Impacts of environmental variation on the fitness of the pygmy bluetongue lizard *Tiliqua adelaidensis*

Leili Shamiminoori

M.Sc. in Biodiversity and Conservation
University of Leeds UK

Presented for the degree of Doctor of Philosophy

School of Biological Sciences

Flinders University of South Australia

December 2015

Table of Contents

Summary 4

Declaration 6

Acknowledgments 7

Candidate Contribution 8

Chapter 1. General Introduction 10

1.1. Body condition 15

1.1.1 Use of body condition in conservation monitoring 16
1.1.2 Variation in body condition 17

1.2. Developmental Stability and Fluctuating Asymmetry 19

1.2.1 Fluctuating Asymmetry (FA) 19
1.2.2 Ontogeny of Fluctuating Asymmetry 20
1.2.3 Fluctuating asymmetry and Stress 22
1.2.4 Fluctuating asymmetry and fitness 24

1.3. The pygmy bluetongue lizard 26

1.4. Overall aims 27

1.5. Thesis structure 28

Chapter 2. Biology and Ecology of the pygmy bluetongue lizard 29

2.1 Description of species 29

2.2 Conservation issues 32

2.3 Site description and general methodology 34

2.3.1 Study period 35
2.3.2 Climate during the study 35
2.3.3 Specimen collection 36
2.3.4 Statistical analysis 38

Chapter 3. Variation in the body condition of adult pygmy bluetongue lizards.....................48

Chapter 4. Variation in body condition of neonate pygmy bluetongue lizards, *Tiliqua adelaidensis*..80

Chapter 5. The head scale symmetry in pygmy bluetongue lizards, *Tiliqua adelaidensis*...122

Chapter 6. Ontogenetic changes in head scale symmetry in the pygmy bluetongue lizards 163

Chapter 7. General Overview 183

7.1 Further research..184

Supplementary Materials 186

References...197

Summary

The alarming rate of species extinction has urged ecologists and conservation managers to identify species at risk, obtain information on the causes of decline and find efficient techniques to reverse or halt the declining trend. This thesis explores two potential indicators of population stress, individual body condition and levels of asymmetry in an endangered Australian lizard, *Tiliqua adelaidensis*, the pygmy bluetongue lizard. The pygmy bluetongue lizard is an endangered scincid lizard that is endemic to mid-North region of South Australia. There are currently 31 known populations, in a small geographic range and all restricted to small fragments of native grassland. For endangered species, identifying the causes of changes in the level of fitness overtime is an essential component of conservation management of that population.

Chapter 1 introduces the general topic of monitoring populations, and particularly populations of endangered species for fitness. Chapter 2 introduces the study species, the study sites and the general methodology used in the study.

The results chapters are all published or submitted manuscripts. Because they have more than one author, the terminology used is in the plural “we” and “our” instead of the singular “I” and “my”, although the majority of the practical work and analysis was conducted by me. Chapter 3 and 4 of the thesis explore the changes in the body condition of both adult and neonate pygmy bluetongue lizards. We considered the relative importance of phenotypic (plain and patterned morphs), temporal (sampling year and activity period), biological (sex) and climatic factors (rainfall and temperature) that can affect the body condition of lizards. Our results indicated that body condition changed significantly over sampling years in both adults and neonates. In addition, in adults, body condition was different between early (September- December) and late (January-April) activity periods within a season. In neonates, we also looked at the relationship between presence/absence of mothers and the size of litter on body condition of lizards. None of those factors showed any association with neonate body condition. The substantial annual variation in body condition of pygmy bluetongue lizards can provide important insights into the persistence of these fragile populations of this enigmatic species.

Chapter 5 and 6 of this thesis investigate the relationship between the level of symmetry in the head scales of adult (Chapter 5) and neonate (Chapter 6) pygmy bluetongue lizards and two parameters of their fitness, body condition and jaw width. We developed an index of symmetry using the digital photos of the head scales of lizards. The results of this section demonstrated a negative relationship between jaw width and the symmetry index in adult lizards over sampling years. More symmetrical lizards had narrower jaws. We provide some possible explanations of this relationship. However, body condition did not correlation with the symmetry index.

For the long-term conservation of pygmy bluetongue lizards, the findings of this study can assist in monitoring the fragmented populations and to identify any deteriorating trend in the body condition. Further study needs to explore the underlying causes of the changes in the body condition in this species. In addition, in order to use symmetry index as an indicator of fitness, future studies need to look at symmetry index in multiple traits in pygmy bluetongue lizards in order to shed light on the association between asymmetry and fitness in this species.

This thesis also contains two published papers as appendices (I, II) which are collaborative effort between my study and Damien Tohl's (School of Computing Science, Engineering and Mathematics, Flinders University) PhD thesis in image analysis. The collaboration resulted in development of the Symmetry Index of head scalation (Appendix I) and an automated photo identification technique (Appendix II) for pygmy bluetongue lizards.

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 does not contain any material previously published or written by another person except where due reference is made in the text.

Leili Shamiminoori

Acknowledgments

I would firstly like to thank my supervisor Professor Michael Bull for accepting me into this amazing project and giving me the opportunity to work with such an enigmatic species. I would like to thank him for all of his support, guidance, time, patience and advice he gave me throughout this journey. I would like to thank him for believing in me and giving me hope when I had lost my drive while pregnant with my little boy. Without his support and encouragement this work could never have been completed.

I would like to thank my co-supervisor, Dr. Mark Hutchinson for his useful guidance and advice throughout my candidature.

I would like to thank the Australian Research Council, the Holsworth Wildlife Research Endowment Fund and the Nature Foundation of South Australia for their funding and support of this project. I would also like to thank the Australian Society of Herpetologist and the Flinders University research travel grant that have provided funding for me to travel and present my work at conferences.

I also thank the landholders who allowed me access to their properties and allowed me to conduct my fieldwork. For without their continued support and interest in this species, this project could not have existed. I am particularly grateful to Chris Reed for allowing access to his property, Wandillah, which subsequently has become the Tiliqua Reserve of the Nature Foundation of SA, where the majority of my field work was conducted. His continuous support and tolerance of my presence with my equipments and car in his paddock was essential to this project.

I would like to thank Dr. Jimmy Li and Damian Tohl from the School of Computing Science, Engineering and Mathematics at Flinders University for their collaboration on parts of this thesis. Thank you for the productive, yet fun meetings we had and your invaluable work in developing the symmetry index for our lovely lizard species.

A big thank you to my lizard lab members for their support, advice, laughs, nice food and wine! Without them this work could not have been as joyful as it was. Thank you Aaron Fenner, Julie Schofield, Melissa Pettigrew, Stephanie Godfrey and Mehregan Ebrahimi for being there for me and helping and tolerating me throughout my field work and for your friendship! A special thank to Dale Burzacott for logistical support he provided that made our field trips more comfortable.

The last but not least, I would like to thank my husband Arash Haddadi for his unconditional love and support throughout this research. His encouragements, his laughs and his optimism made this journey much more fun and endurable. And a special thank to my gorgeous little boy, Dara, for your sweet cuddles, kisses and your understanding. These were the main drive behind this thesis. I love you!

Candidate Contribution

This thesis is composed predominantly of manuscripts that have been published, accepted for publication or submitted to the peer reviewed journals. I conducted all of the field work, data collection, and major parts of the analyses and interpretation of the results. I benefited hugely from the insights and advice of my supervisor Prof. C. M. Bull who helped with advice on interpreting the trends and thoroughly reviewed all the manuscripts and chapters. All the co-authors have given me permission for their collaboration to be included in this thesis. Estimates of the contribution of each co-author to each manuscript are provided.

Appendix note: The thesis also contains two published manuscripts (Appendix I, II) for which I have provided my contributions below. Although these are not to be considered as part of the assessment of this thesis, they are presented to give additional information on the ongoing studies on pygmy bluetongue lizards and to provide some background to the methodology derived by Damien Tohl and used in chapters 5 and 6.

Chapter 3

Shamiminoori, L., Fenner, A. L. & Bull, C. M. (2014) Weight watching in burrows: variation in body condition in pygmy bluetongue lizards. *Australian Journal of Zoology*, 62 (4) 284-293.

LS 80%, ALF 10%, CMB 10%

Chapter 4

Shamiminoori, L., Fenner, A. L., Schofield, J. A. & Bull, C. M. (2015) Variation in size and condition of neonate pygmy bluetongue lizards*, Tiliqua adelaidensis. Transactions of the Royal Society of South Australia.* doi: 10.1080/03721426.2015.1045312

LS 80%, ALF 5%, JAS 5 %, CMB 10%

Chapter 5

Shamiminoori, L., Bull, C. M. (2015) Can we use head scale symmetry in endangered pygmy bluetongue lizards to alert managers to population condition?

Status at the time of the thesis submission: Submitted; Herpetological Conservation and Biology

LS 80%, CMB, 20%

Chapter 6

Shamiminoori, L., Bull, C. M. (2015) Ontogenetic changes in head scale symmetry in the pygmy bluetongue lizards.

Status at the time of the thesis submission: Accepted; Transactions of the Royal Society of South Australia

LS 80%, CMB, 20%

Appendix I

Tohl, D., Li, J.S.J., Shamiminoori, L. & Bull, C. M. (2013) Image asymmetry measurement for the study of endangered pygmy bluetongue lizard. *IEEE Xplore. 28th International Conference of Image and Vision Computing New Zealand, Wellington, NZ, Nov 2013*, 76-81.

LS 10%, DT 80%, JL 5%, CMB 5%

Appendix II

Li, J.S.J., Tohl, D., Randhawa, S., Shamiminoori, L. and Bull, C.M. (2009) Non-invasive Lizard Identification using Signature Curves. *IEEE, Proceedings of TENCON 2009, Singapore*.

LS 5%, DT, 80% JL 5%, SR 5%, CMB 5%

Chapter 1. General Introduction

Global biodiversity is fast diminishing under the influence of human activity. Where individual populations come under stress from threatening processes, conservation mangers need tools that will allow them to recognise that stress while there is still time to intervene. This thesis explores two potential indicators of population stress, individual body condition and levels of asymmetry in an endangered Australian lizard, *Tiliqua adelaidensis*, the pygmy bluetongue lizard.

The pygmy bluetongue lizard is an endangered scincid lizard found exclusively in a few, small isolated remnants of native grasslands that were once more widely distributed in the mid north of South Australia (Armstrong et al., 1993; Hutchinson et al., 1994). Historical records showed the geographical distribution of this species extended over at least 150 km, from the Adelaide Plains in the south to Burra in the north (Ehmann, 1982). However, only 20 specimens had ever been collected, and there was no recording of the species, despite extensive search, after 1959. The pygmy bluetongue lizard was thought to be extinct (Cogger, 1992). There was then considerable excitement when, in 1992, the twenty first specimen was found dead inside a brown snake, *Pseudonaja textilis*, which had been killed by the car on the outskirts of Burra (Armstrong et al., 1993). The rediscovery of the pygmy bluetongue lizard prompted new search in the vicinity of the discovery and resulted in the first capture of a live specimen for more than thirty years (Armstrong and Reed, 1992). At the moment, the locations of 31 populations of pygmy bluetongue lizards are now known in the mid north of South Australia. Its very restricted distribution, confined to small habitat patches within a restricted area, has resulted in it being classified as an Endangered species. The rediscovery of the population of pygmy bluetongue lizard has enabled researchers to undertake studies to understand the basic biology and ecology of this enigmatic species. This thesis is the continuation of those studies investigating the fitness of populations of pygmy bluetongue lizards near the town of Burra in mid north of South Australia.

Biodiversity is under threat globally and many species are pushed to the brink of extinction. Factors such as introduced species (Vitousek 1988; Paulay 1994), land-use intensification, climate change (Dawson, Jackson et al. 2011) and habitat fragmentation (Haila 2002; Fahrig 2003) are some of the main driving forces behind declining populations and rapid biodiversity loss. This has drawn attention of conservation biologists and policy makers to the process of population extinction (May, Lawton et al. 1995; Sala, Chapin et al. 2000; Pullin 2002). For instance, one factor, the introduction of invasive species has had a devastating impact on native biodiversity, especially on populations now in isolation from habitat fragmentation (Vitousek 1988; Paulay 1994; Steadman 1995; Banko, Oboyski et al. 2002; Drake, Mulder et al. 2002; Hansen, Olesen et al. 2002; Peacock and Abbott 2014). The introduction of mammals to New Zealand has resulted in severe contraction of the geographic range of native reptiles and birds (Daugherty, Gibbs et al. 1993). Elsewhere, the dodo (*Raphus cuculatus*) and other smaller flightless birds on islands were the first victims of species introduced by men (e.g. mice, rats and cats) (Roberts and Solow 2003). In Australia the introduction of rabbits for food and foxes for sport hunting have had a catastrophic impact on populations of marsupials both through fierce competition for food and also predation. Hansen et al. (2002) examined the potential of alien and endemic animals to compete for floral resources on endemic trees in Mauritius. Here, the introduced bees *Apis millifera L.* compete with endemic birds over the nectar availability; this negatively impacts the pollination of the endemic trees as bees are less efficient pollinators than are the birds. Banko et al. (2002) showed that the endangered Hawaiian honey-creeper population can be at risk of extinction because their range has been reduced following the habitat fragmentation and introduction of alien species. This endangered bird is now unable to forage for seasonal seed and insect food resources over its full elevational range. Subsequently, their foraging has become concentrated into a subset of both space and time that they previously used which in turn increases their vulnerability to extinction.

The impact of invasive species has been amplified by the habitat fragmentation that now inhibits the ability of native species to respond. Habitat fragmentation by humans has been identified as one of the largest causes of loss of biodiversity (Haila 2002; Pullin 2002; Fahrig 2003). It changes the structure of the landscape by dividing a continuous habitat into small fragments which are separated by a less hospitable matrix. Once continuous habitat is eroded by destruction, and the remaining fragments differ from the original habitat in four major ways: (1) the total remaining area is smaller; (2) the proportion of edge in relation to the area is greater; (3) any given point within a fragment is closer to the edge; (4) on average each fragment is more isolated from the other fragments than before. Each one of these characteristics has a profound effect on the population (Pullin 2002). Theory predicts that small, isolated populations have a higher risk of extinction for reasons such as, (1) genetic stress, that is the loss of population genetic variation through inbreeding and genetic drift; (2) demographic stochasticity, random variation in birth and death rates; (3) limited dispersal from nearby populations; and (4) increased edge effect (McArthur and Wilson 1967; Soule 1986). In particular populations in isolated fragments are increasingly vulnerable to other threatening processes because they cannot rely on dispersal of new recruits or new genetic material to supplement their demographic losses.

Many conservation programmes are currently aimed at identifying and protecting species that only exist in isolated patches and small fragments of appropriate habitat (Lindenmayer and Possingham 1995; Anciaes and Marini 2000; Moore, Miller et al. 2008; Rocha, Siqueira et al.

2009). Within a disturbed landscape, the size of remnant fragments of the previous habitat is an important determinant the diversity they can maintain. Marini (2001) found that the species richness of the forest-dependent bird community increased with the fragment size in the Cerrado region of central Brazil. Ross et al. (2002) found that plant species richness in eucalypt forest fragments of east Australia was determined mainly by interaction between patch size, age and especially the nature of any disturbance. The native species richness increased after a natural disturbance like fire, but dramatically decreased following anthropogenic disturbance. An implication of these and related biodiversity studies, is that populations of individual species are more likely to persist in larger fragments.

The level of genetic diversity is also crucial in determining the potential of populations to adapt to the changing environments (Pertoldi, Bijlsma et al. 2007; Rivera-Oritz, Aguilar et al. 2015). High rates of fragmentation of land use change create potential barriers to genetic exchange. Federman et al. (2014) found high levels of near-neighbour pollination and spatial and temporal genetic differentiation within isolated populations of the Amazonian palm on the island of Trinidad. They hypothesised that the anthropogenic fragmentation of the habitat has hindered gene flow through a reduced distance of animal-mediated seed dispersal and cross – pollination. In a broader recent study, Rivera-Oritz et.al (2015) found that habitat fragmentation reduced overall allelic diversity of a wide range of tetrapod species (amphibians, reptiles, mammals and birds). Negative effects of fragmentation on genetic diversity were stronger for amphibians, birds and mammals, and within each taxonomic group, species with larger body sizes were more affected by habitat fragmentation. One impact of the loss of genetic diversity is the reduced ability to evolve defences against parasites and pathogens. Whitehorn et al. (2014) investigated the relationship between genetic diversity and prevalence of mites in multiple populations of two species of bumblebee (*B. mascorum* and *B. jonellus*) that were isolated due to fragmentation of their natural habitat in Scotland. They found higher prevalence of mites in populations of *B. mascorum* with lower genetic diversity, but there was no significant relationship in the more genetically diverse *B. jonellus.*

Nevertheless, different species can respond differently to habitat fragmentation based on their body size, mobility rate and trophic level. For instance, predators, higher in the trophic food web, are more susceptible to isolation than the species at lower trophic level (Holt 1996). Among different taxa of vertebrates, reptiles with their naturally limited dispersal and sensitivity to environmental conditions (Gibbons, Scott et al. 2000) may be more susceptible to the effects of habitat fragmentation than other taxa. In the New Zealand reptiles, tuatara, Moore et al. (2008) found fine-scale genetic structuring driven by the habitat fragmentation and the limited dispersal of this species.

Given the gradual effects of fragmentation, that could take decades to manifest at the population level, conservation managers need to find ways to assess the impact of stress from fragmentation on endangered species and to identify vulnerable populations before such effects could plunge them into an irreversible demographic or genetic turn down. Studies of natural history and ecological morphology and of life history factors such as mortality, fecundity, and recruitment provide the background factual information for conservation managers. However, deriving these parameters is mostly cumbersome, lengthy and expensive; hence many endangered populations may lose the race by the time the relevant issues are identified. Therefore, conservation managers need faster monitoring methods that are inexpensive, easy and repeatable by less trained monitors. That is why indirect measures

of well being of the populations are more practical than direct measures in many cases (Clarke and McKenzie 1992; Clarke 1995; Alford, Bradfield et al. 1999).
Can we detect whether populations of a fragmented species are at risk at an earlier stage and allow for intervention before it is too late? This thesis explores two possible indicators of population stress in an endangered lizard species now restricted to isolated fragments of a once more continuous range, body condition and fluctuating asymmetry (FA). Body condition, can be used as an indicator of more immediate stress, and FA can be used for documenting a longer term stress. Ideally these two should be used in combination (Lens, Van Dongen et al. 2002). The aim of this thesis is to explore the usefulness of these two indicators for wider monitoring of population condition, using one endangered lizard species as a case study.

1.1. Body condition

Ultimately, the fitness of an individual is a measure of its ability to survive and to contribute to offspring in the next generation. Body condition is an indirect indicator of fitness that is often used when longer term data on survival and fecundity are not available. It usually refers to the amount of energy stored in the body relative to some measure of body size (Green 2001; Waye and Mason 2008). Individuals with higher body condition, derived from these estimates, are considered to have higher energy reserves to contribute towards the direct fitness generating processes of reproduction (Loehr, Henen et al. 2004; Coates, Wylie et al. 2009; Vitousek 2009), growth (Madsen and Shine 2002), and survival (Shine, LeMaster et al. 2001). If a population has many individuals in poor condition it could indicate that reproductively aged adults have reduced reproductive activity, hence potentially leading to reduced population growth or even population decline (Waye and Mason 2008). Body condition can be used to quantify the well being of an individual, and average body condition can reflect the well being of a population. Estimates of body condition are typically derived from a ratio of body mass to body size, or from the residuals in regressions of body mass against body size (or in regressions of log transformed values of those parameters) (Jakob, Marshall et al. 1996; Green 2001; Connolly and Cree 2008).

The use of mass-body size residuals as an index of body condition has been criticised by some researchers for generating spurious relationships between body condition and other measures of body size, particularly when the relationship between mass and body size is non-linear (Green 2001; Schulte-Hostedde, Zinner et al. 2005). However, for many species, reproductive data are difficult or time consuming to derive, and body condition estimates can provide preliminary comparative clues about fitness profiles among different populations, or at different times for the same population. This can be particularly important in conservation programs where assessments of various management interventions and their consequences on the fitness of target species are needed quickly.

1.1.1. Use of body condition in conservation monitoring

Body size and condition are measures that can be obtained easily during a routine monitoring of a population and they can be effective means to assess the health of the population through time. Body condition indices have been used widely in environmental and conservation biology on a variety of threatened, endangered and non-threatened taxa. Examples include studies of Galapagos marine iguanas (Romero and Wikelski 2001), desert tortoises (Nagy, Henen et al. 2002), polar bears (Humphries, Umbanhowar et al. 2004), caribou (Gerhardt, White et al. 1996; Konfias, Russell et al. 2002), and the Adder viper (Bonnet and Naulleau 1995). Some studies have reported that body condition indices are lower following environmental degradation and habitat loss (Carter 1997; Hoare, Pledger et al. 2006), when there has been overharvesting of the resources used by the population (Rosen and Trites 2000), or after measurable climate change (Boersma 1998). Other studies have established that body condition indices are related to more direct measures of fitness (reproduction and survival) (Bonnet and Naulleau 1994; Aubret, Bonnet et al. 2002; Nagy, Henen et al. 2002), or to specific increases in threatening ecological interactions, such as increased parasite loads (Whiteman and Parker 2004; Godfrey, Moore et al. 2010), across a range of different taxa (Stevenson and Woods 2006).

Body condition can be a useful tool in monitoring temporal variation in the fitness of individuals or of populations overall (Bradshaw, Davis et al. 2000). If data are available over a range of environmental conditions at different times it can be used to explore the influence of alternative environmental factors in determining individual fitness in the population. That can in turn provide management clues about moderating specific environmental conditions to reduce their adverse impacts on the fitness of an endangered species. This monitoring approach can also be used to gauge the range of body conditions that can be tolerated in a normally variable environment, so that times for appropriate conservation action can be more reliably identified.

1.1.2. Variation in body condition

The principle of variation in fitness (Kitcher 1984) argues that individuals in a population differ in ways that affect their overall fitness. Some individuals have characteristics that better enable them to survive and reproduce than others (Endler 1986; Matthen and Ariew

2002). The relationship between phenotypes and fitness is critical for evaluation of mechanisms through which natural selection acts (Garland Jr. and Losos 1994). To evaluate the strength and direction of selection, the impact of different phenotypes on fitness should be assessed, and also the source of phenotypic variation (genetic or environmentally induced) must be known (Warner and Andrews 2002).

The genetic control of phenotypic differences is already well documented, but equally important can be the impact of the environment, particularly during early development. Studies of lizards have demonstrated a significant influence of the hatchling phenotype on its subsequent fitness, but also a dramatic plasticity in phenotype depending on the environmental conditions experienced during early development. Therefore, the environmental determinants of offspring phenotype are of key interest in life history theory (Olsson, Gullberg et al. 1996). In the viviparous scincid lizard *Eulamprus heatwolei,* the physical conditions that the embryo experiences prior to hatching subsequently influence characteristics of the offspring such as its time of hatching, size, shape, behaviour, thermoregulatory behaviour, and performance ability (Shine and Harlow 1993). In particular, the thermal environment of the developing embryo can be affected by the amount of time the gravid female lizards spends basking. Females that bask more produce larger and faster juveniles. Other studies have shown that larger juvenile lizards have characteristics that allow better survival than smaller lizards (Ferguson and Fox 1984; Warner and Andrews 2002). In the lizard *Eublepharis macularius*, the level of agonistic behaviour in juveniles is associated with incubation temperature. They have more aggressive behaviour and stronger competitive ability if they were exposed to higher temperatures (Gutzke and Crews 1988). Other evidence that incubation temperatures can influence the phenotype of hatchling reptiles, and consequently their fitness and survival, comes from three Australian scincid lizards, *Saproscincus mustelina*, and *Nannoscincus maccoyi*. In these lizards incubation-induced changes affect both the chemosensory responses of hatchlings to snake scent and their locomotor performance, influencing their vulnerability to predation (Downes and Shine 1999). In all of these cases, phenotypic measures relate to survival and thus can be used as indirect measures of fitness.

1.2. Developmental Stability and Fluctuating Asymmetry

1.2.1. Fluctuating Asymmetry (FA)

Another component of the phenotype of an individual that might reflect its fitness and that might be influenced both by genetic and environmental factors, is the degree of bilateral symmetry of the body. A lack of symmetry is often considered to reflect errors occurring during the development of the body. Fluctuating asymmetry is defined as the degree of random difference between left and right sides in bilateral structures, and is the most common index of developmental stability, with more symmetry implying greater developmental stability (Van Valen 1962; Palmer and Strobeck 1986). Developmental stability is defined as the ability of the organism to shield its development against environmental perturbations to produce a predetermined phenotype (Waddington 1942). Because corresponding body sides presumably have cells with the same genetic characteristics and experience similar external environmental effects, any difference in their development cannot be attributed directly to genetic or environmental differences between the two sides (Reeve 1960). Rather, asymmetrical differences are believed to demonstrate the inability of individuals to buffer their development against small perturbations (Palmer and Strobeck 1986).

In addition to fluctuating asymmetry there are two other types of bilateral asymmetry: directional asymmetry and antisymmetry (Van Valen 1962). Directional asymmetry occurs where one side is consistently biased in all individuals to become larger than the other side (e.g., the mammalian heart; Van Valen 1962). Antisymmetry is characterised by a bimodal distribution of the frequency of the difference in size between right and left structures with a mean of zero (e.g., the signalling claws of the male fiddler crab; Graham et al. 1993). There are conflicting reports in the literature regarding which types of asymmetry reflect developmental instability. Palmer and Strobeck (1992) argued that only FA is useful for studying developmental stability. In order to discern which type of asymmetry can reflect developmental stability, we need to understand the origins of asymmetry of various types (Kellner and Alford 2003).

1.2.2. Ontogeny of Fluctuating Asymmetry

There are seven hypotheses that attempt to explain how asymmetry arises during growth and development (Swaddle and Witter 1997; Kellner and Alford 2003).

1) The directional external cues hypothesis suggests that environmental influences can impose side-biased asymmetrical growth in a trait, such as the "handedness" bias in the preferential usage of right or left hand on the development of bones and muscles in primates and humans (Hallgrimsson 1998).
2) The "coin-toss" hypothesis suggests that structures grow by accumulation of morphological subunits, therefore asymmetry between two traits is the cumulative results of the difference in sizes between two traits, which are determined by chance (Hallgrimsson 1998; Hallgrimsson 1999). This hypothesis predicts that the level of

asymmetry within individuals should vary early, and then decrease throughout most of ontogeny (Soule 1982).

3) The magnification of asymmetry hypothesis posits that small variations in the initial growth conditions of a structure can be magnified by subsequent morphogenesis (Emlen, Freeman et al. 1993; Hallgrimsson 1999).
4) The accumulation of accidents hypothesis suggests that the developmental programme does not target perfect symmetry but aims for a range of *Right minus Left* values (Hallgrimsson 1998; Hallgrimsson 1999). As long as the asymmetry does not depart from this range, asymmetry will follow a random path through time.
5) The persistent asymmetry hypothesis suggests that asymmetry in a structure is determined genetically or through environmental effects early in ontogeny and the magnitude of asymmetry should persist over time (Chippendale and Palmer 1993).
6) The compensatory growth hypothesis suggests that because large random differences in size of the bilateral structures are not normal, feedback compensation mechanisms must operate. Negative feedback among cells might suppress the growth on one side that is too large and positive feedback between right and left side would promote catch-up growth on the smaller side (Emlen, Freeman et al. 1993). Hence, the right and left side oscillate in asymmetry and the magnitude of this could be the result of an individual's growth rate or the strength of the feedback signals.
7) The residual asymmetry hypothesis is a subset of the compensatory growth hypothesis and it predicts that there are simple compensatory mechanisms that counteract the effects of developmental noise and they respond continuously to the morphology of an individual. This hypothesis posits that the level of asymmetry in an individual is the residual of developmental noise minus correction and the level of FA in the

population reflects only recent levels of developmental noise (Van Valen 1962; Kellner and Alford 2003).

Three of these hypotheses, the directional external cues hypothesis, magnification of asymmetry hypothesis, and the persistent asymmetry hypothesis, each suggest that asymmetry levels in adult individuals can reflect short-term perturbations during early development. In the accumulation of accidents hypothesis, either a short period of strong stress or a prolonged period of less intense stress would increase the final level of fluctuating asymmetry. Under the compensatory growth hypothesis and the residual asymmetry hypothesis differences among individuals in FA at any given time reflect recent differences in the stress levels they have experienced, while under the coin-toss hypothesis FA largely reflects recent stress levels and should decrease as the individual grows (Kellner and Alford 2003). One theory, discussed below, is that stress, whether it is environmental or genetic, can lead to higher levels of asymmetry.

1.2.3. Fluctuating asymmetry and Stress

Environmental stress can increase phenotypic variation in populations by affecting developmental stabilities of individuals (Leary and Allendorf 1989; Graham, Freeman et al. 1993; Keiser 1993). Understanding the implications of differences in asymmetry levels in populations is important because monitoring programmes that utilise developmental stability and FA over time may provide an early warning system that would alert biologist and conservation managers to the presence of stress and, potentially a population in decline (Leary and Allendorf 1989; Parsons 1992; Alford, Bradfield et al. 1999; Lens, Van Dongen et al. 2002).

Fluctuating asymmetry (FA) at both individual and population levels has been shown to correlate positively with environmental stress from factors including climate change, habitat degradation and inadequate nutrition (Skogland 1983; Parsons 1992; Polak and Trivers 1994; Sarre 1996; Moller and Swaddle 1997; Wright and Zamudio 2002; Helle, Huhta et al. 2011; Vangestel and Lens 2011). Fluctuating asymmetry increased in the thrush *Turdus helleri,* when it was subjected to high levels of habitat disturbance (Lens et al. 2002). Red squirrels in small fragmented woodlands were shown to have higher levels of FA and smaller body size than those in larger forests (Wauters, Dhondt et al. 1996). Tsubaki (1998) investigated the changes in FA in vein length of the wings of fruit flies during the eradication programme from Okinawa islands in Japan. The study found significant variation in FA among years with the highest level of FA recorded on the year before the population went extinct. Tsubaki (1998) concluded that the increased stress on the remaining population has led to high levels of FA and suggested that FA was a useful indicator of stress. A study on shrews also showed the increased mandible asymmetry in shrews born under stressful conditions resulted from vegetation removal (Badyaev, Forseman et al. 2000).

Genetic stress can also increase the fluctuating asymmetry through loss of genetic diversity due to inbreeding or genetic drift on individual or population level (Soule 1979; Leung and Forbes 1997; Clarke 1998; Lens, Van Dongen et al. 2000; Lens, Van Dongen et al. 2002; Learny and Klingenberg 2005; Pertoldi, Kristenssen et al. 2006). Lovatt and Hoelzel (2011) found that fluctuating asymmetry and morphological variation in the skull increased in the bottleneck population of reindeer on the island of South Georgia. Also, the impact was stronger in a founder population of fewer females. Waldmann (2001) examined the effects of one-generation of selfing or outcrossing on asymmetry of flower structure of *Scabiosa canescens*, a locally rare plant species in Southern Sweden. He found higher levels of FA in the flowers of the inbred progeny than the outcrossed pollinated ones.

1.2.4. Fluctuating asymmetry and fitness

While the examples above show that levels of FA increase in conditions of environmental and genetic stress, they do not clearly establish whether there is any cost to an individual to be less symmetric. Many researchers have suggested this is the case, and that fluctuating asymmetry may also relate to individual quality and fitness (Leary and Allendorf 1989; Parsons 1992; Houle 1997; Moller 1997; Brown and Brown 1998; Clarke 1998; Alford, Bradfield et al. 1999; Leung, Forbes et al. 2000). There is considerable empirical support for a negative correlation between FA and an organism's fitness, especially where the trait measured is one that directly affects performance. For example, in some species of birds with sexually dimorphic traits, males exhibit sexual ornaments to attract females. In swallows, males that are most successful in mating have longer and more symmetrical pairs of tail feathers (Moller 1991; Moller 1992a). Martin and Lopez (2000) showed that in the lizard *Lacerta monticola*, females were able to discriminate among chemical signals of different males, and preferred to be in areas marked by males that were more symmetric in femoral pore counts. Gemsbok males (*Oryx gazella*) with asymmetric horns had poorer body condition than more symmetric individuals (Moller, Cuervo et al. 1996). In two other species of ungulates, individuals with more asymmetric antlers had lower social dominance (Moller, Cuervo et al. 1996; Putman, Sullivan et al. 2000; Mateos, Alarcos et al. 2008). Equivalent results have been reported in European starlings, *Sturnus vulgaris* (Swaddle and Witter 1994); red-winged blackbirds (Dufour and Weatherhead 1998a); mallards (Hoysak and

Ankney 1996); and Japanese scorpionfly (Thornhill 1992). However, the relationship between FA and fitness is not always clear, and other studies on this relationship have yielded heterogeneous results (See (Bjorksten, Fowler et al. 2000; Lens and Eggermont 2008). Some have reported no detectable association between FA and fitness (Merila, Forsman et al. 1992; Lindell, Forsman et al. 1993; Shine, Langkilde et al. 2005). For example, Shine et al. (2005) found no significant association between the levels of asymmetry in ventral scale count and fitness- related parameters such as body size, body condition, or locomotor speed in garter snakes, *Thamnophis sirtalis parietalis*. It has been suggested that the extent to which FA is related to fitness will depend on the character chosen for analyses, on the species under study (Clarke 1995; Bjorksten, Fowler et al. 2000; Hosken, Blanckenhorn et al. 2000) and on the magnitude of stress. Some studies have shown that relationships between FA and fitness that may be apparent under stressful conditions cannot be detected under low levels of stress (Lens, Van Dongen et al. 2002).

Nevertheless, FA has been found to be a useful monitoring tool to detect populations under stress. Alford et al. (1997) demonstrated that declining populations of two frog species (*Litoria nanotis* and *L. genimaculata*) showed large increases in FA two years before standard monitoring techniques identified the declines in the populations. Subsequently, Alford et al. (1999) suggested incorporating FA measurements into routine monitoring for frog populations for early detection of cases where conservation intervention may be required. Also, FA in otoliths is used as an indicator of stress in fish larvae in aquatic ecology (Gagaliano, Depczynski et al. 2008; Lemberget and McCormick 2009). Higher levels of otolith asymmetry have been demonstrated to affect dispersal and recruitment of fish larvae by reducing their ability to navigate towards coral reefs. Tsubaki (1998) showed an increase in FA of wing vein lengths in fruit flies as a Japanese population approached extinction, and again recommended the use of FA as an early indicator of populations requiring conservation action. FA has also been used as an early warning system to identify vulnerable populations of birds (Anciaes and Marini 2000; Lens, Van Dongen et al. 2002; Lens and Eggermont 2008). Lens et al. (2002) measured tarsus asymmetry in the taita thrush, *Turdus helleri,* inhabiting three differentially disturbed forest fragments of Kenya, and found higher levels of asymmetry in populations of more highly deteriorated fragments. In addition, within fragments, asymmetric thrushes showed lower probabilities of survival than more symmetric ones. Although, Lens et al. (2002), in this example, showed that an indication of environmental stress can be derived from levels of fluctuating asymmetry, they suggested that a better estimation could be achieved more directly from body condition, but that FA may still be used in the selection of suitable individuals (those with lower FA) in any translocation program. Ancias and Marini (2000) also used FA in wing and tarsus of passerine birds to explore the effects of fragmentation. They showed that FA was significantly higher in fragments than continuous Brazilian forest areas.

1.3. The pygmy bluetongue lizard

This thesis explores aspects of body condition and FA in an endangered Australian scincid lizard, the pygmy bluetongue lizard, *Tiliqua adelaidensis*. The pygmy bluetongue lizard is an endangered skink that now lives in a few isolated remnant patches of native grassland in the mid-north region of South Australia. Its native grassland habitat was once more widespread, but has now been mostly taken over for cropping and grazing, and this lizard species is now protected under the Australian Environment Protection and Biodiversity Conservation Act 1999. Australian native grasslands in this region are now severely fragmented and heavily degraded by agriculture. Grassland fragmentation and land use alteration are major threats to the endangered pygmy bluetongue lizard. A few populations are persisting in small fragmented remnant grasslands, and conservation managers need easy and inexpensive monitoring tools to enable them to identify the condition and environmental stress of the isolated populations in this species. In this thesis, I explore the efficiency of two indirect measures of fitness (body condition and fluctuating asymmetry) as easy, non-invasive assays to monitor isolated populations of pygmy bluetongue lizards.

1.4. Overall aims

The overall objectives of this project are:

- To investigate if there are natural variations in body condition (as a measure of fitness) among years and between sexes in pygmy bluetongue lizards, and if there are, to determine the underlying causes of the variation.
- To investigate if there is fluctuating asymmetry present in pygmy bluetongue lizard populations;
- To investigate any relationships between body condition and fluctuating asymmetry in these lizards;

This information should contribute to a broader understanding of this enigmatic species, should provide information relevant to any population monitoring programs, and should provide useful insights to the long-term management plan to conserve the pygmy bluetongue lizard in South Australia.

1.5. Thesis structure

The first two chapters of the thesis introduce the topic under investigation and give details of biology and ecology of pygmy bluetongue lizards and a description of the study sites and general methodology of the study. These chapters are structured in traditional thesis manner. Chapter 3, 4 have been formed into separate manuscripts (both are already published). Chapter 3 looks at the variation in body condition of adult pygmy bluetongue lizards and the factors contributing to those changes. Chapter 4 explores the body condition of neonate pygmy bluetongue lizards and its relationship to the female condition.

Chapters 5 and 6 investigate the correlation between the symmetry index of the head scales of pygmy bluetongue lizards and its usefulness as an indicator of fitness in adults (Chapter 5) and neonate (Chapter 6) lizards. These two chapters have been structured into separate manuscripts (Chapter 5 is submitted for publication and Chapter 6 is accepter for publication). Chapter 7 gives an overview of the thesis and is formatted in a traditional manner.

Chapter 2. Biology and Ecology of the pygmy bluetongue lizard

2.1 Description of species

The pygmy bluetongue lizard, *Tiliqua adelaidensis*, is an endangered scincid lizard from the Mabuya group (formerly *Egernia* group) and the genus *Tiliqua*. Three genera of the Mabuya group occur in Australia, *Egernia, Tiliqua*, and *Cyclodomorphus*, and these are generally regarded as primitive scincids (Chapple 2003; Greer 1979). Recent genetic study has split *Egernia* into four genera, *Egernia, Liopholis, Bellatorias and Lissolepis. Tiliqua* remains the sister lineage to *Egernia* (Gardner et al. 2008).

The pygmy bluetongue lizard is currently listed as endangered under the Australian Environment Protection and Biodiversity Conservation Act 1999. The historical geographical distribution of pygmy bluetongue lizards was thought to have stretched from the Adelaide Plains in the south to Burra in the north of South Australia (Ehmann 1982). However, for 32 years from 1960 there were no recorded collections and pygmy bluetongue lizard was thought to be extinct (Cogger 1992). In October1992, a specimen of pygmy bluetongue lizard was recovered from the stomach of a recently road killed eastern brown snake, *Pseudonaja textilis* during a vertebrate survey of the South Olary Plains region, near Burra (Armstrong, Reid & Hutchinson, 1993), and subsequently a viable population was discovered at that location. The rediscovery of pygmy bluetongue lizard has enabled extensive studies on the biology and ecology of this species, previously known only from 20 museum specimens (Ehmann 1982; Hutchinson, Milne et al. 1994). Since the initial discovery of pygmy bluetongue lizard in 1992, the location of 31populations of this species are currently known from a few isolated fragments of remnant native grassland, all owned privately, in a small area in the mid-north of South Australia (Armstrong and Reid 1992; Duffy et al. 2012; Fenner 2009; Milne 1999; Souter 2003) (Figure 2.1).

The pygmy bluetongue lizard is the smallest member of the genus *Tiliqua* with a snout-vent length (SVL) of 38-107 mm (Hutchinson et al., 1994). There is sexual dimorphism in this species with adult males and females having different head and body proportions. Males generally have shorter body length and larger and wider heads whereas adult females have more elongated bodies and narrower heads (Hutchinson, Milne & Croft, 1994). The relatively large head size in males is common in skinks (Simbotwe 1985).

The pygmy bluetongue lizard has a dorsal colouration of pale green grey to yellowish brown (Figure 2.2). The dorsal surface is either plain or with irregular blackish lateral spotting which can form a longitudinal series of bars or scattered blotches sometimes coalescing into ragged vertebral stripes. The lower lateral surfaces are pale greyish becoming off-white ventrally (Hutchinson, Milne & Croft, 1994). There is a considerable variation in colour and pattern within individuals of this species. However, there have been no detailed studies focused on colour pattern variations in the genus *Tiliqua*. Pygmy bluetongue lizards do not show the common body cross-bands that occur in other species in the genus *Tiliqua* (Cogger 1992). Polymorphism has not previously been recorded in pygmy bluetongue lizards (Hutchinson, 2009, pers. comm.), although, it has been observed that the species exhibits various intermediate patterning morphs. Anecdotal evidence has also suggested that there is variation in colour pattern both within and among populations, with some individuals having a plain phenotype while others are patterned with the lateral spotting (Fenner, personal communication). One observation has been that only populations around the Burra region are dimorphic (plain and patterned), while elsewhere in its distribution all lizards in the population are patterned with lateral spotting (Fenner, personal communication), but this notion has not been tested by definitive sampling.

The pygmy bluetongue lizard inhabits single entrance vertical burrows constructed by trapdoor spiders in the genus *Blakistonia*, and wolf spiders, *Lycosa stilingi* and *Lycosa gilberta*, withn one adult lizard per burrow (Fellows, Fenner et al. 2009; Milne 1999).They spend almost all of their time associated with their burrows either retreated down the burrow or basking at the burrow entrance. They use the burrows both as shelters from extremes of climate, and from predators, and as vantage points for ambushing their invertebrate prey. Lizards prefer burrows that are deeper than 24cm and with an entrance diameter of 16-22 cm (depending on the head width of the lizards) (Fellows, Fenner *et al.* 2009; Hutchinson, Milne *et al.* 1994; Milne 1999). Diet is mainly insects but there is evidence that vegetation is also consumed (Ehmann 1982; Milne, Bull *et al.* 2003a).

The pygmy bluetongue lizard is a solitary species that rarely encounters its conspecifics. Males interact agonistically with each other during the breeding season (early spring), one of the rare times when they leave their burrows to move around, looking for potential females to mate. They appear to use scats as social signals to indicate burrow occupancy (Fenner and Bull 2010). Both males and females deposit piles of scats in the direction of the nearest neighbour, and this shows the importance of the burrow ownership in this species. Fenner and Bull (2011a) demonstrated that pygmy bluetongue lizards defend their burrows with intense aggression against closely approaching conspecifics. Studies on social structures of pygmy bluetongue lizards also showed that lizards use olfactory cues to recognise a burrow which had been previously occupied by a lizard as a suitable refuge (Fenner and Bull 2011). This finding is vital for the future translocation release program which is considered as the optimal long-term conservation management for pygmy bluetongue lizards (Fordham et al. 2013). Stimulated translocations on a small captive population of pygmy bluetongue lizards in South Australia have shown the necessity of abundant refuge burrows, supplementary feeding, and denser vegetation in the release site to persuade individuals not to disperse from that site (Ebrahimi 2013).

