The ecological and genetic risks of wildlife translocation

by

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THESIS SUMMARY

Native species increasingly need management. Common species need to be controlled and rare species need to be conserved. There are many management strategies which can be used to achieve these goals. One species management tool is translocation, the human mediated movement of living organisms from one area to another, which can be used to resolve human-wildlife conflict, biological control of pest species or to mitigate against anthropogenic changes such as renewable energy developments. Conservation translocations are specifically targeted to conserving a focal species or ecosystem and this approach is often successful, as seen with high profile examples such as reintroduction of grey wolves (*Canis lupus*) into Yellowstone National Park and reintroduction of California condors (*Gymnogyps californianus*) into the wild.

While there are many success stories, there are also many reported translocation failures or uncertain outcomes due to inconsistent criteria for success and insufficient species knowledge to shape the way a translocation is carried out. On the other hand, concern of perceived risks can lead to management inaction, even in the absence of empirical evidence to validate these concerns. My study is one of the first pre-emptive studies to quantify potential areas of risk so that, should risks be detected - they can be mitigated. There is very little literature on this topic and this study uses an endangered species to conduct performance assessments, assess population health risks, genetic risks and intra and interspecific competition risks. Here, I conducted an experimental translocation in enclosures built around an existing wild pygmy bluetongue lizard population. I moved individuals from two genetically distinct populations at the edges of the current species range and mixed them with a third recipient population. Through monthly monitoring over three consecutive activity seasons I was able to accurately, and

repeatedly, assess the ecological and genetic risks associated with mixing source populations during population augmentation on both resident pygmy bluetongues and other lizards.

Before carrying out a translocation, in addition to having sufficient species knowledge to facilitate a successful movement, it is important to know what effect the translocation will have on resident species at the recipient site to ensure that efforts to conserve one species do not detrimentally impact others in the local area. I found that augmenting pygmy bluetongue populations does not negatively affect the abundance and health of co-occurring lizard species in the recipient ecosystem.

Monitoring population health is an important step in conservation of endangered species to allow action to be taken when decline is detected, to avoid extinctions or extirpations. The ability of an animal to perform an ecologically relevant task is key to their fitness but can be invasive and time consuming to collect data. Being able to comment on the performance of an animal through non-invasive data collection, such as body condition, would be important in monitoring of vulnerable populations and potentially identify the beginning of decline allowing action to be taken pre-emptively. Although this suggestion is incumbent upon those measures of condition accurately identifying animals that were more likely to survival and reproduce. My results showed, using bite force as the measure of performance, that both males and females in better body condition performed better. These results provide confidence that for this species, body condition is a good measure of population health and can be used to assess not only small isolated populations but also populations after receiving management actions such as a reinforcement or reintroduction.

Using polymorphic microsatellite loci, I genotyped each pygmy bluetongue and conducted parentage analysis to identify lineages of offspring according to the source population of the parents. The offspring were raised in semi-wild conditions where they had to compete for dispersed prey and burrow ownership. I used three fitness-related traits to compare those lineages in terms of growth (body condition and growth rate) and performance (bite force) to assess whether mixed lineages grew or performed differently to unmixed lineages. I found that individuals from genetically differentiated populations do interbreed which is a key finding if population augmentation is to be a conservation strategy for this species. Mixed-lineage offspring have similar levels of growth and performance to unmixed offspring suggesting that mixed-lineage offspring are unlikely to outcompete unmixed lineages for resource such as burrows or prey. There also was no evidence of outbreeding depression as shown by the fact that mixed-lineage individuals were not noticeably inferior to unmixed offspring in terms of their growth or performance. I have also found that body condition plays an integral part in the reproductive success of female lizards which highlights the importance of supplementary feeding to support population establishment and growth in years of low prey abundance.

The results of this study inform a broader question of whether mixed-lineage source populations would fare better at a new site in terms of population growth. Establishment of new populations, and successful reproduction at a new site, without detrimentally affecting co-occurring species are both fundamental to the success of a translocation and the present study provides empirical data on both areas. The results of this study will be integral to conservation of species existing in small isolated populations and increase the success rate of subsequent movements.

DECLARATION

‘I certify that this thesis does not incorporate without acknowledgement 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.’

Lucy Clive

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Mike Bull with Sir David Attenborough during the filming of Life in Cold Blood

I began my candidature with Mike Bull as my primary supervisor. I remember first experiencing his efficient work ethic when I emailed him from the depths of the Kalahari Desert in South Africa, only to receive a reply the very next day! I knew then that I had found a kindred spirit I would work well with and I will never forget walking into his office the following year to meet him for the first time as his new PhD candidate. He took a chance on me and, following his passing, I have spent the past few years making sure he wasn't mistaken. He always had a (truly terrible) dad joke to hand and a sparkly eyed grin he got immediately after telling it which made it even funnier. I feel very fortunate to have worked with him & aspire to work as efficiently as him one day, even if it takes me a lifetime to get there!

And finally, thanks to all the pygmy bluetongue lizards. You will forever be my favourites.

Adult pygmy bluetongue lizard *(Tiliqua adelaidensis*) (Photo: Peter Matejcic)

AUTHOR CONTRIBUTIONS

CHAPTERS 1 & 6 – LC (100%)

CHAPTER 2 – Population augmentation had no effect on the abundance or body condition of conspecifics and co-occurring lizard species in a native grassland community

Authors: Clive LFR, Gardner MG, Clayton JL, Baring R, Hutchinson MN, Fenner A, Bull CM

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LC:75%, MG: 7.5%, MH: 7.5%, JC: 5%, RB: 3%, AF: 2%

MB: funding & concept (80%)

CHAPTER 3 – Body condition is a good indicator of bite force performance: an assessment tool for endangered species populations

Authors: Clive LFR, Barnett LK, Jones MEH, Hutchinson MN, Bull CM, Gardner MG

Status: Prepared for submission to Journal of Wildlife Management

LC: 75%, MG: 8%, MH: 8%, MJ: 5%, LB: 4%

MB: funding & concept (50%)

CHAPTER 4 – The size and performance of a weapon does not predict reproductive success

Authors: Clive LFR, Jones MEH, Hutchinson MN, Bull CM, Gardner MG

Status: Prepared for submission to Conservation Genetics

LC: 75%, MG: 12%, MH: 8%, MJ: 5%

MB: funding & concept (50%)

CHAPTER 5 – Genetic admixture has no negative effect on three fitness-related traits in an endangered species

Authors: Clive LFR, Hutchinson MN, Jones MEH, Bull CM, Gardner MG

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We, the undersigned have agreed to co-authored work being included in this thesis, and agree with the above stated contribution to thesis chapters:

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ORGANISATION OF THIS THESIS

This thesis contains four data chapters (Chapters 2-5) that communicate research undertaken as part of a doctoral program. Statements connecting the work of previous and subsequent chapters are provided between chapters. Each chapter was written to stand alone, therefore references are provided at the end of each chapter as well as a full bibliography at the end of the thesis. Formatting of each chapter has been standardised for thesis submission and, to aid readability, tables and figures have been embedded in each chapter where appropriate. Supplementary tables and figures relevant to the chapter will be included after the references for that chapter. One chapter has been published (Chapter 2) and three chapters are prepared for publication after submission of this thesis (Chapters 3, 4 & 5). There is a thesis appendix which is a published short natural history note.

Although I conducted the majority of the work, the chapters were written as manuscripts where "we" is used more commonly than "I" due to the contribution of the co-authors. The contribution of each co-author has been included in the statement of authorship. The overall order of the thesis, by chapter, is below:

Chapter 1 - General Introduction

Chapter 2 - Clive et al., (2019) Published. Population augmentation had no effect on the abundance or body condition of conspecifics and co-occurring lizard species in a native grassland community

Chapter 3 - Clive et al., (*in prep*) Body condition is a good indicator of bite force: an assessment tool for endangered species populations

Chapter 4 - Clive et al., (*in prep*) Size doesn't matter: Weapon size and performance are not correlated with reproductive success

Chapter 5 - Clive et al., (*in prep*) Genetic admixture has no negative effect on three fitness-related traits in an endangered species

Chapter 6 - Discussion & Conclusions

The following chapter (Chapter 1) places the work of this thesis in a broad conservation focussed context, providing the necessary background information and theory relating to the thesis. It also details the overall aims of the work. Due to each chapter being written as a manuscript, each contains its own introduction and discussion so naturally there will be some unavoidable repetition.

CHAPTER 1

GENERAL INTRODUCTION

Pygmy bluetongue lizard *(Tiliqua adelaidensis*) in an artificial burrow (Photo: Carmel Maher)

INTRODUCTION

Increasingly native species require management. For example, common species are culled (Debus 2008) or contracepted (Miller and Fagerstone 2000) to control their numbers whereas - at the other end of the spectrum - less common species need intervention for their conservation (Seddon *et al.* 2012). There are many types of management strategies, each specific to the conservation issue being addressed, and an understanding of the species' biology and life history requirements is needed (Table 1). Protection of habitat and the species populations within can provide time and opportunity to build the necessary species knowledge for decision-making analysis or modelling predictions of future responses to either climactic changes or management strategies. Many areas of knowledge are required in order to conserve or manage a species and the most effective management strategies use an integrated multi-skill approach that combine many of the below examples (Table 1).

Table 1: Table showing examples of species management strategies

Action	
Habitat restoration	Conserve areas of degraded habitat (Miller and Hobbs 2007)
Ecological management	Use of alternative species for ecological replacements to restore ecological function (Griffiths *et al.* 2013)
Protection of land	Areas of land can be purchased and turned into a National parks to protect both the habitat and the species within (Le Saout *et al.* 2013)
Captive maintenance	Housing species to act as insurance populations for species facing extinction in the wild. Captive breeding programs can also help to reinforce wild populations (Foose and Wiese 2006)
Targeted Research	Research examining aspects of species biology, ecology, physiology and genetics will help to support management actions and ensure they are appropriate for the species and its threats
Population Modelling	Demographic or population viability analysis to simulate extinction processes and assess the long-term viability of small populations (Lindenmayer *et al.* 1993)
Translocation	Movement of animals in response to ecological crisis' or climate change (Armstrong and Seddon 2008; Germano *et al.* 2015)

One method of species management that this thesis focuses on is that of translocation, the over-arching term for the human-mediated movement of an organism, which can be carried out in a variety of ways for a variety of reasons (Table 2). Translocations can used to resolve human-wildlife conflict (Linnell *et al.* 1997); for commercial purposes (Horwitz 1990); biological control of pests (Phillips *et al.* 2007) or for purely aesthetic reasons (Seddon *et al.* 2012). Translocations can also be carried out to militate against anthropogenic change such as development of solar farms, wind farms and hydroelectric projects, all of which contribute to habitat loss (Germano *et al.* 2015). Populations threatened by climate change can be relocated to new more favourable areas of habitat using translocation (McLaughlin *et al.* 2002; McLachlan *et al.* 2007) and, finally, translocations can be conducted purely for conservation benefit, termed ‘conservation translocations’ (IUCN 2013).

Table 2: Table showing the types of translocations and their definitions (IUCN 2016)

		Definition
Translocation		Human mediated movement of living organisms from one area with release in another
Mitigation translocation		The removal of organisms from habitat due to be lost through anthropogenic land use change and release at an alternative site.
Conservation translocation		The intentional movement and release of a living organism where the primary objective is a conservation benefit
Within indigenous range		
Population restoration		Any conservation translocation to within a species' indigenous range
	a) Reinforcement	The intentional movement and release of an organism into an existing population of conspecifics (Also known as population augmentation or supplementation)
	b) Reintroduction	The intentional movement and release of an organism inside its indigenous range from which it has disappeared
Outside indigenous range		
Conservation Introduction		The intentional movement and release of an organism outside its indigenous range
	a) Assisted colonisation	The intentional movement and release of an organism outside its indigenous range to avoid extinction of populations of the focal species
	b) Ecological replacement	The intentional movement and release of an organism outside its indigenous range to perform a specific ecological function

CONSERVATION TRANSLOCATIONS

Conservation translocations are increasing in frequency due to the wide-ranging effects of human impact, such as urban spread (Seddon *et al.* 2012; Chauvenet *et al.* 2013). Development of barriers to dispersal, such as roads, impede gene flow between populations leading to altered population structure (Taylor and Goldingay 2010) leading to the development of road crossing structures, such as rope bridges or road underpasses, which facilitate wildlife movement while reducing road mortality (Woltz *et al.* 2008). A lack of continuous habitat, or the presence of dispersal corridors, can result in population isolation and the onset of inbreeding depression (Lesbarrères *et al.* 2003).

A lack of genetic diversity, resulting from inbreeding depression, will limit the adaptive capacity of a population and its ability to withstand impending climatic stochasticity caused by climate change (Weeks *et al.* 2011). Although climate change is a well-documented cause of species decline, few mitigation translocations have been carried out and, of those that have, many are unsuccessful due to the lack of post-movement planning or monitoring (Sullivan *et al.* 2015; Hughes 2018). Species or population declines result from multiple threats and management strategies, such as translocations, are most effective when a systematic 'step-by-step' approach is applied (Hughes 2018; Baling *et al.* 2013). Intensive prey eradication programs have been undertaken in New Zealand due to the threat to their native species including bird (Miskelley and Powlesland 2013) and reptile (Towns and Ferreira 2001) species. New Zealand island translocations are an example of where selection of a release site is largely driven by the capacity to eliminate or control the active threat to those species, such as invasive mammal pests. Establishment of island population to safeguard species while feral pests are targeted on the mainland is another example of a multi-faceted approach to species management through use of translocation, habitat management or restoration and genetic research to maximise genetic diversity of the island population. If the threats cannot be controlled, the species may require translocation to a new area where the threat is absent.

Translocation aims will be specific to the set of issues facing the species in question. The most common, and basic, aim to create a self-sustaining population has been described by Seddon (2015) as "aspirational and unquantifiable" due to the unique set of circumstances each translocation faces, affecting the point at which that translocation can be classified as a success. Long-lived species may not sexually mature for decades and so classifying those translocations as a success only through creation of a self-sustaining populations could take a lifetime and, thus, is not an appropriate measure (Monks *et al.* 2012; Bell and Herbert 2017). A population demographic framework suggested by Sarrazin (2007) defined the three stages of population that can be used to assess translocation outcome: establishment, growth and regulation. Reporting on translocation outcomes using this terminology could be more beneficial as it identified which population stage has been achieved post-release rather than declaring the translocation a success or failure in the short-term. While initial post-translocation assessments could indicate a favourable result, early-stage success to turn into failure with time, highlighting the need for long-term monitoring. Monitoring does not guarantee success, however it does enable conservation managers to pinpoint the timing and causes of failure and quantify the inherent value of additional data to increase future chances of success (Canessa *et al.* 2016). This raises a commonly debated question of ‘How do you measure translocation success?’. Criteria for success in published studies include: abundance (Weeks *et al.* 2011; Beck *et al.* 1994); survival or body condition (Strum 2005) and reproduction by the F1 generation of wild-born offspring (Sarrazin and Barbault 1997). There is no single term to define translocation success as it is relative to the aim of the translocation and the life history of the species in question. Translocations, growing in popularity, are reported as having varying levels of success often due to poor planning and monitoring (Germano *et al.* 2014; Dodd and Seigel 1991; Fischer and Lindenmayer 2000; Armstrong and Seddon 2008). Planning of wildlife movements involves assessing the potential for failure which involves identification of factors that could influence the ability of those individuals to establish and survive at the new site. By identifying those areas of risk, it allows for mitigation to control their impact before the movement occurs (Leighton 2002).

Risks of conservation translocations

All wildlife movements carry an element of risk and, while identification of risk factors is important, the overall risk landscape depends upon the number of potential risks identified, the likelihood of their occurrence and the potential severity of their impact. Movements carry risk of failure, risk of success and risk of failing to act at all. All of these elements of risk must be considered and prepared for when planning a wildlife movement.

Some sources of risk associated with wildlife movements include: disease or parasite transmission to conspecifics or other species (Cunningham 1996); human-wildlife conflict presenting socio-economic risks (Treves and Karanth 2003); financial risks (Malik and Johnson 1994); risk of invasive behaviour at the release site (Mueller and Hellmann 2008); genetic risks (Weeks *et al.* 2011); and ecological risks (Hewitt *et al.* 2011). When designing a translocation, it is important to consider all factors that could affect the outcome of a release including: the source of the animals i.e. wild or captive (Letty *et al.* 2007; Snyder *et al.* 1996; van Heezik *et al.* 1999; Griffin *et al.* 2000), the sex and age of the individuals, group composition of social species (Sullivan *et al.* 2015), time of year (Eastridge and Clark 2001; Clark *et al.* 2002; Ebrahimi, Ebrahimie, *et al.* 2015), method of release i.e. hard or soft (Santos *et al.* 2009; de Milliano *et al.* 2016; Knox and Monks 2014; Hunter *et al.* 2007) as well as whether to conduct a once off-release or multiple augmentations over subsequent years (Weeks *et al.* 2011). Each course of action has its own impact on the overall success of the movement. Decision-trees aid identification of the optimal release strategies that will maximise the chances of success while taking into account the potential risks of each action (Ebrahimi, Ebrahimie, *et al.* 2015; Canessa *et al.* 2014). The chosen strategy should reflect the life-history needs and behavioural ecology of the focal species and this species knowledge comes from targeted experimental research to avoid unnecessary failures (Sarrazin 2007; Letty *et al.* 2007; Galloway *et al.* 2016).

Ecological risks

There is theoretical discussion surrounding ecological risks arising from translocations (Seddon *et al.* 2014) but little empirical data, with many citations arising from invasion ecology examples such as the invasive brown snake in Guam (Fritts and Rodda 1998) or the invasive cane toad in Australia (Phillips *et al.* 2007). Research concerning competition between, and displacement by, native species following wildlife movements are a gap in the literature that this thesis addresses by providing empirical data and a novel methodological approach for examining these risks (Habgood 2003).

Predation is a natural mechanism but intense predation pressure caused by poor habitat quality and lack of available refuge points is a risk that can be avoided with adequate pre-release site selection assessment and habitat augmentation (Bennett *et al.* 2013). Reducing vulnerability to predation is key during the population establishment phase post-release as the individuals settle in to the new habitat. Loss of individuals through predation is to be expected as predation is a natural mechanism regulating population size and it is important that translocation planning accounts for initial losses when choosing how many individuals to translocate so that it does not result in an inability of the remaining individuals to establish and reproduce. The ability of the translocated animals to exhibit the appropriate anti-predator behaviours necessary for survival in the wild is also important to translocation success. The translocated species may be vulnerable to novel or exotic predators although this risk is unlikely to occur in a reinforcement as, ideally, a release would not occur with an unmitigated threat present (Rominger *et al.* 2004; McKinney *et al.* 2006). Pest eradication programs through trapping or chemical baits could help to remove this threat although complete eradication is sometimes required which can be costly in time and money (Parkes *et al.* 2014). Demographic modelling could identify the necessary level of pest removal required for a successful translocation without repeatedly sacrificing animals in failed attempts (Tang *et al.* 2010). Pre-release predator training can also help to combat prey naivety and reduce post-release predation (Moseby *et al.* 2015, 2011).

On some occasions however, translocations can surpass expectations and population growth can succeed to a level where the focal species, termed a 'native invasive', begins to impact resident species (Mueller and Hellmann 2008; Sodhi and Ehrlich 2010). Niche partitioning allows species in an ecological community to exist in the same location while remaining spatially or temporally separate (Albrecht and Gotelli 2001). The introduction of a new species into a community via a conservation introduction, or a sudden increase in a species abundance due to a population reinforcement, could result in an imbalance in interspecific competition for habitat, prey or mates (Brown 1964; Jeffries and Lawton 1984). This imbalance could cause a reduction in the health of the resident species at the release site depending on the extent of shared life history traits and the amount of resource available at the site (Falk-Petersen *et al.* 2006). Ecological disruption is possible; however, it is more likely to occur as a result of assisted migration than a reintroduction or reinforcement. Published literature discussing invasiveness arising from wildlife movements seem to focus on assisted migrations rather than movements within the indigenous range of a species, perhaps indicating the low risk associated with such movements (Mueller and Hellmann 2008; Thomas 2011; Loss *et al.* 2011).

Genetic risks

A species requiring conservation management may encounter genetic risks in different ways. The selected management action will depend upon the threats facing the species and each strategy comes with its own set of risks. Species existing in only one place, or few isolated populations, are vulnerable to the onset of inbreeding depression and reduced genetic diversity (Charlesworth and Charlesworth 1999), both of which can lead to reduced fertility and, as a result, reduced population recruitment (Edmands 2007). Inbreeding depression can result in rare mutations becoming homozygous, reduced allelic diversity and a reduction in the adaptive capacity to environmental stressors (Newman and Pilson 1997; Weeks *et al.* 2011; McLaughlin *et al.* 2002; Eizaguirre *et al.* 2009). An increase in population size, through population reinforcement (Table 2), could be a potential solution to these issues as new genetic material would be introduced to the population and contribute toward an increase in genetic diversity.

Population reinforcements (or ‘augmentations’) are defined as “the intentional movement and release of an organism into an existing population of conspecifics” (IUCN 2016) and can affect offspring fitness in two ways. The offspring could exhibit higher fitness than their parents (‘heterosis’) due to increased genetic diversity, which is usually seen in the F1 generation but is not usually permanent (Rius and Darling 2014). On the other hand, they could exhibit reduced fitness (‘outbreeding depression’) resulting in diminished survival and reproduction, although outbreeding depression may take 2-3 generations to be detected and has also been found to be temporary (Frankham *et al.* 2011; Frankham 2016, 2015; Tallmon *et al.* 2004; Edmands 2007). It is important to point out here that studies of outbreeding depression are often based on intraspecific crosses between adaptively divergent populations (Goto *et al.* 2011; Erickson and Fenster 2006) rather than outbreeding between conspecific populations of a single species.

Therefore, the fitness of the F1 generation can be positive or negative depending on whether the effect of hybrid vigour outweighs the negative effects associated with the loss of beneficial genetic local adaptations (Tallmon *et al.* 2004). Weeks et al., (2011) advocated the creation of genetically diverse source populations, through strategic mixing of populations in order to maximise the genetic diversity of the founding population and ensure their adaptive potential to future climatic stochasticity. The risks of altered offspring fitness are reduced when mixing genetically similar populations due to the probable absence of locally adapted traits (Raabová *et al.* 2009). In addition to reinforcements, other movements pose their own genetic risks. Relocating animals to entirely new sites may result in exposure to novel pathogens, a lack of prior exposure leading to increased susceptibility to disease, as well as being vulnerable to a lack of adaptation to new environmental conditions (Ward 2006; Storfer 1999; Allendorf *et al.* 2001; Hall and Willis 2006).

History of reptile translocations

Many of the published translocations were conducted on bird or mammal species as evidenced in reviews such as those by Griffith et al., (1989), Fischer and Lindenmayer (2000), Short (2009) and Sheean et al., (2012) (Table 3). Many reptile translocations have been conducted using tortoises (Pille *et al.* 2018), whose strong homing behaviours present an interesting challenge in translocations, with few translocations conducted on lizards. In addition to published reptile translocations being in fairly short supply there is an additional bias in the literature for successful translocations, failed translocations being less likely to get published (Miller *et al.* 2014). This publication bias prevents conservation managers avoiding previously identified issues, slowing progress in this field. Translocation failures are just as valuable as the successes as they identify potential and highlight the next steps needed to uncover a more optimal translocation strategy.

The reviews listed here illustrate a pattern of variable success in translocations and inconsistency in the criteria used to determine translocation success.

Table 3: Cross-taxa translocation reviews which show a lower success rate for reptile translocations than those of birds or mammals

Citation	**Animal group (% success rate)**	**Success rate**	**Cause of failure**	**Country**
(Griffith *et al.* 1989)	Birds & Mammals	Threatened - 44%, Native game sp - 86%	Habitat quality/ Captive or wild bred	World-wide (1973-1986)
(Dodd and Seigel 1991)	Amphibian & Reptile	45%	Dispersal, predation, habitat unsuitability	World-wide (1970's & 80's)
(Fischer and Lindenmayer 2000)	Birds, Mammals	26%	Captive or wild-bred	World-wide (1979-1998)
(Rummel *et al.* 2016)	Mammals, Birds, Reptiles, Amphibians		Program duration, No. of release animals, Captive or wild-bred	Spanish Mediterranean regions (1980's - 2013)
(Short 2009)	Mammals, Birds, Reptiles, Amphibians	Mammals (62%), Birds (38%), Reptiles (33%), Amphibians (10%)	Predation caused 80% of failures	Australia
(Germano and Bishop 2009)	Amphibian & Reptile	41%	Dispersal, predation, habitat unsuitability	World-wide (1991-2006)
(Sheean *et al.* 2012)	Plants, Mammals, Birds, Amphibians, Fish, Invertebrates	46%	Predation & habitat unsuitability	Australia (1983 - 2009)
(Romijn and Hartley 2016)	Reptile (lizards)	33%	Predation	New Zealand (1988-2013)

In a time of advancing climate change and habitat degradation, species ranges are shifting as they are forced to find new and more favourable areas to inhabit (Loss *et al.* 2011). However, some species cannot move adapt or disperse quickly enough (Besson *et al.* 2011) and, as a result, biodiversity in those areas is affected and species rely on human-mediated movements to persist. As discussed in the "Risks of conservation translocations" section of this Introduction, the way in which a translocation is conducted will affect the likelihood of success. Reptiles are a diverse class of animals and include a wide range of groups including snakes, crocodiles, lizards and tortoises. Each of these groups will have their own behavioural response to movement and, as such, strategies used differ among the published translocation attempts (Table 4).

Table 4: Examples of reptile translocations conducted over the past 20 years

Year	**Citation**	**Species**	**Outcome**	
1999	(Platenberg and Griffiths 1999)	Slow worms (*Anguis fragilis*)	Failure	Mitigation translocation to locally unoccupied site. Habitat augmentation pre-release. Declining capture rates and no evidence of reproduction over the 2yrs post-release suggesting failure of translocation
2002	(Nelson *et al.* 2002)	Tuatara (*Sphenodon guntheri*)	Success	Wild adults & captive juveniles translocated to a predator free island. Used a female biased sex ratio and habitat augmentation
2005	(Butler *et al.* 2005)	Tigersnake (*Notechis scutatus*)	~	Population reinforcement for research purposes - no measure of success. Translocated snakes exhibited home ranges 6 times that of resident snakes
2011	(Christie *et al.* 2011)	Napoleons skink (*Egernia napoleonis*)	Success	Experimental reintroduction using wild caught adults into previously mined/restored habitat and unmined habitat. All surviving skinks moved into unmined habitat
2012	(Hare *et al.* 2012)	Otago skinks (*Oligosoma otagense*)	Success	Reintroduced to a mammalian-predator free sanctuary (avian predation still possible)
2014	(McCoy *et al.* 2014)	Sand lizards (*Plestiodon reynoldsi*)	Success	Mitigation translocation (Introduction) to unoccupied site. Habitat augmentation experimental treatments showed that heterogeneity of available habitat is important

Reducing dispersal

Dodd and Seigel (1991) cited dispersal and poor quality habitat as being the main contributing factors to failure which was reinforced by Germano and Bishop (2009) who additionally highlighted dispersal away from the release site as being a prominent cause of failure (Rittenhouse *et al.* 2009). High dispersal activity has been reported for many species including: the Florida sand skink (McCoy *et al.* 2014); three toed box turtles (Rittenhouse *et al.* 2009) and jewelled geckos (Knox and Monks 2014). Habitat augmentation (Ebrahimi and Bull 2014; Milne, Bull, *et al.* 2003; Kyek *et al.* 2007; Goldstein *et al.* 2018) and supplementary feeding (Ebrahimi and Bull 2012; Hardman and Moro 2006) are strategies that have been shown to reduce dispersal behaviours post-translocation in addition to temporary penning in soft-release enclosures (Ebrahimi and Bull 2014; Attum and Cutshall 2015; Knox and Monks 2014).

Sedentary vs mobile species

Translocation of more mobile species presents a more challenging issue, especially in the case of migratory species or species with large territories. Migratory species move through multiple habitat types each year which presents a more complex issue in terms of translocation release strategies and protection of important habitat (Orloff 2011). Additionally, time of year was an important factor in the reduction of dispersal following translocation especially for hibernating or brumating species (Ebrahimi, Ebrahimie, *et al.* 2015). Interestingly, in a population reinforcement of Hermann tortoises (*Testudo hermanni hermanni*) individuals confined in soft-release enclosures for a mean of seven years still exhibited high dispersal behaviours upon release (Pille *et al.* 2018). It took 1-2 years to establish in the new location, an area of favourable habitat, suggesting that releasing into high quality habitat alone may not be enough to dampen dispersal behaviours for highly mobile species.

Wild vs captive sourced animals

In an early review of translocations, Griffith et al., (1989) did not comment on reptiles but did highlight that translocations using wild caught birds and mammals had twice the success than using captive-reared individuals. However, Rumer et al., (2016) found that translocation success was higher for reptiles when using captive-reared individuals, in terms of establishment and reproduction, possibly due to their higher body weight and faster growth rate in captivity which would reduce their vulnerability to predation and transition into the wild. Captive head-started green smooth snakes (*Opheodrys vernalis*) did not benefit from soft-release confinement and their dispersal behaviours were similar to those of head started conspecifics (Sacerdote-Velat *et al.* 2014). However, snakes aggregate for breeding and so release in an area with other conspecifics may have contributed toward the high level of site fidelity seen in this study. Santos et al., (2009) found that captive-born lacertids, *Psammodromus algirus*, dispersed more than the native lizards but they were also larger and had better survival. However, the high activity levels of the captive-born individuals were actually viewed as beneficial as those individuals could colonise nearby unoccupied patches of habitat, thus expanding the population range. Head-started juvenile tuatara were behaviourally similar to wild juveniles in terms of landscape use and selected retreat sites indicating that head-starting could be a useful management strategy as the juveniles would benefit from higher condition when released in addition to having the necessary ‘wild’ behaviours (Jarvie *et al.* 2016).

In order to persist, a population must first be able to successfully establish at the site which requires research into ways to minimise dispersal away from the release site. Secondly, in order to achieve population growth, we must identify factors maximising reproductive success in the target species. And thirdly, in order to assess whether the population is self-regulating through mechanisms such as immigration, emigration, births and deaths we must spend an adequate amount of time monitoring population demographics post-translocation (Bell and Herbert 2017). Success in any of these steps depends upon having extensive species knowledge to highlight critical gaps that additional research can address to increase translocation success when it is attempted (Baling *et al.* 2013). The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is an ideal study species due to the comprehensive species knowledge accumulated during 30 years intensive research into its biology, ecology and behaviour including its behavioural response to relocation. With strategies to limit dispersal post-translocation already identified, we are now able to examine other factors that could affect the success of translocations in this species.

STUDY SPECIES, THE PYGMY BLUETONGUE LIZARD (*TILIQUA ADELAIDENSIS)*

Background

The smallest of the *Tiliqua* genus, the pygmy bluetongue lizard (*Tiliqua adelaidensis*) is a medium sized skink endemic to South Australia. It was thought extinct until rediscovery in 1992 (Armstrong *et al.* 1993), after which extensive research was conducted into aspects of its life history and ecology. Approximately 34 populations have been found since the rediscovery of the species, on small remnant patches of native grassland on privately owned sheep-grazed farmland.

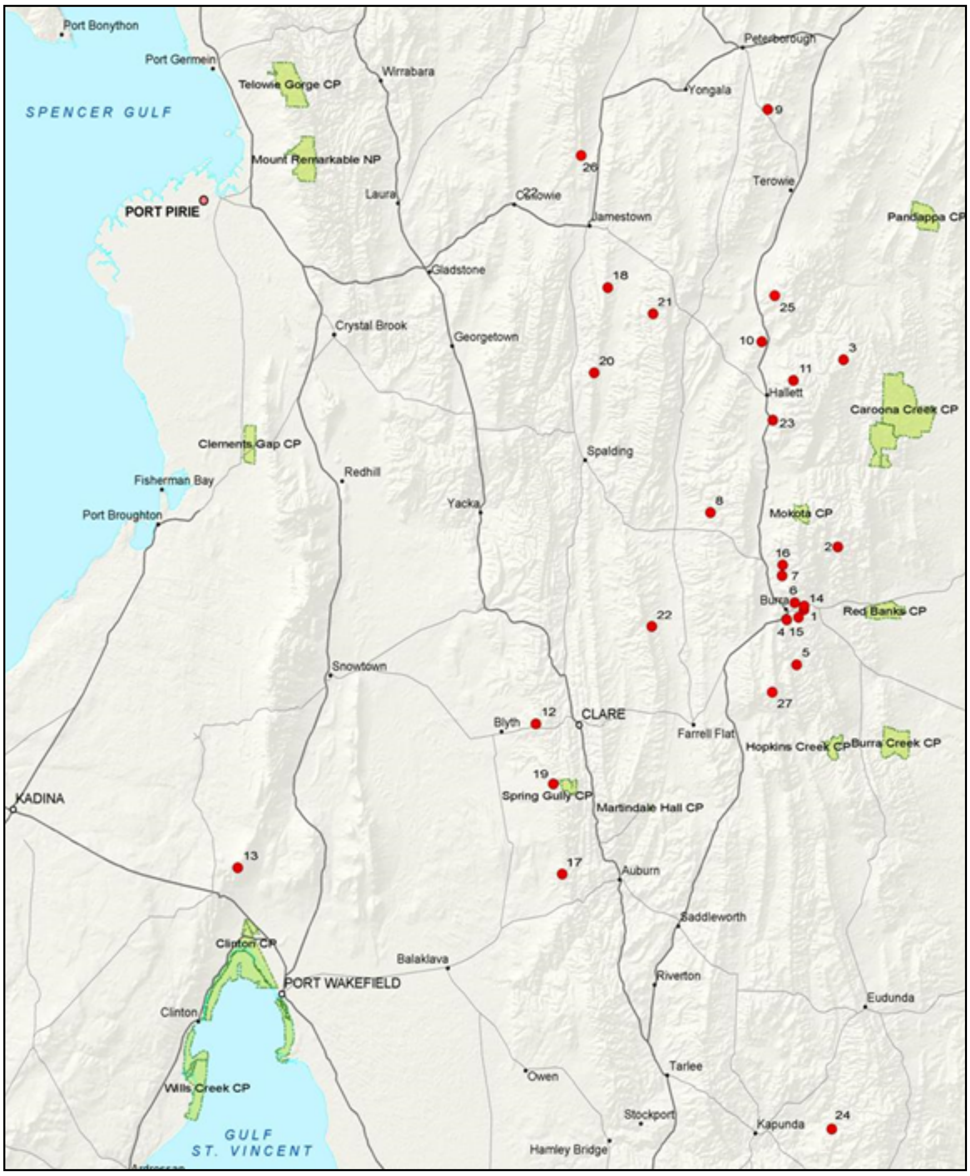

Figure 1: Map of known pygmy bluetongue populations (●) (2012)

Pygmy bluetongue lizards live in abandoned spider burrows built by various species of wolf and trapdoor spiders (Milne, Bull, *et al.* 2003; Clayton 2018). They do not actively maintain the burrows and are reliant upon the presence of spiders in the habitat to provide burrows as refuge. The lizards spend the majority of their time at the burrow entrance either basking or, being a sit and wait predator, using it as an ambush point to catch passing prey (Pettigrew and Bull 2014; Ebrahimi, Godfrey, *et al.* 2015a).

Figure 2: Pygmy bluetongue lizard basking at the entrance to its burrow

Although *Tiliqua adelaidensis* is a predominantly solitary species and individuals can be found in neighbouring burrows although they vigorously defend their burrow against conspecifics (Fenner and Bull 2011). Scat piles are also placed in the direction of the nearest occupied burrow as a social signal and chemical cue to notify nearby conspecifics of their presence (Fenner and Bull 2010). The species exhibits low vagility with individuals reported to only move an average of 27 m during mating season (Schofield *et al.* 2014). Low to moderate levels of genetic differentiation have been found resulting from a lack of gene flow between northern and southern populations (Smith *et al.* 2009) and all populations studied appear to contain high levels of allelic diversity. Pygmy bluetongue lizards exhibit a promiscuous mating system with male biased dispersal with females also having been shown to accept matings from multiple males in one season as evidenced by the detection of multiple paternity litters (Schofield *et al.* 2014, 2012). As yet, there has been little evidence of mate choice with mated pairs seeming to select partners irrespective of relatedness (Schofield *et al.* 2014) which has also been reported in the promiscuous grand skink, O*ligosoma grande* (Berry 2006). A lack of female mate choice is a generalised pattern in lizards (Olsson and Madsen 1995).

Populations have become isolated through agricultural practices such as ploughing of land for cropping which destroys burrows, kills their inhabitants and leaves any surviving lizards vulnerable to predation (Hutchinson *et al.* 1994). Lizards do not disperse across ploughed land which leads to populations becoming stranded within habitat surrounded by ploughed land (Ebrahimi and Bull 2015). Habitat suitability modelling, with respect to predicted climatic changes, highlighted the likelihood that climate change would alter the structure and composition of the native grasslands to such an extent that long-term persistence of the species would require the use of managed relocations (Fordham *et al.* 2012). Availability of suitable habitat was predicted to decline more quickly for the northern populations, leading to the need for research examining the risks associated with relocating animals from the northern populations further south toward the centre of the species current range.

