviour over the entire activity season. Filling in such knowledge gaps on the behaviour in this species will help inform conservation efforts that include future translocations, providing information that may also be applicable to other similar species.

Methods

Enclosures

At Monarto Zoo, South Australia, PBT were housed outdoors in six raised enclosures situated within a 15 m diameter circular caged area with a netted roof and wire meshed walls to exclude predators. The average number of lizards per enclosure was 5.3, ranging from 1 to 11; there were one to three adults per enclosure, and zero to eight neonates per enclosure at the time of filming. Enclosures were 0.65 m high, 2.4 m long, 1.2 m wide and filled with sandy loam to a depth of 0.4 m. Each enclosure contained 18 adult sized artificial burrows spaced approximately 30 cm apart in three rows of six and 10 neonate sized burrows in two rows of five placed within the outer rows of adult burrows (Figure 1). Adult burrows were wooden dowel 300 mm long, 30 mm in diameter with a 20 mm hole drilled through the centre. Neonate burrows were 200 mm long, 28 mm in diameter with a 14 mm hole. Each enclosure had 12 native grasses (*Austrostipa* sp. and *Austrodanthonia* sp.) to provide shade.

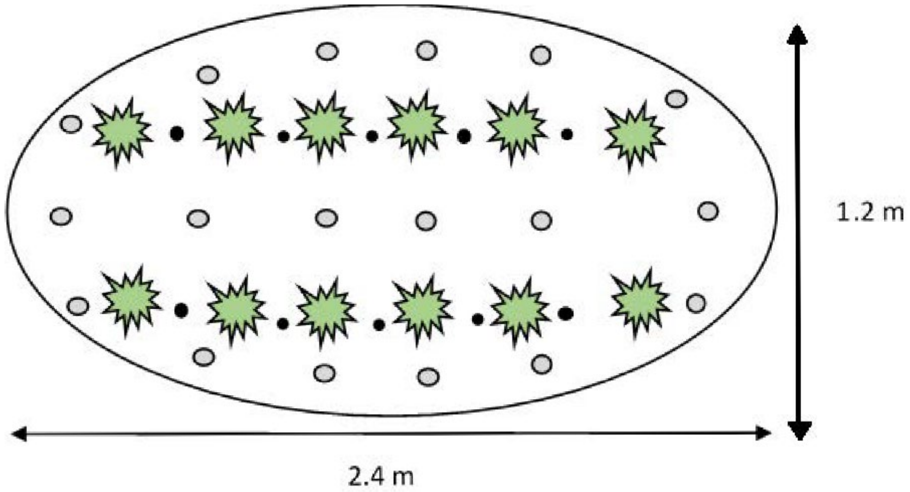

Figure 1: Diagram of enclosure layout, large grey circles represent adult burrows, small black circles represent neonate burrows and green clumps represent grass tussocks.

Lizards

The study population consisted of a captive breeding population of 14 adult PBT (i.e. seven male, seven female) and 12 neonate offspring born from three different mothers. An additional two females produced three offspring each, however these births occurred two to three weeks later than the first births and as such were omitted from the analyses. Females that gave birth were all from single male enclosures, allowing paternity to be confirmed in all cases. Maternity was not able to be determined in one case as two adult females were housed together with a single adult male, and the females gave birth the same day to four offspring each. In this case, the offspring dispersed from the natal burrow prior to their first capture for identification purposes. All lizards were captured once a month over a seven-month period (three months for neonates) to obtain body weight (g), snout-vent length (mm), and to apply an individual coloured non-toxic paint spot to the dorsal surface of the head for identification purposes. Photographs were taken to identify lizards based on specific patterns of individuals i.e. spotted, striped or plain colour morphs, which given the small number of individuals in our sample, has proven to be a reliable method of identification as an alternative to toe-clipping in the PBT (Tohl et al., 2015). Adult lizards were hand fed live crickets two times per week, while neonates were hand fed three times per week. Crickets were lightly dusted with Reptile One calcium powder +D3 and Aristopet Repti-vite vitamin supplements once per week.

Filming behaviours

To observe behaviour, lizards were filmed over the activity season which spanned the austral spring to autumn (October 2016 to April 2017) for the two components investigating; (1) neonate and adult behaviour and (2) adult behaviour over the entire season. For both components, six Movii Neo action cameras with an external battery charger were used to film three of the six enclosures at a time, alternating enclosures each day, with two cameras positioned at each end of the enclosure to provide coverage of the entire enclosure. Filming hours were usually between 10 am and 2 pm, however this varied on days with rainy periods when filming started one to two hours later or extremely hot days above 35°C and below 40°C when filming was started at 9 am. Filming was postponed when the temperature was forecast to exceed 40°C. First, neonates and adults were filmed from the birth of neonates to compare behaviour between age classes. Lizards were filmed for six weeks, from the birth of neonates in February 2017 until early April 2017. Filming was split over four days per week with half the enclosures filmed per day and alternated so that each lizard was filmed twice per week for four hours per day. Second, adults were also filmed over the entire season to investigate seasonal changes in behaviour, with filming occurring from October 2016 to April 2017, for two days per month per lizard. The average temperature during filming hours was calculated for each filming day.

Four behavioural parameters were recorded for the two components, measured as the total minutes or occurrences per day (four hours) for each individual lizard:

1. Basking time in minutes: defined as the time a lizard spent basking at the entrance of its burrow. PBTs bask at their burrow entrance for thermoregulation, and to watch for passing prey (Milne et al., 2003). Previous studies on adult PBT have shown a decline in basking time when lizards were confined to the release area by a plastic wall for a short period in a simulated translocation (Ebrahimi and Bull, 2013), and later in the activity season (Ebrahimi and Bull, 2014).
2. Number of burrow exits: defined as a lizard exiting a burrow to either walk around, defecate, enter grass or change burrows. This parameter was considered important as lizards moving around on the surface are exposed to potential predators, and the more times they exit burrows, the greater the risk.
3. Number of burrows occupied: defined as the total number of burrows occupied for any length of time (e.g. minutes or hours) during the daily four hour filming period for each lizard. The number of burrows occupied can be used to indicate stable burrow occupancy and the likelihood of dispersal. In wild populations, there is a mix of residents and dispersers, with some lizards occupying one burrow across multiple activity seasons and others occupying a burrow briefly before dispersing (Bull et al., 2015).

4. Number of occasions walking the perimeter wall: defined as a lizard walking back and forth around the wall or attempting to climb the wall. Walking the wall was assumed to be an attempt to disperse from the enclosure area.

Statistical analysis

Primer v7/PERMANOVA+ was used to conduct univariate multifactorial repeated measures PERMANOVA tests based on Euclidean distance matrices, which accounts for the repeated measurements of individuals over time by treating time as a fixed factor (Anderson et al., 2008, Clive et al., 2020). The behavioural parameters tested were basking time (minutes), number of burrow exits, number of burrows used, and occasions walking the perimeter wall each filming day for each individual lizard. All data underwent square root transformations except for number of burrows occupied as the data were normally distributed. Average temperature (°C) during filming was obtained from the BOM Pallamana station in South Australia which is located 11 km from Monarto Zoo and was used as a covariate for analyses.

For neonate and adult analyses, month (fixed factor) and day (nested in month; random factor) were within subjects factors, and age class (fixed factor) was a between subjects factor. Two-way interactions between month and age and day and age were included to determine interaction effects between the factors.

For adult analyses, month (fixed factor) and day (nested in month; random factor) were within subjects factors, and sex (fixed factor) were between subjects factors. Month and sex and day and sex were included as two-way interactions.

As a measure of effect size, we conducted an analysis of similarities – ANOSIM in Primer v7. The ANOSIM R statistic provides a measure of the strength of differences between groups and is scaled between -1 and +1, with values toward +1 representing greater strength of differences between groups (Anderson et al., 2008).

Results

1. PBT neonate and adult behaviour

Overview

Over the entire six-week filming period for the neonate and adult comparison, a total of 34,560 minutes of neonate PBT footage, and 40,320 minutes of adult PBT footage was collected (2,880 minutes of footage per lizard). The adult seasonal behaviour analysis covered two days per month from October to April and a total of 47,040 minutes of adult footage was collected (3,360 minutes per lizard).

Basking

Basking time (minutes) was significantly different between the two PBT age classes (Table 1) with neonates (114.35 min. ± 4.55 SE) having a higher mean basking time than adults (38.53 min. ± 3.84 SE) (Figure 2). There was a significant interaction effect between month and age class for PBT basking time, with a significant difference between the age classes in both March (PERMANOVA, pair-wise test, $p < 0.001$) and April (PERMANOVA, pair-wise test, $p = 0.024$) when neonates basked more than adults as adult basking decreased (Figure 3). On the finer temporal scale, there was significant variation in mean PBT basking time between days nested within months (Table 1).

Temperature had no effect on mean basking time among months, days or between age classes (Appendix S1).

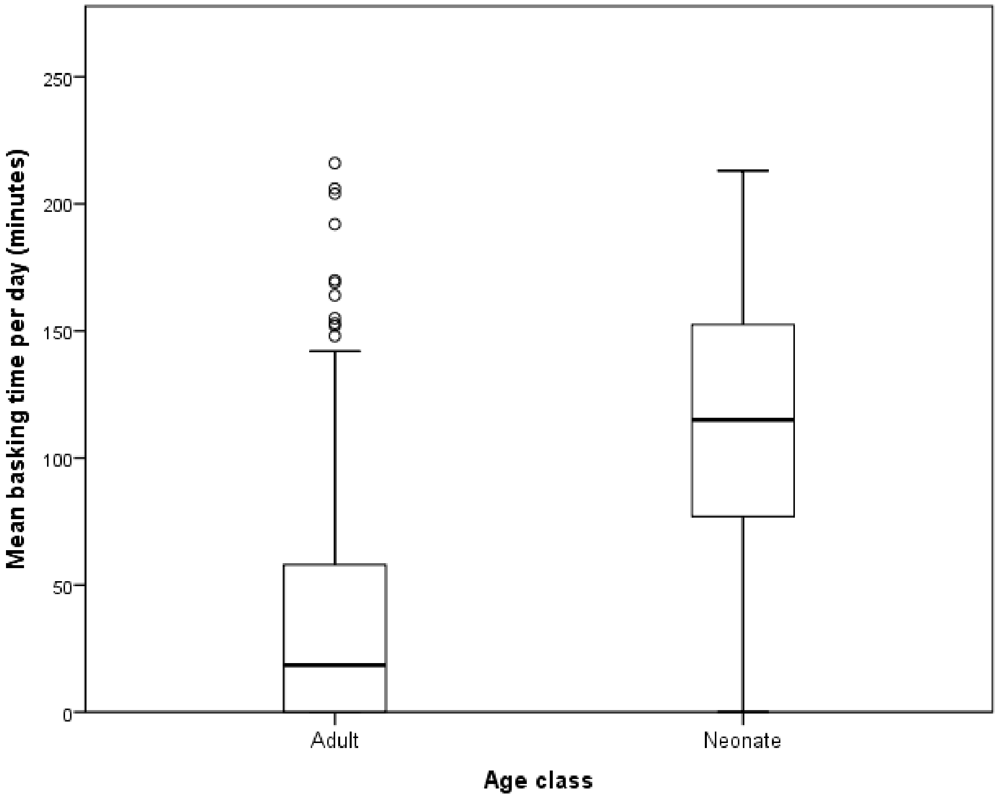

Figure 2: Mean time (minutes; ± SE) spent basking at the burrow entrance during the daily four-hour filming period for adult and neonate pygmy bluetongue lizards, *Tiliqua adelaidensis*. Adults n = 14, neonates n = 12.

Burrow movements – exits and burrows occupied

The mean number of burrow exits by PBTs was significantly different between age classes with greater movement by neonates (1.47 exits ± 0.14 SE) compared to adults (0.21 exits ± 0.06 SE) (Table 1; Figure 4a). There was also a significant difference among days and an interaction effect between day and age class for the number of times lizards exited their burrows (Table 1).

The mean number of burrows occupied by PBTs was significantly greater for neonate lizards (1.39 burrows ± 0.77 SE) compared to adults (1.08 burrows ± 0.36 SE) (Table 1; Figure 4b). However, day also had a significant effect on the number of burrows used by PBTs. Temperature had no effect on the number of burrow movements or burrows occupied (Appendix S1).

Walking perimeter wall

The mean number of occasions lizards walked the perimeter wall was significantly greater for neonates (0.06 occasions ± 0.03 SE) than adults (0.00 occasions ± 0.00 SE) (Table 1; Appendix S2). The mean number of occasions walking the perimeter wall was not affected by temperature (Appendix S1).

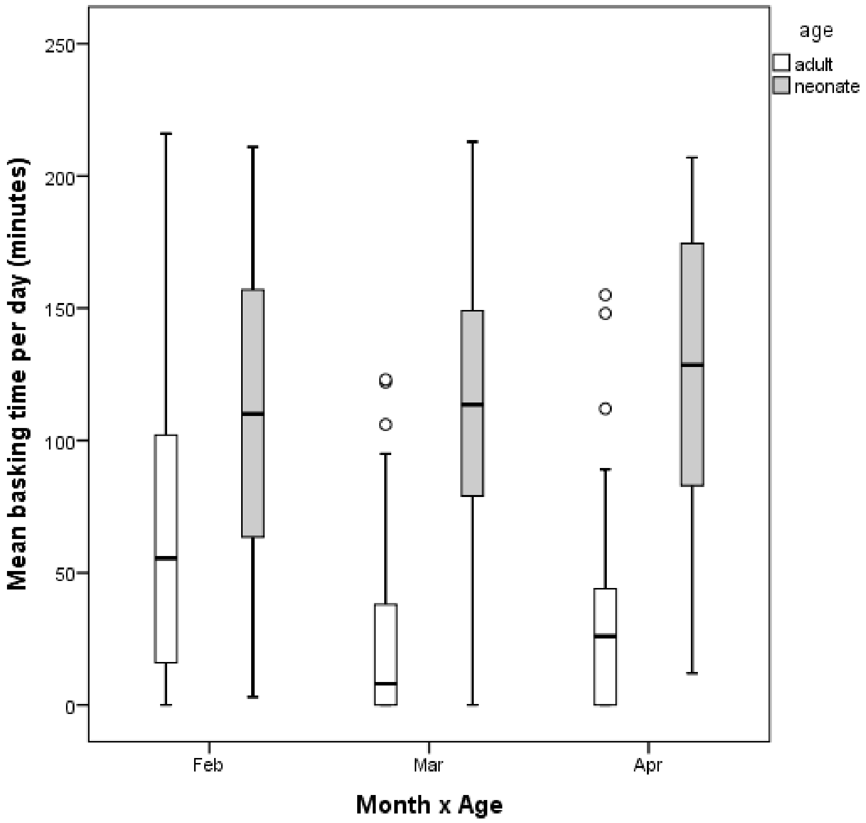

Figure 3: Mean time (minutes; ± SE) spent basking at the burrow entrance in February, March and April for adult and neonate pygmy bluetongue lizards, *Tiliqua adelaidensis*. Adults n=14, neonates n = 12.

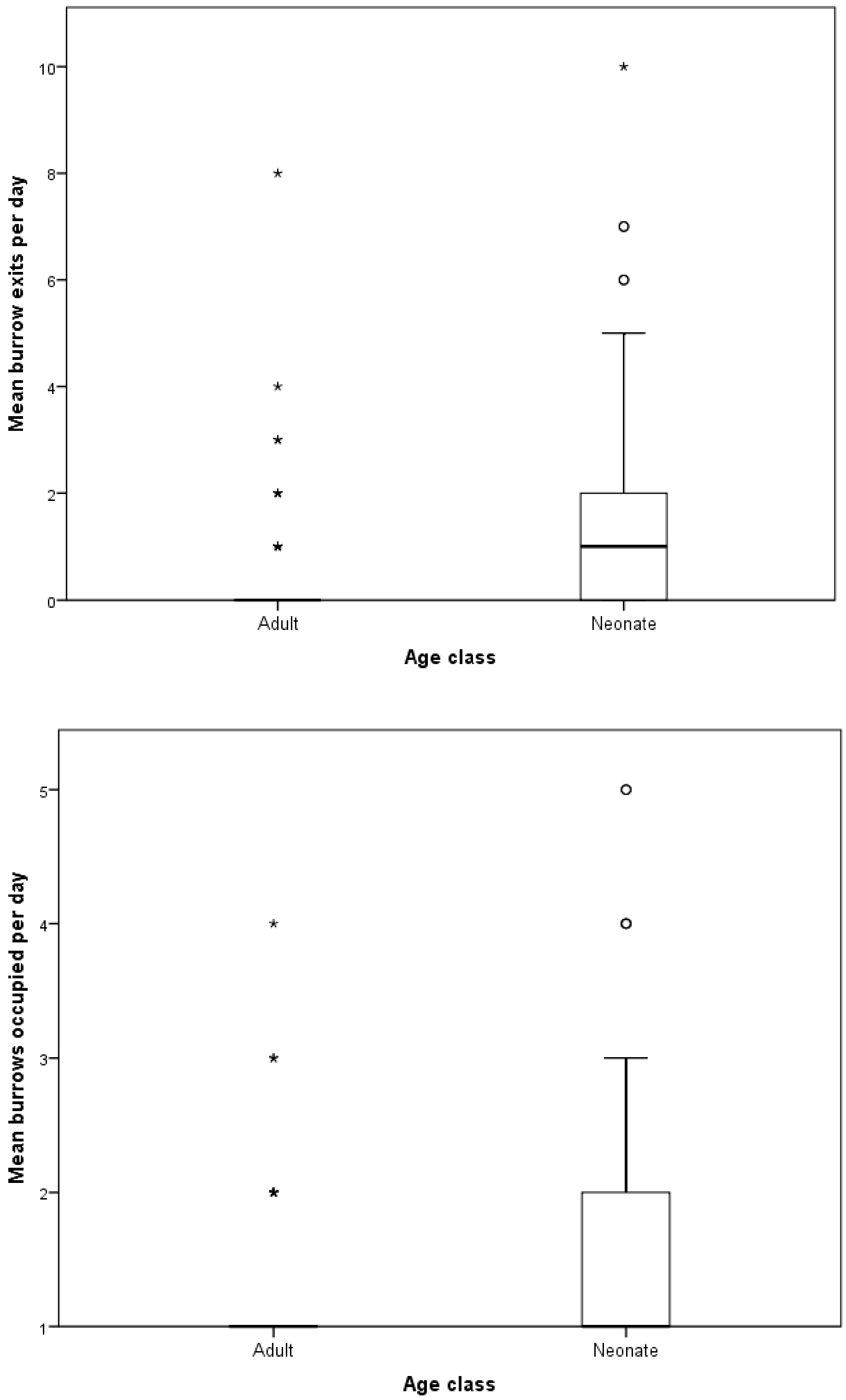

Figure 4: a) Mean number (± SE) of burrow exits for adult and neonate pygmy bluetongue lizards, *Tiliqua adelaidensis*. Adults n = 14, neonates n = 12. b) Mean number of burrows occupied (± SE) during daily filming (four hours) by adult and neonate pygmy bluetongue lizards. Adults n = 14, neonates n = 12. Days n = 24.

Table 1: PERMANOVA results for the behavioural variables; basking time, burrow exits, walking perimeter wall and number of burrows used. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations, ANOSIM R = used as a measure of effect size; Age = age class; Day = filming day. Bold indicates significant P value.

Basking time

Source	df	SS	MS	Pseudo-F	P(perm)	perms	ANOSIM R
Month	2	191.05	95.52	2.28	0.133	9955	0.17
Age	1	1423.00	1423.0	133.93	**<0.001**	9839	
Day	21	918.22	43.73	4.57	**<0.001**	9908	
Month x age	2	207.77	103.89	9.78	**0.001**	9958	
Day x age	21	224.38	10.69	1.12	0.338	9909	
Res	264	2524.90	9.56				
Total	311	6671.20					

Burrow exits

Source	df	SS	MS	Pseudo-F	P(perm)	perms	ANOSIM R
Month	2	2.25	1.12	1.04	0.370	9944	0.08
Age	1	28.49	28.49	39.78	**<0.001**	9832	
Day	21	23.70	1.13	3.62	<**0.001**	9921	
Month x age	2	0.27	0.14	0.19	0.829	9945	
Day x age	21	15.52	0.74	2.37	**0.001**	9921	
Res	264	82.29	0.31				
Total	311	169.19					

Walk perimeter wall

Source	df	SS	MS	Pseudo-F	P(perm)	perms	ANOSIM R
Month	2	0.10	0.05	2.14	0.115	9954	-0.04
Age	1	0.19	0.19	7.88	**0.010**	9850	
Day	21	0.51	0.02	0.85	0.636	9894	
Month x age	2	0.10	0.05	2.14	0.150	9952	
Day x age	21	0.51	0.02	0.85	0.642	9932	
Res	264	7.50	0.03				
Total	311	8.80					

Burrows used (data not transformed)

Source	df	SS	MS	Pseudo-F	P(perm)	perms	ANOSIM R
Month	2	0.28	0.14	0.27	0.773	9949	0.14
Age	1	4.99	4.99	10.44	**0.005**	9788	
Day	21	11.37	0.54	1.64	**0.048**	9923	
Month x age	2	0.30	0.15	0.32	0.738	9955	
Day x age	21	10.21	0.49	1.47	0.096	9906	
Res	264	87.20	0.33				
Total	311	115.74					

2. PBT adult seasonal behaviours

Mean basking time of adult PBTs was lower in March (25.68 min. ± 8.61 SE) and April (36.61 min. ± 8.33 SE) compared to previous months i.e. October (119.75 min. ± 15.23 SE), November (137.71 min. ± 9.16 SE), December (105.04 min. ± 13.34 SE), January, (51.93 min. ± 11.15 SE) and February (83.50 min. ± 12.83 SE) (Appendix S3). There was a significant difference in mean basking time (minutes) between sexes of adult PBTs and an interaction between month and sex (Table 2). Females (90.62 min. ± 7.13 SE) basked longer on average than males (69.44 min. ± 7.31 SE) (Appendix S4), however males basked slightly more than females in October and November, while females basked more in other months (Appendix S5). There were also finer temporal significant differences in adult PBT basking time between days. (Table 2). Temperature had no effect on mean basking time among months (Appendix S6).

Additionally, for adult PBTs, there was no significant difference between months for the behavioural parameters of burrow exits or walking the perimeter wall. However, there was a significant difference in adult PBT burrow exits among the finer temporal scale of days (Table 2).

Table 2: PERMANOVA results table for adult behaviours over the activity season. Behaviours include basking time (minutes), number of burrow exits and number of occasions walking the perimeter wall. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation, P(perm)= p-values based on more than 9000 permutations, Perms= number of permutations; P(MC) = p-value calculated using Monte Carlo projected estimate, ANOSIM R = used as a measure of effect size; Bold indicates significant P value.