Pygmy bluetongue lizards are slow growing. Individuals reach sexual maturity at 2-3 years. Mating usually takes place in spring, from late October to early November, and involves males dragging females out of their burrows to mate with them on the surface (Milne et al., 2003). Females give birth in late January until mid-March. Litter size can vary between 1-4 juveniles which may stay in the mother's burrow for up to six weeks, although many disperse within one week (Hutchinson, Milne *et al.* 1994; Milne, Bull *et al.* 2003a).

2.2. Conservation issues

The currently known populations of pygmy bluetongue lizard are restricted to isolated fragments of native grasslands ranging from Auburn in the south, to Peterborough in the north of South Australia (Duffy *et al.* 2012; Fenner 2009; Milne 1999; Souter 2003) (Fig 2.1). The main reason for the decline of this species is land use alteration from native grassland to croplands and sheep grazing. Less than 1% of the original native grassland habitat remains in the previous species range (Milne 1999, Souter 2003). Agricultural activities, such as ploughing can be detrimental to pygmy bluetongue lizard by changing the composition and depth of soil and vegetation. Studies have shown that soil composition and depth affect the distribution of pygmy bluetongue lizards (Souter 2003; Souter, Bull et al. 2007). Also, ploughing can kill lizards directly or destroy their refuge burrows (Hutchinson, Milne et al. 1994). Studies on burrow longevity in pygmy bluetongue lizards have shown that they will not use burrows in ploughed fields as their refuge, as the burrows collapse easily in these areas (Souter 2003). Hence, pygmy bluetongue lizards are restricted to small fragments of remnant native grasslands, and these are surrounded by now inhospitable agricultural habitat.

Genetic analyses of adjacent populations (Smith, Gardner et al. 2009) have shown there is no detectable gene flow, and thus by inference no lizard movement even between geographically close populations. Within populations, pygmy bluetongue lizard individuals show high site fidelity with lizards using the same burrow for periods of four weeks to over one year (Fellows, Fenner et al. 2009). Resident lizards will normally move less than 20 cm from their burrows in their daily activity (Milne 1999; Milne, Bull *et al.* 2003a). Recent investigations into movement patterns of pygmy bluetongue lizards as detected by pit fall trapping have shown there is some local movement of males in the breeding season in early spring and some dispersal of neonates in late summer (February/March) (Schofield, Fenner et al. 2012).

All known populations of pygmy bluetongue lizards are on privately owned land and have been subjected to extensive grazing by cattle and sheep for the past few decades. Pettigrew and Bull (2012) found that simulated heavy grazing influenced the burrow choice of pygmy bluetongue lizards. Lizards were shown to avoid burrows where there was no vegetation around the entrance of the burrow. On the other hand, lizards in burrows with simulated grazing, basked more and captured prey more efficiently (Pettigrew and Bull 2012), so it appears that some grazing might be beneficial in allowing more basking opportunity and clearer sight of passing prey.

Because of this grazing, and additional incursion of exotic weeds into the native grasslands, no current population is likely to inhabit an area with habitat as it was before Europeans arrived in SA. A component of conservation management must be the continued monitoring of the known populations. This thesis addresses questions of what to look for in the lizards in a monitoring program.

2.3 Site description and general methodology

The study was carried out around Burra (33° 42'S, 138° 56'E) in the mid-north of South Australia (Fig 2.1). The region contains a few relict patches of the once more widespread native grassland and is home to ten out of the 31 known populations of pygmy bluetongue lizards. Samples were taken from these 10 sites, over five years (Figure 2.3). Some more intensive sampling took place in one study site, known as Site 2 (Fenner and Bull 2007) which has been the main study site for pygmy bluetongue lizards over the past decade. This site is located 10 km east of Burra, South Australia (33° 42'S, 138° 56'E) in semi-arid native grassland. At all of the sampled sites, populations of pygmy bluetongue lizards occupy small isolated relicts of the once more widespread native grassland. These sites have remained relatively undisturbed because they are in areas that are difficult to plant crops in, although all of them have been used for sheep grazing and all are predominantly farmed land. The native vegetation in these grasslands is dominated by endemic species such as, irongrass (*Lomandra spp.)*, spear grass (*Austrostipa spp*.), wallaby grass (*Danthonia spp.)* and New Holland daisies (*Vittadinia spp*.) (Souter 2003). The sites have also been heavily invaded by exotic annual grasses and weeds including wild oats *(Avena barbata*) and wild sage (*Salvia verbenaca*) (Souter 2003). The area has hot, dry summers and cool, moist winters. The long-term average annual rainfall is between 300 and 500 mm in the Burra region.

Figure 2.4 shows the average maximum and minimum temperature per month for the Burra region for the last 45 years (1965- 2010). Temperature data for the Burra region come from Eudunda, which is approximately 50 kilometres SSE of Burra. Average daily maxima for each month in Burra region ranged from 15.5 C° in June to 34.5 C° in January. Average daily minima for each month were lowest in July (11.3 C°) and highest in January (26.0 C°). The long-term average rainfall per month in the Burra is shown in Figure 2.5. Long-term average annual rainfall was 431.1 mm for Burra over 45 years (http://www.bom.gov.au).

2.3.1 Study period

Lizards are active between September and April (the austral spring/summer) of each year (Milne 1999) and populations were sampled during this period. Each sampling season included months in two calendar years, but in this thesis I refer to each separate sampling season as a 'sampling year'. Samples were collected from the ten Burra sites over five sampling years (2005/2006-2009/2010). Some data and some samples used in the analyses in this thesis were collected by other researchers before this thesis study started (see below), but all of the data collation and analysis was by me.

2.3.2 Climate during the study

The average annual rainfall at Burra for the five year study period was 378.4 mm. Year 2006 was particularly dry with an annual rainfall of 225 mm, and 2010 was the wettest with an annual rainfall of 443.3 mm (Figure 2.6). Figure 2.7 shows the rainfall during the spring/summer period of each sampling year. During this spring/summer sampling period, 2007/2008 was the driest sampling year with the September/March rainfall of 19.08 mm and 2009/2010 was the wettest sampling year with September/ March rainfall of 37.27 mm (Figure 2.7).

There were incidents of locust plagues in 2009/2010 and 2010/2011 in the study area, supported by high summer and autumn rainfall. The Australian plague locust (*Chortoicetes* *terminifera*) forms plagues after wet conditions in western Queensland that promote high levels of hatching in those regions far distant from the pygmy bluetongue population sites. After hatching, locusts form swarms and move into South Australia independent of local temperatures and rainfall. Alternatively, local grasshopper species such as *Austroicetes cruciata* that swarm after successive years of locally high rainfall in winter and spring allow large numbers of adults to get to egg laying stages. The locust plagues provided abundant food for lizards in those years, but made sampling more difficult because lizards were less interested in the mealworms used to lure them out of their burrows (see below).

2.3.3 Specimen collection

Some of the data in this study came from samples collected by Dr. Aaron Fenner from the 10 sites from 2005-2008 for his research on the conservation and behaviour of *Tiliqua adelaidensis* (Fenner 2009). Data that he collected included the date and location of the capture, the sex, phenotype (plain or patterned), mass and various body measurements (see below) of each individual sampled. His data made up the first two years of the data set I analysed. I collected data over three sampling years 2008/09, 2009/10 and 2010/2011 from two sites around Burra previously known as Site 1(Milne 1999) and Site 2 (Fenner 2009). Details of sampling effort carried out by myself are listed in Table 1. During my surveys lizards were sampled over six days within a two week period of each month from September to March in each sampling year. Sampling took place for three days at each site. Site 1 has been subjected to intensive cattle grazing which has resulted in a scattered population of pygmy bluetongue lizards, whereas the population at Site 2 is larger and more clustered. Study sites were searched intensively for burrows on the first day of a sampling period. Burrows were inspected with an optical fibrescope (Provision Elite) (Milne 1999) for lizards, and occupied burrows were marked with a peg. Attempts were then made to capture as many lizards as possible from the marked burrows over the following days. Pygmy bluetongue lizards were collected using the fishing technique (Fenner 2009; Milne 1999) by luring them out of their burrows with the help of a mealworm tied to fishing line at the end of a fibreglass fishing rod. The mealworm was lowered to the ground in front of, or was dangled into the entrance of the lizard burrow. The lizard would then normally come out of the burrow and seize the mealworm. When the lizard had seized the mealworm it could either be lifted into the air and away from the burrow and be captured by hand or could be dragged away from its burrow. The lizards had to be lured out of the burrow completely before they were allowed to bite on the mealworm; otherwise they could hold onto the rim of the burrow and avoid capture. The burrow from which the lizard was captured was marked with a red peg with a unique identification code written on it. For each lizard, the date of capture was recorded, the sex was deduced from the sexually dimorphic morphology, or was determined when possible by cloacal examination and eversion of male hemipenes. As mentioned before, male lizards have shorter body length and wider heads and females have longer bodies and narrower heads. This is not a completely reliable diagnostic tool on its own, as there are overlaps; e.g. adult females with wide heads and adult males with small heads. Body measurements taken were snout to vent length (SVL), head length (the distance from snout to axilla) (HL) , head width (HW), jaw width (JW), total body length (from axilla to groin) (TBL), and tail length (TL). All measurements were taken using vernier callipers and were made to the nearest 0.1 mm. Lizards were weighed on a spring field balance (Pesola Precision lightline spring scale) to the nearest 0.05 gram. The position of the burrow where the lizard was captured was also registered by GPS unit. Lizards were marked by toe clipping, were photographed from the dorsal surface and released back into the same burrows.

Lizards were categorised into three age groups based on SVL: adults >80mm, subadults 60-80 mm, and juveniles <60 mm (Hutchinson, Milne et al. 1994). Each lizard was assigned to one of two phenotypic groups, ‘Patterned’ or ‘Plain’, based on the presence or absence of dark spotting on the dorsal and lateral surfaces (Hutchinson, Milne et al. 1994) (Figure 2.2).

Photographs of the dorsal scales on the head were subsequently used to assess the level of symmetry between left and right hand sides. This is described in detail later in the thesis.

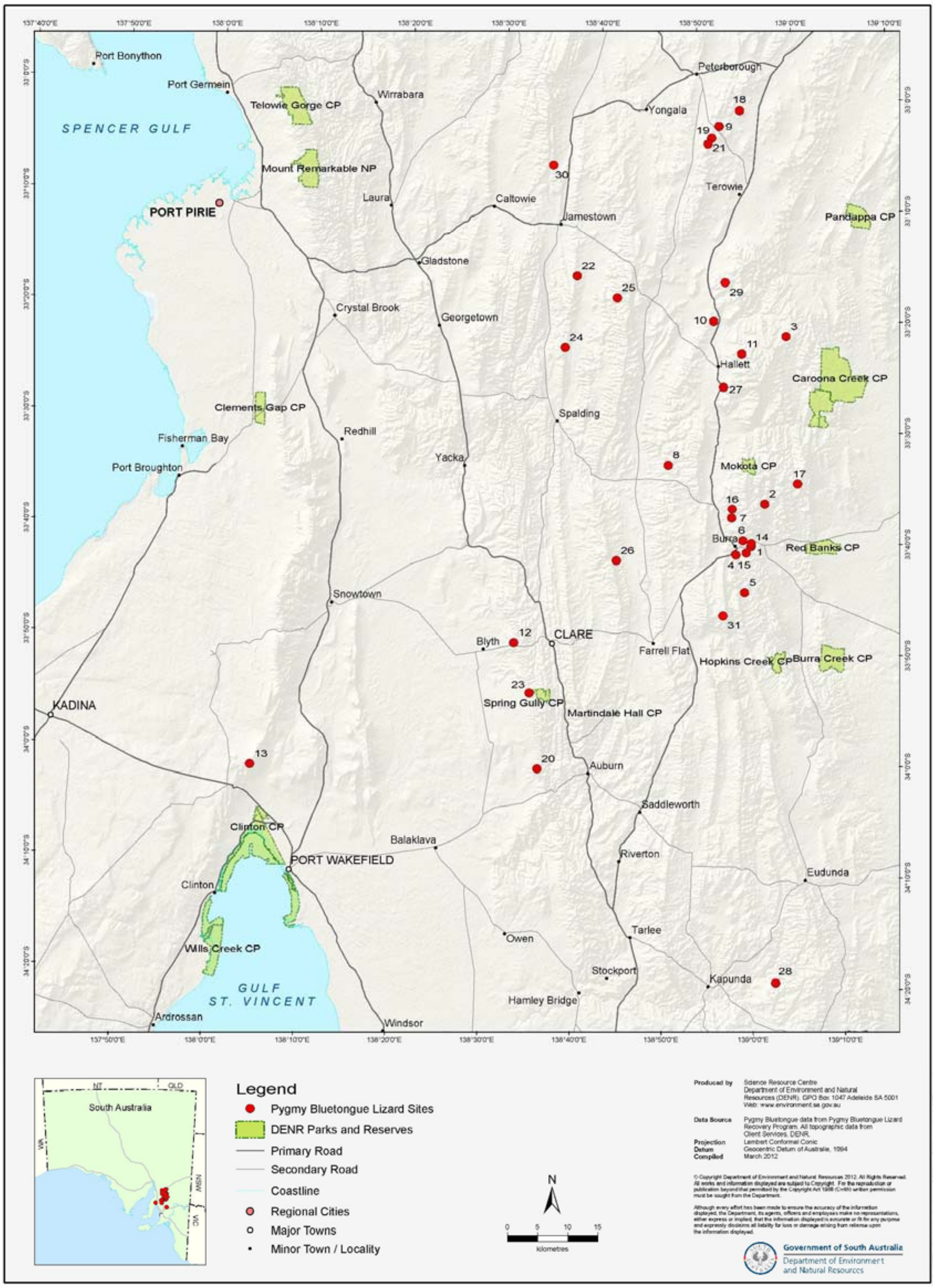
2.3.4 Statistical analysis

Statistical analyses in this thesis were conducted using ‘R’, version 2.13.0, and SPSS version 20. Details of specific tests used are described in the relevant chapters.

Table 2.1. Details of lizards captured during my study period from 2008-2011.

		Sep	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Total**
Site 1									
2008/2009	Male					8	5		**13**
	Female					10	9		**19**
	Neonate					1	8		**9**
	Total					**19**	**22**		**41**
2009/2010	Male	2	9	13	10	6	5	2	**47**
	Female	1	7	12	12	4	3	1	**40**
	Neonate					2	4		**6**
	Total	**3**	**16**	**25**	**22**	**12**	**12**	**3**	**93**
2010/2011	Male			6		4	5		**15**
	Female			4		8	3		**15**
	Neonate					0	6		**6**
	Total			**10**		**12**	**14**		**36**
Site 2									
2008/2009	Male		13	15	11	11	8	0	**58**
	Female		10	14	15	15	11	1	**66**
	Neonate					5	13	2	**20**
	Total		**23**	**29**	**26**	**31**	**32**	**3**	**144**
2009/2010	Male	3	21	25	19	15	12	10	**105**
	Female	1	14	22	18	25	11	16	**107**
	Neonate					10	40	8	**58**
	Total	**4**	**35**	**47**	**37**	**50**	**63**	**34**	**270**
2010/2011	Male		2	12		8	5		**27**
	Female		1	10		8	12	4	**35**
	Neonate					10	32	11	**53**
	Total		**3**	**22**	**-**	**26**	**49**	**15**	**105**

Figure 2.1. Map of distribution of known populations of *Tiliqua adelaidensis* in mid-north of South Australia. Figure taken from Department for Environment and Natural Resources, Government of South Australia, 2012.



Produced by Science Resource Centre Department of Environment and Natural Resources (DENR). GPO Box 1047 Adelaide SA 5001 Web: www.environment.sa.gov.au

Data Source Pygmy Bluetongue data from Pygmy Bluetongue Lizard Recovery Program. All topographic data from Client Services, DENR.

Projection Lambert Conformal Conic

Datum Geocentric Datum of Australia, 1994

Compiled March 2012

Government of South Australia
Department of Environment and Natural Resources

Figure 2.2. Illustrations showing the variation in spotting patterns and colours in *Tiliqua adelaidensis.* Picture (a) is an example of a plain lizard and pictures (b-e) show different spotting and marks on patterned lizards.

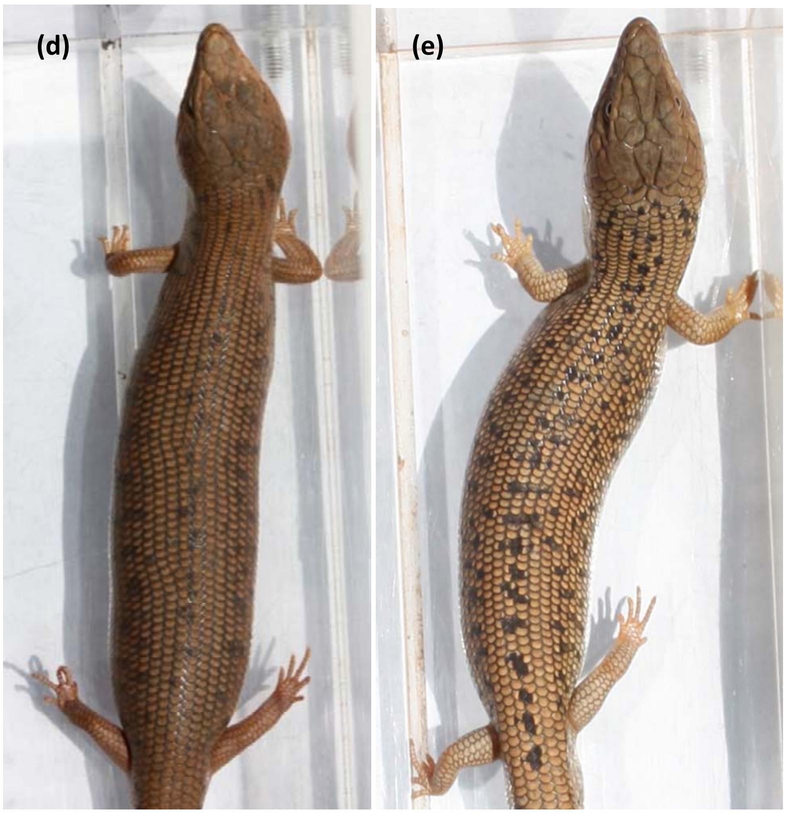

(d)
(e)

Figure 2.3. Location of the ten study sites in mid-north of South Australia. Numbers refer to populations in the larger distribution map (Fig 2.1)

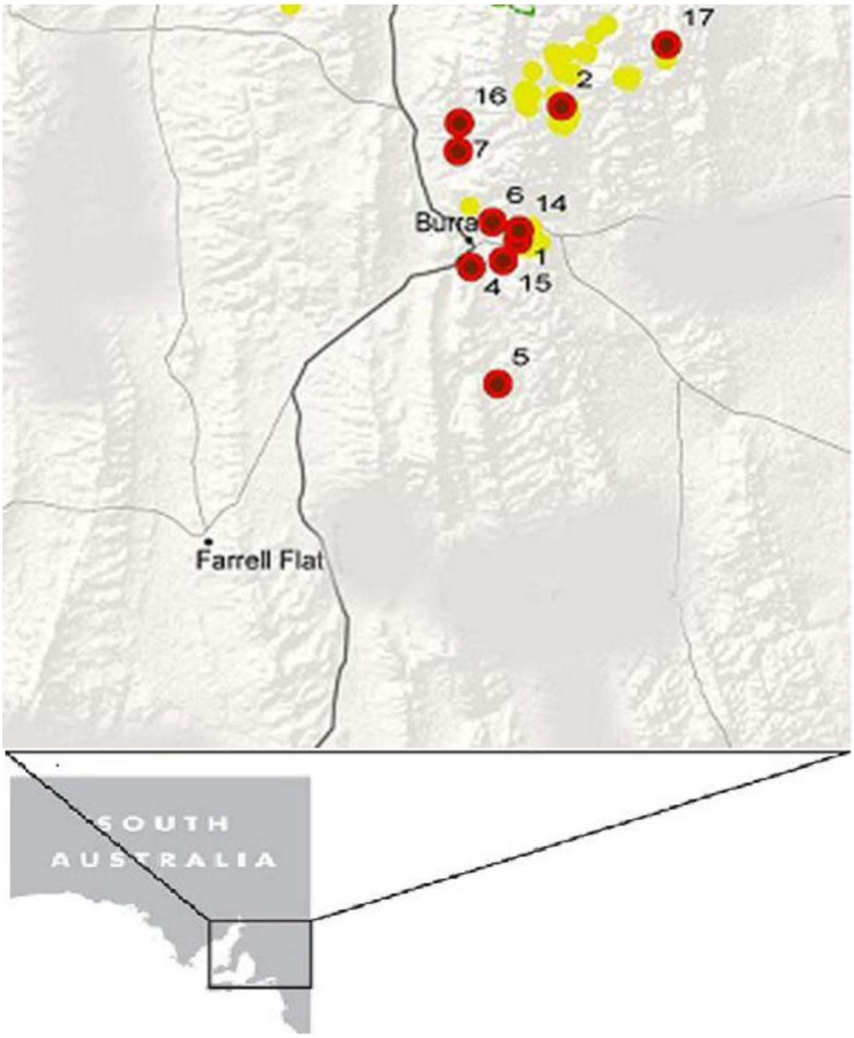


Figure 2.4.Long-term average temperature for Eudunda (1965-2010).

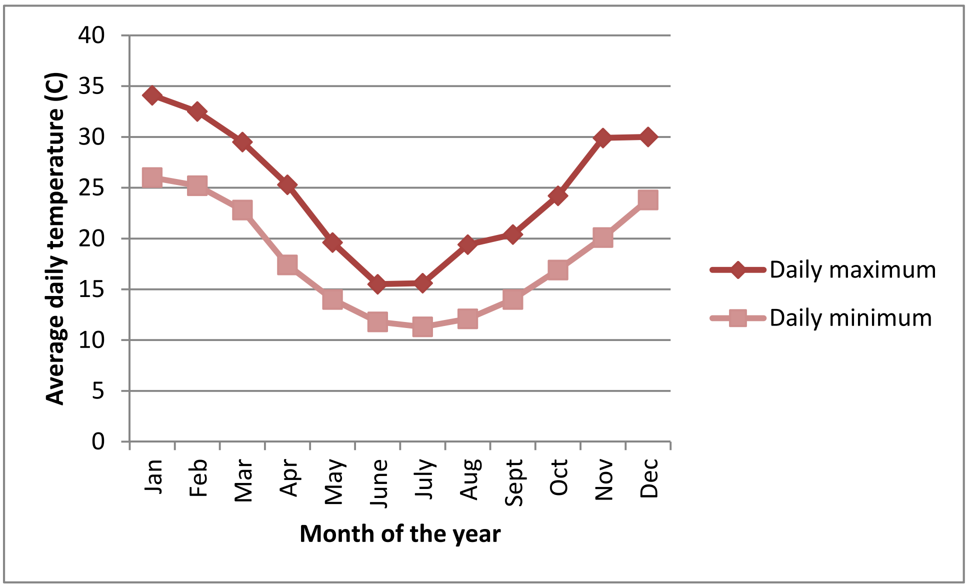


Figure 2.51. Long-term average monthly rainfall for Burra (1961-2010).

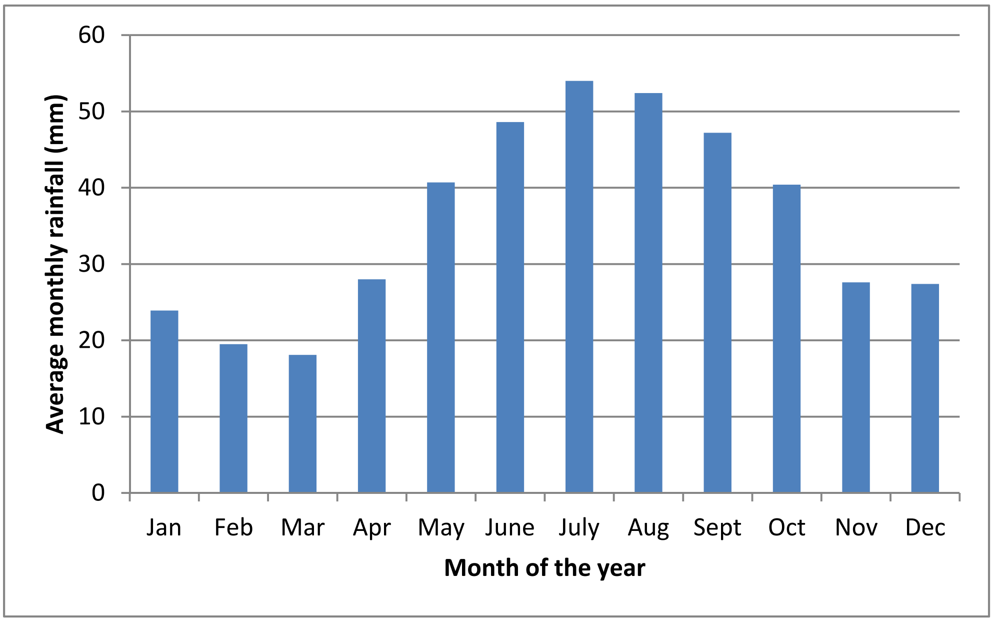


Figure 2.6. Total rainfall each year for the duration of study for Burra (2005-2010).

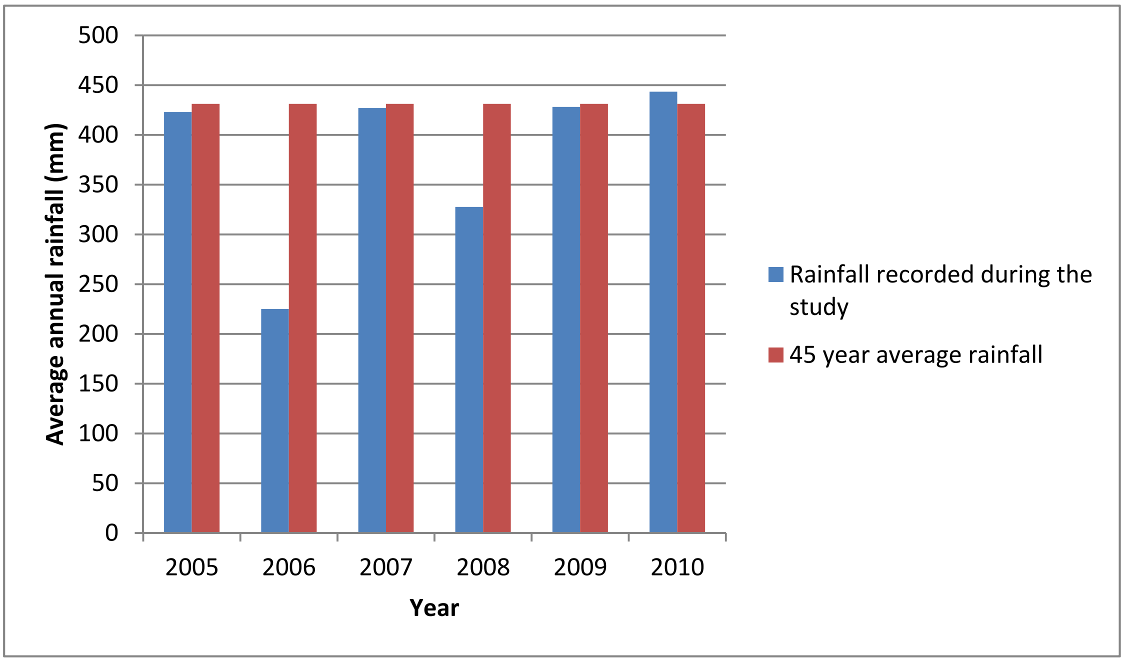
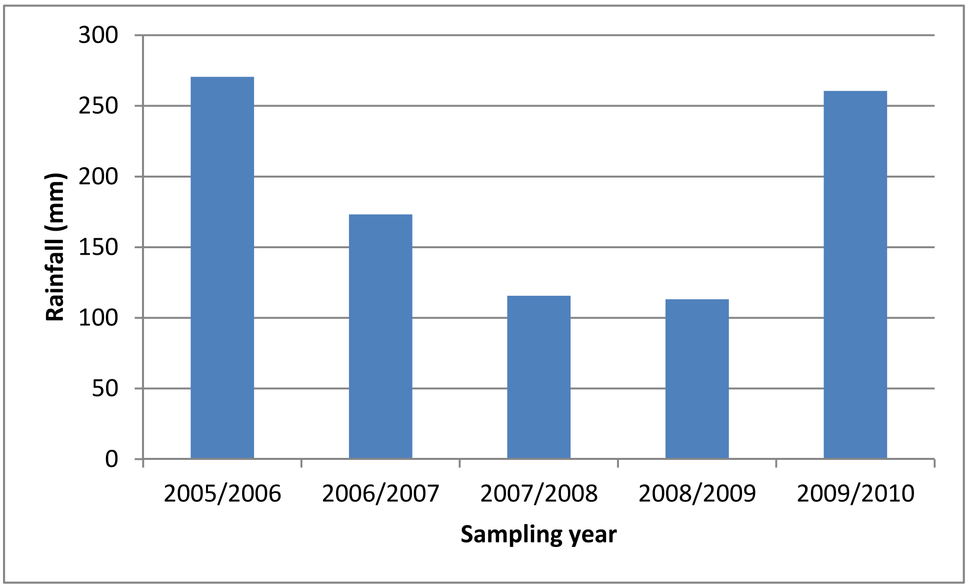


Figure 2.7. Annual spring/summer (September-March) rainfall over the study period in Burra region.



Chapter 3. Variation in the body condition of adult pygmy bluetongue lizards

Biologists have developed a wide range of morphological and physiological metrics to assess the health of animals at both individual and population levels. Body condition is one indirect indicator of fitness. It represents the amount of energy stored in the body relative to some measure of body size. Individuals with higher body condition are considered to have higher energy reserves to invest into reproduction, survival and growth. Changes in body condition are shown to correlate with the number of climatic and seasonal factors, as well as life history parameters.

This chapter explores the changes in body condition index of adult pygmy bluetongue lizards over the study period and investigates the underlying causes of variation. The analysis of changes of body condition among years may provide insights into external factors that influence the survival and demography of the pygmy bluetongue lizard.

This chapter has been published in the ***Australian Journal of Zoology*** (2014), Vol. 62 (4), pp.284-293.

Weight watching in burrows: variation in body condition in pygmy bluetongue lizards. *Australian Journal of Zoology,* 62(4) 274-293.

Leili Shamiminoori[A,B], Aaron L. Fenner[A] and C. Michael Bull[A]

[A]School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001

[B]Corresponding author: sham1012@flinders.edu.au

Telephone: 61 8 82012805

Fax: 61 8 8210 3015

Abstract

The pygmy bluetongue lizard, *Tiliqua adelaidensis*, is an endangered scincid lizard which occurs in remnants of natural grasslands in the mid-north of South Australia. We assessed the factors affecting body condition of male and female lizards, using body size residuals as an index, over five sampling years. We included sex, phenotypic (patterned or plain morphs), temporal (sampling year and activity period within year), and climatic factors in our analyses. The results indicated that sampling year and activity period within the year were the two most important factors influencing variation in body condition of both male and female lizards over the period of the study. There were similar trends when we considered females separately in each of three stages of their reproductive cycle (pre-partum, gravid, post-partum). None of the analyses showed any significant effect of phenotype on body condition. Winter-spring rainfall was positively correlated with body condition of females in the pre-partum period, but showed no significant effect on mean body condition of any other grouping of adult lizards, nor a consistent direction of correlation among the different subsets of adult lizards that

we considered. The substantial annual variations in the body condition of lizards, although of uncertain cause, provide important information for conservation managers who monitor persisting populations of this endangered species.

Introduction

In ecological studies, fitness is considered a fundamental factor in assessing the long term survival of a species. For endangered animal species, identifying the underlying causes of any observed changes in the mean levels of fitness over time is an essential component in the conservation management of natural populations (Sarre *et al.* 1994). Body condition is an indirect indicator of fitness that is often used when longer term data on survival and fecundity are not available. It usually refers to the amount of energy stored in the body relative to some measure of body size (Green 2001; Waye and Mason 2008). Estimates of body condition are typically derived from a ratio of body mass to body size, or from residuals in regressions of body mass against body size (Jakob *et al.* 1996; Green 2001; Connolly and Cree 2008). Individuals with higher body condition, derived from these estimates, are considered to have higher energy reserves to contribute towards the direct fitness generating processes of reproduction (Loehr *et al.* 2004; Coates *et al.* 2009; Vitousek 2009), growth (Madsen and Shine 2002), and survival (Shine *et al.* 2001). Although reproductive fitness is essential for population viability, and although each sex relies on energy reserves for activities related to reproduction, males and females employ different strategies in allocating their reserves (Aubret *et al.* 2002), and may have different allometric relationships. This means that direct comparisons of body condition between males and females, based on ratios of body mass and size, do not always translate into real fitness differences, and any comparative analyses are most relevant within a sex. A further complication is that reproductive females carrying developing eggs or young will weigh more than non-reproductive females with equivalent energy reserves. In that case, fitness comparisons within a sex, using ratios of body mass and size, are most effective when comparing individual females at similar stages in their reproductive cycle. Despite these issues, body condition can still be used as an indirect indicator of fitness, for comparative purposes, within males, within reproductive females and within non-reproductive females.

The use of mass-body size residuals as an index of body condition has been criticised by some researchers for generating spurious relationships between body condition and other measures of body size, particularly when the relationship between mass and body size is non-linear (Green 2001; Schulte-Hostedde *et al.* 2005). Ultimately the fitness of individuals of each sex must be judged by their reproductive success. However, for many species, reproductive data are difficult or time consuming to derive, and body condition estimates can provide preliminary comparative clues about fitness profiles in populations. This can be particularly important in conservation programs where assessments of the consequences of various management interventions are needed quickly.

Body condition indices have been used as a substitute for fitness in a wide variety of contexts in conservation. Some studies have reported reductions in body condition indices with environmental degradation, resulting from habitat loss (Carter 1997; Hoare *et al.* 2006), from overharvesting of the resources used by the population (Rosen and Trites 2000) or from climate changes (Boersma 1998). Other studies have related body condition indices to more direct measures of fitness (reproduction and survival) (Bonnet and Naulleau 1995; Nagy *et al.* 2002) or to specific threatening ecological interactions, such as increased parasite loads (Whiteman and Parker 2004), across a range of different taxa (Stevenson and Woods 2006).

For endangered species, conservation managers also need to have signals of when intervention will become important. One signal might be a decline in overall fitness of individuals in the population. Body condition can be useful as an indirect assay of temporal trends in the fitness of individuals or of populations overall (Bradshaw *et al.* 2000; Hoare *et al.* 2006; Loehr *et al.* 2007). That can in turn provide management clues about moderating or adjusting environmental conditions for optimal persistence of an endangered species. This monitoring can also be used to gauge the range of body conditions that can be tolerated in a normally variable environment, so that times for appropriate conservation action can be more reliably identified. In the current study we use body condition to assess the impact of biological, temporal and climatic factors on the fitness of an endangered Australian scincid lizard.

The pygmy bluetongue lizard *Tiliqua adelaidensis* is the smallest member of the genus *Tiliqua* and is currently listed as endangered under the *Australian Environment Protection and Biodiversity Conservation Act 1999.* The known populations of the species are restricted to a few isolated fragments of remnant native grassland in a small area in the mid-north of South Australia. Individual lizards occupy burrows constructed by lycosid and mygalomorph spiders (Hutchinson *et al.* 1994; Fellows *et al.* 2009). Adult males are on average shorter than females and have wider heads (Hutchinson *et al.* 1994), a common phenomenon in skinks (Simbotwe 1985; Hutchinson and Donnellan 1992).

Annual rainfall within the current range varies substantially among years (http://www.bom.gov.au), and individual populations have undergone major fluctuations in density in the period since the species was re-discovered in 1992 (J. Schofield, personal communication, 2011). A priority for conservation management is to understand how climate and other factors impact demography. Our study uses variation in body condition across five sampling years to provide an indirect indicator of fitness, and a pointer to factors that might influence reproduction and survival in this endangered lizard.

Additionally, the lizard has two distinctive phenotypes, patterned and plain (with and without dorsal and lateral melanistic spots) (Hutchinson *et al.* 1994; Milne 1999). We included phenotype in the analyses to determine if body condition differences could be detected between the two forms. In ectotherms, darker coloured or more pigmented individuals may absorb heat more rapidly and perform better in cooler conditions. This is the thermal melanism hypothesis (Gates 1980). Other studies have reported that darker more melanistic individuals are more frequent in populations occupying cooler habitats (Forsman 1995a; Forsman 1995b). We compared body condition of the two phenotypes in the pygmy bluetongue lizard to provide possible additional insights into the responses of each phenotype to variable climatic conditions. Our analyses were designed to determine how body condition was influenced by varying conditions, and to infer fitness consequences for future conservation management.

Materials and methods

Study sites and field methods

Sampling was conducted at 10 populations of pygmy bluetongue lizards in remnant patches of native grasslands close to the town of Burra (33° 42’S, 138° 56’E) in the mid-north of South Australia (Fig.1). The population sites were separated from each other by 1 – 15 km of agricultural land, unsuitable for lizard occupancy. Sample sizes from many of the ten sampled populations were too small to explore geographic variation across the sampled sites, and we pooled the data from the ten populations. The area has hot, dry summers and cool, moist winters. The long-term average annual rainfall at Burra is 431.1 mm, although actual rainfall each year can range between 300 and 500 mm.

Pygmy bluetongue lizards are active and can be sampled in the period between September and April (the austral spring/summer) of each year (Milne 1999). Populations were surveyed during this sampling period over five seasons (2005/06 -2009/10). Although each sampling period contained months in two calendar years, in our analyses we refer to the separate sampling periods as ‘sampling years’. We further divided each sampling year into two activity periods early (September-December) and late (January-April).

Lizards were located in each population by looking down their refuge burrows with an optical fiberscope (Provision Elite), and were captured by luring them out of their burrows with a mealworm (Strong *et al.* 1993; Milne 1999; Fenner 2009). For each lizard we recorded the date of capture (and its activity period), its sex as determined by relative head size and cloacal examination, and its mass and snout to vent length (SVL). We only included adult lizards, defined as having SVL> 80mm (Milne 1999) in this study. Each lizard was assigned to one of two phenotypic groups, ‘Patterned’ or ‘Plain’, based on the presence or absence of dark spotting on the dorsal and lateral surfaces (Hutchinson *et al.* 1994). The lizard was given a unique toe clip identification marking, and then released back to its burrow. The data were collected by random sampling and the recapture rate within and between sampling years was low. Each of the 783 adult lizards sampled (Table 1) was included in the analysis only once. For the 11 lizards that were captured more than once we used data from the first occasion the lizard was captured.

Body condition analyses were conducted on the sample of all adult lizards, then on male and female lizards separately, then on females separately from each of three different times within the sampling period to allow for different reproductive condition. In this lizard, mating occurs in early November, and litters of live young are produced in early February (Hutchinson and Donnellan 1992; Milne 1999). We considered females sampled before November 7 as pre-partum, and after February 15 as post-partum. The sample of females from between those dates was considered to contain some gravid females, with those females at different stages of litter development during that period. In each analysis for each sub-set of adult lizards, body condition was calculated separately, from the residuals of the linear regression between natural log-transformed mass and natural log-transformed SVL for all lizards in that subset. In our study, there were highly significant positive relationships between ln mass and ln SVL in each subset of the data (Fig. 2). Under these conditions this index can be a powerful indicator of spatial and temporal differences in body condition that does not require destructive sampling (Bradshaw *et al.* 2000; Hoare *et al.* 2006; Peig and Green 2010). This body condition index has been commonly used in studies of other reptile species (Lebas and Marshall 2001; Connolly and Cree 2008; Godfrey *et al.* 2010) and of other vertebrates (Jakob *et al.* 1996; Dubiec and Cichon 2001; Blackwell 2002; Cattet *et al.* 2002).

Statistical analysis

All adult lizards

Analyses were designed to determine factors that influenced patterns of variation in body condition among the sampled lizards. Data were checked for normality and are presented with standard errors where applicable. We used the general linear model in ‘R’, version 2.13.0. We first investigated the variation in body condition of all adult lizards. Body condition was the dependent variable and fixed factors were sex, sampling year, lizard phenotype (plain, patterned), and activity period (early season, late season).

This led to 23 models to explain variation in body condition of adult pygmy bluetongue lizards. The full list of models is summarised in Appendix 1. They included all combinations of factors, and all of their two-way and three-way interactions, and the null model of intercept only. Akaike’s Information Criterion (AIC) was used to rank the models for their ability to explain the observed variation in body condition index (Burnham and Anderson 2002). Subsequently we used analyses of variance to explore the interactions between factors that appeared in the best AIC model.

Male and female lizards

We next analysed separately the subsets of all adult males and all adult females. We used the same approach as before, but omitted phenotype, which had been shown to play no role in any of the top ranked models used to explain variation of body condition of the whole sample. For males and for females we considered only two explanatory factors, sampling year and activity period (early or late season), and developed five alternative models (Appendix 2) including all combinations of the two factors, their interaction and the null model of intercept only. Akaike’s Information Criterion (AIC) was used, as before, to rank the models for their ability to explain the observed variation in body condition index.

Pre-partum, post-partum and gravid female lizards

We performed separate analyses of covariance (ANCOVA) to explore the variation in body condition for each of the three groups of females sampled at different reproductive times (pre-partum, post-partum and gravid). Body condition was the dependent variable, and sampling year was a factor. In these analyses we included day of sampling (number of days after Sept 1) as a covariate, to allow for changes in body condition over time within each reproductive phase. This provided a finer scale of temporal resolution than the use of early and late activity period categories in the previous analyses. Note that no females were collected in the pre-partum period (before Nov 7) in the 2008/2009 sampling year so only four years were included for that analysis. Similarly, for the gravid period, only three females were collected, all very late in the gravid period of 2007/2008, so that year was omitted from that analysis (although those females are included in the graphical representation of the trend). And in both 2006/2007 and 2007/2008 only three and two females were collected in the post-partum period, in each case at one time in that period, so the analysis of this group of females included data from only three years.

Rainfall

Because sampling year was a significant factor contributing to all of the analyses (see results), we considered that climatic factors within each year may have influenced body condition. We did not have enough sample years to explore multiple climatic indicators, but our prediction was that rainfall during the winter and early spring (May - October), before and at the beginning of the sampling period each year, might influence both germination of annual plants, and subsequently the invertebrate food levels for the lizards. Thus, indirectly, rainfall could influence lizard body condition, and we expected body condition to be higher in years with more winter and spring rainfall. Rainfall data from Burra for May/Oct in each sampling year of the study were obtained from the Bureau of Meteorology website (http://www.bom.gov.au) (Fig.3). We explored correlative relationships between the winter-spring rainfall and mean lizard body condition in that year for all adult lizards and for each subset of adult lizards separately, to help explain observed patterns in body condition.

Results

All adult lizards

The best model to explain variation in body condition among all adult lizards contained the three way interaction between sampling year, sex and activity period (Table 2). The Akaike weight ($w_i = 0.9946$) of this model was considerably higher than the next best model, and we rejected all of the alternative models as having low support (Appendix 1).