Ecological risks for *Tiliqua adelaidensis*

Pelgrim et al., (2014) assessed the dynamics of the co-occurring reptile community at known pygmy bluetongue populations in order to identify the species that co-existed in the immediate area and identify any potential species that may compete with *Tiliqua adelaidensis* for prey. Comparing 13 different species they only found one species with a comparable gape size to *T. adelaidensis* but due to considerable body size differences between the two species it was concluded that there was little scope for competition between the species in terms of targeted prey species. In addition, Ebrahimi et al., (2015b) studied 16,000 hours of video footage to determine whether *T. adelaidensis* interacted with any of the previously identified co-existing reptile species in the local ecosystem, which they did not. Based on these findings it was deemed that increasing the density of pygmy bluetongue lizards through population reinforcement would be unlikely to have a negative impact on the sympatric reptile species in the ecological community at the recipient site. However, only an assessment of an actual population augmentation would provide the necessary evidence to support this hypothesis. Ricciardi and Simberloff (2009) suggest that the risks posed to biodiversity at the recipient site cannot be fully anticipated, although I argue that experiments such as those laid out in this thesis do exactly that and provide an important tool for further development of such pre-emptive investigative studies.

Genetic risks for *Tiliqua adelaidensis*

Integration of new, or 'foreign', genetic material into a population can be described as invasive if it swamps the genetic material of the recipient population (Champagnon *et al.* 2012). Although low to moderate levels of genetic differentiation have been detected between populations of *T. adelaidensis* (Smith *et al.* 2009), no fixed chromosomal differences are expected due to moderate mtDNA differences among populations (Schofield 2015) which indicates that populations of this species remain conspecific with population reinforcement unlikely to result in outbreeding depression. Maintenance of high heterozygosity by a promiscuous mating system may explain why inbreeding depression has not been detected in the species (Smith *et al.* 2009), which combats the impact of minimal dispersal activity exhibited by the species.

Translocation as a conservation strategy for pygmy bluetongue lizards

Since translocation was identified as the optimal conservation strategy for the pygmy bluetongue lizard, research has been conducted to examine the behavioural response elicited by moving individuals to a novel area (Ebrahimi, Ebrahimie, *et al.* 2015). Dispersal away from the release site has been highlighted as a major contributing factor to reptile translocation failure (Germano and Bishop 2009) as well as habitat quality at release site and source of translocated individuals i.e..

captive or wild caught (Dodd and Seigel 1991). A translocation simulation experiment, using wild caught lizards housed captively, used decision-making analysis (Ebrahimi, Ebrahimie, *et al.* 2015) to show that dispersal of *Tiliqua adelaidensis* individuals can be reduced by: short term confinement at the release site, i.e. a soft-release (Bull and Ebrahimi 2013); habitat augmentation (Ebrahimi and Bull 2014; Milne and Bull 2000; Souter *et al.* 2004; Milne, Bull, *et al.* 2003); supplementary feeding (Ebrahimi and Bull 2012) and translocating adults in later summer before torpor (Ebrahimi and Bull 2014).

In order to build upon the findings of the captive translocation simulation, the crucial next step was to conduct an *in-situ* experimental translocation, using wild conspecifics from existing populations in order to more comprehensively assess the potential risks of such an action, in a controlled setting. From this point on, when I use the term 'translocation' I specifically refer to population reinforcement, also known as population augmentation or supplementation.

CONCLUSION AND SCOPE OF THIS THESIS

The overall aim of this thesis is to examine two main categories of risk associated with population reinforcement: the risk to the wider ecological lizard community and the genetic risk of mixing individuals from genetically differentiated conspecific populations. This will be achieved through monitoring of conspecifics, and sympatric species, pre and post translocation in both experimental and control enclosures as well as the housing and monitoring growth and performance of offspring produced post translocation. We put forward bite force as an ecologically relevant measure of whole-organism performance. We assessed the suitability of bite force to reflect body condition which has been widely used to indicate, as an average, population health.

The four main aims of this thesis are:

1. To assess the impact of population reinforcement on the body condition and abundance of resident sympatric species at the release site. *Rationale: A risk associated with reinforcements is that increasing the population density of one species could negatively impact other resident species at the recipient site through increased competition for food or refugia. It is important to identify potential negative impacts in an experimental setting before reinforcements are conducted in wild populations.*
2. To investigate whether bite force was a good indicator of whole-organism performance. *Rationale: Many published studies have highlighted the ecological relevance of bite force in allowing animals to acquire larger prey items (ecologically relevant as that animal may experience better success at energetically demanding tasks than rivals). Establishing whether bite force was an honest signal of an individuals body condition allows us to comment on the potential health of that individual, reflected by its body condition.*
3. To establish whether weapon size or performance (i.e. bite force) was associated with individual reproductive success. *Rationale: Identifying factors maximising reproductive success of endangered species can impact the success of conservation efforts. The study species occurs in many small, isolated populations and is vulnerable to stochastic events. Therefore, identifying a link between reproductive success and physical size (weapon size) or ability (bite force) could aid captive breeding of the species and long-term conservation efforts.*
4. To assess whether admixed offspring grew or performed differently compared to unmixed offspring. *Rationale: Before you can conserve species through reinforcement of wild populations, you must be able to empirically show regulatory government bodies (such as Department for Environment and Water, DEW) that mixing populations will not negatively impact on the fitness of the resulting mixed-lineage*

offspring. This chapter serves this purpose and provides empirical data relating to the mixing of three populations.

This thesis is intended to fill gaps in the current knowledge about the impact of population reinforcement on co-existing species at the recipient site. Population augmentation has never been attempted in this species and even though we anticipate the likelihood of such deleterious effects to be low we must test it empirically in a wild population to truly assess the impact of this conservation strategy. While efforts are focused on conserving a focal species it is important to ensure we do not disrupt the balance of the wider ecosystem as a result, nor negatively impact the fitness of the offspring produced after the translocation (Moehrenschlager *et al.* 2013).

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CHAPTER 2

ECOLOGICAL RISKS

A sleepy lizard (*Tiliqua rugosa*) gaping as a threat display: one of the co-occurring species

PREFACE

Clive, LFR., Gardner, MG., Clayton, JL., Baring, R., Hutchinson, MN., Fenner, A. and Bull, CM. (2019) Population augmentation had no effect on the abundance or body condition of conspecifics and co-occurring lizard species in a native grassland community. Austral Ecology, doi: 10.1111/aec.12704

This chapter (Chapter 2) examines the effect of population reinforcement on the sympatric lizard species at the release site and contributes to a gap in the literature regarding this topic. Native grasslands are a rapidly declining habitat (Hyde 1995) and habitat suitability modelling has shown that persistence of pygmy bluetongues will rely on managed relocations due to the effect of climate change (Fordham *et al.* 2012). Habitat suitability has been predicted to decline most rapidly for northern areas, so conservation research has focussed on potential risks associated with relocation of individuals from northern populations further south within the existing species distribution. While the focus of this management strategy is to conserve the pygmy bluetongue lizard, it is important to ensure that it does not impact the rest of the ecological community at the release site. Many studies focus on the survival and reproduction of the focal species receiving the translocation but very few, if any, consider the co-existing species at the release site that may be affected by this conservation action. My study builds upon previous research identifying the co-existing reptile species (Pelgrim *et al.* 2014) and the level of interaction between those species and the pygmy bluetongue lizard (Ebrahimi, Godfrey, *et al.* 2015b).

POPULATION AUGMENTATION HAD NO EFFECT ON THE ABUNDANCE OR BODY CONDITION OF CONSPECIFICS AND CO-OCCURRING LIZARD SPECIES IN A NATIVE GRASSLAND COMMUNITY

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ABSTRACT

Wildlife translocations have been historically plagued by failure. As more species gain endangered status, we must increase the success rate through use of pre-emptive empirical, evidence-based research. The main ecological risk is the potential for the relocated individuals to have competitive advantage at the recipient site, acting invasively and, potentially, outcompeting native fauna for food, shelter or other resources. Here we investigated the ecological risk of increased population density, following a population augmentation of the pygmy bluetongue lizard (*Tiliqua adelaidensis)* an endangered lizard, endemic to South Australia. To ascertain if a sudden increase in *T. adelaidensis* density would negatively affect the abundance or body condition of the resident conspecifics and co-existing lizard species at the recipient site, twenty-four individuals, from two populations, were relocated into previously built enclosures at a recipient site in grassland habitat, north of Adelaide, South Australia. For one activity-season pre-and-post, the augmentation, co-existing lizard species were sampled in an effort to detect any changes in abundance or body condition. Comparisons were also made between experimental enclosures, containing residents and translocated individuals, and control enclosures, containing only residents. Using *Menetia greyii* as a proxy for all of the competing species, our results show no reduction in abundance or body condition post-augmentation. Our finding that there was no negative impact of the *T. adelaidensis* translocation on the body condition and abundance of the resident lizard species is a positive outcome for future conservation of this species. This study provides a new way of approaching wildlife movements, through identification of potential risks using small-scale translocations in an enclosed area before conducting large-scale releases into unfenced areas. The results of this study are intended to facilitate higher translocation success rates and limit the negative effects upon the wider ecological community at recipient sites.

INTRODUCTION

Translocations, as a conservation strategy, have increased in popularity over the past few decades (Germano *et al.* 2014), however, they have also been plagued by a high rate of failure. Regular reviews of translocation outcomes are noticeably absent in current literature, however, of the reviews published, success rates are found to be low for birds and mammals, (44% Griffith *et al.* 1989), and herpetofauna, (19% - Dodd and Seigel 1991; 33% Romijn and Hartley 2016: 41% Germano and Bishop 2009; 51% Miskelly and Powlesland 2013). Specifically in the case of herpetofaunal translocations, the rate of success doubled in the 17 years between reviews by Dodd & Seigel (1991) and Germano & Bishop (2009)(Sheean *et al.* 2012). It is important to note, however, that translocations not reported as successful may not necessarily be classified as a failure, assessment of the outcome may be ongoing. Another important consideration is the lack of consistent criteria for evaluation of translocation success between publications, making it difficult to confidently analyse translocation success rates over recent years. Historically, translocations were analysed retrospectively, limiting potential for uncovering key factors influencing their success or failure (Seddon *et al.* 2007; Knox and Monks 2014); translocations often having been seen as a last resort rather than a powerful tool requiring extensive planning and foresight (Chauvenet *et al.* 2013). While some authors have suggested an inability of researchers to be accurate in measuring the risks associated with wildlife movements (Cunningham 1996) others have, more recently, noted an increase in monitoring targeted to addressing *a priori* questions highlighting a shift in the way translocation science research is conducted (Germano *et al.* 2014; Armstrong and Seddon 2008; Sheean *et al.* 2012). In light of such a high historic rate of failure, with increasing numbers of species acquiring endangered status (Czech and Krausman 1997; Jetz *et al.* 2007; Pimm and Raven 2000), there is a growing need to identify potential risks before carrying out wildlife movements so as to avoid negatively impacting both the species being conserved, but also the wider ecological community at the release site.

In this study, we use the term translocation to discuss the movement of individuals between populations even though a more specific, and accurate, definition would be population ‘reinforcement, augmentation or supplementation’. The literature regularly uses the broader term translocation when discussing the relocation of individuals or populations and we continue this theme in this paper (Sheean *et al.* 2012; IUCN 2013).There are many risks associated with movement of organisms including pathogen risk (Kock *et al.* 2010; Cunningham 1996); genetic risk (Johnson 2000; Jamieson and Lacy 2012; Keller *et al.* 2012; Frankham *et al.* 2011); and ecological risks (Jeffries and Lawton 1984; Kaspersson *et al.* 2010; Frair *et al.* 2007). A key ecological risk is that the translocated species could begin acting invasively at the recipient site (Ricciardi and Simberloff 2009) and, while this risk is low (Mueller and Hellmann 2008), possible negative outcomes include rapid growth in population numbers resulting in a negative impact on co-existing species at the recipient site (Ricciardi and Simberloff 2009). Co-existing species could be negatively impacted through an increase in competition for resources, such as shelter, food and mates (Brown 1964; Jeffries and Lawton 1984; Langkilde and Shine 2004; Sanchez-Zapata *et al.* 2007). The likelihood of invasive behaviour occurring would depend on the composition of the community at the recipient site and the presence of ecological overlaps between resident species (Chauvenet *et al.* 2013). There is a lack of empirical literature concerning the ecological impact of wildlife movements, specifically at the site of release, which this study hopes to address and potentially motivate other researchers to follow suit.

Study species

The pygmy bluetongue lizard (*Tiliqua adelaidensis),* is a predominantly solitary, medium sized skink, endemic to South Australia that was once thought extinct until its rediscovery in 1992 ; now classified as endangered (Hutchinson *et al.* 1994). *Tiliqua adelaidensis* are found in small fragments of native grassland; a rapidly declining habitat currently at 0.33% of its original distribution (Hyde 1995). They shelter in burrows built by various species of mygalomorph and lycosid spiders, relying on these burrows for protection against predation and thermal extremes as well as an ambush site from which to capture passing prey (Hutchinson *et al.* 1994; Milne and Bull 2000).

Fordham *et al*. (2012) highlighted the need for managed relocations of *T. adelaidensis* to ensure future sustainability of the species, through climatic modelling and, as a result, research efforts were focussed on identifying the optimal release conditions to increase the chance of a successful translocation (Bull and Ebrahimi 2013; Ebrahimi and Bull 2012; Ebrahimi and Bull 2014; Schofield *et al.* 2012). In-situ research of an existing population found no evidence to suggest competition between *T. adelaidensis* and co-existing reptile species (Pelgrim *et al.* 2014) nor evidence of interactions between *T. adelaidensis* and those co-existing reptile species (Ebrahimi, Godfrey, *et al.* 2015b). However, these conclusions were based on natural patterns of presence and absence of a similar suite of species occurring with *T. adelaidensis* across its range. The effects, if any, of a sudden increase in its density through a translocation, are as yet unstudied.

This study aimed to investigate whether body condition was a good indicator of bite force as an indirect measure of population health through assessing the impact of increased population density of *T. adelaidensis* on the body condition and abundance of resident *T. adelaidensis* conspecifics along with the sympatric reptile species at the recipient site. This was achieved through monthly pre-and-post treatment monitoring of the co-existing species, at a site within a thriving pygmy bluetongue population using a before-after control-impact (BACI) design

(McDonald *et al.* 2000; Eggleston *et al.* 2008). We hypothesised that there would be no adverse effect of the increased *T. adelaidensis* density on the body condition or abundance of any co-existing lizard species sampled. While we cannot directly discuss the impact upon the fitness of the co-existing species, we use this approach to examine fitness metrics, such as body condition, as indirect indicators of body health as a proxy for fitness (Bonnet and Naulleau, 1994; Nagy et al, 2002 both cited by Shamiminoori *et al.* 2014).

METHODS

The study was conducted over two consecutive Austral spring/summer periods (Oct – Feb) of 2015 - 2017; the study site being the Nature Foundation 'Tiliqua' site just outside the town of Burra in the mid north of South Australia. Six enclosures (3 pairs of 30 x 30m enclosures) were constructed at the field site, surrounding an existing population (resident *T. adelaidensis*), during the winter prior to data collection to prevent escape of lizards during the study.

A set of enclosures at the field-site, experimental and control sides sharing an adjoining wall

The walls, made from lengths of sheet metal, were 20cm high with a 5cm lip facing inwards at a 45-degree angle, and buried 10cm into the ground. The enclosure fences were high enough to ensure that *T. adelaidensis* could not escape, but low enough to permit large synoptic reptile species, such as *Tiliqua rugosa* and *Pseudonaja textilis*, to move in and out of the enclosures. Each pair of enclosures consisted of a control enclosure (only resident lizards), and an adjoining experimental enclosure (resident and translocated lizards). Each enclosure contained a unique *T. adelaidensis* density, as is reflected naturally in wild populations due to the patchy, uneven, distribution of spider burrows. This remained unaltered to provide a clearer understanding of the effects of translocation on a naturally occurring population. Enclosures were monitored monthly to monitor resident *T. adelaidensis* and co-existing reptile species naturally occurring within control and experimental enclosures.

Enclosure wall made of galvanised steel with an inward facing lip

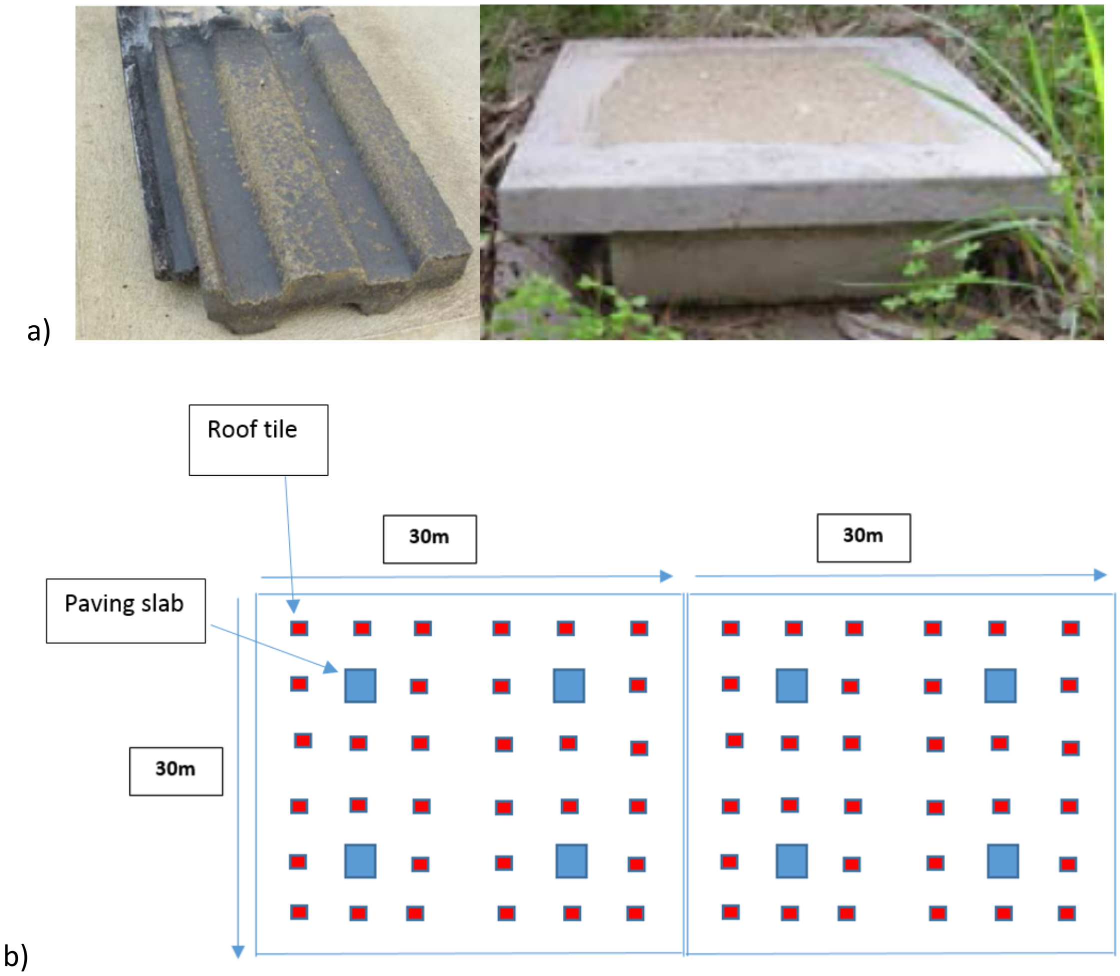
Both experimental (n=3) and control (n=3) enclosures were each augmented with 12 artificial burrows made from wooden dowel, 300mm long x 32mm wide with an internal diameter of 18mm, to ensure burrow availability did not affect the results of the experiment; high-quality burrows to a depth of 30cm are a limited resource in the wild (Clayton and Bull 2016). *Tiliqua adelaidensis* have been shown to readily use the artificial burrows (Souter *et al.* 2004; Milne and Bull 2000) and holes were dug to the appropriate depth using a mechanised auger with the wooden dowel being inserted and edges of the hole being filled in. Some co-existing reptile species have been sighted down spider burrows (L. Clive 2015/16, personal observation) and so by increasing the density of the high-quality burrows within the enclosures we ensured burrow availability was not a limiting factor of the study.

Pygmy bluetongues basking in a natural spider burrow (left) and an artificial burrow (right)

In February 2016, we collected 24 adult *T. adelaidensis* from two populations, one ~ 70km north and one ~ 30km west of the recipient site. Reproduction in *T. adelaidensis* involves mating in early spring, gestation of 1-4 embryos, with birth from mid-January to mid-February, hence collecting in late February enabled us to avoid capturing gravid females. To ensure sampling only sexually mature animals, all individuals had a SVL of 80mm or greater (Milne 1999), but sex ratio of the individuals could not be determined as external indicators of sex are not always apparent. In February 2016, at the recipient site, each experimental enclosure received eight *T. adelaidensis* lizards, four from each population, resulting in experimental enclosure *T. adelaidensis* density being increased by 53% - 73% (Table A1) while the control enclosures remained unaltered.

Sampling of *T. adelaidensis* at the recipient site occurred monthly with lizards being caught directly from their burrow using a previously described technique using a fishing rod and mealworm lure (Milne and Bull 2000). Upon capture, each *T. adelaidensis* individual was toe clipped, providing them with a unique ID and enabling collection of blood and tissue samples. Toe clipping is commonly used in herpetofaunal studies and has been shown to have no effect on movement speed (Borges-Landaez, 2003). Blood was collected onto Whatman® Classic and Elute FTA paper while toes were stored in vials of 70% ethanol and refrigerated. Body mass of *T. adelaidensis* was recorded using a 50g spring loaded Pesola scale while, for smaller sympatric species, a 10g spring loaded Pesola scale was used (to the nearest 0.05g). Body length, determined by snout-vent-length (SVL), was recorded using a transparent plastic ruler, to the nearest 1mm. Within each enclosure, each burrow had a unique burrow coordinate, allowing the pygmy bluetongue to be returned to the burrow it was caught from post-sampling.

Previously, 13 reptile species have been detected at multiple patches of native grassland, including our study site, which provided a predictive list of species that we expected to sample during this study (Pelgrim *et al.* 2014). Artificial cover objects were used to sample the lizard community by acting as a form of refuge. The artificial cover object's ability to heat up gradually, and hold that heat over time, also authentically represented naturally occurring refuges at the field site i.e. large rocks. Cement roof tiles, (L) 50cm (W) 25cm, and cement paving slabs raised at one end on a block of wood, (L) 50cm (W) 50cm (H) 10cm, were placed into the enclosures two months before sampling began to allow acclimatisation of the refugia as recommended by previous literature (Grant *et al.* 1992; Monti *et al.* 2000; Hampton 2007). Each enclosure contained 36 artificial cover objects: one paving slab surrounded by eight roof tiles at regular intervals in each of the four corners of the enclosure. Within each enclosure, each roof tile & paving slab was individually numbered to enable the return of individuals to the location they were sampled from. Through use of the numerous roof tiles, we ensured sampling of the smaller species such as *Menetia greyii*, whereas the larger paving slabs enabled sampling of the larger species such as the sleepy lizard, *Tiliqua rugosa*, or the common bluetongue, *Tiliqua scincoides*. We monitored each of the artificial cover object's at sunrise to ensure the lizards were cool, slow and easier to catch and each individual was then returned to the artificial cover object it was caught under. Sampling of the co-existing lizard species occurred each month in line with *T. adelaidensis* sampling (activity-season 1 Oct'15 – Feb'16; activity-season 2 Oct'16 –Feb'17) and capture effort was consistent throughout the study.



c) 

Artificial cover objects. a) Roof tile (left) and paving slab (right), b) diagram of positioning within each enclosure, c) photograph of tiles within the enclosures at the field site

Weather data

For the two seasons in this study, monthly maximum air temperature (^{o}C) and total monthly rainfall (mm), were recorded; activity-season 1 being hotter than average, with high temperatures being reached early in the season, and activity-season 2 being wetter than average with consistent rainfall each month (Figure A2). Air temperatures were recorded at Clare high school, the closest BOM weather station, 35.6km away from the field-site while rainfall data were collected from the bureau station at Burra community school, 0.3km away from the field-site (Commonwealth of Australia, Bureau of Meteorology, 2015-16).

Data analysis

Due to low sampling numbers of six of the seven species caught, analysis has focused on the most abundant species, *M. greyii*, with only descriptive statistics discussed for the other six species. Analysis in this paper is restricted to interspecific comparisons as we focus on the effect of increased density of *T. adelaidensis* density on the co-existing species in the immediate vicinity at the recipient site.

A body condition index was calculated by generating the residuals of mass against SVL and was only used for within species comparisons over time due to body size, and proportion differences between species. Treatment, experimental and control enclosures, was analysed over two consecutive activity-seasons with each activity-season containing five monthly sampling periods. Activity-season one (Oct'15-Feb'16) represented sampling pre-translocation, as the translocation was carried out in the second half of Feb'16 after sampling had been carried out, while activity-season two (Oct'16-Feb'17) was sampled post translocation. Analysis of these data for an interaction effect of activity-season and treatment enabled us to test for an effect of the increased *T. adelaidensis* density on abundance or body condition. Due to the non-normal nature of the data and the repeated sampling over time, we used a repeated measures PERMutational ANalysis Of VAriance (PERMANOVA) in the software PERMANOVA + version 1.0.6 add-on to PRIMER version 7.0.13 (Anderson *et al.* 2008). The experimental design consisted of four factors: activity-season (two levels: activity-season 1 pre-translocation; activity-season 2 post-translocation; fixed factor), month nested in activity-season (five levels: Oct – Feb; random factor), treatment (two levels: experimental & control; fixed factor) and enclosure number nested in treatment (six levels; random factor). We also included weather data (mean monthly temperature and total monthly rainfall) as covariates. Where necessary, we square root transformed these data to ensure they were normally distributed with PERMANOVAs done on Euclidean distance similarity matrices. Even though March is part of the overall activity-season, it was left out of the analysis to allow for an even balance of months pre and post translocation.

Due to low levels of sexual dimorphism in *T. adelaidensis*, we pooled males and females together in these analyses to prevent mis-identification of sexes which would affect the outcome of the analysis. As we didn't mark individuals of co-existing species, we have assumed in our analysis that the same individual may be captured multiple times and therefore we have used repeated measures PERMANOVA for our analyses. As the purpose of this study was to examine the effect of increased population density on the abundance and body condition of the co-existing species, for the purposes of analysis, we have pooled the resident *T. adelaidensis* together with those conspecifics translocated from the two source populations.

RESULTS

Abundance

Lizard community

At the beginning of spring 2016, following the translocation, a check showed that the majority of translocated *T. adelaidensis* individuals had survived the winter and were resident at the start of the breeding season.

In addition to the 118 pygmy bluetongue lizards sampled within the enclosures, a total of 445 captures, across seven reptile species, were made during the study (Table A2). A total of 216 artificial cover objects were sampled each month, with *M. greyii* being the most commonly caught (420 captures representing 94.4% of total captures) (Table A2). The second most commonly sampled species, *Morethia adelaidensis,* was sampled in 6 out of 10 months and accounted for 2.02% of total captures (Table A2). The other five species were infrequently caught, each with fewer than 6 captures during the 10-month study period (Table A2). There were 247 *M. greyii* sampled pre-translocation (Table A2), accounting for 97.2% of all captures during the pre-translocation period. Post translocation, there were 191 captures across 7 species, with *M. greyii* accounting for 90.6% of all captures. *Delma molleri*, *Lerista bougainvillii* and *Tiliqua scincoides* were only sampled in one of the two treatments and so they were excluded from our statistical analysis as we could not test for an effect of treatment (Table A3).

Menetia greyii

There was no significant interaction effect of activity-season and treatment, indicating abundance of *M. greyii* was unaffected by the translocation (Table 1). There was a significant main effect of month, with *M. greyii* being most abundant in December of each activity-season (Table 1, Figure 1).

Table 1. Results of repeated-measures PERMANOVA for effects of month, activity-season (pre or post translocation) and treatment (experimental or control treatments) on abundance of Menetia greyii. Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Activity-season	1	1.577	.276
Treatment	1	1.313	.279
Month	4	3.016	**.010**
Enclosure	4	0.731	.585
Activity-season*treatment	4	0.511	.792
Activity season * enclosure	1	1.269	.303
Month*treatment	4	0.867	.556

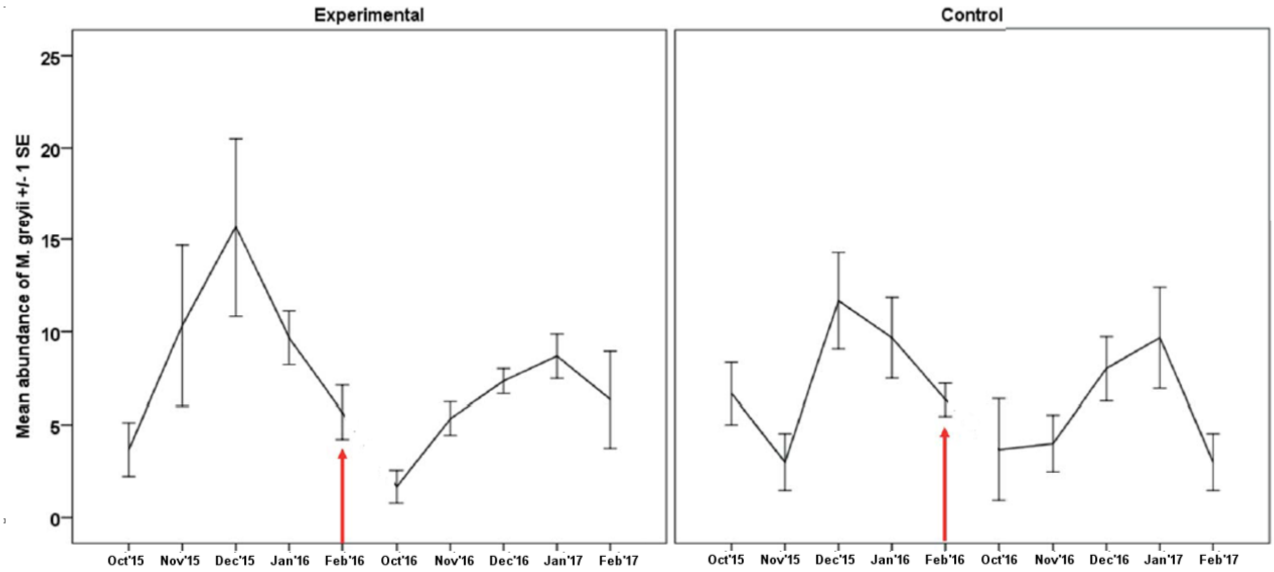


Figure. 1. Mean monthly abundance of Menetia greyii across all enclosures. Activity-season 1 (Oct'15 – Feb'16), Activity-season 2 (Oct'16 – Feb'17) split by treatment. Translocation occurred at the end of Feb'16 (post-data collection) as indicated by the arrow. Error bars represent +/- 1 standard error.

There was no main effect of treatment; *M. greyii* abundance not differing significantly between experiment and control enclosures (Table 1). There were also no significant interaction effects between month*activity-season, month*treatment, activity-season*treatment or month*activity-season*treatment (Table 1). When temperature was included as a covariate there was still a non-significant result for the interaction effect of season*treatment. The only significant effect was that of temperature on its own which fluctuated throughout the study (Table A7, Figure A2). When rainfall was added as a covariate the interaction effect of season*treatment remained non-significant but there was a main effect of month, with the volume of rain fluctuating throughout the study period (Table A8, Figure A2).

Body condition

Tiliqua adelaidensis

The mean adult pygmy bluetongue body condition index was higher than the other co-existing species sampled (Table A4). There was no significant interaction effect for activity-season*treatment, indicating no effect of translocation on the body condition of *T. adelaidensis* (Table 2).

Table 2. Results of repeated-measures PERMANOVA for effects of time (month), activity-season (pre or post translocation) and treatment (experimental or control enclosures) on body condition of Tiliqua adelaidensis. Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Activity season	1	0.454	.525
Treatment	1	4.419	.081
Month	8	6.406	**.0001**
Enclosure	4	2.916	**.021**
Activity season * treatment	1	0.805	.236
Activity season * enclosure	4	0.653	.633
Month * treatment	8	1.569	.127

The body condition of translocated lizards increased following their arrival at the recipient site, but not to the detriment of resident lizards within the experimental enclosures (Figure 2 and 4). There was a significant effect of month, with body condition declining throughout the first activity season and increasing throughout the second (Table 2, Figure 3).

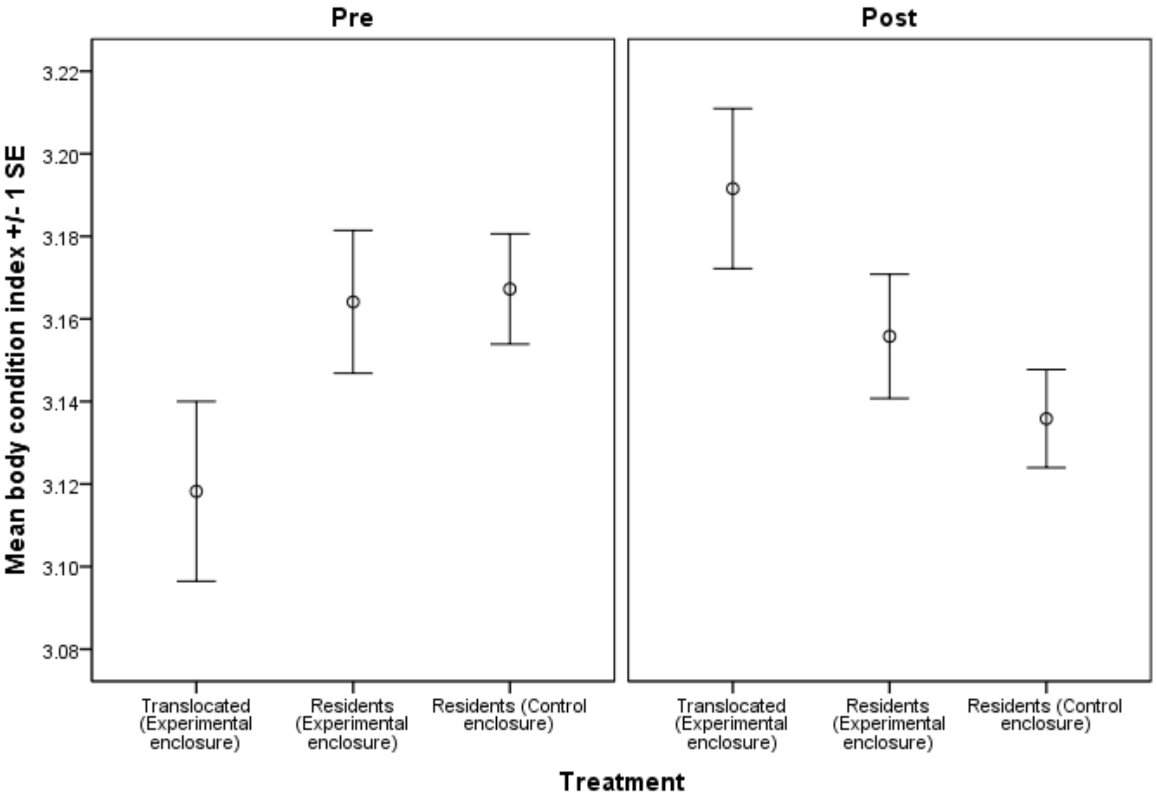


Figure 2. Mean body condition (BCI) of Tiliqua adelaidensis before and after augmentation. BCI of translocated lizards was recorded upon capture at source population before being introduced to enclosures at recipient site. Error bars represent +/- 1 standard error.

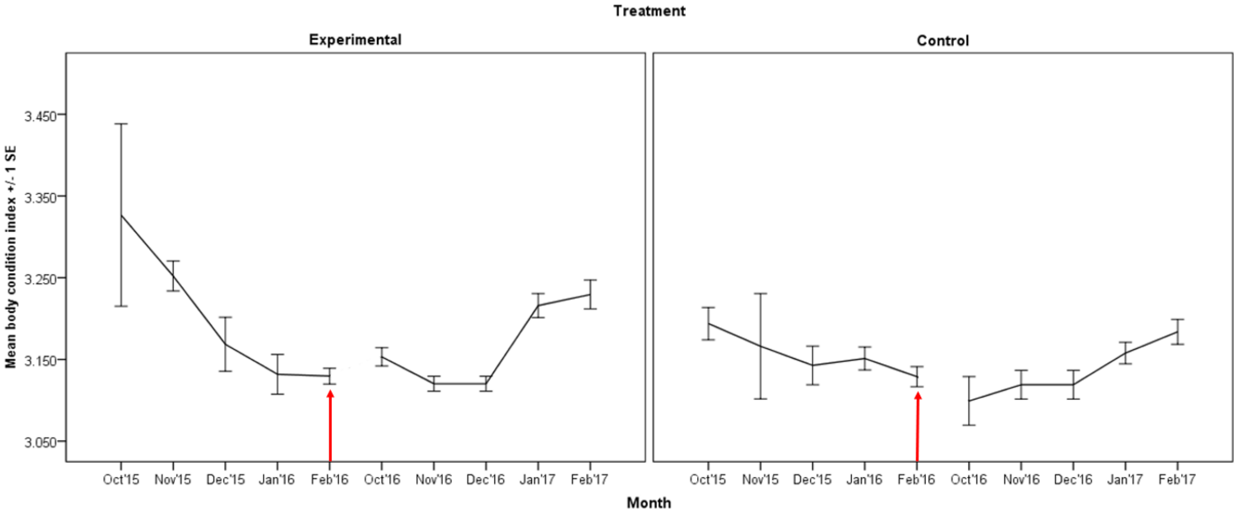


Figure 3. Mean body condition of Tiliqua adelaidensis. Activity-season 1 (Oct’15 – Feb’16), Activity-season 2 (Oct’16 – Feb’17). Translocation occurred at the end of Feb’16 (post-data collection) as indicated by the red arrow. Error bars represent +/- 1 standard error.