Basking								
Source	df	SS	MS	Pseudo-F	P(perm)	perms		ANOSIM R
Month	6	1476.70	246.12	5.75	**0.023**	9637		0.47
Sex	1	150.29	150.29	9.69	**0.002**	9828		
Day	7	299.80	42.83	2.76	**0.009**	9936		
Month x sex	6	263.21	43.87	2.83	**0.012**	9944		
Res	175	2714.90	15.51					
Total	195	4904.90						
Exits								
Source	df	SS	MS	Pseudo-F	P(perm)	perms		ANOSIM R
Month	6	5.24	0.87	0.85	0.514	1408		-0.03
Sex	1	0.36	0.36	1.89	0.207	9823		
Day	7	7.22	1.03	3.29	**0.003**	9928		
Month x sex	6	3.94	0.66	3.45	0.057	9959		
Day x sex	7	1.33	0.19	0.61	0.752	9924		
Res	168	52.70	0.31					
Total	195	70.79						
Walk perimeter wall								
Source	df	SS	MS	Pseudo-	P(perm)	perms	P(MC)	ANOSIM
Month	6	1.01	0.17	F	0.153	4	0.219	R
Sex	1	0.17	0.17	1.84	0.174	2099		0.07
Day	7	0.64	0.09	2.35	0.388	9909		
Month x sex	6	1.01	0.17	1.07	0.053	9036		
Day x sex	7	0.50	0.07	2.35	0.632	9926		
Res	168	14.36	0.09	0.84				
Total	195	7.69						

Discussion

Understanding what life stage to use as a source of individuals for translocations is a fundamental question which faces conservation managers. Our aims were to identify if behaviour differs between adult and neonate PBTs and observe changes in adult behaviour over the entire activity season. These aims were investigated in order to better understand the PBT neonate life stage and inform future translocation efforts. We found that neonate and adult behaviours varied considerably, and that adult basking behaviour changed over the activity season. Of the adult behaviours analysed over the activity season – basking time, burrow exits and walking the perimeter wall, only basking time differed among months, being lower in March and April. Our study had three main findings; (1) neonate PBT basked more than adults in late summer and autumn, (2) neonates exited burrows more and occupied more burrows than adults and (3) neonates walked the perimeter wall more often than adults. The higher activity levels of neonates suggest that in a translocation, neonates may be at more risk of predation and dispersal from the release site compared to adults.

Neonates spent marginally more time on the surface than adults however they exited burrows nearly six times more than adults. Increased exits are likely to attract attention from aerial (birds) and ground dwelling (snakes) predators. In the PBT, the more time spent out of burrow exposed on the surface, the greater the predation risk, thus neonates are more likely to be predated upon than adults assuming that predators can easily spot small prey such as neonate PBT. Therefore, in a translocation of neonates, the number of lizards released would need to be higher to account for predation losses. Although due to high juvenile mortality in wild PBT populations, juveniles can be considered as a harvestable resource for translocations, selecting the juveniles with fewer chances of survival for translocation is not possible as we do not know what factors reduce survival, thus alternative methods that increase individual survival should be considered. For example, in reptile species that have a harvestable resource (e.g. eggs), head-starting can be used to improve both hatching success and survival of juveniles by raising individuals in a predator-free environment (Heppell et al., 1996, Nelson et al., 2002). Therefore, translocating neonate PBTs may not be the most optimal strategy — and either selecting adults as an alternative, or head-starting neonates under captive conditions may be more viable.

We found neonate PBTs basked more than their adult counterparts in late summer and autumn. Basking at the burrow entrance is both a means of thermoregulation, and a method of ambush predation for the PBT, thus one possible explanation is that neonates bask more than adults later in the activity season due to differences in metabolic requirements and growth rates. Adults were found to bask at comparable levels in the spring as neonates basked in late summer and autumn, suggesting that adults are focussed on increasing body condition in spring after emerging from torpor and during the mating period before the birth of offspring. Basking lizards are still partly within the burrow and can rapidly retreat into the safety of the burrow when threatened, hence the seasonal differences in basking between age classes is likely to have limited effect on predation risk. Adult basking time also differed between the sexes, with females basking more than adults in most months, but males basking slightly more than females in the spring months. This difference between sexes could be a result of females basking more in the summer months due to reproductive requirements. PBT neonates have a relatively short length of time from birth to their first period of torpor, to gain sufficient body condition for an extended period of reduced activity and feeding. Neonates of the iguanid lizard *Sceloporus jarrovi* have also been found to spend more time running and foraging than adults, likely a result of the higher metabolic needs of neonates (Watters, 2009). Similarly, neonate water pythons (*Liasis fuscus*) move shorter distances but more often within their floodplain habitat compared to adults, possibly due to difficulty finding suitable sized prey (Pizzatto et al., 2009). Higher activity levels observed in juvenile reptiles could be a result of juveniles taking advantage of suitable thermal ranges to forage and bask in order to increase growth. For example, a study on hatchling *Sceloporus* spp. lizards, found growth rates were plastic and increased with potential activity times according to the thermal environment (Sinervo and Adolph, 1994). On the other hand, in the nocturnal broad-headed snake (*Hoplocephalus bungaroides*), juveniles take shelter during the day to avoid avian predation whereas adults can be found basking or moving around (Webb and Whiting, 2005). This behavioural variation between age classes in the broad-headed snake contributes to its slow growth to reach maturity but presumably lowers mortality from predation (Webb and Whiting, 2005).

PBT neonates were found to exit burrows more often and occupy more burrows than adults late in the activity season. Lizards that are spending more time exposed on the surface outside of burrows are more at risk of predation. Therefore, if the behaviours we observed in captive lizards also occur in wild lizards, it could explain the low rate of juvenile survival from birth to the following spring in PBTs of 6.7% for the 1993/94 season and 36% for the 1995/96 season (Milne, 1999). Using the juvenile survival rates from the 1993/94 and 1995/96 seasons, it was estimated that 6.6% and 19.4% respectively of juvenile females may reach adulthood (Milne, 1999). If there is a large enough source population of neonate lizards, then mortalities during translocation may be acceptable as juvenile mortality is naturally high for this species. However, head-starting projects may be a more viable option for reducing juvenile mortality and increasing success of potential translocations. Head-starting has proven effective in reptile species such as the tuatara (*Sphenodon punctatus*) (Nelson et al., 2002, Jarvie et al., 2015) and gopher tortoise, (*Gopherus polyphemus*) (Quinn et al., 2018). However, there are monetary costs involved in captive husbandry and risks of habituation to captivity the longer individuals are raised in captivity

(Seddon et al., 2007). To minimise the negative effects of captivity, rearing in semi-natural conditions that allow natural behaviours is beneficial (Nelson et al., 2002). Furthermore, head-starting to improve survival in the first year is unlikely to be the most effective method in conserving long-lived species such as turtles that also suffer mortalities in older age classes (Heppell et al., 1996).

In addition to basking and moving between burrows more when compared with adult PBT, neonates also walked the perimeter wall more frequently than adult lizards. Although this behaviour was infrequent (occurred on nine occasions out of a total of 34,560 mins), it only occurred in neonates and walking the perimeter wall could be considered a proxy for dispersal attempt from the enclosure, as lizards would pace along the wall and sometimes try to climb the sides presumably in an attempt to exit. PBT neonates are known to be actively dispersing during the period after birth in late summer (Schofield et al., 2012) and the result that captive neonates walk the perimeter wall more than adults could support this. If this wall-walking behaviour does accurately represent a dispersal attempt, then this indicates neonates may be more likely than adults to disperse from a translocation site in late summer or autumn. Short term confinement at the release site is one method of mitigating dispersal in translocations. However a previous study with adult PBTs has shown that a confinement time of one day is better than five days, as the five day confinement increases stress and the risk of dispersal for this species (Ebrahimi and Bull, 2013). This short confinement period may not be long enough to prevent dispersal of neonates, as our results indicate that they have a dispersal period spanning late summer through to autumn. Differences in dispersal between age classes during translocations have been observed in other species. Captive bred juvenile eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) have shown lower site fidelity in translocations compared to wild born translocated adults (60% to 90% respectively) (McCallen et al., 2018).

The results from our study showed that PBT neonates basked longer, made more burrow movements, occupied more burrows, and walked the perimeter wall more than adults from late February to early April. However, the behavioural comparison between neonate and adult lizards in this study focused on general behaviour in a captive environment and cannot be compared to a translocation. In a translocation, adults may display increased activity and dispersal behaviour as a consequence of the translocation and the new environment, much like neonate lizards exploring their new environment. A previous study investigated changes in adult PBT behaviour and dispersal over the months of the activity season in simulated translocation experiments carried out over two years (Ebrahimi and Bull, 2014). Ebrahimi and Bull (2014) showed a consistent decline in basking, burrow movements, and dispersal from the central release area from October to March, and suggest that translocations that occur later in the season would have a better chance of success. As the declines in adult activity observed in our study are similar to Ebrahimi and Bull's (2014) translocation study, evidence would suggest that neonates translocated later in the season would be more active than adults and thus more likely to disperse or be preyed upon.

Previous studies with other species have compared age classes in translocations with findings showing that the most successful age class varies. A study comparing translocation of wild adult and captive-bred juvenile eastern hellbenders found survival rates were lower in juveniles (Kraus et al., 2017). Site fidelity was also lower in juvenile eastern hellbenders compared to adults (McCallen et al., 2018). However in a translocation of wild adult and captive-reared juvenile tuatara, recapture rates were similar; 56% for juveniles and 61% for adults (Nelson et al., 2002). In our study, we found that PBT neonates moved around more than adults, which is likely to result in lower survival and site fidelity during translocation. The most suitable age class to translocate will depend on the species, and our study provides another method to assist in determining which age is appropriate. Our method of observing differences in behaviour between age classes is especially applicable in species where there is a knowledge gap in age-specific behaviour, such as the PBT.

This study was limited by small sample size, however, working with endangered species poses limits — this study is the first to observe neonate PBT behaviour in detail in a captive environment. The results support field observations on PBT neonate dispersal patterns (Schofield et al., 2012) as neonates were found to be highly active around the time when natal burrow dispersal occurs and for six weeks after. It is difficult to conclusively infer dispersal behaviour from walking the perimeter wall as observed in this study, as the enclosures were small, and our sample size was also small. Future studies in larger enclosures or in the wild could determine if walking the perimeter wall does result in dispersal from the site or alternative exploratory behaviour within the site. Although this study used the PBT as the focal species, it could be replicated in other species with known knowledge gaps in juvenile age classes, particularly in species with high juvenile mortality such as the PBT.

In conclusion, PBT neonates were more active than adult lizards, spending more time basking, moving between burrows and potentially attempting to disperse in a captive environment. This may indicate that neonates are less suitable than adults for translocation projects. For future conservation management of the PBT, we suggest selecting adults or sub-adults for translocation, or raising neonates in captivity until they pass their dispersal stage. Although studies in other species are required, these findings of PBT behaviour can be used cautiously to help decide on suitable age classes for translocations. Other species that are characterised by small juvenile life stages with a lack of parental care are likely to face similar risks, especially if the neonate age class are more active than adult counterparts. Future studies specifically on PBTs and other similar species could compare adult and neonate age classes in a translocation to determine if survival is affected by behavioural variations between age classes.

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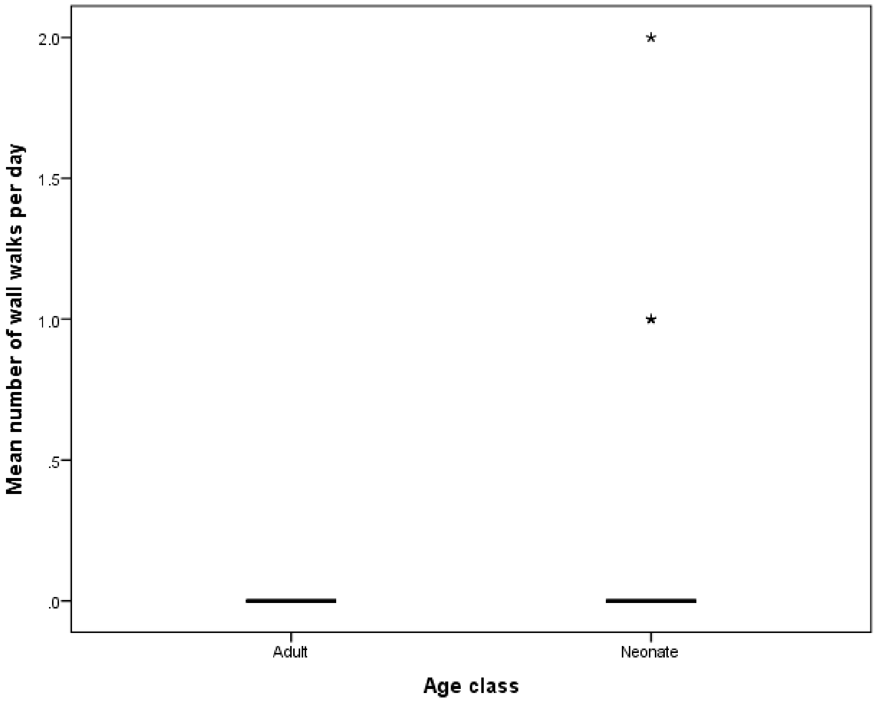
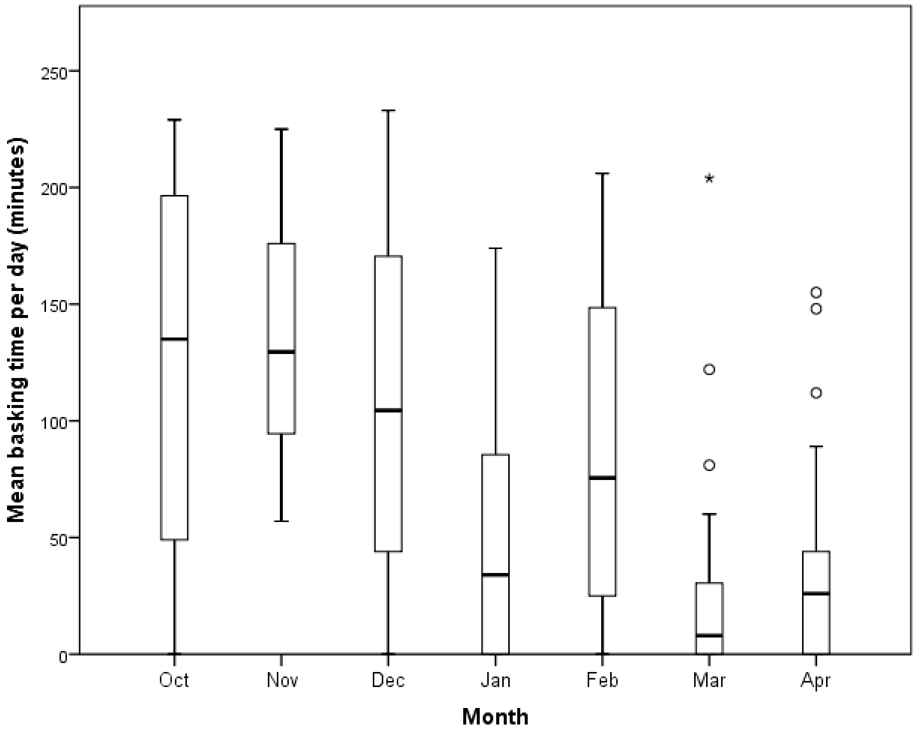
Supporting Information

Appendix S1: PERMANOVA results for the behavioural variables; basking time, burrow exits, walking perimeter wall and number of burrows used with temperature as a covariate. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations; Temp = temperature; Age = age class; Day = filming day. Bold indicates significant P value.

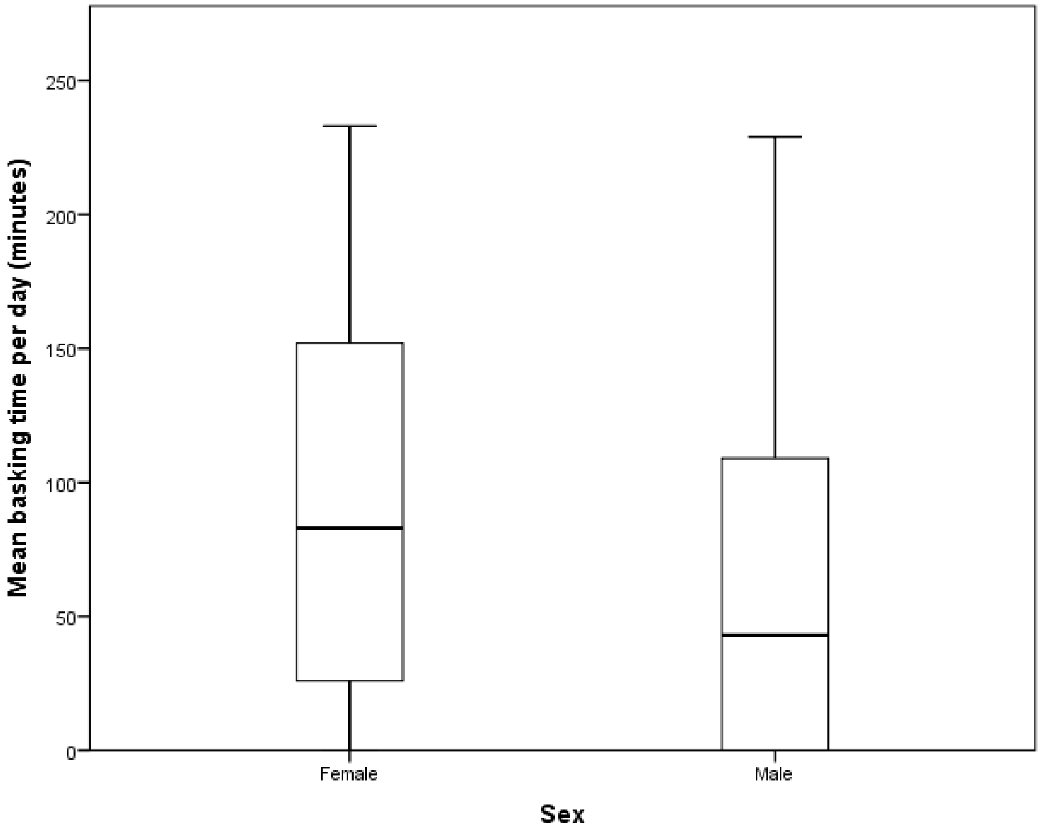
Temperature co-variate basking						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temp	1	136.38	136.38	3.36	0.080	9827
Month	2	195.05	97.53	2.21	0.131	9948
Age	1	2540.00	2540.00	209.27	<**0.001**	9843
Day	20	833.94	41.70	4.36	**<0.001**	9905
Month x age	2	216.55	108.28	9.46	**0.001**	9943
Day x age	21	224.38	10.69	1.12	0.324	9917
Res	264	2524.90	9.56			
Total	311	6671.20				
Temperature co-variate burrow exits						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temp	1	2.95	2.95	3.04	0.093	9829
Month	2	1.69	0.85	0.82	0.454	9949
Age	1	46.59	46.59	57.01	<**0.001**	9825
Day	20	19.94	1.00	3.20	<**0.001**	9913
Month x age	2	0.21	0.11	0.16	0.852	9960
Day x age	21	15.52	0.74	2.37	**0.001**	9913
Res	264	82.29	0.31			
Total	311	169.19				
Temperature co-variate walking the perimeter wall						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temp	1	0.02	0.02	1.03	0.314	9834
Month	2	0.08	0.04	2.10	0.125	9944
Age	1	0.23	0.23	8.61	**0.005**	9845
Day	20	0.38	0.02	0.68	0.888	9905
Month x age	2	0.09	0.05	1.86	0.180	9959
Day x age	21	0.51	0.02	0.85	0.654	9923
Res	264	7.50	0.03			
Total	311	8.80				
Temperature co-variate burrows used						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temp	1	1.34	1.34	2.95	0.103	9852
Month	2	0.16	0.08	0.21	0.811	9955
Age	1	7.36	7.36	13.80	**0.001**	9832
Day	20	9.14	0.46	1.38	0.127	9918
Month x age	2	0.34	0.17	0.37	0.683	9953
Day x age	21	10.21	0.49	1.47	0.093	9926
Res	264	87.20	0.33			
Total	311	115.74				



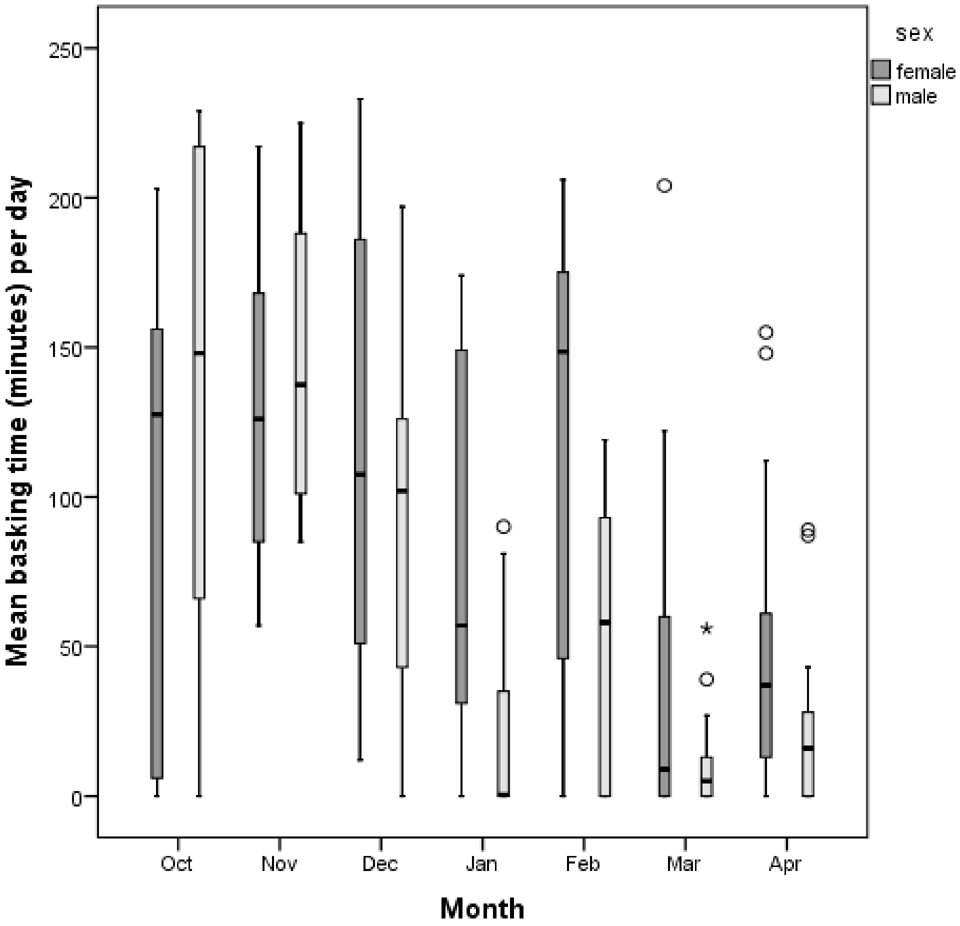
Appendix S2: Mean number (± SE) of occasions a lizard walked the perimeter wall per day for adult and neonate pygmy bluetongue lizards, *Tiliqua adelaidensis*. Adults n=14, neonates n = 12.



Appendix S3: Mean time (minutes; ± SE) spent basking at the burrow entrance per day by adult pygmy bluetongue lizards, Tiliqua adelaidensis over the seven month filming period October to April. Lizards were filmed for two days per month, 4 hours per day, over the seven months. N = 14 lizards.



Appendix S4: Mean time (minutes; ± SE) spent basking at the burrow entrance by adult male and female pygmy bluetongue lizards, *Tiliqua adelaidensis*. Lizards were filmed for two days per month, 4 hours per day, over seven months. N = 14 lizards.