Analysis of Variance including lizard body condition as the dependent variable and sex, sampling year and activity period as fixed factors, exhibited two significant two-way interactions (Table 3). The interaction between sampling year and lizard sex resulted from females having poorer body condition than males in each of four sampling years, according to the index derived from the pooled sample, but equivalent body condition to males in the 2005/2006 sampling year (Fig. 4).

The interaction between sampling year and activity period, resulted because mean body condition was normally higher in samples from early than from late in the season, but the seasonal decline in body condition was greater in some sampling years (e.g. 2008/2009) than in others (e.g. 2006/2007) (Fig. 5).

Male and female lizards

Each of the analyses, for males and for females, identified two models involving combinations of, and interactions between sampling year and activity period, which together accounted for over 99% of the Aikake weight (Table 4; Appendix 2). For each sex, the independently derived body condition varied both between activity periods and among years with patterns similar to those derived from the analysis of all adults (Fig. 6).

Pre-partum, post-partum and gravid female lizards

Analysis of covariance (ANCOVA) revealed a significant effect of day of sampling for gravid females, but not for pre-partum or post-partum females (Table 5). For females sampled during the gravid period body condition tended to decline with later days of sampling (Fig 7). There were no significant main effects of sampling year, nor any interaction effects between sampling year and day of sampling for any of the three groups of females (Table 5).

Rainfall

Correlation analyses showed a significant positive association between mean body condition and total winter-early spring rainfall in pre-partum female lizards (Table 6; Fig. 8). No other group of adult lizards showed any significant correlation with rainfall; nor was there any consistent trend for correlations to be positive or negative among the different groups of adults analysed (Table 6). Further analysis (data not shown) of males caught before November, and of males and females (separately) in the early activity period, failed to show any significant correlation with winter – spring rainfall.

Discussion

In our study the model that best explained variation in body condition of the sample that included all adult pygmy bluetongue lizards, *Tiliqua adelaidensis*, included lizard sex, and the activity period and sampling year when the lizard was sampled. The analysis indicated that this model was substantially better than any of the many alternative models, and we have inferred that these three factors were more important in determining body condition over the period of the study than the other factors we considered.

Effect of lizard sex

In general, males of *T. adelaidensis* displayed a higher body condition index than females (Fig.4). There are three probable sources of intersexual variation in our measure of body condition. One is based on the differences in body and head size between male and female pygmy bluetongue lizards. Male lizards have a larger head and a shorter and bulkier body than female lizards (Hutchinson *et al.* 1994). A shorter male will record a higher ratio of mass to length and thus a higher body condition than a female of equivalent mass. Hence, the allometric size difference, rather than any difference in overall fitness, will be reflected in the body condition index we have used.

The second source of intersexual variation comes from female fecundity. Gravid females, with developing embryos contributing to their body mass, are expected to register higher body condition than males of equivalent SVL with the index we have used. However, our results showed a consistently higher body condition for males than for females. The overall mean body condition for females will be influenced by the proportion of females that are gravid in our sample, but the trend in our data suggests female fecundity has a relatively low influence on the patterns of intersexual variation.

A third possible source is the real differences in the body condition between males and females resulting from differences in their responses to environmental conditions. Because we cannot separate the three explanations for this intersexual variation, we do not explore these patterns any more deeply, except to suggest that females may suffer greater loss of body condition each season as a result of producing litters. A demographic study of *T. adelaidensis* reported that females do not always reproduce in successive years, and that in each year, some females are non-reproductive (Milne 1999). This suggests that there are significant fitness costs for females in reproduction, and that they may not have recovered sufficiently from previous litters to be able to reproduce every year. That explanation is consistent with the observed lower mean body condition of females in the sample. In other reptiles, body growth is related to the availability of food (Cox *et al.* 2007), and females with below-average body condition show lower sexual receptivity (Aubret *et al.* 2002).

Effect of activity period

In four of the five sampling years, body condition of *T. adelaidensis* was higher in the early activity period (September-December) than in the late activity period (January-April), although there was annual variation in the extent of the difference. This result was consistent whether we analysed all adults together, or adult males and females separately. Within females there was also a significant decline in body condition over the gravid period. The latter result is surprising in that we expected gravid females to become increasingly heavy over that period as their embryos developed, leading to predicted increases in body condition index. However, not all females sampled in the gravid period were necessarily gravid, and even gravid females may be transferring their own body reserves to the developing embryos rather than increasing body mass (Wapstra and Swain 2001; Cadby *et al.* 2011; Itonaga *et al.* 2012).

The general seasonal decline in body condition can be explained by ecological factors. Spring growth of annual grassland plants, and moister conditions during spring , may encourage higher invertebrate prey abundances earlier in the season (Milne 1999; Souter 2003). Also during spring, there are fewer days with high temperatures, allowing lizards to remain emerged at their burrow entrances, and able to detect passing prey for longer periods

(Milne *et al.* 2003). Thus lizards could have more feeding opportunities in the early than the late activity season.

Effect of sampling year

An over-riding feature of the analyses, whether they were of all adult lizards or of males and females separately, was the substantial variation in the body condition from one year to the next.

Among the factors that have varied across the study years, the most obvious was climate (for instance rainfall) (Fig.3), and we asked whether climatic variation may explain some of this temporal variation in body condition of pygmy bluetongue lizards. A climate effect may have been direct, through the influence of climate on how often conditions were suitable for lizard activity, or indirect by influencing the levels of plant growth or invertebrate prey at the population sites. Many studies have demonstrated that annual climatic variation can impact body condition of other reptiles (Nagy and Bradshaw 1995; Wapstra 2000; Nagy *et al.* 2002; Lourdais *et al.* 2004; Shine 2005; Chamaille-Jammes *et al.* 2006) either by shifts in the mean temperature or through year to year variation in thermal regimes (Aubret and Shine 2010). Rainfall and temperature are sometimes used as indirect indicators of food availability for reptiles (Hanson and Weltzin 2000; Loehr *et al.* 2007). Nagy and Bradshaw (1995) found seasonal differences in body condition in an agamid lizard *Ctenophorus nuchalis* that were significantly correlated with rainfall. Their lizards had significantly lower body condition in years with low winter rainfall. Similarly, Madsen and Shine (2000) found year to year body condition variation in Arafura filesnakes (*Acrochordus arafurae*) that correlated with varying amount of rainfall and food availability in different years. Loehr *et al.* (2007) showed that rainfall, through its impact on food resources, impacted body condition of male and female

Hompus signatus tortoises, but reported that the timing and frequency of rainfall events were important as well as the amount of rainfall. Both male and female tortoises had low body condition in years with low rainfall.

We chose one rainfall parameter, rainfall in winter and early spring, as the most likely to affect the fitness of the lizards in our study. Despite the small sample size of years, we found one group of lizards, adult females sampled in the pre-partum period, to have a strong positive correlation between body condition and winter spring rainfall, consistent with the patterns found for other reptiles. More rainfall in the period just before lizards start to become active led to females being in better condition in the early part of the season, perhaps because of higher prey densities. However, with a limited sample of only five years of data, we detected no significant effect of winter-spring rainfall on the body condition of any other grouping of adult lizards, and the directions of correlations of body condition with rainfall showed no consistent pattern among the different subgroups of adult lizards. Perhaps the lack of response by males early in the season reflects the fact that female condition is lower than males, and that they can respond more positively to periods of resource abundance. We did not explore the impact of other climatic parameters.

Some factors not influenced by local climate may also have varied sufficiently among years to have generated the substantial annual variation in body condition that we observed. Invertebrate prey abundance may not always be driven by local climate. In the years 2010 and 2011, after our sampling was completed, pulses of plague locusts, *Chortoicetes terminifera*, invaded the study sites, providing abundant food. Those locust pulses were generated by rainfall events in western Queensland, hundreds of kilometres away and many months earlier. Additionally, local stochastic events, such as a grass fire in one population in December 2005, can cause immediate and dramatic reductions in the invertebrate prey, in the amount of time lizards spend at their burrow entrance, and in lizard body condition (Fenner and Bull 2007).

Lack of effect of phenotype

Whether lizards were the patterned or the plain phenotype had no impact on their body condition, suggesting no obvious advantage for one or the other form in gaining resources for growth in the years of our sampling. That leaves unanswered the questions of how this polymorphism is maintained within populations, and why the frequency of patterned individuals varies among populations. The thermal melanism hypothesis of Gates (1980) is not supported by our analyses. Given that the darker spots of the patterned individuals in *T. adelaidensis* are relatively small and positioned laterally as well as dorsally, they are probably unlikely to have much influence on thermoregulation.

Conservation implications

Other reptile studies have shown that body condition is dependent on a number of climatic and seasonal factors, and that body condition is positively correlated with fitness parameters such as survival (Shine *et al.* 2001), and reproduction (Naulleau and Bonnet 1996). Identifying factors that affect variation in body condition within and among seasons will shed light on the demographic processes of reptile populations over time.

The conservation implications from this study are that there are substantial annual variations in the body condition of *T. adelaidensis*, and presumably in the factors that influence body condition. The body condition in populations and its variation should be monitored by conservation managers in order to recognise what are the normally tolerated ranges of body condition, and to identify any deteriorating trend in the body condition of the current populations of *Tiliqua adelaidensis*. To do that we need to know more about what causes the variation that we have observed.

An immediate concern is to determine any impact of changing climate that might affect these lizards either directly, through thermoregulatory constraints on the amount of time lizards can remain at their burrow entrances, or indirectly by climatic influences on surrounding vegetation and available prey. The impact of changes in climate on survival and persistence can only be determined with longer-term field studies that document annual climatic variation and demographic traits and other associated variables such as food availability over more years than the current study (Brown and Shine 2007). A future development with practical application for *T. adelaidensis* would be to determine any influence of the body condition index as measured in this study, and fitness parameters such as survival and fecundity of the lizards. The results of this study stress the need of monitoring by conservation managers to determine any possible declining trend in fitness of the current populations of pygmy bluetongue lizards. Those monitoring programs may, additionally detect body condition declines that indicate population sites that can no longer support viable populations, and where translocation of individuals at those sites might be considered (Fordham *et al.* 2012).

Acknowledgements

This research was funded by the Australian Research Council, Nature Foundation of South Australia and the Holsworth Wildlife Research Endowment Fund. We thank the landholders, Chris and Maria Reed, for allowing access to their property. The study was conducted according to the guidelines of Flinders University Animal Welfare Committee (approval no. E206) and in compliance with the Australian Code of Practice for the Use of Animals for Scientific Research.

References

Aubret, F., Bonnet, X., Shine, R., and Lourdais, O. (2002) Fat is sexy for females but not males: the influence of body reserves on reproduction in snakes (*Vipera aspis*). *Hormones and Behavior* **42**, 135-147.

Aubret, F., and Shine, R. (2010) Thermal plasticity in young snakes: how will climate change affect the thermoregulatory tactics of ectotherms? *Journal of Experimental Biology* **213**, 242-248.

Blackwell, G.L. (2002) A potential multivariate index of condition for small mammals. *New Zealand Journal of Zoology* **20**, 195-203.

Boersma, P.D. (1998) Population trends of the galapagos penguins: impacts of El Nino and La Nina *Condor* **100**, 245-253.

Bonnet, X., and Naulleau, G. (1994) Use of a body condition index (BCI) for the study of the reproduction in snakes. *Compte rendus de l'Academie des Sciences III* **317,** 34-41.

Bradshaw, C.J.A., Davis, L.S., Lalas, C., and Harcourt, R.G. (2000) Geographic and temporal variation in the condition of pups of the New Zealand fur seal (*Arctocephalus foresteri*): evidence for density dependence and differences in the marine environment. *Journal of Zoology* **252**, 41-51.

Brown, G.P., and Shine, R. (2007) Rain, prey and predators: climatically driven shifts in frog abundance modify reproductive allometry in a tropical snake. *Oecologia* **154**, 361-368.

Burnham, K.P., and Anderson, D.R. (2002) 'Model selection and multimodel inference.' 2nd edn. (Springer: New York)

Cadby, C.D., Jones, S.M., and Wapstra, E. (2011) Potentially adaptive effects of maternal nutrition during gestation on offspring phenotype of a viviparous reptile. *Journal of Experimental Biology* **214**, 4234-4239.

Carter, S.I. (1997) 'The habitat ecology of bog turtles (*Clemmys muhlenbergii)* in southwestern Virginia ' (Blacksburg, VA: Virginia Polytechnic Institute and State University)

Cattet, M.R.L., Caulkett, N.A., Obbard, M.E., and Stenhouse, G.B. (2002) A body condition index for ursids. *Canadian Journal of Zoology* **80**, 1156-1161.

Chamaille-Jammes, S., Massot, M., Aragon, P., and Clobert, J. (2006) Global warming and positive fitness response in mountain populations of common lizards Lacerta vivipara. *Global Change Biology* **12**, 392-402.

Coates, P.S., Wylie, G.D., Halstead, B.J., and Casazza, M.L. (2009) Using time-dependent models to investigate body condition and growth rate of the giant gartersnake. *Journal of Zoology* **279**, 285-293.

Connolly, J.D., and Cree, A. (2008) Risks of late start to captive management for conservation: phenotypic differences between wild and captive individuals of a viviparous endangered skink (*Oligosoma otagense*). *Biological Conservation* **141**, 1283-1292.

Cox, R.M., Butler, M.A., and John-Alder, H.B. (2007) The evolution of sexual size dimorphism in reptiles. In 'Sex, size, and gender roles: evolutionary studies of sexual size dimorphism.'. (Eds. DJ Fairbarin, WU Blanckenhorn and T Szekely). pp. 38-49 (Oxford University: Oxford).

Dubiec, A., and Cichon, M. (2001) Seasonal decline in health status of Great Tit (*Parus major*) nestlings. *Canadian Journal of Zoology* **79**, 1829-1833.

Fellows, H.L., Fenner, A.L., and Bull, C.M. (2009) Spiders provide important resources for an endangered lizard. *Journal of Zoology* **279**, 156-163.

Fenner, A.L. (2009) The biology, ecology and social structuring of the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis:* considerations for long term conservation. Ph.D. Thesis., Flinders Unversity Adelaide

Fenner, A.L., and Bull, C.M. (2007) Short-term impact of grassland fire on the endangered pygmy bluetongue lizard. *Journal of Zoology* **272**, 444-450.

Fordham, D.A., Watts, M.J., Delean, S., Brook, B.W., Heard, L., and Bull, C.M. (2012) Managed relocation as an adaptation strategy for mitigating climate change threats to the persistence of an endangered lizard. *Global Change Biology* **18**, 2743-2755.

Forsman, A. (1995a) Opposing fitness consequences of colour pattern in male and female snakes. *Journal of Evolutionary Biology* **8**, 53-70.

Forsman, A. (1995b) Heating rates and body temperature variation in melanistic and zigzag *Vipera berus*: does colour make a difference? *Annales Zoologici Fennici* **32**, 365-374.

Gates, D.M. (1980) 'Biophysical Ecology.' (Springer: New York)

Godfrey, S.S., Moore, J.A., Nelson, N.J., and Bull, C.M. (2010) Unravelling causality from correlations: revealing the impacts of endemic ectoparasites on a protected species (tuatara). *Parasitology* **137**, 275-286.

Green, A.J. (2001) Mass/lenght residuals: measures of body condition or generators of spurious results? *Ecology* **82**, 1473-1483.

Hanson, P.J., and Weltzin, J.F. (2000) Drought disturbance from climate change: response of United States forests. *Science of the Total Environment* **26**, 205-220.

Hoare, J.M., Pledger, S., Keall, S.N., Nelson, N.J., Mitchell, N.J., and Daugherty, C.H. (2006) Conservation implications of a long-term decline in body condition of the Brothers Island tuatara (*Sphenodon guntheri*). *Animal Conservation* **9**, 4456-462.

Hutchinson, M.N., and Donnellan, S.C. (1992) Taxonomy and genetic variation in the Australian lizards of the genus *Pseudemoia* (Scincidae: Lygosominae). *Journal of Natural History* **26**, 215-264.

Hutchinson, M.N., Milne, T., and Croft, T. (1994) Redescription and ecological notes on the pygmy bluetongue, *Tiliqua adelaidensis* (Squamata:Scincidae). *Transactions of the Royal Society of South Australia* **118**, 217-226.

Itonaga, K., Edwards, A., Wapstra, E., and Jones, S.M. (2012) Interpopulation variation in costs of reproduction related to pregnancy in a vivparous lizard. *Ethology Ecology and Evolution* **24**(367-376).

Jakob, E.M., Marshall, S.D., and Uetz, G.W. (1996) Estimating fitness: a comparison of body condition indices. *Oikos* **77**, 61-67.

Lebas, N.R., and Marshall, N.J. (2001) No evidence of female choice for a condition-dependent trait in the agamid lizard, *Ctenophorus ornatus. Behaviour* **138**, 965-980.

Loehr, V.J.T., Henen, B.T., and Hofmeyr, M.D. (2004) Reproduction of the smallest tortoise, the Namaqualand speckled padloper, *Homopus signatus signatus*. *Herpetologica* **60**, 444-454.

Loehr, V.J.T., Hofmeyr, M.D., and Henen, B.T. (2007) Annual variation in the body condition of a small, arid-zone tortoise, *Homopus signatus signatus*. *Journal of Arid Environments* **71**(4), 337-349.

Lourdais, O., Shine, R., Bonnet, X., Guillon, M., Naulleau, G., and (2004) Climate affects embryonic development in a viviparous snake, *Vipera aspis*. *Oikos* **104**, 551-560.

Madsen, T., and Shine, R. (2002) Short and chubby or long and slim? Food intake, growth and body condition in free-ranging pythons. *Austral Ecology* **27**, 672-680.

Milne, T. (1999) Conservation and ecology of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis*. PhD Thesis, Flinders University, Adelaide

Milne, T., Bull, C.M., and Hutchinson, M.N. (2003) Use of burrows by the endangered pygmy bluetongue lizard *Tiliqua adelaidensis. Wildlife Research* **30**, 523-528.

Nagy, K.A., and Bradshaw, S.D. (1995) Energetics, osmoregulation, and food consumption by free-living desert lizards, *Ctenophorus* (equals *Amphibolorus nuchalis*). *Amphibia-Reptilia* **16**, 25-35.

Nagy, K.A., Henen, B.T., Vyas, D.B., and Wallis, I.R. (2002) A condition index for the desert tortoise (*Gopherus agassizii*). *Chelonian Conservation and Biology* **4**, 425-429.

Naulleau, G., and Bonnet, X. (1996) Body condition threshold for breeding in a viviparous snake. *Oecologia* **107**, 301-306.

Peig, J., and Green, A.J. (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and lenght. *Functional Ecology* **24**, 1323-1332.

Rosen, D.A., and Trites, A.W. (2000) Pollock and the decline of Steller sea lions: testing the junk food hypothesis *Canadian Journal of Zoology* **78**, 1243-1250.

Sarre, S., Dearn, J.M., and Georges, A. (1994) The application of fluctuating asymmetry in the monitoring of animal populations. *Pacific Conservation Biology* **1**, 118-122.

Schulte-Hostedde, A.I., Zinner, B., Millar, J.S., and Hickling, G.J. (2005) Restitution of mass-size residulas: validating body condition indices. *Ecology* **86**, 155-163.

Shine, R. (2005) Life-history evolution in reptiles. *Annual Review of Ecology, Evolution, and Systematics* **36**, 23-46.

Shine, R., LeMaster, M.P., Moore, I.T., Olsson, M.M., and Mason, R.T. (2001) Bumpus in the snake den: effects of sex, size, and body condition on morality of red-sided garter snakes. *Evolution* **55**, 598-604.

Simbotwe, M.P. (1985) Sexual dimorphism and reproduction of *Lampropholis guichenoti* (Lacertilia: Scincidae). In 'The biology of Australian frogs and reptiles '. (Eds. G Grigg, R Shine and H Ehmann) pp. 11-16. (Surrey Beatty & Sons: Sydney)

Souter, N.J. (2003) Habitat requirements and conservation of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis*. PhD Thesis, Flinders University of South Australia, Adelaide.

Stevenson, R.D., and Woods, J., W. A. (2006) Condition indices for conservation: new uses for evolving tools. *Integrative and Comparative Biology* **46**, 1169-1190.

Strong, D., Leatherman, B., and Brattstrom, B.H. (1993) Two new simple methods for catching small fast lizards. *Herpetological Review* **24**, 22-23.

Vitousek, M. (2009) Investment in mate choice depends on resource availability in female Galápagos marine iguanas (*Amblyrhynchus cristatus*). *Behavioral Ecology and Sociobiology* **64**, 105-113.

Wapstra, E. (2000) Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology* **14**, 345-352.

Wapstra, E., and Swain, R. (2001) Reproductive correlates of abdominal fat body mass in *Niveoscincus ocellatus,* a skink with asynchronous reproductive cycle. *Journal of Herpetology* **35**, 403-409.

Waye, H.L., and Mason, R.T. (2008) A combination of body condition measurements is more informative than conventional condition indices: temporal variation in body condition and corticosterone in brown tree snakes (*Boiga irregularis). General and Comparative Endocrinology* **155**, 607-612.

Whiteman, N.K., and Parker, P.G. (2004) Body condition and parasite load predict territory ownership in the Galapagos hawk. *Condor* **106**, 915-921.

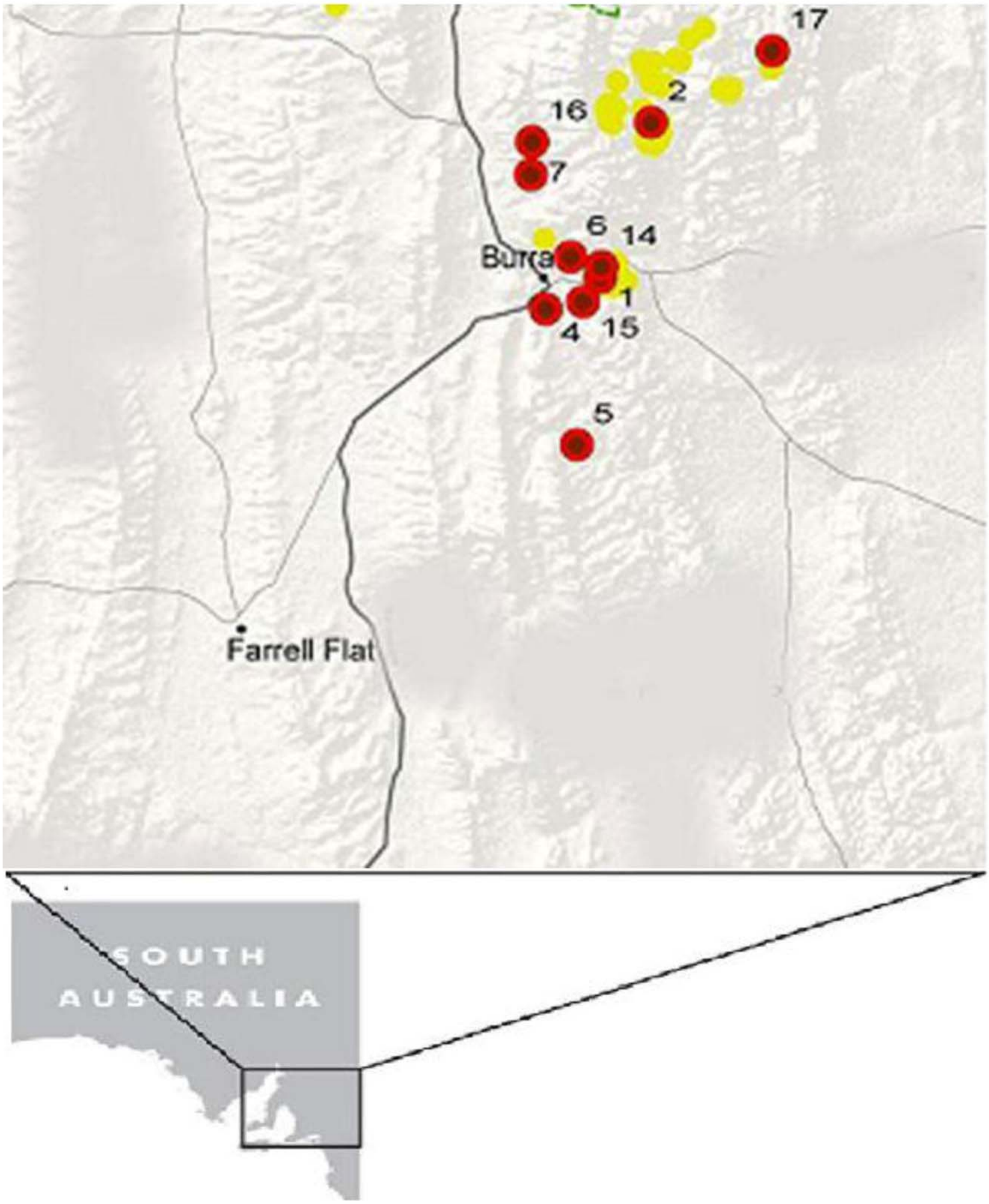
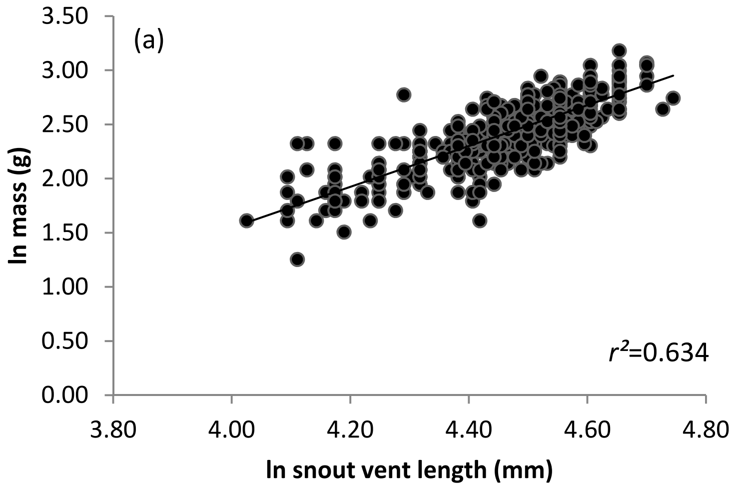
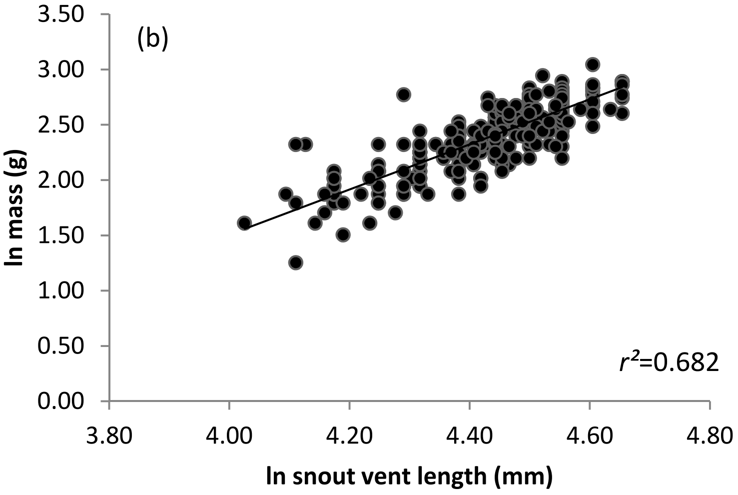
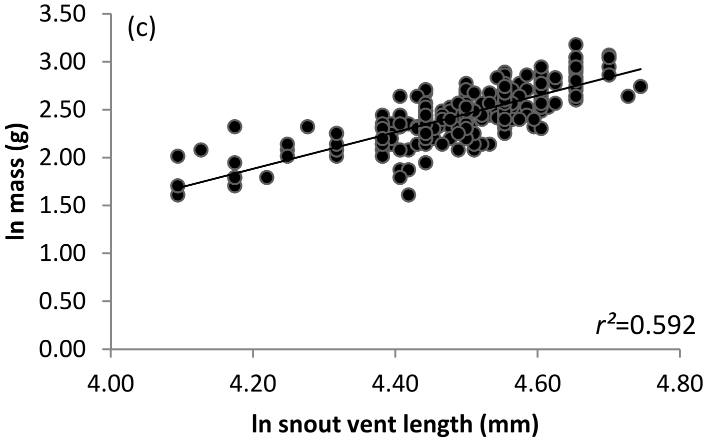


Fig. 1. Locations of all currently known population sites of pygmy bluetongue lizards near Burra. Black circles indicate the populations sampled in this study.


(a)
r²=0.634
ln mass (g)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
3.80
4.00
4.20
4.40
4.60
4.80
ln snout vent length (mm)


(b)
r²=0.682
ln mass (g)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
3.80
4.00
4.20
4.40
4.60
4.80
ln snout vent length (mm)


(c)
r²=0.592
ln mass (g)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
4.00
4.20
4.40
4.60
4.80
ln snout vent length (mm)

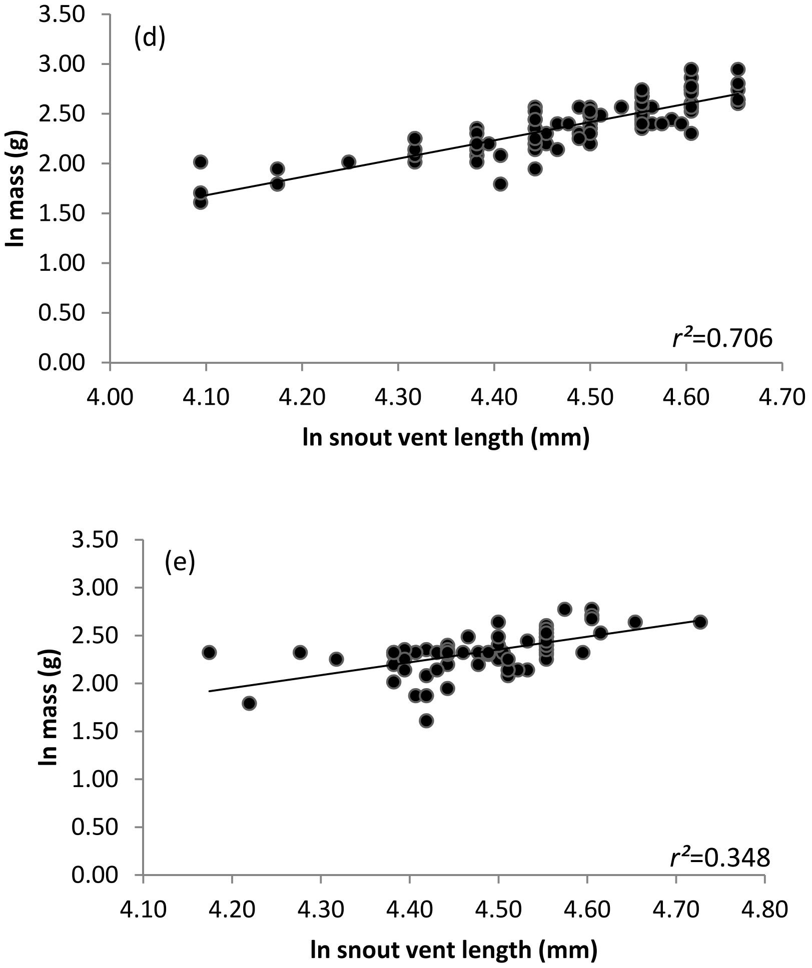


Fig. 2. Relationship between body mass and snout–vent length in different studied groups of pygmy bluetongue lizards: (*a*) all adults, (*b*) males, (*c*) females, (*d*) prepartum females, and (*e*) postpartum females. $P < 0.001$ for all relationships.

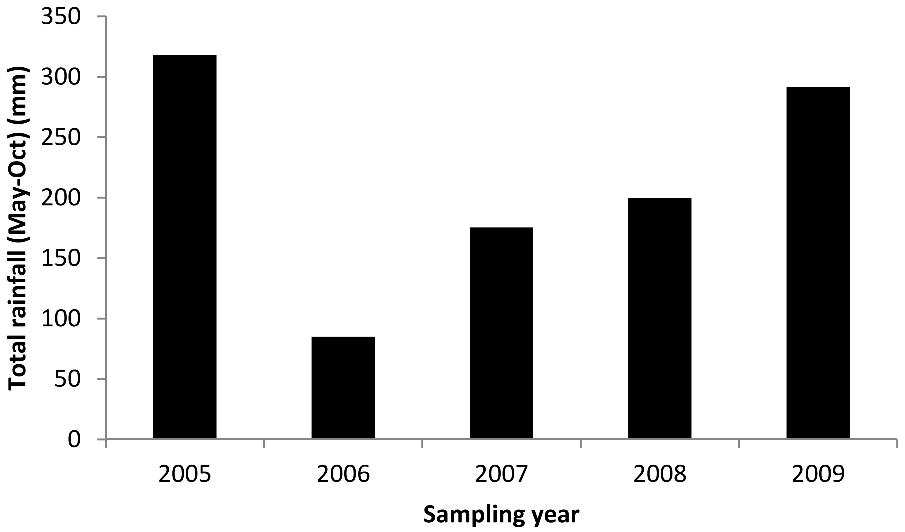


Fig. 3. Total rainfall between May and October for Burra region (2005–09).

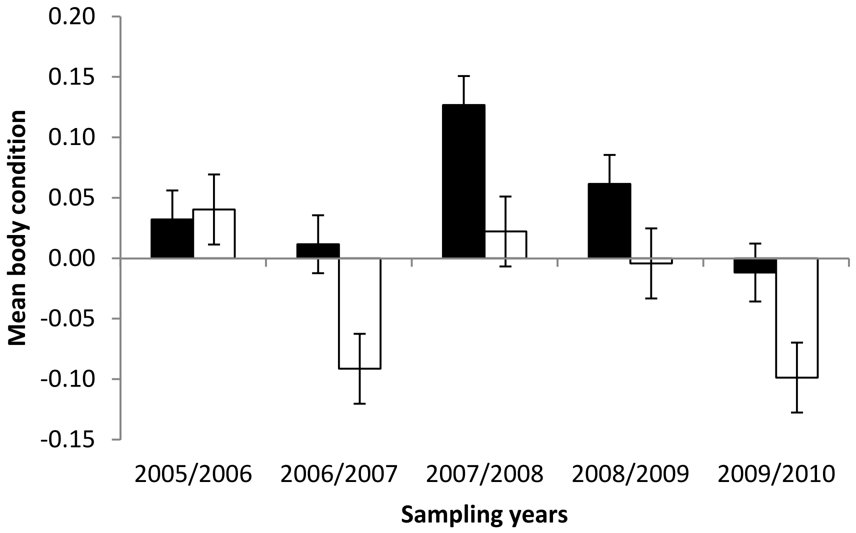


Fig. 4. Mean (s.e.) body condition of males (black bars) and females (white bars) in each sampling year.

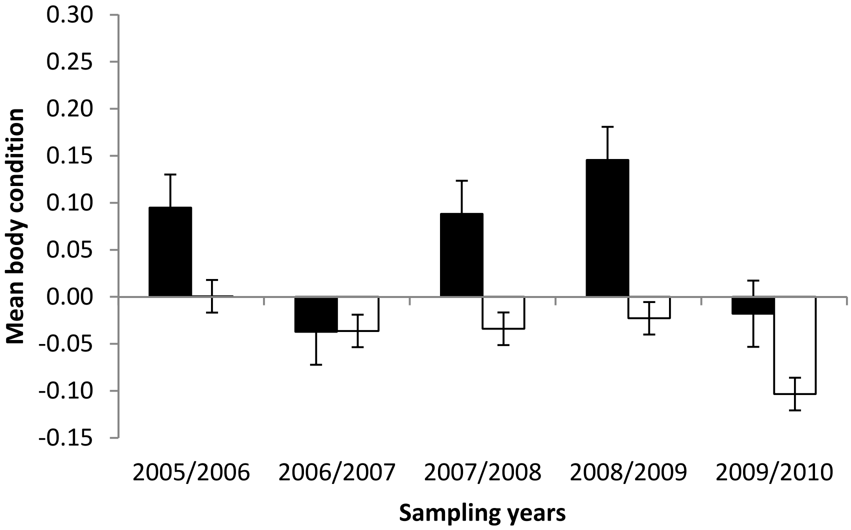


Fig. 5. Mean body condition (s.e.) of adult lizards in early-season (black bars) and late-season (white bars) activity periods in each sampling year.

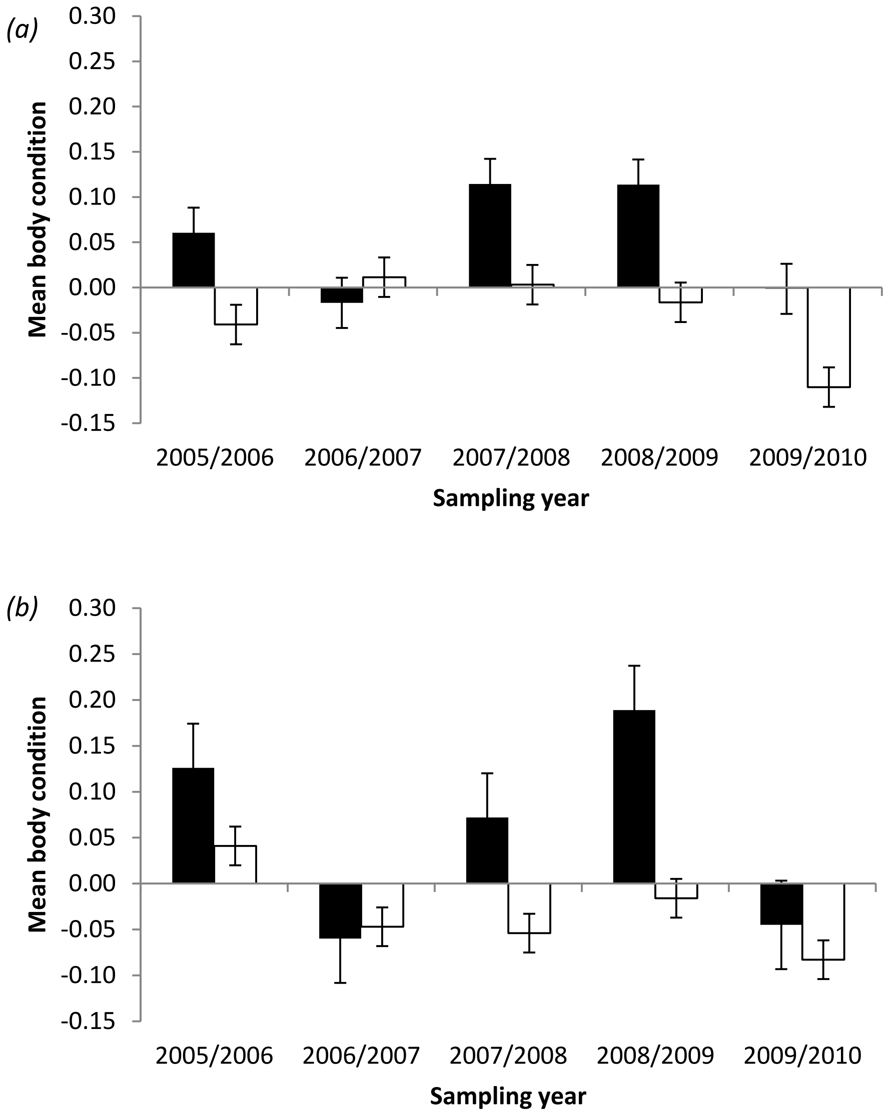


Fig. 6. Mean (s.e.) body condition of (*a*) males, and (*b*) females in early-season (black bars) and late-season (white bars) activity periods in each sampling year.

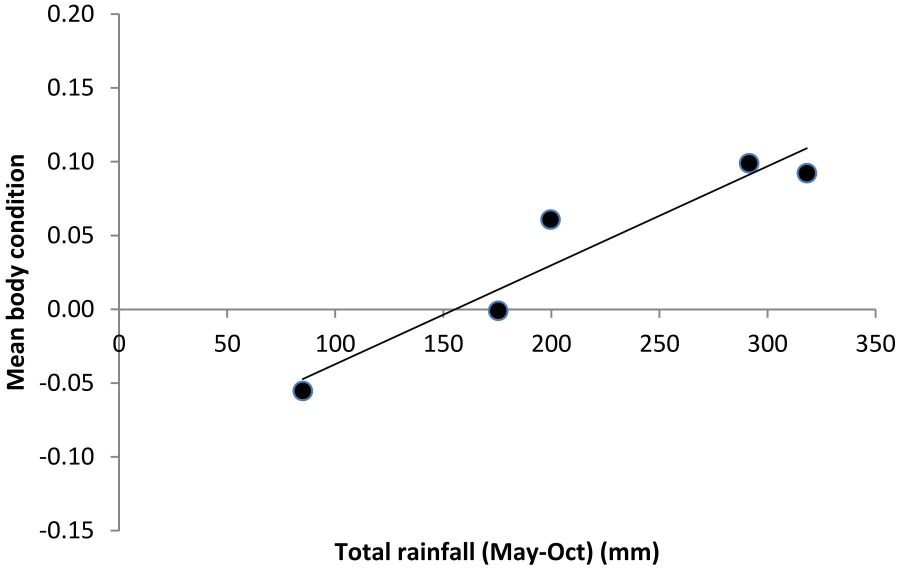


Fig. 7. Correlation between mean body condition and total rainfall (May–October) of prepartum female pygmy bluetongue lizards.

Table 1. Sex and phenotype of adult pygmy bluetongue lizards captured from Burra during the study.

