

# Demographic and life history variation in two sky-island populations of an endangered alpine lizard

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## Keywords

threatened species; fecundity; growth rate; longevity; vulnerability; life-history traits; *Liopholis guthega*; geographic variation.

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## Abstract

A species' life history is a strong determinant of its risk of extinction; traits such as body size, growth rate, age at maturity and fecundity influence population viability and persistence, as well as capacity for dispersal and colonisation of new habitats. Yet, despite the potential for substantial geographic variation in life history, most conservation programmes rely upon the species average rather than information specific to individual populations. We use the Guthega Skink (*Liopholis guthega*), a threatened alpine endemic lizard restricted to two geographically isolated locations in south-eastern Australia, as a case study to demonstrate how geographic variation in life history traits may better inform conservation management. *Liopholis guthega* has a relatively short life span, with only a few years of reproductive activity, inter-annual variation in reproductive output, and less-than-annual reproduction. We show that the Victorian population has a significantly slower growth rate, attains a smaller maximum size, reaches reproductive maturity later, and produces significantly smaller litters, despite no difference in longevity suggesting that it may be more vulnerable to extinction. We, therefore, suggest evaluating genetic rescue from the New South Wales population to improve recruitment and longevity of Victorian *L. guthega*, ongoing population monitoring in both locations, and a dedicated pest control programme to reduce pressure on this population. Our study has far reaching impacts; primarily demonstrating how knowledge of geographic variation in life history has the potential to improve conservation management of threatened species.

## Introduction

Understanding the life histories of threatened species is fundamental for effective conservation management (Dubey *et al.*, 2013; Marín-Moratalla, Jordana & Köhler, 2013). Life history traits such as growth rate, fecundity, age at maturity and longevity can be governed by both extrinsic and intrinsic factors, with phenotypes subject to natural selection (Bronikowski, 2000; Zamudio, Bell & Mason, 2016). Spatial and temporal variability in selection pressures, coupled with phenotypic plasticity, are drivers of intraspecific geographical variation in life history traits, a common natural phenomenon (Du *et al.*, 2005; Roitberg *et al.*, 2013). These phenotypes differ in how they contribute to processes such as dispersal, colonisation and persistence, and collectively affect population size, turnover,

genetic variability and, ultimately, susceptibility to disturbance (Webb, Brook & Shine, 2002; Du *et al.*, 2005; Zamudio *et al.*, 2016).

Threatened species are often restricted and fragmented in their distributions (Frankham *et al.*, 2012), therefore the capacity for substantial variation in life history traits across a geographical gradient means that populations may differ in key facets of their ecology. Assuming identical life histories between populations may leave more susceptible populations increasingly vulnerable to extinction (Fritz, Bininda-Emonds & Purvis, 2009). Additionally, life history parameters are a key input in both population viability analysis (PVA), and assessments of threatened status (e.g. those conducted by the International Union for Conservation of Nature, IUCN), so use of broad species averages, as opposed to a population specific

approach, has the potential to misrepresent populations and alter risk evaluations. Thus, understanding detailed life history across a species' entire range is an essential first step in successful conservation management of threatened taxa (Marín-Moratalla *et al.*, 2013).

Determining life history variables such as growth, age structure and longevity relies on mark-recapture studies, which typically require long-term monitoring (Chinsamy & Valenzuela, 2008; Marín-Moratalla *et al.*, 2013). Alternatively, prompt and accurate demographic information for amphibian and reptile populations can be obtained via skeletochronology (Sinsch, 2015). This technique estimates age via histological analysis of growth markers in skeletal bone (Chinsamy & Valenzuela, 2008; Dubey *et al.*, 2013). Successful application of this technique requires prominent lines of arrested growth (LAGs) as a result of circannual endogenous rhythm, which may be reinforced by annual climate variation (Castanet *et al.*, 1993). Skeletochronology is, therefore, a particularly useful tool for vertebrates found in temperate environments that are exposed to marked seasonal variation (e.g. Dubey *et al.*, 2013). The capacity for rapid data collection without specimen sacrifice makes skeletochronology extremely valuable for prompt threatened species risk assessment (e.g. Marín-Moratalla *et al.*, 2013), and has provided valuable life history information for ectotherms in temperate climates (e.g. Scheele *et al.*, 2015), and in the tropics (e.g. Sinsch & Dehling, 2017).

The Guthega Skink, *Liopholis guthega*, is a medium-sized, viviparous scincid lizard listed as Endangered under the IUCN Red list, and Australian Federal Legislation (the *Environmental Protection and Biodiversity Conservation Act* 1999), with conservation efforts currently including captive husbandry. Threats to the species include climate change and alpine recession, invasive species (e.g. horses, foxes, cats) and anthropomorphic habitat changes. It is a true alpine specialist whose entire range is restricted to two geographically isolated sky islands above 1600 m in south-eastern Australia: the Bogong High Plains (BHP) in Victoria (Vic), and Kosciuszko National Park (KNP) in New South Wales (NSW) (Donnellan *et al.*, 2002; Atkins *et al.*, 2018). The two populations are separated by ~100 km of lowland valleys that completely impede gene flow, and represent an estimated one million years of allopatry (Atkins *et al.*, 2019; Chapple, Keogh & Hutchinson, 2005). Furthermore, at a local scale, discrete colonies exhibit high genetic structure due to natural habitat barriers (alpine bog systems), further reducing gene flow and population viability (Atkins *et al.*, 2019). In populations that remain isolated are predicted to diverge progressively over time as a consequence of selective and stochastic processes (Zamudio *et al.*, 2016). Accurate life history information at a population level will therefore identify the geographic variation in susceptibility of this species to extinction and allow for tailored management at a local scale.

Here, we investigate life history differences between the two geographically isolated *L. guthega* populations. We used phalangeal skeletochronology to estimate the growth rate, age structure and longevity of these lizards, coupled with extensive reproductive and morphological data to examine recruitment. We aim to assess the importance of life history differences

between the two populations to improve conservation efficacy for threatened species.

## Materials and methods

### Study area

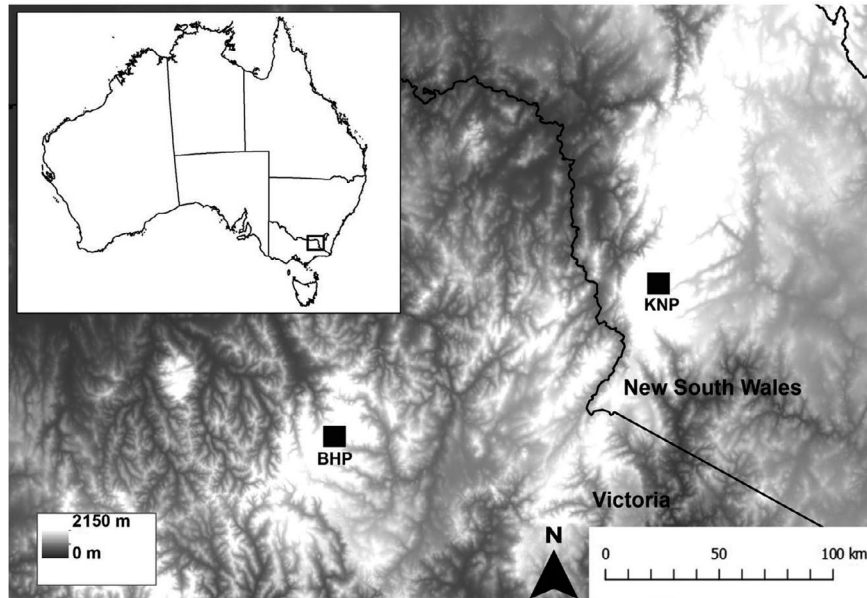
*Liopholis guthega* were collected from 32 colonies within Vic ( $n = 11$ ) and NSW ( $n = 21$ ) (Fig. 1), spanning the species' known range. Sampling within Vic (approximately 50 km<sup>2</sup>) was conducted at 1600–1700 m elevation and within NSW (approximately 160 km<sup>2</sup>) at ~1650–2170 m elevation (Fig. 1). Vic and NSW sites receive a mean annual rainfall of 1200 mm and 1750 mm, respectively [Bureau of Meteorology, <http://www.bom.gov.au/australia/alpine/>, accessed (14/02/2017)], and are characterized by alpine and sub-alpine grasslands, herbfields, heathlands and snow gum woodlands interspersed with granite and basalt (Vic only) boulder fields, as well as small streams and associated alpine-bog systems.