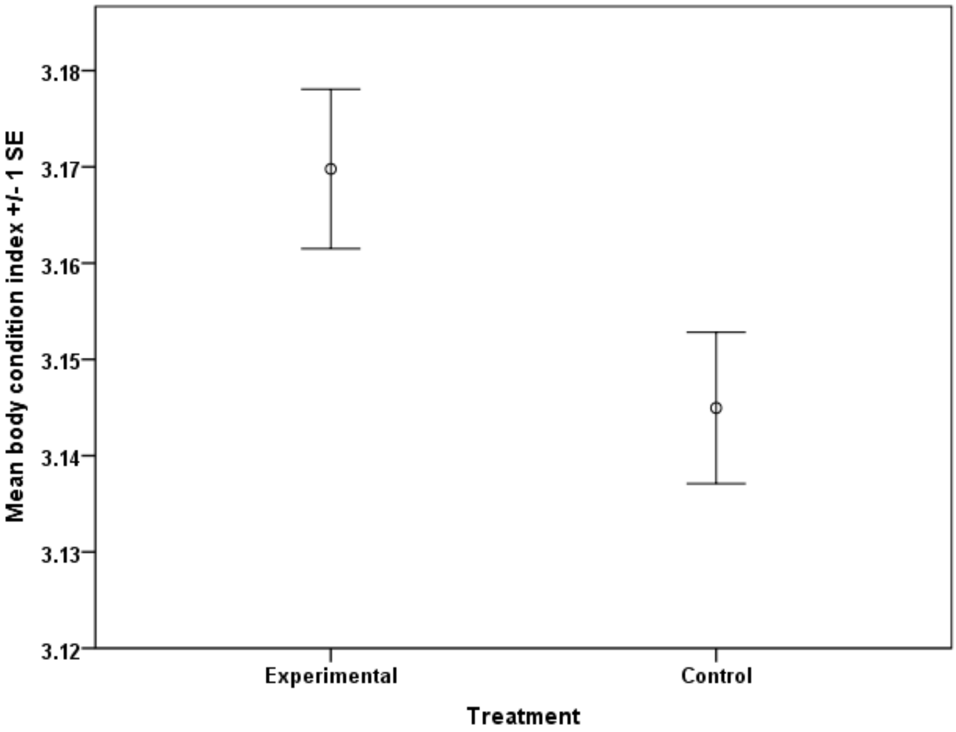


Figure 4. Mean body condition of *Tiliqua adelaidensis* split by treatment. Data includes body condition measurements over the two activity-seasons (activity season 1: Oct'15 – Feb'16, activity-season 2: Oct'16 – Feb'17). Error bars represent +/- 1 standard error.

There was a significant effect of enclosure, highlighting the variation in body condition between the enclosures over the entire study period. There was no effect of activity-season or treatment, nor were there significant interaction effects of activity-season*treatment, activity-season*enclosure or month*treatment. When temperature was included as a covariate, the results were unchanged as indicated by the non-significant interaction effect of activity-season*treatment although there was a significant effect of enclosure (Table A5). When rainfall was added as a covariate, all main effects and interaction effects were not significant (Table A6).

Menetia greyii

There was no significant interaction effect of activity-season*treatment indicating that *M. greyii* body condition was unaffected by the augmentation of *T. adelaidensis* individuals (Table 3). There was a significant main effect of month; *M. greyii* showing a loss of body condition during December, in both activity-seasons (Table 3, Figure 2).

Table 3. Results of repeated-measures PERMANOVA for effects of time (month), activity-season (pre or post translocation) and treatment (experimental or control enclosures) on body condition of Menetia greyii. Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Activity season	1	1.851	.251
Treatment	1	0.144	.719
Month	4	37.565	**.0001**
Enclosure	4	5.183	**.0006**
Activity-season*treatment	4	0.311	.477
Activity-season*enclosure	1	3.898	**.004**
Month*treatment	4	0.143	.997

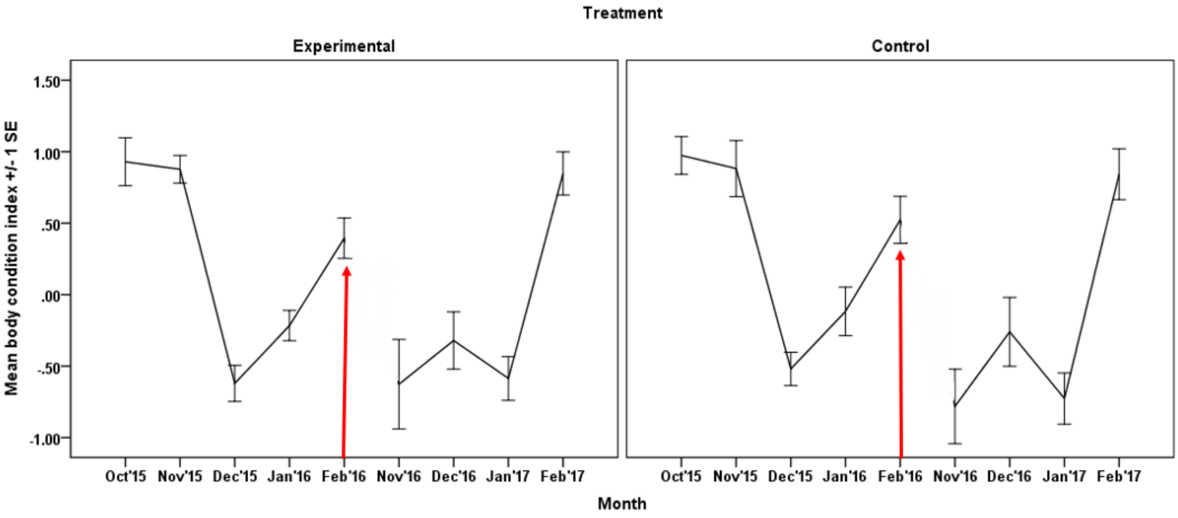


Figure 5. Mean monthly body condition index of Menetia greyii. Activity-season 1 (Oct'15 – Feb'16), Activity-season 2 (Oct'16 – Feb'17) split by treatment. Translocation occurred at the end of Feb'16 (post-data collection) as indicated by the red arrow. Error bars represent +/- 1 standard error.

There was also a significant main effect of enclosure, reflecting variation in body condition occurring between enclosures; in addition to a significant interaction effect of activity-season*enclosure (Table 3). When temperature and rainfall were added as covariates, there still was not a significant interaction of activity-season*treatment indicating that these environmental factors had no effect on our findings. However, there were significant effects of enclosure and an interaction effect of activity-season*enclosure (Table A9).

DISCUSSION

Our study aimed to assess the impact of an experimental translocation of the pygmy bluetongue lizard on the body condition, and abundance, of the resident conspecifics and co-existing reptile species at the recipient site where *Menetia greyii* were used to represent the other competing species within the grassland habitat due to the lack of data on the other species due to infrequent detection. There were two main findings in our study. First, there was no detectable impact on the body condition of resident *T. adelaidensis* within the experimental enclosures post translocation. Second, there was no negative effect on the body condition or abundance of coexisting species sampled within the study. The lack of an interaction effect between activity-season as shown by the lack of a significant activity-season*treatment interaction for all analyses. Body condition of *T. adelaidensis* was subject to monthly fluctuations (Figure 3), but not fluctuations between seasons; the lack of an interaction effect being important in highlighting a lack of post-translocation impact for the target species. Our findings show clear temporal patterns in both abundance and body condition of *M. greyii,* with a lower amplitude of those patterns in activity-season 2. Further research into the life history of *Menetia greyii* would be a valuable addition to the present study to more fully understand the ecological needs of the most abundant species in the native grasslands.

It is challenging to examine these results in the context of existing research due to the lack of empirical research in the literature. Our results are in accordance with the findings of both

Ebrahimi et al., (2015b) and Pelgrim et al., (2014) who respectively indicated a low probability of interaction or competition between *T. adelaidensis* and other sympatric species, potentially due to the low abundance of many of the co-existing species within the native grassland habitat. The lack of detectable impact of translocation on the reptile community may be due, in part, to the low vagility of pygmy bluetongues. The infrequency of dispersal, and *T. adelaidensis* reliance on spider burrows, may result in infrequent encounters between species due to differentiated niche use and, therefore, result in a low likelihood of interaction or competition. Additionally, the lack of regular movement exhibited by *T. adelaidensis* could indicate the species' capacity to withstand situations of relative crowding during years of high survivorship. Continued monitoring of *T. adelaidensis,* and its coexisting species, during periods of low prey abundance would help to confirm this hypothesis. However, competition over prey items is unlikely between these co-occurring species; research has shown *Tympanocryptis lineata* to possess a similar gape size but competition between species has previously been thought to be unlikely due to large differences in body size (Pelgrim *et al.* 2014). Future studies would need to ensure inclusion of sympatric species occupying more similar ecological niches, thus, identifying a stronger effect of the altered ecological community.

While this study did find a reduction in body condition of *M. greyii* post-translocation, this reduction was seen in both experimental and control enclosures indicating the causal factors were possibly external to the parameters examined in this study. The likelihood of locally translocated species, such as *T. adelaidensis*, behaving in an invasive manner at the release site was low due to the species already being present within that ecosystem (Mueller and Hellmann 2008). However, the results from the present study should be treated with caution due to the low sample sizes of co-existing species captured. Addressing this limitation would have required an increase in the sampling frequency per month although frequent disturbance of artificial cover objects can have lasting effects on the suitability of that refuge and actually result in a reduction of animals sampled perhaps suggesting the use of artificial cover objects for this study was not ideal (Pike *et al.* 2010). Disturbance has been shown to change the size and temperature of the crevice, both of which are key selection criteria for reptiles, reducing the quality of that habitat and, ultimately, resulting in those species becoming more susceptible to predation (Pike *et al.* 2010). An extended period of monitoring post-translocation would help to detect possible impacts of translocation over time. Howeverthe time frame of the study may not have been long enough to detect invasive behaviour especially when dealing with low sample sizes (Mehta *et al.* 2007). Dodd & Seigel (1991) highlight a lack of long-term monitoring in the available literature regarding the success or failure or herpetological translocations, the suggestion being that the length of monitoring should be species specific according to life-span, age of sexual maturity and time taken to raise one generation of progeny. While the latest review of herpetological translocation success shows an apparent increase in the number of translocation successes we must be mindful that this review was done almost a decade ago and so, more up to date information is required (Germano and Bishop 2009). The level of post-release monitoring has been reported to be increasing since this review was done, highlighting a comprehensive shift in attitudes during the past decade (Seddon *et al.* 2007; Germano *et al.* 2014).

This manuscript forms part of a larger, ongoing study using these enclosures, and the scope of this manuscript is restricted to assessing the effect upon only the sympatric squamate species. The results of our study are valuable to members of the conservation community looking to trial translocations through highlighting questions for future research surrounding the effects of wildlife movements on the wider ecosystem at the recipient site (Sheean *et al.* 2012). An understanding of factors such as the density of co-existing species and the trap-ability of those species is crucial before research can be conducted, as this study has shown.

Findings from small-scale translocations, such as this, can prove extremely valuable; increasing the chances of success when those movements are carried out on a larger scale, while protecting the wider ecosystem from potential risks. Translocations will, ideally, result in a self-sustaining population of (Griffith *et al.* 1989) while not at the detriment of the resident ecological community. Certain actions, such as translocation, may enable the conservation of specific species, but if the impacts of such actions are not considered in the broader environmental context, there can detrimental outcomes for other species within that ecosystem. Our study also demonstrates the synergy between research on a conservation ‘flagship’ species and the resulting contribution to the knowledge base on the co-existing species sampled in that ecosystem. This research shows how an anticipatory study of this kind can be devised, although it also highlights how complex, time consuming, and potentially impractical, it may be for a habitat of diverse fauna and large seasonal variations.

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APPENDICES

TABLES

Table A1. Number of *Tiliqua adelaidensis* (PBT) in the six enclosures pre & post translocation. Three experimental enclosures (2, 4 and 5) received 8 donor lizards each while control enclosures (1, 3 and 6) remained unaltered.

Enclosure	Original number of PBT	Translocated PBT's (Feb'16)	Final number of PBT	Increase in number of PBT
1	13	-	13	
2	15	8	23	53 %
3	21	-	21	
4	11	8	19	73%
5	14	8	22	57%
6	10	-	10	

Table A2. Summary of total *Tiliqua adelaidensis* and sympatric species captures throughout the two-consecutive activity- seasons. Activity-season 1: Oct'15 – Feb '16; activity-season 2: Oct'16 – Mar'17.

	Activity-season 1					Activity-season 2					
	Oct	Nov	Dec	Jan	Feb	Oct	Nov	Dec	Jan	Feb	**TOTAL**
Tiliqua adelaidensis (resident)	41	34	47	46	48	32	46	46	50	50	440
Tiliqua adelaidensis (translocatee)					24	11	14	14	14	16	93
Menetia greyii	31	40	82	58	36	16	28	46	55	28	420
Morethia adelaidensis	0	0	1	2	0	0	1	2	1	2	9
Delma molleri	1	1	0	0	0	2	1	0	0	0	5
Lerista bougainvillii	0	1	0	0	0	0	0	0	0	2	3
Aprasia pseudopuchella	0	0	0	0	0	2	0	0	0	0	2
Tiliqua rugosa	0	0	0	0	1	1	2	0	0	0	4
Tiliqua scincoides	0	0	0	0	0	0	2	0	0	0	2

Table A3. Mean captures (abundance) per enclosure of co-existing lizards in experimental and control enclosures (Treatment)

	Mean captures per enclosure (± 1 SD)	
Species	**Experimental**	**Control**
Menetia greyii	223 (± 5.15)	197 (± 4.17)
Morethia adelaidensis	3 (± 0.31)	6 (± 0.48)
Delma molleri	5 (± 0.38)	0
Lerista bougainvillii	3 (± 0.40)	0
Aprasia pseudopuchella	1 (± 0.18)	1 (± 0.18)
Tiliqua rugosa	2 (± 0.25)	2 (± 0.25)
Tiliqua scincoides	0	2 (± 0.25)

Table A4. Mean body condition index (BCI) of sympatric species in experimental and control enclosures (pre and post translocation) +/- 1 standard deviation.

	Mean BCI (± 1 SD)			
	Experimental		**Control**	
Species	**Pre**	**Post**	**Pre**	**Post**
Tiliqua adelaidensis (resident)	0.137 (± 0.029)	0.140 (± 0.030)	0.137 (± 0.03)	0.137 (± 0.030)
Tiliqua adelaidensis (translocated)	0.128 (± 0.0238)	0.155 (± 0.028)		
Menetia greyii	0.021 (± 0.0067)	0.021 (± 0.0089)	0.021 (± 0.0065)	0.021 (± 0.0089)
Morethia adelaidensis	0.042 (± 0.0015)	0.031	0.055	0.048 (± 0.004)
Delma molleri	0.051 (± 0.0031)	0.051 (± 0.0074)	0	0
Lerista bougainvillii	0.048	0.041 (± 0.00021)	0	0
Aprasia pseudopuchella	0	0.007	0	0.0077

Table A5. Results of repeated-measures PERMANOVA for effects of time (month), activity-season (pre or post translocation) and treatment (experimental or control enclosures), with temperature as a covariate, on body condition of *Tiliqua adelaidensis*. Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Temperature	1	0.231	.795
Activity season	1	0.133	.680
Treatment	1	1.480	.116
Month	7	7.884	1.000
Enclosure	4	2.509	**.036**
Activity season * treatment	1	0.557	.334
Activity season * enclosure	4	0.451	.771
Month * treatment	8	1.569	.132

Table A6. Results of repeated-measures PERMANOVA for effects of time (month), activity-season (pre or post translocation) and treatment (experimental or control enclosures), with rainfall as a covariate, on body condition of *Tiliqua adelaidensis*. Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Rainfall	1	0.013	1.000
Activity season	1	0.218	.570
Treatment	1	1.466	.108
Month	7	8.052	1.000
Enclosure	4	2.509	**.039**
Activity season * treatment	1	0.557	.324
Activity season * enclosure	4	0.451	.766

Month * treatment	8	1.569	.134

Table A7. Results of repeated-measures PERMANOVA for effects of time (month), activity-season (pre or post translocation) and treatment (experimental or control enclosures), with temperature as a covariate, on abundance of *Menetia greyii*. Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Temperature	1	10.806	**.012**
Activity season	1	0.407	.056
Treatment	1	1.313	.286
Month	7	1.971	.093
Enclosure	4	0.731	.575
Activity season * treatment	1	0.511	.791
Activity season * enclosure	4	1.269	.307
Month * treatment	8	0.867	.551

Table A8. Results of repeated-measures PERMANOVA for effects of time (month), activity-season (pre or post translocation) and treatment (experimental or control enclosures), with rainfall as a covariate, on abundance of *Menetia greyii*. Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Rainfall	1	0.000	.992
Activity season	1	1.673	.257
Treatment	1	1.313	.283
Month	7	3.989	**.003**
Enclosure	4	0.731	.582
Activity season * treatment	1	0.511	.785

Activity season * enclosure	4	1.269	.303
Month * treatment	8	0.867	.560

Table A9. Results of repeated-measures PERMANOVA for effects of time (month), activity-season (pre or post translocation) and treatment (experimental or control enclosures), with temperature as a covariate, on body condition of *Menetia greyii*. Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Temperature	1	0.030	.958
Activity season	1	0.324	.496
Treatment	1	0.338	.464
Month	7	40.429	.999
Enclosure	4	4.223	**.002**
Activity season * treatment	1	0.609	.312
Activity season * enclosure	4	3.970	**.004**
Month * treatment	8	0.143	.997

Table A7. Results of repeated-measures PERMANOVA for effects of time (month), activity-season (pre or post translocation) and treatment (experimental or control enclosures), with rainfall as a covariate, on body condition of *Menetia greyii.* Bold denotes significant effects (p< .05).

Effect	d.f.	F	p
Rainfall	1	0.041	1.000
Activity season	1	0.583	.371
Treatment	1	0.434	.400
Month	7	38.663	.999
Enclosure	4	4.223	**.003**

Activity season * treatment	1	0.609	.314
Activity season * enclosure	4	3.970	**.004**
Month * treatment	8	0.143	.997

FIGURES

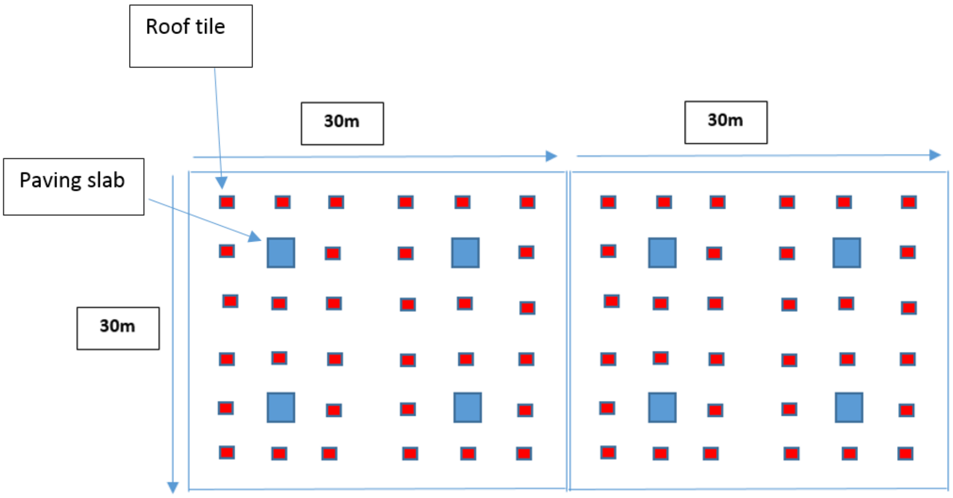

Fig. A1. Diagram depicting ACO placement in a set of enclosures. The large paving slab is surrounded on all four sides by eight evenly placed smaller roof tiles. The paving slabs were approximately 50cm x 50cm with a 10cm high wooden block propping up one side. The roof tiles were corrugated cement approximately 50cm x 25cm.

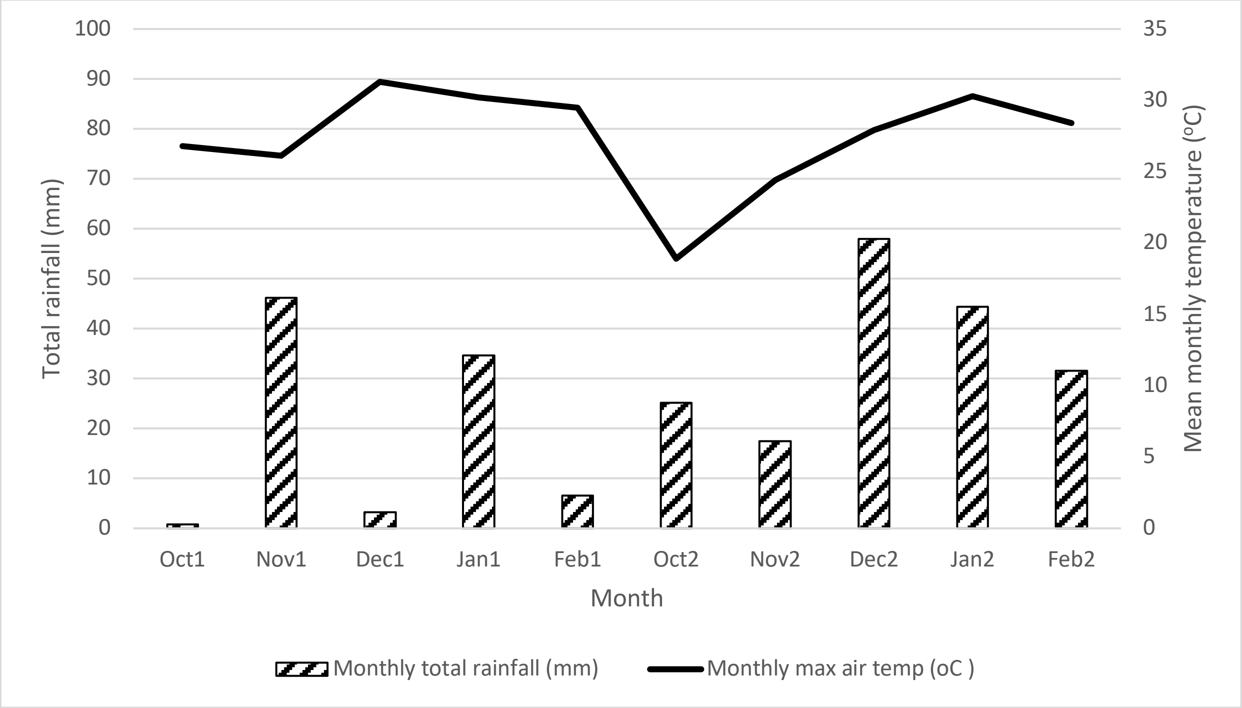

Fig. A2. Monthly total rainfall (mm) for Burra and the mean maximum temperatures (oC) for Clare (closest weather station) between October 2015 and February 2017. Monthly maximum air temperatures (oC) recorded at Clare high school bureau station and rainfall data recorded at Burra community school bureau station (Commonwealth of Australia, Bureau of Meteorology, 2015-16). Data downloaded from www.bom.gov.au/sa/warnings.

Pygmy bluetongue lizard

Morethia adelaidensis

Lerista bougainvillii

Menetia greyii

Tiliqua rugosa

Tiliqua scincoides

Image removed due to copyright restriction.

Delma molleri

Aprasia pseudopuchella

Fig A3: Sympatric lizard species found within the enclosure walls in this study

CHAPTER 3

POPULATION HEALTH

Pygmy bluetongue lizard having its bite force performance recorded *in-situ*

PREFACE

This chapter has been written in manuscript format for publication in the Journal of Wildlife Management after thesis submission.

In the previous chapter I assessed the impact of population reinforcement on the abundance and body condition of co-existing lizard species in order to comment on the health of these individuals after the sudden increase in pygmy bluetongue lizard density following the reinforcement. In this next chapter I investigate the link between body condition and a measure of performance, bite force, in order to ascertain the degree to which population health can be interpreted through collection of body condition data.

Populations of endangered species can exist in very small patches of habitat and remain vulnerable to stochastic events such as disease outbreaks or reduced food availability, both of which may influence body mass and, therefore, standard measures of body condition. The ability to monitor population health for signs of decline could be a useful tool to enable proactive intervention before extirpations occur. Body condition is a widely used, non-invasive method to assess the health of an animal as it is perceived to represent lipid stores in the body which can be an indirect indicator of fitness. However, measuring performance (the ability of an animal to carry out an ecologically relevant task) is growing in popularity but is a more time consuming and invasive method of monitoring. In this chapter, I examine whether body condition is associated with performance and if body condition can be used to act as an easily accessible proxy for the overall health of a population.

BODY CONDITION IS A GOOD INDICATOR OF BITE FORCE: AN ASSESSMENT TOOL FOR ENDANGERED SPECIES POPULATIONS

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ABSTRACT

Populations of endangered species can exist in small remnant patches of habitat, under environmental pressures, which can result in extirpations. The ability to assess the health of small vulnerable populations, with minimal invasiveness, could allow management action to be taken when a decline is observed. Body condition is inferred as a positive indicator of fitness due to an individual's 'plumpness' being representative of lipid stores in the body which enable it to perform fitness related activities such as survival or reproduction. The present study aims to determine whether body condition is indicative of an individual’s bite force performance, which could indicate that individuals ability to perform an ecologically relevant trait such as acquire prey or win fights with conspecifics. While often assumed to be the case, it has seldom been empirically tested. We used bite force as to indicate performance as it is important in this species for feeding and social interactions (home-site defence and breeding behaviours). We sampled 119 adult pygmy bluetongue lizards (*Tiliqua adelaidensis*) from a single population over two consecutive activity-seasons. We collected data of bite force performance, head measurements, body size (SVL) and mass. We found that individuals in better body condition bit more forcefully, larger lizards bit more forcefully, and all lizards had more forceful bites in summer although not as an effect of temperature. Although there are additional factors contributing toward bite force, our study confirmed a strong link between bite force performance and body condition, increasing confidence that this minimally invasive measure is an honest signal of an individuals condition.

INTRODUCTION

Populations of endangered species can exist in small remnant patches of habitat, isolated through human mediated activities such as construction of roads and agricultural activity. Many of these populations are under environmental pressures, such as a reduction in high quality habitat availability, which can result in the extirpation of small populations in a relatively short period of time. Thus, the ability to estimate population health to identify potentially struggling populations is an important tool to allow action to be taken before the population is lost (Sainsbury *et al.* 2001). In the case of the Fraser River sockeye salmon (*Oncorhynchus nerka*) where the development of a fish health standard could have facilitated the identification of risks before its disappearance (Cohen 2012; Stephen 2014).

A tool to proactively identify reductions in population health would be useful in protecting endangered species that are already at risk so management action can be taken to reduce the chance of extirpation occurring (Sarre *et al.* 1994). Fluctuating asymmetry has been suggested as an early warning system used to rank populations in order of needing management although it is indicative of historic stressors rather than present decline (Sarre *et al.* 1994; Knierim *et al.* 2007; Lens *et al.* 2002). Here we must make a distinction between health and fitness. While evaluating measures of such as lifetime reproductive success, is optimal it can require long-term monitoring across multiple generations and can be difficult to apply to an individual at a given time (Frankham 2016; Tallmon *et al.* 2004). However, health, likely linked to fitness, can provide a snapshot of the status of that individual or population at any given point in time. There are inconsistencies regarding how health is measured (Stephen 2014; Hanisch *et al.* 2012) and recent health assessments suggest use of more than one criteria as taxa respond to environmental stress differently (Stephen 2014). For example, in response to food shortages spiders preserve lipid reserves by reducing their metabolic rate (Wilder *et al.* 2016; Wilder 2011). Evidently some species are able to prioritise maintenance of key behaviours, as in this example, which indicates that body condition alone may not give the full picture of organism health across different taxa and raises the question of what measure of performance provides us with an honest signal of health? It is possible that body condition interpreted in conjunction with activity level or performance ability of an individual may provide a more comprehensive view. In addition to use of multiple measures in health assessments, researchers now consider that health should include not only an absence of disease or abnormality, but also evidence that an organism is thriving in its environment

(Depledge and Galloway 2005). Therefore, this allows not only identification of declining populations but stable and prospering populations as well.

First, health can be assessed through biochemical indicators such as stress hormones which could indicate a populations potential vulnerability to disease as a result of immunosuppression (Schreck *et al.* 1989). Second, health can be inferred through the abundance and body condition of a population, both of which can be assessed visually from a distance to reduce stress caused to the wildlife (Pettis *et al.* 2004). Physical condition can include both visual assessments (Pettis *et al.* 2004) or use of body condition indices (Shamiminoori *et al.* 2014). Body condition indices are commonly used to assess health, likely due being minimally invasive and easy to measure in many species (Wilder *et al.* 2016; Vervust *et al.* 2008). However, it is important to acknowledge alternative explanations for high body condition (Madsen & Shine 2002). The third way to assess organism health is through its behaviour. Reduced physical activity, a change in foraging behaviour or increased aggression could signal the need for conservation intervention (Berger-Tal and Saltz 2016). And finally, a fourth way to assess organism health is through monitoring performance measures, such as bite force, which reflect the ability to perform an ecologically relevant task (Anderson *et al.* 2008), however collection of these data can be more invasive than collection of body condition measurements. Reduced performance could signal declining health before observations of diminished physical condition are made, acting as an early indicator of decline. Bite force and sprint speed are two commonly used, energetically demanding, measures of performance. Bite force can indicate the potential for an organism's ability to capture harder or larger prey (Dollion *et al*. 2017; Herrel et al. 1999) or fight rivals for mating opportunities (Husak *et al*. 2006; 2009) while sprint speed, often termed locomotor performance, can signal an animal's ability to evade predators (Miles 2004; Byers *et al.* 2010; Vervust *et al.* 2008; Huyghe *et al.* 2013).

Herrel et al. (1996) found that *Podarcis hispanica (p. h. atrata)* with more forceful bites were able to prey upon larger and harder prey items and determined bite force to possibly be an ecologically relevant trait. While this study highlights existing literature investigating the link between bite force and the ecologically relevant ability to access larger prey items, it does not provide empirical data on this topic. Although commonly discussed, the idea that individuals in good body condition are more adept at performing ecologically relevant tasks has rarely been tested (Vervust *et al.* 2008) and is not consistently validated (Husak 2006).

Study species

The pygmy bluetongue lizard (*Tiliqua adelaidensis*) is an endangered skink endemic to patches of native grassland in South Australia (Hutchinson *et al.* 1994) a habitat that has been almost entirely eradicated (Hyde 1995). Habitat suitability modelling has shown that managed relocations will be required for long-term persistence of the species (Fordham *et al.* 2012), so using body condition to comment on the health of a population would aid identification of populations needing management intervention. Body condition can also help to assess how translocated individuals are responding at a new site post-release. The pygmy bluetongue lizard is a good study system as threat of extinction is medium-term rather than short-term which allows time to develop monitoring tools and explore management options to allow more effective conservation of the species when required. *Tiliqua adelaidensis* is a sit-and-wait predator living in abandoned spider burrows built by various species of wolf and trapdoor spiders (Clayton 2018), largely preying on invertebrate species passing their burrow entrance (Fenner *et al.* 2007). A primarily solitary species, individuals rarely interact except in acquisition and defence of burrows (Fenner and Bull 2011), and for mating (Fenner and Bull 2011; Ebrahimi *et al.* 2014). Adult males disperse in early spring to search for prospective mates (Schofield *et al.* 2012) and during dispersal there is an increased likelihood of encountering conspecifics and engaging in agonistic interactions, which can result in significant injuries (Figure A1). *Tiliqua adelaidensis* exhibit a low level of sexual size dimorphism, sexual size dimorphism being a trait prevalent in skinks, with the females possessing larger body size (Table 1) and males possessing larger heads relative to body size (Hutchinson *et al.* 1994). The jaws serve as the pygmy bluetongue's primary weapon, thus the bite force of the jaws is considered an ecologically relevant measure of performance (Irschick *et al.* 2007).

Table 1: Summary statistics (Mean ± SE) for body size and head measurements (to the nearest 0.01mm) for adult male and female *T. adelaidensis.* Adult lizards being identified using a criteria of >80 mm SVL (Milne 1999)

		Mean ± SE	
		Males (n = 43)	Females (n = 76)
SVL (mm)		91.3 ± 0.77	95.6 ± 0.76
Mass (g)		12.4 ± 0.38	12.9 ± 0.40
Bite force (N)		13.6 ± 0.39	14.4 ± 0.37
Head length (mm)	Ros - Ret	20.4 ± 0.22	19.7 ± 0.16
	Ros - Qu	19.8 ± 0.22	19.1 ± 0.15
Head Width (mm)	Qu - Qu	11.0 ± 0.13	10.8 ± 0.13
	Ju - Ju	13.1 ± 0.15	12.7 ± 0.12
Head depth (mm)		11.3 ± 0.14	11.0 ± 0.11
Temporal length (mm)		9.03 ± 0.11	8.9 ± 0.09

The aim of the present study was to investigate whether body condition was positively associated with bite-force performance as a minimally-invasive way to assess population health. This was achieved through monitoring body condition and bite force (performance) of males and females across two consecutive activity seasons. We also included additional variables that may have contributed to performance such as body size (SVL), sex, season and ambient temperature. Firstly, we hypothesised that individuals in better body condition would perform better, as evidenced by a more forceful bite. Secondly, we hypothesised that larger individuals would perform better, as is commonly the case with measures of performance. Our third and final hypothesis was that males would perform better than females, due to their larger relative head size. Overall, we predict that bite-force performance will be a good indicator of organism health, for both males & females, reflected through body condition, as it indicates that animals' ability to perform an ecologically relevant task.

METHODS

The study was conducted over two consecutive Austral spring/summer seasons (Oct – Mar) of 2015 – 2017 at the Nature Foundation of South Australia's 'Tiliqua' reserve just outside Burra, South Australia. We measured weapon size and performance in 119 adult pygmy bluetongues (males: n = 43, females: n = 76) over a two-year period; adult lizards being identified using a criteria of >80 mm SVL (Milne 1999). Lizards were caught monthly using a previously described technique using a fishing rod and mealworm lure (Milne and Bull 2000). Upon initial capture each lizard was toe clipped, allowing collection of blood and tissue, and providing that individual with a unique ID for future recognition. Snout-vent-length (SVL) was measured to the nearest 1mm and body mass was measured to the nearest 0.25 g using spring loaded 50 g Pesola scales. After processing, each lizard was put back in the burrow from which it was caught.

Data collection occurred in spring and summer for two consecutive activity seasons. Year one, spring data were collected in October 2015 (n=14), summer data were collected in March 2016 (n=33). Year two, spring data were collected in December 2016 (n=38) and summer data were collected in March 2017 (n=34). To have a fair comparison between activity-seasons, year two spring data collection was delayed from October to December due to unseasonably low spring temperatures (Table A1). It is important to note that data collection in spring occurred while males were dispersing to find mates, and the data collection in summer occurred after females had given birth so our body condition data did not reflect gravid females.

Bite force performance

Bite force was recorded using a Kistler force transducer (Type 9203, Kistler Inc, Switzerland), connected to a charge amplifier (Type 5995, Kistler Inc, Switzerland), that consisted of two parallel plates for the animal to bite (Figure A2). To protect the lizards' teeth during sampling, strips of leather were attached to the end of the metal bite plates (measuring (L) 15 mm x (W) 5 mm x (D) 0.35 mm) (Anderson et al., 2008; Jones & Lappin, 2009; Lappin & Jones, 2014). The leather also helped localise the bite force to the terminal tips of the bite plates. The amplifier was calibrated as per Lappin and Husak (2005) with readings following a linear relationship ($r^2 = 0.99$). The bite position was standardised through calculation of the bite-out lever using ImageJ software (https://imagej.net) (Jones and Lappin 2009). Four defensive bites were recorded for each individual and the maximal value (after lever correction) was used for analysis.

Head Dimensions

Using digital callipers, to 2 decimal places, we took six external head measurements including: three measures of head length (Ros-Ret, Ros-Qu, and temporal length (or post-orbital length)); two measures of head width (Qu-Qu and Ju-Ju) and one measure of head depth (Figure A3). We additionally calculated the size of the adductor chamber by multiplying the head width (Ju-Ju) with head depth and head length (temporal). To correct each measurement for overall head size we generated Mosimann size adjusted shape variables (Mosimann 1970; Sakamoto and Ruta 2012), also known as Log shape ratios, from individual head measurements. This was achieved by dividing each measurement by the geometric mean for individual lizards. Mosimann shape variables are regarded as more reliable than residuals when analysing morphometric measurements (Jungers *et al.* 1995).

Statistical Analysis

All of the following analyses were conducted using R version 1.0.143 (R Core Team 2016). We scaled and mean-centred each predictor variable to aid interpretation of effect size in model output. We used the *princomp* function on the head shape measurements to generate principal components that explained most variation in the head shape data. We then used linear mixed effects models within the *lmer* package, to assess whether these components or other factors affected bite force. Lizard ID was included as the random variable in all models to account for individual differences in performance.

