# Title

# Abstract

# Keywords

# Introduction

In many species, body size is a key fitness-related trait as it strongly predicts survival and fitness. Body size shows ontogenetic variation (growth) mediates various life history traits (Ricklefs & Wikelski, 2002; Stamps, 2007). Generally, organisms with fast growth rates, tend to mature more quickly and have shorter lifespans (slow-fast continuum, Bauwens & Diaz-Uriate, 2010; Sæther, 1987). However, growth is further shaped by developmental environments such as temperature, thus promoting variation in life history strategies which can result in changes in population demography and dynamics (Coulson et al., 2010; Kearney, 2012; Noble et al., 2017). Developmental plasticity is an important mechanism that enables organisms, particularly ectotherms to cope with unpredictable temperature changes as modified phenotypes may be better suited to the environment (West-Eberhard, 2003). Accordingly, theoretical work suggests that plasticity may allow populations to track closely with the environment as they slowly undergo genetic assimilation of the adaptive phenotype (‘plasticity-first’ hypothesis, Lande, 2009). As temperature and thermal variability continues to increase, understanding developmental responses in body size and growth is necessary to predict population persistence, particularly in ectotherms.

Organisms that develop in cooler environments tend to mature at larger body sizes compared to those inhabiting warmer environments (‘temperature-size rule’, Angilletta Jr et al., 2017). This ubiquitous pattern suggests there is commonality in the mechanisms that give rise to body size variation, however these are still not well understood (Angilletta, Jr., & Dunham, 2003). Larger body sizes could be achieved by prolonging growth rates and/or delaying reproduction (Angilletta Jr et al., 2017). In support of this, a recent meta-analysis showed that reptiles that experience cold incubation temperatures have been shown to have slower growth rates compared to those that experienced hot incubation temperatures (Noble et al., 2017). However, previous studies typically compare body size over a short interval at early life stages or at only few ages throughout ontogeny (Le Henanff et al., 2013; Pearson & Warner, 2016; Verdú‐Ricoy et al., 2014). This tends to simplify non-linear growth trajectories, making it difficult to assess when growth is maximised and when it begins to slow down. Thus, to understand developmental plasticity of body size and growth, it is necessary to quantify the full ontogenetic trajectory.

Developmental responses to temperature can facilitate adaptive evolution in body size however this relies on presence of heritable variation. The evolutionary potential of a phenotypic trait is usually measured as heritability (*h2*) and expressed as a ratio of additive genetic variation to non-genetic variance (Kruuk, 2004; Wilson et al., 2010). In some cases, high developmental temperatures increased heritability of morphological traits, however the relationship is not straightforward (Charmantier & Garant, 2005; Hoffmann & Merilä, 1999). Most studies that have investigated the effects of developmental temperature on morphological traits are in invertebrate systems, as such we lack an understanding on how vertebrate systems may respond (but see Uller et al., 2002). Moreover, environmental comparisons of heritability has been criticised as estimates may show no changes but the relative contributions of variance (maternal and environmental effects) may actually differ (Rowiński & Rogell, 2017). One study showed that environmental variance of morphological traits was higher in low stress environments compared to high stress environments but h2 showed no differences (Rowiński & Rogell, 2017). This suggests that the speed of evolutionary change may be higher in high stress environments due to less environmental variance. To gain a better understanding of how developmental plasticity can guide adaptive evolution in body size, evaluating genetic as well as phenotypic variance components is essential.

While body size typically increases with age, its genetic and phenotypic components of variance can also display ontogenetic variation. Researchers have suggested that directional or stabilising selection at certain life stages can change variance components of body size at different ages (Wilson et al., 2005, 2007). Indeed, body size at sexual maturity is likely a major target of selection as it strongly predicts reproductive success and survival (Bartheld et al., 2015; Calsbeek & Sinervo, 2002). Maternal effects such as nutrient provisioning or nest site selection should have the most influence on offspring body size at early life stages (Cheverud, 1984; Wilson & Réale, 2006). Accordingly, maternal contributions decline with age in mammals presumably because mothers cannot continue to influence her offspring’s phenotype post-weaning (Krist, 2010; Wilson et al., 2005). Alternatively, compensatory growth strategies can also promote to ontogenetic patterns in body size variance (Hector & Nakagawa, 2012). Namely, individuals that have experienced unfavourable conditions at an early stage may accelerate growth rate at a later age to catch up to the size of unrestricted conspecifics when conditions are more amenable (Radder et al., 2007). While these proposed mechanisms can all partly explain age-dependent patterns in quantitative parameters, how they respond under different developmental temperatures remains poorly understood.