### Morphology

During the 2013–2016 Australian summers (December–March), we captured 450 *L. guthega* (Vic (2013–2015): 234; NSW (2014–2016): 216), of which, 298 were considered mature (Vic: 135; NSW: 163). The smallest gravid *L. guthega* captured throughout this study was 74.6 mm snout-vent length (SVL) in Vic and 77.5 mm SVL in NSW. Paternity analysis was undertaken in NSW, and confirmed reproductive activity in males > 90 mm SVL (R. Hayes unpublished data). However, signs of reproductive activity (semen) were observed for smaller males from both regions (~80 mm SVL). Thus, 75 mm SVL was the size used to differentiate between juvenile and adult *L. guthega*, with only the latter used for morphological comparisons. This excluded three advanced one-year old individuals in NSW that were >75 mm SVL, but <20 g in mass, with prominent juvenile colouration. These individuals had not undergone the obvious ontogenetic colour change characteristic of this species (Wilson & Swan, 2017; R. Makkdissi, Z.S. Atkins, N. Clemann, & K.A. Robert, unpublished data).

Lizards were captured via noosing, or opportunistically by hand; all lizards were released after processing. Upon capture, we determined sex via cloacal examination and eversion of the hemipenes of males; we palpated females to detect developing embryos to determine their reproductive status [Gravid (G); non-gravid (NG)] (Melville & Swain, 1999). Mass (g) and standard morphometric data were collected using digital scales and calipers (to the nearest millimetre), respectively, and included: snout-vent length (SVL); tail length (TL); head length (HL); head width (HW); front limb length (FLL); hind limb length (HLL). Any individuals with missing or regenerated tails were not used in tail length analyses.

To identify recaptures, we marked lizards using Trovan nano Passive Integrated Transponder (PIT) tags (Microchips Australia Pty Ltd, Keysborough, Australia). PIT tags were inserted under the skin between the epidermis and the muscle, dorsolaterally above the right hind limb. Visible Implant Elastomer



**Figure 1** The allopatric distribution of *Liopholis guthega* to the two sky-islands; the Bogong High Plains (BHP) in Victoria and Kosciuszko National Park (KNP) in New South Wales.

(VIE) tags (Northwest Marine Technology, Washington, DC, USA) (Penney *et al.*, 1999) were used on neonate animals weighing <5 g. Mark-recapture data were also used to verify the age analysis.

### Skeletochronology

Toe-clips were collected from 134 individuals (Vic: 57; NSW: 77) representing the complete size range of this species across both geographic locations. The complete fifth toe from the right hind limb was removed and stored in 90% ethanol in 2.0 ml microcentrifuge tubes for subsequent skeletochronological age estimation.

Laboratory protocols for processing the second phalanx of each toe followed standard methods of skeletochronology (e.g. Sinsch, 2015). The samples were embedded in Historesin™ (JUNG) and stained with 0.5% cresylviolet (details in Dubey *et al.*, 2013). Diaphysis was cross-sectioned at 12 µm using a JUNG RM2055 rotation microtome. Cross sections were examined under an OLYMPUS BX 50 light microscope (Olympus Corporation, Tokyo, Japan) for the presence of growth marks, at magnifications of 400×. We distinguished two types of growth marks in the periosteal bone: (1) Birth line (BL), a narrow to broad line separating the embryonic bone from the postnatal bone. Embryonic bone stains stronger than postnatal bone; (2) strongly stained single LAGs, separated by faintly stained broad postnatal annuli (growth zones). Endosteal bone was present in all individuals, but resorption never affected the first LAG, whereas BL was partially or completely resorbed in some of the older individuals.

Lines of arrested growth were counted in the periosteal bone of those diaphysis sections in which the size of the medullar cavity was at its minimum and that of bone at its maximum.

The number of usable diaphysis sections per individual varied between 4 and 12. All were considered for LAG counting and two of the authors (US, AMS) independently obtained age estimates for each individual.

Growth following birth was estimated using the Von Bertalanffy (1938) equation:  $SVL_t = SVL_{\text{asympt}} - (SVL_{\text{asympt}} - SVL_{\text{birth}}) * e^{-k*t}$ , where  $SVL_t$  = average body length at age  $t$ ;  $SVL_{\text{asympt}}$  = asymptotic body length;  $SVL_{\text{birth}}$  = body length at birth (= smallest size of a neonate captured in field, 43 mm);  $t$  = number of growing seasons experienced ( $n$  LAGs), and  $k$  = growth coefficient (i.e., shape of the growth curve). The von Bertalanffy growth model was fitted to the average growth curve using least-squares (nonlinear regression). Estimates of  $SVL_{\text{asympt}}$  and  $k$  are given with corresponding 95% confidence intervals. Absence of overlap between two confidence intervals was considered a significant difference at least  $P < 0.05$  (e.g. Schoener & Schoener, 1978).

### Reproductive output

To complement the field data on *L. guthega* reproduction, confirm the accuracy of palpation estimates, and to accurately investigate reproductive output, gravid females were collected in March (end of gestation period) and temporarily held captive until parturition (Vic: 9; NSW: 8). Mass (g) and SVL (mm) were recorded, and each female housed individually at field site accommodation in enclosures (600 mm × 400 mm). Heat was provided by a 42 W incandescent light bulb suspended from the roof of the enclosure at one end, providing a thermal gradient of 22–40°C for a 12 h period (06:00–18:00 h). All lizards were provided with water *ad libitum*, and offered four mealworms, *Tenebrio molitor*, every other day. Each female was weighed following parturition, with neonate

number and size (mass and SVL) recorded as a measure of maternal investment.

### Statistical analysis

All analyses were performed in R (R Core Team, 2015); with the exception of skeletochronological analysis, which was performed using STATGRAPHICS (Centurion, version XVI; StatPoint Technologies Inc., Warrenton, VA, USA). Reported variances are standard deviations.

### Morphology

A two-way ANOVA was used to examine the effects of population and sex on body size (SVL).

Principal component analysis (PCA) on morphological variables was used to examine patterns of sex and site-related variation in multivariate morphology.