We first fit a global model for bite force using SVL, body condition index (the residuals of a linear regression between SVL and mass), sex, season (i.e. spring or summer), mean maximum ambient temperature and the three principal components for head shape as predictors. We then performed automated model selection on this global model using the *dredge* function in the *MuMIn* package (Barton 2018) and used the best fitting model to predict bite force.

Global model parameters: Bite force ~ PC1 + PC2 + PC3 + SVL + Sex + Season*BCI + Temperature + (1| lizard ID)

RESULTS

There were two models shown to fit equally well. One model included SVL, body condition, season and a body condition*season interaction and the other model also included SVL, body condition and season but not the body condition*season interaction (Table 2). As the inclusion of the body condition*season interaction did not improve the model fit, and the model weight was the same, we chose to run the simpler model. The results of this model (Table 3) showed that lizards in better body condition bite more forcefully (Fig 1, $t = 6.93$, $p < .001$) as do larger lizards (Fig 2, $t = 11.15$, $p < .001$). However larger lizards are not automatically in better body condition (Fig 4). Season was also positively associated with performance, lizards biting more forcefully in summer, at the end of the activity season, than in spring (Fig 3, $t = 5.77$, $p < .001$). However, this was not due to ambient temperature as this factor was only in the ninth best fitting model as selected by the automated model selection. Head shape was not an important factor for performance, PC1 only being included in the third best fitting model (Table 2).

Table 2: Table showing the results of our model selection using the dredge function. The two best models include body condition (BCI), body size (SVL) and Season

Model	logLik	AIC_c	ΔAIC_c	$wAIC_c$
Bite force ~ BCI + SVL + Season + BCI*Season + (1|lizard ID)	-282.7	580.2	0.00	0.16
Bite force ~ BCI + SVL + Season + (1|lizard ID)	-283.8	580.2	0.00	0.16
Bite force ~ BCI + SVL + Season + PC1 + BCI*Season + (1|lizard ID)	-282.4	581.8	1.6	0.07

Bite force ~ BCI + SVL + Season + PC1 + (1|lizard ID)	-283.6	582.0	0.2	0.07
Bite force ~ BCI + SVL + Season + Sex + (1|lizard ID)	-283.7	582.2	0.2	0.06

Table 3: Table showing the results of the model run using the variables suggested by the dredge (model selection) function.

	Estimate	Standard error	df	t-value	p-value
Intercept	12.973	0.296	107.047	43.798	**<.001**
SVL	1.973	0.177	83.63	11.151	**<.001**
BCI	1.472	0.213	129.533	6.929	**<.001**
Season (summer)	2.301	0.399	131.212	5.774	**<.001**

Table 4: Result of the Principal Components Analysis (three extracted components with eigenvalues above 1, cumulatively explaining 0.80 of the variance)

	Component 1 (PC1)	Component 2 (PC2)	Component 3 (PC3)
Head length 1 (Ros-Ret)	0.517	0.368	0.157
Head length 2 (Ros-Qu)	0.565	0.221	0.132
Head length 3 (Temporal)	0.239	-0.723	-0.295
Head width 1 (Qu-Qu)	-0.426	0.407	-0.543
Head width 2 (Ju-Ju)	-0.299	0.189	0.496
Head depth	-0.293	-0.302	0.575
Eigenvalue	2.36	1.33	1.12
Proportion of variance explained	0.39	0.22	0.19

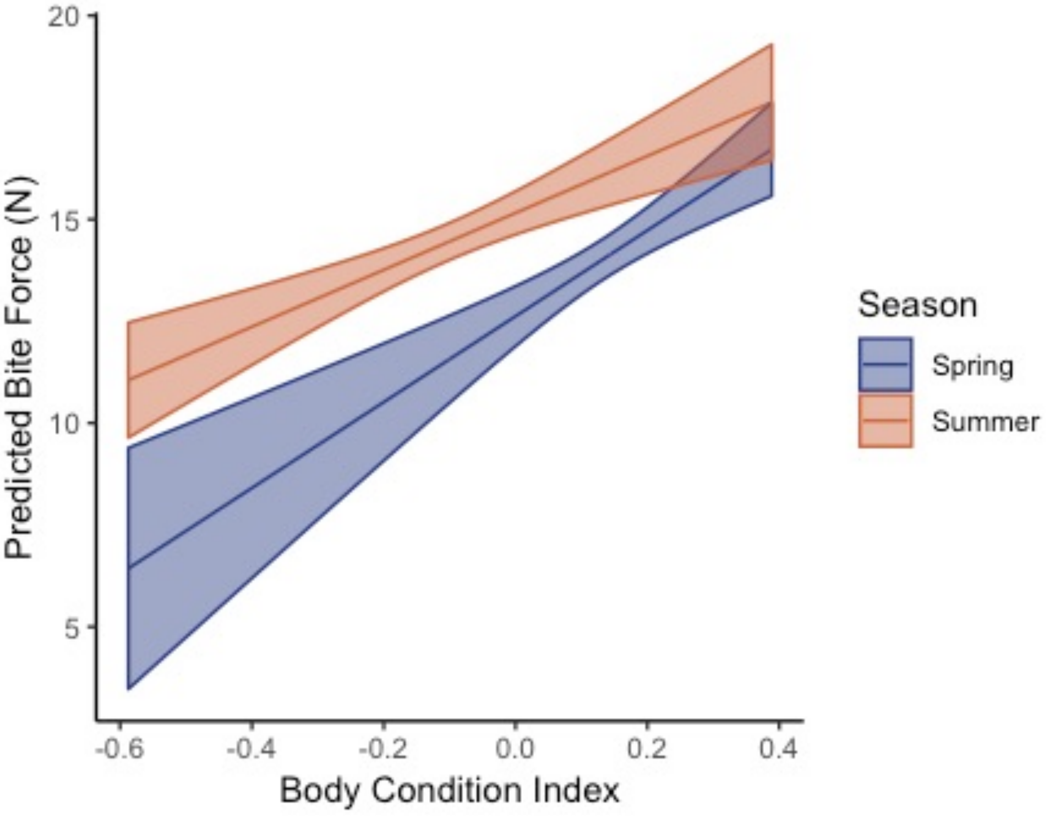

Figure 1: Predicted bite force against body condition index (BCI; the residuals of a linear regression between snout-vent length and mass) in *T. adelaidensis* for lizards of average snout-vent length

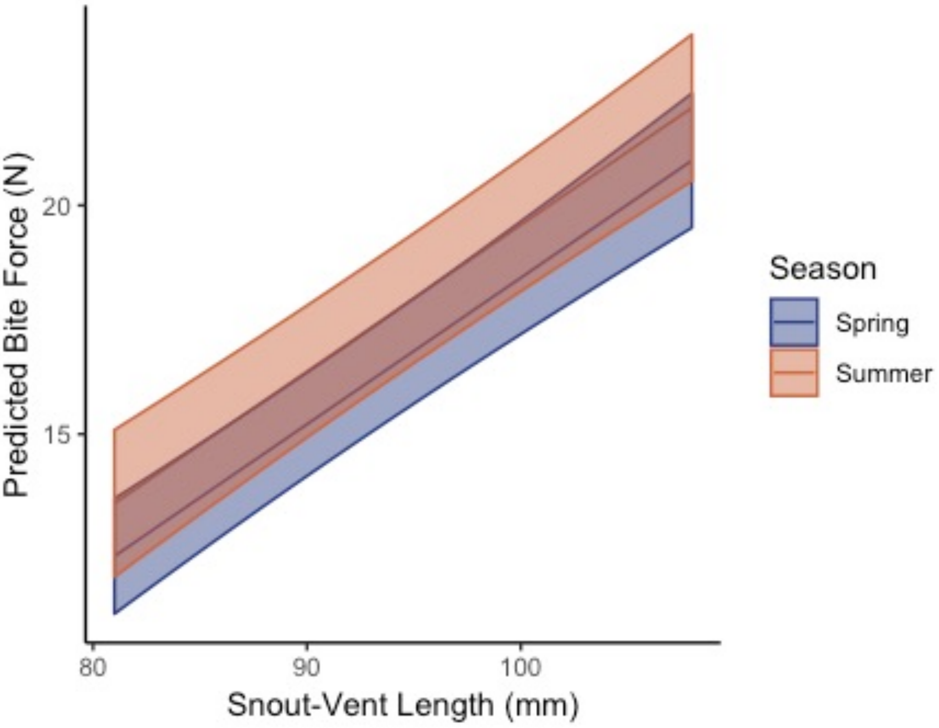

Figure 2: Predicted bite force against snout-vent length (SVL) in *T. adelaidensis* of maximum body condition.

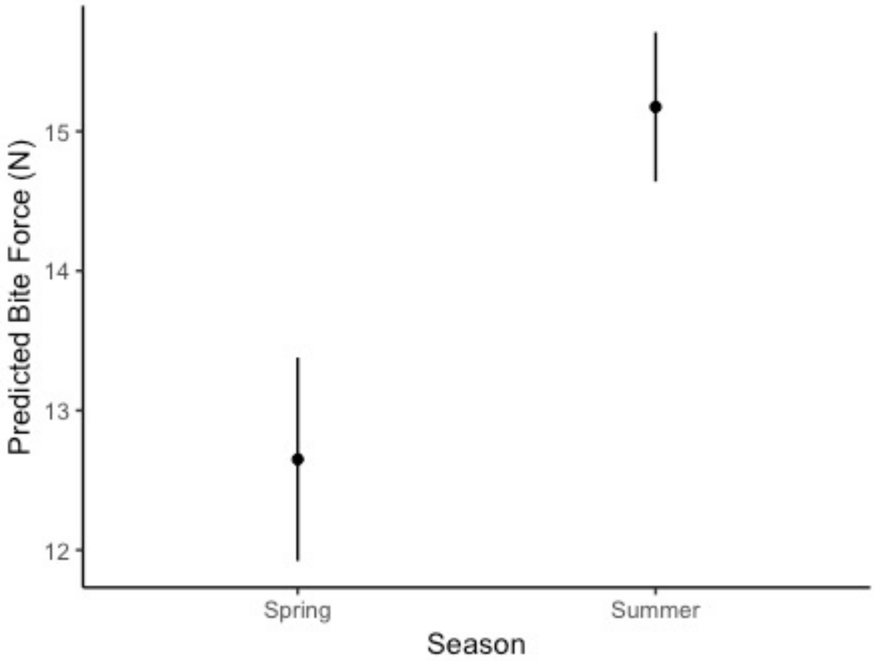

Figure 3: Predicted bite force (Newtons) in *T. adelaidensis* by season, at the average snout-vent length and body condition

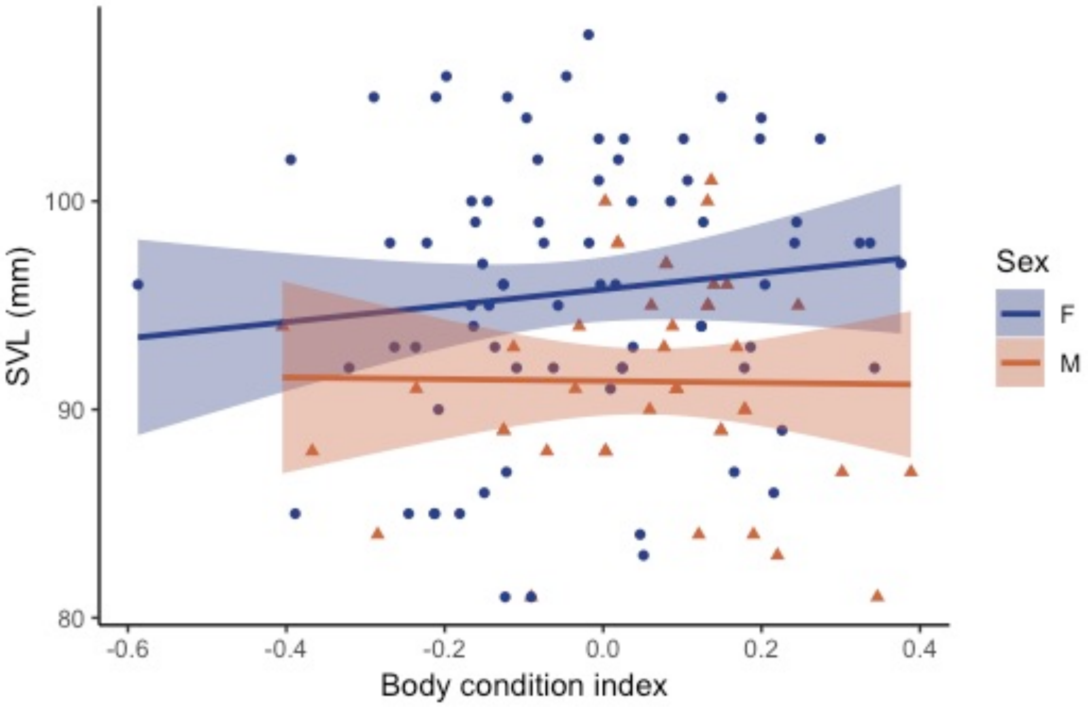

Figure 4: Scatter plot showing the lack of relationship between body condition index and SVL for both male and female *Tiliqua adelaidensis* (males = filled triangles, females = filled circles)

DISCUSSION

The aim of the present study was to investigate whether body condition was positively associated with bite force performance as a minimally-invasive way to assess population health. This study is also the first to document the bite force capabilities of this species. Our work had three important findings. First, lizards in better body condition had a more forceful bite. Second, larger lizards performed bit more forcefully than smaller lizards, although large lizards did not necessarily have better body condition. And third, all lizards bit more forcefully in summer rather than spring, yet body condition was lower in summer although this wasn't a significant result.

The finding that lizards in better body condition bit more forcefully better provides support to our prediction that body condition is indicative of bite force performance for both sexes and, as such, may be an indicator of overall health due to the ecological relevance of the task. The fact that larger lizards bit more forcefully than smaller lizards is a well-documented pattern (Anderson *et al.* 2008). However, the fact that lizards bit more forcefully in summer rather than spring was surprising. We included ambient temperature in our model and it was not selected as a factor strongly associated with bite force performance which would imply energetic motivational state was similar in both sampling periods. However, body condition may have been lower in summer due to the reduced abundance of prey items at that time of year (Fenner *et al.* 2007). Thermoregulation does not explain the more forceful bites shown in summer as the automated model selection only included temperature in the fifth best model and there was no interaction between temperature and season which would indicate temperature differences between spring and summer.

The results of this study support our hypothesis that body condition positively indicates bite force performance, but this finding is not consistently supported in the literature. Herrel et al., (2010) found that body condition was positively correlated with bite force performance in male tuatara (*Sphenodon punctatus*) whereas Vervust et al., (2008) found that Adriatic lizards (*Podarcis sicula*) in good body condition did not always perform better, using print speed as a measure of performance . Husak (2006) investigated the link between locomotor performance (sprint speed) and survival in collared lizards (*Crotaphytus collaris)* and found that although hatching survivors performed better than hatchling non-survivors they did not have significantly different body condition. While survivors and non-survivors did not significantly differ in their body condition, Husak did not comment on the relationship between body condition and sprint speed although the lack of relationship is implied. Sprint speed was the performance measurement used in Husak's study and hindlimb length, morphological trait associated with sprint speed, was found to be associated with survival. My study used bite force as the measure of performance although head shape, the morphological trait associated with bite force, was not found to be associated with performance, likely due to the ecological constraints of crevice-dwelling on cranial development. Additionally, in another study Husak, Lappin and Van Den Bussche (2009) failed to include body condition as a factor contributing to bite force performance and reproductive success rather only including body size (SVL) and head measurements.

Sinervo, Hedges and Adolph (1991) found that female western fence lizards (*Sceloporus occidentalis*) performed poorly following oviposition likely due to their reduced body condition, a finding that was supported by Murray (2002) who found that individuals that had experienced a recent decrease in body condition were more likely to be predated than individuals with consistently low body condition. Decreases in body condition can be indicative of other factors such as disease or age, thus, monitoring of endangered species populations need to happen over consecutive seasons to monitor for changes in body condition which may be more informative than the level of condition itself. While not included in the present study, our approach can be applied to future studies in conjunction with parasite load data to confirm our hypothesis that these measures are indicative of overall health.

The present study examined the in-situ bite force performance of a wild population over time and it is important to note that the studies by Vervust et al., (2008) and Husak (2006) were both laboratory based studies which may have impacted their results. Studies involving wild subjects may be a more accurate representation of the animal's performance ability but also provides a snapshot of the condition and health of the animal, and thus the population, at that point in time. While there is value to be had from laboratory studies in terms of standardised testing in a controlled setting to ascertain baseline performance levels, we feel that sampling of wild populations is more appropriate for the interpretation of the data (Miles 2004). It is also important to ensure that there is thorough sampling of both sexes and individuals of all sizes to get an accurate representation of the population at that time (Figure A4).

While outside the scope of the current study, future research would benefit from investigating the relationship between body condition and performance with parasite load of health animals to form baseline data that can later be used to identify potential declines as a result of disease outbreaks. Each taxon will have its own unique way of responding during times of stress (Wilder 2011) which it is why it is important to establish baseline patterns of health in order to detect future declines more accurately. Future studies would also benefit from extending this investigation by linking measures of health to reproductive success in a population which could identify optimal physical condition to maximise reproduction, a very important area of knowledge for conservation of endangered species with vulnerable populations (Clive *et al.* 2019). This direction of research would also benefit from resource allocation experiments to analyse how condition, performance and reproductive success respond in times of prey shortage, a response that may differ between taxa as highlighted by Wilder (2011). In times of stress such as a shortage of prey, bite force performance could decline before physical condition and so a disparity between the two variables could be an early warning system of impending decline, a thorough understanding of this mechanism could be key in identifying potential patterns of fluctuation that could precede population decline. However, depending on how crucial the performance measure is to survival, an animal may preferentially allocate energy to that trait rather than maintaining condition. Testing these hypotheses would help to build a clearer picture of what a stable or a declining population actually looks like in terms of condition, performance and reproduction (Deem *et al.* 2008; Morris *et al.* 1994; Rodwell *et al.* 2001).

The present study determined that body condition may be a good proxy for ecologically relevant traits such as bite force performance. Bite force is not being maintained at low body condition, suggesting it is an honest signal in that respect. However, body condition is a measure of health that will be less honest at certain times of the year, such as breeding season, when increased female mass would inflate the results. Although if body condition is to be used a decrease in condition at certain times of the year will be expected. Thus, longitudinal year-round monitoring will enable identification of seasonal fluctuations in performance and body condition and a baseline to be established for subsequent comparison. This study provides the first records of bite force performance in this species and establishes a baseline of information for future studies to build on.

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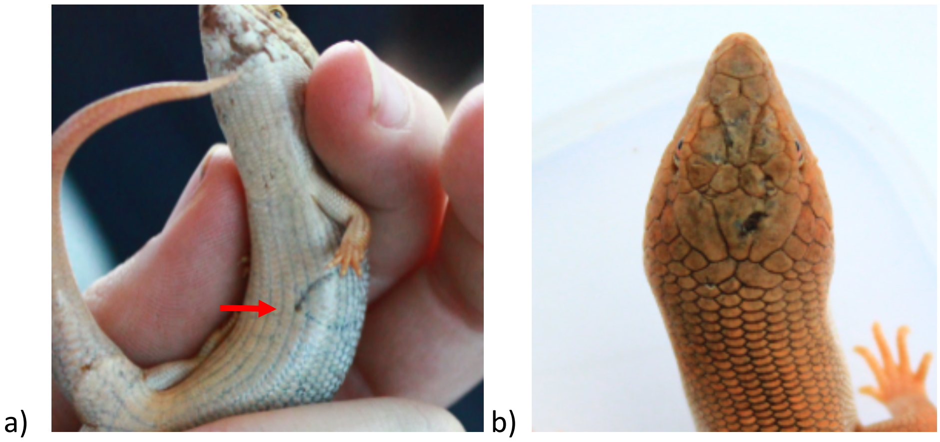
APPENDICES

TABLES

Table A1: Monthly mean maximum temperature for Clare high school BOM station 21131 (closest weather station to Burra) for the months of data collection to highlight the season that October 16 data collection (spring Year 2) was delayed to December 2016. Data downloaded from www.bom.gov.au (Commonwealth of Australia, Bureau of Meteorology, 2015-2017)

	Oct-15	Mar-16	Oct-16	Dec-16	Mar-17
Mean monthly maximum temperature	26.8	28.0	18.9	27.9	29.1

FIGURES



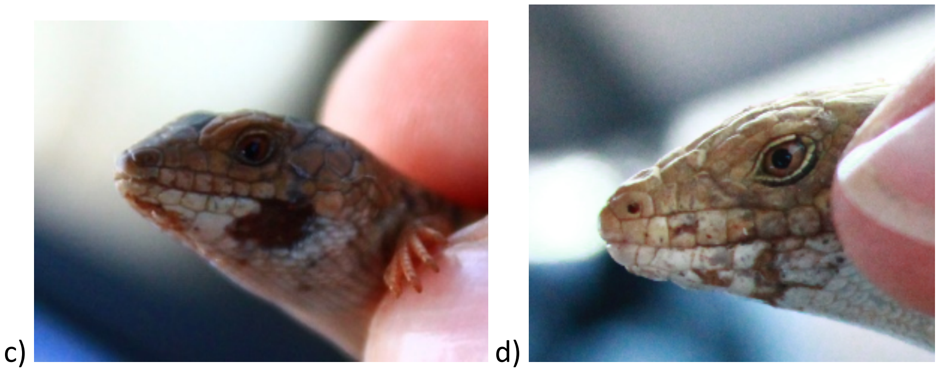


Figure A1: Photograph examples of recorded *T. adelaidensis* injuries. a) bite mark on flank; b) damaged head scales; c) extensive damaged scales around mouth of neonate; d) dirt and scale damage around mouth of adult male.

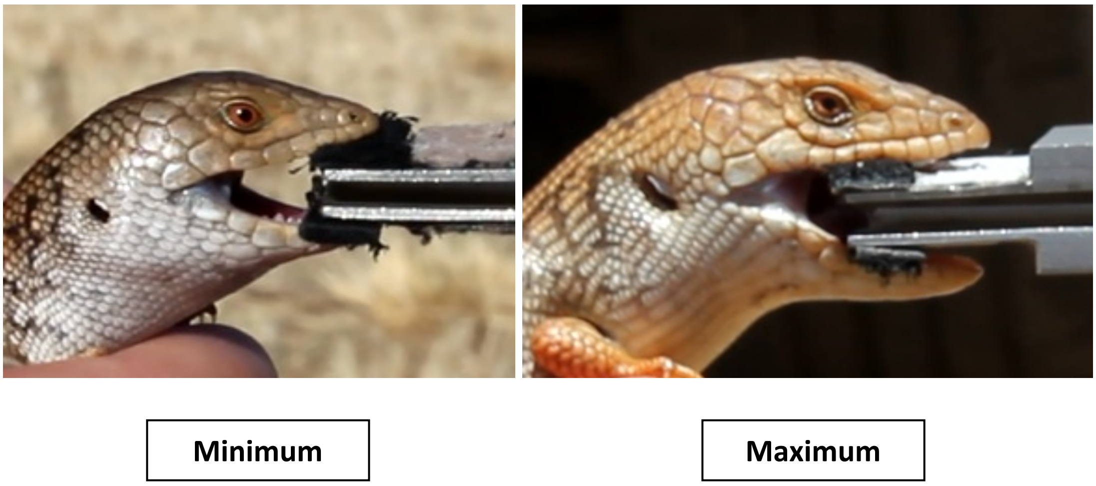


Figure A2: Position of the bite force transducer in the mouth of an adult *T. adelaidensis* to illustrate the minimum and maximum bite positions.

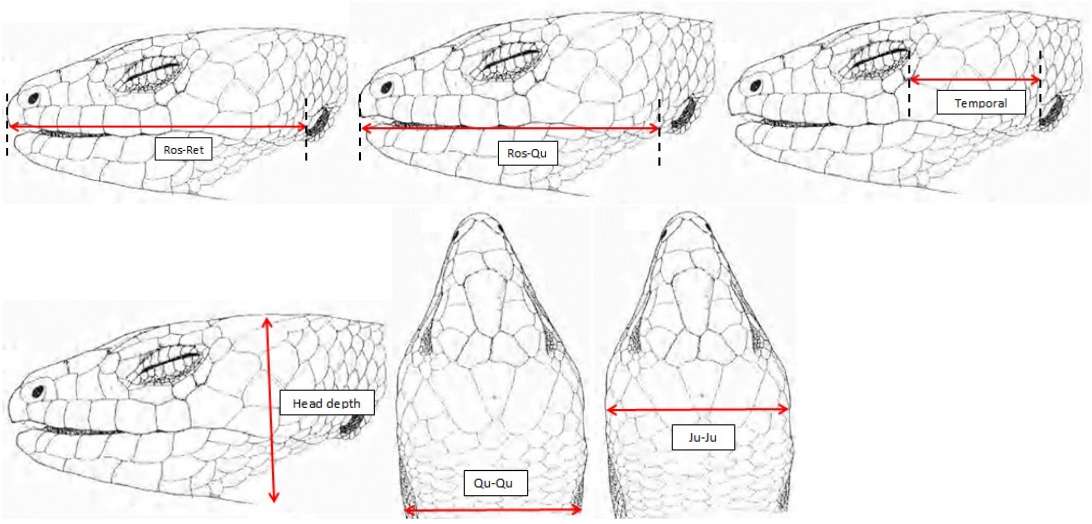


Figure A3: Locations of external head measurements taken during data collection (Scientific illustrations taken from Hutchinson et al., 1994). Ros: Rostrum, Ret: Reticulum, Qu: Quadrate, Ju – Jugal, T: post-orbital (temporal) length

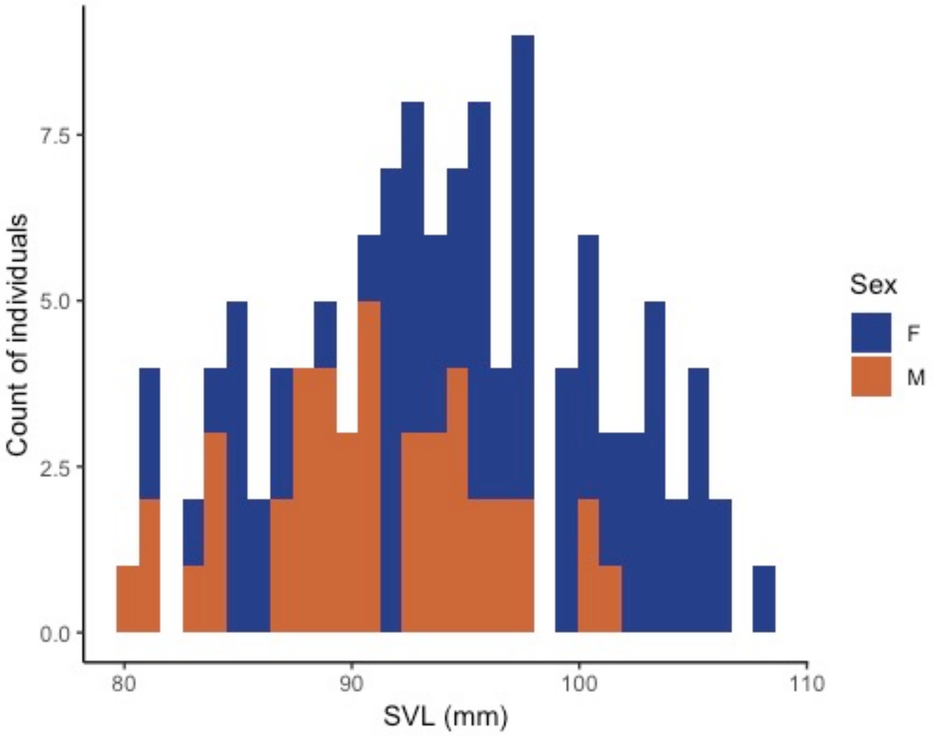


Figure A4: Frequency of sampling across various size classes of *T. adelaidensis*

CHAPTER 4

REPRODUCTIVE SUCCESS

Three sibling neonate pygmy bluetongue lizards

PREFACE

This chapter has been prepared for publication in Conservation Genetics.

In the previous chapter I examined the usefulness of performance and body condition to assess population health. I established that animals in better body condition performed better and, as such, body condition provides information on the ability of animals to perform ecologically relevant tasks, indicative of overall health. In this chapter, I explore how various physical characteristics, including body condition and performance, may be associated with reproductive success.

When looking to use managed relocations as a future conservation strategy, identifying characteristics that increase reproductive success will improve the chances that optimal individuals are selected for a translocation to maximise the likelihood of successful reproduction at the new site. Increasing the likelihood of reproductive success in an establishing population at a new site will help to ensure that population enters the population ‘growth’ phase as described by Sarrazin (2007). Small, isolated populations can be vulnerable to extirpation by stochastic events and research identifying characteristics or mechanisms that can increase the effective population size of endangered species could help to not only prevent extirpations but possibly help to stabilise those populations through increases in population size.

Through previous research we know that pygmy bluetongue lizards mate in early spring (Schofield *et al.* 2012, 2014) and we know that body condition is higher in spring (Chapter 3) (Shamiminoori *et al.* 2014). Through the findings of my previous chapter we know body condition is positively associated with performance, but this chapter will help to establish if any of these characteristics determine reproductive success for males or females. Pygmy bluetongue lizards exhibit sexual size dimorphism where the males have larger heads relative to body size (Hutchinson *et al.* 1994) and females are more elongate (Greer 1989). Linking male head size to bite force performance and reproductive success as evidenced by published studies, raises the question of whether larger headed males are more reproductively successful? Additionally, linking female body size to reproductive success as evidenced in the literature, do larger females have a higher reproductive output? It is our assumption that a healthy population will breed more, due to the connection between body condition, lipid stores and performance, although this link has yet to be made. Understanding if lizards with better body condition have higher reproductive output will help establish this link.

THE SIZE AND PERFORMANCE OF A WEAPON DOES NOT PREDICT REPRODUCTIVE SUCCESS

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ABSTRACT

Weapon size and performance are commonly cited as being important factors in reproductive success although studies commonly use species exhibiting territoriality and male-male combat. Few studies ask the same question of species that are dimorphic but are not territorial or exhibit well documented male-male combat. We measured weapon (head) size and performance (bite force) in 41 adult pygmy bluetongue lizards (*Tiliqua adelaidensis*) during spring, the time of year when mating occurs. We genotyped all adults and offspring using seven polymorphic microsatellite loci and used those genotypes to perform parentage analysis, allowing us to attribute reproductive success to each adult male and female based on the number of offspring produced, using reproductive output as a proxy for reproductive success. We also included additional variables such as body size (SVL), mass and microsatellite heterozygosity to see if these were contributing factors in reproductive success. We found that body condition was the best predictor of reproductive success in females whereas reduced microsatellite heterozygosity predicted reproductive success in males, although there was a lot of variation in these data so this finding should be treated with caution. Neither weapon size, nor performance, were predictors of reproductive success which is in-keeping with findings of other studies in non-territorial, promiscuous species. Our findings indicate that the presence of dimorphic morphology in itself is not necessarily an indicator that it plays an essential role in reproductive success.

INTRODUCTION

Evolution favours individuals that maximise their reproductive success which can be achieved through mechanisms such as: larger body size (Dosen and Montgomerie 2004; Sokolovska *et al.* 2000); alternative mating strategies (Johnson and Brockmann 2012); ornate physical secondary characteristics (Andersson 1992; Siefferman and Hill 2003); song repertoire and complexity

(Doutrelant *et al.* 2000; Seddon *et al.* 2004); attaining and defending dominance in social groups (Wroblewski *et al.* 2009); territory size and quality (Hiebert *et al.* 1989); or weapon size (Preston *et al.* 2003). Males and females differ in their strategies with males preferentially investing in traits linked to male-male competition, with fecundity selection driving females to invest in larger body size — also known as the *fecundity-advantage* hypothesis (Cox *et al.* 2007). These differences, and their effect on that individual's fitness, have resulted in the evolution of sexual dimorphism's such as body size (Hormiga *et al.* 2000), head size (Kratochvíl and Frynta 2008), and colour dimorphism (Owens and Hartley 1998). Alternatively, individuals could choose mates based on a genetic component such as scent or diversity of the MHC genes (Pearson *et al.* 2017). Honest signals reflective of the individual's overall health will be selected for such as: colourful plumage (Hill and Montgomerie 1994; Siefferman and Hill 2003); long tail feathers (Andersson 1992); song duration (Clutton-Brock and Albon 1979); antler size (Vanpé *et al.* 2007); weaponry (Lailvaux *et al.* 2009; Lappin *et al.* 2006).

For species requiring male-male combat to win access to females, the development of large weaponry, i.e. fighting apparatus, is key to increasing the likelihood of a successful agonistic interaction. Weaponry can be developed by males either to improve fighting ability (Vanpé *et al.* 2007) or to bluff competitors to hide their lack of competitive ability (Lailvaux *et al.* 2009). Weapon size alone can, in some species, provide individuals with the opportunity to gain a higher degree of reproductive success (Huyghe *et al.* 2013; Lebas 2001).

For some species, it is not the size of the weapon, but the weapon performance itself, that results in reproductive success (Husak *et al.* 2009; Huyghe *et al.* 2008; Lappin and Husak 2005). However, weapon performance, i.e. bite force, is not always a positive predictor of reproductive success (Huyghe *et al.* 2014; Lailvaux and Irschick 2006). Dalmatian wall lizards (*Podarcis melisellensis)* exhibit male-biased dimorphism and, even though the dimorphism is associated with the successful displacement of rivals, it is not correlated with reproductive success. In fact, bite force was negatively correlated with reproductive success indicating female selection for males with lower bite forces, possibly due to injuries sustained during mating.

Study species

The endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) is a medium sized skink, endemic to small, isolated remnants of native grassland in South Australia (Hutchinson *et al.* 1994). *Tiliqua adelaidensis*, a predominantly solitary species, spend most of their time in their spider burrow (Milne, Bull, *et al.* 2003) and show limited dispersal. Pygmy bluetongue lizards exhibit a promiscuous mating system (Schofield *et al.* 2014), promiscuous in the true sense of the word (Elgar *et al.* 2013) as both males and females have been shown to mate with multiple individuals in one breeding season. Males disperse in early spring (Schofield *et al.* 2012) and have been observed approaching burrows following scent trails laid by the female, suggesting that females may be using chemical cues to attract mates (Ebrahimi *et al.* 2014). Males will mate with females they encounter above ground or alternatively will pull a female from her burrow to mate with her (Ebrahimi *et al.* 2014). Bite force is considered an ecologically relevant measure of performance for both males and females (Herrel and Gibb 2005; Huyghe et al. 2014; Irschick and Meyers 2007; Vervust et al. 2008) in this species, as it is used for prey capture (Milne, Bull, *et al.* 2003), fighting/ burrow defence (Fenner and Bull 2011) and mating (Ebrahimi *et al.* 2014).

Tiliqua adelaidensis exhibits relative head size dimorphism where males have larger heads relative to body size as is commonly seen in skinks, a group of lizards that are almost ubiquitously sexually dimorphic (Clemann *et al.* 2004; Cooper and Vitt 1988; Olsson *et al.* 2002; Schwarzkopf 2005; Shine 1989; Vitt and Cooper 1986). Published literature examining bite force focuses mainly on male-biased sexually size dimorphic species that exhibit high levels of territoriality or male-male combat (Husak *et al.* 2009; Huyghe *et al.* 2013) with little known about skinks (D'Amore *et al.* 2008; Noble *et al.* 2014; Vanhooydonck *et al.* 2011) or crevice-dwelling species which represent an interesting study species due to the additional pressure of ecological constraints (Lappin *et al.* 2006). Although *T. adelaidensis* males have relatively larger heads, overall this species shows a female-biased dimorphism in body size (Hutchinson *et al.* 1994), in part due to a higher presacral vertebral count in females compared to males (Greer 1989; pp171, Table 3). We undertook this study to test whether bite force performance was correlated with mating success in non-territorial species in which males possess larger heads relative to their body size than females, but which has a promiscuous mating system and exhibits female-biased sexual size dimorphism.

We investigated the relationship between weapon size, performance and reproductive success in pygmy bluetongue skinks. We measured weapon size (head dimensions) and performance (bite force) in both males and females and measures body condition in both sexes over the course of a single breeding season. We then assigned paternity to the offspring born that breeding season. We hypothesised that body size would predict reproductive success for females, and that bite force and head width would predict reproductive success for males.

METHODS

Study site

Data collection took place across two consecutive Austral spring/summer periods (Oct – Mar) of 2015 – 2017 at the Nature Foundation of South Australia ‘Tiliqua’ site in Burra, approximately 160km north of Adelaide, South Australia. Six enclosures (three pairs of 30 x 30 m enclosures), were built at the field site, surrounding an existing population of resident lizards, during the winter months prior to data collection to prevent lizards escaping during the study. The enclosures were constructed with 20cm high sheet metal walls and an inward facing 5cm lip at a 45-degree angle. All data were collected in accordance with Flinders University Animal Welfare Committee ethics (E417-15, E453-17) and Department for Environment and Water (permit G25011-10) with permission from the Nature Foundation of Australia.

Translocation

In February 2016, at the end of the activity season, we collected 24 adult lizards following Milne & Bull (2000) and translocated them to the recipient site in Burra. The translocated lizards were sourced from two populations, from the Jamestown area, (70km north of Burra), and from the Clare area, (30km west of Burra). Adults were identified as individuals with a SVL of 80 mm or greater (Milne 1999) and we ensured that none of the females were gravid upon capture. Each experimental enclosure received eight translocated lizards, four from each population, while the control enclosures remained un-augmented.