Appendix S5: Mean time (minutes; ± SE) spent basking at the burrow entrance per day by male and female adult pygmy bluetongue lizards, *Tiliqua adelaidensis* over the seven month filming period October to April. N = 14 lizards.

Appendix S6: PERMANOVA results table for adult behaviours over the activity season with temperature as a covariate. Behaviours include basking time (minutes), number of burrow exits and number of occasions walking the perimeter wall. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation; P(perm)= p-values based on more than 9000 permutations; Perms= number of permutations; Temp = Temperature; Bold indicates significant P value.

Temperature co-variate basking						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temp	1	153.13	153.13	3.95	0.074	9851
Month	6	1333.70	222.28	4.76	**0.041**	9963
Sex	1	151.20	151.20	9.78	**0.002**	9832
Day	7	308.36	44.05	2.85	**0.009**	9924
Month x sex	6	267.62	44.60	2.88	**0.012**	9952
Res	174	2690.90	15.47			
Total	195	4904.90				
Temperature co-variate exits						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temp	1	1.20	1.20	2.24	0.161	9842
Month	6	7.34	1.22	1.99	0.192	9968
Sex	1	0.34	0.34	1.78	0.229	9863
Day	7	4.10	0.59	1.86	0.078	9935
Month x sex	6	3.94	0.66	3.48	0.054	9954
Day x sex	7	1.32	0.19	0.60	0.771	9949
Res	167	52.55	0.31			
Total	195	70.79				
Temperature co-variate walk perimeter wall						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temp	1	0.16	0.16	2.79	0.121	9842
Month	6	1.25	0.21	4.00	**0.009**	9922
Sex	1	0.16	0.16	2.27	0.173	9486
Day	7	0.35	0.05	0.59	0.832	9938
Month x sex	6	1.01	0.17	2.37	0.053	9939
Day x sex	7	0.50	0.07	0.84	0.636	9923
Res	167	14.25	0.09			
Total	195	17.69				

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Chapter 4.

Retention of foraging ability in captive lizards managed by a hand-feeding regime.

Hand-feeding a neonate pygmy bluetongue lizard at Monarto Zoo.

Chapter Preface

The previous chapter identified age-specific behavioural differences between neonate and adult lizards, which could affect translocation success. I now investigate another important factor for translocation success – foraging ability. The captive environment is usually less complex than wild habitats, with limited foraging opportunities, an abundant food supply and no predators. Although captive conditions are beneficial for protecting and raising captive animals, the lack of exposure to predators and natural foraging opportunities can result in captive animals that lack important life skills. Lacking the ability to forage can result in starvation or poor condition in animals released into the wild, which can in turn reduce translocation success. This chapter addresses a key knowledge gap — the effect of the captive environment and husbandry methods on lizard foraging ability. There is often a compromise to be made by managers of captive populations —ensuring individuals are in optimal condition and not interfering too much in the natural behaviours of captive animals. In this chapter, I compare the effect of hand-feeding versus self-feeding (lizards capture their own prey) regimes on lizard body condition, foraging ability and behaviour in the pygmy bluetongue lizard. The findings have important implications for the husbandry of captive lizards that may be used in translocations.

Retention of foraging ability in captive lizards managed by a hand-feeding regime.

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Abstract

Captivity can affect animal behaviour and may reduce the chances of wild survival upon release. Specifically, husbandry practices such as captive feeding regimes could have a negative impact on an animal's ability to forage for food, where they may lack foraging ability when released into the wild – however the impact of feeding regimes has received limited research. Our study aimed to determine whether hand-feeding alters body condition and foraging ability in the pygmy bluetongue lizard, *Tiliqua adelaidensis*, a species under captive breeding for conservation. We investigated the effect of a hand-feeding regime on a lizard's prey capture ability, by comparing hand fed lizards against self-fed (i.e. allowed to capture their own prey) captive lizards. Prey capture assays were conducted before the feeding trial commenced and again one year later to determine if foraging ability differed between the two feeding groups. In both feeding treatment groups, lizards were filmed for two hours to observe their behaviour and prey capture responses to a standard number of prey items. Effects of the two feeding regimes on lizard body condition were generally similar, apart from the immature cohort in which a small advantage in the hand-fed group was recorded. The prey capture assays and behavioural trials revealed that hand-fed and

self-fed animals showed mostly similar responses and feeding efficiency, but the hand-fed group basked longer and spent more time in grass tussocks in October than self-fed lizards. Overall, we observed little difference in foraging ability and body condition between the hand-feeding or self-feeding treatment groups, but hand-fed animals displayed less wariness which could increase predation risk in captive animals once released into the wild. Our results provide evidence that approaches to feeding facilitation should simulate wild situations to minimise adverse behavioural changes in captive-release management programs.

Introduction

Around the world, many conservation efforts are based on captive breeding and maintenance of threatened species, in order to enable them to survive destruction and reduction of habitat, and to set up translocation or reintroduction of species into ameliorated wild areas. However, when such captive-reared or maintained animals are returned to a wild situation, the results are often disappointing (Fischer and Lindenmayer, 2000, Moseby et al., 2011). Captive individuals can lack the necessary skills to survive in the wild, interact with conspecifics, avoid predators and forage successfully (Miller et al., 1999, Fischer and Lindenmayer, 2000, Jule et al., 2008). There are now numerous cases where such returns end in failure, or are only maintained by intensive and expensive interventions (Fischer and Lindenmayer, 2000, Letty et al., 2007). The captive environment, often more simplistic than the natural environment, is often identified as the cause of these failures.

Animal husbandry methods are aimed at improving the welfare and wellbeing of animals kept in captivity and captive husbandry strategies that train an animal to respond in a certain way can be beneficial from a zoo perspective. Captive animals can be habituated to allow daily husbandry and veterinary procedures to be performed safely and efficiently, and improve animal welfare (Hellmuth et al., 2012, Burghardt, 2013, Emer et al., 2015). However, practices beneficial for the care of captive animals may affect the ability of animals to survive if released into the wild. Captive-born animals released into the wild have showed lower survivorship than wild-born animals (Letty et al., 2007), and starvation has been recorded as a major cause of death in translocated captive-bred carnivores (Jule et al., 2008) and desert tortoises (*Gopherus agassizii*) (Nafus et al., 2017). More nuanced examples include low growth rates of captive-bred animals once released, possibly due to inadequate captive diets (Roe et al., 2015), and observations that foraging ability of captive-reared animals is improved if the captive environment is enriched (Alberts, 2007). However few studies have examined if intensive hand feeding can be replaced by wild-simulated feeding without sacrificing body-condition and subsequent foraging ability required for release.

The pygmy bluetongue (PBT), *Tiliqua adelaidensis* is an endangered lizard endemic to grasslands in a limited region just to the north of Adelaide, South Australia. This species is threatened by climate change, habitat loss and fragmentation due to agricultural practices and changing land use (Souter et al., 2007, Smith et al., 2009, Delean et al., 2013) and captive populations may form part of future release strategies to mitigate against the decline of the species (Fordham *et al.* 2012). The species occupies vertical burrows constructed by lycosid and mygalomorph spiders (Hutchinson et al., 1994) which provide protection from temperature extremes, predators and a site to ambush prey (Milne et al., 2003). Pygmy bluetongues are omnivorous, mostly consuming invertebrates caught by a sit-and-wait ambush strategy and some plant matter (Milne et al., 2003, Fenner et al., 2007). This lizard therefore provides an excellent case to determine how captive feeding regimes may affect the sit-and-wait prey capture ability of the species, as reduced ability to perform such ambush feeding behaviour would likely lead to compromised captive-bred release animals.

When releasing animals for translocations, it is important that animals have both good body condition and the ability to forage. For this reason, we needed to investigate if we could simulate natural foraging conditions by having lizards self-feed whilst maintaining body condition. Previously, captive breeding of pygmy bluetongue lizards entailed hand-feeding to ensure good body condition and growth rates. We therefore first examined if the body condition of self-fed captive lizards were comparable to those lizards hand-fed. Next, we aimed to determine the effect of captivity on foraging ability in the pygmy bluetongue lizard, an ambush foraging lizard species, as a lack of foraging ability is a major cause of mortality when captive animals are released into the wild. Thus, a feeding trial was conducted to compare hand-fed and self-fed treatments to determine if; (1) body condition differed between the two treatment groups (2) prey capture and foraging ability differed between treatments and, (3) general behaviours differed between the two treatment groups. Although our study species was the pygmy bluetongue lizard, the findings could also be applicable to other reptile species with similar ambush foraging behaviours.

Methods

Enclosures

Pygmy bluetongue lizards were housed in ten raised enclosures situated within a 15 m diameter circular caged area, at Monarto Zoo, South Australia. The circular cage had a netted roof and wire meshed walls to exclude predators. Enclosures were 0.65 m high, 2.4 m long, 1.2 m wide and filled with sandy loam to a depth of 0.4 m. Each enclosure was further divided into three sections, with six artificial burrows per section spaced approximately 30 cm apart. Adults were housed singly (7 hand-fed, 7 self-fed), while juveniles (8 hand-fed, 10 self-fed) and immatures (6 hand-fed, 7 self-fed) were housed in pairs. Adult burrows were constructed from wooden dowel 300 mm long, 30 mm in diameter with a 20 mm hole drilled through the centre. Juvenile and immature wooden dowel burrows were 200 mm long, 28 mm in diameter with an 18 mm hole. Each enclosure section had four individual native grasses (*Austrostipa* sp. and *Austrodanthonia* sp.) to provide shade.

Lizards

The captive population of PBT at Monarto Zoo consisted of 45 individuals; 14 wild born adults in captivity since spring 2014, 13 immatures born in 2016 and 18 juveniles born in 2017. Lizards were randomly assigned to a treatment group; hand-fed or self-fed. Lizards were kept in the same treatment group throughout the experiments. All lizards had been hand-fed in previous lizard activity seasons. Feeding in spring 2017 did not commence until after the first prey capture assay was conducted in early October 2017 and then lizards were fed immediately according to their treatment group prior to the commencement of behavioural filming. Feeding treatments were maintained over the 2017/18 activity season and recommenced once lizards became active in September 2018 prior to the second prey assay.

Feeding and body condition

Adults were fed crickets twice a week, and juveniles and immatures were fed three times a week over the lizard activity season (October 2017 to April 2018). Crickets were lightly dusted in Reptile One Calcium powder + D3, and Aristopet Repti-vite once per week as a dietary supplement. Hand-fed lizards were fed crickets individually using a pair of tweezers. The self-fed group were given double the number of crickets as hand-fed lizards, and crickets were chilled in a fridge prior to release into lizard enclosures to slow their movements. Lizards were captured once a month to measure weight (g) and length (snout-vent length, SVL) to calculate body condition (g/SVL) for comparison of feeding treatments and to monitor lizard welfare. Those measurements taken in March 2018 were analysed to compare differences between the two treatment groups after lizards had experienced the feeding treatment for an entire spring/summer season and differences in body condition between treatment groups would be most evident.

Lizard behaviour

All lizards were filmed for four hours per day across four days per month with 15 Movii Neo action cameras with external battery, from October 2017 to March 2018. Half of the enclosures e.g. adult enclosures, were filmed one day and the other half e.g. juvenile / immature enclosures, filmed another day, alternating so that all lizards were filmed 4 times per month. Filming hours were usually 10 am to 2 pm, except on cool rainy days when filming started later or hot days over 35°C when filming commenced earlier, at 9 am. Behavioural assays included; time spent basking, number of burrow exits, burrow changes and time spent on the surface either sitting, in grass or walking the perimeter wall.

Prey capture assays

Assays were conducted twice; in October 2017 before feeding treatments commenced and finally during September 2018 after lizards had experienced the treatment for a year. This time frame allows for any sustained changes in foraging ability to be detected as lizards had a year to adapt to the feeding treatment. Assays were all conducted outdoors within the lizard enclosure. A container 57 x 38 x 36 cm was filled with sand to a depth of 10 cm, which was refreshed between each of the lizards. Each lizard had a single artificial burrow 300 mm long with a hole inserted into the sand on an angle. Lizards were placed into the arena and allowed to become acclimatised for one hour. After the acclimatisation period, five crickets were released into the arena and lizards were filmed for two hours. Parameters included successful capture attempts, unsuccessful capture attempts, time to first attempt, time out of burrow capturing prey, basking time and time spent on the surface walking or sitting (but not foraging for prey). After the assay concluded, lizards were returned to their home enclosures.

Ethics statement

The research was conducted according to the approval of the Flinders University Animal Welfare Committee (project number E453/17) and the Wildlife Ethics Committee (project number 28/2017).

Statistical analysis

Primer-e v7/PERMANOVA+ was used to conduct univariate, multifactorial, repeated measures PERMANOVA tests based on Euclidean distance matrices on square-root transformed data. . Feeding treatment (hand-fed, n = 21 or self-fed, n = 24) and age (juveniles, immatures, or adults) were classified as the between subjects fixed factors for both prey assay analysis and behavioural analyses. For the prey analyses, trial was a fixed within subjects factor. Interaction effects were tested for trial x treatment, trial x age and treatment x age. For the behavioural analyses, month was a fixed within subjects factor and day was a random within subjects factor. Interaction effects were tested for month x treatment, month x age, day x treatment and treatment x age. Temperature was analysed as a covariate in both prey assay and lizard behaviour analyses. IBM SPSS Statistics 23 was used to conduct independent T-tests on body condition data.

Results

Body condition

Body condition of PBTs did not differ significantly between feeding groups for adult or juvenile cohorts, but there was a significant difference between feeding groups for the immature cohort (Table 1). In the immatures, hand-fed lizards had a higher g/SVL than self-fed lizards, while g/SVL was similar between feeding groups for adults and juveniles (Table 1)).

Table 1: Independent t test results for March mean g/SVL comparing the two feeding treatments, self-fed and hand-fed in the adult, immature and juvenile age classes.

Age	Group	Mean	SD	df	t value	P value
Adult	Self-fed	0.18	0.014	12	-1.178	0.262
	Hand fed	0.19	0.014			
Immatures	Self-fed	0.13	0.015	11	-2.837	**0.016**
	Hand fed	0.15	0.010			
Juveniles	Self-fed	0.11	0.015	16	-0.778	0.448
	Hand fed	0.12	0.010			
Juveniles combined	Self-fed	0.12	0.017	29	-1.867	0.072
	Hand fed	0.13	0.019			

Prey assays

The total filming time over the two prey assays was 10,800 minutes. Lizards spent 2,595 minutes basking, 1,270 minutes sitting or walking on the surface and 182 minutes out of their burrows capturing prey. Lizards took on average 9.83 minutes to make their first capture attempt, made a total of 185 successful captures and 146 unsuccessful capture attempts. The average temperature in the first (before treatment) trial was 29.5 ° C and 20.8°C in the second (after treatment) trial.

Time to first capture

The time to first capture attempt by PBTs was significantly different between feeding treatments (Table 2), with the hand-fed group (2.22 min. ± 0.87 SE) making their first capture attempt in less time than the self-fed group (16.84 min. ± 5.07 SE) . Lizards took less time to make their first capture attempt in the ‘before’ trial (5.36 min. ± 2.85 SE) than the ‘after’ trial (16.24 min. ± 5.27 SE) although this was not significant (Table 2). There was no significant interaction effect between trial and treatment (Table 2, Figure 1). Temperature did not have a significant effect on time to first capture attempt (Table S2).

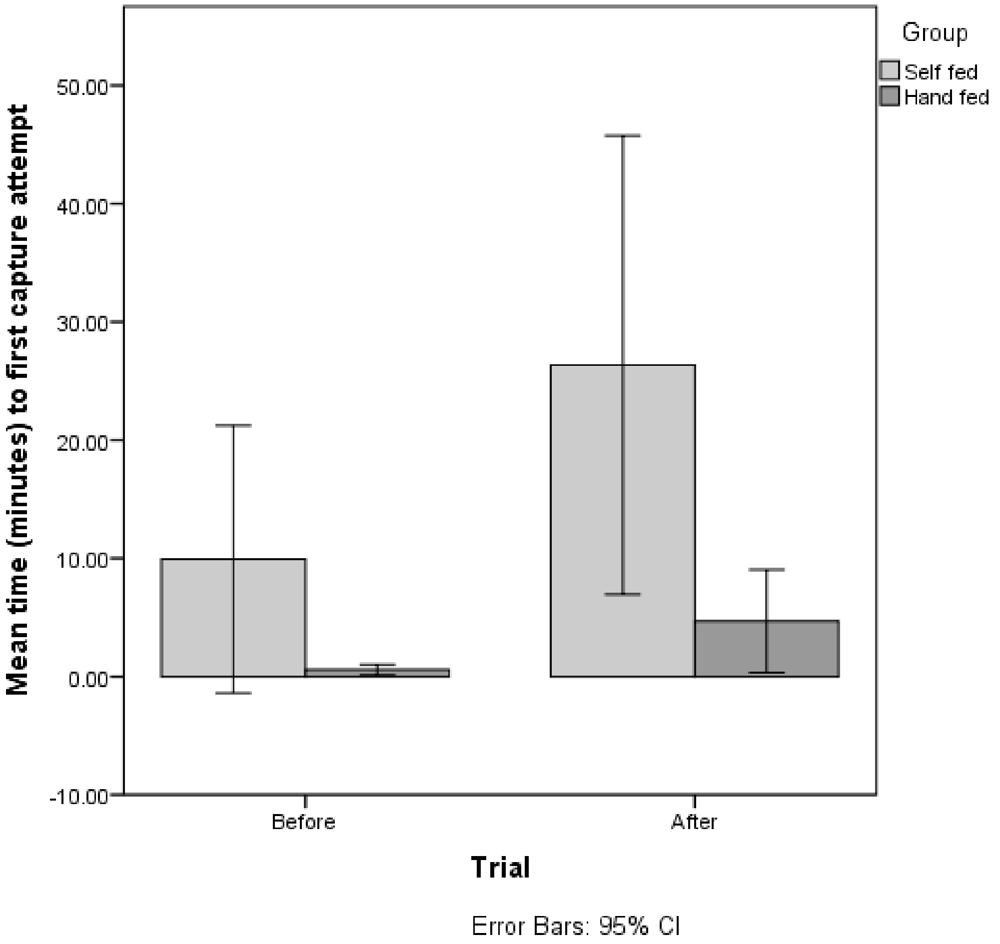

Figure 1: Mean time in minutes to first capture attempt (95% CI) by lizards in the self-fed and hand-fed groups before and after feeding treatments commenced.

Capture attempts

The number of successful captures by PBTs differed significantly between trials (Table 2). However, there was no significant interaction effect between trial and feeding treatment (Table 2, Figure 2). Lizards from both groups made more successful attempts in the 'before' trial (3 attempts ± 0.26 SE) than in the 'after' trial (1.07 attempts ± 0.18 SE). There was no significant difference between the number of unsuccessful attempts between the 'before' trial (2.27 attempts ± 0.43 SE) and 'after' trial (0.98 attempts ± 0.22 SE) or interaction effect between trial and treatment although temperature had a significant effect on the number of unsuccessful captures between trials (Supporting information: Table S1). There were no significant differences between feeding groups or among age classes.

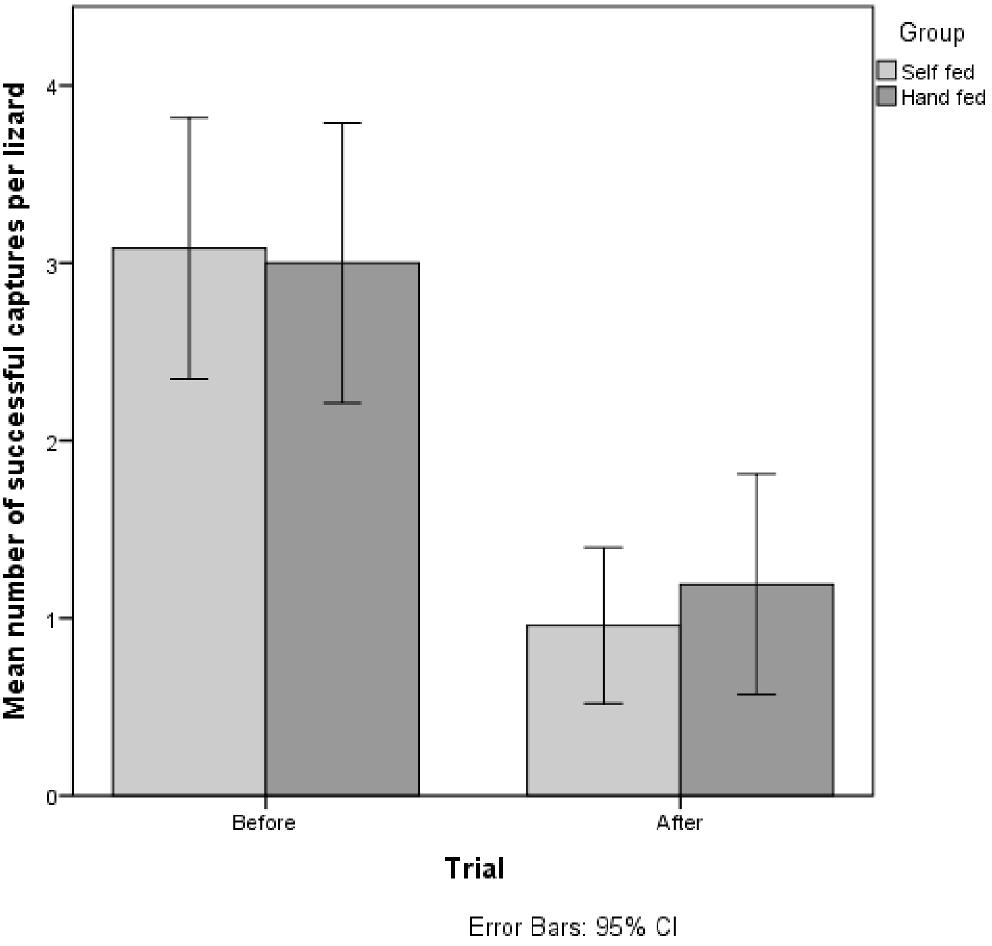

Figure 2: Mean number of successful captures (95% CI) per lizard before (first trial) and after (second trial) feeding treatments were implemented for the two treatment groups – hand-fed and self-fed.

Time out of burrow capturing prey

There was a significant difference between trials in time PBTs spent out of burrows capturing prey (Table 2). In the 'before' trial, lizards spent more time capturing prey outside of their burrow on the arena surface (3.60 min. ± 0.36 SE), than in the 'after' trial (0.9 min. ± 0.20 SE). There was no significant interaction effect between trial and treatment (Table 1, Figure 3). There were no significant differences between hand-fed or self-fed lizards or among age classes and temperature did not affect the time lizards spent capturing prey outside of the burrow (Table S2).