### Skeletochronology

Intraspecific variation in demography and growth patterns was analysed in eight traits: (1) minimum age at maturity ( $n$  LAGs) = age of the youngest adult of the sample, (2) minimum SVL (mm) of first breeders = size of the smallest adult irrespective of age; (3) median age of all adults sampled ( $n$  LAGs); (4) median SVL (mm) of all adults sampled; (5) longevity = maximum age detected within a population ( $n$  LAGs); (6) maximum SVL (mm) of adults sampled irrespective of age; (7) asymptotic maximum SVL (mm), estimated by the von Bertalanffy growth model; (8) growth coefficient  $k$ , estimated by the von Bertalanffy growth model.

As age distributions were significantly skewed, descriptive statistics included median, minimum and maximum. Statistical comparison between sex and between locations were based on the non-parametric Mann-Whitney-Wilcoxon  $W$ -test. Size distributions (SVL) were normalized by  $\log_{(10)}$ -transformation. Sexual size dimorphism was examined using analyses of covariance (ANCOVA) with sex as a categorical factor and age as continuous covariate. Age-adjusted size estimates are given as least square means.

### Reproductive output

Prior to analysis, maternal SVL and offspring mass were log-transformed. Likelihood ratio tests were used to determine whether inclusion of a population (BHP or KNP) effect in the regression models relating offspring number and mass led to significantly improved model fit compared to models including only the effect of (log-transformed) maternal SVL.

To test whether there was a trade-off between offspring size and number within litters, we used a linear mixed model, with offspring size as the response variable and maternal size (SVL) and the number of offspring in the litter as covariates, and litter size as the random effect. If females are trading off offspring size and number, then a positive effect of maternal size, and a negative effect of the number of offspring in the litter is

to be expected. Models with and without the effect of the number of offspring in the litter were fitted, and the hypothesis that the number of offspring in the litter influenced offspring size after maternal size had been accounted for by conducting a likelihood ratio test between models with and without the effect of offspring number.

We used Fisher's exact tests to determine whether the proportion of gravid *L. guthega* females observed in the field differed between the two populations, and across both years for each population. A logistic regression model was used to determine the relationship between SVL, population and the probability that each individual adult female was gravid. A linear model was used to determine whether the number of embryos per litter differed between the two populations, while accounting for body size. Both litter size and maternal SVL were log-transformed prior to analysis.

## Results

### Morphology

NSW *L. guthega* had a significantly larger SVL than Vic animals ( $t = 20$ ,  $P < 0.001$ ), averaging  $99.9 \pm 9.21$  mm, compared with  $86.5 \pm 5.67$  mm for Vic. The large size difference was clearly reflected in the PCA of morphological characteristics; the first component, PC1, accounted for 86.7% of the total variation (Table 1). Character loadings from PC1 were all positive and fairly uniform, indicating isometry among characters. TL was the only character that departed from multiple isometry (expected character loading at perfect isometry =  $(1/6)^{0.5} = 0.408$ ), suggesting the tails tend to be proportionally smaller in larger lizards.

The second principal component, PC2 (5.54% of total variation), mainly reflected the variation in TL (Table 1). The loadings on PC2 show a contrast between TL and all other morphological variables, supporting tail length departure from isometry.

Overall, the results of the PCA suggest that morphological traits (other than tail length) scales uniformly with animal size. Tail lengths were highly variable independent of overall body size, however there was no consistent difference in tail length between the two populations, or between males and females (Fig. 2).

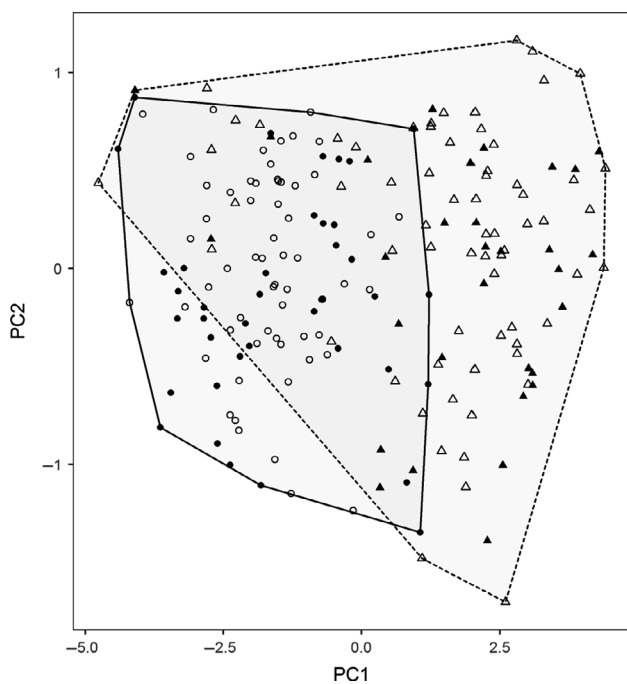
### Skeletochronology

Skeletochronological age estimation was successful in 134 individuals (Table 2). Both types of growth marks were evident, the birth line (BL) in 65.7% of individuals and LAGs in 84.3%. The BL was indistinguishable from the outer bone edge in seven neonates (42.2–48.9 mm SVL; Fig. 3a). In 14 juveniles (42.7–49.6 mm SVL) that were collected days or weeks after birthing, but before their first hibernation, BL was visible (Fig. 3b). The presence of BL in the bone sections of individuals that had hibernated at least once ( $\geq 1$  LAG) decreased with age (82.0% in age classes 1–2 LAGs to 36.4% in age classes 5–6 LAGs). Periods of growth (faintly stained

**Table 1** Importance of components and character loadings for the principal component analysis of morphological variables of six components for *Liopholis guthega*

Principal component	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	2.28	0.58	0.42	0.35	0.31	0.27
Per cent variance explained	86.65	5.54	2.96	2.02	1.64	1.18
Cum. per cent	86.65	92.20	95.15	97.17	98.82	100.00
Factor loadings						
SVL	0.419	-0.023	0.270	-0.222	-0.807	-0.224
TL	0.372	0.916	-0.077	0.044	0.117	0.041
HW	0.416	-0.172	0.482	-0.070	0.544	-0.514
HL	0.420	-0.195	0.354	0.188	0.072	0.788
FLL	0.410	-0.211	-0.577	-0.641	0.167	0.126
HLL	0.411	-0.222	-0.481	0.705	-0.075	-0.219

FLL, forelimb length; HL, head length; HLL hindlimb length; HW, head width; SVL, snout-vent length; TL, tail length.