Data collection

Sampling of lizards within the enclosures occurred on a monthly basis, lizards being caught directly from their burrow as per Milne & Bull (2000). For each individual sampled, body mass was recorded using 50 g spring loaded Pesola scales (to the nearest 0.25 g) and body length, determined by snout-vent-length (SVL), was recorded (to the nearest 1 mm) using a transparent plastic ruler with these measurements being used to calculate body condition indices. Upon first capture, each individual was toe clipped providing them with a unique ID number for future identification. Toe clips were stored in 100% ethanol and refrigerated, in addition to blood being collected using Whatman® Classic and Elute FTA paper. Within each enclosure, every burrow had a unique burrow coordinate, allowing the lizard to be returned to the burrow it was caught from post-sampling.

Gravid females were sighted every second day in January 2017 in order to sample offspring prior to dispersal from the natal burrow. Offspring were first detected on 10th February 2017 with captures starting the same day. Neonate sampling followed the above protocol detailed for the adults. Where neonates were caught from the burrow of an adult female, we presumed the female was the mother and that was the natal burrow and used this information in the parentage analysis.

Weapon size and performance data were collected in spring of the activity-season following the translocation, December 2016, and represents the first opportunity the three populations had of breeding together. Six external head measurements were recorded for each individual: head length from the tip of the snout to the back of the lower jaw (Rost-Ret); head length from the tip of the snout to the base of the external ear (Rost-Qu); head width at the external ear (Qu-Qu); head width at the mid-point of the temporal region (Ju-Ju); head depth at the mid-temporal region (HD-mt) and the length of the temporal region defined between the back of the eye and base of the external ear (Porb or Temporal length). Bite force was recorded using a Kistler bite force transducer (Type 9203, Kistler Inc, Switzerland) in a custom build holder, connected to a charge amplifier (Type 5995, Kistler Inc, Switzerland), that consisted of two parallel plates for the animal to bite. Leather plates were fitted onto the plates to protect the animals' teeth during sampling and four defensive bites (trials) were recorded for each animal with at least a few seconds rest in between. Trials were filmed in side view and the output of each trial was standardised by measuring the bite-out lever (Jones and Lappin 2009). The greatest bite from the four trials was used in the statistical analysis.

Microsatellite amplification

All adults and offspring were genotyped using seven previously described, polymorphic microsatellite loci: TrL14, TrL16, TrL 21, TrL28, TrL29, TrL32 and TrL34 (Gardner *et al.* 2008). Loci were amplified using two multiplex reactions, each 23 µl in volume. Multiplex one contained 1 X MRT buffer, 0.08 µM TrL14 forward & reverse primers, 0.12 µM TrL16 forward & reverse primers, 0.08 µM TrL32 forward & reverse primers, 1U Immolase enzyme and 2 µl of DNA. Multiplex two contained 1 X MRT buffer, 0.24 µM TrL21 forward & reverse primers, 0.12 µM TrL28 forward & reverse primers, 0.12 µM TrL29 forward & reverse primers, 0.12 µM TrL34 forward & reverse primers, 1U Immolase enzyme and 2 µl of DNA. The forward primer of each pair was labelled using the fluorescent dyes FAM, VIC or PET (Applied Biosystems). Thermal cycling conditions for all loci consisted of denaturisation at 95 ^{o}C, followed by 35 cycles of 94 ^{o}C for 30 s, 60 ^{o}C for 60 s, and 72 ^{o}C for 60 s, with a final extension at 72 ^{o}C for 45 min and 25 ^{o}C for 2 min. The PCR amplicon was then cleaned using MultiScreenHTS 384-well filter plates on a vacuum manifold (Merck Millipore) and diluted to 1/50 and sent to the Australian Genome Research Facility (AGRF) for capillary separation. Fragments were called using GS500(-250) LIZ size standard in 'GeneMapper 4' (Applied

Biosystems) and microsatellite profiles were scored using Geneious software version R8 (Kearse *et al.* 2012) providing each animal sampled with a unique genotype. Alleles were pooled at certain fragment sizes in loci TrL 21 and TrL29 due to a lack of certainty when distinguishing between alleles which were often 1bp apart. Pooling alleles is a conservative approach to reduce the likelihood of false paternity assignment.

Parentage analysis

Parentage-offspring combinations were identified using Cervus version 3.0.7 (Kalinowski *et al.* 2007) which takes a maximum likelihood approach to assigning parent-offspring relationships. We accepted parent-offspring matches with a 95% confidence interval or above to reduce the likelihood of a Type II error. All adults sampled during the study were included in the analyses. Parentage analysis was run by enclosure, including all adults sampled within that enclosure during the three years of data collection. Due to the cryptic nature of the species it is possible that some individuals contributing to the gene pool were not sampled, although due to the thorough sampling conducted over a three-year period, we envisage this number to be minimal. As a result, we used a 0.8 probability that all fathers had been sampled; survival of males being lower than females due to increased chance of predation during dispersal to find mates (Schofield *et al.* 2012). The majority of litters were found in the natal burrow soon after birth, allowing us to confirm the known mother in the parentage analysis. Where lone neonates were found, we assigned parentage using ‘parents unknown’ parameters, including all adults sampled within that enclosure. The enclosure walls were deemed high enough to prevent escape during the study as no lizard was ever found in the wrong enclosure, so prospective parents were restricted to the enclosure of birth for each neonate.

Statistical analysis

In this paper, we characterise reproductive success as the number of offspring sired by each individual, with individuals producing more offspring being described as more successful. A body condition index was calculated through generating residuals of body size (SVL) against body mass with a higher body condition score being interpreted as ‘better’ body condition. Data were collected in early spring, soon after the lizards became active post-torpor, to ensure collection of pre-gravid female mass. By collecting data when matings were occurring, we ensured our data accurately reflected the lizards condition at the relevant point in time, allowing us to answer our question. We also included individual heterozygosity as a predictor in the analyses, calculated as the proportion of heterozygous loci (PHt) (Coulon 2010).

To determine which factors were predictors of reproductive success, we conducted a linear regression (*lm*) in R version 1.0.143 (R Core Team 2016) with the analysis being done separately for each sex. The response variable was reproductive success (i.e. number of offspring), and the predictor variables, for males, included SVL, body condition index, bite force, heterozygosity, source population and the six head measurements: Ros-Ret, Ros-Qu, Qu-Qu, Ju-Ju, head depth & temporal length (Fig. 1). For females we included: SVL, body condition index, bite force, heterozygosity and source population.

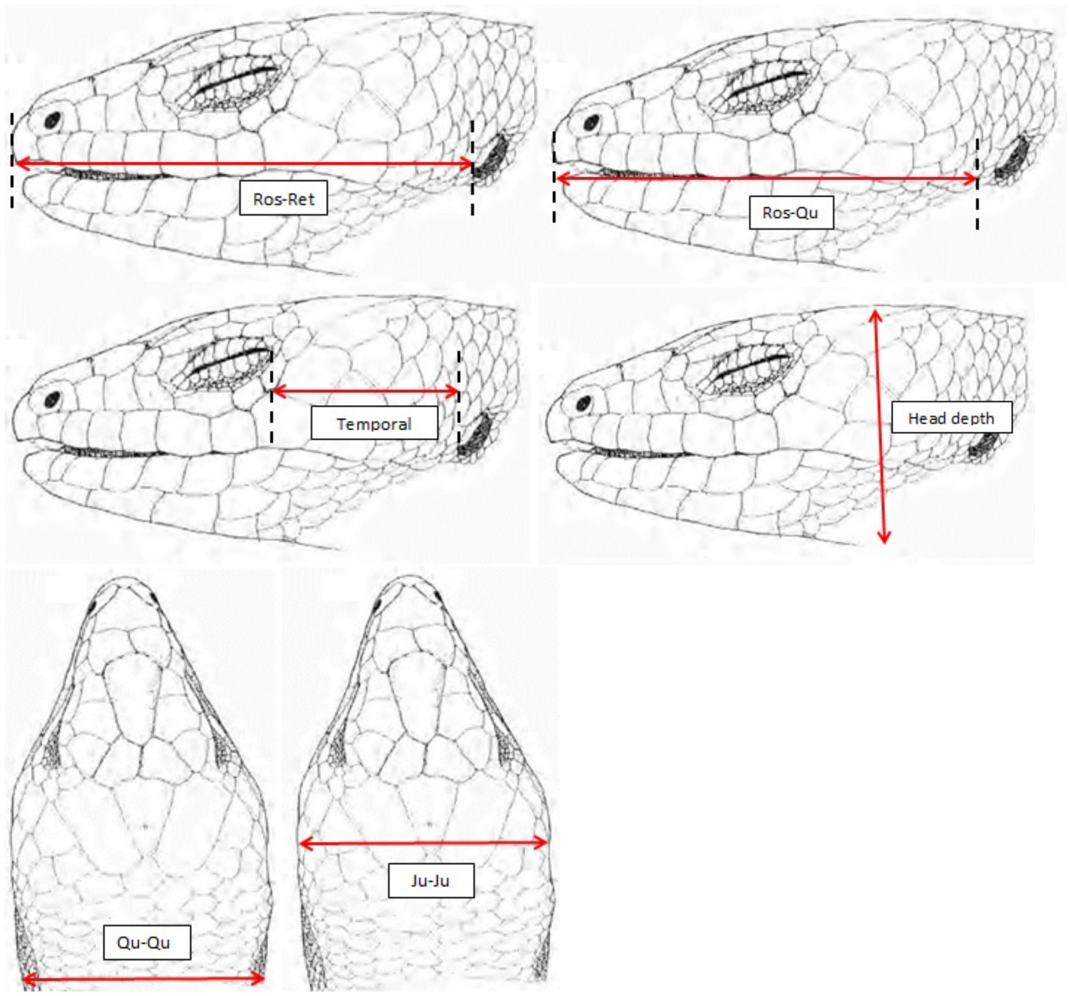

Fig. 1: Locations of external head measurements taken during data collection (Scientific illustrations taken from Hutchinson et al., 1994). Ros: Rostrum, Ret: Reticulum, Qu: Quadrate, Ju – Jugal, T: post-orbital (temporal) length

RESULTS

In total, we sampled and successfully genotyped 106 neonates, from 26 litters, from February to March 2017. Of those, 80 neonates were found in the natal burrow and 26 single neonates were found after dispersing from the natal burrow. Of the 106 neonates genotyped, 97 were assigned to a mother (91.5%), 80 were assigned to a father (75.5%) and 93 were assigned to a parent pair with a trio confidence level of 95%. The term “trio confidence level” refers to the probability that the two adults are the parents of the particular neonate in question. During parentage analysis, when parent pairs were assigned to each offspring, it was also discovered that males and females from different source populations successfully mated, producing admixed offspring. Of the 27 confirmed fathers in the study, 13 (48%) were found to have sired offspring in multiple litters. The largest litter of a female *T. adelaidensis* was five neonates, the maximum litter size recorded to date for this species. The highest reproductive output for a *T. adelaidensis* male was 14 neonates with a total of five females, with the second highest male reproductive output being seven neonates with four different females. Of the 93 parent-offspring assignments retained in the analysis, multiple paternity was found in 11 of the 32 litters (34%). The mean neonate body size (SVL) was 43.17mm +/- 4.92 and the mean neonate mass was 1.49g +/- 0.19.

Predictors of female reproductive success

Thirty-two females were confirmed as mothers and of these 32, only 25 were caught in spring allowing bite force data to be recorded (female n = 25). The most promiscuous females produced offspring by 3 different males and the mean clutch size per female (+/- 1 SD) was 2.88 (SD = 1.04, range = 1-5).

Body condition was the only significant predictor of reproductive success for females *T. adelaidensis* in this study, with females in higher body condition producing more offspring (Table 1, Fig. 2). As the lone predictor in a linear model, there was a significant effect of body condition on reproductive success, explaining 45% of the variation ($F_{1,23} = 18.72$, $p = 0.0002$, $R^2 = 0.45$). However, the model that explains the largest proportion of variation in reproductive success (indicated by the R^2 value) included SVL, bite force, body condition, population, heterozygosity and head length (RR) (Model: $F_{6,18} = 3.526$, $p = 0.017$, $R^2 = 0.54$) although body condition remained the only significant predictor ($p = 0.003$).

Table 1: Results of a linear regression to identify factors associated with reproductive success as indicated through number of offspring produced by female *T. adelaidensis*. Bold p-value denotes a significant result, p< .05.

Effect	**Estimate**	**Std error**	**t-value**	**p-value**
SVL	0.023	0.047	0.480	0.637
Bite Force	-0.085	0.095	-0.891	0.385
Body condition	5.988	1.781	3.363	**0.003**
Heterozygosity	0.759	1.747	0.435	0.669
Population - Clare	0.954	0.615	1.552	0.138
Population - Jamestown	0.618	0.525	1.176	0.255

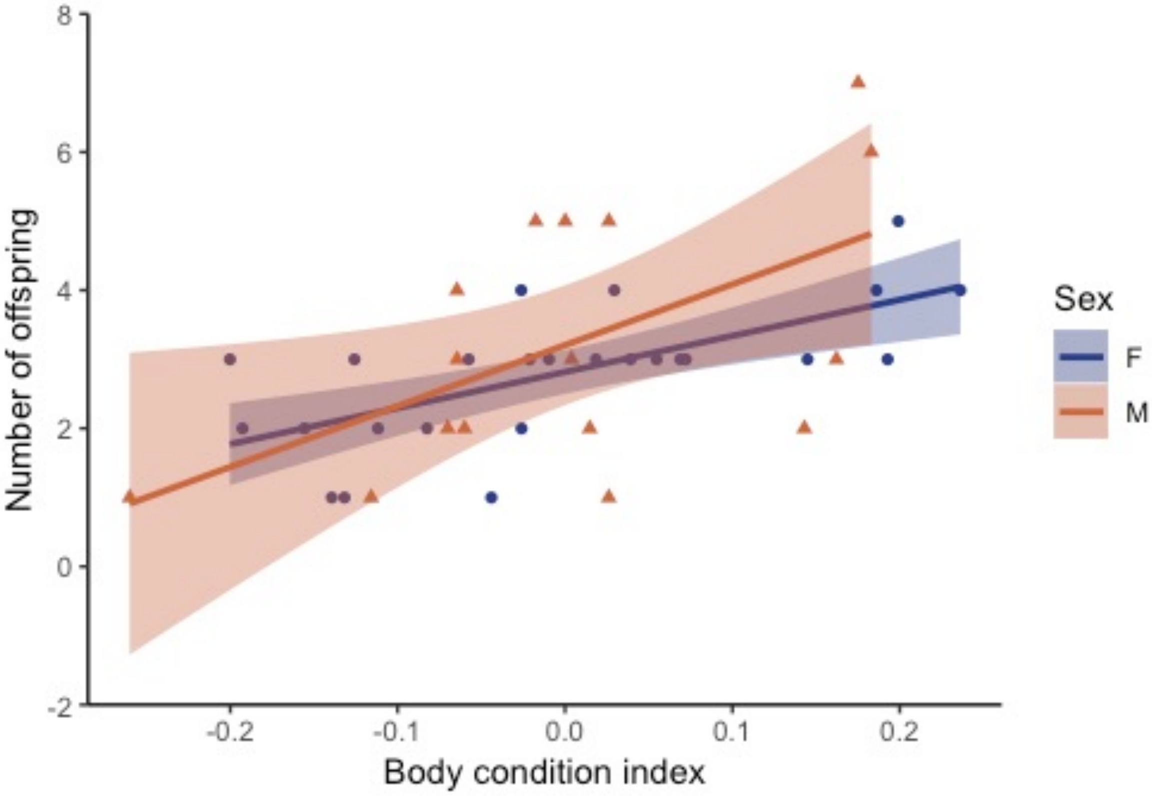

Fig. 2: Scatter plot showing the positive relationship between body condition index and reproductive success in *T. adelaidensis*. Males: filled triangles. Females: filled circles

Females with longer heads had higher body condition (Fig. A1) and females with higher body condition exhibited higher reproductive success (Fig. 2) but females with longer heads do not have higher reproductive success (Fig. A2), body condition is the driving factor in reproductive success for female *T. adelaidensis*. Larger females, in terms of SVL, develop longer heads (Fig. A3) but larger females do not have higher body condition (Fig. A4) or higher reproductive success (Fig. A5). And whereas larger females do bite more forcefully (Fig. A6), they do not benefit from higher reproductive success (Fig. A7), highlighting the importance of body condition in female reproductive success.

Predictors of male reproductive success

As with the females, males were confirmed as fathers using microsatellite genotypes in parentage analysis. Twenty-eight males were confirmed as fathers and of these, 16 were sampled in spring and retained in this analysis (male n=16). The most promiscuous male sired 14 neonates with 5 separate females (the second most promiscuous male fathered 7 neonates by 4 females) and the mean number of offspring sired by a male in (+/- 1 SD) was 3.36 +/- 2.71 (range 1-14).

For male *T. adelaidensis*, individual heterozygosity was negatively associated with reproductive success. Males with lower levels of heterozygosity sired more offspring although the relationship was weak with a lot of statistical variation in the data (Table 2, Fig. 3). The model including SVL, bite force, body condition and population was significant (Model: $F_{6,9} = 4.273$ $p = 0.026$, $R^2 = 0.74$), with heterozygosity being the only significant predictor of reproductive success for male *T. adelaidensis* ($p = 0.017$). Although our data showed that males with lower individual heterozygosity had higher reproductive success, females do not appear to be selecting potential mates based on this characteristic (Fig. A8).

Table 2: Results of a linear regression to identify factors associated with reproductive success as indicated through number of offspring sired by male *T. adelaidensis*. Bold p-value denotes a significant result (p< .05)

Effect	Estimate	Std error	t-value	p-value
SVL	-0.160	0.080	-1.998	0.077
Bite Force	0.149	0.197	0.758	0.468
Body condition	5.134	3.985	1.289	0.230
Heterozygosity	-7.173	2.444	-2.935	**0.017**
Population - Clare	1.938	1.119	1.732	0.117
Population - Jamestown	1.898	1.107	1.714	0.121

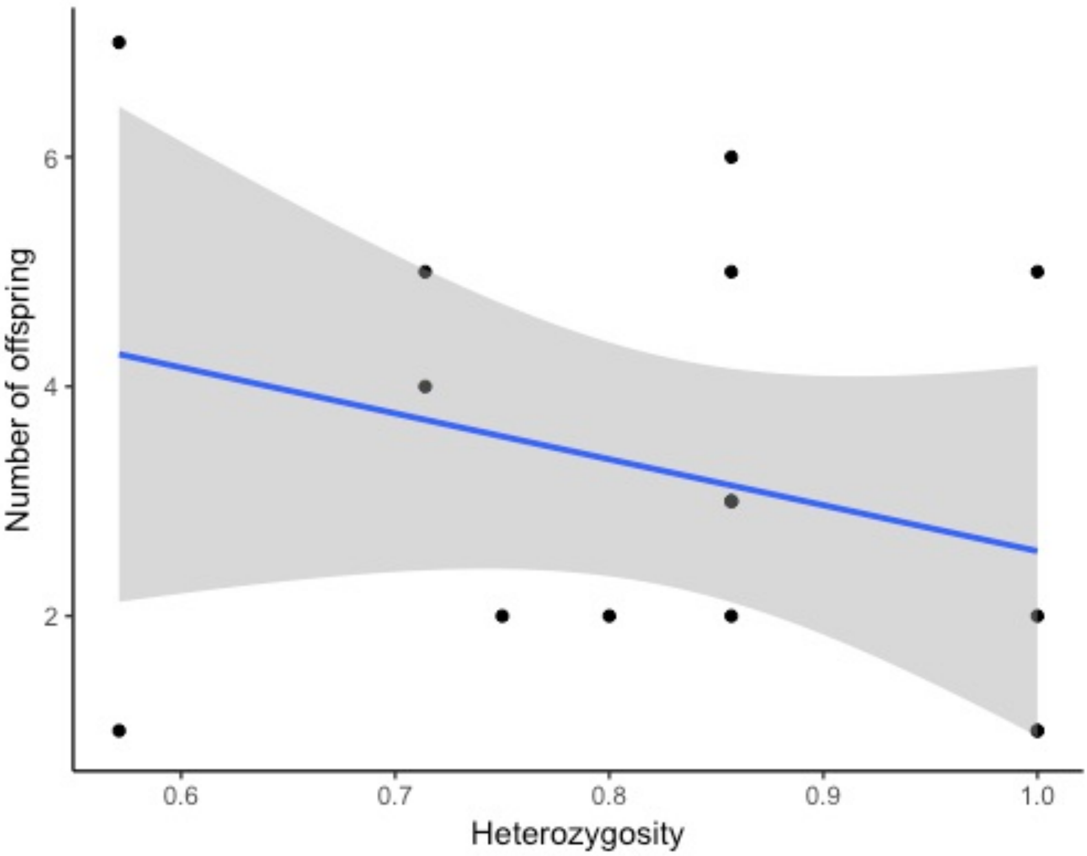

Fig. 3: Scatter plot showing the negative relationship between microsatellite heterozygosity and reproductive success of male *T. adelaidensis*.

DISCUSSION

The aim of our study was to assess the relationship between weapon size, performance and reproductive output (our proxy for reproductive success) in a lizard lineage (skinks) that is commonly found to exhibit sexual dimorphism. We found that predictors of reproductive success varied between the sexes in *Tiliqua adelaidensis*. We did not find support for the hypothesis that larger females would have higher reproductive success. Instead, we found that females with better body condition produced the most offspring. We also found no support for our hypotheses that more forcefully biting males, with wider heads, would sire more offspring. Weapon size, weapon performance and body size did not predict reproductive success for either sex in this study. Instead we found a weak negative relationship between individual male microsatellite heterozygosity and reproductive output, with more heterozygous males fathering fewer offspring. However, we must treat this finding with caution. Alleles were pooled at loci TrL21 and TrL29 in order to avoid type 1 errors, and this will have resulted in a reduction in heterozygosity estimates. Due to overlapping size distributions in the species it was also difficult to identify the sex of non-breeding individuals which prevented us from asking deeper questions regarding which individuals did not successfully reproduce and relating those data to the numerous factors we measured in this study. *Tiliqua adelaidensis* is an outbred species due to high heterozygosity, which is maintained by its promiscuous mating system, and as a result heterozygosity-based mate choice may not be especially advantageous in this species. There is low variation in the reproductive success of males and females which is consistent with the findings of Schulte-Hostedde, Millar and Gibbs (2004) who also examined reproductive success in a promiscuous species with female-biased sexual size dimorphism.

Bite force performance was not a predictor of reproductive success for male *T. adelaidensis* in this study (Fig. A7) which is consistent with the findings of Huyghe et al. (2014) in their study of another promiscuous lizard (*Podarcis melisellensis*). However, there is a weak positive relationship and a larger sample size may have helped us to investigate more conclusively. Future studies would need to obtain a larger male sample size, but this is problematic as adult males disperse to find mates during the breeding season which makes them very difficult to detect. Our data accord with those from other studies in finding that bite force performance is not always a predictor of reproductive success among lizards where male-male combat is not an activity essential to achieving mating success (McBrayer and Anderson 2007). Huyghe et al. (2012) showed that, whereas a forceful bite may enable that individual to win agonistic interactions with rivals, it does not mean that females will preferentially mate with those individuals possibly due to potential physical injury to themselves from male delivered bites during coitus.

Many species exhibit female-biased sexual size dimorphism as explained by Darwin's *fecundity-advantage* hypothesis (Becker and Paulissen 2012; Cox *et al.* 2007; McBrayer and Anderson 2007). However, in *T. adelaidensis*, it is body condition rather than body size that is the key indicator of female reproductive success which is in contrast to previous findings in this species that larger bodied females (SVL) produced larger litters (Shamiminoori *et al.* 2015). The ability of a female to produce larger or more numerous offspring is linked to her energy reserves during gestation (Castellano *et al.* 2004; Guinet *et al.* 1998). In our study, we found that females in better body condition had higher reproductive output while the offspring size (Fig. A10) and body condition (Fig. A9) remained comparable to offspring produced by females with lower body condition. In the face of limited resources, females will need to make an energetic investment trade-off between the number of offspring and the size (or "quality) of those offspring (Lawson *et al.* 2012). Larger offspring may have a higher chance of survival (Fischer and Fiedler 2001; Milligan *et al.* 2002) and so the ability of female *T. adelaidensis* to increase the number of offspring produced, while not reducing the size of those offspring, should be selectively advantageous.

Research suggests that individuals would select more genetically diverse mates to increase the fitness of the offspring; the *good genes as heterozygosity* hypothesis predicting a preference for genetic diversity linked to fitness-related traits (Landry *et al.* 2001; Pearson *et al.* 2017). It is currently unclear whether the loci in the present study are representative of whole genome-wide diversity. Although Vali et al., (2008) suggest the possibility of a link between microsatellite and genome-wide diversity, it is not corroborated by Huchard et al. (2010). There is a well-documented link between fitness and MHC gene diversity, with sexual selection favouring optimally diverse individuals rather than maximally diverse, meaning the combination of genes could more important than diversity itself (Kalbe *et al.* 2009). However, this link has not been made in all species and Huchard et al. (2010) suggest that some species may not be able to discriminate a potential mates genetic makeup based on pheromone odours. Lizards have a well-developed sense of olfaction and *T. adelaidensis* use scat piles for social signalling to nearby lizards (Fenner and Bull 2010) and during the breeding season female *T. adelaidensis* have been observed to use chemical cues to attract males (Ebrahimi *et al.* 2014). As a result, we expect that recognising a chemical cue of this nature would be possible for this species although it is unclear if this is how female *T. adelaidensis* decide which males to lay scent trails for. Conducting mate choice experiments with individuals of known MHC genotypes would enable us to clarify whether MHC-based mate selection exists in this species or if it is a more random process, influenced by proximity, as suggested by Schofield et al. (2014).

Potentially each sex has developed their own strategy to increase reproductive success while not selecting for particular characteristics during mate choice. Mate guarding has been observed in *T. adelaidensis* by Ebrahimi et al., (2014) and could be a strategy employed by males in poor body condition to obtain a successful mating. Males in better body condition are more promiscuous (Fig. A14) and attain higher reproductive success (Fig. A12). Future research examining the frequency of mate guarding in years of food abundance and scarcity would allow the testing of this hypothesis. Lailvaux and Irschick (2006) found that in the promiscuous sand lizard (*Lacerta agilis*), females did not select males based on weapon size or performance. Olsson and Madsen (1995) did not find a definitive form of mate choice and suggest that in a non-territorial species without parental care, the only resource available for a female to base mate choice on is the perception of 'good genes'. If the weapon itself plays little part in the mating process, it will provide little advantage to a male securing a mating. Olsson et al. (2002) were also unable to find evidence of selection for head size in the snow skink (*Niveoscincus microlepidotus) but* did find positive selection for trunk length in females which is similar to the findings of this study where female *T. adelaidensis* are known to be more elongate than males due to the presence of an extra presacral vertebrae (Greer 1989; pp171, Table 3). However, the extra presacral vertebrae present in females is one of the sources of sexual dimorphism in the species but this is true of females of all sizes; larger females do not have more vertebrae than smaller females.

Identifying factors that result in increased reproductive success is key for the conservation of small populations, vulnerable to stochastic events in a fluctuating environment. Although this study did not find a link between weapon size and weapon performance with reproductive success, it represents valuable information regarding factors that could be key in increasing the reproductive capacity of captive and wild populations.

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APPENDICES

FIGURES

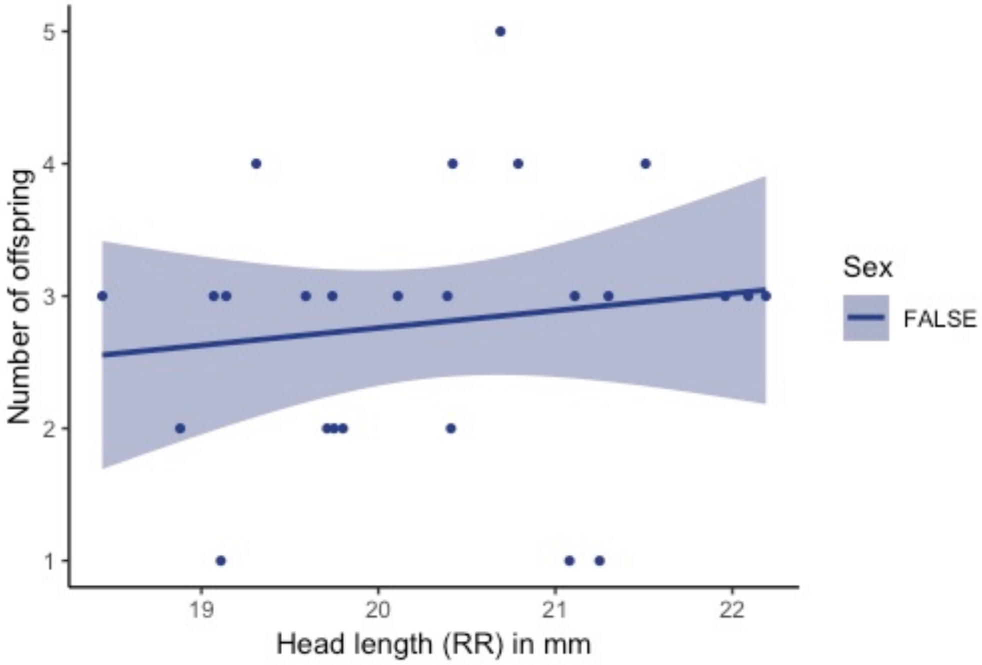

Fig. A1: Scatterplot showing the positive relationship between head length (RR) and body condition index in female *T. adelaidensis*

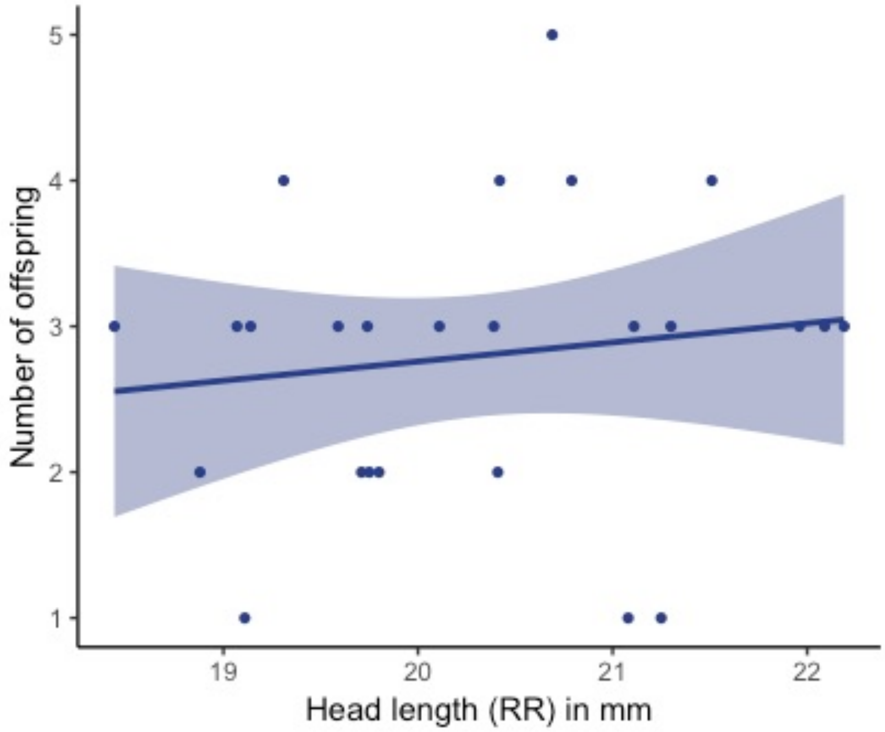

Fig. A2: Scatterplot showing the lack of relationship between head length (RR) and reproductive success (indicated by number of offspring) in female *T. adelaidensis*

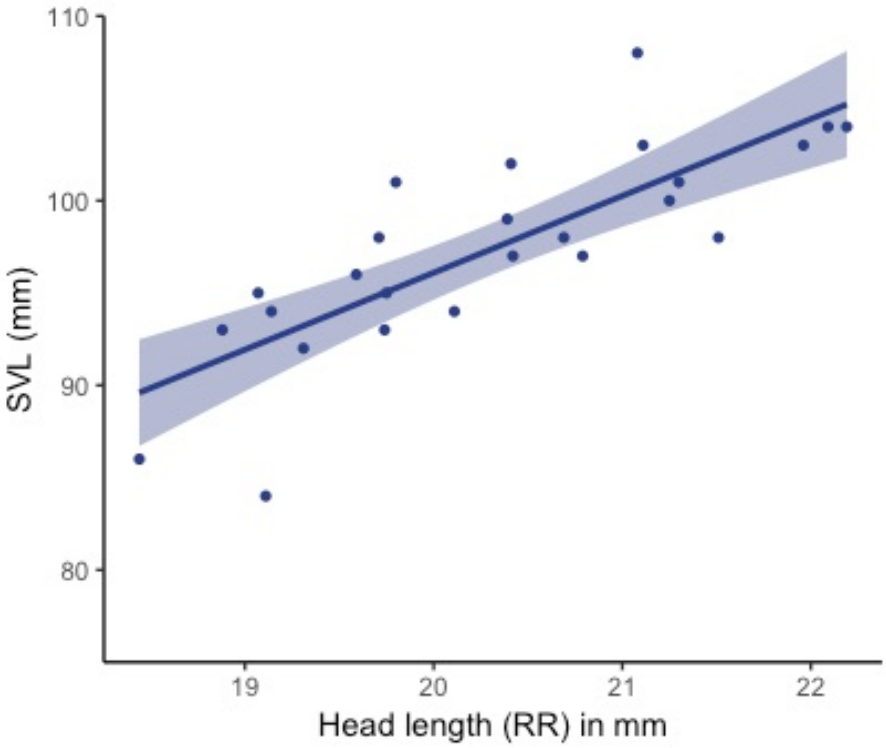

Fig. A3: Scatterplot showing the positive relationship between head length (RR) and body size (SVL) in female *T. adelaidensis*

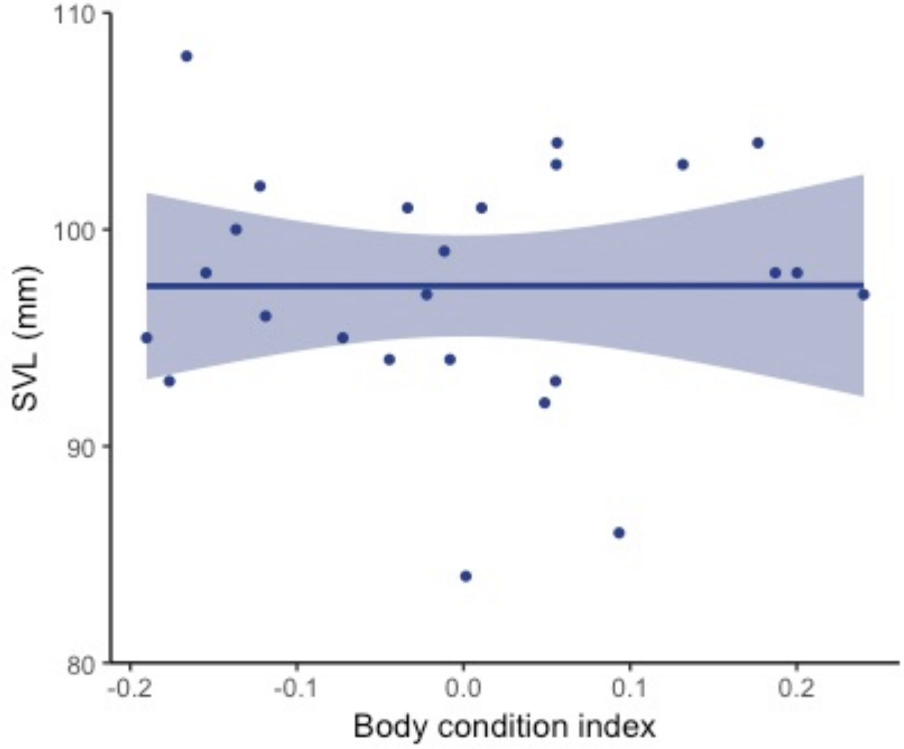

Fig. A4: Scatterplot showing the lack of relationship between body condition and body size (SVL) in female *T. adelaidensis*

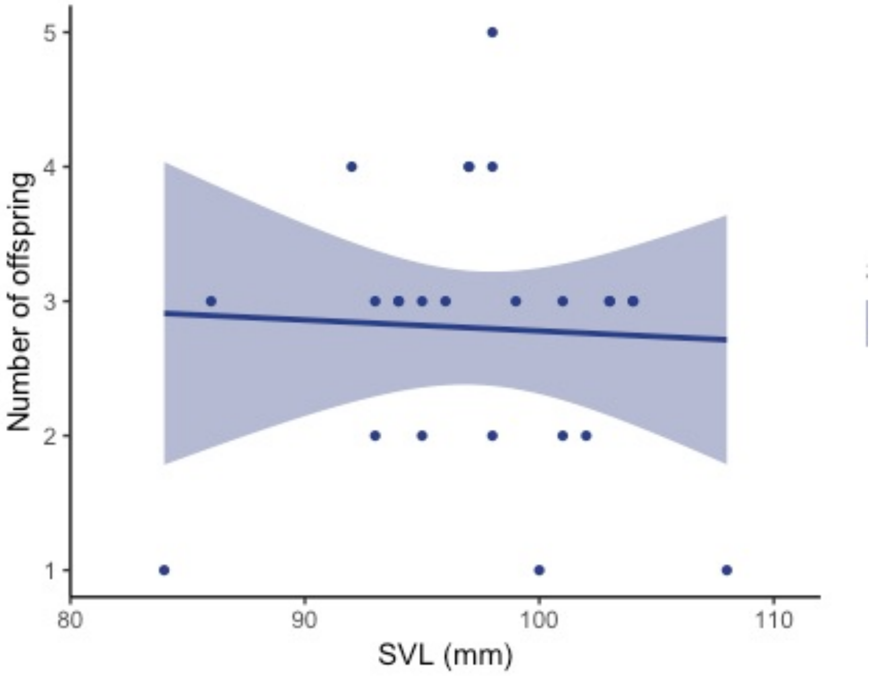

Fig. A5: Scatterplot showing the lack of relationship between body size (SVL) and reproductive success in female *T. adelaidensis* (indicated by number of offspring)