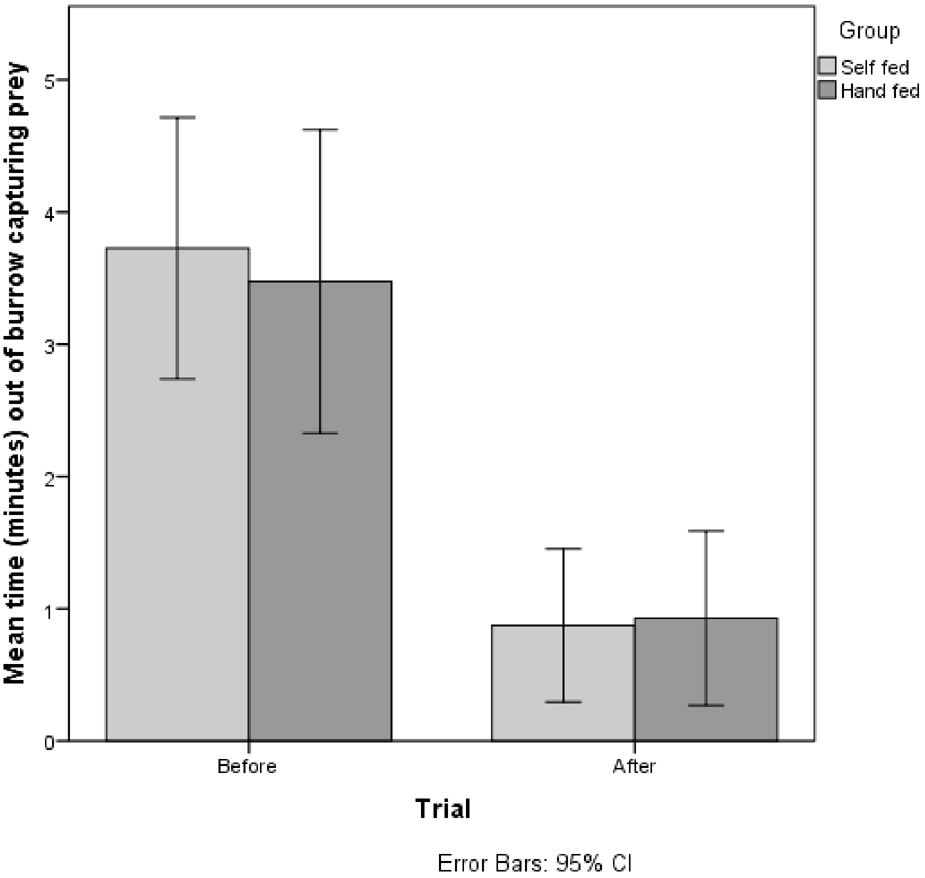

Figure 3: Mean time in minutes (95% CI) lizards spent out of burrow capturing prey for the self-fed and hand fed groups in the before and after feeding treatments commenced.

Basking time

Basking time of PBTs did not differ significantly between feeding groups or trials (Table 2). There was a significant difference in basking time among age classes (Table 2) with juveniles basking 33.36 min. ± 5.30 SE, immatures basking 36.69 min. ± 6.62 SE and adults basking 15.71 min. ± 4.16 SE. There was also a significant interaction effect between trial and age (Table 2, Figure S3), with juveniles and adults basking longer in the 'after' trial compared to the 'before' trial. Temperature did not affect basking time (Table S2).

Walking or sitting on surface

Time spent walking or sitting on the sand surface by PBTs did not differ significantly between trials — 'before' trial 16.38 min. ± 2.50 SE and 'after' trial 11.84 min. ± 2.93 SE or between feeding groups — self-fed 14.29 min. ± 2.52 SE and hand-fed 13.90 min. ± 3.00 SE (Table S1). Adults spent more time on the surface (17.18 min. ± 3.98 SE) compared to the juvenile (12.56 min. ± 2.63 SE) and immature (12.96 min. ± 3.66 SE) cohorts, but this difference was not significant (Table S1). There was no significant effect of temperature (Table S1).

Table 2: PERMANOVA results for the prey assay variables; time to first capture attempt, successful capture attempts, time out of burrow capturing prey and time walking or sitting on the surface. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations; Age = age classes – adults, immatures and juveniles. Bold indicates significant P value.

Time to first attempt						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Trial	1	47.45	47.45	7.47	0.058	9962
Treatment	1	48.28	48.28	348.27	**0.003**	9994
Age	2	-0.35	-0.17	0.22	0.690	9972
Trial x treatment	1	0.17	0.17	4.59	0.095	9971
Trial x age	2	-6.68	-3.34	negative		
Treatment x age	2	-6.76	-3.38	negative		
Resolution	54	185.26	3.43			
Total	89	386.41				
Successful captures						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Trial	1	14.62	14.62	19.57	**0.002**	9933
Treatment	1	0.04	0.04	0.52	0.484	9848
Age	2	2.51	1.25	3.76	0.099	9955
Trial x treatment	1	0.09	0.09	0.53	0.523	9943
Trial x age	2	0.09	0.05	0.86	0.568	9961
Treatment x age	2	0.76	0.38	0.71	0.494	9951
Resolution	59	33.82	0.57			
Total	89	60.93				
Time out burrow captures						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Trial	1	16.53	16.53	103.81	**0.003**	9979
Treatment	1	-0.03	-0.03	0.17	0.721	9971
Age	2	0.63	0.32	0.71	0.557	9969
Trial x treatment	1	0.06	0.06	0.35	0.544	9981
Trial x age	2	2.92	1.46	2.37	0.190	9970
Treatment x age	2	1.17	0.58	1.82	0.237	9977
Resolution	59	17.83	0.30			
Total	89	44.40				
Basking						
source	df	SS	MS	Pseudo-F	P(perm)	perms
Trial	1	42.78	42.78	1.57	0.245	9948
Treatment	1	0.76	0.76	0.50	0.497	9817
Age	2	110.09	55.04	28.81	**0.001**	9953
Trial x treatment	1	16.54	16.54	3.09	0.105	9936
Trial x age	2	25.08	12.54	8.42	**0.015**	9953
Treatment x age	2	5.47	2.73	0.29	0.749	9939
Resolution	59	630.83	10.69			
Total	89	1078.50				

Behavioural assays

During the October to March filming period (3 days per lizard in October and 4 days other months) lizards basked for a total of 65,043 minutes, exited burrows 1110 times, spent 1,856 minutes in grass tussocks, spent 703 minutes sitting on the surface and spent 320 minutes walking the enclosure wall.

Basking

Basking time differed significantly between feeding treatments (Table 3). Self-fed lizards basked for 59.93 min. ± 2.50 SE and hand-fed lizards basked for 66.17 min. ± 2.74 SE. There was a significant interaction effect between treatment and age (Table 3). In the immature age class, self-fed lizards basked less than the hand-fed group (Figure 4). Basking time was not significantly different between feeding groups for adults and juveniles (Figure 4). There were significant differences among months, days and age classes (Table 3; refer to chapter 2). Temperature had no significant effect on basking time.

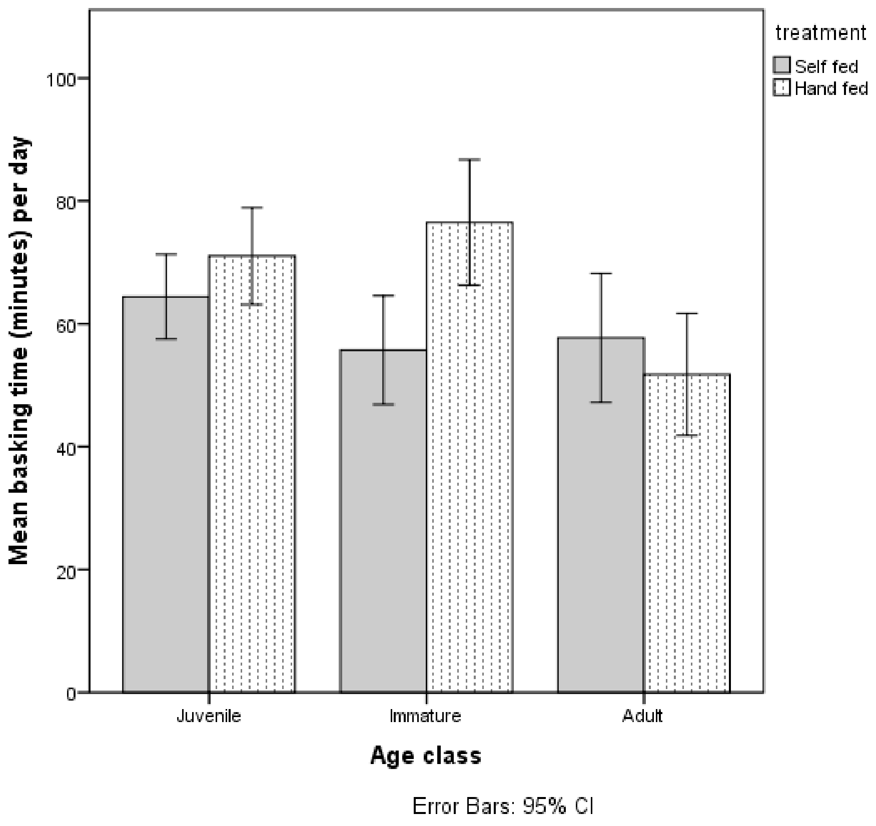

Figure 4: Mean time (minutes, 95% CI) spent basking per day by juvenile, immature and adult lizards in the self-fed and hand-fed treatment groups.

Grass use

The mean time PBTs spent in grass tussocks did not differ significantly between feeding treatments (Table 3). Hand-fed lizards spent 2.23 min. per day ± 0.53 SE in grass tussocks compared to 1.41 min. per day ± 0.20 SE for self-fed lizards. There was a significant interaction between month and treatment, with hand fed lizards spending more time in grass tussocks in October compared to self-fed lizards (Figure 5). Month, day and age were significantly different, and there was a significant interaction between month and age (Refer to chapter 2). Temperature had no significant effect on time lizards spent in grass.

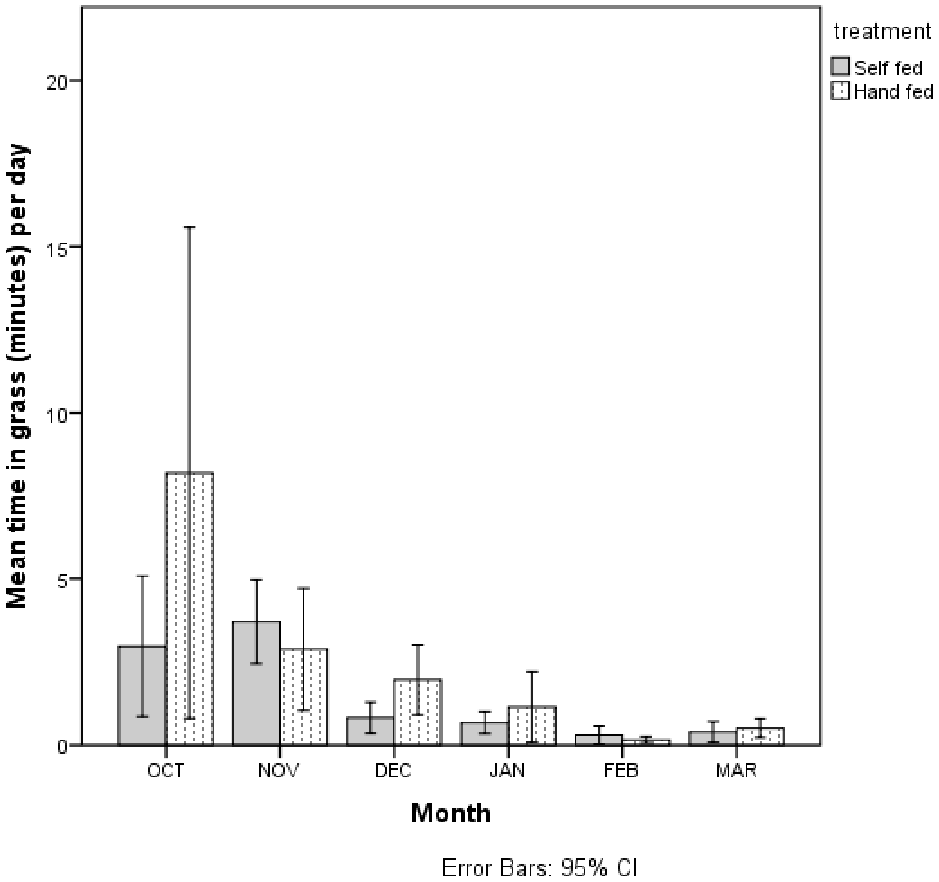

Figure 5: Mean time (minutes, 95% CI) lizards spent in grass tussocks per day for hand-fed and self-fed treatments over the months of the activity season.

Exits

Treatment did not have a significant effect on the number of burrow exits made by lizards — self-fed lizards made 1.11 exits ± 0.10 SE and hand-fed lizards 1.02 exits ± 0.09 SE (Table 3). There was a significant interaction effect between treatment and age (Table 3). Self-fed lizards made slightly more exits than hand-fed lizards in juvenile and immature age classes, and hand-fed adults exited burrows more than self-fed adults (Figure 6). Month, day and age differed significantly, and there was a significant interaction between month and age (Refer to chapter 2). Temperature did not have a significant effect on burrow exits.

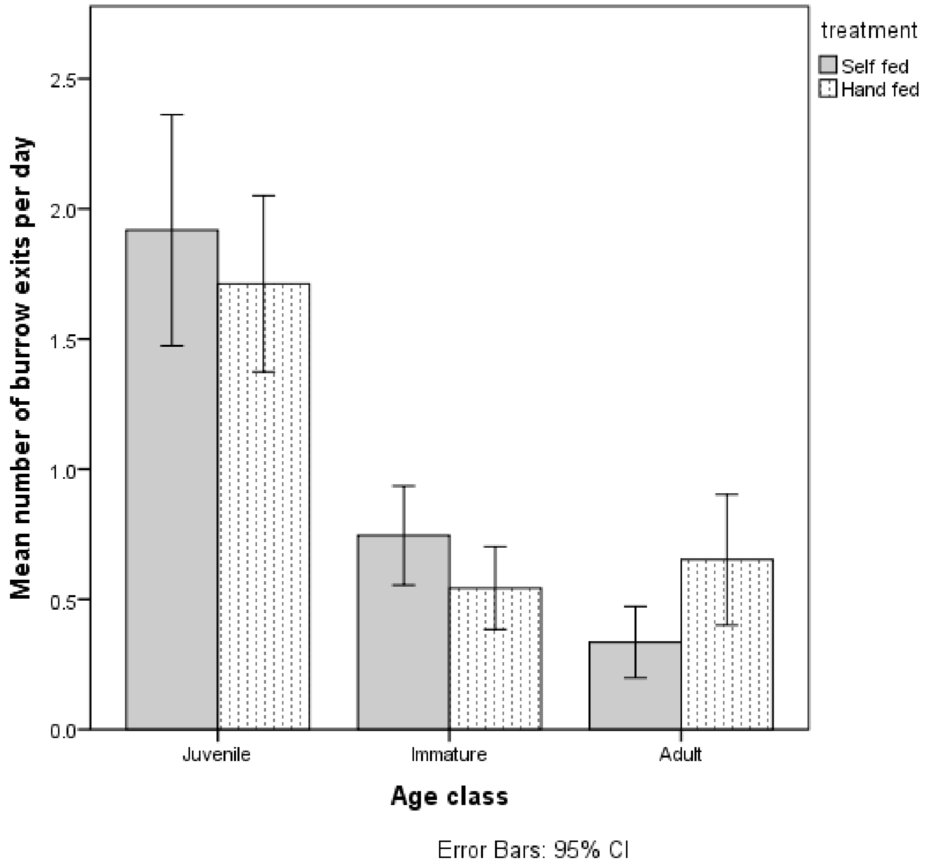

Figure 6: Mean number of burrow exits per day by lizards in juvenile, immature and adult age classes for self-fed and hand-fed groups.

Other behaviours

There was no significant effect of feeding treatment on the number of burrows occupied, or time spent sitting on the surface or walking the enclosure wall by pygmy bluetongue lizards (Table 3). For significant differences among age classes and months and interaction effects between month and age, refer to chapter 2.

Table 3: PERMANOVA results for the behavioural variables; basking, exits, time spent in grass, sitting on surface, walking perimeter wall, and burrows occupied. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations; Day = filming day, Age = age class. Bold indicates significant P value.

Bask						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	7669.80	1534.00	16.14	**<0.001**	9948
Treatment	1	40.15	40.15	4.56	**0.038**	9840
Day	40	3378.80	84.47	9.21	**<0.001**	9872
Age	2	278.26	139.13	11.26	**<0.001**	9943
Month x treatment	5	43.97	8.79	1.12	0.369	9947
Month x age	9	196.41	21.82	1.73	0.124	9954
Day x treatment	40	316.93	7.92	0.86	0.707	9895
Treatment x age	1	22.70	22.70	4.43	**0.045**	9831
Resolution	882	8085.30	9.17			
Total	1034	20485.00				
Exits						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	119.91	23.98	6.13	**<0.001**	9938
Treatment	1	0.42	0.42	1.35	0.251	9816
Day	40	139.29	3.48	7.90	**<0.001**	9885
Age	2	38.05	19.02	38.62	**<0.001**	9947
Month x treatment	5	2.43	0.49	1.54	0.185	9948
Month x age	9	23.02	2.56	5.16	**0.001**	9942
Day x treatment	40	12.48	0.31	0.71	0.917	9868
Treatment x age	1	3.19	3.19	7.25	**0.009**	9821
Resolution	904	398.40	0.44			
Total	1034	750.71				
Grass						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	130.24	26.05	5.48	**0.001**	9935
Treatment	1	5.43	5.43	3.49	0.069	9820
Day	40	169.79	4.24	3.57	**<0.001**	9877
Age	2	24.11	12.06	11.64	**0.001**	9950
Month x treatment	5	17.85	3.57	2.34	**0.047**	9940
Month x age	9	42.55	4.73	4.61	**0.003**	9932
Day x treatment	40	55.86	1.40	1.17	0.217	9879
Treatment x age	1	0.07	0.07	0.06	0.810	9829
Resolution	904	1075.10	1.19			
Total	1034	1548.40				
Sitting						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	31.65	6.33	4.85	**0.001**	9946
Treatment	1	1.36	1.36	2.76	0.105	9844
Day	40	46.86	1.17	2.11	**<0.001**	9864
Age	2	0.18	0.09	0.11	0.881	9955
Month x treatment	5	1.22	0.24	0.62	0.723	9935
Month x age	9	7.28	0.81	0.96	0.463	9942
Day x treatment	40	18.21	0.46	0.82	0.770	9866
Treatment x age	1	0.01	0.01	0.02	0.886	9837
Resolution	904	502.03	0.56			
Total	1034	632.09				

Walking						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	21.06	4.21	8.17	**<0.001**	9942
Treatment	1	0.02	0.02	0.18	0.679	9828
Day	40	18.45	0.46	1.81	**0.003**	9891
Age	2	2.08	1.04	5.46	**0.017**	9942
Month x treatment	5	2.85	0.57	1.79	0.119	9941
Month x age	9	4.66	0.52	2.78	**0.024**	9954
Day x treatment	40	11.84	0.30	1.16	0.236	9884
Treatment x age	1	0.02	0.02	0.06	0.809	9858
Resolution	904	230.34	0.25			
Total	1034	296.17				
Burrows						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	5	287.31	57.46	7.20	**<0.001**	9944
Treatment	1	2.33	2.33	0.99	0.332	9856
Day	40	284.72	7.12	3.60	**<0.001**	9872
Age	2	90.11	45.06	14.96	**<0.001**	9944
Month x treatment	5	22.18	4.44	1.78	0.124	9947
Month x age	9	141.81	15.76	5.11	**0.003**	9951
Day x treatment	40	92.64	2.32	1.17	0.223	9887
Treatment x age	1	0.21	0.21	0.11	0.744	9845
Resolution	904	1785.20	1.97			
Total	1034					

Discussion

We aimed to determine the effect of two captive feeding regimes (hand-feeding versus self-feeding) on body condition, foraging ability and general behaviours in the pygmy bluetongue lizard (*Tiliqua adelaidensis*) as poor body condition and a lack of foraging ability are potential reasons for low survival of captive animals released into the wild. We found that body condition and foraging ability were not affected by the captive feeding regime, but behaviour differed between the hand-fed and self-fed groups. Our study had three main findings; (1) lizards that were self-fed were able to maintain adequate body condition, (2) hand-feeding did not affect prey capture success or the number of capture attempts made by lizards, and (3) hand-fed lizards basked for longer than self-fed lizards and spent more time in grass tussocks in October compared to self-fed lizards. These findings suggest that hand-fed lizards would be able to forage successfully if released into the wild but may be more likely to be preyed upon due to spending more time out of the burrow. Therefore, as self-feeding lizards were able to maintain good body condition without displaying negative behavioural changes, we have some confidence that captive maintenance with self-feeding would be a suitable strategy for head-starting future releases of PBTs.

Our study found that hand-feeding was not required in order to maintain adequate body condition. Pygmy bluetongue lizard body condition as measured by g/SVL was similar between hand-fed and self-fed groups for the juveniles and adults. The hand-fed group of the immature cohort had a significantly higher g/SVL than the self-fed group, however it was still within the normal range reported from wild populations (Fenner and Bull, 2007, Shamiminoori et al., 2014). Overall, we suggest that captive lizards released into the wild would be able to maintain a healthy body condition based on immediate foraging ability, or at least not succumb to starvation (i.e. dependent on prey availability), as the self-fed lizards in our feeding trial were able to forage successfully. However, as this was a short-term captive study, a long-term study after release into the wild would be required to investigate foraging ability and survival in the wild.

We found that pygmy bluetongue lizard foraging ability was not affected by hand feeding in captivity. This could be due to pygmy bluetongue lizards having an innate foraging ability, which has been reported in other vertebrate species such as the Tasmanian devil (*Sarcophilus harrisii*) (Rogers et al., 2016). Furthermore, we did not detect differences between wild-born captive adults and captive-born lizards, which could further support that foraging ability is innate, and that the number of years in captivity has not altered adult foraging behaviour. Previous studies have varied results, with foraging behaviour maintained in pupfish (*Cyprinodon bovinus*) (Black et al., 2017) but not in ratsnakes (*Elaphe* *obsoleta*) (DeGregorio et al., 2013). Alternatively, the semi-naturalistic enclosures which allow invertebrate species to enter could provide potential foraging opportunities more akin to what their natural environment provides even for hand-fed lizards (Fenner et al., 2007). A captive breeding population of the sand lizard (*Lacerta agilis),* maintained in naturalistic enclosures that allow the colonisation of invertebrate prey to supplement the supplied diet, has provided juveniles for successful reintroductions (Woodfine et al., 2017). Providing natural foraging opportunities may better prepare animals for release into the wild.

A potential cost of the hand-feeding strategy in terms of captive-breeding and release was that behaviour of hand-fed lizards differed from self-fed lizards . Our results show that hand-fed lizards spent longer basking and in grass tussocks in the month of October than self-fed lizards. Lizards in both feeding treatments exited their burrow around once per day, however there were interaction effects among age classes. Hand-fed adults exited their burrows more than self-fed adults, although the opposite was true for juvenile and immature age classes, albeit not significantly. This could indicate that hand-feeding has altered lizard behaviour, and those lizards have become less cautious. Hand-feeding requires lizards to take the cricket from the tweezers held by researchers, potentially resulting in habituation to humans as food providers. This altered behaviour could increase the predation risk to hand-fed lizards if they were released into the wild in a translocation — however, this interpretation should be taken cautiously, as burrow exits, and time spent in grass were relatively infrequent behaviours. For example, pygmy bluetongue lizards are preyed upon by brown snakes (*Pseudonaja textilis*) and a number of bird species in their natural habitat (Hutchinson et al., 1994, Fenner et al., 2008) and any additional time outside of the safety of their burrow would increase the risk of encountering predators. Similar changes in animal behaviour in which animals have lost their fear of humans have been identified in captive raptors (Park, 2003) and wild reef fish (de Paula et al., 2018), and habituation to humans can lead to increased boldness and decreased wariness towards predators (Geffroy et al., 2015).