**Figure 2** Scatterplot of first and second individual's scores on principal components of morphological variables in relation to population and sex of *Liopholis guthega*. Circles indicate Victorian animals and triangles indicate NSW animals. Open shapes indicate females and closed shape indicate males.

annuli) alternated with strongly stained LAGs in 113 individuals (Fig. 3c, d). Periosteal bone growth was greatest in the period between birth and first hibernation (= broad annulus) decreasing considerably during the later years of life (= narrow annuli). In the individuals with completely resorbed BL the first LAG always remained identifiable because of the preceding extensive growth period. Age estimation by counting LAGs as a proxy for hibernation events seemed reliable in *L. guthega* because the growth trajectories of 15 Vic skinks that were captured at least twice during this study (identified

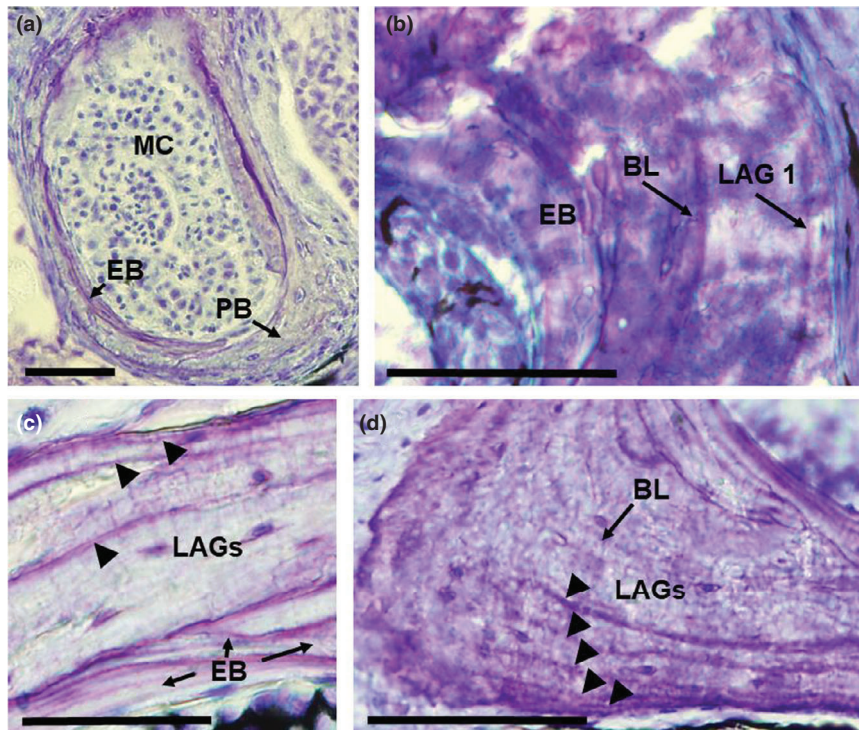
**Table 2** Demographic life history traits of *Liopholis guthega* in two populations: the Bogong High Plains in Victoria, and Kosciuszko National Park in New South Wales. LAGs, lines of arrested growth from skeletochronological analysis

	Victoria	New South Wales
<b>Males</b>		
	<i>N</i> = 17	<i>N</i> = 22
Age at maturity ( <i>n</i> LAGs)	3	2
Minimum size at maturity (mm)	81.6	77.0
Median age ( <i>n</i> LAGs)	4	3
Median size (mm)	88.5	99.4
Maximum observed longevity ( <i>n</i> LAGs)	5	6
Maximum size (mm)	98.5	105.5
<b>Females</b>		
	<i>N</i> = 18	<i>N</i> = 33
Age at maturity ( <i>n</i> LAGs)	3	2
Minimum size at maturity (mm)	77.3	83.0
Median age ( <i>n</i> LAGs)	4	3
Median size (mm)	86.7	103.2
Maximum observed longevity ( <i>n</i> LAGs)	5	6
Maximum size (mm)	97.2	113.8
<b>Juveniles</b>		
	<i>N</i> = 22	<i>N</i> = 18
Age range ( <i>n</i> LAGs)	0–1	0–1
Size range (mm)	44.9–74.1	42.2–72.6

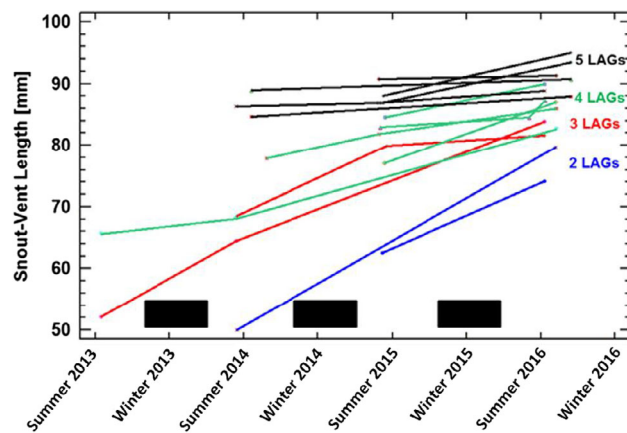
via marked recapture±) were consistent with the estimated age at final capture in 2016 (Fig. 4).

**Age structure**

NSW *L. guthega* reached sexual maturity at 2 years of age, whereas Vic lizards matured at 3 years, irrespective of sex (except for one female at 2 years; Fig. 5a). Despite a 2-year old Vic skink being identified as ‘mature’ based on a >75 mm SVL, this female was non-gravid, suggesting the animal was probably not reproductively mature. Maximum observed longevity was 6 years in NSW and 5 years in Vic. Median age did not differ significantly between males and females in the NSW population (3 LAGs; Mann-Whitney-Wilcoxon *W*-test, *W* = 359.5,



**Figure 3** Bone sections of *Liopholis guthega* from (a) a new-born neonate (46.2 mm SVL, Vic), (b) a 1-LAG old juvenile with birth line (89.6 mm SVL, NSW), (c) a 3-LAGs female with birth line replaced by endosteal bone (106.8 mm SVL, NSW), and (d) a 5-LAGs female with remains of the birth line (108.8 mm SVL, NSW). Vic, Victoria, Australia; NSW, New South Wales, Australia; LAG, line of arrested growth; SVL, snout-vent length; MC, medullary cavity; EB, endosteal bone; PB, periosteal bone; BL, birth line. Black bars indicate the 50 µm. [Colour figure can be viewed at [zslpublications.onlinelibrary.wiley.com](http://zslpublications.onlinelibrary.wiley.com)]



**Figure 4** Growth trajectories of mark-recaptured *Liopholis guthega* from the Victorian Bogong High Plains population that were skeletochronologically aged at their 2016 recapture. SVL increase is large during the first 2 years of life and then slows down. The age classes are colour-coded according to number of lines of arrested growth (LAGs) detected. Black blocks represent annual hibernation periods. [Colour figure can be viewed at [zslpublications.onlinelibrary.wiley.com](http://zslpublications.onlinelibrary.wiley.com)]

$P = 0.955$ ), or in Vic (4 LAGs;  $W$ -test,  $W = 143.0$ ,  $P = 0.737$ ). However, on average Vic adults were one year older than NSW adults (4 vs. 3 LAGs;  $W$ -test,  $W = 1403.5$ ,  $P = 0.0001$ ).