Sampling year	Sex		Phenotype	
	Male	Female	Patterned	Plain
2005/06	143	117	161	99
2006/07	114	102	165	51
2007/08	31	31	62	0
2008/09	50	36	69	17
2009/10	79	80	120	39
Total	417	366	577	206

Table 2. Summary of first three models for AIC model selection investigating the effects of year, sex, activity period and phenotype on body condition index of the pygmy bluetongue lizards

The first model is the most parsimonious model. K, the number of fitted parameters; AIC, Akaike's Information Criteria; Δ AIC, difference between AIC value of the best model and AIC value of other models; w_i, Akaike model weight; *cum* w_i, Cumulative Akaike weights; '×' refers to the interaction between factors; '+' represents the inclusion of main factors in the model

Candidate model	K	AIC	Δ AIC	w_i	*cum* w_i
Year x Sex × Activity period	21	–766.1897	0.0000	0.9946	0.9946
Year + Activity period + Sex	8	–755.7547	10.4350	0.0054	1.0000
Year × Sex	11	–738.8413	27.3485	0.0000	1.0000

Table 3. Results of the analysis of variance of the body condition of pygmy bluetongue lizards with sex, sampling year and activity period as factors

P values in bold indicate results that are statistically significant at $P < 0.05$

	d.f.	*F*	*P*
Sex	1	37.68	**0.001**
Year	4	21.38	**0.001**
Activity period	1	52.71	**0.001**
Year × Sex	4	4.39	**0.001**
Activity period × Sex	1	0.29	0.766
Year × Activity period	4	4.96	**0.001**
Year × Activity period × Sex	4	0.59	0.664
Error	814		

Table 4. Summary of the best models for AIC model selection investigating the effects of year, and activity period on body condition index of male and female pygmy bluetongue lizards

K, the number of fitted parameters; AIC, Akaike's Information Criteria; Δ AIC, difference between AIC value of the best model and AIC value of other models; w_i, Akaike model weight; *cum* w_i, Cumulative Akaike weights; '×' refers to the interaction between factors; '+' represents the inclusion of main factors in the model

Candidate model	K	AIC	Δ AIC	w_i	*cum* w_i
Males					
Year + Activity period	7	–422.9524	0.0000	0.7121	0.7121
Year × Activity period	11	–421.1408	1.8116	0.2879	1.0000
Females					
Year × Activity period	11	–349.6167	0.0000	0.7579	0.7579
Year + Activity period	7	–347.3305	2.2863	0.2416	0.9995

Table 5. Results of the analysis of covariance of the body condition of female pygmy bluetongue lizards during two periods (prepartum and postpartum) with sampling year as a factor and day of sampling as a covariate.

	d.f.	*F*	*P*
Prepartum			
Year	3	1.62	0.187
Day of sampling	1	0.02	0.877
Year × Day of sampling	3	1.21	0.306
Error	115		
Postpartum			
Year	2	2.38	0.101
Day of sampling	1	1.51	0.223
Year × Day of sampling	2	2.72	0.074
Error	59		

Table 6. Correlations between mean body condition and total rainfall (May–October) for five studied groups of pygmy bluetongue lizards

Asterisk indicates correlation is significant at the 0.05 level (2-tailed)

Lizard group	*r*	N	*P*
Adults	0.042	5	0.947
Males	–0.287	5	0.639
Females	0.305	5	0.618
Prepartum females	**0.953***	4	**0.012**
Postpartum females	–0.439	5	0.561

Appendix 1. The full list of models for AIC model selection investigating the effects of year, sex, activity period, and phenotype on body condition index of the pygmy bluetongue lizards

K, the number of fitted parameters; AIC, Akaike's Information Criteria; Δ AIC, difference between AIC value of the best model and AIC value of other models; w_i, Akaike model weight; *cum* w_i, Cumulative Akaike weights; '×' refers to the interaction between factors; '+' represents the inclusion of main factors in the model

Candidate model	K	AIC	Δ AIC	w_i	*cum* w_i
Year × Sex × Activity period	21	–766.1897	0.0000	0.9946	0.9946
Year + Activity period + Sex	8	–755.7547	10.4350	0.0054	1.0000
Year × Activity period	11	–738.8413	27.3485	0.0000	1.0000
Year + Activity period	7	–734.3585	31.8312	0.0000	1.0000
Year × Activity period × Phenotype	19	–726.0781	40.1117	0.0000	1.0000
Year × Sex	11	–724.1189	42.0709	0.0000	1.0000
Year × Sex × Phenotype	19	–717.9687	48.2210	0.0000	1.0000
Year + Sex	7	–712.2252	53.9646	0.0000	1.0000
Year + Sex + Phenotype	8	–710.6168	55.5729	0.0000	1.0000
Year	6	–685.5839	80.6058	0.0000	1.0000
Year + Phenotype	7	–684.0801	82.1096	0.0000	1.0000
Year × Phenotype	10	–680.3160	85.8737	0.0000	1.0000
Activity period × Sex	5	–674.3874	91.8023	0.0000	1.0000
Activity period × Phenotype × Sex	9	–672.6001	93.5897	0.0000	1.0000
Sex + Activity period	4	–671.8253	94.3644	0.0000	1.0000
Sex	3	–657.9201	108.2697	0.0000	1.0000
Phenotype × Sex	5	–657.7948	108.3950	0.0000	1.0000
Sex + Phenotype	4	–656.2693	109.9204	0.0000	1.0000
Activity period	3	–647.4754	118.7143	0.0000	1.0000
Activity period + Phenotype	4	–645.5916	120.5982	0.0000	1.0000
Activity period × Phenotype	5	–643.7091	122.4806	0.0000	1.0000
intercept	2	–631.1395	135.0502	0.0000	1.0000
Phenotype	3	–629.5465	136.6433	0.0000	1.0000

Appendix 2. The full list of models for AIC model selection investigating the effects of sampling year, and activity period on body condition index of male and female pygmy bluetongue lizards.

K, the number of fitted parameters; AIC, Akaike's Information Criteria; Δ AIC, difference between AIC value of the best model and AIC value of other models; w_i, Akaike model weight; *cum* w_i, Cumulative Akaike weights; '×' refers to the interaction between factors; '+' represents the inclusion of main factors in the model

Candidate model	K	AIC	Δ AIC	w_i	*cum* w_i
Males					
Year + Activity period	7	–422.9524	0.0000	0.7121	0.7121
Year × Activity period	11	–421.1408	1.8116	0.2879	1.0000
Activity period	3	–397.5558	25.3966	0.0000	1.0000
Year	6	–393.6921	29.2603	0.0000	1.0000
intercept	2	–377.6812	45.2712	0.0000	1.0000
Females					
Year × Activity period	11	–349.6167	0.0000	0.7579	0.7579
Year + Activity period	7	–347.3305	2.2863	0.2416	0.9995
Year	6	–335.0809	14.5358	0.0005	1.0000
intercept	2	–284.7405	64.8763	0.0000	1.0000
Activity period	3	–284.1339	65.4835	0.0000	1.0000

Appendix 3. A diagram showing the mean body condition of male and female pygmy bluetongue lizards between the months of September and October as they emerge from hibernation.

Chapter 4. Variation in body condition of neonate pygmy bluetongue lizards, *Tiliqua adelaidensis*

Offspring phenotypic variation is an important element of the life history of a species. In viviparous lizards, the environment experienced by mothers during gestation as well as the genetic variation among offspring could result in different phenotypes which subsequently affect their survival. Understanding the fitness of juveniles and factors affecting their survival is highly important in studies of endangered species such as pygmy bluetongue lizard.

This chapter explores variation in phenotype and survival of neonate pygmy bluetongue lizards in field populations. It explores changes in body condition and body size among years. It also investigates correlations between mother and offspring fitness. In addition, it explores the effect that competition among siblings can have on the fitness of neonates.

This chapter has been published on-line in the *Transactions of the Royal Society of South Australia* (2015), DOI:10.1080/03721426.2015.1045312

VARIATION IN SIZE AND CONDITION OF NEONATE PYGMY BLUETONGUE LIZARDS, *TILIQUA ADELAIDENSIS*.

Transactions of the Royal Society of South Australia (2015), DOI. 10.1080/03721426.2015.1045312

LEILI SHAMIMINOORI[A,B], AARON L. FENNER[A], JULIE A. SCHOFIELD[A] AND C. MICHAEL BULL[A]

[A]School of Biological Sciences,

Flinders University, GPO Box 2100,

Adelaide, SA 5001, Australia.

[B]Corresponding author. Email: sham1012@flinders.edu.au

Abstract

Phenotypic variation among offspring of individual animals is an important life-history trait. In viviparous lizards, it could result from genetic variation of individuals and also differences in environments experienced by mothers during gestation. We investigated variation in phenotype and survival of neonate pygmy bluetongue lizards, *Tiliqua adelaidensis,* over three sampling years. We explored changes in body condition, using body size residuals as an index, and body size (snout-vent length (SVL) and mass) of neonates. For litters that were captured with their mothers, we hypothesised that body condition and size of offspring are influenced by the condition and size of their mothers, the presence of mothers in the burrow and litter size. We found that there were significant variations in body condition and mass of neonates among years. There was no significant relationship between neonate body condition, mass and SVL and post-partum body condition of their mothers. Results revealed that females with higher SVL produced larger litters. None of the analyses showed any effect of mother's presence on neonate body condition, SVL or mass. Neither was there any effect of observed litter size on these parameters. Our findings have important conservation implications for this endangered species, given that the aim of conservation managers is to encourage conditions that promote optimum body condition and fitness in offspring in order to maximise their survival in fragmented habitats.

Introduction

Neonate offspring of individual animal species vary in their phenotype as a result of their own genotypic variation and of variation in the environment experienced by their mothers during gestation, or by the developing embryo in its egg (Qvarnstrom & Price, 2001). The environment in which the embryo develops can profoundly influence its morphology, behaviour (Burger, 1989; Du, Shou, & Liu, 2003; Hare & Daugherty, 2002; Shine, Elphick,

& Harlow, 1997) and postnatal growth rate (Rhen & Lang, 1995). In this paper we explored the role of environmental factors in influencing phenotypic features that might subsequently affect survival among neonates of an endangered Australian scincid lizard. Specifically we investigated inter-annual variation in neonate size and condition in the viviparous pygmy bluetongue lizard, *Tiliqua adelaidensis*, and looked for factors that might explain this variation.

In viviparous lizards, annual variation in neonate body condition could be attributed to the different environmental conditions experienced by gravid females during embryonic development in each year (Lourdais et al., 2004; Reinhold, 2002; Wapstra, 2000). Climatic conditions, especially the temperature experienced by gravid female lizards (Hubert, 1985) can influence the duration of gestation (Atkins, Swain, Wapstra, & Jones, 2007) and the phenotype and fitness of offspring (Bernado, 1996; Mousseau & Fox, 1998; Olsson, Wapstra, & Olofsson, 2002). Under warmer conditions, increased basking opportunities and higher food availability for gravid female lizards can increase the fitness and survival of their offspring (; Bernado, 1996; Itonaga, Jones, & Wapstra, 2012; Massot & Clobert, 1995; Olsson, et al., 2002; Shine, 1980; Shine & Harlow, 1993; Sorci & Clobert, 1997; Wapstra, 2000). For example, Itonaga et al. (2012) showed that females of the Australian scincid species *Pseudemoia entrecasteauxii*, when given extended basking opportunities with higher food availability during gestation, produced larger offspring with larger fat reserves and a higher sprint speed. All of these factors are expected to contribute to higher fitness. Similarly, Wapstra (2000) showed that female *Niveoscincus ocellatus* lizards with longer access to thermal basking conditions gave birth significantly earlier than those with shorter basking opportunities and they also gave birth to larger offspring in better body condition.

After birth, any variation in survival in neonate lizards among years could result from different opportunities to survive and grow in each year. Differences in the available microhabitat may have a direct effect on survival of neonates. Some microhabitats provide better refuges against predators or supply higher quality food (Anders Forsman, 2000; Fox, 1978; Martin & Lopez, 1998). Fox (1978) showed that home ranges of surviving juvenile *Uta stansburiana* had more access to food and shelter sites compared to non-survivors. However, individual neonate phenotype may affect survival more profoundly than local heterogeneities in microhabitat quality. Body size at birth has an important impact on fitness of juveniles (Blanckenhorn, 2000; Dmitriew, 2011) with larger juveniles having survivorship advantage over small ones (Ferguson & Fox, 1984). For example, Civantos and Forsman (2000) found that larger *Psammodromus algirus* juveniles survived better than smaller juveniles. However, there is a trade-off between growth rate and energy storage or body condition in young lizards (A. Forsman & Lindell, 1996). Larger individuals may benefit from better body condition but rapid growth and better body condition may reduce survival due to increased energy demands for locomotion (Pond, 1978) and from a decrease in speed (Garland, 1985)

In many studies of lizards, body condition of neonates has been used as a predictive measure of future fitness. Body condition is a reflection of the mass per unit body length of an individual lizard and is often used as an indicator of food intake rate, energy storage and general health (Civantos & Forsman, 2000; A. Forsman & Lindell, 1996). Information about the longer-term reproductive recruitment for an endangered lizard species is often difficult to acquire directly, but growth rates and body conditions indices among neonates are valuable indicative parameters that are relatively more easily derived over a shorter time frame. Here, we use body condition and body size (snout-vent length and mass) of neonate pygmy bluetongue lizards (*Tiliqua adelaidensis*) and their mothers to investigate factors affecting variation in their condition over the study period.

Tiliqua adelaidensis is the smallest member of the genus *Tiliqua* and is currently listed as endangered under the *Australian Environment Protection and Biodiversity Conservation Act 1999*. It is a viviparous Australian member of the family Scincidae. All known populations of the species are restricted to a few isolated fragments of remnant native grassland in a small area in the mid-north of South Australia. Individual lizards occupy burrows constructed by lycosid and mygalomorph spiders (Fellows, Fenner, & Bull, 2009; Hutchinson, Milne, & Croft, 1994). Litters of one to four neonate pygmy bluetongue lizards are born between late January and mid February (Hutchinson, et al., 1994). Neonates briefly share the natal burrow with their mother and siblings, but independently leave the burrow, usually between one and five weeks after birth. Sometimes, the mother leaves the burrow before the neonates disperse (Milne, Bull, & Hutchinson, 2002). Pygmy bluetongue lizards feed by ambushing invertebrate prey that pass by the burrow entrance, and the more occupants in the burrow the fewer opportunities for prey capture by each individual. In that case, dispersal away from the natal burrow may reduce competition among siblings or between mother and offspring.

The aims of this study were (i) to provide parameters of neonate size and condition for conservation managers to explain how populations respond to annual changes in environmental conditions, and (ii) to derive background information relevant to understanding annual recruitment dynamics in this species. To achieve these aims we (i) explored the variation in body condition, SVL and mass of neonate pygmy bluetongue lizards over three sampling years, and (ii) investigated relationships between neonate body condition, SVL and mass and the relative clutch mass (RCM) of their mothers. We predicted that females in better body condition would contribute a higher proportion of their body mass to their clutches. Additionally we considered some indirect behavioural influences on neonate fitness, asking whether there were differences in neonate phenotypes between those that did or did not stay with their mother or that did or did not stay with siblings. We made no specific predictions for these questions recognising that there could be benefits of remaining in a secure burrow with related conspecifics, balanced by costs from increased competition for the invertebrate prey close to the burrow.

Materials and Methods

Study site and field methods

The study site was the "Tiliqua" property of the Nature Foundation of South Australia, previously described as Site 2 (Fenner & Bull, 2007), and was located about eight kilometres from Burra in South Australia (33° 42'S, 138° 56'E) in a remnant patch of native grassland. The area has hot, dry summers and cool, moist winters. The long-term average annual rainfall at Burra is 431mm and normally ranges between 300 and 500 mm. Table 1 summarises the rainfall and temperature data in each of four years. For the three years of our study (2009 – 2011) and the one year preceding our study, annual rainfall was the highest in 2011 (561.4 mm) and lowest in 2008 (327.6 mm). The rainfall during late summer and early autumn (Jan – April), the months when neonates are born and start to grow, was highest in 2011 (193.4 mm) and lowest in 2009 (48.8 mm). Total winter-spring and early summer rainfall (June-December), the period leading up to the birth of neonates in summer, was highest in 2009 (370.5 mm) leading up to the birth of neonates in 2010 and lowest in 2008 (274.5 mm) leading up to the birth of neonates in 2009.

The average annual temperature over the study period was highest in 2009 (22.2℃) and lowest in 2010 (21.2℃). The average daily temperature over the winter-spring- early summer period (June-December) was highest in 2008 (18.6°C), and lowest in 2010 (17.5°C). Average temperature in the summer months (January-April) was highest in 2010 (28.2°C) and lowest in 2011 (26.0°C).

We sampled 116 neonate lizards over three years 2009 – 2011 from mid January to early March in each year. Our searching effort of 34 -36 days within that period was the same each year and covered the same six week period that included the time from when litters were first detected each year to when lizards normally became inactive with the approaching winter (Milne, et al., 2002). The mean snout-vent length (SVL) of neonate pygmy bluetongue lizards is less than 55 mm (Milne, et al., 2002), and we used 55 mm as an upper SVL limit to distinguish neonates from sub-adult and adult lizards.

Lizards were captured using the fishing method as previously described by Milne (1999). We deduced that most neonates were captured in their natal burrows, either because an adult female was also present in the burrow, or because 2 – 4 neonates were found together. Genetic studies on mother-offspring relatedness in pygmy bluetongue lizards have shown that females captured with neonates in a burrow are almost always mothers and offspring, and litters found in the same burrow are almost always siblings (Schofield, Gardner, Fenner, & Bull, 2013). Some neonates begin dispersing from the natal burrow within a week after birth and by 5 weeks most of the neonates have left the natal burrow (Milne, et al., 2002). Therefore, the number of neonates described as a litter in this study was not necessarily the complete litter size, since some young may already have dispersed from the burrow at the time a litter was detected (Table 2).

In some cases females leave their intact litters and disperse to another burrow soon after giving birth (Schofield, et al., 2013), and in this study we found some litters without an adult female present. For each lizard, we recorded the date of capture and measured the mass, snout to vent length (SVL), and total body length (TBL). Lizards were weighed on a field balance to the nearest 0.01 g, and were given a unique toe clip identification marking, and then released back to their burrows. Marking was used to prevent multiple observations of the same individuals.

Analysis

Neonate body condition was calculated from the residuals of the linear regression between natural log-transformed mass and natural log-transformed SVL for the complete sample of neonate lizards. Although there has been debate about the validity of various indices of body condition (Green, 2001; Schulte-Hostedde, Zinner, Millar, & Hickling, 2005) in our analysis, there was a significant linear relationship between ln mass and ln SVL of the neonates (R^2=0.158, $F_{1,102}$=19.12, P<0.001) (Fig.1), an appropriate condition to justify the use of this index (Bradshaw & De'ath, 1991; Green, 2001; Peig & Green, 2010).

The analyses were designed to investigate variation in body condition, SVL and mass among neonate individuals over the six weeks late summer and autumn period when neonates could be sampled each year, and to determine whether those three parameters varied among the three sampling years. For those neonates that were captured with siblings, we used mean values per litter for each parameter under investigation to ensure independence of data points. Because neonates were captured from January to March, and because they may have altered their body condition during growth in the period between when they were born and when they were captured and measured, we included the day of the year (after January 1) when the lizard was captured as a covariate. Analysis of covariance (ANCOVA) was performed on the body condition indices per litter of neonates, with year as the factor and day of capture as the covariate. We also investigated growth in snout-vent length (SVL) and mass per litter, by comparing neonates captured at different times from January to March over three sampling years. There were no recaptures of neonates within a year in our study. We assumed that sizes of neonates captured later in the season would reflect a period of growth since birth.

On a subset of 23 litters, where both mother and offspring neonates (range 1-3 neonates per litter) were found together, we investigated whether post-partum maternal body condition was related to neonate body condition, SVL or mass. We hypothesised that females with better body condition would have produced offspring with better body condition or larger litters with greater overall mass. For those litters, we performed three Pearson's correlation tests between post-partum body condition of mothers and (a) the mean body condition of neonates in the litter, (b) the mean SVL of neonates in the litter SVL, and (c) the mean mass of neonates in the litter. Similarly we performed Pearson's correlation tests comparing post-partum body condition, mass and SVL of mothers with total litter mass and RCM. We also carried out one-way ANOVAs on mean body condition, SVL and mass of mothers with litter size (1-3 young) as a factor to investigate whether different numbers of young in a litter came from mothers of different sizes.

In addition, we compared the mean body condition, SVL and mass of neonates in litters that were sampled with their mothers, to those that were found without an adult female present. This analysis might suggest, if there were a positive association, that the presence of mothers helps neonates to achieve better body condition, or, if there were a negative association, that mothers and their co-habiting offspring compete for prey (or alternatively that neonates in better body condition are more likely to disperse earlier) (Le Galliard, Ferriere, & Clobret, 2003). Analysis of covariance (ANCOVA) was conducted with body condition of neonates as the dependent factor, year and the presence or absence of mothers as factors, and day of capture as a covariate. The same analyses were performed with neonate SVL and mass per litter as dependent variables.

We also examined the differences in body condition, SVL and mass of neonates in litters of different sizes to determine any role of competition among neonates in the same burrow. We performed ANCOVA analysis with mean body condition of neonates in the litter as the dependent variable, the number of neonates in the litter (1-3) and sampling year as factors, and day of capture as a covariate. The same analyses were executed on SVL and mass of neonates in the litter.

We used SPSS version 22 to conduct analyses in this study. Data were checked for normality and are presented with error bars of one standard error where appropriate.

Results

We collected a total of 116 neonate pygmy bluetongue lizards from 84 litters over the study period (Table 2). Of those, 37 neonates (23 litters) were captured with their mothers in their natal burrows, while 79 neonates (61 litters) were found in burrows without an adult lizard present. Mean detected litter size was 1.58 (SE= 0.15; range 1-3). The mean neonate SVL was 44.6 mm (SE=0.2) and mean neonate mass was 2.7g (SE= 0.07). Mean relative clutch mass (RCM) was 0.29 and varied between 0.13 and 0.84. The lower values may not be true measures of RCM if some of the litter members had already dispersed before the litter was measured; the first litter of the year was detected on January 26 in 2009, on February 10 in 2010, and on January 17 in 2011.

Analysis of covariance (ANCOVA) tests showed significant variation in body condition and mean mass among years in neonate pygmy bluetongue lizards (Table 3). Neonates had the highest body condition and mass in 2011 and the lowest in 2010 (Figure 2(a), 2(b)). Day of capture did not have an effect on the body condition or mass.

Results also revealed that SVL increased with days into the season. Lizards that were captured later in the season were longer (Figure 3) but not heavier. The results were consistent across years (no significant year x day of capture interaction; Table 3).

The presence of mothers in the maternal burrow did not significantly influence the body condition, mass or SVL of neonate litters (Table 4). Similarly those three parameters were not found to be influenced by detected litter size (Table 5), with results consistent over the three sampling years (Table 5).

Pearson's correlation tests revealed no significant relationship between post-partum body condition of mothers and mean body condition of their litters, mean litter mass or SVL (Table 6).

There was a significant difference in the mean female SVL among litters of different sizes (Table 7; Figure 4). Post hoc comparisons using Tukey HSD test indicated that the mean SVL of females with three or more young (Mean=97 cm, SD=6.0) was significantly higher than mothers with one or two young in their litter (Mean=84 cm, SD=3.0). Body condition and mass of mothers did not vary among those detected with litters of different sizes.

Neither total litter mass nor RCM were significantly correlated with mother's body condition, SVL or body mass (Table 8).

Discussion

Our first aim was to provide parameters for conservation managers to explain how populations respond to annual changes in environmental conditions. We found significant variation in the body condition of neonate pygmy bluetongue lizards over the three sampling years. Neonates had the lowest body condition in 2010 and the highest body condition in

2011 (Fig.2a). Although we have insufficient temporal or spatial replicates for a rigorous analysis, we comment below on some possible mechanisms for the trends we observed.

Variation in the body condition could be attributed to the different environments experienced by gravid females during embryonic development in each year (Lourdais, et al., 2004; Reinhold, 2002; Wapstra, 2000). Climatic conditions, especially the temperature experienced by gravid female lizards (Hubert, 1985) can influence duration of gestation (Atkins, et al., 2007) and the phenotype and fitness of offspring (Bernado, 1996; Mousseau & Fox, 1998). In our study, summer in 2010 was preceded by a cold and wet spring. Females may have had less basking opportunities prior to and during their gestation periods, leading to later births and to neonates with lower body condition. This may have impacted on the fitness of their offspring in that year. Pygmy bluetongue lizards normally give birth by mid to late January (Hutchinson, et al., 1994), but births were unusually late in 2010, with the first litters not detected until February 8. This delay in parturition may have had an adverse effect on the body condition of offspring that year. Past studies have shown that viviparous lizards can delay parturition if subjected to unfavourable environmental conditions (Atkins, et al., 2007). Although the delay might reduce overall fitness, it might still ensure that offspring are born under more favourable conditions for their subsequent survival (Olsson & Shine, 1998; Shine & Olsson, 2003; Swaine & Jones, 2000). Climatic and environmental variation among years may also influence the body condition and size achieved by female lizards. One of our results showed females with longer SVL could produce larger litters. However those litters did not have greater overall mass in our study. Our finding is consistent in part with that of Milne and Bull (2002) who found that larger pygmy bluetongue females produced larger litters with greater overall mass. Maternal size and age has been related to offspring size in other taxa such as fish (Kindsvater, Rosenthal, & Alonzo, 2012) and birds(Parker & Begon, 1986). One possible interpretation of the pattern for larger body sizes later in the year is that larger babies were born later, that is, there is a tradeoff between gestation period and offspring size.

Another possible explanation for the variation among years in neonate body condition and size could be that neonates had different opportunities to survive and grow in each year. Our results showed a consistent pattern across the three years for significantly longer SVL of neonate lizards captured later in the season (Figure 3). We interpreted this to indicate lizards were increasing in length through natural growth. An alternative interpretation, because we were not recapturing individual lizards, is that neonate lizards were not growing over the last few weeks of the lizard activity period after they are born, but that there was differentially higher survival of longer lizards over the first few weeks. A lack of growth may result from a relative shortage of the invertebrate prey late in the summer. This interpretation might explain why mass of neonates did not change among captures at different times within the sampling period. However, mean mass of neonates was significantly different among years. This may be because of differences in food availability, in basking opportunities, or in microhabitat conditions for either neonates after they have been born or for their mothers before the birth. Our data do not allow us to differentiate among these explanations, but suggest strong difference in one or more of that set of conditions.

Our second aim was to derive background information relevant to understanding annual recruitment dynamics in this species. Specifically we hoped to find trends that would indicate how various maternal and neonate behaviours affected those neonates. One problem with our analyses is that we have tried to infer recent past conditions from snap shot observations of lizards. Thus, although we located some litters or individual neonates without females, we had no data on how long they had been separated from their mothers. And if we had found differences in size or body condition between litters with or without mothers present, interpretation would have been speculative. Thus if we had showed that neonates with mothers present were larger or in better condition, this may have been explained by mothers assisting growth of neonates in some way, or by mothers or litter mates differentially forcing dispersal of the weakest litter members, In fact our analyses showed no impact in any year of the presence or absence of mothers in the maternal burrows, or of the number of litter mates sharing the burrow. The only conclusion that can be derived is that we found no evidence to suggest neonate growth was influenced by burrow sharing, and that apparently, during the early weeks of neonate development, competition for insect prey passing the burrow entrance did not influence the growth and body condition of litters of different sizes.

In summary, our study found significant variation in the body condition of neonate pygmy bluetongue lizards over three sampling years. This finding has important conservation implications for this endangered species, given that the aim of conservation managers is to encourage conditions that promote optimum body condition and fitness in offspring in order to maximise their survival. Future studies will need to consider long term effects of body condition on survival of neonate pygmy bluetongue lizards, and how changing climatic conditions affect the fitness of neonate lizards.

Acknowledgements

This research was supported by the Australian Research Council; the Nature Foundation of South Australia; and the Holsworth Wildlife Research Endowment Fund. We thank the landholders, Chris and Maria Reed, for allowing access to their property. The study was conducted according to the guidelines of Flinders University Animal Welfare Committee (approval no. E206) and in compliance with the Australian Code of Practice for the Use of Animals for Scientific Research.

References

Atkins, N., Swain, R., Wapstra, E., & Jones, S. M. (2007). Late stage deferral of parturition in the viviparous lizards Niveoscincus ocellatus (Gray 1845): implications for offspring quality and survival. *Biological Journal of the Linnean Society, 90*, 735-746.

Bernado, J. (1996). Maternal effects in animal ecology. *American Zoologist, 36*, 83-105.

Blanckenhorn, W.U. (2000). The evolution of body size: what keeps organisams small? *The Quarterly Review of Biology, 75*, 385-407.

Bradshaw, S. D., & De'ath, G. (1991). Variation in condition indices due to climatic and seasonal factors in an Australian desert lizard, *Amphibolusus nuchalis*. *Australian Journal of Zoology, 39*, 373-385.

Burger, J. (1989). Incubation tempertaure has long-term effect on behaviour of young pine snakes (*Pituophis melanoleucus*). *Behavioral Ecology and Sociobiology, 24*, 201-207.

Civantos, E., & Forsman, A. (2000). Determinants of survival in juvenile *Psammodromus algirus* lizards. *Oecologia, 124*, 64-72.

Dmitriew, C.M. (2011). The evolution of growth tractories: what limits grwoth rate? *Biological Review, 86*, 97-116.

Du, W.G., Shou, L., & Liu, J.K. (2003). The effect of incubation tempertaure on egg survival, hatchling traits and embryonic use of energy in the blue-tailed skink, *Eumeces elegans*. *Animal Biology, 53*, 27-36.

Fellows, H.L., Fenner, A. L., & Bull, C. M. (2009). Spiders provide important resources for an endangered lizard. *Journal of Zoology, 279*, 156-163.

Fenner, A. L., & Bull, C. M. (2007). Short-term impact of grassland fire on the endangered pygmy bluetongue lizard. *Journal of Zoology, 272*, 444-450.

Ferguson, G.W., & Fox, S.F. (1984). Annual variation of survival advantage of large juvenile side-blotched lizard, *Uta stansburiana*: its causes of evolutionary significance. *Evolution, 38*, 342-349.

Forsman, A., & Lindell, L.E. (1996). Resource dependent growth and body condition dynamics in juvenile snakes: and experiment. *Oecologia, 108*, 669-675.

Forsman, Anders. (2000). Some like it hot: intra-population variation in behavioural thermoregulation in colour-polymorphic pygmy grasshoppers. *Evolutionary Ecology, 14*, 25-38.

Fox, S.F. (1978). Natural selection in behavioral phenotyped of the lizard *Uta stansburiana*. *Ecology, 59*, 834-847.

Garland, T. (1985). Ontogenetic and individual variation in size, shape and speed in the Australian agamid lizard *Amphibolurus muchalis. Journal of Zoology, 207*, 435-439.

Green, A. J. (2001). Mass/lenght residuals: measures of body condition or generators of spurious results? *Ecology, 82*, 1473-1483.

Hare, K.M., & Daugherty, Cree, A. (2002). Incubation regime affects juvenile morphology and hatching success, but not sex, of the oviparous lizard *Oligosoma suteri* (Lacertilia: Scincidae). *New Zealand Journal of Zoology, 29*, 221-229.

Hubert, J. (1985). Embryology of the squamata. In C. Gans & F. Billet (Eds.), *Biology of the Reptilia 15* (pp. 1-55): Wiley and Sons.

Hutchinson, M. N., Milne, T., & Croft, T. (1994). Redescription and ecological notes on the pygmy bluetongue, *Tiliqua adelaidensis* (Squamata:Scincidae). *Transactions of the Royal Society of South Australia, 118*, 217-226.

Itonaga, K., Jones, S. M., & Wapstra, E. (2012). Effects of maternal basking and food quantity during gestation provide evidence for the selective advantage of matrotrophy in a viviparous lizard. *Plos One, 7*(7). Retrieved from

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0041835 doi:10.1371/journal.pone.0041835

Kindsvater, H.K., Rosenthal, G.G., & Alonzo, S.H. (2012). Maternal Size and Age Shape Offspring Size in a Live-Bearing Fish, *Xiphophorus birchmanni. Plos One, 7*(11). Retrieved from **http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0048473** doi:doi:10.1371/journal.pone.0048473

Le Galliard, J. L., Ferriere, F., & Clobert, J. (2003). Mother-offspring interactions affect natal dispersal in a lizard. *Proceedings of The Royal Society B:Biological Sciences, 270*, 1163-1169.

Lourdais, O., Shine, Richard, Bonnet, X., Guillon, M., Naulleau, G., & (2004). Climate affects embryonic development in a viviparous snake, *Vipera aspis*. *Oikos, 104*, 551-560.

Martin, J., & Lopez, P. (1998). Shifts in microhabitats use by the lizard *Psammodromus algirus:* responses to seasonal changes in vegetation structure. *Copeia, 1998*, 780-786.

Massot, M., & Clobert, J. (1995). Influence of maternal food availability on offspring dispersal. *Behavioral Ecology and Sociobiology, 37*, 413-418.

Milne, T. (1999). Conservation and ecology of the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*. PhD Thesis, Flinders University, Adelaide, South Australia.

Milne, T., Bull, C. M., & Hutchinson, M. N. (2002). Characteristics of litters and juvenile dispersal in the endangered Australian skink *Tiliqua adelaidensis*. *Journal of Herpetology, 36*, 110-112.

Mousseau, T. A., & Fox, C.W. (1998). The adaptive significance of maternal effects. *TREE, 13*(10), 403-407.

Olsson, M., & Shine, R. (1998). Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution, 52*, 1861-1864.

Olsson, M., Wapstra, E., & Olofsson, C. (2002). Offspring size-number strategies: experimental manipulation of offspring size in a viviparous lizard (*Lacerta vivipara*). *Functional Ecology*(16), 135-140.

Parker, G.A., & Begon, M. (1986). Optimal egg size and clutch size - effects of environment and maternal phenotype. *American Naturalists, 128*, 573-592.

Peig, J., & Green, A. J. (2010). The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Functional Ecology, 24*, 1323-1332.

Pond, C.M. (1978). Morphological aspects and the ecological and mechanical consequences of fat deposition in wild vertebrates. *Annual Review of Ecology, Evolution, and Systematics, 9*, 519-570.

Qvarnstrom, A., & Price, T. D. (2001). Maternal effects, paternal effects and sexual selection. *Trends in Ecology & Evolution, 16*, 95-100.

Reinhold, K. (2002). Maternal effects and the evolution of behavioural and morphological characters: a literature review indicates the importance of extended maternal care. *Journal of Heredity, 93*, 400-405.

Rhen, T., & Lang, J.W. (1995). Phenotypic plasticity for growth in the common snapping turtle: effects of incubation temperature, clutch and their interaction. *The American Naturalist, 126*, 726-747.

Schofield, J. A., Gardner, M. G., Fenner, A. L., & Bull, C. M. (2013). Promiscuous mating in the endangered Australian lizard *Tiliqua adelaidensis*: a potential windfall for its conservation. *Conservation Genetics, 15*, 177-185. doi: 10.1007/s10592-013-0529-0

Schulte-Hostedde, A. I., Zinner, B., Millar, J. S., & Hickling, G. J. (2005). Restitution of mass-size residulas: validating body condition indices. *Ecology, 86*, 155-163.

Shine, R. (1980). "Costs" of reproduction in reptiles. *Oecologia, 46*, 92-100.

Shine, R., Elphick, M.J., & Harlow, P.S. (1997). The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology, 78*, 2559-2568.

Shine, R., & Harlow, P.S. (1993). Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia, 96*, 122-127.

Shine, R., & Olsson, M. (2003). When to be born? Prolonged pregnancy or incubation enhances locomotor performance in neonatal lizards (Scincidae). *Journal of Evolutionary Biology, 16*, 823-832.

Sorci, G., & Clobert, J. (1997). Environmental maternal effects on locomotor performance in the common lizard (*Lacerta vivipara*). *Evolutionary Ecology, 11*, 531-541.

Swaine, R., & Jones, S. M. (2000). Maternal effects associated with gestation conditions in a vivparous lizard, *Niveouscincus metallicus. Herpetological Monographs, 14*, 432-440.

Wapstra, E. (2000). Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Functional Ecology, 14*, 345-352. doi: 10.1046/j.1365-2435.2000.00428.x

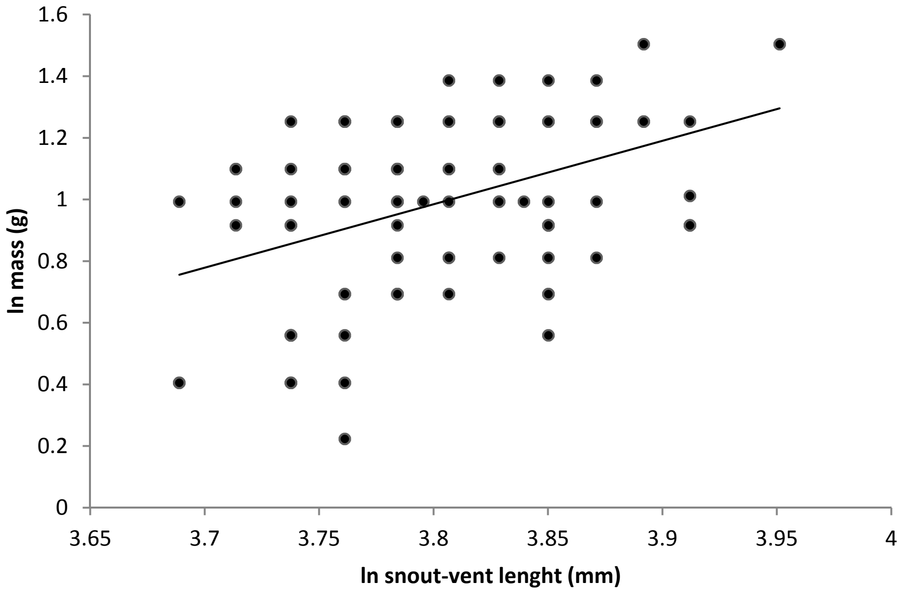


Figure 1. Relationship between body mass and snout-vent length in neonate pygmy blue tongue lizards (R2=0.158). (There are overlaps of data points in the figure.)

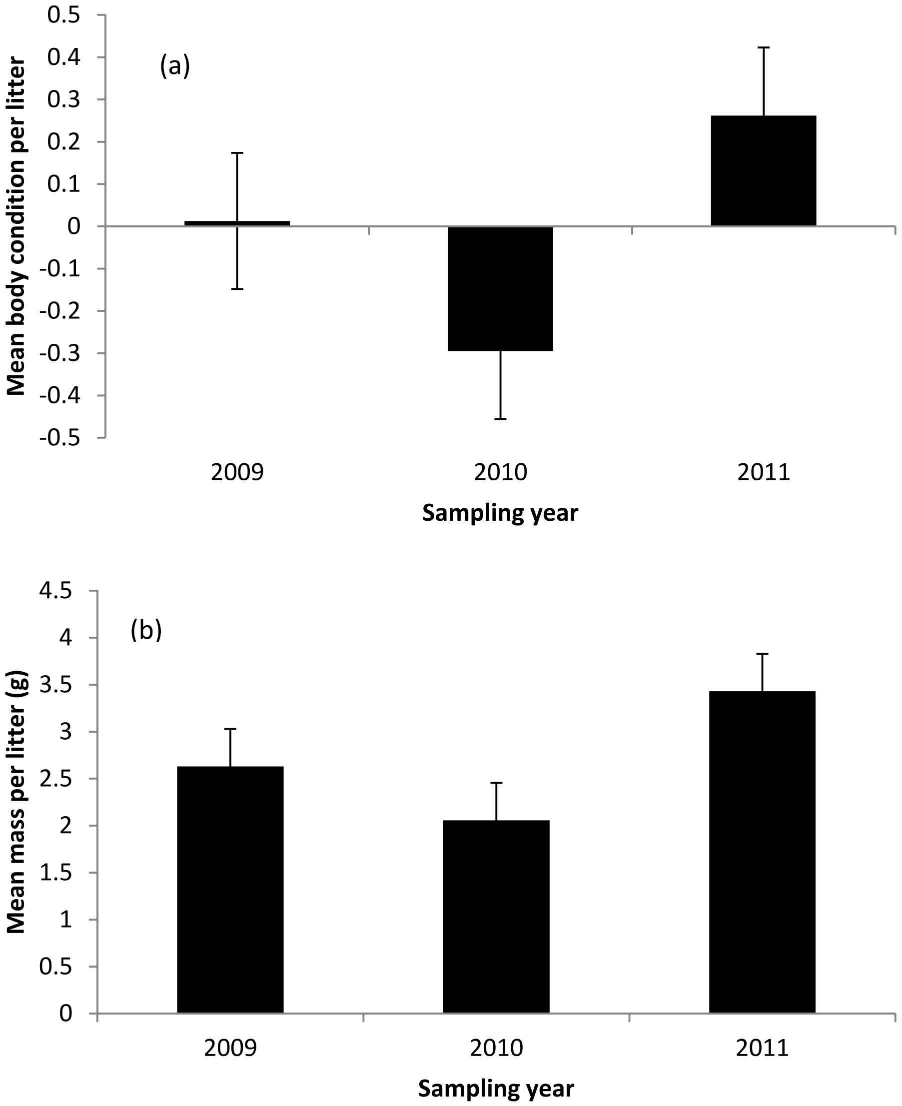


Figure 2. Mean (SE) (a) body condition, and (b) mass per litter in each sampling year.

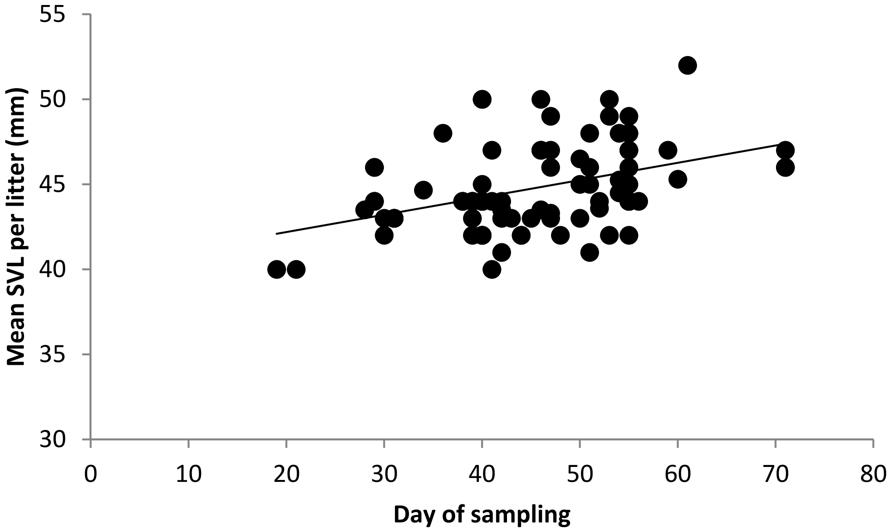


Figure 3. Mean snout-vent length per litter as a function of the date of sampling (days after January 1).

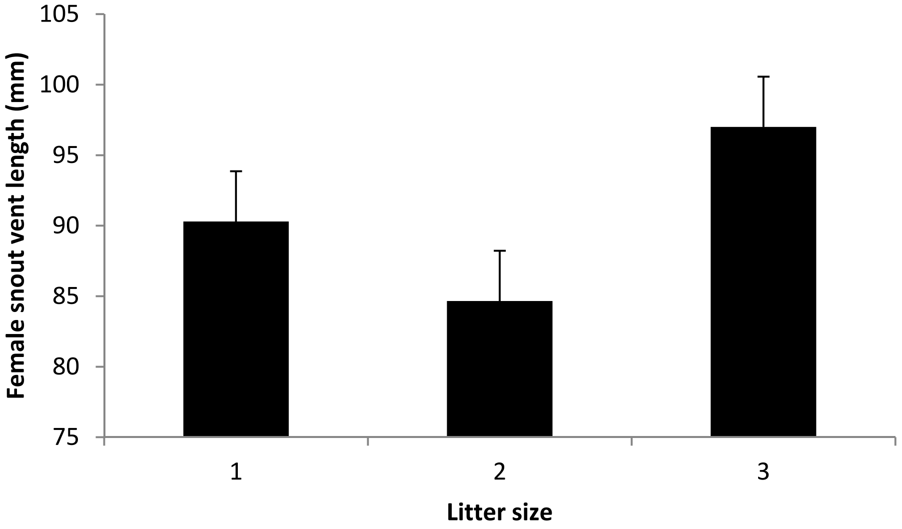


Figure 4. Mean (SE) snout-vent length (SVL) of mothers of different litter size (1-3).

Table 1. Rainfall and temperature for Burra region in each of four years 2008 - 2011.