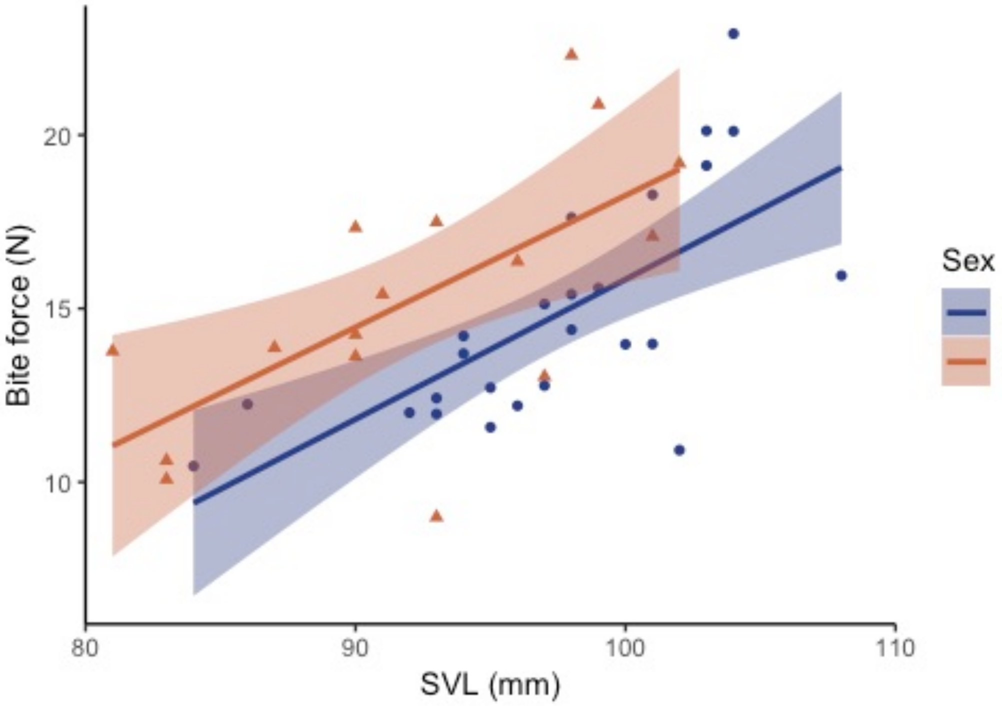

Fig. A6: Scatterplot showing the positive linear relationship between bite force (N) and body size (SVL) in *T. adelaidensis.* Males: filled triangles. Females: filled circles

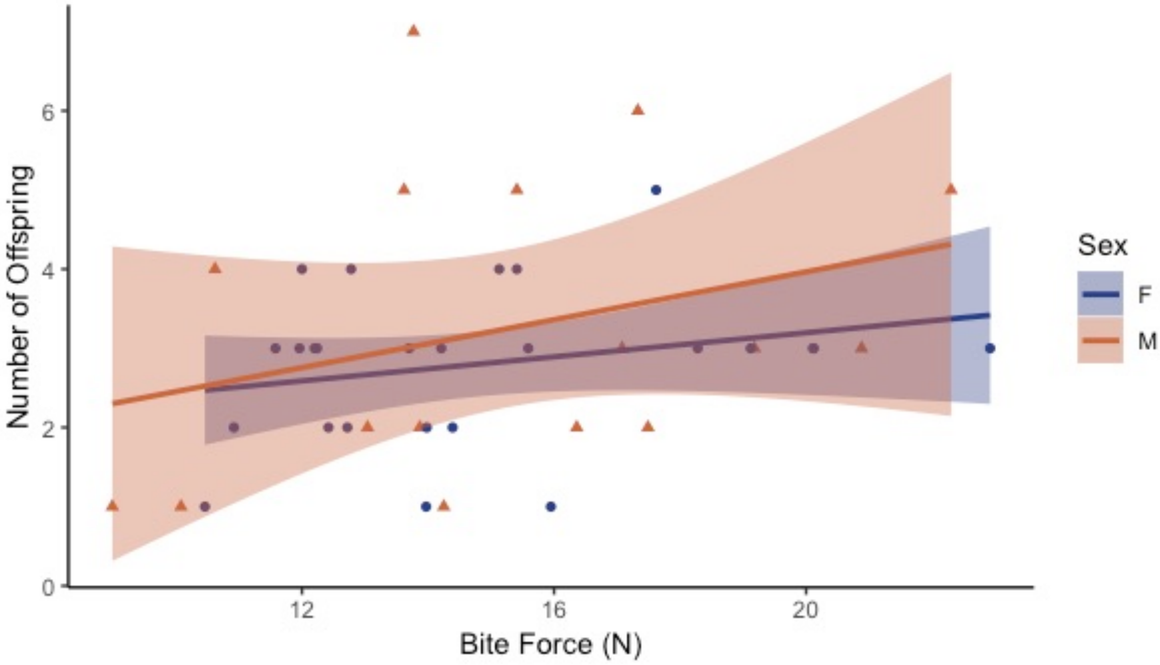

Fig. A7: Scatterplot showing the relationship between bite force (N) and reproductive success (indicated by number of offspring). Males: filled triangles. Females: filled circles

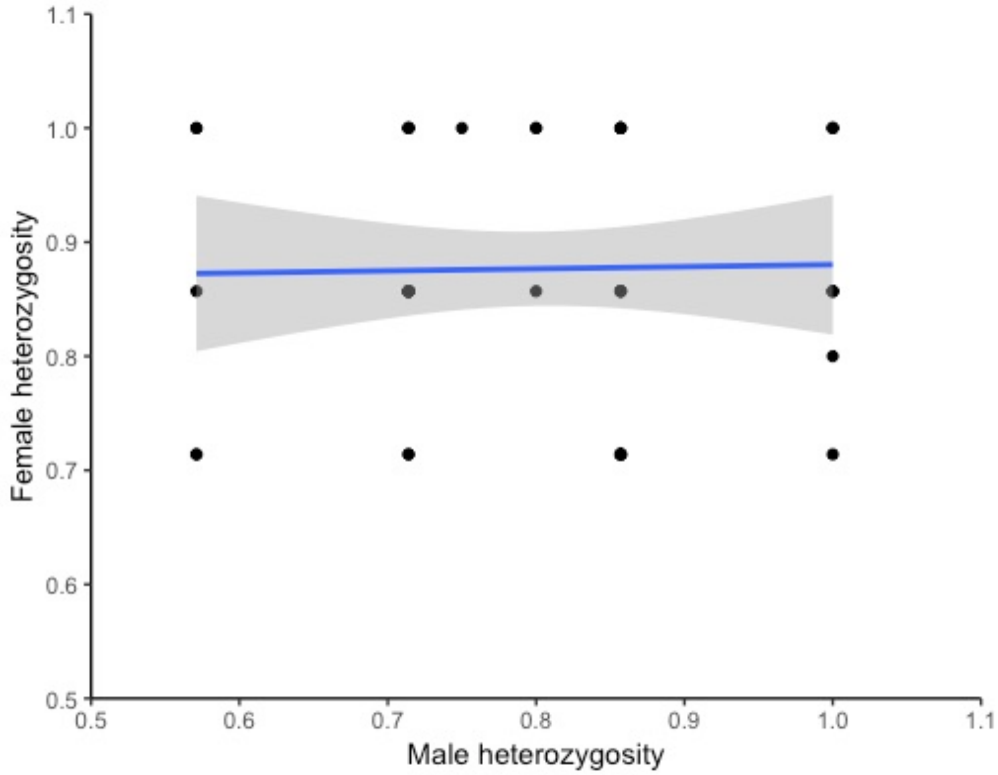

Fig. A8: Scatter plot showing the lack of relationship between the microsatellite heterozygosity of mated male and female pairs. Individual heterozygosity was calculated as the proportion of heterozygous loci (PHt)

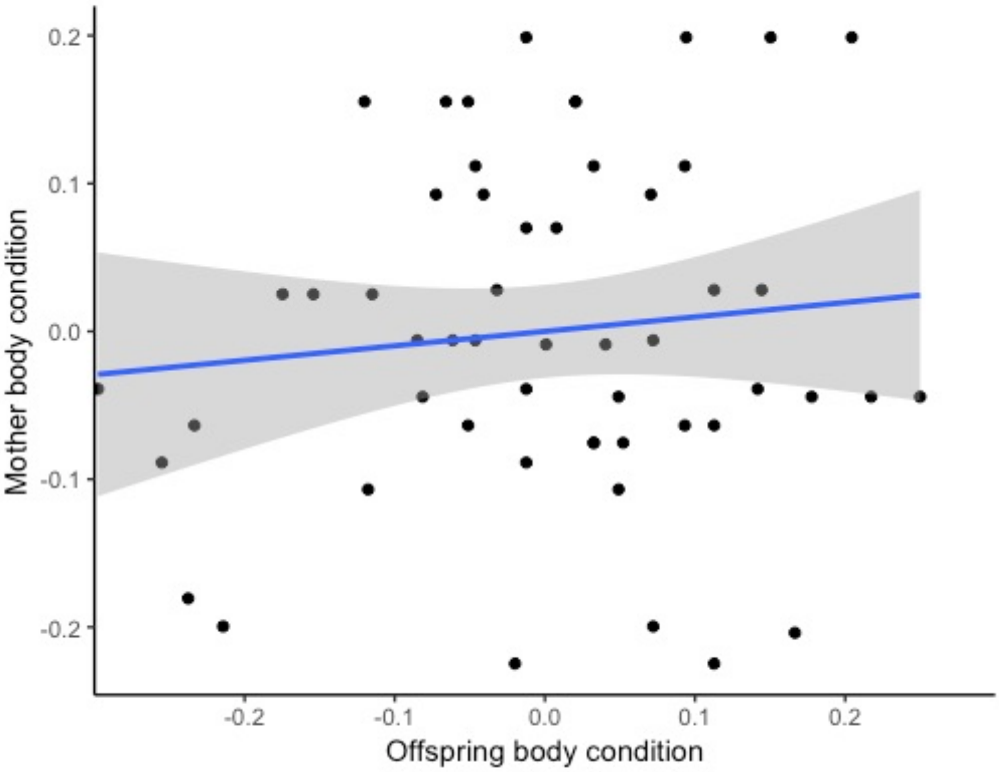

Fig. A9: Scatterplot showing the lack of relationship between mother's body condition index and offspring body condition.

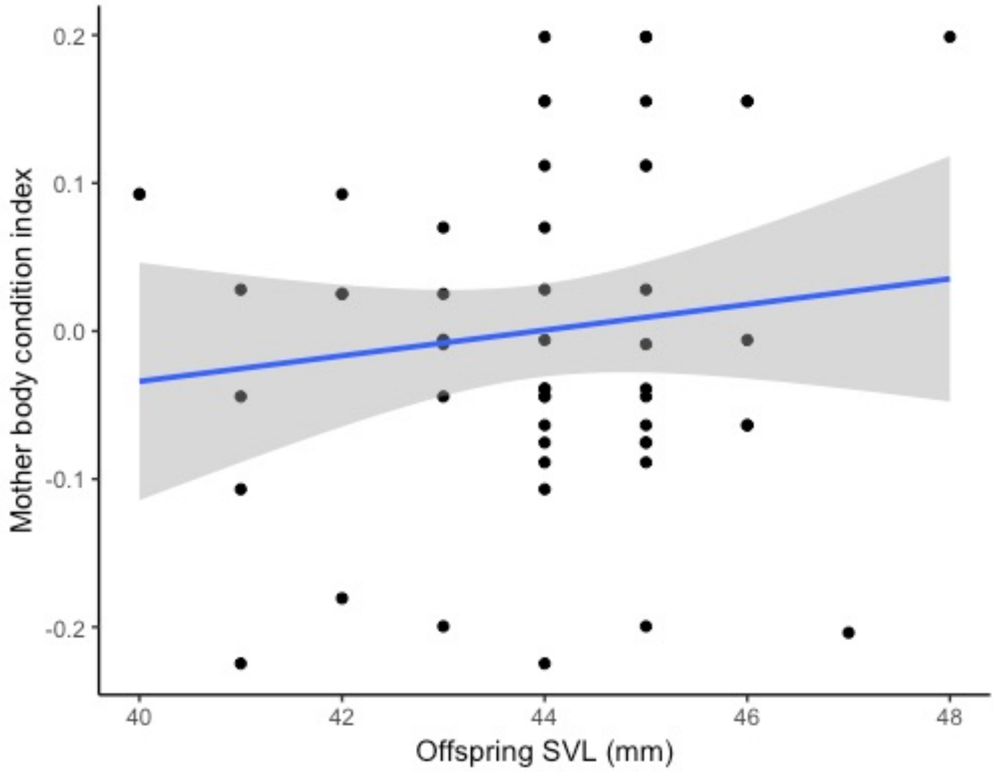

Fig. A10: Scatterplot showing the lack of relationship between mother's body condition and offspring body size (SVL).

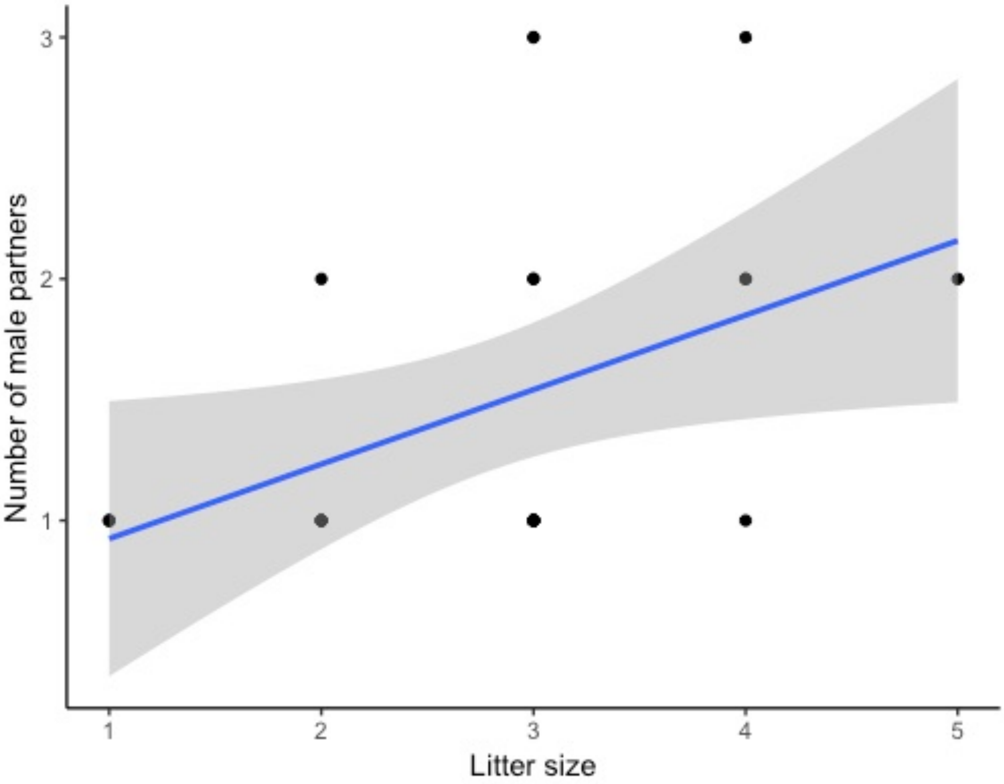

Fig. A11: Scatterplot showing the positive relationship between number of male partners and litter size produced by female *T. adelaidensis*.

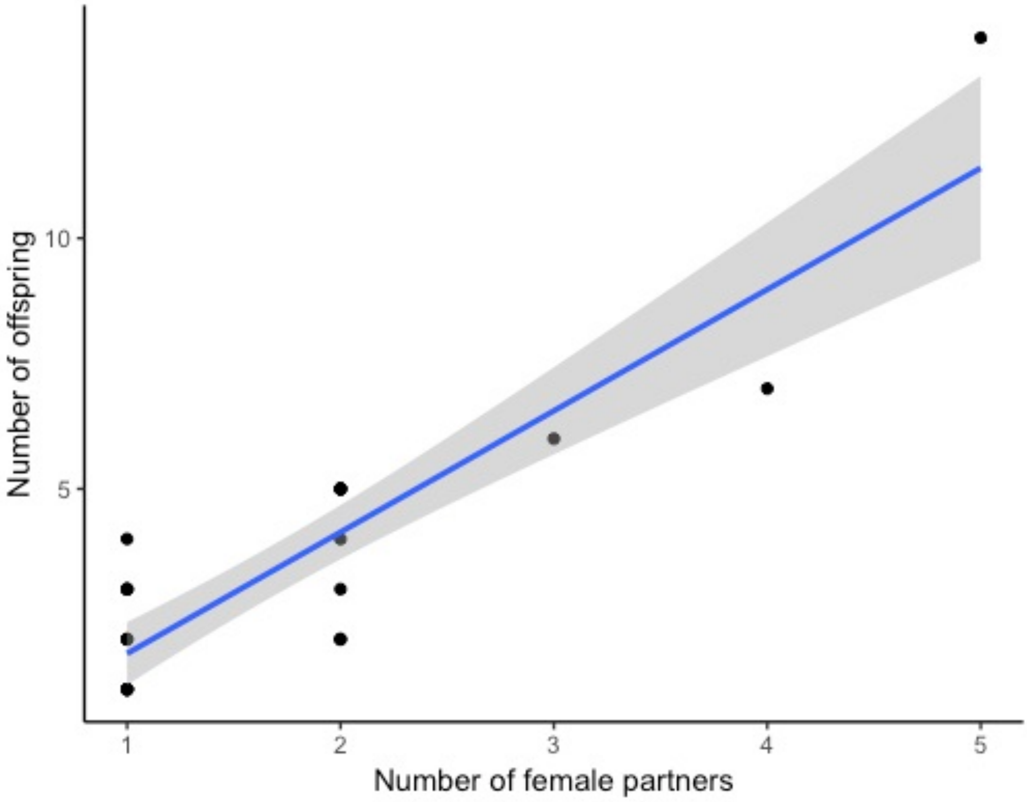

Fig. A12: Scatterplot showing the positive relationship between the number of female partners and reproductive success in male *T. adelaidensis* with more promiscuous males exhibiting higher reproductive success

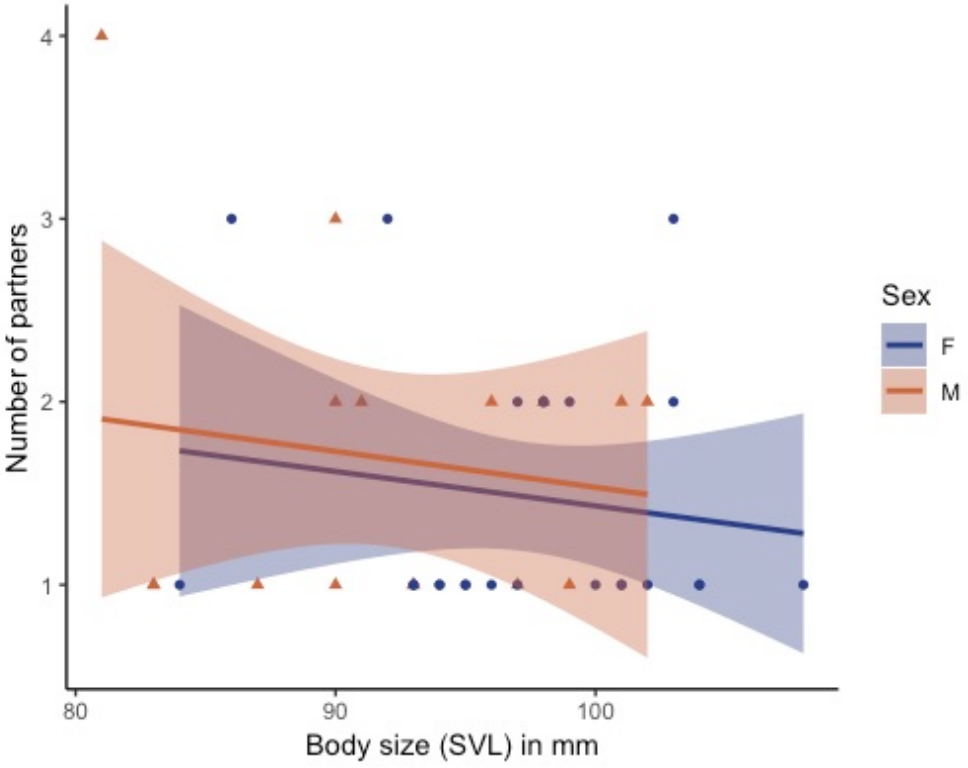

Fig. A13: Scatter plot showing the lack of relationship between number of partners an individual mates with against body size (SVL in mm) showing that larger individuals are not more promiscuous. Males: filled triangles. Females: filled circles

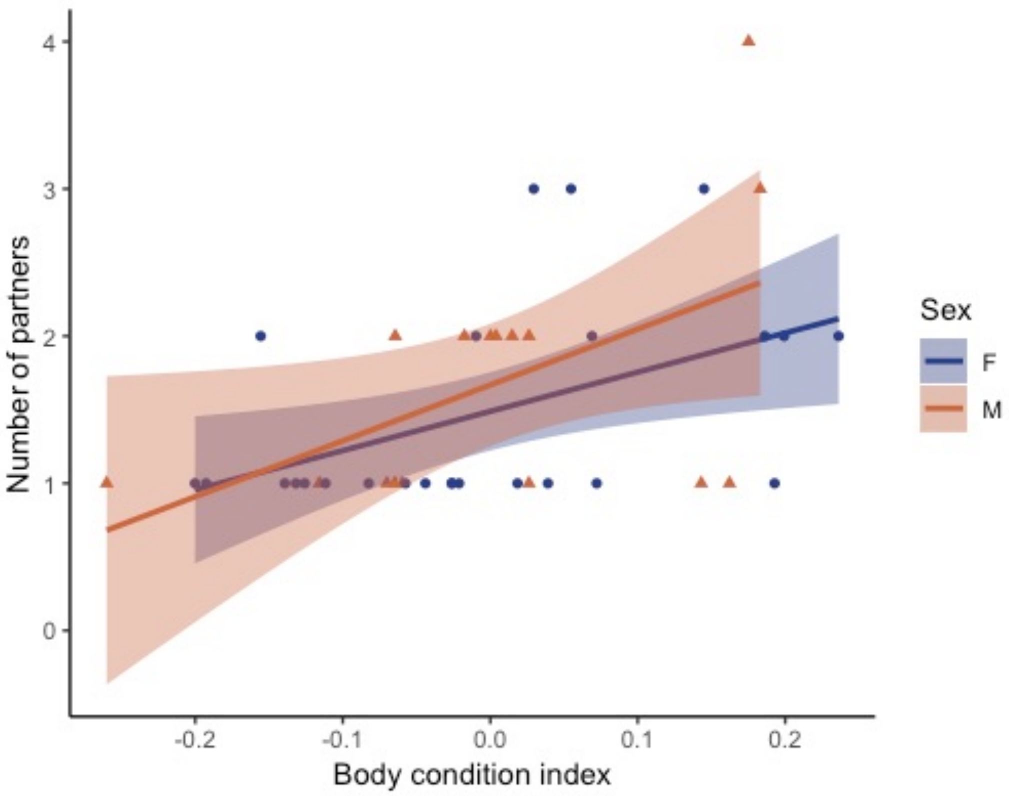

Fig. A14: Scatter plot showing the positive relationship between body condition and the number of partners mated with indicating that individuals in better body condition are more promiscuous. Males: filled triangles. Females: filled circles

CHAPTER 5

ADMIXTURE

A pair of sibling neonate pygmy bluetongues. Colour patterns exhibited are representative of the colour pattern polymorphism within the Burra population of this species

PREFACE

This chapter has been prepared for publication in Conservation Genetics.

In the previous chapter, I established that individuals from genetically differentiated source populations would successfully breed, an important finding if population reinforcements are to be used for conservation of this species. In addition, my results showed successful reproduction in the first-year post-translocation which is another positive sign for use of reinforcement as a conservation strategy. In this chapter, I explore the indirect fitness of the resulting offspring in order to determine whether admixed offspring grow and perform differently to unmixed offspring.

Concerns about the genetic risks of mixing populations are well documented although rarely tested. Frankham et al., (2011) deemed the likelihood of admixed offspring exhibiting reduced fitness unlikely without the presence of fixed chromosomal differences between the parent source populations. The likelihood of fixed chromosomal differences in pygmy bluetongue populations is very low due to previous research detecting only low levels of genetic differentiation among populations (Smith *et al.* 2009), and small mtDNA differences among populations (Schofield 2015). The results of my study will provide empirical evidence to support or reject the suggestions made by Frankham (2011), Smith (2009) and Schofield (2015).

GENETIC ADMIXTURE HAS NO NEGATIVE EFFECT ON THREE FITNESS-RELATED TRAITS IN AN ENDANGERED SPECIES

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In preparation for submission to Conservation Genetics.

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ABSTRACT

Translocations for conservation purposes do not have a high rate of success, underpinning the need for anticipatory research to investigate potential sources of failure, or risk, before movements are carried out. Here we investigated the risk posed by population reinforcement by augmenting an existing population of an endangered species with individuals from two geographically and genetically distinct populations. We moved 24 individuals of the pygmy bluetongue lizard (*Tiliqua adelaidensis*), from two separate populations into *in-situ* enclosures at a third site containing resident conspecifics in a patch of native grassland habitat north of Adelaide, South Australia. The populations were allowed to freely interbreed, and the progeny monitored for potential adverse impacts by assessing whether growth and performance differed between admixed and unmixed lineages. In the activity-season following the translocation, we collected all offspring produced and transferred them off-site to a captive holding area for monitoring. We assessed three fitness-related traits: body condition, growth rates, and bite force, and found no evidence to suggest that admixed lineages perform differently to unmixed, intrapopulation conspecifics in the short term. Although this study uncovered slight differences in performance, the extent of variation in mixed lineage offspring remains comparable with that in unmixed offspring. This novel, pre-emptive study can be used as an approach for other practitioners considering population augmentation where concerns exist regarding the potential negative impacts such movements may cause.

INTRODUCTION

Populations of organisms can become isolated through many mechanisms, existing in small fragments of habitat with little to no gene flow occurring between them (Kronenberger et al., 2018). As a result, these populations can suffer from inbreeding depression and genetic bottlenecks, in addition to being vulnerable to stochastic events (Becker *et al.* 2016; Seddon 2010). Consequently, reinforcements (“population augmentations”) where individuals are moved to supplement existing populations, can be undertaken to restore gene flow and maintain the genetic diversity and adaptive potential of that population, while preserving the genetic integrity of the population itself (IUCN 2013; Day *et al.* 2017; Swaisgood 2007; Rius and Darling 2014; Frankham 2016). Although use of conservation translocations is increasing, many movements are conducted without anticipatory research to inform the decision-making process and failures are common (Hoegh-Guldberg *et al.* 2008; Fischer and Lindenmayer 2000; Weeks *et al.* 2011; Fawcett *et al.* 2010).

Population augmentation produces positive outcomes such as hybrid vigour or increased genetic diversity (Rius and Darling 2014), or alternatively the outcomes may be negative such as the introduction of disease to the recipient population (Aiello *et al.* 2014). Negative impacts such as the creation of invasive genotypes and outbreeding depression of fitness have been reported but found to be temporary, reduced fitness increasing over time (Frankham *et al.* 2011; Hamilton and Miller 2015; Mock *et al.* 2004). Outbreeding depression, a greatly feared but rarely recorded risk, is entirely measurable and predictable (Frankham *et al.* 2011; Edmands 2007) and has been commonly discussed but rarely empirically tested (Facon *et al.* 2005; Storfer 1999; van Oppen *et al.* 2014; Weeks *et al.* 2011; Wolfe *et al.* 2007). Many species in need of population reinforcement may only have genetically differentiated donor populations, highlighting the need for the risks of such movements to be empirically tested, so that action may be taken with confidence rather than inaction due to fear of the unknown (Kronenberger et al., 2018).

Assessing fitness requires long-term monitoring, over the lifespan of the individuals. However, the use of fitness-related traits (“proxies”) is more tractable for short-term studies (Clutton-Brock and Sheldon 2010). To date, researchers have used a variety of ecologically relevant traits as potential indicators of fitness including: survival (Violle *et al.* 2007; Edmands *et al.* 2005); reproductive success (Le Galliard *et al.* 2004); bite force (Huyghe *et al.* 2014); body condition and growth rates (Civantos and Forsman 2000) or genetic diversity (Averill-Murray and Hagerty 2014). Organisms with high growth rates can move through vulnerable life stages more quickly and reach sexual maturity sooner (Sinervo and Adolph 1994; Civantos and Forsman 2000). Identification of traits allowing offspring to reach sexual maturity earlier, and possibly having a higher chance of reproductive success should be key for conservation of vulnerable species as mortality at the juvenile life-stage prevents individuals from reaching sexual maturity (Audet *et al.* 2017).

Study species

The endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*, exists in small, isolated patches of native grassland, north of Adelaide, South Australia (Hutchinson *et al.* 1994). Gene flow is restricted between populations more than 1.7km apart (Smith *et al.* 2009). While low to moderate levels of genetic differentiation have been found (Smith *et al.* 2009), fixed chromosomal differences are unlikely and no fitness or phenotypic differences have been detected (Schofield, 2015; Shamiminoori, Fenner, & Bull, 2014), indicating a low risk of outbreeding depression for this species. Habitat suitability modelling, in response to climate change, has highlighted the need for managed relocations due to likely extinction of the species by the end of this century, with northern populations being most vulnerable (Fordham *et al.* 2012). In view of the predicted decline of available habitat, future persistence of the species will likely depend on the use of managed relocations (Fordham *et al.* 2012). Evidence-based decision making to assist conservation of the species is facilitated by extensive a priori species knowledge from 25 years of research, to use population augmentation to supplement declining southern populations with northern animals adapted to hot and dry conditions.

However, research to understand the potential risks associated with population augmentation needs to be conducted before this strategy should be implemented. To that end, this study aimed to assess the impact of mixing *T. adelaidensis* populations using three ecologically relevant fitness-related traits: growth rates, body condition and bite force as an indicator of whole organism performance. We compared these three fitness proxies between mixed-lineage (“admixed”) offspring and un-mixed recipient population conspecifics through regular monitoring of the individuals in a controlled setting. Admixed and intrapopulation offspring were identified through microsatellite genotyping parentage analysis with parental source population determining the subsequent lineage each offspring was assigned. We hypothesised that there would be no difference in these traits among the lineages. This study presents a novel approach that can be used by conservation practitioners to pre-emptively assess the risks posed by population reinforcement.

METHODS

Study site

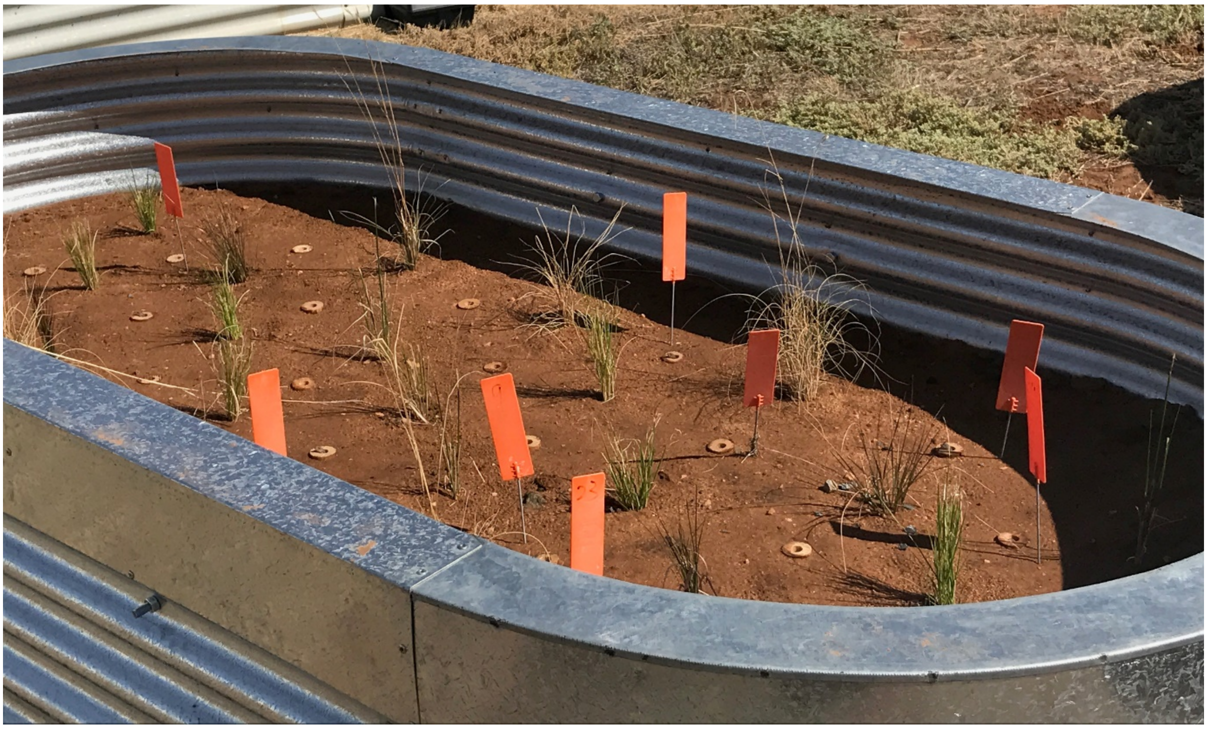
The study was carried out over two consecutive Austral spring/summer periods (Oct – Feb) of 2015 - 2017; at the Nature Foundation 'Tiliqua' site, Burra approximately 160km north of Adelaide, South Australia. Enclosures (3 pairs of 30 x 30m enclosures) were constructed at the field site, around an existing population of resident lizards, during the winter prior to data collection to prevent lizards from escaping during the study. Each pair of enclosures consisted of a control enclosure (only resident lizards), and an adjoining experimental enclosure (resident and translocated lizards). The roofless enclosures were constructed from sheet metal with walls 20cm high with in inward facing 5cm lip at a 45-degree angle. Field methods and handling were all as approved by Flinders University Animal Welfare Committee (E417-15 & E453-17), Wildlife Ethics Committee (project number 22/2016) and Department for Environment and Water (scientific licence G25011-10 and G25011-14).

Translocation

Following Ebrahimi and Bull (2014), at the end of the activity season in February 2016 we collected adults lizards from the Jamestown area (70km north of Burra) and the Clare area (30km west of Burra) and translocated them to the recipient site in Burra. Adults were identified as individuals with a SVL of 80mm or greater (Milne 1999) and we ensured that none of the females were gravid at the time of capture. Each experimental enclosure received eight translocated lizards, four from each population, with lizard density in those enclosures being increased by 53% - 73%. Control enclosures remained unaugmented.

Data collection

The lizards in the experimental enclosures mated in spring of the following activity season, October 2016. Gravid females were sighted every second day during January 2017 in anticipation of offspring, resulting in neonates often being collected from the natal burrow the day after birth. The first offspring were detected and caught on 10th February 2017. Lizards were caught directly from their natal burrow using a previously described technique using a fishing rod and mealworm lure (Milne and Bull 2000). Each animal was toe clipped, providing a unique ID, as well as allowing blood and tissue samples to be taken. Blood was collected onto Whatman® Classic and Elute FTA paper while toes were stored in vials of 95% ethanol and refrigerated post sampling. After capture the neonates were transferred to Monarto Zoological Park (n = 81) and housed in custom built enclosures containing artificial burrows and planted native grasses with their feeding regime controlled and standardised for all individuals.



Custom built enclosures at Monarto zoo containing 8 neonate pygmy bluetongues (each burrow containing a lizard is marked with an orange marker)

After torpor during the winter months, each lizard was monitored at the beginning (October 2017), and the middle (December 2017), of the following activity season. During each sampling period, mass, body size, head measurements and bite force performance were recorded to assess three proxies for fitness: body condition, growth rate and bite force. Mass was measured with a 50g spring loaded Pesola scale, and snout-vent-length (SVL) was recorded (to the nearest 1mm) using a transparent plastic ruler. Six external head measurements were recorded using digital callipers, to the nearest 0.01mm (Fig. A1). Bite force, measured in Newtons, was recorded using a Kistler force transducer, connected to a charge amplifier, that consisted of two parallel plates. To protect the animals' teeth during testing, the plates were covered with leather strips, measuring (L) 15 mm x (W) 5 mm x (D) 0.35 mm (Jones and Lappin 2009; Anderson *et al.* 2008; Lappin and Jones 2014). Amplifier output of the assembled transducer was calibrated as per Lappin & Husak (2005) and with readings following a linear relationship ($r^2 = 0.99$). Bite forces were standardised through calculation of the bite out-lever (Jones and Lappin 2009) using ImageJ software (https://imagej.net). Four defensive bites were recorded for each animal, with a 1-minute rest between bouts, the maximal bite recorded being used in the analysis.