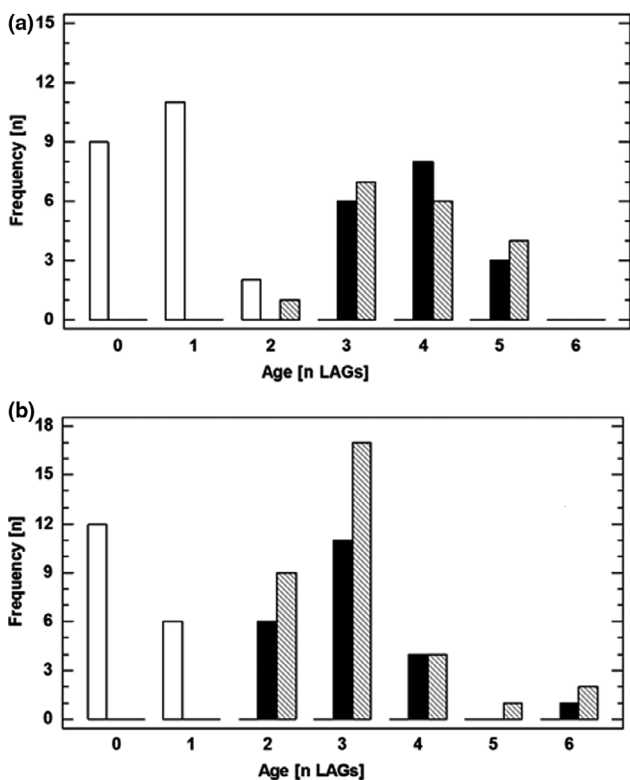
### Growth pattern

$\log_{10}$ -transformed SVL of adult skins did not differ significantly between males and females ( $F = 1.00$ ,  $P = 0.32$ ), but did differ between localities ( $F = 121.49$ ,  $P < 0.0001$ ), if adjusted for age variation ( $F = 11.70$ ,  $P = 0.001$ ). There were no significant interactions between sex and population ( $F = 2.00$ ,  $P = 0.16$ ).

The sex-specific von Bertalanffy growth models estimated the same asymptotic SVL and growth rate ( $k$ ) for males and females, and there were no significant differences between the two populations ( $P > 0.05$ ; overlap among CIs; Table 3). The pooled age-size data set for each population showed different growth patterns (Fig. 6a, b). Estimated asymptotic SVL was 96.4 mm (CI: 90.3–102.5 mm) in Vic, and 108.0 mm (CI: 104.0–112.0 mm) in NSW, differing significantly ( $P < 0.05$ ; no overlap between CIs). The corresponding growth coefficient  $k$  in Vic (0.487; CI: 0.351–0.624), as compared to that in NSW, (0.796; CI: 0.640–0.951) indicated significantly slower growth ( $P < 0.05$ ; no overlap between CIs).

### Reproductive output – captive females

Mean litter sizes of *L. guthega* from Vic and NSW were  $1.55 \pm 0.17$ , and  $2.5 \pm 0.18$ , respectively (Table 4). Across both populations, larger females produce more offspring



**Figure 5** Age distribution of juvenile (white), male (black) and female (hatched) *Liopholis guthega* from (a) the Bogong High Plains in Victoria, and (b) Kosciuszko National Park in New South Wales, where LAGs is lines of arrested growth from skeletochronological analysis. For statistical details see text.

( $t_{15} = 4.82$ ,  $P < 0.01$ ), and heavier litters ( $t_{15} = 6.01$ ,  $P < 0.01$ ). Likelihood ratio test revealed that regression models for offspring number and litter mass that included an effect of population did not fit the data significantly better than models including (log-transformed) maternal SVL alone (Table 5).

The average size (SVL) of neonate *L. guthega* born in Vic and NSW is  $45.35 \pm 0.29$  mm and  $42.12 \pm 0.38$  mm, respectively; while average offspring mass was  $2.55 \pm 0.06$  g and  $2.38 \pm 0.05$  g, respectively. Comparison of linear mixed models with and without an effect of offspring number on offspring size, while accounting for the effect of maternal SVL and site, showed no evidence to support the inclusion of an offspring number effect in the model ( $\text{Pr}(\chi^2_{4,5} > n) = 0.46$ ). Mothers with a larger SVL produced larger offspring ( $t = 4.3$ ). However, mothers in NSW produced smaller offspring than mothers in VIC, once maternal SVL was accounted for ( $t = -2.3$ ).

### Reproductive output – field captures

There was no significant difference between the proportion of gravid females at each site during the 2015 summer period, with 81.8% gravid in Vic and 77.1% gravid in NSW (Fisher’s exact test, odds ratio = 0.75,  $P = 0.78$ ). Gravid females had an average number of 1.56, and 2.14 embryos, in the Victorian and NSW populations, respectively. Results of a binomial generalized linear model showed that SVL, population and their interaction did not influence the probability of being gravid ( $Z_{1,123} = 0.17$ ,  $P = 0.863$ ). Results suggest that larger females, across both sites, had more offspring as a function of SVL (Fig. 7).

Reproductive status and investment of female *L. guthega* varied considerably across years in both Vic and NSW, with reproductive output in 2015 exceeding that in other years (Table 4). Both populations had proportionally more gravid females during 2015 than other years, which coincided with an increase in number of offspring produced by the Vic mothers, from 1.28 in 2014 to 1.55 in 2015. The NSW mothers also had a higher number of offspring in 2015; producing an average of 2.14 in 2015, and 1.93 in 2016, and, however this difference was only marginally significant (Table 4). Also, while the NSW population average number of embryos was only marginally higher in 2015, the number of mothers with three embryos was significantly higher in 2015 than 2016 (odds ratio = 0.05,  $P < 0.01$ ).

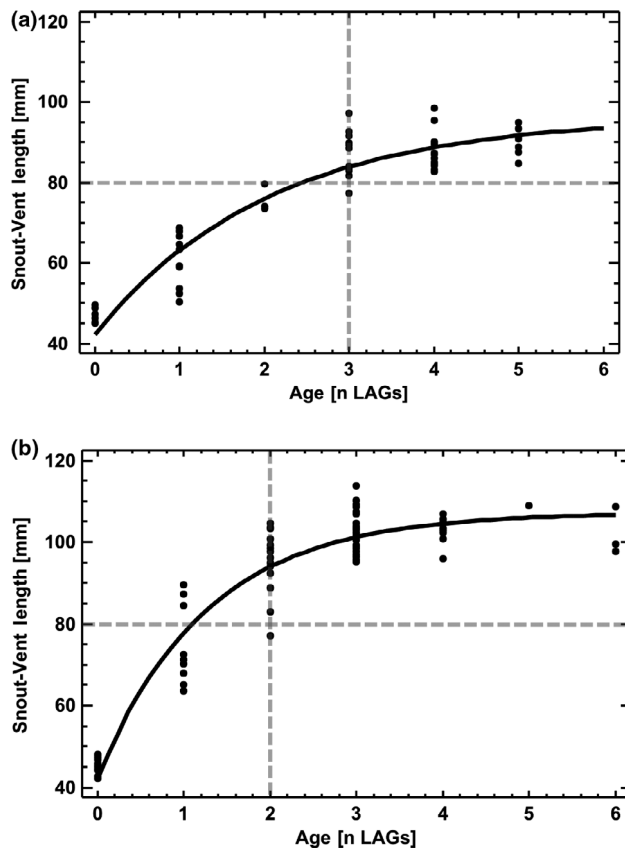
### Discussion

Here, we show differences between the sky-island populations of the threatened *L. guthega* to the extent of requiring population-level conservation. NSW animals had a significantly higher growth rate, attained a greater maximum size, reached reproductive maturity earlier, and these larger animals produced significantly larger litters. This species has a relatively short life span, with only a few years of reproductive activity, generally exhibiting less-than-annual mode of reproduction. The substantial geographic variation in life history traits between the two *L. guthega* populations likely reflects local adaptation to their respective habitats (Dubey & Shine, 2011), and clearly highlights the need for population-level (as opposed to species-level) considerations when developing effective management strategies.