	Rainfall (mm)			**Temperature (C°)**		
Year	Annual	Jan-Apr	June-Dec	Annual	Jan-Apr	June-Dec
2008	327.6	45.6	247.8	21.5	18.6	27.3
2009	428.1	48.8	370.5	22.2	18.45	27.8
2010	539.2	83.8	357.8	21.2	17.54	28.2
2011	561.4	193.4	331.5	21.4	19.58	26

Table 2. Number of neonate *Tiliqua adelaidensis* captured from Jan – March in each of three years 2009-2011.

Year	**NEONATE LIZARDS**			**NEONATE LITTERS**		
	N lizards	*N* with mother	*N* without mother	*N* litters	*N* with mother	*N* without mother
2009	23	7	16	20	6	14
2010	44	19	25	35	13	22
2011	49	11	38	29	4	25
Total	**116**	**37**	**79**	**84**	**23**	**61**

Table 3. Results of the analyses of covariance of body condition, SVL, and mass of neonate pygmy bluetongue lizards, with sampling year as a factor and day of sampling as a covariate.

	Body condition			**SVL**			**Mass**		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Year	2	3.825	**0.026**	2	2.587	0.637	2	3.123	**0.05**
Day of capture	1	3.393	0.069	1	6.377	**0.014**	1	1.415	0.238
Year x Day of Capture	2	1.799	0.172	2	0.384	0.682	2	1.05	0.355
Error	78			78			78		

Note: P values in bold indicate results that are statistically significant at $P < 0.05$.

Table 4. Results of the analyses of covariance of body condition, SVL,and mass of neonate pygmy bluetongue lizards with sampling year and mother presence or absence as factors and day of sampling as a covariate.

	Body condition			**SVL**			**Mass**		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Year	2	3.897	**0.025**	2	1.221	0.301	2	2.857	**0.062**
Day of capture	1	1.493	0.226	1	1.779	0.186	1	1.057	0.307
Mother presence	1	0.389	0.535	1	2.187	0.143	1	0.010	0.920
Year x Day of Capture	2	1.886	0.159	2	1.029	0.362	2	1.600	0.209
Mother presence x year	2	0.387	0.681	2	0.601	0.616	2	0.085	0.919
Mother presence x Day of capture	1	0.306	0.582	1	1.107	0.336	1	0.002	0.964
Error	74			74			74		

Note: P values in bold indicate results that are statistically significant at $P < 0.05$.

Table 5. Results of the analyses of covariance of body condition, SVL, and mass of neonate pygmy bluetongue lizards in the litter with sampling year and litter size as factors and day of sampling as a covariate.

	Body condition			**SVL**			**Mass**		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Year	2	4.246	**0.018**	2	0.868	0.424	2	3.299	**0.043**
Day of capture	1	0.013	0.910	1	0.087	0.769	1	0.456	0.502
Litter size	2	1.044	0.357	2	0.673	0.514	2	1.413	0.250
Year x Day of Capture	2	2.689	0.075	2	0.920	0.403	2	1.600	0.209
Litter size x year	3	0.169	0.917	3	0.601	0.616	3	0.516	0.672
Litter size x Day of capture	2	0.975	0.382	2	0.094	0.963	2	1.395	0.255
Error	71			71			71		

Note: P values in bold indicate results that are statistically significant at $P < 0.05$.

Table 6. Results of the Pearson's correlation between post-partum body condition of mothers and mean body condition, SVL, and mass of pygmy bluetongue neonate litters.

	r	*N*	*p*-value
Body condition	0.13	23	0.953
SVL	0.030	23	0.890
Mass	-0.044	23	0.843

Table 7. Results of one-way ANOVAs comparing mean body condition, SVL and mass of mother pygmy bluetongue lizards that were detected with litters of one two or three neonates.

	df	*F*	*P*
Body condition	2,18	0.067	0.935
SVL	2,18	4.730	**0.022**
Mass	2,18	1.472	0.256

Note: P values in bold indicate results that are statistically significant at $P < 0.05$.

Table 8. Results of the Pearson's correlation between post-partum body condition, SVL and mass of mothers and total litter mass and RCM of neonate pygmy bluetongue lizards.

	Total litter mass			**RCM**		
	r	*N*	*P*	*r*	*N*	*P*
Body condition	0.05	21	0.826	-0.67	21	0.773
SVL	0.28	21	0.218	0.13	21	0.553
Mass	0.25	21	0.268	0.04	21	0.986

Chapter 5. The head scale symmetry in pygmy bluetongue lizard, *Tiliqua adelaidensis*

With the alarming rate of species extinction, conservation biologist and managers are in need of new tools to identify populations in decline. Fluctuating asymmetry (random deviation from perfect symmetry in otherwise bilaterally symmetrical traits), and has become a commonly used indicator of developmental stability and might be linked to environmental or genetic stress conditions during growth.

The aim of the following two chapters is to explore the usefulness of a symmetry index of the head scalation of pygmy bluetongue lizard as an indicator of stress. They investigate the relationship between the symmetry index and two indirect measures of fitness (body condition and jaw width) in this lizard. These chapters consist of two manuscripts (both submitted for publication). Chapter 5 looks at the correlation between symmetry index and body condition and jaw width in adult pygmy bluetongue lizards and its possible implications in monitoring programmes. This paper is submitted to the journal of *Herpetological Conservation and Biology***.** The second paper (Chapter 6) explores the ontogenetic changes in symmetry index in pygmy bluetongue lizards from neonate to adult stage. It has been submitted to *Transactions of the Royal Society of South Australia* for publication.

Can we use head scale symmetry in endangered pygmy bluetongue lizards to alert managers to population condition?

Leili Shamiminoori[A,B], and C. Michael Bull[A]

[A] School of Biological Sciences

Flinders University

GPO Box 2100

Adelaide, SA 5001

Australia

[B] Corresponding author: L. Shamiminoori

Email: sham1012@flinders.edu.au

Phone: +61 8 8201 2805

Fax: +61 8 8201 3015

Submitted: ***Herpetological Conservation and Biology (May 2015)***

Abstract.- Different levels of symmetry of individuals within a population, or among populations are suggested to correlate positively with environmental or genetic stress by affecting the developmental stability of an organism during growth. However, the studies on the relationship between asymmetry and fitness have shown heterogeneous results. If stress increases the level of symmetry of an individual in a population, the measurement of asymmetry can be a valuable tool to assess the wellbeing of that population. In this paper we used symmetry in head scales of Pygmy Bluetongue Lizards to establish if there was a relationship between symmetry index and three indirect fitness measures, body condition, jaw width and activity period (early and late). We found that lizards captured in the late activity period had more symmetric head scales than those captured in early activity period. Our results also demonstrated that females were less symmetric than males. Jaw width had a negative significant relationship with symmetry index for all groups of lizards, where lizards with wider jaws were less symmetric. We did not find a significant correlation between body condition with either jaw width or with the symmetry level for any of the groups of lizards. The significant differences in symmetry of the head scalation between sexes and between samples collected at different periods of the year indicate that there is fitness differences associated with symmetry in Pygmy Bluetongue populations. Nevertheless, we did not find consistently strong evidence of an association between asymmetry and poor body condition in pygmy bluetongue lizards; therefore, at present there is little value in assessing population well being using asymmetry measures of head scalation in this species.

Key words.—fluctuating asymmetry; developmental stability; body condition; conservation, endangered species; *Tiliqua adelaidensis*

INTRODUCTION

The growing rate of species extinction has urged wildlife conservation managers to use biological indicators to identify species at risk, find underlying causes of decline and implement appropriate management programs to halt or reverse the decline. For endangered species that are restricted to small isolated patches of habitat, populations will face multiple threatening processes. These include ecological stresses from crowding, from habitat deterioration, and from climatic changes, and genetic stresses from elevated levels of inbreeding within small isolated populations.

Fluctuating asymmetry has been considered as one indicator of long-term stress (Sarre 1996). It is defined as random deviation from perfect symmetry in otherwise bilaterally symmetrical traits, and has become a commonly used measure of developmental stability that might be linked to stress conditions during growth (Palmer 1996; Palmer and Strobeck 2003; Van Valen 1962). As the two corresponding body sides share a single genome and experience similar external factors, differences in their development are unlikely to have resulted from environmental or direct genetic effects. Rather, it is believed that asymmetry reflects the inability of individuals to buffer their development against small, random perturbations (Sarre, Dearn, and Georges 1994; Palmer 1996). Different levels of asymmetry of individuals within a population, or among populations are sometimes shown to correlate positively with environmental stress, from factors such as extreme climate, degraded, fragmented or polluted habitat, or inadequate nutrition (Polak and Trivers 1994; Parsons 1992; Moller and Swaddle 1997; Helle et al. 2011; Wright and Zamudio 2002; Sarre 1996; Vangestel and Lens 2011), with higher levels of asymmetry in more stressful conditions. Fluctuating asymmetry increased in Taita Thrush *Turdus helleri,* which were subjected to high levels of habitat disturbance (Lens et al. 2002), and Red Squirrels in small fragmented woodlands had higher levels of fluctuating asymmetry and smaller body size than those in larger forests (Wauters et al. 1996).

Similarly, it has been suggested that genetic stress resulting from the loss of genetic diversity either by inbreeding or genetic drift, might increase the incidence and level of asymmetry in populations (Lens, Van Dongen, and Matthysen 2002; Leung and Forbes 1997; Lens et al. 2000; Clarke 1998; Pertoldi et al. 2006; Learny and Klingenberg 2005; Soule 1979; Lovatt and Hoelzel 2011). *Drosophila melanogaster*, individuals with lower heterozygosity at major gene loci were more asymmetric in sternopleural bristle number (Reeve 1960), and a bottlenecked population of Reindeer *Rangifer tarandus,* on the island of South Georgia had increased levels of fluctuating asymmetry in skull measurements (Lovatt and Hoelzel 2011).

If stresses increase asymmetry of individuals in a population, then measurements of symmetry might be a valuable tool to quickly assess the well-being of the population. A high proportion of asymmetric individuals may indicate that the population has experienced or is experiencing stress, and that management intervention is required. And if there is an established relationship between fluctuating asymmetry and fitness, it can be a useful monitoring tool to detect changing condition in populations. For example, Alford, Bradfiled and Richards (1999) suggested that measurements of limb symmetry should be included in amphibian monitoring programs to improve the ability of managers to detect declines in the health of populations. Similarly, comparing symmetry of left and right otoliths in larval fish is used as an indicator of larval quality and likely recruitment success in fisheries ecology (Lemberget and McCormick 2009; Gagaliano et al. 2008).

As well as monitoring populations overall, fluctuating asymmetry may also relate to quality and fitness among individuals within a population (Leary and Allendorf 1989; Leung, Forbes, and Houle 2000; Parsons 1992; Clarke 1998; Houle 1997; Brown and Brown 1998; Moller 1997; Alford, Bradfield, and Richards 1999). Less symmetrical individuals may have lower fitness, especially where the trait measured directly affects performance. In some bird species such as swallows *Hirundo rustica,* where males exhibit sexual ornaments to attract females, more symmetrical displays are more attractive (Moller 1991, 1992a). Similarly, females of the lizard *Lacerta monticola* preferred males that were more symmetric in the number and size of their femoral pores (Martin and Lopez 2000). Symmetry might also affect other aspects of fitness. Gemsbok males, *Oryx gazelle*, with asymmetric horns had poorer body condition than more symmetric individuals (Moller et al. 1996).

However, not all studies have reported negative relationships between fluctuating asymmetry and fitness (Bjorksten, Fowler, and Pomiankowski 2000; Lens and Eggermont 2008). Some have reported no association between fluctuating asymmetry in the trait measured and fitness (Lindell, Forsman, and Merila 1993; Merila, Forsman, and Lindell 1992; Shine 2005; Moller 1992a, 1993a). For example, Dufour & Weatherhead (1998) reported no association between asymmetry and social dominance in captive male Red-winged Blackbirds *Agelaius phoeniceus*, and Kark *et al.* (2000) found bilateral asymmetry was not significantly associated with body condition at either the population or the individual level in the Chukar Partridge *Alectoris chukar*.

Other studies have found a positive relationship between fluctuating asymmetry and fitness in Common Starling *Sturnus vulgaris* (Swaddle and Witter 1994), Red-winged Blackbirds *Agelaius phoeniceus* (Dufour and Weatherhead 1998a), Mallards *Anas platyrhynchos* (Hoysak and Ankney 1996), and the Japanese Scorpionflies *Panorpa japonica* (Thornhill 1992). In two species of ungulates, Gemsbok *Oryx gazella*, and Fallow Deer *Dama dama,* the most dominant males had the least symmetrical antlers (Mateos et al. 2008; Moller et al. 1996; Putman, Sullivan, and Langbein 2000). These apparently contradictory findings suggest that the extent to which symmetry relates to fitness, and thus its usefulness as an indicator of population condition, depends on the character trait chosen for analysis and the species under study (Bjorksten, Fowler, and Pomiankowski 2000; Clarke 1995b; Hosken, Blanckenhorn, and Ward 2000). Our aim in this paper was to explore the value of measuring symmetry in the dorsal head scales of an endangered Australian lizard, as a potential tool to monitor individual fitness and population condition.

The Pygmy Bluetongue Lizard *Tiliqua adelaidensis,* is an endangered Australian scincid lizard, now limited to a small number of isolated fragments of previously continuous native grassland in the mid-north region of South Australia (Milne, Bull, and Hutchinson 2003) . The species was thought to be extinct for 30 years until its unexpected re-discovery in the stomach of a brown snake, near the town of Burra, South Australia in 1992 (Armstrong and Reid 1992; Armstrong, Reid, and Hutchinson 1993). Although a promiscuous mating system appears to have retained high levels of genetic diversity within some populations (Schofield et al. 2014) there is now no detectable dispersal between even geographically close population sites (Smith et al. 2009) and a risk of losing genetic diversity in small populations. If head scale symmetry reflected genetic or ecological stress, it would be an easily applied tool for monitoring population condition.

In this paper we measured symmetry in the head scales of the Pygmy Bluetongue Lizard, and made three indirect measures of fitness, related to ecological stress. The first was a comparative measure of the symmetry of individuals caught early or late in the activity period. Any difference in mean symmetry between those two periods might indicate differential mortality, and hence, by implication, different fitness of individuals with different levels of symmetry. The other two measures were correlations between symmetry and other measured parameters that were assumed to reflect fitness. One was a measure of jaw width, and the other was a measure of body condition, derived from the relationship between body mass and snout-to-vent length (SVL). Head size has been identified as important in both intra- and intersexual agonistic behaviors in many lizards (Mouton and Van Wyk 1993; Husack et al. 2006; Herrel, Van Damme, and De Vree 1996). Males with larger heads and larger jaws are more effective in fights with other males (Bull and Pamula 1996), and can hold females more firmly, which gives them an advantage in both competition with other males and in copulation (Vitt and Cooper 1985; Herrel et al. 1999). Among insectivorous lizards, individuals of either sex with wider jaws may also have an advantage in being able to capture and ingest larger prey items (Siqueira et al. 2013; Herrel et al. 1999).

Our aim was to establish if there were relationships between symmetry and fitness among individuals in a population that might allow the degree of symmetry to be used to indicate overall population condition.

MATERIALS AND METHODS

Study site and sampling.— We collected samples from two isolated populations of Pygmy Bluetongue Lizards within 15 km of each other, near Burra, South Australia (33°42′S, 138°56′E). The area has hot, dry summers and cool, moist winters. The long-term average annual rainfall at Burra is 400 mm.

We located lizards in each population by inspecting burrows with an optical fiberscope (Provision Elite), and captured them by hand after luring them out with a mealworm tethered to a fishing line (Strong *et al.,* 1993; Milne, 1999; Fenner, 2009). Pygmy Bluetongue Lizards are active between September and April (the austral spring/summer) (Milne 1999) and we call this seven month period the activity period. Sampling was conducted on 262 days over three activity periods (2008/2009-2010/2011). In the analyses we refer to these as three sampling years (2008, 2009 and 2010) related to the year of the spring start to the season. Following Milne (1999), we defined adults as those with a snout to vent length (SVL) of more than 80 mm, and only included adults in the subsequent analyses. We assessed a total of 163 adult lizards for symmetry (see below), and selected 100 individuals (30% of the most symmetric and 30% of the least symmetric) for some analyses in this paper (Table 1).

For each lizard we recorded the date of capture, its sex, usually by cloacal examination, mass (to the nearest 0.5g), snout to vent length (SVL) and jaw width (JW) (both to the nearest 1.0 mm). Jaw width was measured as the longest distance between left and right ears for each lizard (Fig. 1). We took digital photographs of the scales on the dorsal surface of the head of each adult lizard (Fig. 2) using a Canon SLR 450D camera. The lizard was given a unique toe clip identification marking, and then released back to its burrow. We deliberately searched for independent samples and avoided recaptures by broadening the area of sampling on each new visit. For the small number of lizards that were captured more than once, only data from the first capture were used.

A body condition index was calculated from the residuals of the linear regression for all adults between the natural log-transformed mass and the natural log-transformed SVL (Shamiminoori, Fenner, and Bull 2014). This measure of body condition is commonly used in reptile population studies (Green 2001; Madsen and Shine 2000). In our study system, there was a highly significant positive relationship between (ln) mass and (ln) SVL (Shamiminoori et al. 2014), a condition that makes this index a powerful indicator of fitness (Bradshaw et al. 2000; Peig and Green 2010).

Development of a symmetry index.— We developed a symmetry index by comparing the size and shape of each of six pairs of dorsal head scales (Fig. 2). Before making those comparisons, we needed to correct the digital images for the impact of variation in image size, in horizontal tilt and in luminance. This process has been formally described by Tohl, Li *et al.* (2013). First, color images of the head of each lizard were transformed to grayscale so that any seasonal changes in dorsal color would not affect the measure of symmetry. Then a vertically centered line of symmetry from the tip of the snout to the middle of the neck was inserted for each photo, positioned so that the mirror image of the head scales across the line produced the highest cross-correlations. Next the image was corrected for any angle of head tilt in the posture of the lizard while it was being photographed. To achieve this, the image was split into left and right halves using the line of symmetry, and then tilted through various degrees. The left half of the image was mirrored and then cross-correlated with the right half at each 0.5 degree angle of tilt. The angle with the highest correlation value was deemed as the angle for tilt correlation. Next we applied edge detection techniques to the image to extract scale pattern edges of the head as binary images. Edge detection removed features from in between the scale patterns that might affect individual scale measurements. Finally, we calculated a symmetry index as the normalized correlation between extracted scale outlines of the left and right images. This resulted in a value between one and zero. Values closer to one were more symmetrical, while values closer to zero were less symmetrical (Tohl et al. 2013).

***Statistical analysis.*—**Samples from all years and from both populations were pooled in the analyses, but each lizard was only included once. To investigate changes in the symmetry index among individuals sampled at different times within a sampling year, we divided the data into an early activity period (between September and December) and a late activity period (between January and April the following year), as described previously by Shamiminoori *et al.* 2014. Differential mortality among lizards with different symmetry should be reflected in any temporal changes. We performed a two-way ANOVA of the symmetry index, with sex and activity period as factors.

Pearson's correlation analyses were conducted to determine relationships between the symmetry index and either jaw width or body condition index in the adult lizards. We first analyzed data from all adult lizards, then from adult males and females separately to allow for any effects of sexual size dimorphism (Hutchinson, Milne, and Croft 1994). For analysis of body condition in females, following Shamiminoori *et al.* (2014), we also considered separately individual females at two different reproductive stages, pre-mating (before 7 November), and post-parturition (after 15 February of the following year) to allow for reproductive changes in body condition. We omitted analyses of females collected in-between those dates because inclusion of both gravid and non-gravid individuals might have confused interpretation of any trends in body condition.

In further analyses we used a general linear model in 'R', version 2.13.0, to determine the relative importance of sex, sampling year and symmetry index on jaw width (in one analysis) and body condition index (in a second analysis). In these analyses, jaw width, or body condition index, were the dependent variables and the symmetry index, sampling year and lizard sex were fixed factors. For the factor symmetry index, lizards were categorized into two groups of 'Symmetrical' or 'Asymmetrical' individuals describing the level of symmetry in their head scales. We report analyses using the 30% most symmetrical and the 30% least symmetrical lizards in this paper (Table 1). For separate analyses on only males or only females we chose 30% of the most symmetric and 30% of the least symmetric males or females separately. Equivalent analyses using the 20%, 40% and 50% most and least symmetrical individuals produced similar results and are reported in Appendix 1.

For these general linear model analyses of all adult lizards, we compared ten models for the data set including all three main effects (sex, symmetry level (most symmetric and least symmetric) and year) and different combinations and interactions of those effects, plus a null model with only an intercept. For data sets with just males or just females we compared five models with different combinations of symmetry level and year. Akaike's Information Criterion (AIC) (Burnham and Anderson, 2002) was used to select the models that best explained the observed variation in jaw width or in body condition index. We calculated AIC_c values for all models and then the Akaike weight (*wi*) of each, to rank them and evaluate model fit. For each variable considered we also calculated the model-averaged coefficient and confidence intervals using the *modavg* package in R 2.13.0. Model-averaged coefficients that had confidence intervals that did not overlap zero were considered to have a strong effect on the dependent variable. The full list of models is given in Appendix 2.

RESULTS

The symmetry index calculated from all adults had a mean and SE of 0.389±0.13 (range=0.152-0.648). Males (mean 0.406± 0.13; range=0.181-0.647) were significantly more symmetrical than females (mean 0.368 ± 0.134; range=0.152-0.610) (Table 2; Fig. 3a).

There were also significant differences in symmetry index between early and late activity period (Table 2) with a higher symmetry in lizards sampled in the late activity period (January-April) (Fig. 3b). There was no significant interaction between sex and activity period, indicating that both males and females became equivalently more symmetric in the late activity period.

Jaw width.—There were significant negative correlations between the symmetry index and jaw width for all adults, for all males and for all females (Table 3a). Lizards that were less symmetric in their head scales had significantly wider jaws. Analysis of Covariance for jaw width on all adult lizards using symmetry index as a covariate, also showed a significant effect of the symmetry index ($F_{1,96}$= 13.71; P <00.1) on jaw width, but no effect of sex ($F_{1,96}$

= 1.25; *P* = 0.26), and no significant interaction between sex and symmetry index ($F_{1,96}$=1.88, *P*=0.20). Changes in jaw width with the symmetry index occurred equally in each sex.

The most parsimonious AIC models to explain the variation of jaw width among individuals contained only symmetry level (Table 4). In all lizards and in males and females separately, symmetry level had the highest predictor weight; with negative model average coefficients that were significant for all lizards and for males (Table 5). The 30% of lizards with the least symmetric heads had wider jaws than the 30% of lizards with the most symmetry head (Fig. 4a,b,c). Sampling year appeared in the second best models for all adults, males and females. However, the predictor weight was not high enough in any data set to show a significant effect of sampling year on jaw width.

Body condition.**—**Lizard body condition index was not significantly correlated with either jaw width (Table 3b), or with the symmetry level (Table 3c) for any of the groups of lizards.

For the body condition index the best AIC model for all adults included only sex (Table 6) with females showing lower body condition values than males, as reported in Shamiminoori et al. (2014). The symmetry level appeared as a factor that explained body condition in models with a Δ AIC of less than 2 for all lizards, for males and for females (Table 6), and there were positive predictor coefficients in each case (Table 7). However, none of the coefficients were statistically significant, indicating weak effects of symmetry index on body condition (Fig. 4d, e, f). Symmetry level did not appear in any of the highest ranked models for body condition in either pre-partum or post-partum females.

DISCUSSION

Our study established there was variation in the level of symmetry in the head scalation among adult pygmy bluetongue lizards. There were three significant trends in the data. First, females showed significantly less symmetry than males. One explanation may be that females were under more stress during development. Alternatively, there may be higher selection pressure on males to be symmetric. Other studies, in other species, have reported a female preference for more symmetrical males even when the mating cues are olfactory with no obvious reference to the visual aspects of the symmetry (Thornhill 1992; Lopez, Munoz, and Martin 2002). Pygmy Bluetongue Lizard females can mate multiple times and apparently randomly with respect to genetic relatedness (Schofield et al. 2012), although video recordings have shown that not all courting males are accepted by females (Ebrahimi 2013). Thus, although it is not yet established, there may be some mate choice by females, and, as in other species, symmetrical males may be preferred.

The second trend was that symmetry was lower in lizards sampled in the early active period (September-December) than in those sampled in the late active period (January-April). Previous studies have shown that lizards are more likely to be moving on the surface, either seeking new burrows or looking for mating partners, in the early period (Schofield et al. 2014) and much more likely to remain in their burrow in the late period (Ebrahimi 2013). While they are out of their burrows they are most susceptible to predation (Fenner et al. 2008) and populations suffer a decline in resident adults over the whole activity period (Fellows, Fenner, and Bull 2009). An interpretation for the increase in symmetry over the season is that there is higher mortality among less symmetric individuals. An implication is that more symmetric individuals are fitter. This trend was equivalent for both sexes.

The third trend was that, among the individual adult lizards with variable symmetry, we found a significant negative relationship between the index of symmetry and jaw width.

Lizards with wider jaws were less symmetric in their head scalation. This could be explained by the ontogenetic origins of symmetries. Symmetry relies on developmental stability (Palmer and Strobeck 1986, 2003). Higher investment into growth of one trait, such as the jaw, may reduce control on the stability of development of other traits, allowing developmental noise to cause random asymmetries in biosynthesis of the two sides in those other traits (Aparicio 1998). In that case, variation of symmetry in one trait could correlate with different investments to the growth in another, without directly influencing overall developmental stability. The origin of variation in symmetry of particular traits in a species could be better deduced from patterns of change in symmetry as individuals grow. In this case, lizards that develop wider jaws may do so at the expense of symmetry in other characters, like the head scales.

In some lizards, jaw width is considered an indirect measure of fitness in that lizards of both sexes with wider jaws can handle a wider range of prey item sizes (Siqueira et al. 2013; Vitt and Cooper 1985; Vitt and Pianka 1994; Herrel et al. 1999), and male lizards with wider jaws can fight conspecific rivals better (Bull and Pamula 1996), or can grasp females for mating more strongly (Milne 1999). Thus our finding that the widest jaws belonged to the lizards with the least symmetry might be seen to potentially contradict our earlier conclusion that more symmetric lizards were fitter. Nevertheless, the temporal trend for lizards in the population to show higher symmetry later in the season is a stronger indication of a real fitness advantage for symmetry, than any indirect measure of fitness.

Our measures of body condition similarly provided no support for an association between symmetry and fitness, because there was no relationship between the symmetry index and body condition index. In contrast to some other studies, we found no evidence that individuals that were less symmetric in head scales, in our case, had reduced fitness in terms of body growth or jaw width. In a range of species, the relationship between asymmetry and fitness can be sex, trait or species specific (Leung and Forbes 1997; Clarke 1995b). In our system we did not explore symmetry in other traits (e.g. left and right leg measurement; number of lamellae in toes) because we considered investigating those traits would take more handling time and would be more intrusive. The aim of this study was to develop a simple examination of symmetry, based on body parts, which could be quickly assayed in an endangered species. Our chosen assay was by photography of the dorsal scales, so that population condition, if it was associated with symmetry, could be quickly monitored.

Although the general hypothesis is that symmetry manifests an overall phenotypic quality (e.g. Palmer and Strobeck 2003) the literature shows very mixed results. The lack of correlation between symmetry and indirect fitness measurements that we report is not without precedent in the literature (Dufour and Weatherhead 1998a, 1998b; Moller 1992a, 1993a). In pygmy bluetongue lizards, head scalation is neither a sexual trait nor a performance trait, although it is plausible head scale symmetry may reflect other developmental stabilities, and, more broadly, overall fitness. An explanation for the lack of relationship between body condition and symmetry in head scales may be that all of the lizards in the populations have developed under relatively benign and unstressed conditions. Associations between symmetry and fitness may become stronger under high stress levels (Lens, Van Dongen, and Matthysen 2002; Woods et al. 2002; Hendrickx, Maelfair, and Lens 2003). In the two pygmy bluetongue populations we studied, even though now isolated in small fragments of native grassland and classed as Endangered, the lizards may not be under high levels of stress. Schofield et al. (2014) showed that high genetic heterogeneity was sustained by promiscuous mating patterns in these populations, and a natural cycle of abundance and decline may have evolved in the stochastic natural conditions of their semi-arid habitat, before habitat fragmentation led to their current Endangered status. In conditions where there is little ecological or genetic stress, attempts to use measures of symmetry as assays of population health may be unrewarding.

In conclusion, we found significant differences in symmetry of the head scalation between sexes, and between samples collected at different periods of the year. Both of these relationships indicate that there is fitness differences associated with symmetry in pygmy bluetongue populations. The adjustment of levels of symmetry by sexual and natural selection is probably a continuing normal process in this species, as in most other animal species. Nevertheless, we did not find consistently strong evidence of an association between asymmetry and poor body condition in Pygmy Bluetongue Lizards. The lower symmetry in lizards with wider jaws may indicate that a healthy population of Pygmy Bluetongue Lizards should manifest some level of asymmetry. Current populations of pygmy bluetongue lizards are not inbred or lacking genetic heterozygosity (Schofield et al. 2014), so we do not expect the extreme high levels of asymmetry that might trigger conservation concern. This is an important finding both from the perspective of the biology of isolated populations in general, and from the more specific perspective of conservation management of Pygmy Bluetongue Lizards. In the case of the Pygmy Bluetongue Lizard and at this point in time, there is little value in trying to assess population wellbeing using asymmetry measures of head scalation. Further work may establish a stronger relationship between symmetry and body condition using a multi-trait approach.

Acknowledgements.—This research was funded by the Australian Research Council, the Nature Foundation of South Australia and the Holsworth Wildlife Research Endowment Fund. We thank the landholders, Chris and Maria Reed, for allowing access to their property. The study was conducted according to the guidelines of Flinders University Animal Welfare

Committee (approval E206) and in compliance with the Australian Code of Practice for the Use of Animals for Scientific Research.

LITERATURE CITED

Alford, R. A., K. S. Bradfield, and Stephen Richards. 1999. Measuring and analysing developmental instability as a tool for monitoring frog populations. In *Declines and disappearance of Australian frogs*, edited by A. Campbell. Canberra: Environment Australia.

Aparicio, J.M. 1998. Patterns of fluctuating asymmetry in developing primary feathers: a test of the compensational growth hypothesis. *Proceedings of The Royal Society of London B* 265:2353-2357.

Armstrong, G., and J. Reid. 1992. The re-discovery of the Adelaide pygmy bluetongue, Tiliqua adelaidensis (Peters, 1863). *Herpetofauna* 22:3 – 6.

Armstrong, G., J. Reid, and M. N. Hutchinson. 1993. Discovery of a population of the rare scincid lizard *Tiliqua adelaidensis* (Peters). *Rec. S. A. Mus.* 36:153 – 155.

Bjorksten, T. A., K. Fowler, and A. Pomiankowski. 2000. What does sexual trait FA tell us about stress? *TREE* 15:163 – 166.

Bradshaw, C. J. A., L. S. Davis, C. Lalas, and R. G. Harcourt. 2000. Geographic and temporal variation in the condition of pups of the New Zealand fur seal (*Arctocephalus foresteri*): evidence for density dependence and differences in the marine environment. *Journal of Zoology* 252:41 – 51.

Brown, C. R., and M. B. Brown. 1998. Intense natural selection on body size and winge and tail asymmetry in cliff swallows during severe weather. *Evolution* 52:1461 – 1475.

Bull, C. M., and Y. Pamula. 1996. Sexually dimorphic head sizes and reproductive success in the sleepy lizard *Tiliqua rugosa*. *Journal of Zoology* 240:511 – 521.

Clarke, G. M. 1995b. Relationships between developmental stability and fitness- application for conservation biology. *Conservation Biology* 9:18 – 24.

Clarke, G. M. 1998. The genetic basis of developmental stability. IV. Individual and population asymmetry parameters. *Heredity* 80:553 – 561.

Dufour, K. W., and P. J. Weatherhead. 1998a. Bilateral symmetry and social dominance in captive male red-winged blackbirds. *Behavioural Ecology and Sociobiology* 42:71 – 76.

Dufour, K. W., and P. J. Weatherhead. 1998b. Reproductive consequences of bilateral asymmetry for individual male red-winged blackbirds. *Behavioral Ecology* 9:232 – 242.

Ebrahimi, M. 2013. Optimising translocation strategies in the conservation management of reptiles; a case study of an endangered Australian skink, *Tiliqua adelaidensis.*Ph.D. School of Biological Sciences, Flinders University, Adelaide, Australia.

Fellows, H.L., A. L. Fenner, and C. M. Bull. 2009. Spiders provide important resources for an endangered lizard. *Journal of Zoology* 279:156 – 163.

Fenner, A. L., J. A. Schofield, A. L. Smith, and C. M. Bull. 2008. Observations of snake predation on the pygmy bluetongue lizard, *Tiliqua adelaidensis. Herpetofauna* 35:105 – 109.

Gagaliano, M., M. Depczynski, S. D. Simpson, and J. A. Moore. 2008. Dispersal without errors: symmetrical ears tune into the right frequency for survival. *Proceedings of The Royal Society B* 275:527 – 534.

Green, A. J. 2001. Mass/lenght residuals: measures of body condition or generators of spurious results? *Ecology* 82:1473 – 1483.

Helle, Samuli, Esa Huhta, Petri Suorsa, and Harri Hakkarainen. 2011. Fluctuating asymmetry as a biomarker of habitat fragmentation in an area-sensitive passerine, the Eurasian treecreeper (*Certhia familiaris*). *Ecological Indicators* 11 (3):861 – 867.

Hendrickx, F., J.P. Maelfair, and L. Lens. 2003. Relationship between fluctuating asymmetry and fitness whitin and between stressed and unstressed populations of the wolf spider *Pirata piraticus. Journal of Evolutionary Biology* 16:1270 – 1279.

Herrel, A., L. Spithoven, R. Van Damme, and F. De Vree. 1999. Sexual dimorphism of head size in Gallotia galloti: testing the niche divergence hypothesis by functional analyses. *Functional Ecology* 13:289 – 297.

Herrel, A., R. Van Damme, and F. De Vree. 1996. Sexual dimorphism of head size in *Podarcis hispanica atrata*: testing the dietary divergence hypothesis by bite force analysis *Netherlands Journal of Zoology* 46:253 – 262.

Hosken, David J., W.U. Blanckenhorn, and P.I. Ward. 2000. Developmental stability in yellow dong flies *(Scathophaga stercoraria)*: fluctuating asymmetry, heterozygosity and environmental stress. *Journal of Evolutionary Biology* 13:919 – 926.

Houle, D. 1997. Comment on " a meta-analysis of the heritability of developmental stability" by Moller and Thornhill. *Journal of Evolutionary Biology* 10:17 – 20.

Hoysak, D.J., and C.D. Ankney. 1996. Correlates of behaviour dominance in mallards and American black ducks. *Animal Behaviour* 51:409 – 419.

Husack, J. F., A. K. Lappin, S.F. Fox, and J. A. Lemos-Espinal. 2006. Bite-force performance predicts dominance in male venerable collared lizards (*Crotaphytus antiquus*). *Copeia* 2006:301 – 306.

Hutchinson, M. N., T. Milne, and T. Croft. 1994. Redescription and ecological notes on the pygmy bluetongue, *Tiliqua adelaidensis* (Squamata:Scincidae). *Transactions of the Royal Society of South Australia* 118:217 – 226.

Learny, L.J., and C.P. Klingenberg. 2005. The genetic and evolution of fluctuating asymmetry. *Annual Review of Ecology, Evolution, and Systematics* 36:1 – 21.

Leary, R. F., and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology & Evolution* 4:214 – 217.

Lemberget, T., and M. I. McCormick. 2009. Replenishment success linked to fluctuating asymmetry in larval fish. *Oecologia* 159:83 – 93.

Lens, L., and H. Eggermont. 2008. Fluctuating asymmetry as a putative marker of human-induced stress in avian conservation. *Bird Conservation International* 18:125 – 143.

Lens, L., S. Van Dongen, S. Kark, and E. Mathyssen. 2002. Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? *Biological Reviews* 77:27 – 38.

Lens, L., S. Van Dongen, and E. Matthysen. 2002. Fluctuating asymmetry as an early warning system in the critically endangered Taita Thrush. *Conservation Biology* 16:479 – 487.

Lens, L., S. Van Dongen, E. Matthysen, and T. Van de Casteele. 2000. Developmental instability and inbreeding in natural bird populations exposed to different levels of habitat disturbance. *Journal of Evolutionary Biology* 13:889 – 896.

Leung, B., and M.R. Forbes. 1997. Modelling fluctuating asymmetry in relation to stress and fitness. *Oikos* 78:397 – 405.

Leung, B., M.R. Forbes, and D. Houle. 2000. Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. *The American Naturalist* 155:101 – 115.

Lindell, L.E., A. Forsman, and J. Merila. 1993. Variation in number of ventral scales in snakes: effects on body size, growth rate and survival in the adder, *Vipera berus*. *Journal of Zoology* 230:101 – 115.

Lopez, P., A. Munoz, and J. Martin. 2002. Symmetry, male dominance and female mate preferences in the Iberian rock lizard, *Lacerta monticola*. *Behavioural Ecology and Sociobiology* 52:342 – 347.

Lovatt, F.M., and A. R. Hoelzel. 2011. The impact of population bottlenecks on fluctuating asymmetry and morphological variance in two separate populations of reindeer on the island of South Georgia. *Biological Journal of the Linnean Society* 102:798 – 811.

Madsen, T., and R. Shine. 2000. Rain, fish and snakes:climatically driven populations dynamics of Arafura filesnake in tropical Australia. *Oecologia* 124:208 – 215.

Martin, J., and P. Lopez. 2000. Chemoreception, symmetry, and mate choice in lizards. *Proceedings of The Royal Society of London B* 267:1265 – 1269.

Mateos, C., S. Alarcos, J. Carranza, C.B. Sanchez-Prieto, and J. Valencia. 2008. Fluctuating asymmetry of red deer antlers negatively relates to individual condition and proximity to prime age. *Animal Behaviour* 75:1629 – 1640.

Merila, J., A. Forsman, and L.E. Lindell. 1992. High frequency of ventral scale anomalies in *Vipera berus* populations. *Copeia* 1992:1127 – 1130.

Milne, T. 1999. Conservation and ecology of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis*, Ph.D. School of Biological Sciences, Flinders University, Adelaide, Australia.

Milne, T., C. M. Bull, and M. N. Hutchinson. 2003. Use of burrows by the endangered pygmy bluetongue lizard *Tiliqua adelaidensis. Wildlife Research* 30:523 – 528.

Moller, A. P. 1991. Sexual ornament size and the cost of fluctuating asymmetry. *Proceedings of The Royal Society of London B* 243:59 – 62.

Moller, A. P. 1992a. Female swallow preference for symmetrical male sexual ornaments. *Nature* 357:237 – 240.

Moller, A. P. 1993a. Female preference for apparently symmetrical male sexual ornaments in the barn swallows *Hirundo rustica. Behavioral Ecology and Sociobiology* 32:371 – 376.

Moller, A. P. 1997. Developmental stability and fitness. *American Naturalists* 149:916 – 932.

Moller, A. P., J. J. Cuervo, J. J. Soler, and C. Zamora-Munoz. 1996. Horn asymmetry and fitness in gemsbok, *Oryx g. gazella. Behavioral Ecology* 7:247 – 253.

Moller, A. P., and J.P. Swaddle. 1997. *Asymmetry, developmental stability, and evolution.* Oxford University Press,Oxford, UK.

Mouton, N., and J. H. Van Wyk. 1993. Sexual dimorphism in cordylid lizards: a case study of the Drakensberg crag lizard, *Pseudocordylus melanotus. Canadian Journal of Zoology* 71:1715 – 1723.

Palmer, A.R. 1996. Waltzing with asymmetry. *Bioscience* 46:518 – 532.

Palmer, A.R., and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology, Evolution, and Systematics* 17:391 – 421.

Palmer, A.R., and C. Strobeck. 2003. *Fluctuating asymmetry analyses revisited. In* Developmental instability: causes and consequences. M. Polak (Ed.). Oxford University Press, New York,USA.

Parsons, P.A. 1992. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity* 68:361 – 364.

Peig, J., and A. J. Green. 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and lenght. *Functional Ecology* 24:1323 – 1332.

Pertoldi, C., T.N. Kristenssen, D.H. Anderson, and V. Loeschcke. 2006. Developmental instablility as an estimator of genetic stress. *Heredity* 96:122 – 127.

Polak, M., and R. Trivers. 1994. The science of symmetry in biology. *Trends in Ecology & Evolution* 9:122 – 124.

Putman, R. J., M. S. Sullivan, and J. Langbein. 2000. Fluctuating asymmetry in antlers of fallow deer (*Dama dama*): the relative roles of environmental stress and sexual selection. . *Biological Journal of the Linnean Society* 70:27 – 36.

Reeve, E. C. R. 1960. Some genetic tests on asymmetry of sternopleural chaetae number in *Drosophila*. *Genetical Research* 1:151 – 176.

Sarre, S. 1996. Habitat fragmentation promotes fluctuating asymmtery but not morphological divergence in two geckos. *Research on population ecology* 38:57 – 64.

Sarre, S., J. M. Dearn, and A. Georges. 1994. The application of fluctuating asymmetry in the monitoring of animal populations. *Pacific Conservation Biology* 1:118 – 122.

Schofield, J. A., A. L. Fenner, K. Pelgrim, and C. M. Bull. 2012. Male-biased movement in pygmy bluetongue lizards: implications for conservation. *Wildlife Research* 39:677 – 684.

Schofield, J. A., M. G. Gardner, A. L. Fenner, and C. M. Bull. 2014. Promiscous mating in the endangered Australian lizard *Tiliqua adelaidensis*: a potential windfall for its conservation. *Conservation Genetics* 15:177 – 185.

Shamiminoori, L., A. L. Fenner, and C. M. Bull. 2014. Weight watching in burrows: variation in body condition in pygmy bluetongue lizards. *Australian Journal of Zoology* 62:284 – 293.

Shine, Richard. 2005. Life-history evolution in reptiles. *Annual Review of Ecology, Evolution, and Systematics* 36:23 – 46.

Siqueira, C. C., M. C. Keifer, M. Van Sluys, and C. F. Durate Rocha. 2013. Variation in the diet of the lizard *Tropidurus torquatus* along its coastal range in Brazil. *Biota Neotrop* 13:93 – 101.

Smith, A. L., M. G. Gardner, A. L. Fenner, and C. M. Bull. 2009. Restricted gene flow in the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) in a fragmented agricultural landscape. *Wildlife Research* 36:466 – 478.

Soule, M. E. 1979. Heterozygosity and developmental stability: another look. *Evolution* 33:396-401.

Swaddle, J.P., and M.S. Witter. 1994. Food, feathers, and fluctuating asymmetries. *Proc. R. Soc. Lond. B* 255:147 – 152.

Thornhill, Randy. 1992. Female preference for the pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (*Panorpa japonica*: Mecoptera). *Behavioral Ecology* 3:277 – 283.