Most literature compares foraging ability between enriched and unenriched enclosures and foraging conditions in captive environments (Vargas and Anderson, 1999) and the effects on behaviour and survival once released (Biggins et al., 1999, Lepeigneul et al., 2014, Roe et al., 2015). To our knowledge, our study uniquely combines foraging assays with extensive behavioural observations over an activity season to compare the effect of feeding strategy on foraging ability and risky behaviour in a captive environment. A similar study compared growth rates, foraging ability and refuge emergence between habitat enriched and unenriched box turtles (*Terrapene carolina*) and, unexpectedly, found unenriched turtles performed better than enriched turtles in foraging assays whilst there was no difference in emergence (Tetzlaff et al., 2019). Studies have also shown that exposing captive black-footed ferrets (*Mustela nigripes*) to enriched cages and live prey improves predatory skills (Vargas and Anderson, 1998, Vargas and Anderson, 1999) however a semi-natural rearing environment was more important on post-release behaviour and survival than predatory ability (Biggins et al., 1999). In contrast, post-release foraging ability was not affected by captive rearing method in Tasmanian devils (Rogers et al., 2016), or time in captivity in Hermann’s tortoise (*Testudo hermanni hermanni*) (Lepeigneul et al., 2014).

We found several differences between trials and age groups of pygmy bluetongue lizards, however these differences did not affect foraging success. Successful captures declined in the second trial after the feeding treatment was implemented, (but unsuccessful attempts did not increase) and lizards spent longer capturing prey outside of the burrow on the arena surface in the first trial (before the feeding treatment commenced) compared to the second trial. The lack of interaction effect between trial and treatment suggests that these differences are not related to the feeding treatment. Lizards may have been hungrier in the 'before' trial which was conducted later in the spring than the 'after' trial and thus more active and quicker to capture prey, or the higher temperatures in the 'before' trial (29.5°C) compared to the 'after' trial (20.8°C) may have increased appetite, although temperature was not found to have a significant effect on number of successful captures. European minnows, (*Phoxinus phoxinus*), were found to be more active when energy reserves were low (Metcalfe and Steele, 2001) and appetite was shown to increase with increased temperature in an ambush foraging lizard, *Cordylus melanotus melanotus* (McConnachie and Alexander, 2004). In our study, adult lizards spent longer sitting or walking on the arena's surface in prey assays compared to juveniles and immatures. This could possibly be due to adult lizards being more affected by the disturbance of being relocated into the experimental arenas (Pettigrew and Bull, 2014), or greater difficulty adapting to the diagonal angle of the artificial burrows as PBTs prefer vertical burrows (Hutchinson et al., 1994, Milne and Bull, 2000).

Our study found self-fed pygmy bluetongue lizards maintained adequate body condition and foraging ability was retained in captive lizards managed under a hand-feeding strategy, at least in the short-term. We suggest that captive reptiles could be capable of foraging successfully if released into the wild even if they have been hand-fed in captivity. However, hand feeding resulted in behavioural differences that may increase predation risk for lizards released into the wild. For this reason, we recommend a self-feeding strategy that replicates close-to-normal foraging conditions for animals that are being considered for future translocations. We do emphasise that regardless of the hand-fed or self-fed strategy in captivity, releases of lizards and other reptiles back into natural habitats need to be monitored over the long-term (e.g. months to years) to ensure good animal health and population viability. Future research should investigate survival of hand-fed compared to self-fed lizards once released into the wild, and whether the behavioural differences we observed between the feeding groups results in differences in wild survival. Overall, observational studies like ours can be used to assess if captive feeding strategies alter behaviour in other threatened species held in captivity prior to considering animals for release to the wild.

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Supporting Information

Table S1: PERMANOVA results for the prey assay variables; unsuccessful capture attempts and walking or sitting on surface with temperature covariates. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations. * indicates significant P value. Note that for temperature to have a significant effect, the p value must be significant in the covariate analysis but not the original analysis.

Unsuccessful capture attempts						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Trial	1	5.66	5.66	4.14	0.068	9929
Treatment	1	<0.01	<0.01	0.15	0.706	9818
Age	2	1.99	0.99	2.61	0.229	9966
Trial x treatment	1	0.86	0.86	0.81	0.411	9939
Trial x age	2	2.92	1.46	3.43	0.099	9963
Treatment x age	2	0.95	0.48	0.71	0.501	9940
Resolution	59	42.56	0.72			
Total	89	74.87				
Unsuccessful capture attempts temperature covariate						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	0.25	0.25	0.25	0.861	9946
Trial	1	8.94	8.94	4.83	***0.046**	9954
Treatment	1	0.01	0.01	0.17	0.680	9837
Age	2	0.62	0.31	1.37	0.370	9969
Trial x treatment	1	0.58	0.58	0.66	0.472	9926
Trial x age	2	1.95	0.98	2.87	0.133	9945
Treatment x age	2	1.03	0.52	0.79	0.458	9951
Resolution	58	41.06	0.71			
Total	89	74.87				
Walking or sitting on surface						
source	df	SS	MS	Pseudo-F	P(perm)	perms
Trial	1	26.51	26.51	2.18	0.167	9923
Treatment	1	1.14	1.14	0.31	0.584	9863
Age	2	14.99	7.50	1.00	0.449	9967
Trial x treatment	1	2.01	2.01	0.39	0.620	9939
Trial x age	2	48.08	24.04	2.60	0.161	9964
Treatment x age	2	0.47	0.23	0.05	0.949	9941
Resolution	59	273.78	4.64			
Total	89	536.02				
Walking or sitting on surface temperature covariate						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	1.01	1.01	0.16	0.956	9945
Trial	1	43.16	43.16	3.06	0.099	9946
Treatment	1	1.24	1.24	0.36	0.557	9832
Age	2	20.45	10.23	1.02	0.431	9966
Trial x treatment	1	1.02	1.01	0.28	0.717	9956
Trial x age	2	47.84	23.92	2.11	0.216	9954
Treatment x age	2	0.64	0.32	0.07	0.934	9946
Resolution	58	249.30	4.30			
Total	89	536.02				

Table S2: PERMANOVA temperature covariate results for the prey assay variables; time to first capture attempt, successful capture attempts, time out of burrow capturing prey and time walking or sitting on the surface. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations; Age = age classes – adults, immatures and juveniles. *Bold indicates significant P value.

Time to first attempt temperature covariate

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	12.58	12.58	2.11	0.216	9971
Trial	1	40.34	40.34	6.33	0.061	9971
Treatment	1	48.89	48.89	396.37	**0.003**	9983
Age	2	-0.57	-0.28	0.21	0.666	9978
Trial x treatment	1	-0.02	-0.02	4.44	0.110	9978
Trial x age	2	-8.23	-4.12	negative		
Treatment x age	2	-7.40	-3.70	negative		
Resolution	54	239.77	4.13			
Total	89	386.41				

Successful captures temperature covariate

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	6.92	6.92	4.45	0.051	9938
Trial	1	7.79	7.79	4.96	**0.040**	9949
Treatment	1	0.04	0.04	0.40	0.528	9824
Age	2	0.81	0.41	1.40	0.340	9965
Trial x treatment	1	0.03	0.03	0.25	0.739	9934
Trial x age	2	0.41	0.20	0.97	0.498	9970
Treatment x age	2	0.94	0.47	0.96	0.401	9961
Resolution	58	29.91	0.52			
Total	89	60.93				

Time out burrow captures temperature covariate

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	4.70	4.70	8.60	0.054	9977
Trial	1	13.43	13.43	24.13	**0.014**	9981
Treatment	1	-0.02	-0.02	0.22	0.696	9969
Age	2	1.33	0.66	2.08	0.239	9971
Trial x treatment	1	-0.01	-0.01	0.15	0.643	9985
Trial x age	2	1.19	0.59	1.85	0.232	9976
Treatment x age	2	1.10	0.55	1.97	0.214	9977
Resolution	58	16.38	0.28			
Total	89	44.40				

Basking temperature covariate

source	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	80.30	80.30	1.49	0.256	9954
Trial	1	0.03	0.03	0.05	0.999	9949
Treatment	1	0.88	0.88	0.34	0.574	9842
Age	2	39.81	19.91	48.95	**0.001**	9968
Trial x treatment	1	10.12	10.12	1.46	0.264	9943
Trial x age	2	10.82	5.41	19.30	**0.004**	9953
Treatment x age	2	3.24	1.62	0.20	0.811	9934
Resolution	58	531.31	9.16			

Total	89	1078.50

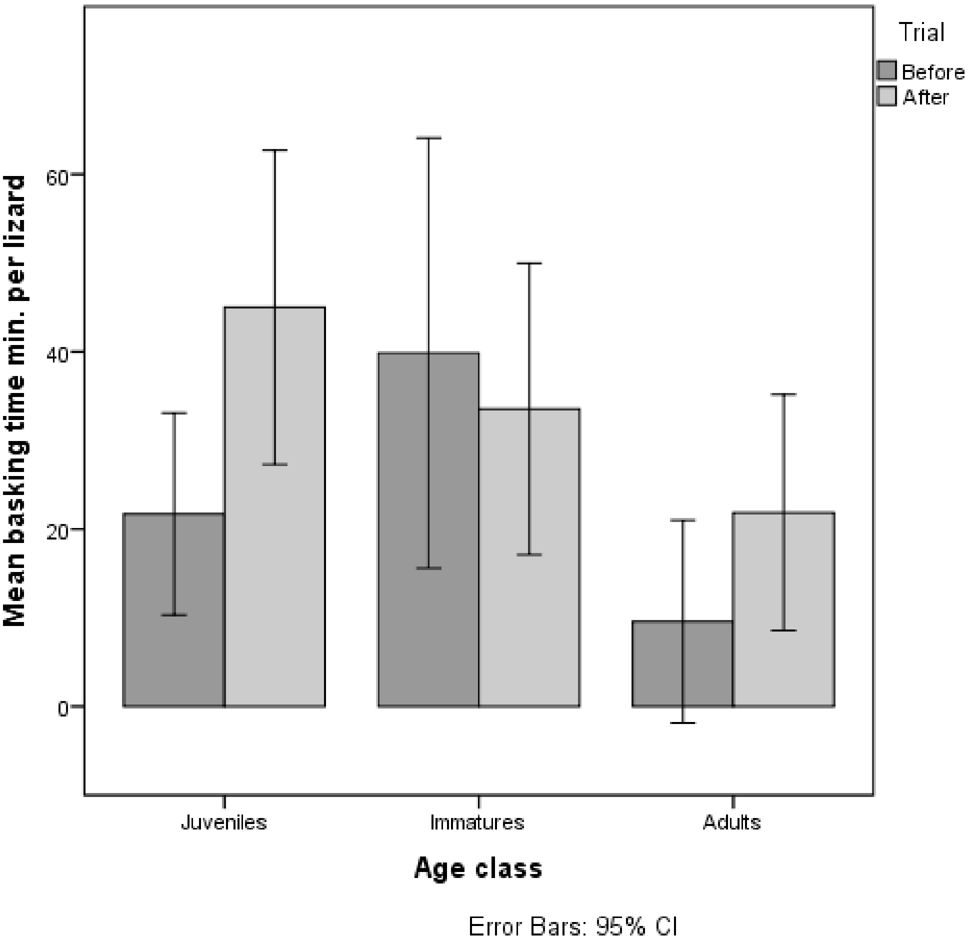

Figure S3: Mean time spent basking (95% CI) for lizard age classes in the first trial and second trial

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Chapter 5.

Recognition of reptile predator scent is innate in an endangered lizard species.

An eastern brown snake attempting to predate a pygmy bluetongue lizard within the burrow at Burra (source: Lucy Clive)

Chapter Preface

Following on from the previous chapter which investigated the effects of captivity on foraging ability, this chapter investigates the effects of the captive environment on predator recognition and avoidance. Prey species rely on predator cues to avoid predators in their environment. Cues can be chemical, visual or auditory in nature and species can rely on one or a combination of cues, depending on the species and the predator. Many captive-bred animals lack the ability to avoid predators when released into the wild, thus affecting translocation success. This chapter investigates the effect of captivity on predator recognition in the pygmy bluetongue lizard and if this species has an innate ability to recognise predator chemical cues. This knowledge is an important first step in determining whether pygmy bluetongue lizards will be able to effectively recognise (and avoid) predators when released in future translocations. My research contributes to our knowledge of the pygmy bluetongue lizard and the use of predator chemical cues for predator avoidance behaviour in lizard species. This chapter has been submitted to the Australian Journal of Zoology.

Recognition of reptile predator scent is innate in an endangered lizard species.

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Abstract

Chemical cues can alert prey to the presence of predators before the predator is within visual proximity. Recognition of a predator's scent is therefore an important component of predator awareness. We presented predator and control scents to wild, wild-born captive, and predator naive captive-born pygmy bluetongue lizards to determine if: 1) captive-born lizards recognise predators innately; 2) captive-born lizards have reduced predator recognition compared to wild lizards and if time spent in captivity reduces responses to predators; 3) captive lizards respond more strongly to a known predator than other predatory reptiles; and 4) the avoidance response to predator detection differs between naive and experienced lizards. There was no significant difference in the number of tongue flicks to predator scent among wild, wild-born and captive-born lizards, suggesting that predator detection is innate in the pygmy bluetongue lizard and time in captivity did not reduce predator recognition. The number of tongue flicks directed toward brown snake scent was significantly higher than to the novel and water controls for all lizard origins. Lizards of all origins continued to bask in the presence of predator scents, suggesting chemical cues alone may be insufficient to instigate an avoidance response and other cues may be required.

Introduction

Prey species have evolved to recognise predator presence and respond with antipredator behaviours to help evade predation attempts (Apfelbach et al., 2005, Atkins et al., 2016). Detection of predator chemical cues is an additional method that prey species can use to alert to predator presence besides visual and auditory cues (Apfelbach et al., 2005, MacLean and Bonter, 2013). Many taxa are known to recognise and respond to chemical stimuli from predators, including reptiles, amphibians, mammals, fish, birds, crustaceans and invertebrates (Kats and Dill, 1998, Apfelbach et al., 2005). Recognition of predator cues can be threat sensitive, as predicted by the threat sensitive hypothesis, whereby prey can differentiate between predators based on the degree of threat posed (Helfman, 1989, Stapley, 2003, Lloyd et al., 2009, Cornelis et al., 2019). Alternatively, predator recognition can be generalised via avoidance of multiple species cues (Blumstein, 2006, Webb et al., 2009, Webster et al., 2018).

When prey species are isolated from predators, such as in captive environments or predator free islands or reserves, predator avoidance behaviours that have evolved over time can be relaxed or lost (DeGregorio et al., 2017, Jolly et al., 2018, Muralidhar et al., 2019). Therefore captive-born animals that have no prior exposure to predators may lack the ability to recognise and respond to predator threats if released into the wild — a major concern for captive conservation projects with intentions for reintroduction. Unsustainable predation is a major cause of mortality in translocation or reintroduction of captive animals (Jule et al., 2008, Aaltonen et al., 2009, Moseby et al., 2011). Hence, it is important to understand the effect of the captive environment on predator avoidance in order to improve survival of animals released back into the wild.

Squamates tongue flick to transport chemical cues to the vomeronasal organ (VNO) in order to detect stimuli that assist in avoiding predators, communicating with conspecifics and foraging (Cooper, 1994). The family Scincidae, including the subfamily Egerniinae, are known to have advanced vomerolfactory abilities (Cooper, 1994, Bull et al., 1999). A member of the Egerniinae, the pygmy bluetongue lizard (*Tiliqua adelaidensis*), is an endangered species rediscovered in 1992, found in native grasslands in mid-north South Australia (Armstrong and Reid, 1992, Hutchinson et al., 1994). These lizards inhabit burrows constructed by spiders, which provide shelter from temperature extremes, a site to ambush prey, and refuge from predators such as eastern brown snakes and various bird species (Hutchinson et al., 1994, Milne et al., 2003, Fenner et al., 2008). Pygmy bluetongue lizards use vomerolfactory cues for social signalling — to communicate burrow ownership to conspecifics and locate mates by following female scent trails, and as such are suitable subjects to study predator scent response (Fenner and Bull, 2011, Ebrahimi et al., 2014). Pygmy bluetongues are threatened by climate change, habitat loss and fragmentation, and a captive population was established as a potential source for translocations (Smith et al., 2009, Fordham et al., 2012, Delean et al., 2013).

Lizards that lack the ability to recognise and avoid predators, or in which avoidance behaviour has been relaxed due to the lack of predation pressure are at greater risk of predation, potentially reducing the success of translocation. Therefore, we investigated the use of chemical cues for predator avoidance in captive and wild pygmy bluetongue lizards. Our aims were to determine; 1) if captive-born pygmy bluetongue lizards recognise predators innately; 2) if captive-born lizards have reduced predator recognition compared to wild lizards and if time spent in captivity reduces response to predators; 3) if captive pygmy bluetongue lizards respond more strongly to a known predator, the eastern brown snake (*Pseudonaja textilis*), than other predatory reptiles; and 4) if the avoidance response to predator detection differ between naive and experienced lizards.

Methods

Study populations

Our study incorporated three treatments groups, wild lizards, wild-born captive lizards and captive-born lizards.

Monarto Safari Park

We used a captive population of 37 pygmy bluetongue lizards at Monarto Safari Park, South Australia. The population was composed of nine potentially predator experienced wild-born adults — captive since spring 2014, and 28 predator naive captive-born offspring born in either 2016 or 2017 — 12 immatures and 16 juveniles respectively. Lizards were housed singly in sections of raised enclosures divided into thirds and situated within two 15 m diameter circular caged areas that had netted roof and wire mesh sides to exclude predators. Lizard enclosures were 0.65 m high, 2.4 m long, 1.2 m wide and filled with sandy loam to a depth of 0.4 m. Each enclosure was divided into three sections to house lizards individually, with six artificial burrows per section spaced approximately 30 cm apart. Artificial burrows were constructed from wooden dowel with a circular hole drilled through the centre — adult burrows were 300 mm long, 30 mm in diameter with a 20 mm hole; juvenile and immature burrows were 200 mm long, 28 mm in diameter with an 18 mm hole.

Four native grasses (*Austrostipa* sp. and *Austrodanthonia* sp.) per section provided shade and potential retreats for lizards venturing on the surface.

Burra

The wild population for this study — 20 adult lizards, was located at Tiliqua Reserve, a Nature Foundation property approximately 10 km from Burra, in mid north South Australia. The site consists of native grassland and exotic weeds usually grazed by sheep. The Burra area experiences hot, dry summers and cool, moist winters with a mean annual rainfall of 421.4 mm for the years 1961 to 2019 (Commonwealth of Australia Bureau of Meteorology, 2020). At the time of scent trials, no grazing was occurring as vegetation levels were low following several years of below average rainfall — 259 mm in 2018 and 220.6 mm in 2019 (Commonwealth of Australia Bureau of Meteorology, 2020). One paddock of the site was searched to locate 20 burrows which we confirmed were occupied by pygmy bluetongue lizards using an optic fibre scope (Medit Inc. FI4-2BDP-1850, Canada) (Milne and Bull, 2000) and the burrows were then marked by numbered plastic pin markers for the study duration.

Scent treatments

Our study tested five scent treatments — three reptile species' scents, a novel control and an odourless water control applied to absorbent paper towel. The first reptile species was the eastern brown snake (*Pseudonaja textilis*), an active forager known to predate the pygmy bluetongue (Hutchinson et al., 1994). The second species was the eastern bluetongue lizard (*Tiliqua scincoides*), a omnivorous species that predates upon small lizards and is sympatric but is not known to predate pygmy bluetongues (Pelgrim et al., 2014).

Lastly, was the Rosenberg's monitor (*Varanus rosenbergi*), a species known to predate upon smaller lizards but not found within the pygmy bluetongue's current range of distribution (King and Green, 1979, Smith et al., 2007). The two controls were a novel odour control of 1:10 diluted eucalyptus oil (Stapley, 2003) and an odourless distilled water control.

All reptile scent was collected by zoo keepers from Adelaide Zoo, South Australia. Scent was collected from one eastern brown snake only. In the case of both the Rosenberg's monitors and eastern bluetongue lizards, individuals were housed in groups in the same enclosures so that the corresponding scent donor to each sample could not be determined but were likely a mixture of different individuals. Clean gloves were worn to place absorbent paper towels, dampened with distilled water, in reptile enclosures for 48 hours to absorb scent. Paper towel was then removed and stored in ziplock bags in a freezer (-20° C) until use (Bourke et al., 2017). Storage time between scent collection and trials did not exceed three weeks.

Scent trial

Scent trials were conducted over a five-day period at both Monarto and Burra. Each individual lizard was exposed to each of the five scents separately with a single scent presented one day at a time until each scent had been presented over the five-day period. The order of scent presentation was randomised among lizards.

Filming was conducted once lizard activity was observed in the late morning or early afternoon between the hours of 10 am and 3 pm at Monarto between 30th of September and 4th of October 2019 and at Burra between the 11th and 15th of October 2019. Filming took place when the temperature was between 16.8°C and 30.1°C and there was no rain.

There was a six-day gap between the Monarto and Burra trials as weather was unfavourable. We used a combination of five GoPro cameras and 15 Movii cameras with external power bank placed 30 cm from the lizard burrow. The Monarto lizards were filmed in two groups daily, one group in the morning and one in the afternoon in a randomised order, as there were not enough cameras to film all lizards at the same time. All lizards were filmed in their home burrows to minimise disturbance — artificial burrows within enclosures at Monarto and natural burrows in situ at Burra. We placed the cameras at ground level and close to burrows to provide clear vision of tongue flicking behaviour or on short tripods when placing on the ground was not practical due to surrounding vegetation. Fresh, single-use scent papers were placed approximately five cm from lizard burrows and pinned down with nails to hold them in place. Clean disposable gloves were worn when handling scent papers and changed for each scent treatment. Lizards were filmed for 30 minutes, after a maximum 30-minute acclimation period after camera setup and placement of scent papers, each treatment day to record the number of tongue flicks directed toward scent paper and minutes spent basking per lizard. Basking was defined as when a lizard had a part of its body (head, forelegs or torso) emerged from the burrow entrance. We chose basking time to measure lizard behavioural response to predator scent, as pygmy bluetongue lizards will cease basking and retreat into the burrow when threatened. Therefore, time spent basking can be used as a measure of predator avoidance in this species.

Statistical analysis

Primer v7/PERMANOVA+ was used to conduct univariate multifactorial repeated measures PERMANOVA tests based on Euclidean distance matrices and PERMANOVA pair-wise tests. The behavioural parameters tested were the number of tongue flicks directed toward scent papers (aims one to three) and minutes spent basking (aim four) at the burrow entrance for each individual lizard and each scent treatment. Lizard origin and treatment were between subjects factors, and day was a within subjects factor. Temperature (C°) during filming was obtained from the Commonwealth of Australia's Bureau of Meteorology (BoM) from the nearest weather stations to Monarto and Burra – Pallamana station 11 km away, and Clare station, 37 km away respectively. We analysed temperature (C°) as a covariate as it may influence lizard tongue flicking and basking.

We tested each of our aims in the following manner:

1) if captive-born pygmy bluetongue lizards recognise predators innately; and 2) if captive born lizards have reduced predator recognition compared to wild lizards and if time spent in captivity reduces response to predators.