NSW lizards have higher growth rates and attain a greater maximum size, which contrasts with the commonly found trend of slower life histories of species in cooler, high elevation environments (e.g. growth rates: Kubisch *et al.*, 2012; Body size: Rohr, 1997; Horváthová *et al.*, 2013). Warmer environments are often associated with longer diel and annual

**Table 3** Sex- and population-specific von Bertalanffy growth models for *Liopholis guthega*. SVL, snout-vent length

	Sex	SVL <sub>asympt</sub> (mm)	SVL ± CI <sub>95%</sub> (mm)	k	k ± CI <sub>95%</sub>
New South Wales	♂	107.8	100.9 ± 114.7	0.658	0.474 ± 0.842
	♀	111.3	105.9 ± 116.6	0.685	0.533 ± 0.837
Victoria	♂	99.4	90.0 ± 108.9	0.421	0.274 ± 0.569
	♀	97.1	88.4 ± 105.8	0.465	0.303 ± 0.627



**Figure 6** Von Bertalanffy growth models for *Liopholis guthega* in (a) the Bogong High Plains, Victoria, and (b) Kosciuszko National Park, New South Wales (a, small-sized morph excluded from analysis). Each dot represents an individual. Dashed lines represent mean size (vertical) and age (horizontal) at maturity. Where LAGs is lines of arrested growth from skeletochronological analysis.

activity periods (Angilletta, 2009), which generally leads to increases in foraging time (e.g. Bronikowski & Arnold, 1999), and therefore increases in annual growth (e.g. Grant & Dunham, 1990) and earlier maturation (Grant & Dunham, 1990; Rohr, 1997; Wapstra, Swain & O'Reilly, 2001; Angilletta,

**Table 5** Comparison of models for the influence of maternal snout-vent length (SVL, log-transformed) and population on the number of offspring and the litter mass in *Guthega Skinks* in two populations: the Bogong High Plains, Victoria and Kosciuszko National Park, New South Wales

	d.f.	Log-likelihood	Chi-sq	P-Value
a. Litter Size Model				
~SVL	3	0.115	0.068	0.794
~SVL + Population	4	0.149		
b. Litter Mass Model				
~SVL	3	0.336	0.111	0.739
~SVL + Population	4	0.391		

Steuery & Sears, 2004). As the NSW population is higher in elevation, and experiences colder climates with more pronounced snow cover, our results were unexpected and contrasted studies undertaken on other Australian temperate zone skinks (e.g. Wapstra & Swain, 2001). The most significant growth in *L. guthega* occurred during the first two years and was more pronounced in NSW lizards, ultimately resulting in significantly larger animals that reached reproductive size/maturity earlier than the Vic lizards. These findings contradict Rohr (1997) life history study on Southern Water Skink, *Eulamprus tympanum*, whose high elevation distribution overlaps with *L. guthega*. Overwinter survival is directly related to body size (*Niveoscincus ocellatus*; Uller *et al.*, 2011), therefore given the higher elevation of the NSW population it is possible that selection is favouring larger juvenile size to survive the longer hibernation period at this elevation. Recent dietary analysis between the two populations revealed an almost identical foraging ecology, with consistent temporal variation (Atkins *et al.* 2018), suggesting that diet is not driving the life history variation seen here. It is possible that the marked differences maybe a result of the phylogeographic differences documented between the two populations (Atkins *et al.*, 2019), as has been suggested elsewhere (e.g. Du *et al.*, 2005).

The larger body size of the NSW lizards allows higher fecundity, affording a maximum of three embryos, as opposed to two in Vic. Litter size has been positively correlated to body size within the subfamily Egeneriinae (Chapple, 2003), as

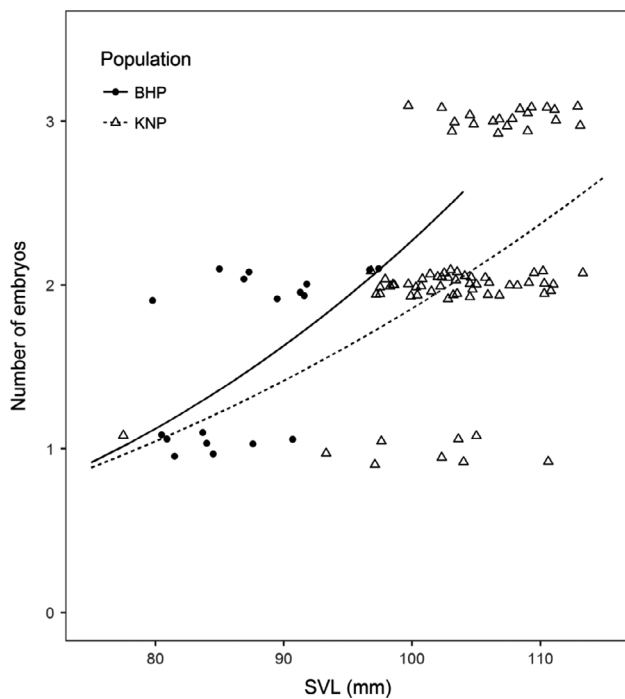
**Table 4** The reproductive status and embryo number of mature female *Liopholis guthega* captured during the 2014 and 2015 summer seasons on the Bogong High Plains, Victoria and 2015 and 2016 summers at Kosciuszko National Park, New South Wales (NSW)

	Victoria		Odds ratio	P-Value	NSW		Odds ratio	P-Value
	2014	2015			2015	2016		
Gravid	32 (55.2%)	18 (81.8%)	0.28	0.038*	81 (77.1%)	28 (59.6%)	2.28	0.03*
Not Gravid	26 (44.8%)	4 (18.2%)			24 (22.9%)	19 (40.4%)		
Embryo #			t-score	P-value			t-score	P-value
1	23 (71.9%)	8 (44.4%)	1.95	0.057	9 (11.8%)	3 (10.7%)	1.76	0.08
2	9 (28.1%)	10 (55.5%)			47 (61.8%)	24 (85.7%)		
3	0	0			20 (26.3%)	1 (3.6%)		

Raw data presented with percentages within parentheses.

\*denotes significant P-value (<0.05).





**Figure 7** Gravid female *Liopholis guthega*, across both New South Wales and Victorian populations in 2015 ( $t_{92,90} = 0.42$ ,  $P = 0.68$ ), produce more offspring as their body size (SVL) increases ( $t_{92,90} = 2.70$ ,  $P < 0.01$ ). Circles indicate Victorian animals, triangles indicate NSW animals. Lines are fitted linear models (with SVL log transformed).

female reproductive output is constrained by body volume (e.g. Roitberg *et al.*, 2013). The smallest *L. guthega* in NSW to produce three embryos was larger than any female captured in Vic during the study period, suggesting the smaller body size in Vic ultimately constrains reproductive output. While body size determines the upper limit of reproductive output in *L. guthega*, fecundity at a population level was variable and not predicted purely by SVL. The closely related, and smaller, *Liopholis montana* and *L. whitii* are capable of producing litter sizes of four and five, respectively (Donnellan *et al.*, 2002; Chapple, 2003). The reduced reproductive capability of *L. guthega*, despite their larger size in comparison to these congeners, further implies costs imposed by a higher elevation environment (Ibargüengoytía & Casalins, 2007). These costs likely extend further again, as biennial/triennial reproductive mode is often seen in squamates living in harsh high elevation environments (e.g. Cree & Hare, 2016). Extensive hibernation periods as a result of marked seasonality in Australian alpine endemic species is well documented (e.g. *Burramys parvus*; Broome & Geiser, 1995), including in *L. guthega* (Atkins, 2018), and is likely the driver of the low annual reproductive output of this species documented here.