Tohl, D., J. S. J. Li, L. Shamiminoori, and C. M. Bull. 2013. Image asymmetry measurement for the study of endangered pygmy bluetongue lizard. Paper read at Proceedings of 2013 IVCN, IEEE; 28th International Conference of Image and Vision Computing, at Wellington, New Zealand.

Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution* 16:125-142.

Vangestel, Carl, and Luc Lens. 2011. Does fluctuating asymmetry constitute a sensitive biomarker of nutritional stress in house sparrows (Passer domesticus)? *Ecological Indicators* 11:389 – 394.

Vitt, L.J., and W.E. Jr. Cooper. 1985. The evolution of sexual dimorphism in the skink *Eumeces laticeps*: an example of sexual selection. *Canadian Journal of Zoology* 63:995 – 1002.

Vitt, L.J., and E. R. Pianka. 1994. *Lizard Ecology: Historical and Experimental perspectives*. Princeton University Press, New Jesrsey, USA

Wauters, L. A., A. A. Dhondt, H. Knothe, and D. T. Parkin. 1996. Fluctuating asymmetry and body size as indicators of stress in red squirrel population in woodland fragments. *Journal of Applied Ecology* 33:735 – 740.

Woods, R. E., C. M. Sgro, M. J. Hercus, and A. A. Hoffman. 2002. Fluctuating asymmetry, fecundity and development time in *Drosophila*: is there an association under optimal and stress condition? *Journal of Evolutionary Biology* 15:146 – 157.

Wright, A.N., and K.R. Zamudio. 2002. Colour pattern asymmetry as a correlate of habitat disturbance in spotted salamanders (*Ambystoma maculatum*). *Journal of Herpetology* 36:129 – 133.

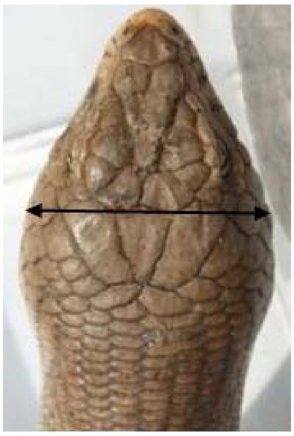


Figure 2. Diagram of the head of pygmy bluetongue lizards showing the measurement of jaw width (JW) as the distance between two ears.

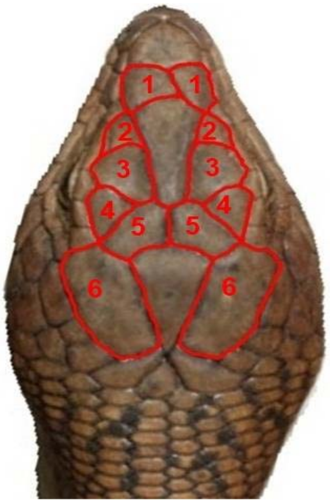


Figure 2. Diagram of the six pairs of scales used in deriving symmetry index (SI) of head in pygmy bluetongue lizards.

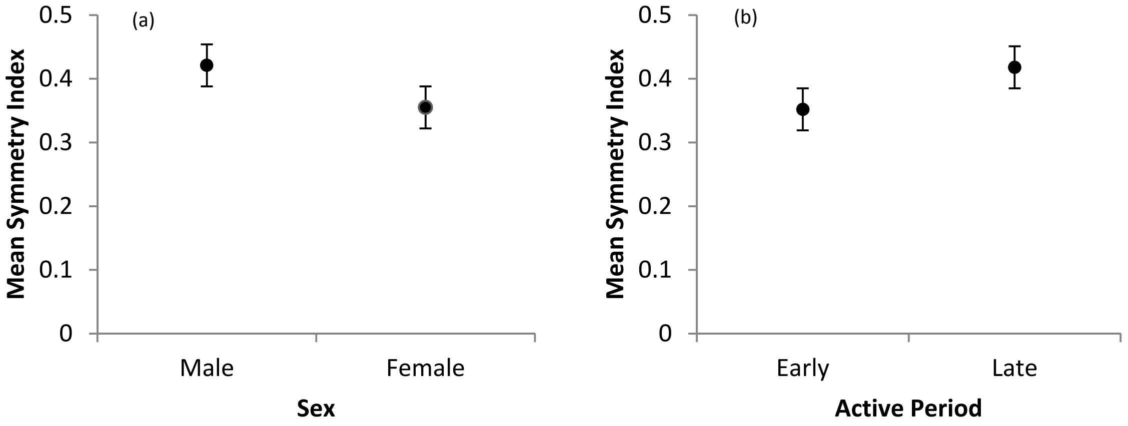


Figure 3. Comparisons of symmetry index between (a) male and female adult lizards, and (b) early and late activity periods.

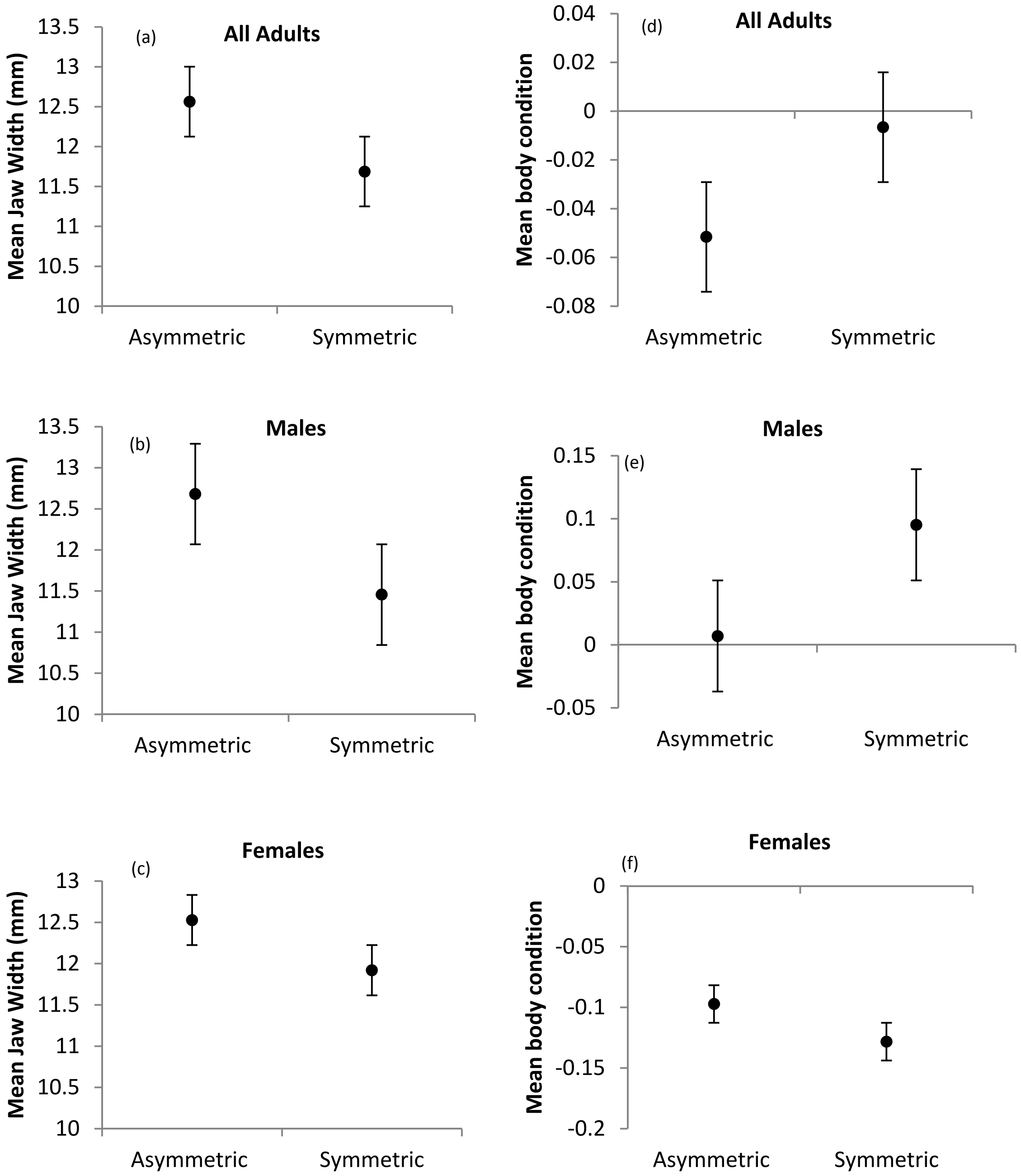


Figure 4. Comparisons of the mean jaw width (a-c) and mean body condition (d-f) between the most symmetric and the most asymmetric adult, male and female pygmy bluetongue lizards.

Table 1. Count of lizards captured during each sampling year and the count of 30% of the most and 30% of the least symmetric lizards used in the analyses in each group.

	2008/2009	2009/2010	2010/2011	Total
All data				
Male	25	55	2	82
Female	24	50	9	81
Total Adults	49	105	11	163
30% of most/least symmetric				
Male	16	32	2	50
Female	18	26	6	50
Total Adults	36	55	9	100

Table 2. Results of two-way ANOVA of symmetry index with sex and activity period as factors.

*The significant results are shown in bold.

	df	**F**	**P**
Sex	1	7.863	**0.006**
Activity period	1	6.829	**0.010**
Activity period x Sex	1	0.007	0.935
Error	96		

Table 3. Results of the correlations between a) jaw width (JW) and symmetry index (SI), b) jaw width (JW) and body condition (BC), and c) body condition (BC) and symmetry index (SI) for different studied groups of pygmy bluetongue lizards.

SI, Symmetry Index; JW, Jaw Width; BC, Body Condition

*Correlation is significant at the 0.05 level (2-tailed).

Lizard group	**r**	**N**	***p*-value**
a) JW-SI			
All adults	-0.370**	100	**0.001**
Males	-0.426**	50	**0.001**
Females	-0.298*	50	**0.035**
b) JW- BC			
All adults	-0.030	100	0.768
Males	-0.021	50	0.886
Females	-0.016	50	0.913
c) BC-SI			
All adults	0.113	100	0.262
Males	0.163	85	0.257
Females	-0.045	80	0.756

Table 4. Summary of the best-ranked AIC models examining the effect of the level of symmetry (sym), sampling year and sex on jaw width in the 30% of the data of (a) all adults, (b) males and (c) females.

K, the number of fitted parameters; AIC, Akaike's Information Criteria; Δ AIC, difference between AIC value of the best model and AIC value of other models; *wi,* Akaike model weight; cum *wi*, Cumulative Akaike weights; '×' refers to the interaction between factors; '+' represents the inclusion of main factors in the model.

Candidate models	***K***	**AIC$_c$**	**ΔAIC$_c$**	***w$_i$***	**Cum. *w$_i$***
(a) All adults					
Sym	3	311.912	0.000	0.4041	0.404
Sym + Year	4	313.719	1.807	0.1637	0.567
Sym+ Sex	4	313.807	1.895	0.1567	0.724
(b) Males					
Sym	3	176.140	0.000	0.491	0.491
Sym + Year	4	176.701	0.560	0.371	0.863
Sym x Year	5	179.056	2.916	0.114	0.977
(c) Females					
Sym	3	128.334	0.000	0.417	0.417
Year	3	129.549	1.214	0.227	0.644
Sym + Year	4	130.136	1.801	0.169	0.814

Table 5. Results of the AIC model averaging for jaw width in 30% of (a) all lizards, (b) males and (c) females.

*The significant results are shown in bold.

	Jaw width		
	Predictor w_i	**Model Av. β**	**95% CI**
(a) All adults			
Sym	**0.995**	**-0.867**	**-1.315, -0.419***
Sex	0.361	-0.124	-0.583, 0.335
(b) Males			
Sym	**0.976**	**-4.552**	**-8.319 , -0.785***
Year	0.497	0.276	-0.133 , 0.687
(c) Females			
Sym	0.645	-1.614	-4.313 , 1.084
Year	0.455	0.265	-0.167 , 0.698

Table 6. Summary of the best-ranked AIC models examining the effect of level of symmetry (sym), sampling year and sex on the body condition of the 30% of the data of (a) all adults, (b) males, (c) females, (d) pre-partum, , and (e) post-partum females.

K, the number of fitted parameters; AIC, Akaike's Information Criteria; Δ AIC, difference between AIC value of the best model and AIC value of other models; *wi*, Akaike model weight; cum *wi*, Cumulative Akaike weights; '×' refers to the interaction between factors; '+' represents the inclusion of main factors in the model.

Candidate model		***K***	**AICc**	**Δ AICc**	***w$_i$***	**Cum. *w$_i$***
(a)	**All adults**					
	Sex	3	-18.369	0.000	0.383	0.383
	Sym+ Sex + Year	5	-17.374	0.994	0.233	0.616
	Sym + Sex	4	-16.316	2.053	0.137	0.753
(b)	**Males**					
	Null	2	-4.219	0.000	0.520	0.520
	Sym	3	-2.496	1.723	0.219	0.740
	Year	3	-1.994	2.225	0.171	0.911
(c)	**Females**					
	Sym + Year	4	-23.135	0.000	0.370	0.370
	Year	3	-22.894	0.241	0.328	0.698
	Sym x Year	5	-22.096	1.039	0.220	0.918
(d)	**Pre-partum**					
	Year	3	-19.426	0.000	0.398	0.398
	Null	2	-19.272	0.153	0.369	0.767
	Sym	3	-16.281	3.144	0.082	0.850
(e)	**Post-partum**					
	Null	2	0.392	0.000	0.604	0.604
	Year	3	2.361	1.968	0.225	0.830
	Sym	3	2.957	2.564	0.167	0.997

Table 7. Results of the AIC model averaging of 30% of data for body condition in (a) all adults, (b) males, (c) females, (d) pre-partum, and (e) post-partum females.

*The significant results are shown in bold.

	Body condition		
	Predictor w_i	**Model Av. β**	**95% CI**
(a) All adults			
Sym	0.623	0.299	-0.656 , 1.256
Sex	0.865	0.112	-0.239, 0.464
Year	0.412	0.100	0.123 , 0.325
(b) Males			
Sym	0.306	0.180	-0.388 , 0.748
Year	0.258	-0.006	-0.075 , 0.063
(c) Females			
Sym	0.610	0.596	-0.427 , 1.620
Year	**0.918**	**0.12**	**0.021, 0.218***
(d) Pre-partum			
Sym	0.306	0.180	-0.388 , 0.748
Year	0.258	-0.006	-0.075 , 0.063
(e) Post-partum			
Sym	0.169	-0.594	-1.506 , 0.316
Year	0.227	0.101	-0.0215 , 0.224

Appendix I

Table 1. Summary of the best-ranked AIC models examining the effect of level of symmetry (Sym), sampling year and sex on body condition of 20% of the most symmetrical and most asymmetrical (a) all adults, (b)male, and (c)female pygmy bluetongue lizards.

Candidate model	***K***	**AICc**	**Δ AICc**	w_i	**Cum.** w_i
(a) All Adults					
Sex	3	-13.535	0.000	0.282	0.282
Sym x Year x Sex	9	-13.313	0.222	0.252	0.534
Sym + Year + Sex	5	-11.996	1.538	0.130	0.665
(b) Males					
Null	2	-5.615	0.000	0.536	0.536
Year	3	-3.849	1.766	0.221	0.757
(c) Females					
Sym + Year	4	-13.933	0.000	0.411	0.411
Year	3	-13.438	0.494	0.321	0.732

Table 2. Summary of the best-ranked AIC models examining the effect of level of symmetry (Sym), sampling year and sex on body condition of 40% of the most symmetrical and most asymmetrical (a) all adults, (b)male, and (c)female pygmy bluetongue lizards.

Candidate model	***K***	**AICc**	**Δ AICc**	w_i	**Cum.** w_i
(a) All Adults					
Sex	3	-32.750	0.000	0.282	0.282
Sym + Year + Sex	5	-31.726	1.023	0.252	0.534
Sym x Year x Sex	4	-30.682	2.067	0.130	0.665
(b) Males					
Null	2	-10.861	0.000	0.522	0.522
Year	3	- 9.045	1.815	0.210	0.733
(c) Females					
Null	2	-17.739	0.000	0.321	0.321
Year	3	-17.712	0.026	0.316	0.637
Sym + Year	4	-16.324	1.414	0.158	0.796

Table 3. Summary of the best-ranked AIC models examining the effect of level of symmetry (Sym), sampling year and sex on body condition of 50% of the most symmetrical and most asymmetrical (a) all adults, (b)male, and (c)female pygmy bluetongue lizards.

Candidate model	***K***	**AICc**	**Δ AICc**	w_i	**Cum.** w_i
(a) All Adults					
Sex	3	-50.936	0.000	0.535	0.535
Sym + Sex	4	-48.848	2.088	0.188	0.723
(b) Males					
Null	2	-15.191	0.000	0.490	0.490
Sym	3	-13.599	1.591	0.221	0.712
Year	3	-13.114	2.076	0.173	0.885
(c) Females					
Null	2	-25.557	0.000	0.345	0.345
Year	3	-25.319	0.237	0.306	0.651
Sym+ Year	4	-23.636	1.921	0.132	0.783

Table 4. Summary of the best-ranked AIC models examining the effect of level of symmetry (Sym), sampling year and sex on jaw width of 20% of the most symmetrical and most asymmetrical (a) all adults, (b)male, and (c)female pygmy bluetongue lizards.

Candidate model	***K***	**AICc**	**Δ AICc**	w_i	**Cum.** w_i
(a) All Adults					
Sym	3	212.204	0.000	0.420	0.420
Sym + Year	4	214.058	1.853	0.166	0.587
Sym x Sex	4	214.241	2.036	0.151	0.739
(b) Males					
Sym	4	122.699	0.000	0.585	0.585
Sym x Sex	3	125.185	2.485	0.168	0.753
(c) Females					
Year	3	83.543	0.000	0.315	0.315
Null	2	83.552	0.008	0.314	0.630
Sym	3	84.056	0.512	0.244	0.874

Table 5. Summary of the best-ranked AIC models examining the effect of level of symmetry (Sym), sampling year and sex on jaw width of 40% of the most symmetrical and most asymmetrical (a) all adults, (b)male, and (c)female pygmy bluetongue lizards.

Candidate model	***K***	**AICc**	**∆ AICc**	***wi***	**Cum. *wi***
(a) All Adults					
Sym	3	411.509	0.000	0.320	0.320
Sym + Year	4	412.410	0.900	0.204	0.525
Sym x Sex	5	412.576	1.066	0.188	0.713
Sym + Sex	4	413.220	1.710	0.136	0.850
(b) Males					
Sym	4	225.132	0.000	0.429	0.429
Sym +Year	3	225.138	0.006	0.428	0.857
(c) Females					
Sym +Year	3	154.001	0.000	0.447	0.447
Sym	4	155.637	1.635	0.197	0.645
Year	3	155.715	1.713	0.190	0.835

Table 6. Summary of the best-ranked AIC models examining the effect of level of symmetry (Sym), sampling year and sex on jaw width of 50% of the most symmetrical and most asymmetrical (a) all adults, (b)male, and (c)female pygmy bluetongue lizards.

Candidate model	***K***	**AICc**	**∆ AICc**	***wi***	**Cum. *wi***
(a) All Adults					
Sym + Year	4	525.663	0.000	0.370	0.370
Sym	3	527.086	1.422	0.182	0.552
Sym + Sex + Year	5	527.565	1.901	0.143	0.695
Sym x Sex	5	527.737	2.073	0.131	0.827
(b) Males					
Sym	3	271.550	0.000	0.505	0.505
Sym + Year	4	272.209	0.658	0.363	0.869
(c) Females					
Year	3	211.734	0.000	0.480	0.480
Sym+ Year	4	213.130	1.396	0.238	0.718

Appendix II

Table 1. A full list of alternative models tested for the effect of level of symmetry and sampling year on body condition of 30% of the most symmetrical and most asymmetrical (a) all adults, (b)male, (c)female, (d) pre-partum, and (e) post-partum pygmy bluetongue lizards.

Candidate model	*K*	AICc	∆ AICc	w_i	Cum. w_i
(d) All adults					
Sex	3	-18.369	0.000	0.383	0.383
Sym+ Sex + Year	5	-17.374	0.994	0.233	0.616
Sym + Sex	4	-16.316	2.053	0.137	0.753
Sym x Sex	5	-15.218	3.151	0.079	0.832
Sym x Year x Sex	9	-14.340	4.028	0.051	0.883
Sym x Year	5	-13.695	4.673	0.037	0.920
Sym + Year	4	-13.818	4.550	0.034	0.956
Year	3	-12.812	5.556	0.023	0.976
Null	2	-11.922	6.446	0.015	0.991
Sym	3	-10.816	7.552	0.008	1.000
(e) Males					
Null	2	-4.219	0.000	0.520	0.520
Sym	3	-2.496	1.723	0.219	0.740
Year	3	-1.994	2.225	0.171	0.911
Sym + Year	4	-0.139	4.074	0.067	0.979
Sym x Year	5	2.236	6.455	0.020	1.000
(f) Females					
Sym + Year	4	-23.135	0.000	0.370	0.370
Year	3	-22.894	0.241	0.328	0.698
Sym x Year	5	-22.096	1.039	0.220	0.918
Null	2	-19.546	3.589	0.061	0.980
Sym	3	-17.302	5.833	0.020	1.000
(g) Pre-partum					
Year	3	-19.426	0.000	0.398	0.398
Null	2	-19.272	0.153	0.369	0.767
Sym	3	-16.281	3.144	0.082	0.850
Sym +Year	4	-16.076	3.349	0.074	0.925
Sym x Year	4	-16.076	3.349	0.074	1.000
(h) Post-partum					
Null	2	0.392	0.000	0.604	0.604
Year	3	2.361	1.968	0.225	0.830
Sym	3	2.957	2.564	0.167	0.997

Sym +Year	4	11.410	11.018	0.002	1.000
Sym x Year	5	29.878	29.485	0.000	1.000

Table 2. A full list of alternative models tested for the effect of level of symmetry and sampling year on jaw width of 30% of the most symmetrical and most asymmetrical (a) all adults, (b)male, (c)female, pygmy bluetongue lizards.

Candidate models	***K***	**AIC$_c$**	**ΔAIC$_c$**	***w$_i$***	**Cum. *w$_i$***
(d) All adults					
Sym	3	311.912	0.000	0.404	0.404
Sym + Year	4	313.719	1.807	0.163	0.567
Sym+ Sex	4	313.807	1.895	0.156	0.724
Symx Sex	5	314.075	2.163	0.137	0.861
Sym x Year	5	315.428	3.516	0.069	0.931
Sym + Year + Sex	5	315.535	3.623	0.066	0.997
Year	3	323.861	11.949	0.001	0.998
Null	2	324.550	12.638	0.000	0.999
Sex	3	324.814	12.901	0.000	0.999
Sex + Year	5	325.706	13.793	0.000	1.000
(e) Males					
Sym	3	176.140	0.000	0.491	0.491
Sym + Year	4	176.701	0.560	0.371	0.863
Sym x Year	5	179.056	2.916	0.114	0.977
Year	3	183.522	7.382	0.012	0.989
Null	2	183.893	7.753	0.010	1.000
(f) Females					
Sym	3	128.334	0.000	0.417	0.417
Year	3	129.549	1.214	0.227	0.644
Sym + Year	4	130.136	1.801	0.169	0.814
Null	2	130.729	2.394	0.126	0.940
Sym x Year	5	132.234	3.899	0.059	1.000

Chapter 6. Ontogenetic changes in head scale symmetry in the pygmy bluetongue lizard

Leili Shamiminoori[A,B], *and C. Michael Bull*[A]

[A]School of Biological Sciences

Flinders University

GPO Box 2100

Adelaide, SA 5001

Australia

[B] Corresponding author: L. Shamiminoori

Email: sham1012@flinders.edu.au

Phone: +61 8 8201 2805

Fax: +61 8 8201 3015

Accepted: ***Transactions of the Royal Society of South Australia (November 2015)***

Abstract

Fluctuating asymmetry (FA, small and random deviations from perfect symmetry in otherwise bilaterally symmetrical traits) has been used as a monitoring tool for species at risk. Differences in the levels of FA in a trait are attributed to the different levels of environmental or genetic stressors during the developmental phase of an organism. In this study we used a symmetry index in the head scales of the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis*, to investigate the relationship between the symmetry index and jaw width (as an indirect measure of fitness) among lizards from different age classes (neonate, subadult and adult) to shed light on the ontogeny of symmetry. Also, we explored changes in the levels of symmetry among different age classes to determine possible selection processes. Our findings showed that there was a positive relationship between the symmetry index and jaw width in neonates (individuals with larger jaws were more symmetric) whereas the trend was inversed in adults (individuals with larger jaws were less symmetric). The relationship was not significant in subadults. This confirms that a transition happened during the growth of lizards. We suggest this resulted from adults allocating more resources to the growth rather than symmetry of head scales. We found no significant differences in the mean symmetry index between adults and neonates, although subadults showed significantly higher values of the symmetry index. This study has revealed some valuable insights into ontogenetic changes in symmetry of head scales in pygmy bluetongue lizards, but it did not provide any evidence that survival from neonate to adult is influenced by head scale symmetry.

Introduction

The current rate of species extinction throughout the world has prompted conservation biologists to look for tools to identify and monitor populations at risk before the threat becomes detrimental. One such tool is fluctuating asymmetry (FA) defined as any random deviation from perfect symmetry in bilaterally symmetrical structures (Van Valen 1962). Fluctuating asymmetry has been widely accepted as an indicator of developmental stability (Palmer and Strobeck 1986) and refers to the ability of an organism to buffer the influence of stress during development (Leary and Allendorf 1989). Developmental stability is believed to decrease with increased levels of either environmental or genetic stress (Leary and Allendorf 1989; Moller 1999; Palmer and Strobeck 1986; Parsons 1992). Differences in the levels of fluctuating asymmetry in a trait are then attributed to different levels of developmental stability (less stability leads to more asymmetry). Thus, asymmetry in a population may be an indirect indicator that the population is under some form of stress, and this could be a valuable monitoring tool for small populations of endangered species.

However, the impact of stress (and reduced developmental stability) on symmetry may vary according to: (1) the trait functionality, (2) the mode of selection, or (3) the nature of the stress during development. Furthermore, the relationship between stress and fluctuating asymmetry is not always clear and has been the subject of controversy (Bjorksten, Fowler et al. 2000). Bjorksten et al. (2000) argued that the relationship was inconsistent and that the genetic basis of FA is poorly understood. In addition, the literature shows heterogeneous results, among species and among different traits, in the response of FA to stress (Bjorksten, David *et al.* 2000; Dufour and Weatherhead 1998a; Hunt and Simmons 1997; Moller 1992). For example, Moller (1992) showed that male swallows, *Hirundo rustica* that were stressed by high mite infestations showed higher levels of asymmetry in their long tail feathers, but no differences in symmetry in their short tail feathers. Similarly Bjorksten et al. (2000) found no evidence of increased FA either in eye stalk length or in wing length or width when stalk-eyed flies, *Cyrtodiopsis dalmanni*, were stressed with reduced larval food. However, other studies have suggested there is an indirect relationship between developmental stability and fluctuating asymmetry (Aparicio 2001; Aparicio and Bonal 2002) taking into account that developmental stability operates at the microscopic level whereas fluctuating asymmetry is a macroscopic manifestation of any smaller discrepancies in the ontogeny of the left and the right side. Aparicio and Bonal (2002) proposed a model based on the origin of fluctuating asymmetry, which suggests that the level of fluctuating asymmetry in a trait could also depend on the amount of structural components allocated to build that trait during development, regardless of the overall degree of developmental stability. Thus, more elaborate characters such as sexual displays like ornamental tails in birds should be more likely to display asymmetry than less elaborate ones.

Additionally, individuals within a population can have different growth rates. We might expect to find increased FA in individuals that grow faster than the evolved optimal rate for their developmental threshold (Leamy and Atchley 1985; Morris, Rios-Cardenas *et al.* 2012). In a laboratory study, Leamy and Atchley (1985) found that faster growing rats had higher levels of fluctuating asymmetry, independent of the level of genetic heterozygosity. Thus, alternative growth rate strategies among individuals in a population could shed light on why some studies did not find a relationship between FA and stress. For instance, in studies on swordtail fish, *Xiphophorus multilineatus*, Morris et al. (2012) reported a relationship between growth rate and symmetry. Males from one genetically influenced size class grew faster and were more asymmetric in their vertical bar sexual display than males in other size classes.

To understand more clearly the relationship between stress and symmetry we need to understand changes in symmetry during ontogenetic development. One way to tackle this question is to explore additional cases, and that will be particularly useful if those cases are of conservation concern, where a link between stress and symmetry might be used to monitor population condition. The pygmy bluetongue lizard, *Tiliqua adelaidensis*, is an endangered skink that lives in remnant patches of grassland in the mid-north region of South Australia. This species is protected under the Australian Environment Protection and Biodiversity Conservation Act 1999. Pygmy bluetongue lizards are solitary skinks which spend most of their life in burrows constructed by spiders (Milne 1999). The dorsal scales on the head normally display bilateral symmetry. We derived a symmetry index as the divergence from symmetry in the shape and size of six pairs of these head scales (Tohl, Li et al. 2013). In a previous analysis of adult lizards (Shamiminoori 2015), we found no relationship between symmetry and lizard body condition, but a significant negative relationship between symmetry and jaw width. Adults with wider jaws had less symmetry in their dorsal head scales. In this paper we used lizard jaw width as an indirect measure of fitness, assuming wider jaws allow a wider range of prey types.

We assessed whether the level of symmetry in the shape and size of dorsal head scales, relates to jaw width, and how that relationship changes during lizard growth. We used our measures of symmetry in neonate, subadult and adult lizards to address two questions concerning the ontogeny of asymmetry.

The first question arose from our interpretation of the earlier results when we suggested that during growth, lizards might trade off an extra input into forming wide jaws against developmental stability for other traits, such as the symmetrical head scales. A hypothesis from that explanation is that the negative relationship between symmetry and jaw width should emerge during growth, and be less apparent among younger lizards than among adults. In this current paper we test that hypothesis by comparing the relationship between symmetry and jaw width among lizards from different age classes.

Our second question concerns another way of assessing the adaptive value of trait symmetry by comparing the degree of symmetry between younger and older age classes, and deducing if there is differential mortality related to symmetry. If we can assume that different age classes that are compared at one time are representative of a single cohort developing through each age, and if there are differences in the level of symmetry between the age classes, one interpretation is that there has been differential mortality of particular classes of symmetry within the population during growth. In this paper, we compared levels of symmetry among age classes to explore this possible selection process.

Materials and methods

Study site

The study site was the "Tiliqua" property of the Nature Foundation of South Australia, previously described as Site 2 (Fenner and Bull 2007). It is located about eight kilometres from Burra in South Australia (33° 42'S, 138° 56'E) in a remnant patch of native grassland. The area has hot, dry summers and cool, moist winters.

We sampled a total of 216 lizards including 142 adults, 29 subadults and 45 neonates over three spring and summer (September to March) lizard activity seasons (2009/2010 – 2011/2012). Neonates were sampled between January and March each year, within two weeks of their live birth. To do this we monitored the burrows of resident adult females from mid-January until their litters were produced. Neonates usually remain in the burrow with their mother for at least a week before dispersing (Milne, Bull *et al.* 2002).

Lizards were captured using the fishing method as previously described by Milne (1999). For each lizard we recorded the date of capture, sex, usually by cloacal examination, mass (to the nearest 0.5g), and snout to vent length (SVL) and jaw width (JW) (measured as the longest distance between left and right ears for each lizard), both to the nearest 1.0 mm. Following Milne (1999), we used SVL to divide lizards into adults (>80mm), subadults (60-80 mm) and neonates (<60mm). In some analyses we considered small (SVL 60-70 mm) and large (SVL 70 – 80 mm) sub-adults. In some other analyses we divided neonates into those captured early (before 15 Feb) or late (after 15 Feb) in the season. We took digital photographs of the scales on the dorsal surface of the head of each lizard using a Canon SLR 450D camera. The lizard was given a unique toe clip identification marking, to avoid resampling the same individuals, and then released back to its burrow.

Development of Symmetry Index (SI)

We developed a symmetry index by comparing the size and shape of six pairs of dorsal head scales on the right and left side (Shamiminoori, Fenner *et al.* 2014; Tohl, Li *et al.* 2013). We used a non-invasive technique of photographing the dorsal head scales of lizards and then, from the image, we derived an asymmetry index (AI) by using an algorithm generated in MATLAB image processing toolbox (Tohl, Li *et al.* 2013). This procedure compared the overall combined outline of the left and right hand representatives of the six pairs of head scales, after correcting the images for the impact of variation in image size, horizontal tilt and luminance. These correction procedures are described in detail in (Tohl, Li *et al.* 2013) but are summarised below.

First, the colour images of the head of the lizards were transformed to greyscale to avoid any impact of seasonal changes in dorsal colour on perceived scale outlines. Then a vertical centred line of symmetry from the tip of the snout to the neck was inserted for each photo so that pairs of scales were separated into left and right side. To correct for tilt, the image was rotated about this line of symmetry until the maximum correlation between left and right side was achieved. Next, an edge detection technique was applied to the image to extract scale pattern edges of the head as binary images, and to remove unwanted image features in between the scales.

Finally, the measure of asymmetry was calculated by the normalized correlation between extracted scale patterns of the left and right image. This resulted in a value between one (strong symmetry) and zero (strong asymmetry).

Statistical analysis

Analyses were conducted to determine whether the symmetry index of head scales differed among adults, subadults and neonate pygmy bluetongue lizards, and to explore whether the relationship between jaw width and the symmetry index varied among age classes. Our specific aim was to detect any ontogenetic changes in symmetry and its relationship with jaw width, and when those changes happened during lizard growth.

We used three analyses of covariance (ANCOVA) with symmetry index as the dependent variable, age class as a factor and jaw width as a covariate. In one analysis we used all of the data and compared the three age classes, adults, subadults and neonates. In a second analysis we only considered subadults and considered younger and older age classes of subadults to be represented by small and large subadult individuals based on their SVL (snout-vent length). In the third analysis we only included neonates and considered those captured early or late in the season. Since most neonates were captured while still with their mothers these may simply represent early or late births, although the late captures may include some individuals born earlier and so may be, on average, older than the early captures.

Results

Table 1 shows the sample size (n) and the mean (SE) values of the symmetry index and the jaw width for all adults, subadults and neonates measured in this study.

The ANCOVA of the symmetry index values of all sampled lizards, showed a highly significant interaction between age class and jaw width (Table2). Figure 1 shows that there was a significant negative relationship between jaw width and symmetry index among adult lizards (r = -0.305; P<0.001), but a significant positive relationship between the same two parameters among neonates (r = 0.412; P=0.005). For subadults, the relationship, while negative, was not significant (r = -0.072; P =0.75). Although the ANCOVA also showed a significant main effect of size class (Table 2), the significant interaction effect of age class and jaw width meant that result could not be interpreted. Nevertheless the differences seemed to result from subadults showing higher mean values of the symmetry index, while there was substantial overlap in symmetry between neonates and adults (Fig 2).

The analyses of symmetry index just considering small and large subadults (Table 3), or early and late born neonates (Table 4) were designed to explore whether the ontogenetic change could be detected during either of those life history stages. They showed no significant interaction between indirectly assessed age class and jaw width. However, within the neonates the positive relationship between jaw width and symmetry index was confirmed by the significant effect of jaw width (Table 4). An Independent-Samples t-test showed neonates had significantly longer SVL among individuals captured late than early in the season (t_{43}= 2.65, P=0.01), implying that neonate lizards had grown over the sampling period (or that later born lizards were larger). We had no recaptures of any individual neonates to explore those alternative explanations.

Discussion

Our results showed changes in the mean symmetry index in head scales of pygmy bluetongue lizards over their life period. We had earlier hypothesised that the negative relationship between jaw width and symmetry index in adult pygmy bluetongue lizards should develop during their growth. Those lizards that invest more into developing wider adult jaws may do so at a cost to developmental stability in other traits such as head scale symmetry. In that case younger lizards should not show the negative relationship between jaw width and symmetry that we found in adults. Our results supported this hypothesis by revealing no evidence for the negative correlation in neonates. In fact the relationship was inversed with jaw width and symmetry positively related in neonates. The lack of any significant relationship between symmetry and jaw width among subadults implied that the switch from a positive to a negative relationship occurred during this phase of growth. However, when we attempted to identify more precisely the transition period, there was no evidence of a switch between smaller (and presumably younger) subadults and larger (older subadults). Nor was there any evidence of a switch during the neonate stage comparing early versus late captured neonates. We cannot be sure that these classifications represent successive age classes within subadults or within neonates, and thus we cannot confidently claim that the switch between a positive and a negative association between jaw size and symmetry did not occur within either of these growth stages. There was an age related transition, but we found no evidence for exactly when it occurred.

Although, we do not know exactly when the switch happened, the pattern of transition is expected if the adult relationship (wider jaws associated with less head scale symmetry) represents a trade off of increasing jaw width at the expense of the control of symmetry in other traits. The structural hypothesis argues that the level of fluctuating asymmetry could depend on the amount of structural components necessary to form a unit of length of a trait

(Aparicio and Bonal 2002). The cost of growth increases with the development of the trait and developmental noise causes asymmetries between the two sides of the trait. In the case of pygmy bluetongue lizards, it may be that genetically more symmetric individuals are born with wider jaws, giving them an early advantage in having access to larger invertebrate prey items. Then, as lizards grow the developmental focus on increasing jaw width disrupts other developmental controls so that by the time they are adults the widest jaws have the most disruption of other processes and so the least symmetry in head scales.

In our second analysis, we found no significant difference in the mean symmetry index between adults and neonates. Although sub adults showed significantly higher values of the symmetry index this could be because of the low sample size, and the direction of change was not sustained consistently into the adult stage. In summary, there was no consistent trend for the mean symmetry index to change when comparing age classes and, by implication, as neonates developed into adults. We had earlier reported no significant relationship between head scale symmetry and adult body condition, and suggested that, for pygmy bluetongue lizards in current conditions, head scale symmetry is not a good indicator of population stress. The current result confirms that conclusion in providing no evidence that survival from neonate to adult is influenced by head scale symmetry. It is possible that contrary evidence may be produced by following a cohort through time, or by examining an alternative trait. Also it may be that the level of stress on individuals may become much more severe, in the future, for instance if there are wind-farm developments near population sites, or as the climate become warmer and drier, and that at those increased stress levels an association between stress and symmetry may become apparent. In summary, although this study has revealed some valuable insights into ontogenetic changes in symmetry of head scales in pygmy bluetongue lizards, it appears the results will be of limited value in terms of monitoring for population stress, at least under current conditions.

Acknowledgements

This research was funded by the Australian Research Council, the Nature Foundation of South Australia and the Holsworth Wildlife Research Endowment Fund. We thank the Nature Foundation of South Australia for allowing access to their Tiliqua property. The study was conducted according to the guidelines of Flinders University Animal Welfare Committee (approval no. E206) and in compliance with the Australian Code of Practice for the Use of Animals for Scientific Research.

References

Aparicio, J.M. (2001) Patterns of growth and fluctuating asymmety: the effects of asymmetrical investment in traits with dominate growth. *Behavioral Ecology and Sociobiology* **49**, 273-282.

Aparicio, J.M., and Bonal, R. (2002) Why do some traits show higher fluctuating asymmetry than others? A test of hypotheses with tail feathers of birds. *Heredity* **89**, 139-144.

Bjorksten, T.A., David, P., Pomiankowski, A., and Fowler, K. (2000) Fluctuating asymmetry of sexual and nonsexual traits in developmental stress and genetic quality. *Journal of Evolutionary Biology* **13**, 89-97.

Bjorksten, T.A., Fowler, K., and Pomiankowski, A. (2000) What does sexual trait FA tell us about stress? *TREE* **15**, 163-166.

Dufour, K.W., and Weatherhead, P.J. (1998a) Bilateral symmetry and social dominance in captive male red-winged blackbirds. *Behavioural Ecology and Sociobiology* **42**, 71-76.

Fenner, A.L., and Bull, C.M. (2007) Short-term impact of grassland fire on the endangered pygmy bluetongue lizard. *Journal of Zoology* **272**, 444-450.

Hunt, J., and Simmons, L.W. (1997) Patterns of fluctuating asymmetry in beetles horns: an experimental examination of the honest signalling hypothesis

Behavioral Ecology and Sociobiology **41**, 109-114.

Leamy, L., and Atchley, W. (1985) Directional selection and developmental stability: Evidence from fluctuating asymmetry of morphometric characters in rats. *Growth, Development and Aging* **49**, 8-18.

Leary, R.F., and Allendorf, F.W. (1989) Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology & Evolution* **4**, 214-217.

Milne, T. (1999) Conservation and ecology of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis*. Flinders University, Adelaide

Milne, T., Bull, C.M., and Hutchinson, M.N. (2002) Characteristics of litters and juveniles dispersal in the endangered Australian skink *Tiliqua adelaidensis*. *Journal of Herpetology* **36**, 110-112.

Moller, A.P. (1992) Parasites differentially increase fluctuating asymmetry in secondary sexual characteristics. *Journal of Evolutionary Biology* **5**, 691-699.

Moller, A.P. (1999) Asymmetry as a predictor of growth, fecundity and survival. *Ecological Letters* **2**, 149-156.

Morris, M.R., Rios-Cardenas, O., Lyons, S.M., Scarlett Tudor, M., and Bono, L.M. (2012) Fluctuating asymmetry indicates the optimization of growth rate over developmental stability. *Functional Ecology* **26**(3), 723-731.

Palmer, A.R., and Strobeck, C. (1986) Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology, Evolution, and Systematics* **17**, 391-421.

Parsons, P.A. (1992) Fluctuating asymmetry an epigenic measure of environmental and genomic stress. *Heredity* **68**, 361-364.

Shamiminoori, L. (2015) Impacts of environmental variation on the fitness of the pygmy bluetongue lizards, *Tiliqua adelaidensis*. Flinders University, Adelaide

Shamiminoori, L., Fenner, A.L., and Bull, C.M. (2014) Weight watching in burrows: variation in body condition in pygmy bluetongue lizards. *Australian Journal of Zoology* **62**, 284-293.

Tohl, D., Li, J.S.J., Shamiminoori, L., and Bull, C.M. Image asymmetry measurement for the study of endangered pygmy bluetongue lizard. In ' Proceedings of 2013 IVCN, IEEE; 28th International Conference of Image and Vision Computing', 2013, Wellington, New Zealand. (Eds. T Rhee, R Rayudu, C Hollitt, J Lewis and M Zhang), pp. 76-81

Van Valen, L. (1962) A study of fluctuating asymmetry. *Evolution* **16**, 125-142.

Table 3. The sample size and mean (SE) of jaw width (JW) and Symmetry Index (SI) in three age classes of pygmy bluetongue lizards.

	N	Mean JW (SE)	Mean SI (SE)
Adult	142	12.52 ± 0.08	0.35 ± 0.008
Subadult	29	10.91 ± 0.2	0.46 ± 0.02
Neonate	45	7.02 ± 0.98	0.37 ± 0.2

Table 2. Results of the Analysis of Covariance of the Symmetry Index of pygmy bluetongue lizards with age class as a factor and jaw width as a covariate.