A second feeding trial was conducted in the second half of this activity season, January–March 2018, to examine how the different lineages would perform in a semi-wild feeding environment. A fixed volume of crickets was released en-masse into the enclosure and the lizards were required to find their own prey mimicking feeding in the wild. The release location of the crickets within the enclosure was randomised so as not to bias results toward lizards in certain areas of the enclosures. Sampling took place at the middle (January) and end (March) of the activity season with body condition and growth data being recorded in the same way as above. Bite force, and head measurements, were not recorded during the second feeding trial.

Microsatellite amplification

All offspring were genotyped using seven previously described polymorphic loci: TrL14, TrL16, TrL 21, TrL28, TrL29, TrL32 and TrL34 (Table A1) (Gardner *et al.* 2008). DNA was extracted from toe clips using the Gentra® Puregene® method (Gentra Systems) and loci were amplified using two multiplex reactions, each reaction being 23µl in volume. Multiplex one contained 1 X MRT buffer, 0.08µM TrL14 forward & reverse primers, 0.12µM TrL16 forward & reverse primers, 0.08µM TrL32 forward & reverse primers, 1U Immolase enzyme and 2µl of DNA. Multiplex two contained 1 X MRT buffer, 0.24µM TrL21 forward & reverse primers, 0.12µM TrL28 forward & reverse primers, 0.12µM TrL29 forward & reverse primers, 0.12µM TrL34 forward & reverse primers, 1U Immolase enzyme and 2µl of DNA. The forward primer of each pair was labelled using the fluorescent dyes FAM, VIC or PET (Applied Biosystems). Thermal cycling conditions for all loci consisted of denaturisation at 95°C, followed by 35 cycles of 94 °C for 30 s, 60 °C for 60 s, and 72 °C for 60 s, with a final extension at 72 °C for 45 min and 25 °C for 2 min. After cleaning the PCR product and diluting the samples, they were sent to the Australian Genome Research Facility (AGRF) for capillary separation. Fragment sizes were called using GS500(-250) LIZ size standard in 'GeneMapper 4' (Applied Biosystems). Microsatellite profiles were scored using the Geneious software version 8.0.5 R8 (Kearse *et al.* 2012) to provide each individual with a unique genotype. Alleles at certain locations in loci TrL21 and TrL29 were pooled due to an inability to distinguish between them, alleles regularly occurring 1bp apart at all loci. While this is a more conservative approach, it reduced the likelihood of false paternity assignment.

Parentage analysis

The number of alleles, the number of individuals typed, the observed and expected heterozygosity's were calculated for each locus using Cervus version 3.0.7 (Kalinowski *et al.* 2007) (Table A2). Paternity assignment was conducted using Cervus with a maximum likelihood approach to assign parent-offspring relationships. For each litter, where the mother was known, a paternity analysis was conducted to identify the father of each neonate. Where the neonate was caught outside the natal burrow, and mother was unknown, we undertook a parent-pair analysis to identify both parents from the total list of adults present in that enclosure. The species exhibits a male-biased dispersal which can result in a higher rate of attrition for male lizards due to the increased vulnerability of dispersing males while travelling above ground (Schofield et al., 2012). As a result, there is a possibility that some potential fathers may have been predated post-mating but prior to sampling and were therefore absent from this dataset. A 95% confidence level ('strict') was used to determine parent-offspring relationships; parent-offspring pairs with an 80% confidence level ('relaxed') or less being excluded from the analysis to reduce the chances of a Type II error.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics 23 and all data were checked for normality prior to analysis and transformed where necessary. We calculated bite force residuals, to control for body size, during analysis involving bite force and a body condition index was calculated by taking the standardised residuals of mass against SVL. Growth rate was calculated as described by Zúñiga-Vega *et al.* (2005):

$$\text{Growth rate} = (\text{SVL}_2 - \text{SVL}_1)/\text{ number of days between captures}$$

When analysing head measurements for each individual, we used Mosimann size adjusted shape variables (Mosimann, 1970), also known as Log shape ratios (Mosimann & James, 1979), which are shown to be more reliable than residuals when analysing morphometric measurements (Jungers *et al.* 1995). Mosimann shape variables are generated by dividing each measurement by the geometric mean for each individual (Sakamoto and Ruta 2012) (Table A2).

We examined the body condition, growth rates and bite force of four offspring lineages: BB (Burra*Burra), BJ (Burra*Jamestown), BC (Burra*Claire) and JC (Jamestown*Claire). Repeated measures ANalysis Of VAriance (ANOVA) was used to investigate the effect of lineage, and month, on bite force performance and body condition over the two sampling periods (period 1- Oct& Dec'17; period 2- Jan& Mar'18). We used Mauchly's test to determine whether these data were spherical, using the Greenhouse-Geisser correction for non-spherical data. Gabriels post-hoc test was performed to further investigate any significant main effects found, chosen due to the unequal sample sizes of each of the four lineages being tested. A one-way ANalysis Of VAriance (ANOVA) was used to investigate differences in head measurements, and growth rate, between lineages.

Genetic diversity

Genetic diversity metrics were calculated to assess whether genetic diversity increased in the admixed individuals. Allelic richness and observed heterozygosity (H_o), were estimated within and compared among populations and lineages using FSTAT software version 2.9.3.2 (Goudet 1995) with analysis run using 10,000 permutations. The within population and lineage number of alleles and number of private alleles were calculated with the Microsoft Excel add-on GenAlEx version 6.5 (Peakall and Smouse 2006). The effective population size (Ne) was calculated using NeEstimator software version 2.1 (Do *et al.* 2014). The linkage disequilibrium method provided results for both Burra and Clare populations but was undefined for Jamestown. However, the heterozygote method provided results for both Clare and Jamestown populations, but for Burra was undefined. A comparison of both methods was used to compare source populations. The four offspring lineages were compared using only the linkage disequilibrium method.

RESULTS

A total of 81 neonates were transferred to Monarto zoo in Feb and Mar 2017, of which 76 survived until sampling began in Oct'17, with 71 being caught in both sampling periods. Of these 71 neonates, 33 (46.48%) were genotypically determined to be pure Burra*Burra offspring whereas the remaining 38 were of mixed-lineage: Burra*Jamestown (BJ), Burra*Claire (BC) or Jamestown*Claire (JC) (n=20; n=9; n=10 respectively). No pure Jamestown*Jamestown or Clare*Clare offspring were detected during this study and, therefore, do not appear in any of our analyses.

Bite force

To account for overall body size we used the residuals of bite force and SVL. Using these residuals, we conducted a repeated measures ANOVA where we found a significant main effect of lineage F(3,67) = 3.637, p = .017 (Table 1); post hoc tests revealing BC lizards exhibiting a greater bite force than BB lizards ($\bar{x}$ = .853, p = .003) and BJ lizards ($\bar{x}$ = .673, p = .026) (Figure 1). There was no significant main effect of month F(1,67) = .035, p=.853, nor was there an interaction effect of month*lineage F(3,67) = .206, p = .892 (Table1).

Table 1: Results of repeated measures ANalysis Of VAriance (ANOVA) for effect of month and lineage on bite force performance of *Tiliqua adelaidensis* F1 generation offspring, by lineage. Bold denotes significant effects (p< .05).

Effect	Month	Lineage	Month*lineage
df	1	3	3
F	0.035	3.637	0.206
p	.853	**.017**	.892

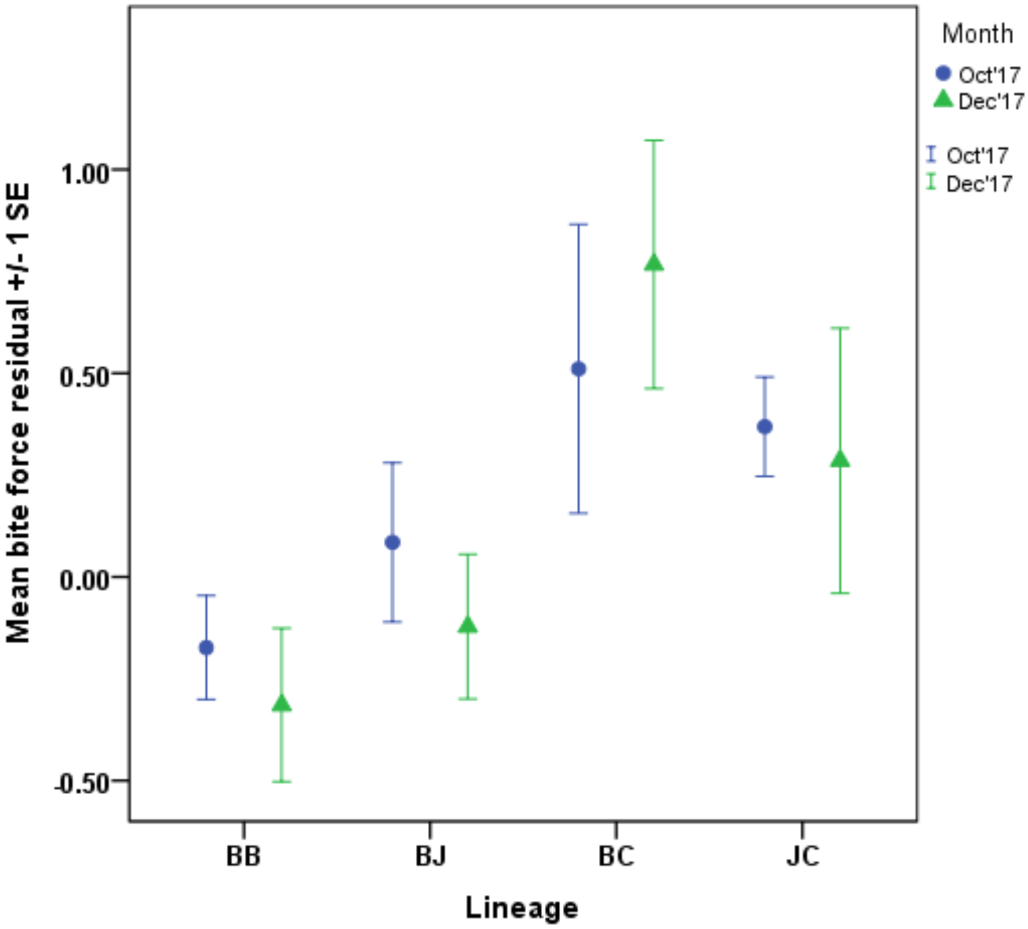


Figure 1: Mean bite force (residual) of four lineages of *Tiliqua adelaidensis* offspring. Error bars represent +/- 1 standard error. No JJ or CC offspring were collected during this study and are, therefore, absent from our analyses.

Head measurements

Through use of Mosimann shape variables, we found a significant difference in relative head width (QQ) between the lineages F(3,136) = 4.144, p = .008 (Table 2) with BC lizards having significantly wider heads than BB lizards ($\bar{x}$ = .027, p = .022), BJ lizards ($\bar{x}$ = .028, p = .048) and JC lizards ($\bar{x}$ = .041, p = .005) (Figure 2). However, BC lizards do not have absolutely wider heads than the other lineages (Figure A4). There was also a significant difference in postorbital length (temporal) between the lineages F(3,136) = 2.900, p = .037 (Table 2) with BB lizards having longer postorbital lengths than BC lizards ($\bar{x}$ = .019, p = .021) (Figure 3).

Table 2: Results of a one-way ANalysis Of VAriance (ANOVA) for effect of lineage on six head measurements. Bold denotes significant effects (p< .05). Head length (RR, RQ, temporal); head width (QQ, JJ) and head depth. RR: Ros-Ret, RQ: Ros-Qu, QQ: Qu-Qu, JJ: Ju-Ju, HD: head depth, Temporal: postorbital length

	RR	RQ	QQ	JJ	HD	Temporal
df	3	3	3	3	3	3
F	1.865	1.220	4.144	1.437	1.613	2.900
p	.139	.305	**.008**	.235	.189	**.037**

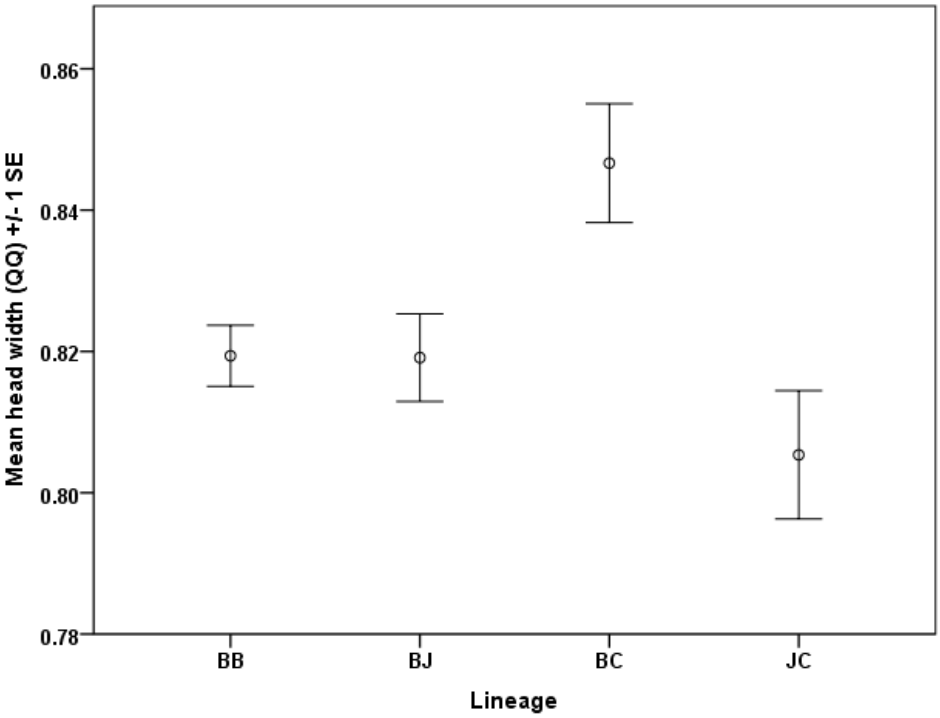


Figure 2: Mean head width (QQ) of four lineages. Head width (mm) portrayed as Mosimann size adjusted shape variable with no unit of measure. Error bars represent +/- 1 standard error.

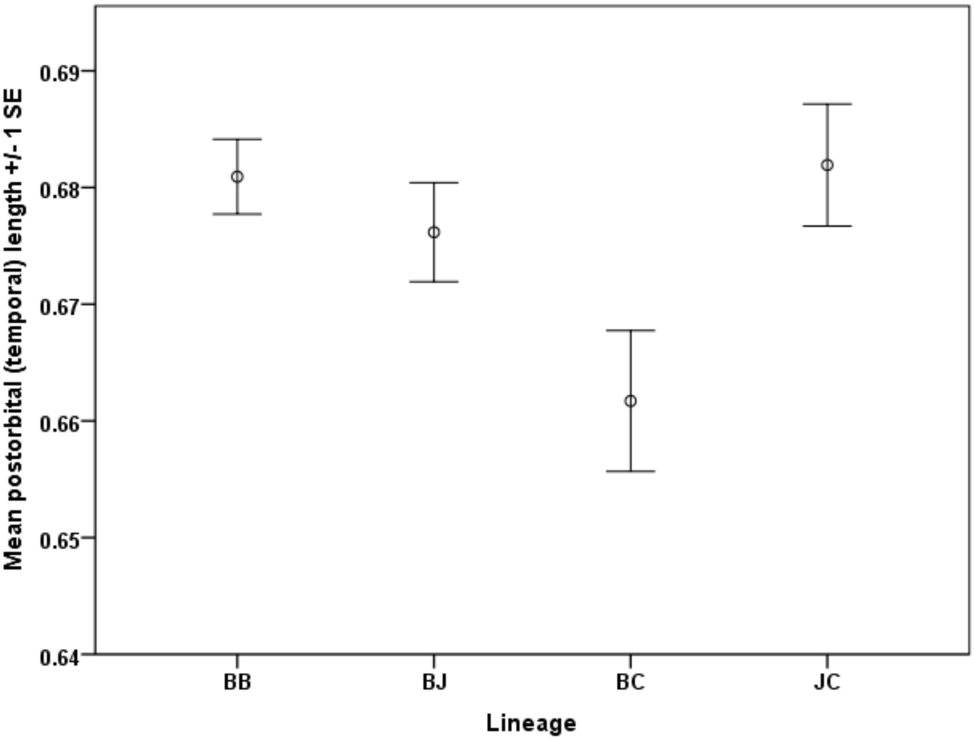


Figure 3: Mean postorbital (temporal) length of four lineages. Temporal length (mm) portrayed as Mosimann size adjusted shape variable with no unit of measure. Error bars represent +/- 1 standard error.

Body condition

During standardised/controlled feeding, there was no main effect of lineage F(3,67) = 2.349, p = .080 (Table 3, Fig 4) . There was also no main effect of month on body condition F(1,67) = .001, p = .980, nor was there a significant interaction effect of month*lineage F(3,67) = 1.638, p = .189 (Table 3). During feeding trial two there was no main effect of month on body condition F(1,67) = .245, p = .623, nor was there a main effect of lineage F(3,67) = .392, p = .759 (Table 3, Fig 4). There also was no significant interaction effect of month*lineage F(3,67) = .924, p = .440 (Table 3).

Table 3: Results of repeated measures ANalysis Of VAriance (ANOVA) for effect of month and lineage on body condition, with (a) Feeding trial 1 (controlled feeding regime) and (b) Feeding trial 2 (scatter feeding). Bold denotes significant effects (p< .05).

Effect	Month	Lineage	Month*lineage
(a)			
df	1	3	3
F	0.001	2.349	1.638
p	0.98	0.08	0.189
(b)			
df	1	3	1
F	0.245	0.392	0.924
p	0.623	0.759	0.434

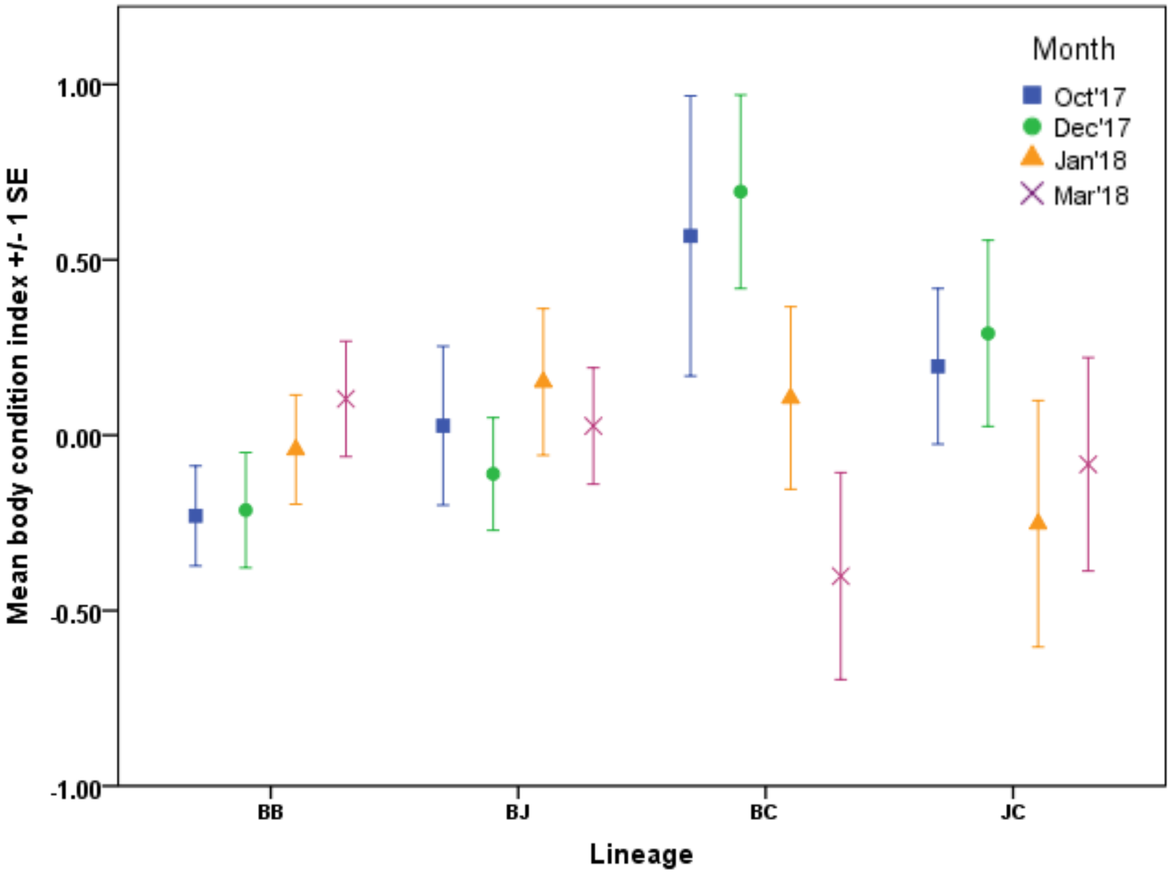


Figure 4: Mean body condition index of four lineages of all juvenile *T. adelaidensis*. Error bars represent +/- 1 standard error.

Growth rate

During standardised/ controlled feeding, there was no main effect of lineage on growth rate F(3,67) = 1.620, p = .193 (Table 4) and the result was the same during feeding trial two F(3,67) = .309, p = .819 (Table 4).

Table 4: Results of a one-way ANalysis Of VAriance (ANOVA) for effect of lineage on growth rate, with (a) Feeding trial 1 (controlled feeding regime) and (b) Feeding trial 2 (scatter feeding). Bold denotes significant effects (p< .05).

Effect	Lineage
(a)	
df	3
F	1.62
p	0.193
(b)	
df	3
F	0.309
p	0.819

Effective population size and genetic diversity

The linkage disequilibrium method identified the Burra population as having a larger estimated effective population size (n = 92, N_e = 61.9) than the Clare population (n = 11, N_e = 44.9). The heterozygote excess method highlighted the Clare population as having a higher estimated effective population size (n = 11, N_e = 12.7) than the Jamestown population (n = 13, N_e = 9.4). As a result, the Jamestown population is expected to have the smallest N_e of all three populations. The Burra population has the highest number of alleles (15.429 +/- 2.44), almost double that of either of the other two populations in the study (Jamestown: 8.429 +/-1.07; Clare: 8.143+/-0.96). There was no significant difference in allelic richness among the populations, although Burra has the highest average of 5.25 alleles per locus (Table 5). Observed heterozygosity was high for all populations sampled, >0.8, observed heterozygosity being higher than expected for the Clare population (H_o = 0.878, H_e = 0.756) (Table 5, Table A3). There was no significant difference in the observed heterozygosity among the populations (Table 5). Inbreeding was not detected in any of the three source populations, with negative F_{IS} values indicating that within Jamestown and Clare samples were each less related than expected under a random mating system (Table A3).

The unmixed BB lineage had the largest effective population size (n = 92, Ne = 24.9) of all four lineages when compared to BJ (n = 22, Ne = 9.9); BC (n = 13, Ne = 3.3) and JC (n = 11, Ne = 2.4). Mixed-lineage F1 offspring showed slightly higher genetic diversity than unmixed individuals, in terms of both allelic richness (mixed 10.77, unmixed 9.505) and observed heterozygosity (mixed 0.906, unmixed 0.855). Mixed and unmixed lineage groups also both had negative F_{IS} values, although mixed-lineage individuals had the lowest (mixed -0.081, unmixed -0.032). There was a significant difference in allelic richness among the four lineages (2-sided p-value, p = .001), the JC lineage having the lowest number of alleles per locus (Table 6). Observed heterozygosity was high for all lineages, the two most heterozygous lineages being BC and JC (Table 5).

Table 5: Results of FSTAT analysis of genetic diversity for three source populations (10,000 permutations). Bold denotes a significant result, p<.05.

	Burra	Jamestown	Clare	2-sided p-value
n (No. of individuals)	92	13	11	
Allelic richness	5.25	4.675	4.551	0.165
Observed heterozygosity	0.84	0.813	0.878	0.067
FIS	0.012	-0.058	-0.081	0.401

Table 6: Results of FSTAT analysis of genetic diversity for four lineages of F1 generation offspring (10,000 permutations). Bold denotes a significant result, p<.05.

	BB	BC	BJ	JC	2-sided p-value
Allelic richness	5.673	5.279	5.463	4.687	0.001
Observed heterozygosity	0.855	0.938	0.881	0.922	0.331
FIS	-0.032	-0.146	-0.102	-0.205	0.249

DISCUSSION

We mixed individuals from three isolated populations of an endangered species in order to achieve our aim of assessing the impact of mixing populations on three ecologically relevant fitness-related traits: body condition, growth rates and bite force performance. This novel study is one of the first to empirically assess the impact of mixing populations on the fitness of the F1 generation and found no immediate negative impact on the three fitness-related traits examined. Our findings are positive for future efforts to use population reinforcements, both for this species but also as a conservation tool to safeguard against genetic bottlenecks or inbreeding in other species.

Neither body condition or growth rate differed significantly between the four lineages in this study, irrespective of feeding method. Our data showed a higher growth rate, for all lineages, in the Oct-Dec period (feeding trial 1) compared to the Jan-Mar period (feeding trial 2) (Fig. 5). While this could be a function of feeding method, higher growth rates in the first half of the activity season have been detected for BB offspring in previous years when subject to a full season of standardised feeding (Fig. 6), possibly highlighting a natural pattern of growth. (L. Clive unpublished data). Although the BC lineage possessed a more forceful bite, and a wider head, it did not afford that lineage a competitive advantage, in terms of higher body condition or faster growth, over the other lineages during this study. BC lineage individuals were consistently larger, in terms of SVL, than the other lineages (Fig. A3) supporting the well documented observation that larger lizards tend to bite more forcefully (Anderson *et al.* 2008; Wainwright 1991; Dufour *et al.* 2018) (Fig. A2). The second feeding trial, a more natural feeding scenario, allowed us to assess how each lineage could perform in the wild, an ecologically important result. The fact that admixed and unmixed lineages performed comparably, in terms of prey acquisition, could indicate that admixed and unmixed individuals may experience similar rates of survival in the wild, although long-term monitoring would be needed to corroborate this supposition. A lack of competitive advantage between the lineages, during this study, indicates support for the hypothesis that mixing *T. adelaidensis* populations does not have a negative effect on selected fitness-related traits when compared between mixed-lineage and unmixed offspring.

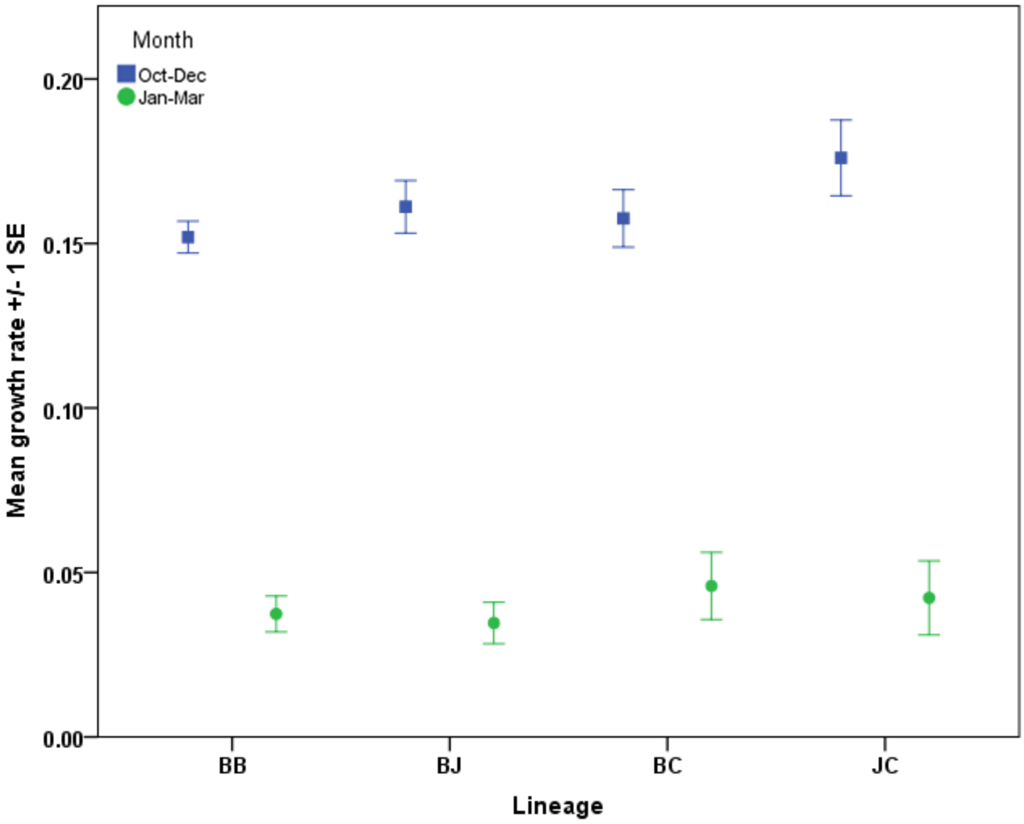


Figure 5: Mean growth rate of four lineages of F1 generation Tiliqua adelaidensis offspring. Error bars represent +/- 1 standard error.

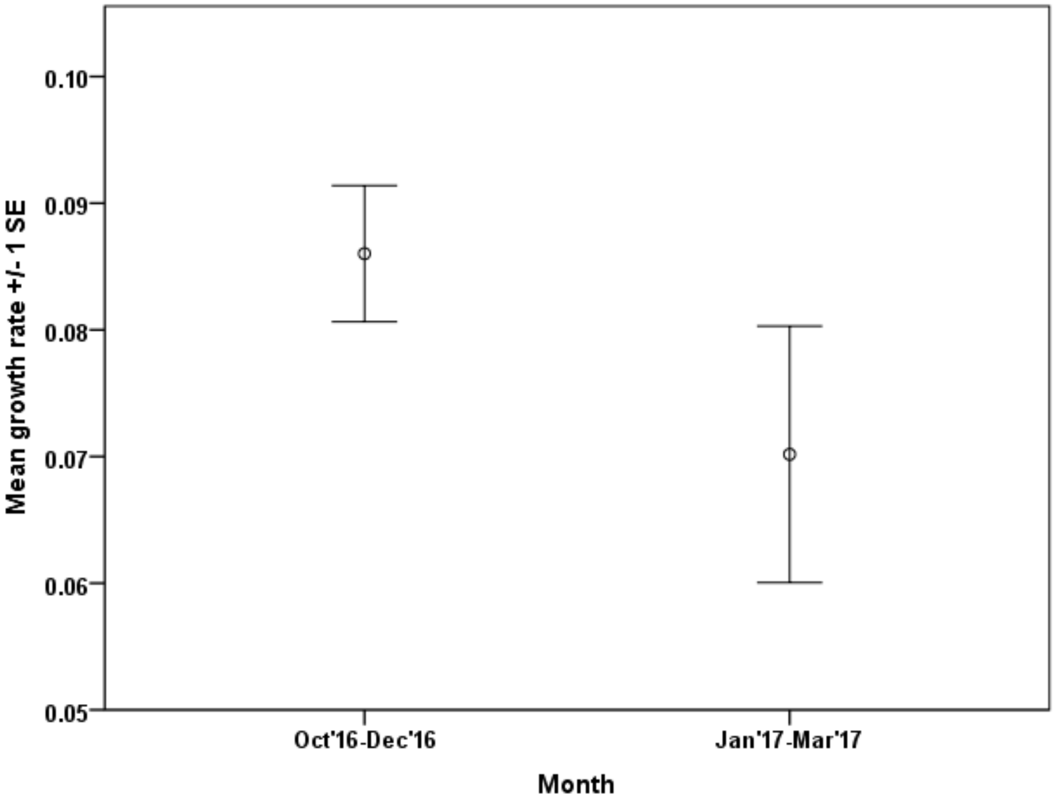


Figure 6: Mean growth rate of BB offspring caught in January 2016, in control enclosures, and housed at Monarto zoo. n = 3. Error bars represent +/- 1 standard error.

Our study found high levels of genetic diversity, in terms of allelic richness and observed heterozygosity, along with no evidence of inbreeding, which are key indicators for selecting source populations for translocation. Our findings built upon existing data on genetic diversity and applied that knowledge to investigate the impact of mixing source populations for a population reinforcement (Schofield, 2015; Smith et al., 2009). The promiscuous mating system exhibited by *T. adelaidensis* likely maintains the high levels of genetic diversity within populations, offsetting the risk of inbreeding within small isolated populations (Schofield 2015; Schofield *et al.* 2014; Smith *et al.* 2009). To our knowledge, the present study is one of few examining the effect of inter-population breeding on fitness, precautionary studies of this kind not being undertaken regularly (van Oppen *et al.* 2014; Averill-Murray and Hagerty 2014).

The fear that mixing diverse populations will result in offspring with altered fitness is one that is often discussed but has seldom been investigated empirically (Edmands 2007; Averill-Murray and Hagerty 2014; Frankham *et al.* 2011; Weeks *et al.* 2011; Raabová *et al.* 2009). Our study provides empirical evidence that mixing populations does not necessarily result in negative fitness consequences and provides reassurance that concerns regarding the genetic hazards of translocation as a conservation strategy may be overstated. It is important to note, however, that the scope of the present study was limited due to the time-frame of a PhD study and so we acknowledge our ability to comment conclusively on this suggestion. Our work adds to the pattern seen in the few other studies that have been conducted, where a negative impact is rarely found, emphasising the potential for population reinforcement as a conservation strategy for many species at risk of inbreeding depression (Averill-Murray and Hagerty 2014; Day *et al.* 2017; Frankham 2015; Kronenberger 2017; Raabová *et al.* 2009; Whiteley *et al.* 2015). When low offspring fitness has been detected, it has been found to recover over time, outbreeding depression likely being a temporary state due to maladapted genes being filtered out of the population by natural selection (Edmands et al., 2005; Erickson & Fenster, 2006). It has been suggested that using local populations in population supplementation scenarios would be optimal, due to fear of outbreeding depression and other risks (Weeks *et al.* 2011). The risk of outbreeding depression in the Mojave Desert Tortoise *(Gopherus agassizii)* is low and yet research is still being conducted on performing translocations that preserves the genetic population structure (Averill-Murray and Hagerty 2014). The present study provides empirical evidence to allay such fears and allow action to be taken, such as population supplementation, to the benefit of the species in question. We used individuals from three of the most diverse lineages within the current *T. adelaidensis* range to document a 'worst case scenario' for augmentation using conspecific populations and found those concerns to be unfounded in this case. We also acknowledge that our species may nevertheless represent a model candidate for such translocations, in that genetic differentiation between the three populations is relatively low, and heterozygosity is high within each of the source populations, making them receptive to the potential genetic benefits of outbreeding (Frankham 2016).

Our three fitness proxies were chosen due to their ecological relevance to the life history of our study species, *T. adelaidensis,* and are commonly used to examine the effects of inter-population breeding; growth and body condition directly impacting survival in many species (Rasmussen *et al.* 2009; Shamiminoori *et al.* 2014). An admixed lineage having a more forceful bite could indicate the potential for that lineage to have greater success when capturing prey, fighting rival males or grasping females during mating, leading to a greater chance of survival or reproductive success later in life (Anderson *et al.* 2008). However, in this study specifically, we did not see the resulting increase in body condition you may expect if higher bite force enabled the acquisition of larger, or more numerous, prey items (Dufour *et al.* 2018). Although this study uncovered slight differences in head shape and performance, the extent of head shape variation in mixed lineage offspring remains comparable with unmixed offspring. Using three proxies for fitness, instead of one, allows a more thorough examination of the overall effect of mixing populations.

This study has shown that there is no evidence to suggest that admixed lineages outperform unmixed, intrapopulation conspecifics in the short term. Moreover, we provide baseline data to show that meaningful rapid cost-effective proxies do exist that might be useful for future studies elsewhere. *Tiliqua adelaidensis* is an outbred species, the promiscuous mating system likely explaining the lack of negative impact mixing populations had on our three fitness proxies. Further studies on a wider array of animal species will allow our approach to be further tested and would also permit the further evaluation of the proxies for fitness that we discuss here to determine if they accurately predict long-term survival or reproductive success (Ranke *et al.* 2017). Additionally, future research of the fitness of F2 and F3 generation offspring would allow further investigation of fitness effects associated with mixing diverse lineages of pygmy bluetongues. While the present study was not able to test for hybrid vigour or outbreeding depression, results indicating an increase or decrease in growth or performance in the F1 offspring would have highlighted the need for caution. Assisted-migration, increasing in popularity, is one way that biodiversity can be protected and conserved in the face of rapidly changing environmental conditions (Thomas 2011; van Oppen *et al.* 2014). In order to persist in the face of climate change, organisms need to adapt to living in a warmer, drier environment. Future research would benefit from identifying diversity at ecologically relevant genes, such as those important for withstanding long periods of drought, allowing source populations to be selected for adaptive potential.

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APPENDICES

TABLES

Table A1: Characteristics of microsatellite loci as described by (Gardner et al. 2008). Size range of each loci as per findings of the current study.