Inter-annual variation in reproductive output coupled with a short lifespan, results in a very low lifetime recruitment rate in *L. guthega*, which differs considerably between NSW and Vic. Maturing at 3 years of age and with a lifespan of

5 years, Vic *L. guthega* appear to have a maximum of three reproductive events (if they reproduced annually). With a mean litter size of 1.27 across seasons, the estimated lifetime reproductive output of skinks within the Vic population is 3.8. Delayed maturity, in conjunction with a shorter lifespan and lower lifetime fecundity, characteristic of Vic *L. guthega*, contrasts with many reptile populations (e.g. Wapstra *et al.*, 2001; Webb *et al.*, 2002). While also very low, a lifespan of six years, coupled with earlier maturation, means NSW *L. guthega* are capable of 5 years of reproductive activity, corresponding to a lifetime reproductive output of 10.2, ignoring the less-than-annual reproductive cycle of this species. Such low recruitment in Vic *L. guthega* leaves this population particularly susceptible to predicted climate change (Pickering, 2007). Low fecundity populations take longer to recover from colony loss during disturbance events, and are, therefore, more likely to go extinct when external pressures elevate mortality rate above fecundity (Bennett & Owens, 1997). Ultimately, the short life span of this species, coupled with small and temporally variable litter sizes, greatly limits recruitment and would affect this species' capacity to recover from disturbances like fire and habitat loss, as well as their competitive ability in the face of novel competitors, predators and congeners encroaching from lower elevations (Green & Pickering, 2002; Dubey *et al.*, 2013; Atkins, Clemann & Robert, 2015).

Life history underlies PVA, and is a central determinant of the time frame over which threatened species assessments are based (e.g. IUCN assessments: Mace *et al.*, 2008). Therefore, if a species' life history is geographically variable, so should be the assessments of extinction risk. Small population size, small geographic range size, specialised habitat requirements, slow life histories and low fecundity are all traits that increase risk of extinction (Purvis *et al.*, 2000; Webb *et al.*, 2002; Sekercioglu *et al.*, 2008); these traits are present in *L. guthega*, particularly in their Victorian range. This species is highly restricted within one of the ecosystems most vulnerable to climate warming (Berg *et al.*, 2010; Dirnböck, Essl & Rabitsch, 2011); how this species will be impacted by changing thermal and hydro regimes associated with climate change is unknown (McCain & Colwell, 2011). This study shows the importance of obtaining life history knowledge from across the entire range of a threatened species, to ensure variations in populations can be incorporated into management. Ultimately, the life history understanding gained from this study will directly inform both *in-situ* and *ex-situ* conservation management and provide greater clarity on local *L. guthega* ecology that will more appropriately guide future decisions regarding the management of Australia's highest elevation reptile.

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## References

- Angilletta, M.J. (2009). *Thermal adaptation: a theoretical and empirical synthesis*. Oxford: Oxford University Press.
- Angilletta, M.J., Steury, T.D. & Sears, M.W. (2004). Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integr. Comp. Biol.* **44**, 498–509.
- Atkins, Z.S. (2018). *Conservation of the Guthega Skink, Liopholis guthega*. PhD thesis, La Trobe University, Bundoora, Australia.
- Atkins, Z., Clemann, N. & Robert, K.A. (2015). Does shelter site selection aid persistence of a threatened alpine lizard? Assessing *Liopholis guthega* populations a decade after severe fire in southeastern Australia. *J. Herpetol.* **49**, 222–229.
- Atkins, Z.S., Clemann, N., Schroder, M., Chapple, D.G., Davis, N.E., Robinson, W.A., Wainer, J. & Robert, K.A. (2018). Consistent temporal variation in the diet of an endangered alpine lizard across two south-eastern Australian sky-islands. *Austral Ecol.* **43**, 339–351.
- Atkins, Z.S., Amor, M.D., Clemann, N., Chapple, D.G., While, G.M., Gardener, M.G., Haines, M.L., Harrison, K.A., Schroder, M. & Robert, K.A. (2019). Allopatric divergence drives the genetic structuring of the endangered alpine endemic lizard, *Liopholis guthega*. *Conserv. Biol.* (in press). <https://doi.org/10.1111/acv.12519>
- Bennett, P.M. & Owens, I.P. (1997) Variation in extinction risk among birds: chance or evolutionary predisposition? *Proc. R. Soc. Lond. B Biol. Sci.*, **264**, 401–408.
- Berg, M.P., Kiers, E., Driessen, G., Van Der Heijden, M., Kooi, B.W., Kuenen, F., Liefjing, M., Verhoef, H.A. & Eilers, J. (2010). Adapt or disperse: understanding species persistence in a changing world. *Glob. Change Biol.* **16**, 587–598.
- Bronikowski, A.M. (2000). Experimental evidence for the adaptive evolution of growth rate in the garter snake *Thamnophis elegans*. *Evolution* **54**, 1760–1767.
- Bronikowski, A.M. & Arnold, S.J. (1999). The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. *Ecology* **80**, 2314–2325.
- Broome, L.S. & Geiser, F. (1995). Hibernation in free-living mountain pygmy-possums, *Burramys parvus* (Marsupialia, Burramyidae). *Aust. J. Zool.* **43**, 373–379.
- Castanet, J., Francillon-Vieillot, H., Meunier, F.J. & de Ricqlès, A. (1993). Bone and individual aging. In *Bone Growth*: 245–283. Hall, B.K. (Ed.). Boca Raton: CRC Press.
- Chapple, D.G. (2003). Ecology, life-history, and behavior in the Australian scincid genus *Egernia*, with comments on the evolution of complex sociality in lizards. *Herpetol. Monogr.* **17**, 145–180.
- Chapple, D.G., Keogh, J.S. & Hutchinson, M.N. (2005). Substantial genetic substructuring in southeastern and alpine Australia revealed by molecular phylogeography of the *Egernia whitii* (Lacertilia: Scincidae) species group. *Mol. Ecol.* **14**, 1279–1292.
- Chinsamy, A. & Valenzuela, N. (2008). Skeletochronology of the endangered side-neck turtle, *Podocnemis expansa*. *S. Afr. J. Sci.* **104**, 311–314.
- Cree, A. & Hare, K. (2016). *Reproduction and life history of New Zealand lizards*. Basel: Springer.
- Dirnböck, T., Essl, F. & Rabitsch, W. (2011). Disproportional risk for habitat loss of high-altitude endemic species under climate change. *Glob. Change Biol.* **17**, 990–996.
- Donnellan, S.C., Hutchinson, M.N., Dempsey, P. & Osborne, W.S. (2002). Systematics of the *Egernia whitii* species group (Lacertilia: Scincidae) in south-eastern Australia. *Aust. J. Zool.* **50**, 439–459.
- Du, W.-G., Ji, X., Zhang, Y.-P., Xu, X.-F. & Shine, R. (2005). Identifying sources of variation in reproductive and life-history traits among five populations of a Chinese lizard (*Takydromus septentrionalis*, Lacertidae). *Biol. J. Lin. Soc.* **85**, 443–453.
- Dubey, S. & Shine, R. (2011). Predicting the effects of climate change on an endangered montane lizard, *Eulamprus leuraensis* (Scincidae). *Climate Change* **107**, 531–547.
- Dubey, S., Sinsch, U., Dehling, M.J., Chevalley, M. & Shine, R. (2013). Population demography of an endangered lizard, the Blue Mountains Water Skink. *BMC Ecol.* **13**, 4.
- Frankham, R., Ballou, J.D., Dudash, M.R., Eldridge, M.D., Fenster, C.B., Lacy, R.C., Mendelson, J.R., Porton, I.J., Ralls, K. & Ryder, O.A. (2012). Implications of different species concepts for conserving biodiversity. *Biol. Cons.* **153**, 25–31.
- Fritz, S.A., Bininda-Emonds, O.R. & Purvis, A. (2009). Geographical variation in predictors of mammalian extinction risk: big is bad, but only in the tropics. *Ecol. Lett.* **12**, 538–549.
- Grant, B.W. & Dunham, A.E. (1990). Elevational covariation in environmental constraints and life histories of the desert lizard *Sceloporus merriami*. *Ecology* **71**, 1765–1776.
- Green, K. & Pickering, C.M. (2002). A scenario for mammal and bird diversity in the Australian Snowy Mountains in relation to climate change. In *Mountain biodiversity: a global assessment*: 241–249. Koerner, C. & Spehn, E.M. (Eds). London: Parthenon Publishing.
- Horvátová, T., Cooney, C.R., Fitze, P.S., Oksanen, T.A., Jelić, D., Ghira, I., Uller, T. & Jandzik, D. (2013). Length of activity season drives geographic variation in body size of a widely distributed lizard. *Ecol. Evol.* **3**, 2424–2442.
- Ibargüengoytía, N.R. & Casalins, L.M. (2007). Reproductive biology of the southernmost gecko *Homonota darwini*: convergent life-history patterns among southern hemisphere reptiles living in harsh environments. *J. Herpetol.* **41**, 72–80.
- Kubisch, E.L., Piantoni, C., Williams, J., Sclaro, A., Navas, C. & Ibargüengoytía, N. (2012). Do higher temperatures increase growth in the nocturnal gecko *Homonota darwini* (Gekkota: Phyllodactylidae)? A skeletochronological assessment analyzed at temporal and geographic scales. *J. Herpetol.* **46**, 587–595.