Aims one and two both tested for differences in tongue flicking behaviour among lizards with different levels of experience with predators and time in captivity. We compared the responses (tongue flicks) of lizards from the three origins — predator naive captive-born lizards, potentially experienced wild-born lizards and wild lizards. Lizard origin and scent treatment were between subjects factors.

3) if captive and wild pygmy bluetongue lizards respond more strongly to a known predator, the eastern brown snake (Pseudonaja textilis), than other predatory reptiles.

For this third aim we compared the tongue flick responses of captive-born, wild-born and wild lizards to the five scent treatments; eastern brown snake — a known predator, the eastern bluetongue lizard — a potential predator, Rosenberg's monitor — a predatory species not in the pygmy bluetongue lizard’s current range, plus water and novel controls. Scent treatments and lizard origin were between subject factors.

4) if the behavioural responses to predator detection differ between naive and experienced lizards.

Here we compared basking time between predator naive captive-born lizards and potentially experienced wild-born and wild lizards to investigate if the predator avoidance response differs. As mentioned above, the PERMANOVA test had lizard origin — captive-born, wild-born or wild and scent treatment as between subjects factors.

Ethics statement

This study conducted according to the approval of the Wildlife Ethics Committee, project number 28/2017.

Results

Innate predator recognition and the effect of captivity on predator recognition (aims 1 and 2)

Tongue flicking did not differ significantly among lizard origin, day or interaction effects between the factors. The mean number of tongue flicks was slightly lower for naive captive-born lizards (10.02 ± 1.99 SE) compared to wild-born (14.49 ± 4.26 SE) and wild lizards (14.67 ± 2.60 SE), but this difference was not significant and wild-born lizards were comparable to wild lizards (Figure 1). The number of tongue flicks varied among lizard origin and day but was not significant (Supporting information, figure S1). Temperature, analysed as a covariate, was found to have a significant effect on the mean number of tongue flicks among days of the trial (Table 1). Temperature varied among days and tongue flicks were lower at temperatures below 19 °C and at 30°C but were variable within this range (Figure 2).

Differential response to scent treatments (aim 3)

The number of tongue flicks lizards directed toward scent papers differed significantly among scent treatments (Table 1, Figure 3). The mean number of tongue flicks toward brown snake scent papers was 24.07 ± 4.93 SE, toward bluetongue lizard was 14.33 ± 2.83 SE, toward Rosenberg's monitor was 12.81 ± 4.04 SE, with tongue flicks to the novel control being 5.81 ± 1.73 SE and 5.42 ± 1.59 SE toward the water control. Response to the brown snake treatment differed significantly from both the water control (PERMANOVA pair-wise test P = 0.005) and novel control (PERMANOVA pair-wise test P = 0.022). There was no significant interaction effect between lizard origin and treatment.

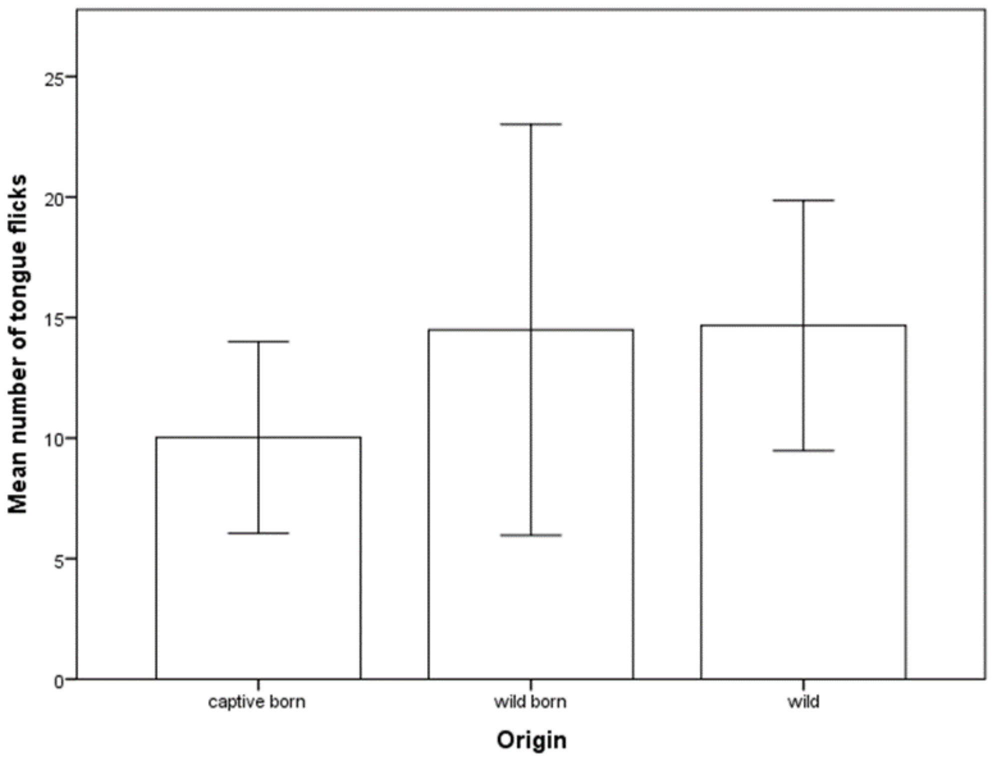

Figure 1: Mean number of tongue flicks (95% CI) made by captive-born, wild-born and wild lizards across all scent treatments.

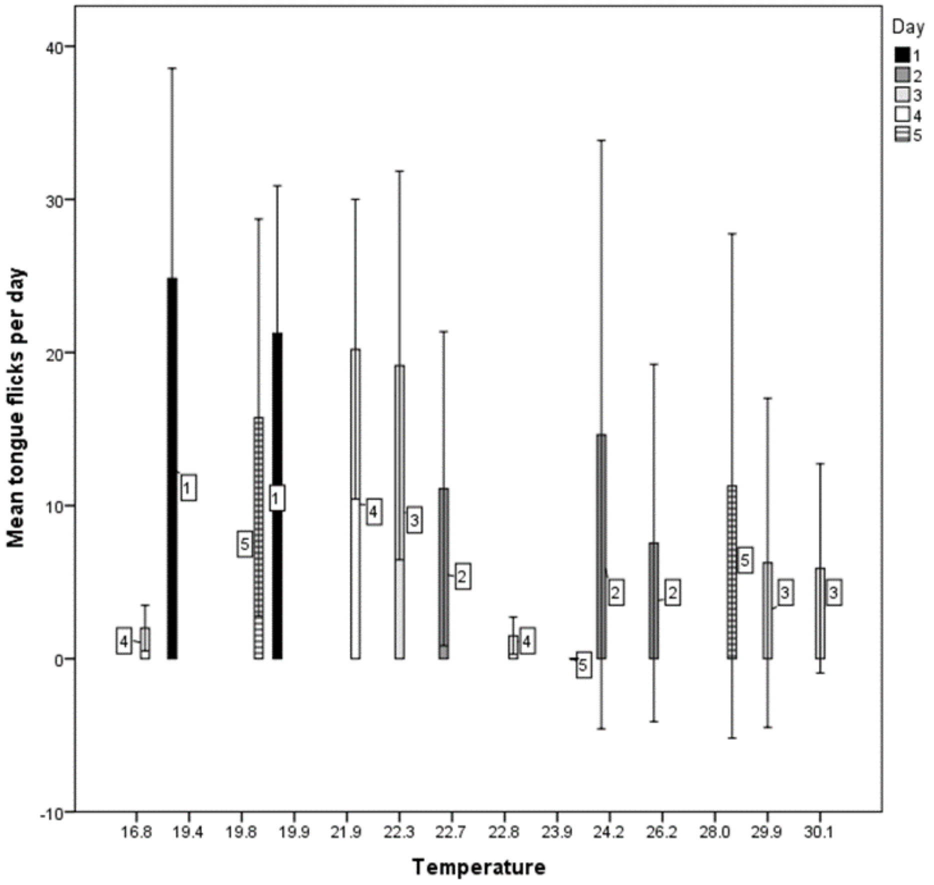

Figure 2: Mean number of tongue flicks (95% CI) directed toward scent paper across the filming temperature range °C by day of trial. Numbered labels on boxplots indicate day of trial.

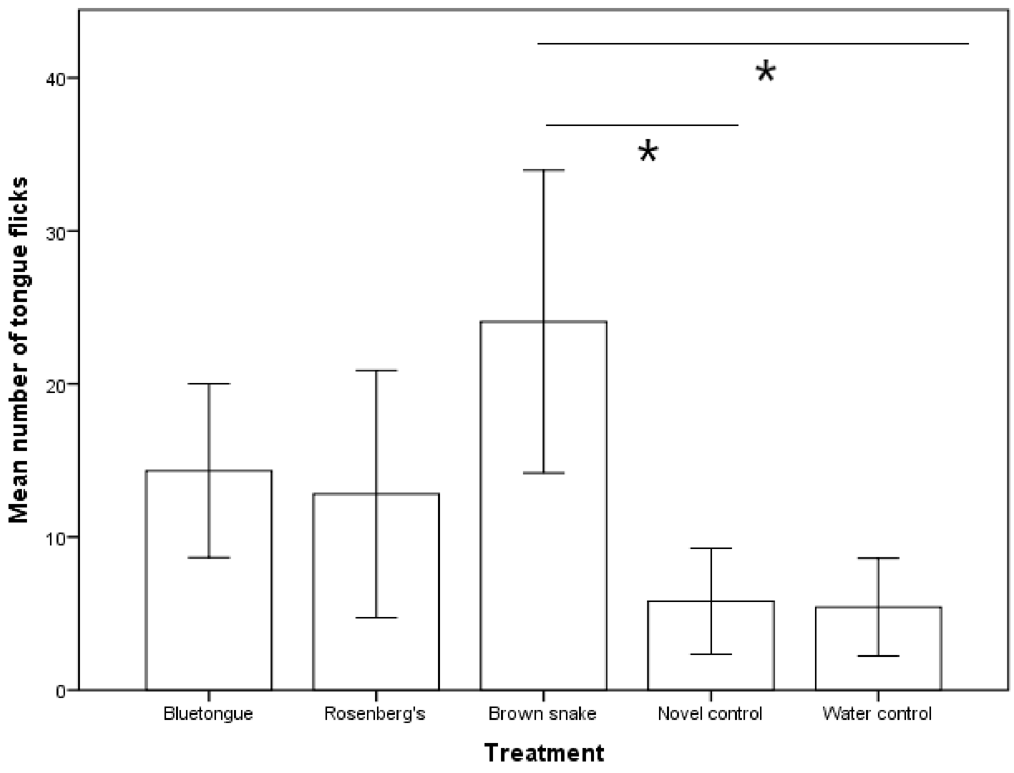

Figure 3: Mean number of tongue flicks (95% CI) made by pygmy bluetongue lizards of all origins (captive-born, wild-born and wild) over a 30-minute period for the five scent treatments: eastern bluetongue lizard, Rosenberg's monitor, eastern brown snake*, novel control* and water control*. * indicates treatments that were significantly different in pairwise comparisons.

Behavioural response to predator detection (aim 4)

There was no significant effect of treatment, lizard origin or day of trial on pygmy bluetongue basking time. Lizards basked for a similar amount of time in the presence of all scent treatments (Figure 4). There was variation in basking time in the presence of the five scent treatments for captive-born, wild-born and wild lizards, but this was not significant (Supporting information, figure S2). However, there was a significant interaction effect of lizard origin and day on basking time (Table 1). Basking time varied among lizard origin and day of trial, with a general trend of basking time decreasing over the five days for captive born lizards and fluctuating with an overall upward trend for both wild born and wild lizards (Supporting information figure S3). The temperature covariate had a significant effect on time spent basking among lizard origins, and an interaction effect between day and treatment however the nature of the interactions were not clear.

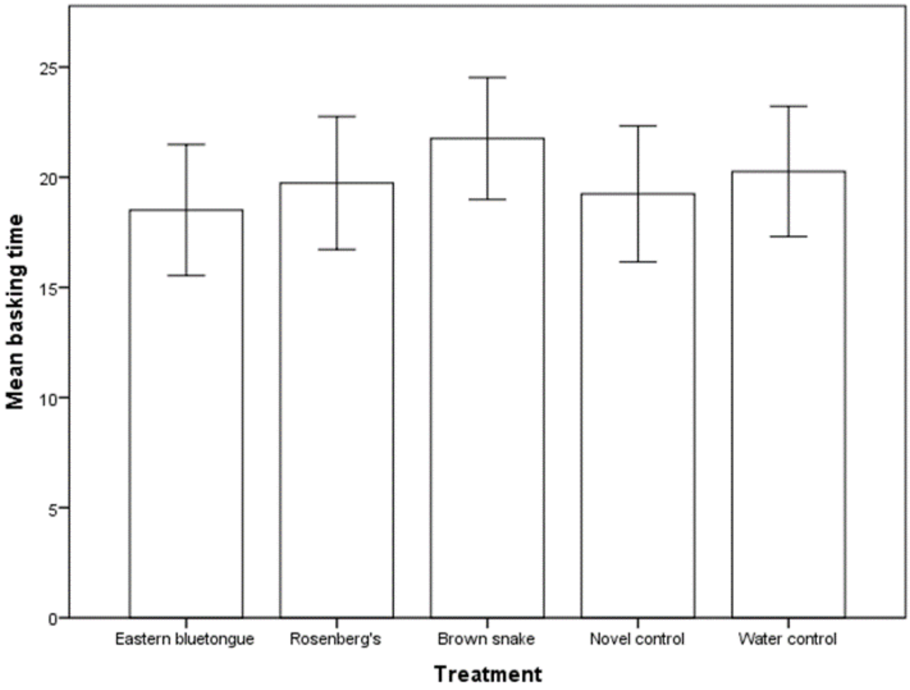

Figure 4: Mean basking time (95% CI) by pygmy bluetongue lizards toward the five scent treatments across all lizard origins.

Table 1: PERMANOVA results for the variables; tongue flicks, temperature covariate (tongue flicks), basking time and temperature covariate (basking time). Data not transformed. Origin = lizard origin; wild, wild-born and captive-born. Treatment = to the five scents presented to lizards. Day = trial day. df = degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F = F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations. Bold indicates significant P value.

Tongue flicks						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Origin	2	1265.4	632.69	1.17	0.313	9960
Day	4	4345.6	1086.4	2.00	0.108	9952
Treatment	4	8382.4	2095.6	3.86	**0.018**	9953
Origin x Day	8	4354.4	544.29	1.00	0.414	9947
Origin x Treatment	8	5017.7	627.21	1.15	0.328	9937
Day x Treatment	16	8629.1	539.32	0.99	0.448	9921
Origin x Day x Treat	23	13843	601.86	1.11	0.327	9910
Res	219	118930	543.08			
Total	284	175700				
Tongue flicks temperature covariate						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	2532.3	2532.3	4.65	**0.035**	9955
Origin	2	583.35	291.67	0.54	0.580	9962
Day	4	5548.1	1387	2.55	**0.043**	9953
Treatment	4	13091	3272.8	6.00	**<0.001**	9939
Origin x Day	8	5075.6	634.45	1.16	0.327	9933
Origin x Treatment	8	5945.3	743.16	1.36	0.224	9942
Day x Treatment	16	10125	632.82	1.16	0.301	9916
Origin x Day x Treat	23	13973	607.54	1.11	0.334	9922
Res	218	118830	545.08			
Total	284	175700				
Basking						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Origin	2	391.46	195.73	1.95	0.146	9946
Day	4	446.43	111.61	1.11	0.361	9958
Treatment	4	622.97	155.74	1.55	0.190	9958
Origin x Day	8	5467.6	683.45	6.8	<**0.001**	9936
Origin x Treatment	8	868.46	108.56	1.08	0.376	9928
Day x Treatment	16	2376.7	148.54	1.50	0.104	9919
Origin x Day x Treat	23	2286	99.39	0.99	0.481	9909
Res	219	22011	100.51			
Total	284	34397				
Basking temperature covariate						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Temperature	1	13.00	13.00	0.13	0.718	9933
Origin	2	928.92	464.46	4.81	**0.008**	9952
Day	4	853.66	213.42	2.21	0.070	9961
Treatment	4	247.27	61.82	0.64	0.639	9960
Origin x Day	8	6050	756.25	7.83	<**0.001**	9935
Origin x Treatment	8	627.49	78.44	0.81	0.582	9947
Day x Treatment	16	2636.6	164.79	1.71	**0.046**	9918
Origin x Day x Treat	23	1989.5	86.5	0.90	0.593	9909
Res	218	21051	96.56			
Total	284	34397				

Discussion

The ability to recognise and respond to predator chemical cues in the natural environment provides prey species the opportunity to become aware of the presence of predators and undertake antipredator behaviours to minimise risk of predation. Prey species can display species-specific recognition of predator cues, or generalised predator recognition. Hence an important question is what type of predator recognition a species displays, as this will determine how the species responds to predators they encounter. Our study had four main findings — 1) tongue flicking behaviour in captive-born lizards was comparable to wild-born and wild lizards suggesting that recognition of predator chemical cues is innate; 2) there was no difference in tongue flicking towards predator scent among lizards that were captive-born, wild-born or wild which suggests that lizards recognise predators regardless of prior experience and that time spent in captivity did not reduce predator recognition; 3) pygmy bluetongue lizards tongue flicked toward the brown snake predator's scent more than other treatments; and 4) unexpectedly, the presence of predator scent did not result in reduced basking or an increase in lizards retreating to their refuges, suggesting that predator chemical cues alone are not enough to prompt predator avoidance behaviour in this species.

We found no significant difference in responses among the captive-born pygmy bluetongue lizards and wild or wild-born adults, which suggests that the recognition of predator chemical cues is innate in this lizard species. The lack of difference in responses between captive-born juveniles and potentially experienced wild-born adults suggests that there is not a learned component in predator recognition of potential versus non-potential predators in this species. Additionally, in the case of wild-born adults, time in captivity did not alter lizard ability to recognise predator scent. In contrast, the survival probability of translocated ratsnakes (*Pantherophis obsoletus*), decreased the longer the snake had been in captivity regardless of environmental enrichment, as concealment behaviour was reduced, likely increasing vulnerability to predation (DeGregorio et al., 2017). Although the exact timeframe required for prey species to lose predator avoidance behaviours is not known and likely variable between species, recent studies have shown that robins reduced antipredator behaviour toward predators within three years (Muralidhar et al., 2019) and quolls lost recognition and avoidance behaviours in 13 generations (Jolly et al., 2018). Although our study found predator recognition was not reduced after five years in captivity, pygmy bluetongues may have other altered behaviours that may reduce survival and were not investigated in this study, such as spending more time exposed on the surface. We also found that temperature had a significant effect on the number of tongue flicks among days, and that low or high temperatures resulted in fewer tongue flicks by lizards. Low and high temperatures have previously been shown to effect tongue flicking (Cooper Jr and Vitt, 1986).

Our findings suggest that, like many other species, pygmy bluetongue lizards have an innate ability to recognise predator chemical cues. For example, cotton-top tamarin monkeys, *Saguinus oedipus,* (Buchanan-Smith et al., 1993) and leopard geckos, *Eublepharis macularius*, (Landová et al., 2016) can innately recognise predator chemical cues regardless of whether they are wild or captive-born. However, juvenile Baltic sturgeon (*Acipenser oxyrinchus*), were found to lack the innate ability to recognise predator chemical cues (Cámara Ruiz et al., 2019). Furthermore, there is likely a learned component in predator recognition for some species, as captive born Iberian wall lizards (*Podarcis hispanicus*), can innately recognise predator scent, but experienced wild-born adults can also differentiate between sympatric and non-sympatric predators (Martín et al., 2015).

We found that pygmy bluetongue lizards tongue flicked toward eastern brown snake scent significantly more than the water and novel odour controls, and more than the eastern bluetongue and Rosenberg's monitor scents — although this difference was not significant. Eastern brown snakes are a known predator of the pygmy bluetongue lizard (Hutchinson et al., 1994), which could explain the higher rate of tongue flicks compared to other scent treatments. Furthermore, the higher number of tongue flicks toward the known predator, compared to the other reptilian scents, provides some support for threat-sensitive or species-specific scent recognition. The pygmy bluetongue lizard potentially recognises the snake as a predator but not the eastern bluetongue which is a sympatric potential predator, nor the allopatric Rosenberg's monitor.

The antipredator response of prey species can be threat sensitive or generalised to a range of predators. The threat sensitive hypothesis predicts prey should assess and respond according to the level of threat posed by the specific predator (Helfman, 1989, Forester et al., 2019). We found that the pygmy bluetongue reacted strongest to a known predator scent, which may suggest this species can distinguish between predators. However, caution is required as the reaction between known and potential predator scents was not significantly different. The ability to differentiate between predators and non-predators and risk levels have been found in reptile and primate species (Buchanan-Smith et al., 1993, Stapley, 2003, Lloyd et al., 2009, Cisterne et al., 2014, Cornelis et al., 2019). The pygmy salamander, (*Desmognathus wrighti*) could differentiate chemical cues from a specialist snake predator and two generalist predators – beetle and salamander species (Forester et al., 2019). Some prey species display a generalised response to predators when antipredator behaviours are not costly or multiple species are potentially dangerous (Blumstein et al., 2006, Webb et al., 2009, Webb et al., 2010). For example, two Australian lizard species were found to recognise and respond to chemical cues of both native and invasive predator species, which could be due to either generalised predator recognition or rapid evolution or learned behavioural response to invasive predators (Webster et al., 2018) and fawn-footed mosaic-tailed rats (*Melomys cervinipes*) recognised but did not discriminate between venomous sympatric and non-venomous non-sympatric snake species (Paulling et al., 2019). The recognition of predators and the response to the recognition of predators appears variable amongst species.

Pygmy bluetongue lizards did not reduce their basking time in the presence of predator scent compared to when presented with control scents — despite our finding of these lizards being able to recognise predator scent. This lack of reduction in basking suggests that chemical cues alone are not enough to elicit the predator avoidance behaviour of retreating into the burrow generally displayed by the pygmy bluetongue lizard when threatened (pers. obs). Perhaps these lizards show a threat-sensitive response to risk from predator cues, as scent alone was not enough to elicit an avoidance response. Lizards are relatively safe when basking at the burrow entrance as they can rapidly retreat into burrows if threatened and choose burrows with diameters close to their head width which would block predators from entering the burrow and snakes are not able to open their mouths to bite the lizards when inside the burrow (Milne and Bull, 2000). Therefore, the presence of predator scent may increase vigilance but further cues — i.e. visual may be required to elicit avoidance behaviour. We have anecdotal evidence from video recordings of wild pygmy bluetongue lizards retreating into burrows when magpies walked near burrows and when an eastern brown snake partially entered a burrow in a predation attempt (pers. obs). Therefore, visual cues or a combination of chemical, visual and auditory cues may be required for these endangered lizards to display avoidance behaviours. Chemical cues can remain in the environment for long periods but this may not be a useful predator cue in the case of active foraging predators (Head et al., 2002). We do not believe that our use of frozen scent was the reason for the lack of avoidance response, as frozen scent has been used effectively previously (Bourke et al., 2017) and our lizards did show increased tongue flicking towards reptile scent compared to controls. In other species, predator avoidance behaviours can be elicited by chemical, visual or auditory cues alone, or a combination of cues and these may be context or habitat specific. For example, wall lizards (*Podarcis muralis*), did not respond more strongly when presented with a combination of visual and chemical cues compared to chemical cues alone, possibly because visual cues are less useful in low visibility conditions present in refuges (Amo et al., 2006). The three-spined stickleback (*Gasterosteus aculeatus*) responded most strongly to visual predator cues whereas responses to chemical cues were weaker, and behavioural responses differed between visual and chemical cues (Landeira-Dabarca et al., 2019). Adult southern water skinks (*Eulamprus heatwolei*) showed no response to chemical cues from a known predator, the red-bellied black snake (*Pseudechis porphyriacus*), possibly due to chemical cues being ineffective in detecting the presence of the snake as it is an active forager (Head et al., 2002). Australian house geckos (*Gehyra dubia*) did not avoid shelters with chemical cues from predatory snake species, which was interpreted as a threat sensitive response and that chemical cues alone were not threatening enough to cause avoidance (Cisterne *et al.* 2014). An example of a general response to predators was found in velvet geckos (*Oedura lesueurii*), that avoided shelters with the scent of both a dangerous snake predator and a less dangerous snake predator, independently of thermal costs associated with shelter avoidance (Webb et al., 2010).