Locus #	Clone name	Genebank accession number	Primer sequences (5’-3’)	Clone repeat sequence	T. a. Multiplex PCR set (primer conc. IM)	Size Range
						T. a.
TrL14	TrP3AC28	EF464754	F:GTTTCCATGGTGTCAAAGTCTTAAC	(AC)34	1 (0.08)	120–160
			R:gttTGACAAGATCTTCAAATCCTACTGC			
TrL16	TrGP2_26	EF464756	F:AAACCCTGGACCTGTCAGTCT	(CAT)19	1 (0.12)	80–150
			R:gttTGGATTACGAACTGGACACATT			
TrL21	TrP1AC19	EF464758	F:CTTCTCTCACAGTTTCCATGAGTTA	(AC)12	2 (0.24)	240–310
			R:gttTCTGTACTGATCCTTACTGCTAATGC			
TrL28	TrP1AC12	EF464760	F:CCAGTTGCAAATTAGAGCTTGA	(CT)3CA	2 (0.12)	130–180
			R:GGTTACTTTCTCATGCAGTTCTGA	(CT)7(CA)12		
TrL29	TrP2AC1	EF464761	F:TCCAGGTATATGCCAACAATAGTCT	(GT)2(TC)12	2 (0.12)	120–180
			R:GGTTACTTTCTCATGCAGTTCTGA	(AC)10		
TrL32	TrP1AC18	EF464763	F:CATTCAAATCACAGTATCTTTCTCG	(AC)13	1 (0.08)	140-180
			R:ACAGGGTTGCTGTTTAGTTTTTGT			
TrL34	TrP3AC36	EF464764	F:GTGTTCCCCTTCCTTCTCCATA	(AC)12	2 (0.12)	110-180
			R:CCCTATGTTTTCCACTGACTTAAAC	(Ag)$_{21}$		

Table A2: Characteristics of microsatellites used in this study, split by source population: Burra Jamestown and Clare.

Locus #	N (No. of individuals typed)			Na (Number of alleles)			Ho (observed heterozygosity)			He (expected heterozygosity)		
	Burra	Jamestown	Clare	Burra	Jamestown	Clare	Burra	Jamestown	Clare	Burra	Jamestown	Clare
TrL14[a]	75	13	11	9	7	5	0.827	0.923	0.818	0.807	0.817	0.674
TrL16[c]	91	12	11	11	6	8	0.791	0.667	0.818	0.875	0.806	0.851
TrL21[b]	91	13	11	23	12	9	0.846	1.000	0.818	0.884	0.893	0.826
TrL28[c]	91	13	11	14	10	10	0.912	0.846	1.000	0.865	0.817	0.843
TrL29[b]	92	13	11	22	10	12	0.957	0.923	1.000	0.925	0.828	0.893
TrL32[c]	73	12	11	8	4	5	0.644	0.750	0.455	0.664	0.719	0.388
TrL34	91	13	11	21	10	8	0.912	0.846	0.909	0.903	0.867	0.814

Table A3: Mean microsatellite characteristics, split by source population: Burra, Jamestown and Clare.

Na = Number of different alleles; Na (Freq>=5%)= No of different alleles with a frequency >= 5%; Ne= No of effective alleles; I= Shannon's information index; No. private alleles= No of alleles unique to a single population; No LComm alleles (<=25%) = No of locally common alleles found in 25% or fewer populations; No LComm alleles (<=50%) = No of locally common alleles found in 50% or fewer populations; He = Expected heterozygosity; uHe = Unbiased expected heterozygosity

	Mean values (+/- 1 SE)		
Population	**Burra**	**Jamestown**	**Clare**
Na	15.429 (+/- 2.44)	8.429 (+/- 1.07)	8.143 (+/- 0.96)
Na Freq. >= 5%	6.429 (+/- 0.649)	5.286 (+/- 0.52)	4.571 (+/- 0.53)
Ne	7.964 (+/-1.26)	6.047 (+/- 0.71)	5.462 (+/-0.95)
I	2.220 (+/-0.18)	1.890 (+/- 0.13)	1.761 (+/- 0.19)
No. Private Alleles	7.143 (+/- 1.28)	1.000 (+/- 0.58)	1.143 (+/- 0.40)
No. LComm Alleles (<=25%)	0.000	0.000	0.000
No. LComm Alleles (<=50%)	0.000	0.000	0.000
He	0.847 (+/- 0.03)	0.821 (+/- 0.02)	0.756 (+/- 0.07)
uHe	0.852 (+/- 0.03)	0.854 (+/- 0.02)	0.792 (+/- 0.07)

FIGURES

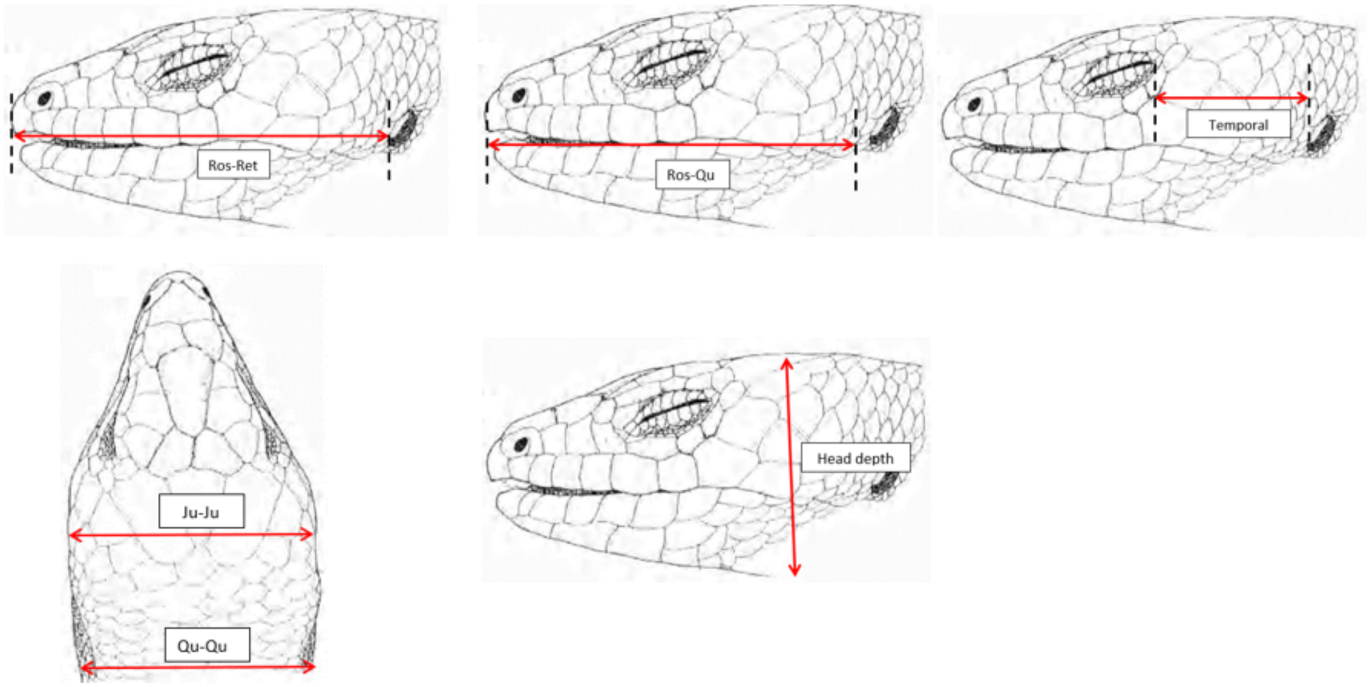

Figure A1: Illustrations showing where each measurement was physically taken. Three measures of head length (Ros-Ret, Ros-Qu and Temporal length), one measure of head depth and two measures of head width (Qu-Qu and Ju-Ju). Illustration credit: Fig.1 (Hutchinson et al. 1994)

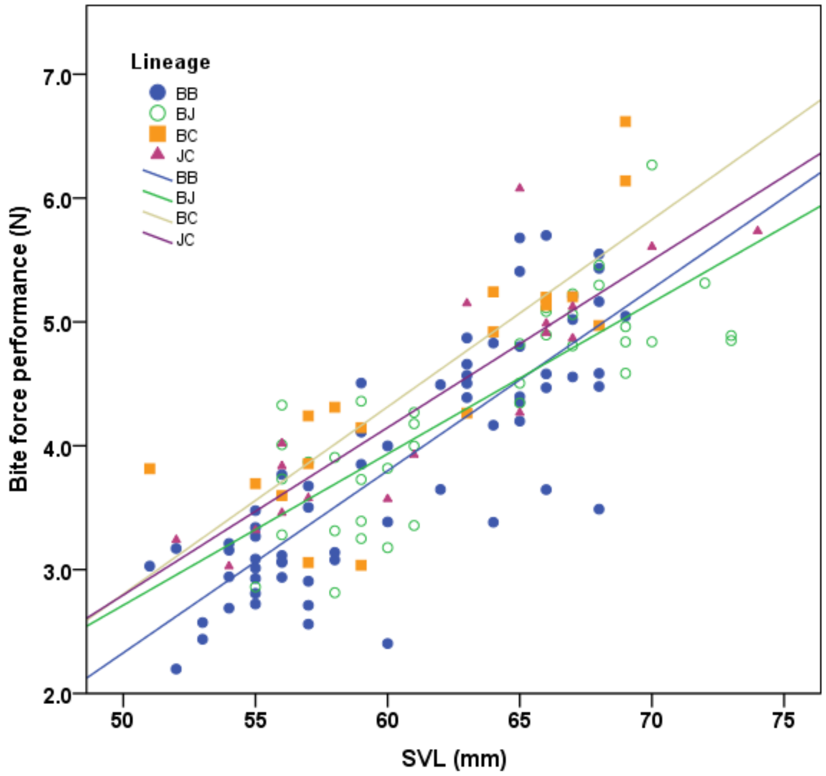

Figure A2: Scatter plot showing the positive linear relationship between bite force performance (N) and SVL (mm) as a measure of body size.

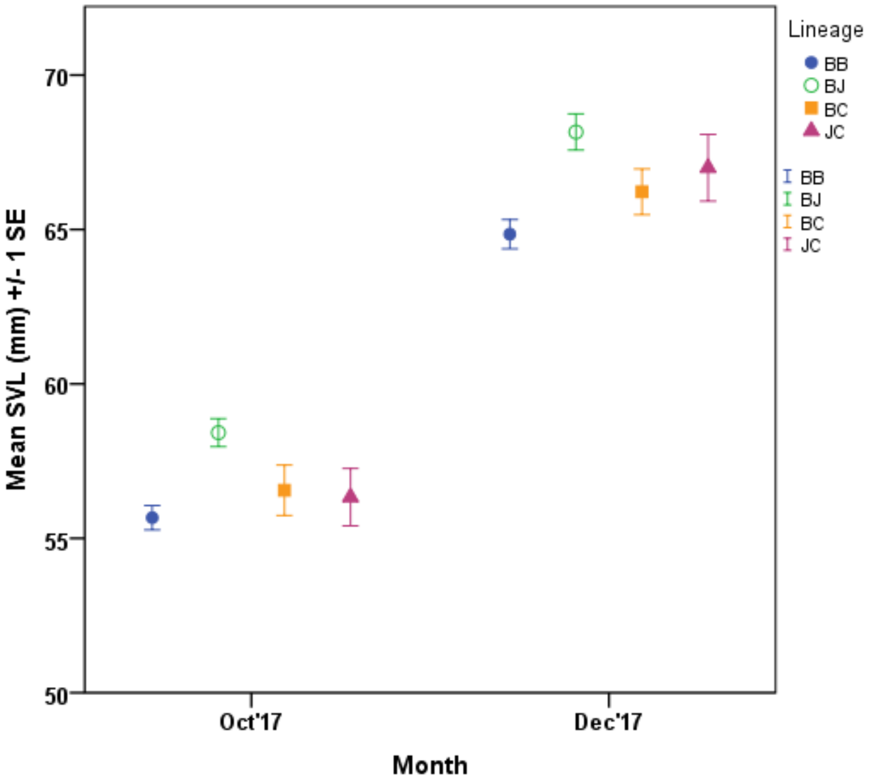

Figure A3: Error bar plot showing mean SVL (mm) in October 2017 and December 2017, split by lineage. Error bars represent +/- 1 standard error.

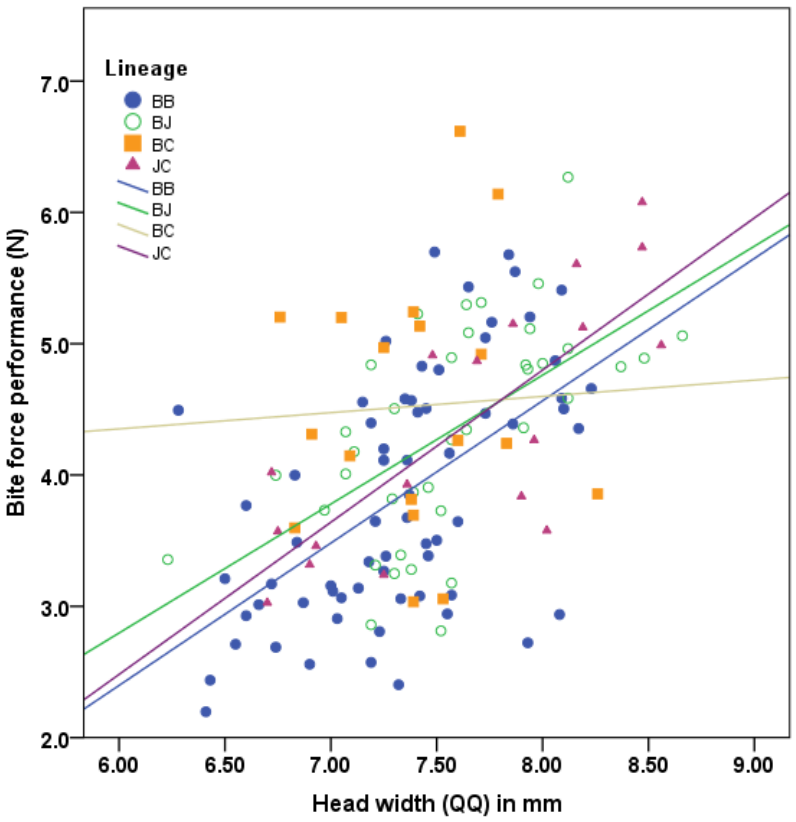

Figure A3: Scatter plot showing the positive linear relationship between bite force performance (N) and head width (QQ) in mm.

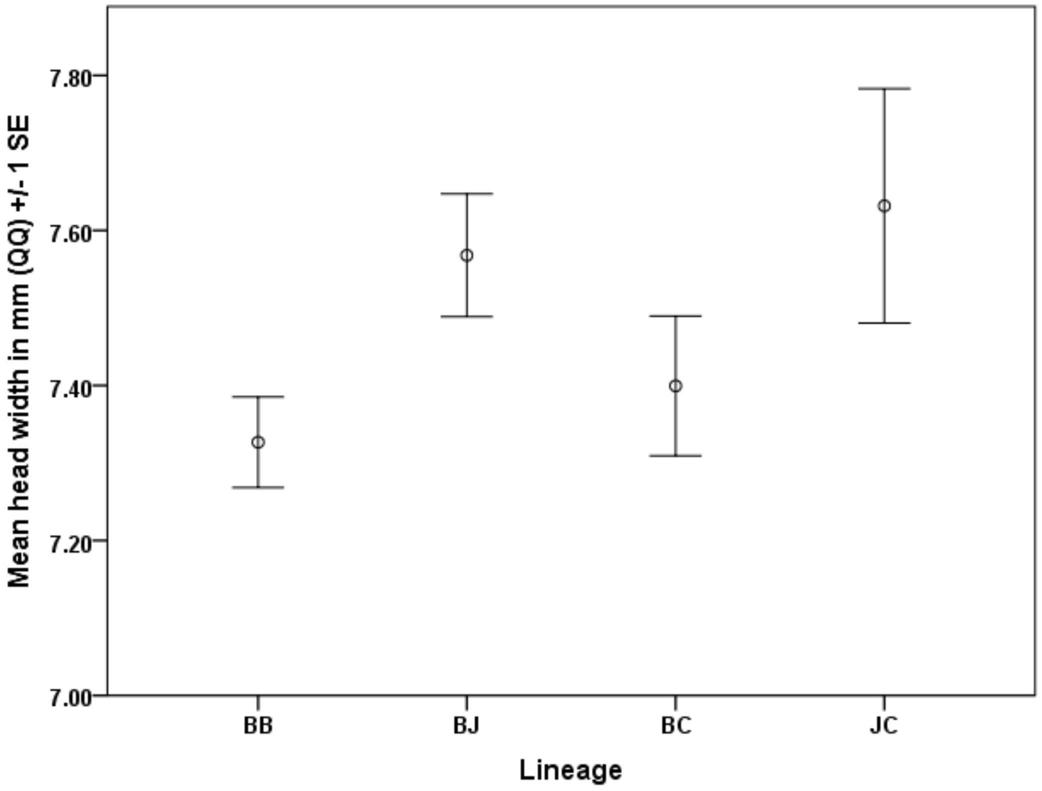

Figure A4: Error bar plot showing mean head width (QQ) in mm, split by lineage. Data shown includes October 2017 & December 2017. Error bars represent +/- 1 standard error

CHAPTER 6

GENERAL DISCUSSION

Pygmy bluetongue lizard about to bite the mealworm lure (Photo: Joyanne Gardner)

GENERAL DISCUSSION

Species' have unique life history requirements and each species will respond differently to translocation. A thorough and complete knowledge of a species' ecological and behavioural needs will guide the best way in which to conduct a translocation in order to maximise the chance of success. However, identification of translocation risk is rarely investigated before the wildlife movement is carried out leading to regularly reported failures which are largely avoidable as, once an area of potential risk has been identified, it can be investigated and mitigated. Studies conducting only post-release monitoring limits the questions that can be asked or answered and can lead to poor wildlife management decisions (Armstrong and Seddon 2008). This trend in the literature highlights the need for targeted *a priori* research to assess potential sources of risk before they occur so they can be mitigated when the time comes (Armstrong and Seddon 2008). The work presented in this thesis addresses that gap through a targeted experimental design dedicated to the identification of ecological and genetic risks associated with population augmentation of an endangered species existing in small fragments of habitat. I addressed four main aims in this thesis which all serve to increase the success rate of conservation translocations and, ultimately, conservation of endangered species. These aims were:

1. To assess the impact of population reinforcement on the resident sympatric species at the release site
2. To investigate whether body condition was a good indicator of performance as a measure of population health
3. To establish whether performance was associated with individual reproductive success
4. To assess whether admixed offspring grew or performed differently compared to unmixed offspring

Below, I discuss the main findings of my research and their broader application to the conservation of endangered species as well as conservation of the pygmy bluetongue lizard.

In Chapter 2, I investigated the potential risks that population reinforcement posed to the wider ecological community at the recipient site, a prominent gap in the literature that my thesis addresses. Ecosystems exist through the delicate temporal and spatial balance that enables numerous species to co-exist in equilibrium with minimal competition for resource (Ricklefs 2008; Gordon *et al.* 2010; Grinnell 1917). Disruption to that equilibrium through the sudden increase in abundance of one species could potentially impact other species in the local area in terms of competition for prey or refuge (Grant 1993; Falk-Petersen *et al.* 2006). My results show that the health of the co-occurring lizard species, as inferred through abundance and body condition, was not negatively impacted by population reinforcement of *Tiliqua adelaidensis.* Observed seasonal fluctuations of abundance were not significantly altered following the translocation and the lower body condition exhibited by *Menetia greyii* post-reinforcement was seen in both experimental and control enclosures which highlights the likely effect of a variable outside those tested by my study. My findings build upon previous research identifying the resident lizard species in known pygmy bluetongue populations (Pelgrim *et al.* 2014) and their level of interaction with the pygmy bluetongue lizard (Ebrahimi, Godfrey, *et al.* 2015b), and it also provides a unique methodology for other researchers to conduct pre-emptive studies of their own. In order to avoid degrading the artificial refugia through too-frequent sampling I monitored the co-existing species once a month which limited my sample size. Many of those species occur in low abundances which, when combined with the relatively small area sampled within each enclosure, resulted in zero-inflated data and limited which statistical tests I could perform.

While conservation efforts are usually targeted toward the focal species, less attention is given to the impact upon the broader ecosystem receiving the translocation, and apparently none regarding the impact of population reinforcements. Similar to my study, Flannagan (2000) used *in-situ* experimental enclosures to examine the ecological impact of translocation on resident species although used activity-levels and behaviour rather than body condition to comment on impact. Another point of difference was that my study compared sympatric species whereas Flannagan examined the effect of introducing a non-sympatric species on a threatened resident species. Even though the study identified a negative impact on the behaviour of the resident species, an introduction was carried out regardless. It seems surprising to conduct this pre-emptive study and ignore the risks that were found, although monitoring body condition as well would have allowed the author to comment on the effect of the behavioural change on the health of the resident species. Habgood (2003) also performed experimental field-based research examining the ecological impact of translocation and included body condition and mortality rate in the analysis which Flannagan did not. Habgood did not find a negative impact on the body condition of the resident or introduced species despite similarities in their ecological life-histories. However, rather than validating their findings by conducting an experimental release, as my study has done, Habgood recommended that future reptile releases be performed away from existing populations. The reluctance of Flannagan and Habgood to integrate their findings into the decision-making process is consistent with other studies that detect no risk and still advocate against the movement (Averill-Murray and Hagerty 2014). This raises the question of 'what is the point of pre-emptive investigatory studies if not to provide empirical evidence upon which to make informed decisions?'

In Chapter 3, I addressed the problem of being able to identify the onset of decline in a population. This could allow earlier intervention to prevent extirpation or extinction, both of which could be a serious threat for species existing in just one locality. Measures of performance can be invasive and difficult to measure *in-situ* and being able to determine performance ability through a well-tested, minimally-invasive, easily collectable measure of body condition will be important for monitoring of endangered species populations. I investigated whether body condition was a good indicator of performance using bite force as a measure of population health. Bite force performance facilitates prey acquisition and territory defence (Fenner and Bull 2011) and the capacity of an animal to perform an ecologically relevant task is likely to be a reliable indicator of fitness (Husak *et al.* 2009). My results show that lizards in better body condition performed better (bit more forcefully) than lizards in lower body condition, the results cutting across patterns in body and head size that correlate with sexual dimorphism. As a result, we confirmed that body condition indices were sufficient to convey an organism's ability to perform ecologically relevant tasks and, as such, is indicative of overall health for both sexes. My results show that larger lizards perform better than smaller lizards, although larger lizards do not automatically have better body condition suggesting that performance ability is tied to factors other than just body size. Many studies have found head size or shape to affect bite force performance, larger lizards having larger heads and a more forceful bite (Anderson *et al.* 2008; Becker and Paulissen 2012; Herrel *et al.* 2001), but the results of this study found that the small individual variations in head shape that we detected were not important influences of bite force in this species. A contributing factor to reducing head dimensions as an influence is likely the ecological constraints of burrow-use which limits all axes of pygmy bluetongue head shape. All lizards performed better in summer, although body condition was lower in summer suggesting that there are other factors responsible for this elevated performance. Ambient temperature was not included as a factor by our automated model selection, only being included in the ninth best fitting model. However, body condition had a larger effect on bite force performance during spring rather than summer possibly due to spring being when dispersal and mating occurs, both activities having high energetic requirements.

In Chapter 4, I examined the relationship between various physical characteristics, including body condition and performance, and reproductive success between individuals from three pygmy bluetongue populations. If the conservation management strategy of population reinforcement is to be successful, it is important to first determine whether genetically different populations will breed with 'foreign' conspecifics i.e. individuals from populations other than their own. A key aim in endangered species conservation is to increase wild population size, and research identifying factors increasing reproductive success are integral to building self-sustaining populations, a common criterion for translocation success. Through identification of parent-offspring pairs using microsatellite loci and parentage analysis, I established that individuals from different source populations successfully reproduced. Although I was not able to test for assortative mating in this study, mixed-lineage offspring of all source population combinations were found which suggests a lack of source-population preference although future research would need to confirm this hypothesis. As well as performing better (Chapter 3), individuals in better body condition were found to achieve higher reproductive success, although this was only statistically significant for females. Performance was not an important factor in reproductive success for either males or females which is an unexpected finding. Individuals in better body condition are expected to perform better potentially due to their presumed energetic reserves. My results show that individuals in better body condition achieve higher reproductive success, likely due to being able to withstand the energetic requirements of dispersal (males) and development of embryos (females) (Shamiminoori *et al.* 2014). Thus, it is to be expected that better performing males and females would achieve higher reproductive success (Shamiminoori *et al.* 2014) but that was not the case in this study. For male pygmy bluetongues, I found that individual microsatellite heterozygosity was negatively associated with reproductive success, males with high heterozygosity siring fewer offspring. This was also an unexpected result and, due to the low variation between individual heterozygosity scores, a larger sample size is needed to confirm this effect. Due to the extensive sexual size dimorphism overlap exhibited by this species, accurately sexing males and females is problematic without physical evidence of breeding such as presence of offspring in the burrow or considerable mass reduction (females) or sight of hemipenes (males). Genetic markers to distinguish sex are currently being developed but were not available for application in this study and so remains a limitation for my study. While not a limitation, it is important to note that the analyses used in this chapter (Chapter 4) and Chapter 5 were conservative as I pooled alleles to avoid type II error and only used parent-offspring combinations with a strict confidence level of 95% compared to the more relaxed level of 80% used in other studies (Huyghe *et al.* 2014; Husak *et al.* 2009). My conservative approach likely reduced the number of offspring included in the analysis and subsequently affected my findings. Although I was able to assign a parent pair to 88% of all offspring sampled to a 95% confidence level, being able to accurately assign sex to all adults sampled would have enabled me to increase the sample size of offspring in the study through more accurate identification of potential mothers and fathers.

There is little research on factors affecting reproductive success in promiscuous lizard species (Coltman *et al.* 1999) and this study provides a unique insight into this gap in our knowledge. The present study investigated the importance of pre-copulatory factors that may have impacted which males successfully reproduced in an activity-season. However, promiscuity in females can result in post-copulation competition between males in the form of sperm-competition (Preston *et al.* 2003). Post-copulatory competition can also occur in the form of behavioural strategies such as mate guarding which serves to increase reproductive success by preventing rival males from mating with that female (Coltman *et al.* 1999). It may be that promiscuous species do not select mates based on specific attributes but rather the diversity of partners allows promiscuous species to maintain the intermediate diversity advantage, although future studies would be required to investigate this hypothesis (Hacking *et al.* 2018; Wegner *et al.* 2003).

Having established in Chapter 4 that genetically differentiated populations would breed successfully, in Chapter 5 I examined the growth and performance of those offspring in order to determine whether admixed individuals, produced from separate source population parent combinations, differed to unmixed offspring. My results showed no evidence that mixed-lineage offspring developed differently to unmixed offspring in terms of body condition, growth rate or performance. Although one lineage did perform better than the other three, it did not afford them a competitive advantage over their counterparts even in a feeding trial where they needed to compete for access to prey. My results confirmed the suggestions by Smith et al (2009) and Schofield (2015), and also provided empirical support for the predictions made by Frankham (2011). This study has created a source of admixed lineages for future translocations, each with high genetic diversity and potentially an increased adaptive capacity to withstand future climatic stochasticity. This experimental translocation is one of the first to experimentally assess the fitness of admixed offspring in comparison to unmixed offspring in order to evaluate source population combination for a population reinforcement. However, my study was only able to examine the indirect fitness of the F1 generation and outbreeding depression may only manifest in later generations so monitoring of F2 and F3 generations would be required to confirm our short-term findings (Tallmon *et al.* 2004; Frankham 2016).

Conservation of threatened species

The core aim of my study was to examine the ecological and genetic risks associated with population reinforcement. The results of my study can be used by the wider conservation community to avoid paralysis through perception of risk which has been shown to limit management action (Weeks *et al.* 2011). These risks can be assessed at relatively low cost and should risk be detected; it can be mitigated. Should no risk be detected, it provides empirical evidence that the chosen management strategy can progress to the next stage with a higher level of confidence regarding the outcome.

When considering population reinforcement as a conservation strategy, it is important to ascertain if the populations will breed or if they will discriminate based on source population. If breeding does not occur, population reinforcement may not be the optimal strategy for that species. However, if breeding does occur, it must also be ascertained if the offspring will have altered fitness as heterosis could result in swamping of a local genotype while outbreeding depression could result in the reduced survival of that F1 generation and subsequent failure of the translocation.

Conservation of pygmy bluetongue lizards

Managed relocations are the selected conservation management strategy for the pygmy bluetongue lizard which will involve either reinforcement of existing populations or reintroductions into previously occupied habitat within the historic range of the species. My study has shown that reinforcement is a viable conservation strategy for this species as populations will interbreed with an absence of hybrid vigour exhibited in the mixed-lineage offspring. The literature calls for caution when mixing genetically differentiated populations due to the risk of outbreeding depression and, by using three of the most genetically diverse populations, I have provided a 'worst case' scenario of the risks for this system. The findings of my study support the prediction that there was little risk posed by population reinforcement of this species which paves the way for future research to build on this, the first experimental population augmentation.

Future directions & conclusions

Climate change

As well as reducing availability of suitable habitat, climate change can indirectly impact species through anthropogenic activities, for example, development of renewable sources of energy such as wind farms. While windfarms are less physically destructive than coal mining, their construction does alter the landscape and displace species through removal of suitable habitat. Species conservation efforts can conflict with land use and the priorities of landowners but, in the instance of pygmy bluetongues and native grasslands, the issue is negligible as the species thrives in moderately sheep grazed land which benefits both farmers and conservationists (Clayton and Bull 2015, 2016; Clayton 2018). Mitigation translocations could be used to move populations out of areas planned for windfarm developments although, in contrast to the usual strategy employed, there would need to be a logical plan for implementation and post-release monitoring program. Adaptive management is an analysis technique that identifies the optimal approach by comparing a selection of alternative strategies, each with different costs and outcomes (Canessa *et al.* 2018). From this, it can be decided which option provides the best outcome while meeting the particular objectives of the project such as cost or minimum population growth required to be classified as a success. Objectives of mitigation translocations are often restricted to successful removal of a population from an area, population establishment and growth at the new site not being a priority which explains the frequent lack of post-release monitoring (Germano *et al.* 2015). Whereas for a conservation translocation, population establishment and growth could be the main criteria for measuring success which highlights the need for a well-constructed targeted monitoring plan designed to provide the required data. Modelling can predict demographic response to variables such as: number of founding individuals, rate of reproduction, sex ratios, age classes, supplementary feeding and habitat augmentation which are all factors that could affect the perceived success of the movement. Modelling could also compare the ecological impact of translocations using either a single large group of individuals or numerous smaller translocations to the same site over subsequent years. Strategy-selection inducing successful establishment and growth at the new site, with minimal ecological disruption, is often a key objective in conservation research, in addition to working within budget constraints. Potential sites should also be selected, not only for their current suitability to support persistence of the species, but also its projected future suitability. Conservation translocations are carried out to mitigate against immediate sources of threat but also to facilitate the long-term survival of a species.

Ecological considerations

Following on from my study examining the risks of population reinforcement, future research should assess the risks associated with reintroductions. Each type of conservation management strategy comes with its own set of risks and introducing a 'new' species to an ecosystem could potentially cause more ecological disturbance than increasing the density of a species already present. In the present study, the limited ecological overlap with the other lizard species in the native grasslands in terms of body size, space use and prey species should be noted as these are all key areas of competition between species (Pelgrim *et al.* 2014; Ebrahimi, Godfrey, *et al.* 2015b; Habgood 2003). Assessments made in other study systems would need to ascertain whether an ecological overlap exists between the species in their system and to what extent as this will impact their findings (Flannagan 2000; Wilson and Joanna 2010; Habgood 2003). Ecological overlap may be more considerable when the focal species is more mobile than the pygmy bluetongue lizard which spends the majority of the time in its burrow leading to limited interaction with other species (Milne, Bull, *et al.* 2003; Ebrahimi, Godfrey, *et al.* 2015b). Published translocations commonly focus on the behaviour, survival and reproduction of the species being translocated but rarely do they consider the ecological community receiving the reinforcement, this thesis managing to cite only two examples of such research (Habgood 2003; Flannagan 2000). It is a surprising gap in the literature considering the documentation of the extensive ecological damage caused by invasive species (Phillips *et al.* 2007; Fritts and Rodda 1998) and the lessons learnt from these case studies serve as a cautionary tale and have contributed to the present level of apprehension regarding risks associated with wildlife movements. The mechanisms contributing to the invasiveness have since been identified for use by future studies to avoid such outcomes in the future and the aim of the present study is to perform a similar service, albeit before negative impacts are apparent and therefore providing a more preventative approach.

Genetic considerations

Use of mixed-lineage founder populations has been recommended for translocation due to their potential vigour resulting from increased genetic diversity, the evidence of which is sustained across multiple generations (Weeks *et al.* 2011; Binks *et al.* 2007; Frankham 2015, 2016). Selecting individuals possessing genes associated with thermal tolerance or disease resistance could also facilitate the creation of a founder population with strong adaptive potential which is key, not just for conservation of the pygmy bluetongue lizard, but all species threatened by climate change. Future research could experimentally examine how well mixed-lineage founder populations establish and grow at new sites in comparison to single-location source population individuals, vigour through increased genetic diversity and adaptive capacity being potentially very important characteristics in a time of such environmental stochasticity. The current study could also be built upon through monitoring of F2 or F3 generations, and backcrossed offspring, in order to more thoroughly investigate the fitness effects of these population combinations.

Disease considerations

Risks regarding potential transmission of parasites to resident conspecifics is currently being investigated using the population reinforcement carried out in the present study. However, it is also important to consider the impact introduction of novel parasites could have on resident species as some parasite lifecycles involve multiple hosts (Kerr and Bull 2006). When planning a wildlife movement, it is important to include an exit strategy so that, should a serious risk occur, you can protect both the remaining individuals but also limit the effect on the surrounding ecosystem. My study was performed inside enclosures that prevented the lizards escaping into the surrounding habitat, thus protecting nearby conspecifics and the immediate ecological community from any sources of risk. However, assisted colonisations and reintroductions may result in exposure to entirely novel parasites so adaptive capacity to disease resistance is an important topic for investigation to benefit future wildlife movements. Including individual MHC gene diversity into future studies examining factors associated with reproductive success or mate choice would enable confirmation of whether selection of genetically diverse individuals is important for conservation of that species.

The pygmy bluetongue lizard provides a good model to understand these ecological and genetic processes. They become sexually mature between 2-3 years old and live for up to 10 years which provides many opportunities for successful reproduction in their lifetime. Conservation of long lived, highly mobile species with delayed sexual maturity, such as whales or elephants, presents a far more complex set of dynamics with more sources of risk. *Tiliqua adelaidensis* is a sedentary species, travelling only 30m – 60m in search of mates, with their burrows and food sources being in the same location. Migratory, or highly mobile species, will often traverse many types of habitat in order to get to breeding or feeding grounds which presents many areas of risk and many types of habitat that are necessary to conserve in order to protect that species.

In conclusion, this thesis details the outcome of the first pygmy bluetongue translocation and, in addition to highlighting the new avenues for *T. adelaidensis* conservation research, provides a framework for conservation practitioners to implement on their own study systems. Using the endangered pygmy bluetongue as a model, I provide tools to assess potential areas of ecological and genetic risk in population reinforcement and contribute to an increase in the success of future translocations.

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THESIS APPENDIX

APPENDIX 1.

Clive, L.F.R and Bull, C.M (2018) Tiliqua adelaidensis (pygmy bluetongue lizard). MORTALITY. *Herpetological Review 49(1) p34*

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The pygmy bluetongue lizard is an endangered skink, endemic to South Australia, living in small fragments of native grassland. Individuals spend the majority of their time in or at the entrance of burrows built by lycosid or mygalomorph spiders (Milne, Bull, *et al.* 2003). Reptiles are often attracted to roads for thermoregulation (Andrews *et al.* 2008), and death on roads from being run over by vehicles is common among species living sympatrically with pygmy bluetongue lizards, including sleepy lizards (*Tiliqua rugosa*) (Bull 1995); bearded dragons (*Pogona vitticeps*) (Taylor and Goldingay 2010); and brown snakes (*Pseudonaja textilis*) (Wotherspoon and Burgin 2011). However, although recorded causes of death of pygmy bluetongue lizards include predation (Hutchinson *et al.* 1994) and intraspecific aggression (Nielsen and Bull 2016) there are no previous records of road-killed mortality for this species. This is probably because surface movements by pygmy bluetongue lizards are rare, and pit-fall trapping is only successful in early spring, as males search for females, and in late summer, as neonates leave their natal burrows (Schofield *et al.* 2012). Herein we report the first road-killed specimen of *T. adelaidensis*.

On 19 October 2015, during the time when males seek females, a dead lizard was found on an unpaved driving track on the Nature Foundation owned Tiliqua property near Burra, South Australia. The track runs in a northerly direction through a sheep-grazed grassland occupied by *T. adelaidensis* and is used regularly by the property managers and researchers. The dead lizard was found lying in the left tyre track with its head pointing to the right, perpendicular to the track. The direction of the body supports the suggestion that the car-lizard encounter occurred while the lizard was starting to cross the track from east to west.

The unpaved track where the dead lizard was found (near a set of enclosures)

The lizard was flattened, with ants feeding on tissue, assumed to be internal organs, lying externally to the body. It was not possible to determine the sex. Although road kills are likely to remain uncommon for this species, they could become an increasing threat with the development of wind tower infrastructure close to known populations. The occurrence of movement across open space may be greater than assumed.

DEDICATION

This short note is dedicated to the late Professor Mike Bull who passed away before publication. He is greatly missed by all who had the privilege of knowing him.

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