- Mace, G.M., Collar, N.J., Gaston, K.J., Hilton-Taylor, C., Akçakaya, H.R., Leader-Williams, N., Milner-Gulland, E.J. & Stuart, S.N. (2008). Quantification of extinction risk: IUCN's system for classifying threatened species. *Conserv. Biol.* **22**, 1424–1442.
- Marín-Moratalla, N., Jordana, X. & Köhler, M. (2013). Bone histology as an approach to providing data on certain key life history traits in mammals: implications for conservation biology. *Mamm. Biol.-Zeitschrift für Säugetierkunde* **78**, 422–429.
- McCain, C.M. & Colwell, R.K. (2011). Assessing the threat to montane biodiversity from discordant shifts in temperature and precipitation in a changing climate. *Ecol. Lett.* **14**, 1236–1245.
- Melville, J. & Swain, R. (1999). Home-range characteristics of an alpine lizard, *Niveoscincus microlepidotus* (Scincidae), on Mt Wellington, southern Tasmania. *Wildl. Res.* **26**, 263–270.
- Penney, K.M., Gianopulos, K.D., McCoy, E.D. & Mushinsky, H.R. (2001). The visible implant elastomer marking technique for small reptiles. *Herpetological Review* **32**, 236–241.
- Pickering, C. (2007). Climate change and other threats in the Australian Alps. *Protected Areas: Buffering Nature Against Climate Change*, **18**, 28.
- Purvis, A., Gittleman, J.L., Cowlishaw, G. & Mace, G.M. (2000). Predicting extinction risk in declining species. *Proc. R. Soc. Lond. B Biol. Sci.*, **267**, 1947–1952.
- R Core Team (2015). *R: a language and environment for statistical computing* [Internet]. Vienna: R Foundation for Statistical Computing.
- Rohr, D.H. (1997). Demographic and life-history variation in two proximate populations of a viviparous skink separated by a steep altitudinal gradient. *J. Anim. Ecol.* **66**, 567–578.
- Roitberg, E.S., Kuranova, V.N., Bulakhova, N.A., Orlova, V.F., Eplanova, G.V., Zinenko, O.I., Shamgunova, R.R., Hofmann, S. & Yakovlev, V.A. (2013). Variation of reproductive traits and female body size in the most widely-ranging terrestrial reptile: testing the effects of reproductive mode, lineage, and climate. *Evol. Biol.* **40**, 420–438.
- Scheele, B.C., Hunter, D.A., Skerratt, L.F., Brannelly, L.A. & Driscoll, D.A. (2015). Low impact of chytridiomycosis on frog recruitment enables persistence in refuges despite high adult mortality. *Biol. Cons.* **182**, 36–43.
- Schoener, T.W. & Schoener, A. (1978). Estimating and interpreting body-size growth in some *Anolis* lizards. *Copeia* **3**, 390–405.
- Sekercioglu, C.H., Schneider, S.H., Fay, J.P. & Loarie, S.R. (2008). Climate change, elevational range shifts, and bird extinctions. *Conserv. Biol.* **22**, 140–150.
- Sinsch, U. (2015). Skeletochronological assessment of demographic life-history traits in amphibians. *Herpetol. J.* **25**, 5–13.
- Sinsch, U. & Dehling, J.M. (2017). Tropical anurans mature early and die young: evidence from eight Afrotropical *Hyperolius* species and a meta-analysis. *PLoS ONE* **12**, e0171666.
- Uller, T., While, G.M., Cadby, C.D., Harts, A., O'Connor, K., Pen, I. & Wapstra, E. (2011). Altitudinal divergence in maternal thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution* **65**, 2313–2324.
- Von Bertalanffy, L. (1938). A quantitative theory of organic growth (inquiries on growth laws. II). *Hum. Biol.* **10**, 181–213.
- Wapstra, E. & Swain, R. (2001). Geographic and annual variation in life history traits in a temperate zone Australian skink. *J. Herpetol.* **35**, 194–203.
- Wapstra, E., Swain, R. & O'Reilly, J.M. (2001). Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* **3**, 646–655.
- Webb, J.K., Brook, B.W. & Shine, R. (2002). What makes a species vulnerable to extinction? Comparative life-history traits of two sympatric snakes. *Ecol. Res.* **17**, 59–67.
- Wilson, S. & Swan, G. (2017). *A complete guide to reptiles of australia*. 5th edn. Sydney: Reed New Holland.
- Zamudio, K.R., Bell, R.C. & Mason, N.A. (2016). Phenotypes in phylogeography: species' traits, environmental variation, and vertebrate diversification. *Proc. Natl Acad. Sci.* **113**, 8041–8048.