Our study found that predator scent recognition is innate in the pygmy bluetongue, that lizards tongue flicked more toward the known predator — the brown snake, compared to other reptile or control treatments, and that the presence of predator scent did not result in reduced basking behaviour. In summary, our results suggest that although the pygmy bluetongue lizard can innately recognise predator scent, chemical cues alone are not enough to elicit predator avoidance behaviour. Future experiments should test responses to multiple snake species of varying degrees of threat level, as our study only tested one snake species. Captivity did not result in a loss of recognition of predator chemical cues for wild born adults in captivity since 2014 – over five years. This retention of recognition suggests that these lizards can maintain predator recognition in a captive environment. We found that captive born pygmy bluetongues can innately recognise chemical cues of predators, although our study focused on first generation captives, and longer generation times may have a different outcome. Furthermore, we cannot confirm that captive lizards released into the wild would display predator avoidance behaviour, as we only tested chemical cues, which did not result in an avoidance response in either captive or wild lizards. Further research is required focusing on visual cues before being able to confirm captive lizards would be able to avoid predators if released into the wild.

Our research further highlights how reactions to predator cues differ between species and contexts, and the need to study predator recognition and avoidance toward multiple cue types, particularly for endangered species that are subject to captive breeding and release programs. Ecological traits of predator and prey species, such as active foraging behaviour of predators, and prey species that are associated with safe refugia, can provide insight and help drive hypotheses for testing of predator cues most likely to elicit predator avoidance.

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Supporting Information

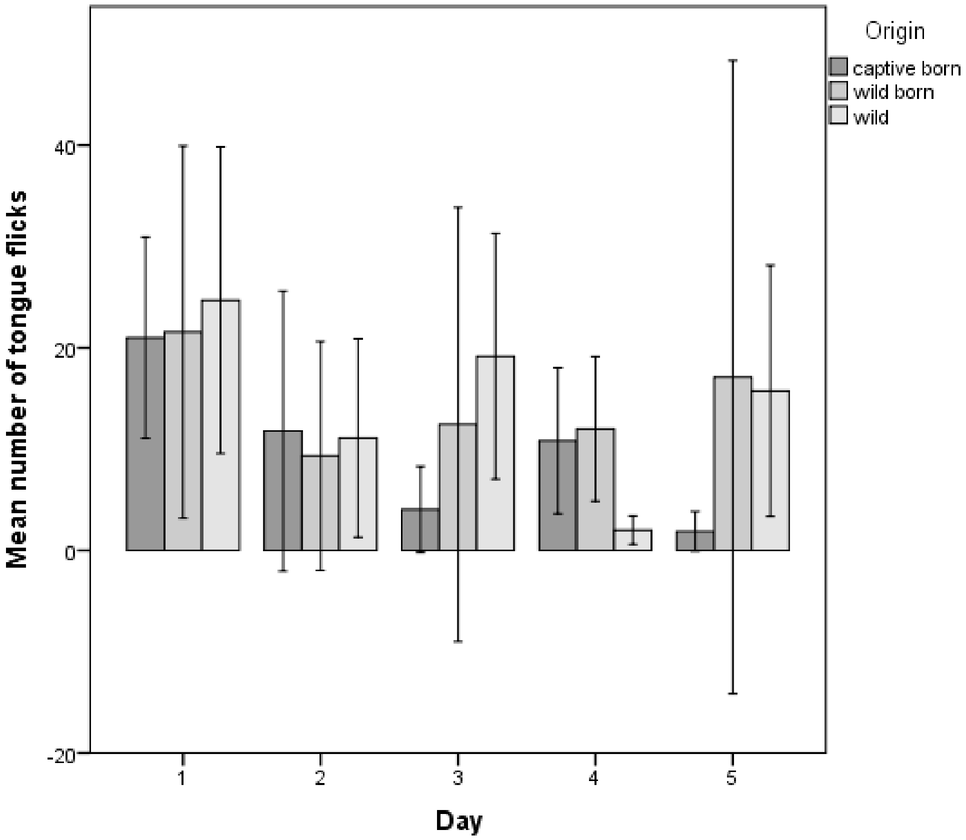

Figure S1: Mean number of tongue flicks (95% CI) for each day of the trial by the three lizard origins; captive born (dark grey), wild born (medium grey), wild (light grey). Data were pooled across scent treatments.

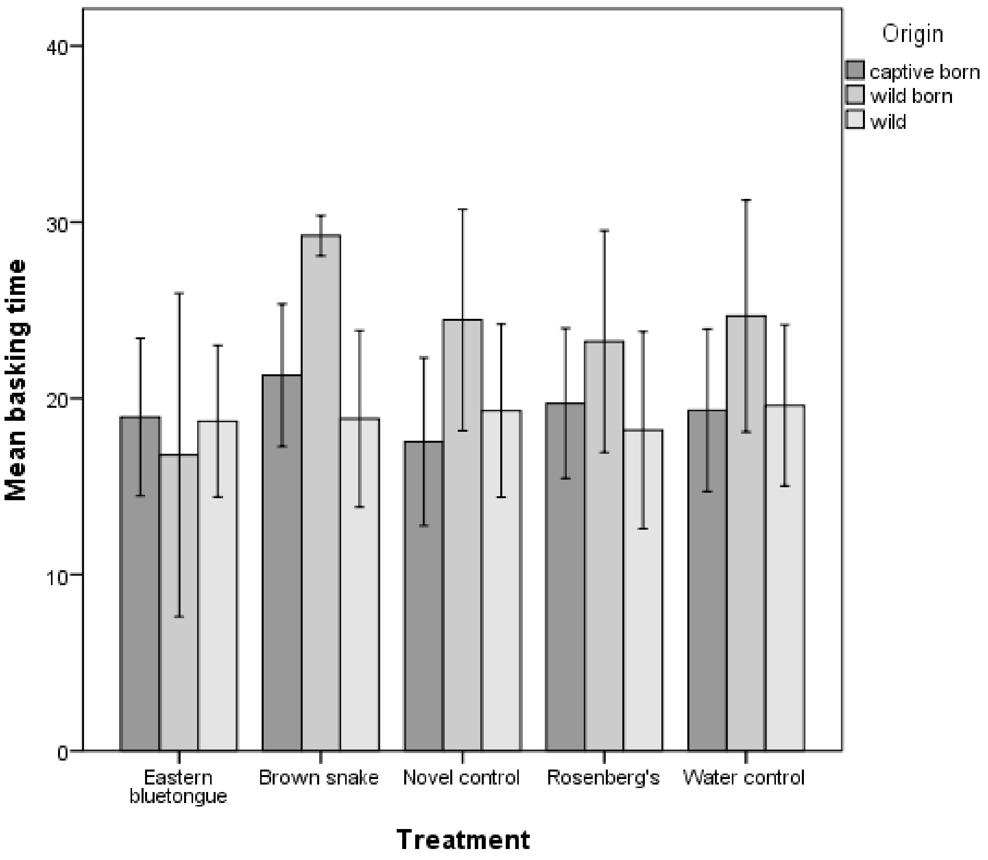

Figure S2: Mean basking time (95% CI) by pygmy bluetongue lizards of the three origins – captive born (dark grey), wild-born (medium grey) and wild (light grey) toward the scent treatments.

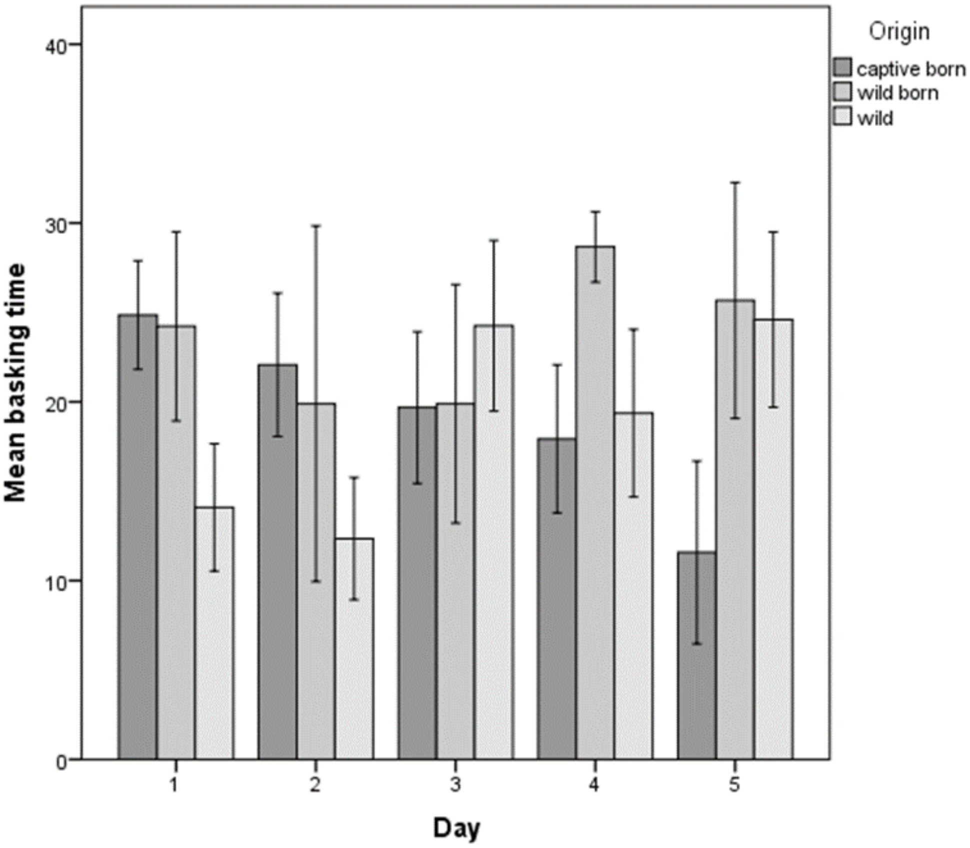

Figure S3: Mean time lizards spent basking (95% CI) over a 30-minute filming session for each day of the trial and three lizard origins; captive born (dark grey), wild born (medium grey), wild (light grey).

Chapter 6.

Tussock grasses are an important resource for captive pygmy bluetongue lizards, *Tiliqua adelaidensis.*

Adult male pygmy bluetongue lizard basking atop a grass tussock at Monarto Zoo.

Chapter Preface

My previous chapters covered age-specific behavioural differences and the effects of captivity on foraging and predator avoidance skills — factors that can affect translocation success. I now investigate another critical factor for translocation success — habitat use. Up to now, research efforts into pygmy bluetongue lizard habitat have focused largely on suitable habitat types, provision of burrows and the effect of sheep grazing on burrows and lizard behaviour. As pygmy bluetongue lizards spend much of their time associated with their burrow, there has been a lack of research into how lizards use surrounding vegetation. Grass tussocks may play an important role as temporary refugia when lizards are dispersing or moving about on the surface to forage or seek mates. The captive environment provides an opportunity to investigate movements of pygmy bluetongue lizards outside of their burrow and in grass tussocks. This chapter investigates the use of grass tussocks by captive pygmy bluetongue lizards to fill this knowledge gap and to provide information on the importance of maintaining some tussock grass cover in lizard habitat for temporary refugia.

Tussock grasses are an important resource for captive pygmy bluetongue lizards, *Tiliqua adelaidensis.*

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Abstract

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is an endangered species found in fragmented native grasslands. They rely on burrows constructed by spiders as refuge from temperature extremes, predators and as an ambush site. Although pygmy bluetongue lizards spend most of their time in their burrow, they can leave their burrow to forage, defecate and seek mates or new burrows during dispersal. The importance of grass tussocks as potential temporary refuges whilst lizards are foraging, or dispersing is likely but unclear. To investigate grass usage, we analysed the time captive pygmy bluetongue lizards spent in grass tussocks over the season, the number of burrow departures, the number of grass entries, and the proportion of time lizards spent in grass tussocks, sitting on the surface or walking the enclosure perimeter. Grass use was higher in spring than summer or autumn months. Approximately half of the burrow departures resulted in lizards entering grass tussocks. Lizards spent longer in grass tussocks than sitting or walking on the surface. Grass tussocks were found to be an important resource for captive pygmy bluetongue lizards, to forage, bask and seek cover while outside of their burrows.

Introduction

Grassland ecosystems are threatened by habitat loss and fragmentation (Hoekstra et al., 2005, Prober and Thiele, 2005), and a number of threatened reptile species inhabit grasslands (Milne et al., 2003, Howland et al., 2014, Melville et al., 2019) often used as grazing land. Species will have different preferences for grass structure and density and grazing intensity may have to be managed differently throughout grassland habitats (Howland et al., 2014). Studies to determine how species use grassland vegetation is therefore important, especially for endangered species which may require conservation management.

The pygmy bluetongue lizard, *Tiliqua adelaidensis*, is an endangered species found in native grasslands in mid-north South Australia. Vegetation consists of native grassland species — spear grasses (*Austrostipa* spp.), wallaby grasses (*Danthonia* spp.), wingless bluebush (*Maireana enchylaenoides*), and brush wire-grass (*Aristida behriana*) and introduced species such as wild oats (*Avena barbata*), thread iris (*Gynandriris setifolia*), onion grass (*Romulearosea* spp.), rats tail (*Vulpia myuros f. myuros*) and storks bills (*Erodium* spp.). The pygmy bluetongue lizard is reliant upon the spider burrows they occupy for their survival — spider burrows provide refuge from temperature extremes, predators and as a site to bask and ambush prey (Milne et al., 2003). Although pygmy bluetongue lizards spend the majority of their time refuged within or at the entrance of their burrow, they do leave their burrow for short periods to forage (Milne et al., 2003) or defecate (Ebrahimi et al., 2016). Males leave their burrows in search of mates in spring (Schofield et al., 2013) and females have been observed laying chemical trails to lead males to their burrows (Ebrahimi et al., 2014). Neonates disperse from the natal burrow soon after birth in late summer (Milne et al., 2002). Grass tussocks may be an important temporary refuge for foraging or dispersing lizards. However, despite pygmy bluetongue lizards being found solely within grasslands, previous research has not investigated tussock use as the burrow is the most important habitat resource for this species (Milne et al., 2003).

All known pygmy bluetongue lizard populations are located on private properties predominantly used as grazing land (Souter et al., 2007) therefore previous research has focussed on the impact of grazing on burrow selection and lizard behaviour. Lizards prefer burrows with surrounding vegetation compared to burrows surrounded by bare ground, (Pettigrew and Bull, 2011) but will remain in burrows as vegetation decreases over the summer (Nielsen et al., 2017). When grass is removed pygmy bluetongue lizards have lower body mass increases, are less likely to move around their burrows, are more likely to disperse from the area, bask longer (Ebrahimi and Bull, 2015) and make more predation attempts (Pettigrew and Bull, 2012, Pettigrew and Bull, 2014) possibly due to a reduction in invertebrate prey abundance (Ebrahimi and Bull, 2012).

As part of a broader study, it was observed that adult and neonate lizards were using grass tussocks as they moved around their enclosures in captivity (Daniell pers. obs. 2018). Although the captive environment is free from predators, the grass tussocks could potentially provide refuge from predators — as a site to hide from birds and gain protection by entering the dense inner areas. Prolonged heavy grazing that reduces grass tussocks could adversely impact pygmy bluetongue lizard populations if tussocks are important temporary refuges. To determine the importance of grass tussocks as potential temporary refuges for lizards, we observed behaviours relating to grass use whilst lizards were emerged from their burrows. Our aims were to 1) quantify grass use by pygmy bluetongue lizards over the activity season and 2) compare the proportion of time lizards spent out of the burrow in grass tussocks, sitting on the surface and walking the enclosure perimeter.

Methods

Captive pygmy bluetongue lizards were filmed over the activity season from October 2017 to March 2018 as part of a broader behavioural study. Filming occurred for four hours per day, eight days per month (each lizard was filmed four times per month). A total of 45 lizards (14 adult lizards at least four years of age and 31 captive-born juveniles) made up the colony. There were two juvenile cohorts; 13 immature animals (born February 2016) and 18 juveniles (born February 2017). Lizards were housed in raised enclosures divided into three sections, filled with sand and artificial burrows, and four grass tussocks (*Austrostipa* sp. and *Austrodanthonia* sp.) per section to provide shade. Grasses were kept trimmed to a height of approximately 30 cm and were not watered to replicate natural conditions over spring and summer. Adults were housed individually, and juveniles and immatures were housed in pairs. The number of grass tussocks per enclosure remained the same throughout the study. Grasses in adult enclosures were older and more established and thus denser than grasses in juvenile and immature enclosures which were not as established.

The parameters recorded were time (minutes) lizards spent in grass; the number of times lizards entered grass; the number of burrow departures; and time (minutes) spent in grass in compared to the other out of burrow activities of sitting or walking the enclosure perimeter. Primer v7/PERMANOVA+ was used to conduct univariate multifactorial repeated measures PERMANOVA tests based on Euclidean distance matrices and SPSS was used to produce graphs. Month and day were within subjects factors and age was a between subjects factor.

Results

Over the six-month filming period, pygmy bluetongue lizards exited burrows a total of 1110 times and entered grass tussocks a total of 613 times. Lizards spent a total of 1,856 minutes in grass tussocks, 703 minutes sitting on the surface, and 320 minutes walking the enclosure perimeter over the six months.

Use of grass tussocks by pygmy bluetongue lizards varied significantly over the six months of filming (Table 1). The monthly mean time in minutes (average calculated from 4 days x 240 minutes filming per lizard per month) lizards spent in grass were as follows: October 5.41 ± 1.82 SE, November 3.32 ± 0.55 SE, December 1.36 ± 0.28 SE, January 0.89 ± 0.27 SE, February 0.23 ± 0.08 SE, March 0.45 ± 0.11 SE. There were also significant differences in time spent in grass among age classes and an interaction between age classes and months (Table 1). Time spent in grass was higher for adults and juveniles compared to immatures in October and highest for juveniles in November, with time spent in grass similar for all age classes from December to March (Figure 1). The mean time spent in grass by the three age classes were; adults 1.80 minutes ± 0.75 SE, immatures 0.99 minutes ± 0.20 SE and juveniles 2.37 minutes ± 0.31 SE. Time in grass also differed among days (Table 1).

Table 1: PERMANOVA results for the variables; number of burrow departures, time in grass (minutes) and number of grass entries. df= degrees of freedom; SS = sum of squares; MS = mean sum of squares; Pseudo-F= F value by permutation, P(perm)= p-values based on > 9000 permutations, Perms= number of permutations; Day = filming day. * indicates significant P value.

Burrow departures						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	4	50.40	12.60	8.08	<**0.001***	9949
Age	1	31.78	31.78	51.04	<**0.001***	9818
Day	39	78.29	2.01	4.58	<**0.001***	9886
Month x age	9	15.74	1.75	3.54	**0.011***	9949
Day x age	27	13.62	0.50	1.15	0.275	9916
Res	951	416.87	0.44			
Total	1034	750.71				
Time in grass						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	4	52.09	13.02	5.29	**0.003***	9950
Age	1	23.40	23.40	18.16	<**0.001***	9831
Day	39	115.50	2.96	2.44	**0.016***	9891
Month x age	9	35.96	4.00	3.71	**0.010***	9939
Day x age	27	28.44	1.05	0.87	0.555	9901
Res	951	1153.20	1.21			
Total	1034	1548.40				
Grass entries						
Source	df	SS	MS	Pseudo-F	P(perm)	perms
Month	4	23.01	5.75	3.47	**0.004***	9931
Age	1	14.83	14.83	42.69	<**0.001***	9817
Day	39	46.39	1.19	4.22	**0.003***	9938
Month x age	9	7.92	0.88	3.12	**0.017***	9953
Day x age	27	7.61	0.28	1.00	0.472	9906
Res	951	268.69	0.28			
Total	1034	452.66				

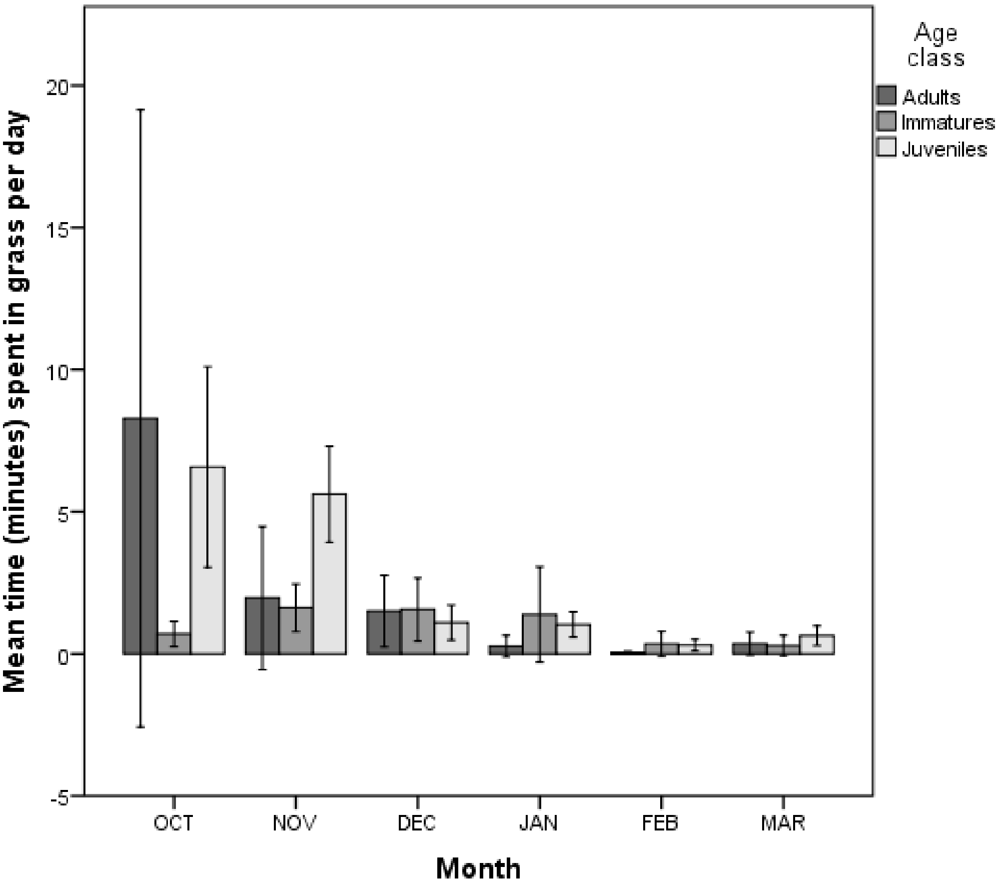

Figure 1: Mean time in minutes (error bars: 95% CI) spent in grass tussocks per day across the months of the lizard activity season for adult (dark grey), immature (medium grey) and juvenile (light grey) age classes.