P values in bold indicate results that are statistically significant at $P < 0.05$.

	df	F	p-value
Age class	2	6.73	**0.001**
Jaw width	1	2.37	0.125
Age class x Jaw width	2	6.30	**0.002**
Error	210		

Table 3. Results of the Analysis of Covariance of the Symmetry Index of subadult pygmy bluetongue lizards with size class as a factor and jaw width as a covariate.

	df	F	p-value
Size class	1	0.002	0.969
Jaw width	1	0.145	0.707
Size class x Jaw width	1	0.009	0.925
Error	25		

Table 4. Results of the Analysis of Covariance of the Symmetry Index of neonate pygmy bluetongue lizards with capture period (early/late) as a factor and jaw width as a covariate. *P* values in bold indicate results that are statistically significant at $P < 0.05$.

	df	F	p-value
Capture period	1	0.51	0.47
Jaw width	1	6.78	**0.013**
Capture period x Jaw width	1	0.60	0.44
Error	41		

Table 5. Correlations between Symmetry Index and jaw width in adult, subadult and neonate pygmy bluetongue lizards**.**

*Correlation is significant at the 0.05 level (2-tailed).

Lizard group	*r*	N	*p*-value
Adults	-0.305**	142	**0.001**
Sub-adults	-0.072	29	0.753
Neonates	0.412**	45	**0.005**

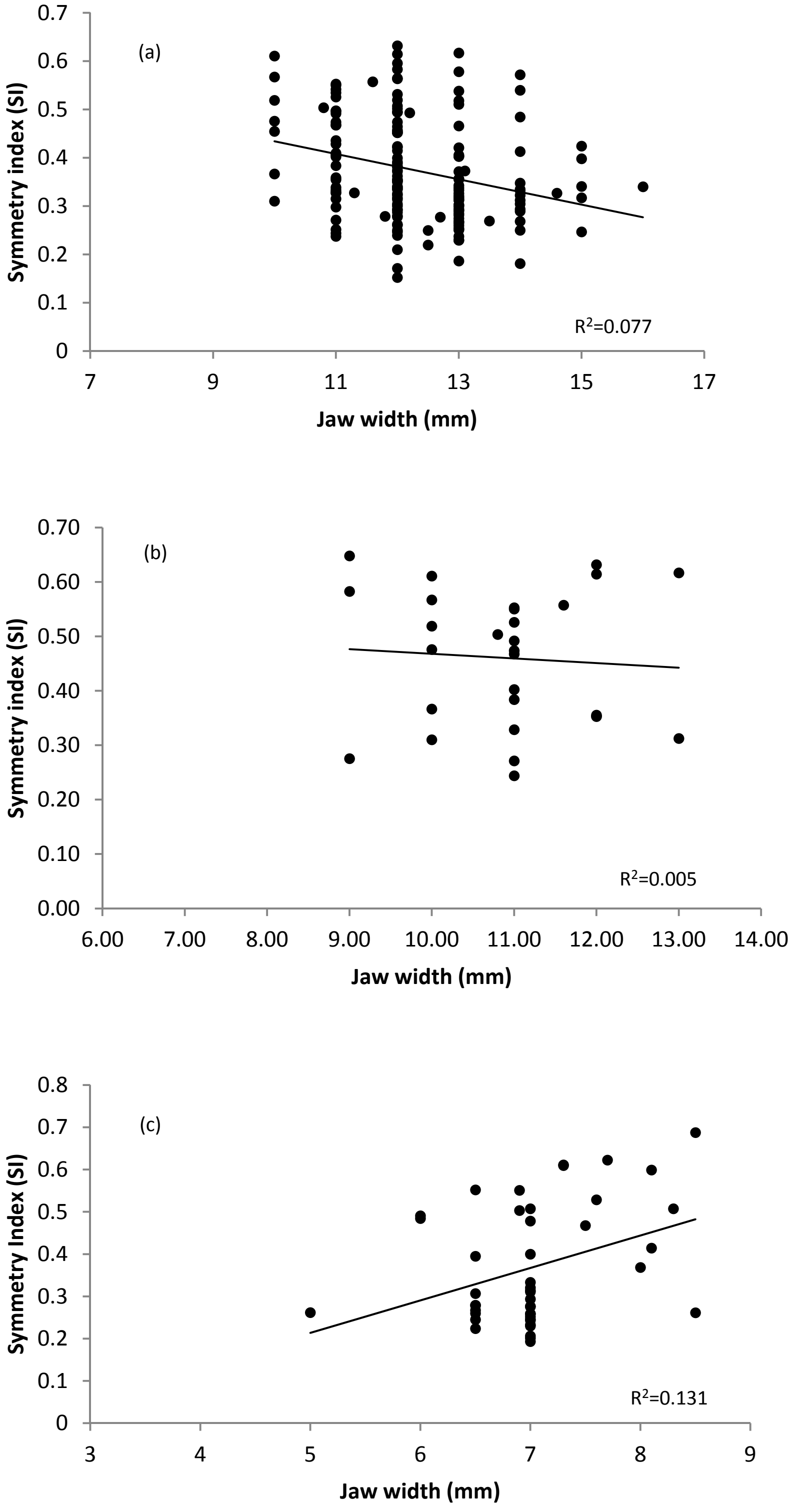


Figure 1. The relationship between symmetry index (SI) and jaw width in *(a)* adults, *(b)* subadults , and *(c)* neonate pygmy bluetongue lizards.

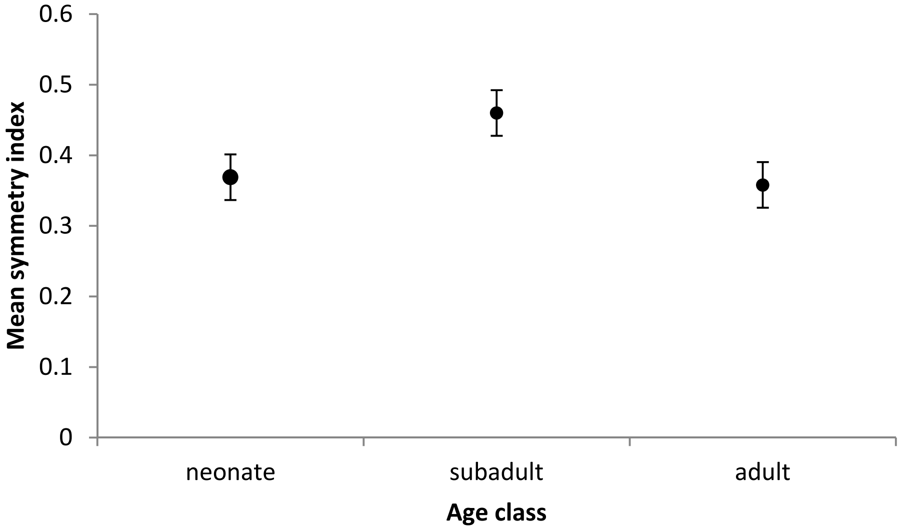


Figure 2. Comparison of mean symmetry index among three different age classes (adult, subadult, neonate) of the pygmy bluetongue lizards.

Chapter 7. General Overview

This thesis had two main aims. The first was to provide insights into the variation of body condition over sampling years and to identify various environmental factors that affected the body condition, as a measure of fitness. The second was to investigate fluctuating asymmetry in head scales of these lizards as an indicator of fitness and to ascertain whether fluctuating asymmetry played a role in the survival of this species. The main goal of this study was to increase our knowledge of the fitness of pygmy bluetongue lizards through variation in their body condition over the study period, so that better management and conservation strategies could be developed to conserve this endangered species.

Chapters 2-4 investigated the variation of body condition in adults and neonate pygmy bluetongue lizards among sampling years and between sexes and regions. It also looked into the effect of climatic factors on the body condition of lizards. Chapter 4 focused mainly on the body condition of neonates and investigated the role of mothers and siblings in the survival of neonates.

Chapters 5-6 focused on the fluctuating asymmetry in the head scales of pygmy bluetongue lizards and its association with body condition in pygmy bluetongue lizards, as the first study on fluctuating asymmetry in pygmy bluetongue lizards. Chapter 5 outlined the details of development of asymmetry index (AI). Chapter six investigated the relationship between AI, body condition, as an indicator of fitness, and jaw width. Chapter 6 explored the symmetry index in head scalation of neonate lizards and its correlation with adult symmetry.

We found significant variation in the body condition of pygmy bluetongue lizards. Sampling year and activity periods were the most important factors affecting the body condition of adults. Neonate lizards also showed significant changes in body condition over three sampling years. The variation in body condition of pygmy bluetongue lizards, although of uncertain cause, provides important information for conservation managers who monitor this population and develop conservation strategies to save this endangered species. Populations with many individuals in poor condition could indicate poor reproductive output; hence the population might be dwindling.

Our results showed that there was a relationship between symmetry index and three indirect fitness measures, body condition, jaw width and activity period (early and late). We did not find a significant correlation between body condition with either jaw width or with the symmetry level for any of the groups of lizards in neither adults nor neonates. Therefore, head scale symmetry in pygmy bluetongue lizards may not be a reliable indicator of fitness in this species. Although, our results did not provide any evidence that survival from neonate to adult was influenced by head scale symmetry, they revealed some valuable insights into ontogenetic changes in symmetry of head scales in pygmy bluetongue lizards.

7.1 Further research

The study and management of endangered populations requires information about the factors affecting the dynamic of the populations. Information on the relative fitness of individuals over time is vital to identify populations in decline. The findings of this thesis have contributed to our knowledge of biology and ecology of pygmy bluetongue lizards and shed light on the condition of this species over time. More generally the detailed study of this endangered species can provide a valuable guide to potential approaches to other endangered species that may be harder to monitor. The impact of changes in climate on survival and persistence can only be determined with longer-term field studies that document annual climatic variation and demographic traits and other associated variables such as food availability over more years than the current study. A future development with practical application for pygmy bluetongue lizards would be to determine any influence of the body condition index as measured in this study, and fitness parameters such as survival and fecundity of the lizards. The results of this study stress the need for monitoring by conservation managers to determine any possible declining trend in fitness of the current populations of pygmy bluetongue lizards. Those monitoring programs may, additionally, detect body condition declines that indicate population sites that can no longer support viable populations, and where translocation of individuals at those sites might be considered.

Supplementary Materials

The following two papers were developed by Damien Tohl and Dr. Jimmy Lee (Supervisor) from the School of Computing Science, Engineering and Mathematics at Flinders University for Tohl's PhD thesis on image analysis. Professor Michael Bull and I co-authored the papers. They are the result of collaboration between our lab and the image processing lab over three years. I provided the data (photos) of pygmy bluetongue lizards and coordinated activities and meetings which resulted in two products; one was the development of Symmetry Index of head scales of lizards (Appendix I) and the second was the development of an automated photo identification technique for pygmy bluetongue lizards (Appendix II).

Appendix I

Image Asymmetry Measurement for the Study of Endangered Pygmy Bluetongue Lizard

Damian Tohl, Jim S. Jimmy Li
School of Computer Science, Engineering & Mathematics
Flinders University
Adelaide, Australia
tohl0003@flinders.edu.au, jimmy.li@flinders.edu.au

Leili Shamiminoori, C. Michael Bull
School of Biological Sciences
Flinders University
Adelaide, Australia
sham1012@flinders.edu.au, michael.bull@flinders.edu.au

***Abstract*— There are applications for the measurement of body asymmetry as some studies have shown a correlation between asymmetry and fitness for some species. In our study of the endangered Pygmy Bluetongue Lizard, the asymmetry of its head is being investigated to see whether this has a correlation with its health and chance of survival in the wild. As there are restrictions on handling the endangered lizards, their digital photos must be taken in the field and therefore it is difficult to impose restrictions on the conditions under which the digital images are acquired. In this paper, we propose a novel automatic technique that is invariant to rotation, size, illumination and tilt, for the measurement of lizard symmetry based on its digital imagery and the resulting symmetry index is used to infer the lizard's asymmetry. The conventional manual methods being used by biologists for fluctuating asymmetry measurement have a number of disadvantages including human errors, and their methods of measurement are based on counting the number of scales and length measurement that do not often agree well with visual assessment. Our proposed image processing technique is non-invasive, robust in a way that will give a similar symmetric index for different images of the same lizard, and more importantly based on the actual image scale pattern of the lizards. Hence our proposed method will also give a better agreement with visual assessment.**

***Index Terms*--Fluctuating Asymmetry, image asymmetry measurement, correlation, mathematical morphology.**

I. INTRODUCTION

The Pygmy Bluetongue Lizard is an endangered species which was thought to be extinct for thirty years. They are found exclusively in remnant fragments of native grassland in South Australia's mid-north [1], [18], [19]. Fluctuating asymmetry is believed to be a valuable indicator of environmental stress on wild populations. It has widely been used as a phenotypic marker of developmental stability; an ability of individuals to undergo identical development on both sides of bilaterally symmetric metrical traits [2]. Asymmetry may be used for monitoring genetic and environmental stress suffered by natural populations [3], [4]. It has been linked to sexual selection and fitness in some taxa [5]. Many studies have shown a correlation between fitness and asymmetry [3], [6], and this correlation has also been shown to be useful in assessing the status of endangered species [7]. Due to the Pygmy Bluetongue Lizards endangered status, a non-invasive method, such as image symmetry measurement, would be preferred.

The current methods for measuring fluctuating asymmetry by biologists involve the measurement of linear lengths of bilateral characters, such as leg length, or more novel characters such as the count of head scales [8], [9], [10], [11]. Both methods have a degree of human error as they require the measurements to be taken manually. They are also invasive as they require a large amount of handling of the lizards, or for the animal to be deceased. Furthermore, measurements such as scale numbers do not always agree with visual perception of asymmetry because the shapes of the scales are not taken into consideration. Our proposed technique is based on the scale pattern of the head, the measurement of which gives a result that better agrees with visual assessment. It is also non-invasive as it is based on the digital images of the lizards and it is an automatic process which eliminates human error.

The lizard, refer to Fig. 1 (a), has a unique scale pattern on the head that displays bilateral symmetry which can be used to give a symmetry measurement which will be used to infer its asymmetry. This is done by finding the line of symmetry in the head of the lizard and comparing the scale pattern on each side of the line of symmetry to obtain a measurement of symmetry. Due to the restrictions on handling the Pygmy Bluetongue Lizard, which is an endangered species, the photos need to be taken in the field and hence it is difficult to impose strict criteria on the method by which the photos are taken. It is therefore necessary that the method proposed is robust and invariant to changes in rotation, size, tilt, and luminance. By automatically detecting the line of symmetry, the method is kept invariant from changes in rotation and scale. As the lizards are alive and their posture cannot be easily manipulated, tilt correction is therefore also required. Edge detection together with morphological filtering is then used to obtain the scale pattern of each lizard while keeping the method invariant to luminance.

978-1-4799-0883-7/13/$31.00

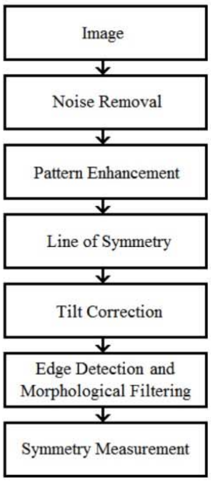


Figure 2. The flow chart of our proposed algorithm for producing image symmetry measurement.

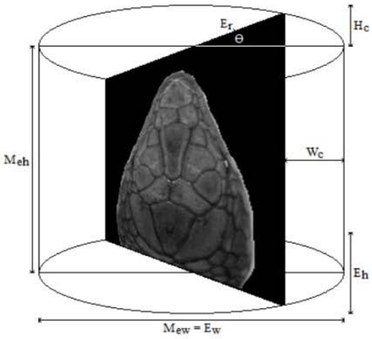


Figure 3. The ellipse used to simulate tilt.

C. *Determining The Line Of Symmetry*

The definition of bilateral symmetry states that the mirror image of an image, M_e, about its symmetrical axis produces an image, M'_e, which is approximately identical to M_e. Therefore the line of symmetry of an image can be found by searching for the orientation that maximises the cross-correlation between the original image and a rotated mirror image of the original image [14].

To find the line of symmetry, the image, M_e, is first reflected about y-axis to produce a mirror image, M'_e. If the line of symmetry of M_e is orientated θ degrees from the vertical, then the line of symmetry of M'_e will be orientated $-\theta$ degrees from vertical. Therefore M'_e would have to be rotated by 2θ degrees for it to have the best cross-correlation with M_e.

To search for the best orientation, θ, of the line of symmetry, M'_e is rotated about the centre of the image and the maximum correlation value, $C(\theta_j)$, form the cross-correlation between M_e and M'_e for each θ_j is recorded. The cross-correlation is performed in the frequency domain, as shown in (3) [14], using the fast Fourier transform (FFT) for improved computational efficiency.

$$C(\theta_j) = max\{F^{-1}(F^*(M_e)rot(2\theta_j,(F(M'_e))))\} \quad (3)$$

Let θ_B, be the angle of the line of symmetry and its optimum value is given by the following equation:

$$\theta_B = \{\theta_j: C(\theta_j) = max\{C(\theta_j)\} \quad (4)$$

After the angle of the line of symmetry is determined, the image is then rotated by $-\theta_B$, such that the line of symmetry is in a vertical position. The horizontal translation offset, t_h, is then evaluated using the phase correlation method [17] and is adjusted accordingly. When calculating the symmetry measurement, it is not necessary to rotate at the centre of the image due to the horizontal translation adjustment that follows.

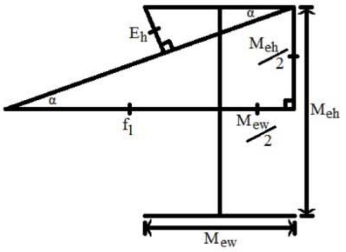


Figure 4. The ellipse height, E_h, is found using the Pythagorean trigonometric identity.

D. *Tilt Correction*

Due to the lizards being live animals, their posture cannot be easily manipulated. Tilt correction is required before symmetry measurement. Refer to Fig. 3, an affine transformation has to be calculated based on the angle of tilt first. The properties of an ellipse are used to estimate the changes in width and height of the image based on the angle of tilt. The width of the ellipse, E_w, is equal to the width of the image, M_{ew}, and the height of the ellipse, E_h, is found with the Pythagorean trigonometric identity shown in Fig. 4 as follows:

$$E_h = M_{ew}cos\alpha \quad (3)$$

$$\alpha = \tan^{-1}(\frac{M_{eh}/2}{f_l + M_{ew}/2}) \quad (4)$$

where M_{eh} is the height of the image and f_l is the focal length of the camera. The height of the ellipse is determined by the distance from which the image is viewed, and the distance from that scene to the sensor is the focal length. Once the width and the height of the ellipse are known, the radius, E_r, can be determined at each angle, θ, of tilt using the following equation:

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1 \tag{5}$$

where

$$a = \frac{E_w}{2}$$

and,

$$b = \frac{E_h}{2}$$

Using the polar coordinates $x = r(\theta)\cos(\theta)$ and $y = r(\theta)\sin(\theta)$ and substituting them into (5) gives the equation for the radius of the ellipse, E_r, as shown below:

$$E_r(\theta) = \frac{ab}{\sqrt{\{(b cos\theta)^2 + (a sin\theta)^2\}}} \tag{6}$$

The change in width, W_c, and height, H_c, needed for the affine transformation at each angle can be found using the Pythagorean trigonometric identity, as the radius of the ellipse is the hypotenuse, W_c is the adjacent edge, and H_c is the opposite edge. The equations for W_c and H_c are then given as follows:

$$W_c = \left(\frac{M_{ew}}{2}\right) - E_r cos\theta \tag{7}$$

$$H_c = E_r sin\theta \tag{8}$$

To determine the tilt angle for correction, the image is tilted through various degrees. The image is divided into its left and right halves, M_L and M_R respectively. The left half of the image is mirrored and then cross-correlated with the right half and the maximum correlation value for each angle is recorded. The angle with the highest correlation value is deemed as the angle for tilt correction. The process described above is tilt correction for the horizontal plane and is then repeated similarly for the vertical plane. An example of tilt correction is shown in Fig. 5, where it can be seen in Fig. 5 (b) that that scale pattern appears to be flatter.

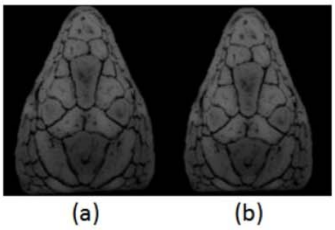


Figure 5. (a) The input image, (b) The tilt corrected image.

E. Edge Detection and Morphological Filtering

Prior to symmetry measurement, edge detection is performed on the pattern enhanced image produced by the enhancement methods described previously to avoid missing weak edges which is important to improve the robustness in the symmetry measurement. Edge detection is used to extract the scale pattern of each lizard to a binary image so that the symmetry rating can be evaluated. The Canny [15], Laplacian, and Sobel edge detection methods were each assessed and it was found through visual inspection that the Canny method provided best edge detection with less unwanted features in the scale pattern. Fig. 6 (a) and (b) show the outputs after edge detection is performed using the Canny method on the original and enhanced image respectively. It is obvious that major scale patterns are preserved in Fig 6 (b) while unwanted features are removed.

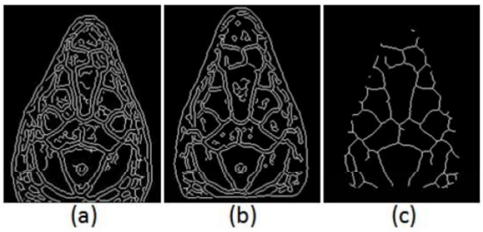


Figure 6. (a) The Canny edge detection output on the original image, (b) the Canny edge detection output on the enhanced image, (c) The final binary image output, M_{10}.

In addition to edge detection, a series of morphological operations are used to strengthen the connecting edges of the scale pattern and remove unwanted features in order to produce a binary image that will give a robust symmetry rating. Dilation, '$\oplus$', is first performed on the edge image, M_1, given by (9), to connect broken edges.

$$M_2 = M_1 \oplus A^s, \text{ where}$$
$$A^s = \{010; 111; 010\} \tag{9}$$

To remove unwanted features from M_2, any connected components with 4-edge connectivity are found, and any components with a size less than the mean size of all the connected components are considered as unwanted features and will be removed to produce, M_3. A logical 'and' is then performed in (10) on the resulting image and the original edge image, M_1, to recover the original edge detection image without the unwanted features, M_4.

$$M_4 = M_3 \bullet M_1 \tag{10}$$

A morphological closing is performed on M_4 in (11) to connect the edges and the 8-edge connected components are found, any components with a size less than the mean size of all the connected components are removed. This is done so that any unwanted features still remaining are removed to produce M_6. A logical 'and' is then performed with the original edge image, M_1, to recover the original edge detection image with all the unwanted features removed to give, M_7 as follows:

$$M_5 = (M_4 \oplus A^s) \ominus A \tag{11}$$

$$S_7 = S_6 \bullet S_1 \tag{12}$$

Another closing is then performed with a larger structuring element in (13) to ensure the edges that form the scale pattern are all connected. A morphological thinning operation, T, [16] is then applied to reduce line thickness in M_8 to a single pixel width producing the image, M_9.

$$M_8 = (M_7 \oplus \mathrm{B}^{\mathrm{s}})\Theta B, \text{ where}$$
$$B^s = \{11111; 11111; 11111; 11111; 11111\} \quad (13)$$

$$M_9 = T\{M_8\} \quad (14)$$

As the scale pattern of the lizard is the major feature for symmetry measurement, the outlier of the lizard has to be removed prior to symmetry measurement using an image screen. A thresholded image, M_o, is first produced by thresholding the original greyscale image with any pixel value greater than zero. A logical 'and' operation is applied to the mirror image of M_o, M_o', with M_o to create a perfectly symmetric image template. This template is then morphologically eroded to produce an image screen, M_s, to exclude the shadow regions and the lizard outlier as follows.

$$M_s = (M_o \bullet M_o')\Theta \mathrm{A}^{\mathrm{s}} \quad (15)$$

The image screen is then applied to M_9 for the extraction of the scale pattern using (15) as shown in Fig. 6 (c).

$$M_{10} = M_9 \bullet \mathrm{M}_{\mathrm{s}} \quad (16)$$

The image symmetry rating is based on the correlation between the left and right half of the image, M_L and M_R, respectively. The two halves of the image are defined using the line of symmetry found in section C as the dividing plane. To maximise the range of the rating, and to reduce errors associated with the reflection axis, the mirror image of one of the halves is first aligned with the other half using the phase correlation method [17].

F. Symmetry Rating

To calculate the final symmetry rating, the normalized correlation between the left and right image halves, namely M_L and M_R, is evaluated by (17) to be used as a symmetry index. A lower correlation value between the left and right images implies greater asymmetry of the lizard. For perfect symmetry, the normalized correlation equation (17) will give a value of unity.

$$S = \frac{\sum_{x,y}[M_L(x,y) - \overline{M_L}][M_R(x,y) - \overline{M_R}]}{\sqrt{\sum_{x,y}[M_L(x,y) - \overline{M_L}]^2 \sum_{x,y}[M_R(x,y) - \overline{M_R}]^2}} \quad (17)$$

where $\overline{M_L}$ and $\overline{M_R}$ are the mean values of the left and right image halves respectively.

III. RESULTS

Samples of the images used for the image symmetry measurement are shown in Fig. 7, they are ranked in order from least symmetric to most symmetric. Included amongst these samples are multiple images of the same lizard. Table I gives the symmetry measurement for each lizard. As an example in Table I, images (j) and (k) having the same code 2224 are different images of the same lizard coded 2224. The symmetry measurement is given to four decimal places for the assessment of the robustness of the technique in the comparison of different images of the same lizard. Whilst there are other methods for measuring asymmetry which involve the measurement of linear lengths of bilateral characters, such as leg length, or other characters such as the count of head scales, these approaches require manipulation of the species and are therefore not applicable to the endangered pygmy bluetongue lizards due to their conservation status. Furthermore other techniques being used by the biologists are measurements based on the count or linear lengths of bilateral characters, and they do not always agree with visual assessment. For the symmetry measurement using different images of the same lizard, the largest standard deviation of errors from the values in Table II is only 0.0060. The number of images for the same lizard, *n*, in Table II is restricted by the lizards' availability in the wild at different periods. The proposed method has also been verified visually by a number of experts by comparing the images with their symmetric indices to confirm that the image symmetry measurement has good consistency with visual assessment.

IV. CONCLUSION

Image asymmetry measurement inferred by a symmetry index for the study of endangered Pygmy Bluetongue Lizard has been developed and found to have very good consistency with visual assessment for the endangered Pygmy Bluetongue Lizards in our experiments. This measurement will be used for the study of how asymmetry in lizards correlates to its health and chance of survival in the wild. Future work will include a quality index for the exclusion of poor quality images in order to give an indication of the accuracy and to improve the robustness of asymmetry measurement.

REFERENCES

[1] J.S. Jimmy Li, D. Tohl S. Randhawa, L. Shamimi, C.M. Bull, "Non-invasive Lizard Identification using Signature Curves," 2009 IEEE Region 10 Conference (TENCON 2009), pp. 1-5, 2009 .

[2] A.R. Palmer, C. Strobeck, "Fluctuating asymmetry analyses revisited. In: Polak, M. (ed) Developmental instability: causes and consequences," (Oxford University Press: New York), pp. 279-319, 2003.

[3] R.F. Leary, F.W. Allendorf, "Fluctuating asymmetry as an indicator of stress: implications for conservation biology," Trends in Ecology & Evolution, vol. 4, pp. 214-217, 1989.

[4] P.A. Parsons, "Fluctuating asymmetry: a biological monitor of environmental and genomic stress," Heredity, vol. 68, pp. 361-364, 1992.

[5] A.P. Moller, "Developmental stability and fitness," American Naturalists, vol. 149, pp. 916-932, 1997.

[6] S. Sarre, J.M. Dearn, A. Georges, "The application of fluctuating asymmetry in the monitoring of animal populations," Pacific Conservation Biology, vol. 1, pp. 118-122, 1994.

[7] R.F. Leary, F.W. Allendorf, "Fluctuating asymmetry as an indicator of stress: implications for conservation biology," Trends in Ecology and Evolution, vol. 4, pp. 214-216, 1989.

[8] B. Verust, S. Van Dongen, I. Grbac, R. Van Damme, "Fluctuating asymmetry, physiological performance, and stress in island populations of the Italian Wall lizard (Podarcis sicula)," Journal of Herpetology, vol. 42, pp. 369-377, 2008.

[9] P. Lopez, J. Martin, "Locomotor capacity and dominance in male lizards Lacerta monticola: a trade-off between survival and reproductive success?," Biological Journal of the Linnean Society, vol. 77, pp. 201-209, 2002.

[10] A.K. Davis, J.C. Maerz, “Spot symmetry predicts body condition in spotted salamanders, Ambystoma maculatum,” Applied Herpetology, vol. 4, pp. 195-205, 2007.

[11] P. Lopez, A. Munoz, J. Martin, “Symmetry, male dominance and female mat preferences in the Iberian rock lizard, Lacerta monticola,” Behav Ecol Sociobiol, vol. 52, pp. 342-347, 2002.

[12] J.S.J. Li, “A class of multi-shell min/max median filters,” IEEE International Symposium onCircuits and Systems Proceedings, vol. 1 of 3, pp. 421-424, 1989.

[13] J.S.J. Li, A. Ramsingh, “The relationship of the multi-shell to multistage and standard median filters,” IEEE Transactions on Image Processing, vol. 4, no. 8, pp. 1165-1169, 1995.

[14] Y. Liu, R.T. Collins, W.E. Rothfus, “Automatic extraction of the central symmetry (mid-sagittal) plane from neuroradiology images,” 1996.http://vision.cse.psu.edu/people/chenpingY/paper/liu_yanxi_1996_1.pdf

[15] J. Canny, “A computational approach to edge detection,” IEEE Transactions on Pattern Analysis and Machine Intelligence, vol. PAMI-8, no.6, pp. 679-698, 1986.

[16] L. Lam, L. Seong-Whan, Y.S. Ching, "Thinning Methodologies-A Comprehensive Survey," IEEE Transactions on Pattern Analysis and Machine Intelligence, vol. 14, no. 9, page 879, 1992.

[17] B.S. Reddy, B.N. Chatterji, “An FFT-based technique for translation, rotation, and scale-invariant image registration”, IEEE Transactions on Image Processing 5, no. 8 pp 1266–1271, 1996.

[18] E.J. Staugas, A. Fenner, M. Ebrahimi, C.M. Bull, “Artificial burrows with basal chambers are preferred by pygmy bluetongue lizards, Tiliqua adelaidensis” Amphibia-Reptilia, vol. 34(1), pp.114-118, 2013.

[19] J. Schofield, M.G. Gardner, A. Fenner C.M. Bull, “Promiscuous mating in the endangered Australian lizard Tiliqua adelaidensis: a potential windfall for its conservation,” *Conservation Genetics,* 2013.

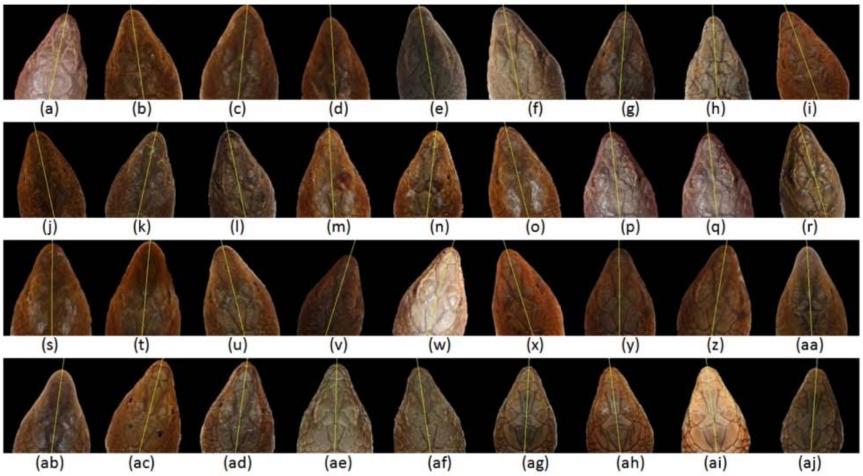


Figure 7. The lizard images used for image symmetry measurement with the line of symmetry generated by our proposed algorithm.

TABLE I. THE SYMMETRY MEASUREMENTS FOR THE IMAGES SHOWN IN FIG. 7.

Image	11067 (a)	11071 (b)	21075 (c)	21075 (d)	11071 (e)	21075 (f)	21062 (g)	21062 (h)	21062 (i)	2224 (j)	2224 (k)	11072 (l)
Symmetry	0.1813	0.2136	0.2142	0.2180	0.2183	0.2199	0.2502	0.2530	0.2617	0.2801	0.2833	0.2910
Image	11072 (m)	11072 (n)	11070 (o)	1183 (p)	1183 (q)	11070 (r)	11070 (s)	11070 (t)	11069 (u)	11070 (v)	11069 (w)	21063 (x)
Symmetry	0.2912	0.2969	0.3016	0.3040	0.3047	0.3051	0.3091	0.3092	0.3093	0.3133	0.3135	0.3310
Image	21063 (y)	21063 (z)	21065 (aa)	21065 (ab)	150 (ac)	150 (ad)	21007 (ae)	21007 (af)	21006 (ag)	21004 (ah)	21015 (ai)	2701 (aj)
Symmetry	0.3368	0.3395	0.3692	0.3715	0.4910	0.4962	0.5275	0.5322	0.6896	0.7223	0.8246	0.9010

TABLE II. THE MEAN AND STANDARD DEVIATIONS FOR DIFFERENT IMAGES OF THE SAME LIZARD.

Image Group	11071	21062	21075	2224	11072	1183	11070	11069	21063	21065	150	21007
No. of images (n)	2	3	3	2	3	2	5	2	3	2	2	2
Mean	0.2160	0.2550	0.2817	0.2817	0.2930	0.3044	0.3077	0.3114	0.3358	0.3704	0.4936	0.5299
Standard Deviation	0.0033	0.0060	0.0029	0.0023	0.0034	0.0005	0.0045	0.0030	0.0043	0.0016	0.0037	0.0033

Appendix II

Non-invasive Lizard Identification using Signature Curves

Jim S. Jimmy Li, Damian Tohl, Sharmil Randhawa
School of Computer Science, Engineering & Mathematics
Flinders University
Adelaide, Australia
jimmy.li@flinders.edu.au, tohl0003@flinders.edu.au,
sharmil.randhawa@flinders.edu.au

Leili Shamimi, C. Michael Bull
School of Biological Sciences
Flinders University
Adelaide, Australia
sham1012@flinders.edu.au, michael.bull@flinders.edu.au

***Abstract*— A novel non-invasive method is proposed to help identify the endangered Pygmy Bluetongue Lizard. This would be preferable to the commonly used, invasive, toe-clipping method, which could be unreliable if the lizard was to lose a toe or foot naturally. Each lizard has a unique and permanent scale pattern which can be used to identify individual lizards. The proposed method involves a novel technique to derive a signature curve from the Hough transform output of the lizard scale pattern such that the signature curve is scale and shift invariant. It has been found that a lizard can be successfully identified by comparing its signature curve with those signature curves of all registered lizards stored in a database.**

Keywords-lizard identification; Hough transform; signature curve; non-invasive

I. INTRODUCTION

The Pygmy Bluetongue Lizard is an endangered species which was thought to be extinct for thirty years. They are found exclusively in remnant fragments of native grassland in South Australia's mid-north. Recognition of individual lizards is essential for ecological studies; therefore a method for identifying each lizard is required. One common method for this is toe clipping, where digits are removed from the feet of the lizards, but this is a highly invasive method. Moreover, natural toe and foot loss is observed in lizards in nature [1] which can impact on the accuracy of the method. Due to the Pygmy Bluetongue Lizards endangered status, a non-invasive identification method, such as photo identification, would be preferred. To our knowledge, the method of photo identification for lizards has not been developed elsewhere.

The unique scale pattern of each lizard is used to identify individual lizards. Edge detection is used to obtain the scale pattern of each lizard while keeping the method independent from variations in luminance. The Hough transform is then used to convert the pattern of each individual lizard to a parameter space matrix. Due to the fact that digital images of the lizards are being used, and it is difficult to maintain the conditions to produce images of exactly the same size with the lizard in the same position, shift and scale invariance is desired in order to make the identification process robust. As the Hough transform is unable to provide shift and scale invariance, a novel technique has been developed to derive a signature curve which provides shift and scale invariance from the Hough transform output.

The signature curves for all registered lizards are stored in a database. When an unknown lizard is to be identified, its signature curve is produced and compared with the signature curves stored in the database. The Mean Square Error (MSE), which is obtained by finding the difference between two signature curves, is used to find a match between two lizards. This is done by comparing the MSE with a threshold value that is required for determining matches. If it is below the threshold value, a match for the unknown lizard is found. Otherwise no match is implied and that means the unknown lizard has not been registered in the database. The threshold value will be discussed in the Results Section.

The organisation of this paper is as follows. In Section II, the lizard identification method is described, including how independence from changes in luminance is achieved through the extraction of the scale pattern using edge detection. The section also describes how the Hough transform is used to detect lines of the scale pattern in the input image and in turn how a signature curve is derived from the Hough transform output. Experimental results are presented in Section III, and Section IV gives the conclusion.

II. IDENTIFICATION METHOD

The input is a digital image of the lizard's head which has been taken in such a way that the head is positioned with the tip of the snout pointing straight up. The identification method has four stages; first an input image has its luminance adjusted by contrast-limited adaptive histogram equalization so the identification method becomes independent of the intensity of the image. In this stage, median filtering is also applied to remove any noise present to produce a clean image for edge detection. In the second stage, edge detection is performed on the adaptive histogram equalised grayscale image so that the unique scale pattern edges of each lizard are extracted. The edge detection image is required for the Hough transform to produce an output in the third stage. The fourth stage describes how a signature curve is derived from the Hough transform output, such that it is scale and shift invariant with respect to the input image.

978-1-4244-4547-9/09/$26.00 ©2009 IEEE

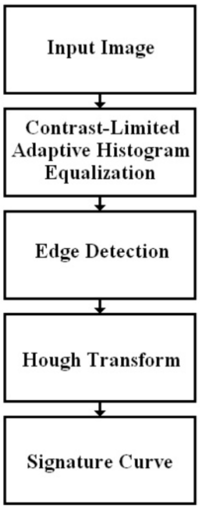


Figure 1. Block diagram of the method to produce a signature curve

A block diagram of the method to produce a signature curve is shown in Fig. 1.

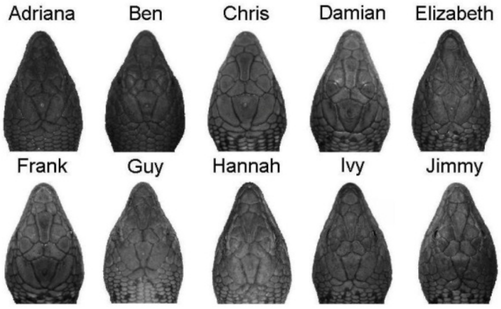


Figure 2. The grayscale images of 10 lizards.

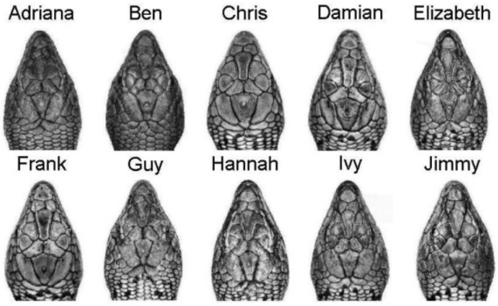


Figure 3. The images of the 10 lizards from Fig. 2 after contrast-limited adaptive histogram equalization has been performed.

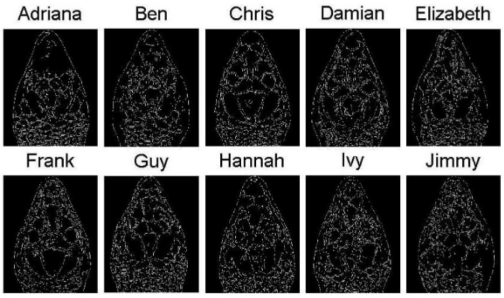


Figure 4. The images of the 10 lizards after edge detection has been performed.

A. Contrast-limited Adaptive Histogram Equalization

Consistent lighting conditions are difficult to maintain when taking the images of the lizards. Hence identification of the lizards needs to be independent from the variation of luminance between images. Fig. 2 shows the grayscale images of 10 lizards where the difference in illuminations can be seen to have had an effect on the images.

Contrast-limited adaptive histogram equalization (CLAHE) [2] is used to equalize the brightness of each image so that identification of the lizards is independent from the variation of luminance between images. The results after applying CLAHE on the images in Fig. 2 can be seen in Fig.3. CLAHE enhances the contrast of images by transforming the values in the intensity image so that the histogram of the output region approximately matches the uniform histogram.

B. Edge Detection

Edge detection is used to extract the scale pattern of each lizard to a binary image that the Hough transform can be performed on. For identification, it is desirable to maximize the minimum MSE error between the signature curves of a pair of lizards. The Canny [3], Laplacian, and Sobel edge detection methods were each evaluated to determine which provided the highest minimum MSE. It was found that the Canny edge detection method provided the largest minimum MSE between lizards as shown in the Results Section. Fig.4 shows the output from the same 10 lizards from Fig.2 and Fig.3 after edge detection is performed using the Canny method.

The Canny method [3] of edge detection finds edges by looking for local maxima of the gradient of the image. The gradient is calculated using the derivative of a Gaussian filter. The method uses two thresholds, to detect strong and weak edges, and includes the weak edges in the output only if they are connected to strong edges.

C. Pattern Recognition

Our pattern recognition technique is based on the number of edge lines having a particular curvature angle within the pattern. Two patterns are deemed to be identical if both patterns have the same number of lines at every curvature angle. As the method depends only the curvature angles, but not on the size and the relative position of features within the image, this comparison method between two patterns is scale and shift invariant. Our proposed signature curve, described in Section C.2, will embody all the required information extracted from the corresponding Hough transform output.

C.1 Hough Transform

The Hough transform [4] is used to detect lines at different angles by using the parametric representation of a line.

$$s = x\cos\theta + y\sin\theta \qquad (1)$$

where the variable *s* is the distance from the origin to the line along a vector perpendicular to the line, and θ is the angle between the x-axis and this vector. The Hough transform output, *H*, relates to a parameter space matrix whose rows and columns correspond to *s* and θ values respectively. The elements in the Hough transform output represent accumulator cells. Peak values in the Hough transform output represent the length of the potential lines in the input image.

C.2 Signature Curves

In order to make the identification process of the lizards more robust, shift and scale invariance are both desired properties. The Hough transform output by itself is not shift or scale invariant, hence the information contained in the Hough transform output needs to be extracted in such a way as to produce a signature curve that is both shift and scale invariant.

Let $H(\theta,s)$ be the length of a line at angle θ and distance *s* from the origin, and $v(\theta)$ be the magnitude of the signature curve at angle θ. Let *f* be an indicator function such that

$$f(x) = \begin{cases} 1 & x \text{ is true} \\ 0 & x \text{ is false} \end{cases} \qquad (2)$$

The signature curve *v* is defined as follows:

$$v(\theta) = \sum_{s=0}^{D} f(H(\theta,s) > \delta) \qquad (3)$$

where *D* is the maximum value of *s* and δ is a threshold value for which only lines of length above this value will be considered. For clean images with high signal-to-noise ratio (SNR), setting δ to zero is adequate, i.e. all lines are included.

Since $v(\theta)$ is no longer a function of *s*, shift invariance is achieved because it does not depend on the perpendicular distance of lines from the origin.

TABLE I. THE MINIMUM MEAN SQUARE ERROR VALUE FOR THE DIFFERENCE BETWEEN ANY TWO SIGNATURE CURVES FOR ALL THE TEN LIZARDS USING DIFFERENT EDGE DETECTION METHODS.

	Canny	**Laplacian**	**Sobel**
Min. MSE	$8.67\text{x}10^{-10}$	$7.97\text{x}10^{-10}$	$8.47\text{x}10^{-10}$

To achieve scale invariance, the indicator function in (2) is applied to $H(\theta,s)$, so that the actual length of lines is irrelevant, as we are only interested in whether a line at that position exists. Hence $v(\theta)$ will not depend on the scale of the input image. The signature curve is then normalized for comparison between different lizards.

Each lizard produces a unique signature curve as shown in Fig.9. In this way, the signature curve can be used to identify each individual lizard. If the MSE of the difference between the signature curves of two lizards is below a certain threshold, a match is deemed to be found. Otherwise no match is found, and the lizard's signature curve will be added to the database as a new lizard.

III. RESULTS

A. Edge Detection

In order to evaluate the performance of various edge detection methods for our application, we investigated which methods would produce a maximum discrimination between lizards, in terms of the MSE of the difference between signature curves. The total combination of any two out of ten lizards is ${}_{10}C_2 = 45$, and the minimum MSE of these 45 combinations of differences between a pair of signature curves is tabulated in Table I for the three edge detection methods, namely Canny, Laplacian and Sobel.

It can be seen from Table I that the Canny edge detection method gives the largest minimum MSE value, which was found to be $8.67\text{x}10^{-10}$. In other words, the Canny edge detection will give the maximum discrimination between lizards for our application.

B. The Criterion for Matching Lizards

The minimum MSE value for the difference between signature curves for all the possible combinations of 10 lizards was found to be $8.67\text{x}10^{-10}$, as seen in Table I. This is also the threshold value used to determine whether a match between two lizards has been found. In order for a correct match to be obtained, the errors arising from differences in shift, scale, and changes in illumination in the original image need to be below this threshold value. If an MSE obtained by comparing the signature curve of an unknown lizard with every signature curve stored in the database is below this threshold value, a match with a registered lizard in the database is found. If the MSE is above this value, no match is found, i.e. the unknown lizard is not in the database.

Fig.5 shows three different images of the lizard Ivy; the first image is the original, the second has been shifted right by 12.5% and scaled down by 25% and the third has been shifted left by 12.5% and scaled up by 25%, with respect to the original image. The signature curves for the three images are shown in Fig.6, where the maximum MSE value that is produced by these shifts and scale variations is $1.69\text{x}10^{-10}$. From this example it is shown that both changes in the shift and scale of an image of the same lizard have only a small effect on the signature curve, and the MSE produced by these changes is well below the threshold for a match.

To illustrate the robustness of the method for variations in illuminations, three images of different illuminations of the lizard Ivy are shown in Fig.7. The signature curves derived from the three images are shown in Fig.8, where the maximum MSE value that is produced by these changes in illumination is 2.71×10^{-10}. From this experimental result, it is shown that maximum difference in the signature curves due to various illumination is also well below the threshold for a match.

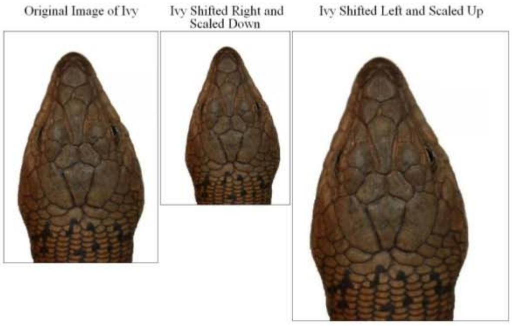


Figure 5. The three images of Ivy with different shifts and scale sizes used to create the signature curves in Fig. 6.

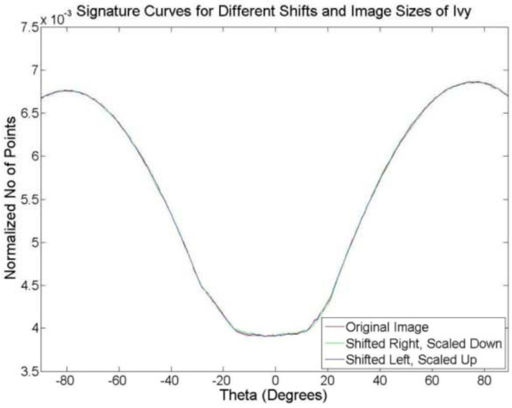


Figure 6. Signature Curves for different shifts and image sizes of the lizard Ivy.

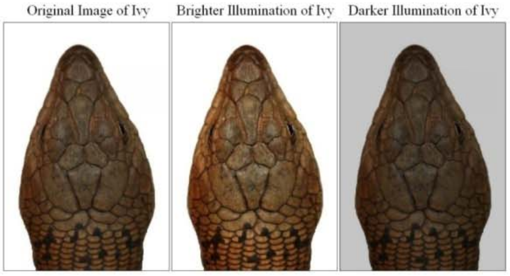


Figure 7. The three images of Ivy with different illuminations used to create the signature curves in Fig. 8

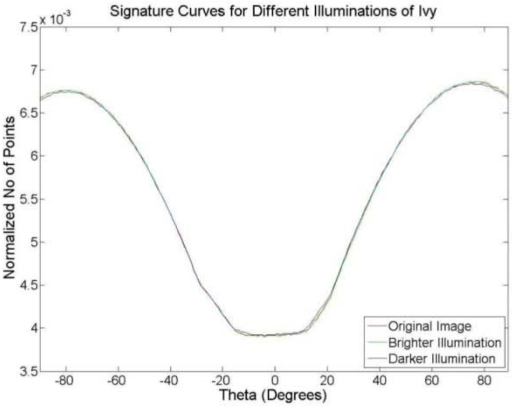


Figure 8. Signature Curves for different illuminations of the lizard Ivy.

These experimental results have shown that the threshold value used to determine whether there is a match between two lizards was found to be 8.67×10^{-10}. The results have also shown the signature curve is invariant to changes in both shift and scale of the original image, as well as being independent from changes in illumination. The MSE produced by these changes has been shown to be sufficiently small enough that they will not affect the identification of lizards based upon the above threshold value.

IV. CONCLUSION

Photo identification for the Pygmy Bluetongue Lizard has been developed and found to be successful for the lizards that have been included in our experiments. Future work will include the extension of the proposed method for use with more lizards whose images will be taken during the next field test. An angle realignment method is also being developed based on the signature curve in order to make the identification process more robust to rotations. The signature curve is also being investigated to see if the symmetry of the curve can help quantify the symmetrical properties of the scale pattern of each lizard.

REFERENCES

[1] Hudson, S., "Natural toe loss in southeastern Australian skinks: implications for marking lizards by toeclipping." *Journal of Herpetology,* No. 30, pp. 106-110, 1996.

[2] K. Zuiderweld, "Contrast Limited Adaptive Histogram Equalisation." *Graphics gem IV*, Academic Press Professional Inc., pp 474-485, 1994.

[3] J. Canny, "A Computational Approach to Edge Detection," *IEEE Transactions on Pattern Analysis and Machine Intelligence,* Vol. PAMI-8, No. 6, pp. 679-698, 1986.

[4] M. Sonka, V. Hlavac and R. Boyle, *Image Processing, Analysis, and Machine Vision*, PWS Publishing, 1999.

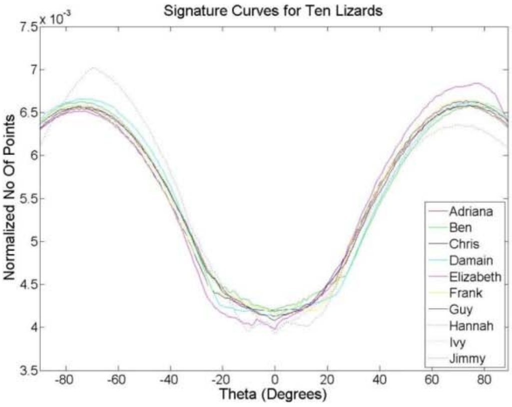


Figure 9. Signature Curves for ten different lizards.

References

Alford, R.A., Bradfield, K.S., and Richards, S. (1999) Measuring and analysing developmental instability as a tool for monitoring frog populations. Environment Australia, Canberra.

Alford, R.A., Bradfield, K.S., and Richards, S.J. (1997) Increasing fluctuating asymmetry precedes frog population declines. In 'Third World Congress of Herpetology Vol. Abstract of talk presented at the Declining Amphibian Symposium.' Prague)

Anciaes, M., and Marini, M.A. (2000) The effects of fragmentation on fluctuating asymmetry in passerine birds of Brazilian tropical forests. *Journal of Applied Ecology* **37**, 1013-1028.

Aparicio, J.M. (1998) Patterns of fluctuating asymmetry in developing primary feathers: a test of the compensational growth hypothesis. *Proceedings of The Royal Society of London B* **265**, 2353-2357.

Aparicio, J.M. (2001) Patterns of growth and fluctuating asymmety: the effects of asymmetrical investment in traits with dominate growth. *Behavioral Ecology and Sociobiology* **49**, 273-282.

Aparicio, J.M., and Bonal, R. (2002) Why do some traits show higher fluctuating asymmetry than others? A test of hypotheses with tail feathers of birds. *Heredity* **89**, 139-144.

Ardovini, A., Cinque, L., and Sangineto, E. (2008) Identifying elephant photos by multi-curve matching. *Pattern Recognition* **41**, 1867-1877.

Armstrong, G., and Reid, J. (1992) The re-discovery of the Adelaide pygmy bluetongue, Tiliqua adelaidensis (Peters, 1863). *Herpetofauna* **22**, 3-6.

Armstrong, G., Reid, J., and Hutchinson, M.N. (1993) Discovery of a population of the rare scincid lizard *Tiliqua adelaidensis* (Peters). *Rec. S. A. Mus.* **36**, 153-155.

Aubret, F., Bonnet, X., Shine, R., and Lourdais, O. (2002) Fat is sexy for females but not males: the influence of body reserves on reproduction in snakes (*Vipera aspis*). *Hormones and Behavior* **42**, 135-147.

Badyaev, A., Forseman, K.R., and Fernandes, M.V. (2000) Stress and developmental stability: vegetation removal causes increased fluctuating asymmetry in shrews. *Ecology* **81**, 336-345.

Banko, P.C., Oboyski, P.T., Slotterback, J.W., Dougill, S.J., Goltz, D.M., Johnson, L., Laut, M.E., and Murray, C. (2002) Availability of food resources, distribution of invasive species, and conservation of a Hawaiian bird along a gradient of elevation. *Journal of Biogeography* **29**, 789-808.

Bjorksten, T.A., David, P., Pomiankowski, A., and Fowler, K. (2000) Fluctuating asymmetry of sexual and nonsexual traits in developmental stress and genetic quality. *Journal of Evolutionary Biology* **13**, 89-97.

Bjorksten, T.A., Fowler, K., and Pomiankowski, A. (2000) What does sexual trait FA tell us about stress? *TREE* **15**, 163-166.

Boersma, P.D. (1998) Population trends of the galapagos penguins: impacts of El Nino and La Nina *Condor* **100**, 245-253.

Bonnet, X., and Naulleau, G. (1994) Use of a body condition index (BCI) for the study of the reproduction in snakes. *Compte rendus de l'Academie des Sciences III* **317**, 34-41.

Bonnet, X., and Naulleau, G. Estimation of body reserves in living snakes using body condition index (BCI). In 'Scientia Herpologica', 1995. (Eds. GS Loirebte, A Montori, X Santos and MA Carretero), pp. 237-240

Bradshaw, C.J.A., Davis, L.S., Lalas, C., and Harcourt, R.G. (2000) Geographic and temporal variation in the condition of pups of the New Zealand fur seal (*Arctocephalus foresteri*): evidence for density dependence and differences in the marine environment. *Journal of Zoology* **252**, 41-51.

Brown, C.R., and Brown, M.B. (1998) Intense natural selection on body size and winge and tail asymmetry in cliff swallows during severe weather. *Evolution* **52**, 1461-1475.

Bull, C.M., and Pamula, Y. (1996) Sexually dimorphic head sizes and reproductive success in the sleepy lizard *Tiliqua rugosa*. *Journal of Zoology* **240**, 511-521.

Carter, S.I. (1997) 'The habitat ecology of bog turtles (*Clemmys muhlenbergii)* in southwestern Virginia ' (Blacksburg, VA: Virginia Polytechnic Institute and State University)

Chapple, D.G. (2003) Ecology, life history, and behaviour in the Australian scincid genus, *Egernia*, with comments on the evolution of complex sociality in lizards. *Herpetological Monographs* **17**, 145-180.

Chippendale, A.K., and Palmer, A.R. (1993) Persistence of subtle departures of symmetry over multiple molts in individual Brachyuran crab: relevance to developmental stability. *Genetica* **89**, 185-199.

Clarke, G.M. (1995) Relationships between developmental stability and fitness- application for conservation biology. *Conservation Biology* **9**, 18-24.

Clarke, G.M. (1998) The genetic basis of developmental stability. IV. Individual and population asymmetry parameters. *Heredity* **80**, 553-561.

Clarke, G.M., and McKenzie, J.A. (1992) The use of fluctuating asymmetry as a quality control indicator for insect mass rearing processes. *Journal of Economic entomology* **85**, 2045-2050.

Coates, P.S., Wylie, G.D., Halstead, B.J., and Casazza, M.L. (2009) Using time-dependent models to investigate body condition and growth rate of the giant gartersnake. *Journal of Zoology* **279**, 285-293.

Cogger, H.G. (1992) 'Reptiles and Amphibians of Australia. .' 5th edn. (Reed Books: Sydney)

Connolly, J.D., and Cree, A. (2008) Risks of late start to captive management for conservation: phenotypic differences between wild and captive individuals of a viviparous endangered skink (*Oligosoma otagense*). *Biological Conservation* **141**, 1283-1292.

Daugherty, C.H., Gibbs, G.W., and Hitchmough, R.A. (1993) Mega-island or micro-continenet? New Zealand and its fauna. *TREE* **8**, 437-442.

Dawson, T.P., Jackson, S.T., House, J.I., Prentice, I.C., and Mace, G.M. (2011) Beyond predictions: Biodiversity conservation in a changing climate. *Science* **332**, 53-58.

Downes, S.J., and Shine, R. (1999) Do incubation-induced changes in lizard's phenotype influence its vulnerability to predators? *Oecologia* **120**, 9-18.

Drake, D.R., Mulder, C.P.H., Towns, D.R., and Daugherty, C.H. (2002) The biology of insularity: an introduction. *Journal of Biogeography* **29**, 563-569.

Duffy, A., Pound, L., and How, T. (2012) Recovery plan for the pygmy bluetongue lizard *Tiliqua adelaidensi.* Department of Environment and Natural Resources, South Australia.

Dufour, K.W., and Weatherhead, P.J. (1998a) Bilateral symmetry and social dominance in captive male red-winged blackbirds. *Behavioural Ecology and Sociobiology* **42**, 71-76.

Dufour, K.W., and Weatherhead, P.J. (1998b) Reproductive consequences of bilateral asymmetry for individual male red-winged blackbirds. . *Behavioral Ecology* **9**, 232-242.

Ebrahimi, M. (2013) Optimising translocation strategies in the conservation management of reptiles; a case study of an endangered Australian skink, *Tiliqua adelaidensis*

PhD Thesis, Flinders University, Adelaide

Ehmann, H. (1982) The natural histroy and conservation staus of the Adelaide pygmy bluetongue, *Tiliqua adelaidensis. Herpetofauna* **14**, 61-76.

Emlen, J.M., Freeman, D.C., and Graham, J.H. (1993) Nonlinear growth dynamics and the origin of fluctuating asymmetry. *Genetica* **89**, 77-96.

Endler, J.A. (1986) 'Natural selection in the wild.' (Princeton University Press: Princeton, New Jersey)

Fahrig, L. (2003) Effects of habitat fragmentatio on biodiversity. *Annual Review of Ecology, Evolution, and Systematics* **34**, 487-515.

Federman, S., Hyseni, C., Clement, W., Oatham, M.P., and Caccone, A. (2014) Habitat fragmentation and genetic structure of the Amazonian palm *Mauritia flexuosa* L.f. (Arecaceae) on the island of Trinidad. *Conservation Genetics* **15**, 355-362.

Fellows, H.L., Fenner, A.L., and Bull, C.M. (2009) Spiders provide important resources for an endangered lizard. *Journal of Zoology* **279**, 156-163.

Fenner, A.L. (2009) The biology, ecology and social structuring of the endangered pygmy bluetongue lizard, *Tiliqua adelaidensis:* considerations for long term conservation. Ph.D. Thesis., Flinders Unversity Adelaide

Fenner, A.L., and Bull, C.M. (2007) Short-term impact of grassland fire on the endangered pygmy bluetongue lizard. *Journal of Zoology* **272**, 444-450.

Fenner, A.L., and Bull, C.M. (2010) The use of scats as social signals in a solitary, endangered scincid lizard, *Tiliqua adelaidensis*. *Wildlife Research* **37**, 582-587.

Fenner, A.L., and Bull, C.M. (2011) Responses of the endangered pygmy bluetongue lizard to conspecific scats. *Journal of Ethology* **29**, 69-77.

Fenner, A.L., Schofield, J.A., Smith, A.L., and Bull, C.M. (2008) Observations of snake predation on the pygmy bluetongue lizard, *Tiliqua adelaidensis. Herpetofauna* **35**, 105-109.

Ferguson, G.W., and Fox, S.F. (1984) Annual variation of survival advantage of large juvenile side-blotched lizard, *Uta stansburiana*: its causes of evolutionary significance. *Evolution* **38**, 342-349.

Gagaliano, M., Depczynski, M., Simpson, S.D., and Moore, J.A. (2008) Dispersal without errors: symmetrical ears tune into the right frequency for survival. *Proceedings of The Royal Society B* **275**, 527-534.

Gardner, M.G., Hugall, A.F., Donnellan, S.C., Hutchinson, M.N., and Foster, R. (2008) Molecular systematics of social skink: phylogeny and taxonomy of the *Egernia* group (Reptilia: Scincidae). *Zoological Journal of The Linnean Society* **154,** 781-794.

Garland Jr., T., and Losos, J.B. (1994) Ecological morphology of locomotor performance in squamate reptiles. . In 'Ecological morphology: integrative organismal biology.'. (Eds. PC Wainwright and SM Reilly) pp. 240-302. (The University of Chicago Press: Chicago, IL)

Gerhardt, K.L., White, R.G., Cameron, R.D., and Russell, D.E. (1996) Estimating fat content of Caribou from body condition scores. *Journal of Wildlife Management* **60**, 713-718.

Gibbons, J.W., Scott, D.E., and Ryan, T.J. (2000) The global decline of reptiles, *deja vu* amphibians. *Bioscience* **50**, 653-666.

Godfrey, S.S., Moore, J.A., Nelson, N.J., and Bull, C.M. (2010) Unravelling causality from correlations: revealing the impacts of endemic ectoparasites on a protected species (tuatara). *Parasitology* **137**, 275-286.

Graham, J.H., Freeman, D.C., and Emlen, J.M. (1993) Developmental stability: a sensitive indicator of populations under stress. In 'Environmental toxicology and risk assessment.'. (Eds. WG Landins, JS Hughes and MA Lewis) pp. 136-158. (American Society for Testing and Materials Philadelphia)

Green, A.J. (2001) Mass/lenght residuals: measures of body condition or generators of spurious results? *Ecology* **82**, 1473-1483.

Greer, A.E. (1979) A phylogenetic subdividision of Australian skinks. *Records of the Australian Museum* **32**, 339-371.

Gutzke, W.H.N., and Crews, D. (1988) Embryonic temperature determines adult sexuality in a reptile. *Nature* **332**, 832-834.

Haila, Y. (2002) A conceptual geneology of fragmentation research: from island biogeography to landscapre ecology. *Ecological Applications* **12**, 321-334.

Hallgrimsson, B. (1998) Fluctuating asymmetry in the mammalian skeleton: evolutionary and developmental implications. . *Evolutionary Biology* **30**, 187-251.

Hallgrimsson, B. (1999) Ontogenetic patterning of skeletal fluctuating asymmetry in rhesus macaques and humans: evolutionary and developmental implications. *International Journal of Primatology* **20**, 121-151.

Hansen, D.M., Olesen, J.M., and Jones, C.G. (2002) Trees, birds and bees in Mauritius: exploitative competition between introduced honey bees and endemic nectarivorous birds. *Journal of Biogeography* **29**, 721-734.

Helle, S., Huhta, E., Suorsa, P., and Hakkarainen, H. (2011) Fluctuating asymmetry as a biomarker of habitat fragmentation in an area-sensitive passerine, the Eurasian treecreeper (Certhia familiaris). *Ecological Indicators* **11**(3), 861-867.

Hendrickx, F., Maelfair, J.P., and Lens, L. (2003) Relationship between fluctuating asymmetry and fitness whitin and between stressed and unstressed populations of the wolf spider *Pirata piraticus. Journal of Evolutionary Biology* **16**, 1270-1279.

Herrel, A., Spithoven, L., Van Damme, R., and De Vree, F. (1999) Sexual dimorphism of head size in Gallotia galloti: testing the niche divergence hypothesis by functional analyses. *Functional Ecology* **13**, 289-297.

Herrel, A., Van Damme, R., and De Vree, F. (1996) Sexual dimorphism of head size in *Podarcis hispanica atrata*: testing the dietary divergence hypothesis by bite force analysis *Netherlands Journal of Zoology* **46**, 253-262.

Hoare, J.M., Pledger, S., Keall, S.N., Nelson, N.J., Mitchell, N.J., and Daugherty, C.H. (2006) Conservation implications of a long-term decline in body condition of the Brothers Island tuatara (*Sphenodon guntheri*). *Animal Conservation* **9**, 4456-462.

Holt, R.D. (1996) Food webs in space: an island biogeographic perspective. In 'Food webs.' (Eds. GA Polis and KO Wine-Miller) pp. 313-323. (Chapman & Hall: New York)

Hosken, D.J., Blanckenhorn, W.U., and Ward, P.I. (2000) Developmental stability in yellow dong flies *(Scathophaga stercoraria)*: fluctuating asymmetry, heterozygosity and environmental stress. *Journal of Evolutionary Biology* **13**, 919-926.

Houle, D. (1997) Comment on " a meta-analysis of the heritability of developmental stability" by Moller and Thornhill. *Journal of Evolutionary Biology* **10**, 17-20.

Hoysak, D.J., and Ankney, C.D. (1996) Correlates of behaviour dominance in mallards and American black ducks. *Animal Behaviour* **51**, 409-419.

Humphries, M.M., Umbanhowar, J., and McCann, K.S. (2004) Bioenergetic prediction of climate change impacts on northern mammals. *Integrative and Comparative Biology* **44**, 152-162.

Hunt, J., and Simmons, L.W. (1997) Patterns of fluctuating asymmetry in beetles horns: an experimental examination of the honest signalling hypothesis

Behavioral Ecology and Sociobiology **41**, 109-114.

Husack, J.F., Lappin, A.K., Fox, S.F., and Lemos-Espinal, J.A. (2006) Bite-force performance predicts dominance in male venerable collared lizards (*Crotaphytus antiquus*). *Copeia* **2006**, 301-306.

Hutchinson, M.N., and Donnellan, S.C. (1992) Taxonomy and genetic variation in the Australian lizards of the genus *Pseudemoia* (Scincidae: Lygosominae). *Journal of Natural History* **26**, 215-264.

Hutchinson, M.N., Milne, T., and Croft, T. (1994) Redescription and ecological notes on the pygmy bluetongue, *Tiliqua adelaidensis* (Squamata:Scincidae). *Transactions of the Royal Society of South Australia* **118**, 217-226.

Jakob, E.M., Marshall, S.D., and Uetz, G.W. (1996) Estimating fitness: a comparison of body condition indices. *OIKOS* **77**, 61-67.

Keiser, J.A. (1993) Evolution, developmental instability and the theory of acquisition. *Genetica* **89**, 219-225.

Kellner, J.R., and Alford, R.A. (2003) The ontogeny of fluctuating asymmetry. *The American Naturalist* **161**(6), 931-947.

Kitcher, P. (1984) 'Valuation Ambition: Sociobiology and the Quest for Human Nature.' (Cambridge Press: MIT, US)

Konfias, G., Russell, D.E., and White, R.G. Monitoring Caribou body condition: Workshop Proceedings. 2002, Ottawa, Ontario, p. 31

Leamy, L., and Atchley, W. (1985) Directional selection and developmental stability: Evidence from fluctuating asymmetry of morphometric characters in rats. *Growth, Development and Aging* **49**, 8-18.

Learny, L.J., and Klingenberg, C.P. (2005) The genetic and evolution of fluctuating asymmetry. *Annual Review of Ecology, Evolution, and Systematics* **36**, 1-21.

Leary, R.F., and Allendorf, F.W. (1989) Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology & Evolution* **4**, 214-217.

Lemberget, T., and McCormick, M.I. (2009) Replenishment success linked to fluctuating asymmetry in larval fish. . *Oecologia* **159**, 83-93.

Lens, L., and Eggermont, H. (2008) Fluctuating asymmetry as a putative marker of human-induced stress in avian conservation. *Bird Conservation International* **18**, 125-143.

Lens, L., Van Dongen, S., Kark, S., and Mathyssen, E. (2002a) Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? . *Biological Reviews* **77**, 27-38.

Lens, L., Van Dongen, S., and Matthysen, E. (2002b) Fluctuating asymmetry as an early warning system in the critically endangered Taita Thrush. *Conservation Biology* **16**, 479-487.

Lens, L., Van Dongen, S., Matthysen, E., and Van de Casteele, T. (2000) Developmental instability and inbreeding in natural bird populations exposed to different levels of habitat disturbance. *Journal of Evolutionary Biology* **13**, 889-896.

Leung, B., and Forbes, M.R. (1997) Modelling fluctuating asymmetry in relation to stress and fitness. *Oikos* **78**, 397-405.

Leung, B., Forbes, M.R., and Houle, D. (2000) Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. *The American Naturalist* **155**, 101-115.

Lindell, L.E., Forsman, A., and Merila, J. (1993) Variation in number of ventral scales in snakes: effects on body size, growth rate and survival in the adder, *Vipera berus*. *Journal of Zoology* **230**, 101-115.

Lindenmayer, D.B., and Possingham, H.P. (1995) Modelling the viability of metapopulations of the endangered leadbeaters possum in South East Australia. *Biodiversity and Conservation* **4**, 984-1018.

Loehr, V.J.T., Henen, B.T., and Hofmeyr, M.D. (2004) Reproduction of the smallest tortoise, the Namaqualand speckled padloper, *Homopus signatus signatus*. *Herpetologica* **60**, 444-454.

Lopez, P., Munoz, A., and Martin, J. (2002) Symmetry, male dominance and female mate preferences in the Iberian rock lizard, *Lacerta monticola*. *Behavioural Ecology and Sociobiology* **52**, 342-347.

Lovatt, F.M., and Hoelzel, A.R. (2011) The impact of population bottlenecks on fluctuating asymmetry and morphological variance in two separate populations of reindeer on the island of South Georgia. *Biological Journal of the Linnean Society* **102**, 798-811.

Madsen, T., and Shine, R. (2000) Rain, fish and snakes:climatically driven populations dynamics of Arafura filesnake in tropical Australia. *Oecologia* **124**, 208-215.

Madsen, T., and Shine, R. (2002) Short and chubby or long and slim? Food intake, growth and body condition in free-ranging pythons. *Austral Ecology* **27**, 672-680.

Martin, J., and Lopez, P. (2000) Chemoreception, symmetry, and mate choice in lizards. *Proceedings of The Royal Society of London B* **267**, 1265-1269.

Mateos, C., Alarcos, S., Carranza, J., Sanchez-Prieto, C.B., and Valencia, J. (2008) Fluctuating asymmetry of red deer antlers negatively relates to individual condition and proximity to prime age. *Animal Behaviour* **75**, 1629-1640.

Matthen, M., and Ariew, A. (2002) Two ways of thinking about fitness and natural selection. *Journal of Philosophy* **99**(2), 55-83.

May, R.M., Lawton, J.H., and Stork, N.E. (1995) Assessing extinction rates. In 'Extinction rates.' (Eds. JH Lawton and RM May) pp. 1-24. (Oxford University Press: New York)

McArthur, R.H., and Wilson, E.O. (1967) 'The theory of island biogeography.' (Princeton University Press: Princeton, New Jersey)

Merila, J., Forsman, A., and Lindell, L.E. (1992) High frequency of ventral scale anomalies in *Vipera berus* populations. *Copeia* **1992**, 1127-1130.

Milne, T. (1999) Conservation and ecology of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis*. Flinders University, Adelaide

Milne, T., Bull, C.M., and Hutchinson, M.N. (2003a) Fitness of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis* in artificial burrows. *Journal of Herpetology* **37**, 762-765.

Milne, T., Bull, C.M., and Hutchinson, M.N. (2003b) Use of burrows by the endangered pygmy bluetongue lizard *Tiliqua adelaidensis. Wildlife Research* **30**, 523-528.

Moller, A.P. (1991) Sexual ornament size and the cost of fluctuating asymmetry. . *Proceedings of The Royal Society of London B* **243**, 59-62.

Moller, A.P. (1992) Parasites differentially increase fluctuating asymmetry in secondary sexual characteristics. *Journal of Evolutionary Biology* **5**, 691-699.

Moller, A.P. (1992) Female swallow preference for symmetrical male sexual ornaments. *Nature* **357**, 237-240.

Moller, A.P. (1993) Female preference for apparently symmetrical male sexual ornaments in the barn swallows *Hirundo rustica. Behavioral Ecology and Sociobiology* **32**, 371-376.

Moller, A.P. (1997) Developmental stability and fitness. *American Naturalists* **149**, 916-932.

Moller, A.P. (1999) Asymmetry as a predictor of growth, fecundity and survival. *Ecological Letters* **2**, 149-156.

Moller, A.P., Cuervo, J.J., Soler, J.J., and Zamora-Munoz, C. (1996) Horn asymmetry and fitness in gemsbok, *Oryx g. gazella. Behavioral Ecology* **7**, 247-253.

Moller, A.P., and Swaddle, J.P. (1997) 'Asymmetry, developmental stability, and evolution.' (Oxford University Press: Oxford)

Moore, J.A., Miller, H.C., Daugherty, C.H., and Nelson, N.J. (2008) Fine-scale genetic structure of a long-lived reptile reflects recent habitat modification. *Molecular Ecology* **17**(21), 4630-4641.

Morris, M.R., Rios-Cardenas, O., Lyons, S.M., Scarlett Tudor, M., and Bono, L.M. (2012) Fluctuating asymmetry indicates the optimization of growth rate over developmental stability. *Functional Ecology* **26**(3), 723-731.

Mouton, N., and Van Wyk, J.H. (1993) Sexual dimorphism in cordylid lizards: a case study of the Drakensberg crag lizard, *Pseudocordylus melanotus. Canadian Journal of Zoology* **71**, 1715-1723.

Nagy, K.A., Henen, B.T., Vyas, D.B., and Wallis, I.R. (2002) A condition index for the desert tortoise (*Gopherus agassizii*). *Chelonian Conservation and Biology* **4**, 425-429.

Olsson, M., Gullberg, A., Shine, R., T., M., and Teglestrom, H. (1996) Parental genotype influences incubation period, offspring size and offspring shape in an oviparous reptile. . *Evolution* **50**(3), 1328-1333.

Palmer, A.R. (1996) Waltzing with asymmetry. *Bioscience* **46**, 518-532.

Palmer, A.R., and Strobeck, C. (1986) Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology, Evolution, and Systematics* **17**, 391-421.

Palmer, A.R., and Strobeck, C. (2003) 'Fluctuating asymmetry analyses revisited. In: Polak, M. (ed) Developmental instability: causes and consequences.' (Oxford University Press: New York) 279-319

Parsons, P.A. (1992a) Fluctuating asymmetry an epigenic measure of environmental and genomic stress. *Heredity* **68**, 361-364.

Parsons, P.A. (1992b) Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity* **68**, 361-364.

Paulay, G. (1994) Biodiversity on oceanic islands: its origin and extinction. *American Zoologist* **34**, 134-144.

Peacock, D., and Abbott, I. (2014) When the 'native cat' would 'plague': historical hyperabundance in the quoll (Marsupialia: Dasyuridae) and an assessment of the role of disease, cats and foxes in its cutailment. *Australian Journal of Zoology* **62**, 294-344.

Peig, J., and Green, A.J. (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and lenght. *Functional Ecology* **24**, 1323-1332.

Pertoldi, C., Bijlsma, R., and Loeschcke, V. (2007) Conservation genetics in a globally changing environment: present problems, paradoxes and future challenges. *Biodiversity and Conservation* **16**, 4147-4163.

Pertoldi, C., Kristenssen, T.N., Anderson, D.H., and Loeschcke, V. (2006) Developmental instablility as an estimator of genetic stress. *Heredity* **96**, 122-127.

Polak, M., and Trivers, R. (1994) The science of symmetry in biology. *Trends in Ecology & Evolution* **9**, 122-124.

Pullin, A.S. (2002) 'Conservation Biology.' (Cambridge University Press: Cambridge, UK)

Putman, R.J., Sullivan, M.S., and Langbein, J. (2000) Fluctuating asymmetry in antlers of fallow deer (*Dama dama*): the relative roles of environmental stress and sexual selection. . *Biological Journal of the Linnean Society* **70**, 27-36.

Reeve, E.C.R. (1960) Some genetic tests on asymmetry of sternopleural chaetae number in *Drosophila*. . *Genetical Research* **1**, 151-172.

Rivera-Oritz, F.A., Aguilar, R., Arizmendi, M.D.C., Kesada, M., and Oyama, K. (2015) Habitat fragmentation and the genetic viability of tetrapod populations. *Animal Conservation* **18**, 249-258.

Roberts, D.L., and Solow, A.R. (2003) Flightless birds: when did the dodo become extinct? *Nature* **426**, 245.

Rocha, C.F.D., Siqueira, C.d.C., and Ariani, C.V. (2009) The endemic and threatened lizard *Liolaemus lutzae* (Squamate: Liolaemidae): current geographic distribution and areas of occurence with estimated population densities. *Zoologia* **26**, 454-460.

Romero, L.M., and Wikelski, M. (2001) Corticosterone levels predict survival probabilities of Galapagos marine iguanas during El Nino events. . *Proceeding of the National Academy of Sciences* **98**, 7366-7370.

Rosen, D.A., and Trites, A.W. (2000) Pollock and the decline of Steller sea lions: testing the junk food hypothesis *Canadian Journal of Zoology* **78**, 1243-1250.

Ross, K.A., Fox, B.J., and Fox, M.D. (2002) Changes to plant species richness in forest fragments: fragment age, disturbance and fire history may be as important as area. *Journal of Biogeography* **29,** 749-465.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Hubersanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., and Wall, D.H. (2000) Global biodiversity scenarios for the year 2100. *Science* **287**, 1770-1774.

Sarre, S. (1996) Habitat fragmentation promotes fluctuating asymmtery but not morphological divergence in two geckos. *Research on population ecology* **38**, 57-64.

Sarre, S., Dearn, J.M., and Georges, A. (1994) The application of fluctuating asymmetry in the monitoring of animal populations. *Pacific Conservation Biology* **1**, 118-122.

Schofield, J.A., Fenner, A.L., Pelgrim, K., and Bull, C.M. (2012) Male-biased movement in pygmy bluetongue lizards: implications for conservation. *Wildlife Research* **39**, 677-684.

Schofield, J.A., Gardner, M.G., Fenner, A.L., and Bull, C.M. (2014) Promiscous mating in the endangered Australian lizard *Tiliqua adelaidensis*: a potential windfall for its conservation. *Conservation Genetics* **15**, 177-185.

Schulte-Hostedde, A.I., Zinner, B., Millar, J.S., and Hickling, G.J. (2005) Restitution of mass-size residulas: validating body condition indices. *Ecology* **86**, 155-163.

Shamiminoori, L., Fenner, A.L., and Bull, C.M. (2014) Weight watching in burrows: variation in body condition in pygmy bluetongue lizards. *Australian Journal of Zoology* **62**, 284-293.

Shamiminoori, L. (2015) Impacts of environmental variation on the fitness of the pygmy bluetongue lizards, *Tiliqua adelaidensis*. Flinders University, Adelaide

Shine, R. (2005) Life-history evolution in reptiles. *Annual Review of Ecology, Evolution, and Systematics* **36**, 23-46.

Shine, R., and Harlow, P.S. (1993) Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96**, 122-127.

Shine, R., Langkilde, T., Wall, M., and Mason, R.T. (2005) The fitness correlates of scalation asymmetry in garter snakes Thamnophis sirtalis parietalis. *Functional Ecology* **19**, 306-314. [In English]

Shine, R., LeMaster, M.P., Moore, I.T., Olsson, M.M., and Mason, R.T. (2001) Bumpus in the snake den: effects of sex, size, and body condition on morality of red-sided garter snakes. *Evolution* **55**, 598-604.

Simbotwe, M.P. (1985) Sexual dimorphism and reproduction of *Lampropholis guichenoti* (Lacertilia: Scincidae). In 'The biology of Australian frogs and reptiles '. (Eds. G Grigg, R Shine and H Ehmann) pp. 11-16. (Surrey Beatty & Sons: Sydney)

Siqueira, C.C., Keifer, M.C., Van Sluys, M., and Durate Rocha, C.F. (2013) Variation in the diet of the lizard *Tropidurus torquatus* along its coastal range in Brazil. *Biota Neotrop* **13**, 93-101.

Skogland, T. (1983) The effects of density-dependent resource limitation of size of wild reindeer. *Oecologia* **60**, 156-168.

Smith, A.L., Gardner, M.G., Fenner, A.L., and Bull, C.M. (2009) Restricted gene flow in the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) in a fragmented agricultural landscape. *Wildlife Research* **36**, 466-478.

Soule, M.E. (1979) Heterozygosity and developmental stability: another look. *Evolution* **33**, 396-401.Soule, M.E. (1982) Allometric variation. I. The theory and some consequences. *American Naturalists* **120**, 751-764.

Soule, M.E. (1986) 'Conservation biology: the science of scarcity and diversity.' (Sinauer Associates: Sunderland)

Souter, N.J. (2003) Habitat requirements and conservation of the endangered pygmy bluetongue lizard *Tiliqua adelaidensis*. Flinders University of South Australia, Adelaide

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

Steadman, D.W. (1995) Prehistoric extinctions of Pacific Island birds: biodiversity meets zooarchaeology. *Science* **267**, 1123-1131.

Stevenson, R.D., and Woods, J., W. A. (2006) Condition indices for conservation: new uses for evolving tools. *Integrative and Comparative Biology* **46**, 1169-1190.

Swaddle, J.P., and Witter, M.S. (1994) Food, feathers, and fluctuating asymmetries. *Proceedings of The Royal Society of London B* **255**, 147-152.

Swaddle, J.P., and Witter, M.S. (1997) On the ontogeny of developmental stability in a stabilized trait. *Proceedings of The Royal Society of London B* **264**, 329-334.

Thornhill, R. (1992) Female preference for the pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (Panorpa japonica: Mecoptera). *Behavioral Ecology* **3**, 277-283.

Tohl, D., Li, J.S.J., Shamiminoori, L., and Bull, C.M. Image asymmetry measurement for the study of endangered pygmy bluetongue lizard. In ' Proceedings of 2013 IVCN, IEEE; 28th International Conference of Image and Vision Computing', 2013, Wellington, New Zealand. (Eds. T Rhee, R Rayudu, C Hollitt, J Lewis and M Zhang), pp. 76-81

Tsubaki, Y. (1998) Fluctuating asymmetry of the oriental fruit fly (*Docus dorsalis*) during the process of its extinction from the Okinawa Islands. *Conservation Biology* **12**, 926-929.

Van Valen, L. (1962) A study of fluctuating asymmetry. *Evolution* **16**, 125-142.

Vangestel, C., and Lens, L. (2011) Does fluctuating asymmetry constitute a sensitive biomarker of nutritional stress in house sparrows (Passer domesticus)? *Ecological Indicators* **11**, 389-394.

Vitousek, M. (2009) Investment in mate choice depends on resource availability in female Galápagos marine iguanas (*Amblyrhynchus cristatus*). *Behavioral Ecology and Sociobiology* **64**, 105-113.

Vitousek, P.M. (1988) Diversity and biological invasions of oceanic islands. In 'Biodiversity.' (Ed. EO Wilson) pp. 181-189. (National Academy Press: Washington)

Vitt, L.J., and Cooper, W.E.J. (1985) The evolution of sexual dimorphism in the skink *Eumeces laticeps*: an example of sexual selection. *Canadian Journal of Zoology* **63**, 995-1002.

Vitt, L.J., and Pianka, E.R. (1994) 'Lizard Ecology: Historical and Experimental perspectives.' (Princeton University Press: New Jersey, USA)

Waddington, C.H. (1942) Canalisation of development and the inheritance of acquired characters. *Nature* **150**, 563-565.

Waldmann, P. (2001) The effect of inbreeding on fluctuating asymmetry in *Scabiosa canescens* (Dipsacaceae). *Evolutionary Ecology* **15**, 117-127.

Warner, D.A., and Andrews, R.M. (2002) Laboratory and field experiments identify soureces of variation in phenotypes and survival of hatchling lizards. *Biological Journal of the Linnean Society* **76**, 105-124.

Wauters, L.A., Dhondt, A.A., Knothe, H., and Parkin, D.T. (1996) Fluctuating asymmetry and body size as indicators of stress in red squirrel population in woodland fragments. *Journal of Applied Ecology* **33**, 735-740.

Waye, H.L., and Mason, R.T. (2008) A combination of body condition measurements is more informative than conventional condition indices: temporal variation in body condition and corticosterone in brown tree snakes (*Boiga irregularis). General and Comparative Endocrinology* **155**, 607-612.

Whitehorn, P.R., Tinsley, M.C., Brown, M.J.F., and Darvill, B. (2014) Genetic diversity and parasite prevalence in two species of bumblebee. *Journal of Insect Conservation* **18**, 667-673.

Whiteman, N.K., and Parker, P.G. (2004) Body condition and parasite load predict territory ownership in the Galapagos hawk. *Condor* **106**, 915-921.

Woods, R.E., Sgro, C.M., Hercus, M.J., and Hoffman, A.A. (2002) Fluctuating asymmetry, fecundity and development time in *Drosophila*: is there an association under optimal and stress condition? *Journal of Evolutionary Biology* **15**, 146-157.

Wright, A.N., and Zamudio, K.R. (2002) Colour pattern asymmetry as a correlate of habitat disturbance in spotted salamanders (*Ambystoma maculatum*). *Journal of Herpetology* **36**, 129-133.