The number of both burrow departures and grass entries varied significantly among months (Table 1). Similarly, there were significant differences among age classes and interaction effects between age and month for both burrow departures and grass entries. The mean number of grass entries were approximately half that of burrow departures, therefore around half of all burrow departures resulted in a lizard entering a grass tussock (Figure 2). Lizards spent a greater proportion of their time when out of burrow in grass tussocks compared to sitting on the surface or walking the enclosure perimeter (Figure 3). This was a consistent trend over the months from October to March, except for February, in which lizards spent a greater proportion of time sitting on the surface than in grass.

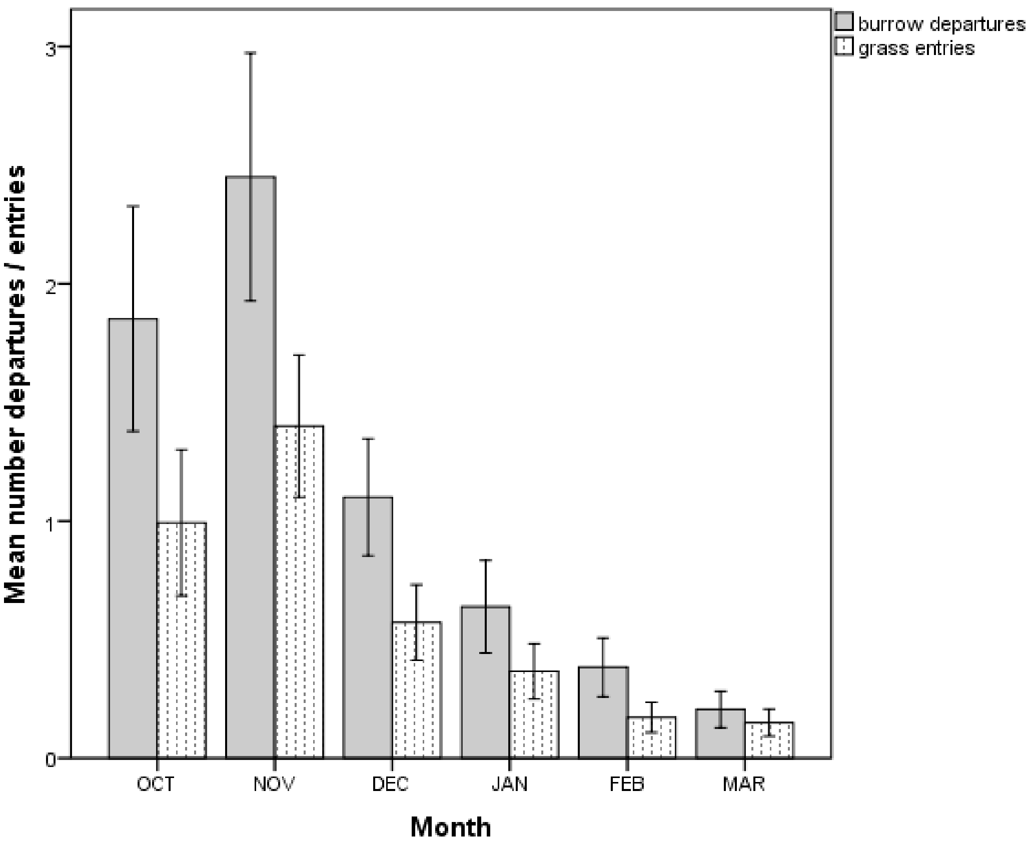

Figure 2: Mean number (95% CI) of burrow exits (grey) and grass tussock entries (patterned) made by pygmy bluetongue lizards over the activity season.

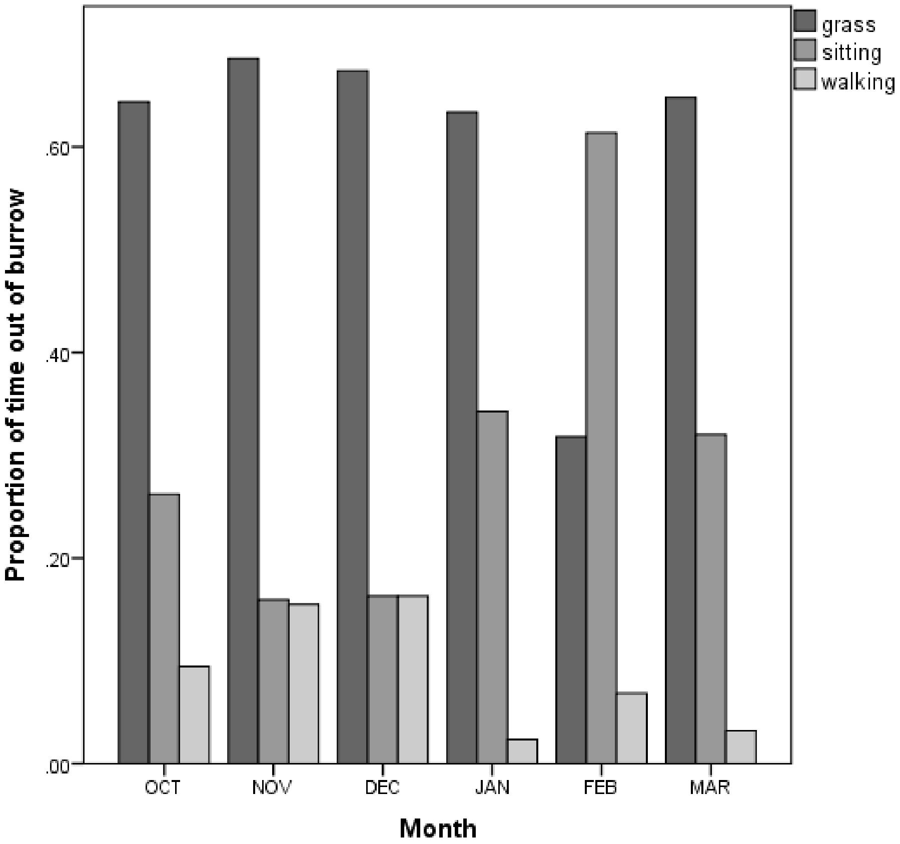

Figure 3: Proportion of the time out of burrow pygmy bluetongue lizards spent in the three activities – in grass (dark grey), sitting on the surface (medium grey) and walking the enclosure perimeter (light grey).

In captivity, we have directly observed juvenile and adult lizards entering grass tussocks for short periods of time for three apparent reasons — for shelter, foraging and basking. First, captive neonate lizards used tussocks as shelter when they were exploring the enclosure and seeking new burrows after dispersal from the natal burrow. The lizards sometimes climbed through the tussocks or sat at the base hidden beneath the stalks. Lizards would occasionally escape from researchers during capture and would try to hide amongst grass tussocks. Second, lizards foraged in grass. One example is on the 27th September 2017 — an immature lizard was observed exiting a burrow to chase a small wolf spider into tussocks and capture it. Finally, during October and November, an adult male PBT was frequently seen perched either atop, or close to the top with the head emerged but torso within, a grass tussock in its enclosure. The enclosure had been divided to separate the male and female lizards to prevent breeding. The lizard appeared to be using the tussock as a basking site. This adult male displayed such perching behaviour most often, but other lizards, both male and female, also perched atop tussocks.

Discussion

We examined the usage of grass tussocks in captive pygmy bluetongue lizards. These lizards spend most of their time retreated within or basking at their burrow entrance (Milne et al., 2003). However, our study revealed that when lizards exit their burrow, they are likely to enter grass tussocks. There were three main findings. First, lizards spent more time in grass in spring than later in the season; second, approximately half of burrow departures resulted in lizards entering grass tussocks; and finally, the proportion of time lizards spent in grass tussocks was higher than other out-of-burrow activities such as sitting or walking the enclosure perimeter.

There were seasonal differences in time spent in grass tussocks and the number of burrow departures and grass entries — all being higher in October and decreasing as the activity season progressed. Lizards may be more active in spring when temperatures are milder than in summer, or it could be due to seasonal differences in vegetation cover. Temperate grasslands with wet winters and dry summers follow annual cycles whereby grass cover is higher in spring than in summer (Pettigrew and Bull, 2011, Nielsen et al., 2017). Previous studies have found that pygmy bluetongue lizards are less likely to move around outside of burrows or change burrows when vegetation cover is reduced (Ebrahimi and Bull, 2015, Nielsen et al., 2017). Differences among age classes could be due to adults seeking mating opportunities in spring (Schofield et al., 2013), as differences among age classes became less apparent after November. The juvenile cohort spent more time in grass than the immature cohort in October and November, which could be related to higher metabolic demands of the juveniles which are still in a period of rapid growth during this time. The density and size of tussocks in our study varied between enclosures, as adult enclosures had more established grasses. This may have influenced grass use among the age classes, but as adults, immatures and juveniles all had high levels of grass use it suggests that even less dense tussocks are a valuable resource.

Of the three out-of-burrow behaviours — in grass, sitting, or walking the enclosure perimeter — captive pygmy bluetongue lizards spent a higher proportion of time in grass tussocks. We did not classify individual cases of grass use into categories such as foraging, seeking refuge or basking, as such detail was not possible from the video recordings — however all are possible explanations for lizards spending time in grass, as captive pygmy bluetongue lizards seem to use grass tussocks as a multipurpose resource. For example, we observed lizards in captivity chasing invertebrate prey into tussocks or hiding within the dense tussocks to avoid capture during monthly weighing procedures. Captive lizards have also been observed to use tussocks as a basking perch. Another possible explanation for this behaviour is the male lizard was using the grass tussock to survey the area in an attempt to locate a female mate (adults were separated to prevent breeding). This perching behaviour could be a result of the captive environment restricting natural mate-seeking behaviour resulting in abnormal behaviours. However, it seems more plausible that the perching behaviour is related to basking as females and juveniles were also observed perching atop grass. Other reptile species are known to bask and shelter within tussocks of *Triodia* spp. including *Delma* spp., eastern three-lined skinks (*Acritoscincus duperreyi)* (Cogger, 2014), and Mallee dragons (*Ctenophorus fordi)* (Olsson, 2001).

This study reports grass use in captive pygmy bluetongue lizards, but it is unknown how significant grass use is for the survival of wild pygmy bluetongue lizards and should be researched further. Adult male pygmy bluetongue lizards are known to move around in search of mates in spring, and neonates disperse in late summer after birth (Milne et al., 2002, Schofield et al., 2013). Grass tussocks are likely to provide shelter for lizards as they move around outside of burrows for foraging or dispersal. As grass cover would naturally be lower during the neonate dispersal period, prolonged heavy grazing or drought could have increased effects on neonate survival by further decreasing grass availability.

Grass tussocks were found to be an important resource for captive lizards while out of their burrow, to forage, bask and seek cover. This finding suggests that grass tussocks play an important role for the pygmy bluetongue, by providing alternate temporary refuges and so has management implications for wild populations and selecting appropriate translocation sites. Prolonged heavy grazing practices may reduce the density and abundance of grass tussocks, which would reduce the availability of grass refuges when a lizard is moving through the habitat. The availability of grass tussocks during mating season when male lizards are moving around, and during neonate dispersal when offspring are searching for their own burrow are likely to be important to lizard survivorship. Further research to determine whether tussocks are commonly used in wild populations is required to support these hypotheses.

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Chapter 7

General Thesis Discussion

Translocations are an important conservation strategy, but efforts are needed to improve success rates. In this thesis, I investigated factors that may affect translocation success in the pygmy bluetongue lizard, an endangered species which will require conservation translocations. I had five primary aims that make a significant contribution to knowledge and address knowledge gaps in the literature to improve our understanding of the pygmy bluetongue lizard and the effect of captivity on animals. These aims were to:

1. Determine how behaviours change ontogenetically in the pygmy bluetongue lizard
2. Determine how behaviour differs between neonates and adults
3. Determine the effect of captivity on lizard foraging ability and behaviour
4. Determine if predator recognition is innate in the pygmy bluetongue lizard, how captivity affects predator avoidance behaviour, and if lizards respond more strongly to a known predator compared to other predatory reptile species
5. Investigate how pygmy bluetongue lizards use grass tussocks

This thesis discussion focuses on the findings of my data chapters (Chapters 2 to 6) and how these results provide a significant contribution to knowledge of the pygmy bluetongue lizard and the broader conservation literature.

Behaviour is known to vary over the life of animals which can have implications when selecting age classes for translocation, as some age classes may be more suitable than others. In chapter 2, I determined how behaviour changes ontogenetically in the pygmy bluetongue lizard for three age classes – juvenile, immature and adult and review ontogenetic behavioural variation in lizard species. I found that like other lizard species, pygmy bluetongue lizard behaviour changes with age. Activity and movement were highest in juvenile age classes, decreasing with age. My findings have important implications for future translocations of pygmy bluetongue lizards, as neonates (Chapter 3) and juveniles are highly active and likely to be more at risk of predation if released into the wild compared to immature and adult lizards.

Pygmy bluetongue lizard research up to now had primarily focused on adult behaviour, and all that was known about neonate lizards was that they dispersed shortly after birth in late summer or early autumn (Milne et al., 2002, Schofield et al., 2013). Neonate or juvenile age classes could be used in translocations after being bred and reared in captivity – a practice known as head-starting. As there is a captive population of pygmy bluetongue lizards, captive bred lizards could be used in future translocations, however, more needs to be known about neonate behaviour to determine if neonate lizards are suitable translocation candidates. Therefore, in Chapter 3 I aimed to determine if behaviour differed between neonate and adult pygmy bluetongue lizards in ways that are relevant to translocations. Pygmy bluetongue lizard neonates were found to bask more than adults late into the activity season when adult basking had decreased. Neonates also moved around on the surface more and changed burrow more than adults. Adult basking and movement behaviour was highest earlier in the activity season and declined in late summer and early autumn, which follows previous research of adult seasonal activity (Ebrahimi and Bull, 2014a). I found that adult females basked more than adult males in summer and autumn, likely due to reproductive requirements. My results have implications for selecting translocation candidates, as the higher activity levels of neonate lizards suggest that they may be at higher predation risk compared to adults. Further research is required to understand if the higher activity levels of neonates would correspond to higher predation in a translocation setting rather than the controlled environment that I used for the experiments.

In Chapter 4 I determined the effect of captivity on lizard foraging ability and behaviour. Gaining this knowledge is important as poor foraging ability has resulted in starvation and mortality in translocations of captive animals, and the simplistic captive environment may impede foraging ability (Jule et al., 2008). Previous research has found that allowing captive animals to forage naturally or with a broader range of food items is beneficial for foraging ability and survival (Vargas and Anderson, 1999, Alberts, 2007). However, in other species the captive rearing environment did not affect foraging ability (Rogers et al., 2016). This variation among studies indicates that responses to captivity and environmental enrichment to promote increased foraging ability may differ among species hence it is important determine suitable feeding practices for captive animals that are potentially going to be used in translocations. Zoos SA were feeding their captive pygmy bluetongue lizards individually by hand to ensure each lizard was adequately fed. However, this intensive husbandry may not be necessary and indeed might be producing animals less suitable for translocations. It was important to understand if a more natural and less labour intensive feeding regime was still consistent with the requirements of keeping lizards in good conditions before husbandry techniques mimicking a more natural approach were introduced. After comparing hand-feeding versus a more natural self-feeding regime, I found that pygmy bluetongue lizard body condition and prey capture ability did not significantly differ. This suggests that time in captivity under a hand-feeding regime did not affect foraging ability, but it did affect behaviour, as hand-fed lizards were found to bask longer and spend more time in grass in October. Lizards that are out of their burrows on the surface are at greater risk of predation, therefore hand-fed lizards may have a greater risk of predation if released into the wild. I therefore recommend a naturalistic self-feeding regime for captive lizards to reduce behavioural changes that may be adverse in a translocation release. As a result of my work the Zoos SA policy on feeding was altered to the self-feeding approach, saving time and money for the Zoo — and likely resulting in a reduction in human induced behaviours in the captive lizards.

Captive animals that are not exposed to predators can lack predator recognition and avoidance behaviours, making them vulnerable to predators when released into the wild (Jule et al., 2008, DeGregorio et al., 2017). It is therefore vital to understand the effect of captivity on predator avoidance and if captive animals can respond successfully to predator threats before releasing captive animals into the wild. Consequently, in Chapter 5 I investigated if pygmy bluetongue lizards can innately recognize predators, discriminate between predatory reptiles of differing threat levels and how captivity affects predator recognition and avoidance. I found that, like in some other lizard species, predator recognition is innate in the pygmy bluetongue lizard as naive captive born lizards responded the same as potentially experienced wild born and wild lizards, and that time in captivity did not affect the response of lizards to predator scent. Lizards also tongue flicked significantly more towards a known predator – the eastern brown snake, compared to the control scents, which indicate that the pygmy bluetongue can discriminate between potential predators. However, I discovered that predator scent alone was not enough to induce avoidance behaviour, thus further research should be conducted to investigate if other cues such as visual or auditory cues are required for lizards to retreat into their burrows.

The pygmy bluetongue lizard is known to spend most of the time associated with spider burrows, either basking at the entrance or retreated within the burrow (Milne et al., 2003). Past research has investigated the effect of grazing on spiders and lizard behaviour related to burrow choice or prey capture ability (Pettigrew and Bull, 2011, Pettigrew and Bull, 2012, Pettigrew and Bull, 2014, Clayton and Bull, 2015, Ebrahimi and Bull, 2015, Clayton and Bull, 2016), however research into how pygmy bluetongue lizards may use grass tussocks as a resource was lacking. One question was whether grass tussocks provide temporary refugia for lizards moving throughout the landscape. I had observed captive pygmy bluetongue lizards basking or hiding within grass tussocks and thus predicted that grass tussocks may be used by lizards when outside of their burrows for shelter, foraging or basking opportunities. To fill this knowledge gap, in Chapter 6 I investigated how lizards use grass tussocks by observing footage of captive lizard behaviour. Lizards were found to use grass tussocks to shelter, forage and bask as predicted, and around half of the occasions in which lizards exited their burrow they would enter grass tussocks. This suggests that tussocks are an important resource for lizards when outside of their burrow. I suggest that tussocks could potentially be used by lizards as a temporary refuge for dispersing lizards, such as dispersing neonates seeking burrows or when lizards are moving around during mating season. Further research is required to determine if wild lizards use grass tussocks in the same way as captive lizards. However, my findings provide a significant contribution to our knowledge of resource use by the pygmy bluetongue lizard and have implications for grazing and conservation management. I suggest that grazing levels should maintain grass tussocks and that when searching for potential translocation sites, the presence of grass tussocks, or planting tussock grasses if absent, must be considered. More research is required to understand the number or density of tussocks that is optimal.

Conclusions and future directions

In this thesis I have investigated factors that may affect translocation success in the pygmy bluetongue lizard, thus contributing to our knowledge of this species and to other similar reptile species. Studies of animal behaviour in a captive environment provide a great opportunity to understand behaviour and ecology, especially in species that are difficult to observe in the wild. The pygmy bluetongue lizard has unique ecology, in that it is solitary, inhabits grasslands and spends the majority of its time within a burrow, with limited dispersal across a small area during mating season and after birth. Therefore, my findings cannot be generalised across a broad range of species, but will be beneficial for similar reptile species, for example small bodied species, those that are solitary and sedentary and disperse over small areas during mating and neonate dispersal and species that are restricted to fragmented grasslands. The finding most applicable to a broad range of species, is that pygmy bluetongue lizards can innately recognise predator scent but may require additional cues to elicit avoidance behaviour. This is likely due to the ranging behaviour of the snake predator resulting in scent not being a good indicator of predator presence, and I suggest that when investigating predator recognition and avoidance multiple cues are tested — chemical, visual and auditory cues, especially for predators that move around.

My results suggest that neonate and juvenile age classes are unsuitable for release in translocations as they are likely at greater risk of predation, because they are more active, spend more time out of their burrow exposed on the surface and are more likely to disperse than immature or adult age classes. It is possible that due to the smaller size of neonate and juvenile lizards, they are less visible to predators thus the higher activity levels may not equal higher predation risk. However, previous research has suggested high mortality in neonate age classes (Milne, 1999), which could be due to the higher levels of movement and activity. Further research is needed to confirm this, but if predation is higher in neonate and juvenile classes, the number of animals released would need to be higher to account for losses to mortality. In the pygmy bluetongue lizard, which currently has a small captive population and fragmented wild populations, sourcing large numbers of juvenile lizards would be difficult. Instead, I recommend translocating immature or adult lizards, or a combination thereof. Neonate lizards can be reared in captivity until they reach immature age (two years old), in which activity levels have reduced and become like adults in behaviour.

Whilst time spent in captivity did not detrimentally affect lizard foraging ability or ability to recognize predators, I suggest that to avoid altering behaviour, the captive environment should simulate the natural environment. Lizards that were hand fed spent more time out of their burrows exposed on the enclosure surface, which could result in hand-fed lizards being more at risk of predation if released into the wild. Furthermore, although I found that predator recognition is innate in the pygmy bluetongue lizard, neither wild or captive lizards avoided predator scent, hence I cannot conclude if captive born lizards would be able to successfully avoid predators if released into the wild. The use of grass tussocks by captive lizards suggest that tussocks are an important resource to this species, hence captive environments should provide tussocks to mimic the natural environment. Grazing management should also ensure that tussock grasses are preserved for lizards and the presence of tussock grasses should be a criterion for selection of potential translocation sites.

My thesis has identified several questions that require future research and important questions that I did not investigate in my research. First, a real translocation is required to compare survival among neonate, juvenile, immature and adult age classes to determine if the differences in activity levels I observed result in higher predation levels or not. Further research should investigate how basking and movement behaviour varies among the age classes in a translocation setting. Previous simulated translocations found that when adult lizards are stressed, basking is reduced, and movements and dispersal increased (Ebrahimi and Bull, 2013, Ebrahimi and Bull, 2014b). Different age classes may be more stressed by the translocation procedure or respond differently as previous simulated translocations only studied adults. Second, we need to identify the predatory cues that elicit a predator avoidance response in the pygmy bluetongue lizard. I suggest testing responses to visual predator cues, auditory cues and chemical cues alone and in combination to determine if lizards respond strongly to a single cue or if multiple cues are required to elicit predator avoidance behaviour. Predator avoidance training using model bird and snake predators could be conducted to test pygmy bluetongue lizard response to simulated predators and whether training improves translocation survival in released captive lizards. Third, research into how wild pygmy bluetongue lizards use grass tussocks is required to determine if wild lizards use tussocks to the same extent as captive lizards. Last, my research did not investigate if behaviour differs among lizard populations and how that may affect survival in translocations. Additionally, lizards may have genetic differences in their behaviours, and research should be directed in understanding what effect, if any, these individual differences could have on the suitability of lizards for translocation.

In conclusion, I have provided new information relating to pygmy bluetongue lizard behaviour and resource use and advanced our knowledge of the effect of the captive environment on captive animal behaviour and life skills. My findings can help inform captive husbandry and selecting appropriate candidates for translocations. I have identified further research questions which are required to continue increasing our understanding of the factors that affect translocation success, with the goal of improving translocation success to conserve our endangered species.

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