Here we investigated the impact of developmental temperature on growth trajectories and the relative contributions of mass variance in an oviparous skink (*Lampropholis delicata*). Using 8,433 SNP markers, we derived a genomic relatedness matrix to estimate quantitative genetics parameters to address the following key questions: 1) How does developmental temperature affect the shape of growth trajectories (initial mass, growth rate and curvature of growth trajectory)?; 2) Do differences in growth result in variation in age at maximum mass?; 3) How does developmental temperature affect genetic and non-genetic variance components as well as heritability overall and across age? Based on the temperature-size rule, we expect lizards that experienced cold developmental temperatures to have larger initial masses, slower growth rates and would reach maximum size at a later age compared to lizards that experienced hot developmental temperatures. We also predict that [Predictions with temperature with genetic and maternal variance].

# Materials and Methods

## Lizard husbandry, Breeding Design and Egg Collection

From 2015 – 2017, we established a breeding colony of adult *L. delicata* (nfemales = 144, nmales = 50) using wild individuals collected across five sites throughout the Sydney region between 28 August and 8 September 2015. We used a half-sib breeding design where up to three females were housed with a single male in an opaque plastic enclosure measuring 35cm 25cm 15cm (L W H). Enclosures were kept under UV lights (12L:12D) in a temperature control room set at 24ºC and they were given access to a heat lamp that elevated temperatures to between 28-32 ºC. Each enclosure was lined with newspaper and lizards had constant access to water and tree bark as refuge. Adult lizards were fed medium sized crickets *ad libitum* (*Acheta domestica*) dusted with calcium powder and multi-vitamin every two days. From the beginning of egg laying seasons (October of each year), we replaced newpaper lining with garden potting mix and placed an opaque plastic box (12 cm 17.5 cm 4.3 cm) containing moistened vermiculite in each enclosure for females to oviposit their eggs. During this time, enclosures were sprayed gently with water every second day to maintain a relatively humid environment. From October to November, egg boxes were checked every day. We took tail tip tissue samples from adults that were from enclosures that produced eggs for DNA extraction (see below). All tissues were stored in 70% ethanol. Animal collection was approved by the New South Wales National Parks and Wildlife Service (SL101549) and all procedures were approved by the Macquarie University Ethics committee (ARA 2015/015) and University of New South Wales Animal Care and Ethics committee (ACEC 15/51A).

## Developmental Temperature Treatments

Eggs were collected over two years from 2016 -2017 and were typically laid between October –March. As soon as eggs were found, they were weighed using a digital scale to the nearest 0.01g (Ohaus Scout SKX123). We also measured egg length (distance between the furthest points along the longest axis of the egg) and egg width (distance between the widest points along the axis perpendicular to the longest axis of the egg) using digital callipers to the nearest 0.01mm. Following measurements, each egg was placed in a plastic cup (80ml) containing three grams of vermiculite and four grams of water. Each cup was then covered using cling wrap and secured by an elastic band. We assumed that eggs that were found together in the substrate box were from the same clutch as eggs from a single female are often stuck together. Each clutch was pseudo-randomly assigned to one of two developmental temperature treatments. We used two incubators to precisely control the temperature of eggs (LabWit, ZXSD-R1090). The ‘hot’ treatment was exposed to a mean temperature of 29ºC whereas the ‘cold’ treatment was exposed to a mean temperature of 23ºC. However, both incubators fluctuated +/- 3ºC over 24 hours. These treatments represent the temperature extremes of natural nest sites of *L. delicata* (Cheetham et al., 2011). Egg cups were rotated within each incubator weekly to avoid uneven heat circulation within incubators. Incubators were also checked daily for dead eggs and emergent hatchlings. On average, the incubation period for the ‘hot’ treatment was 29.36 days (SD = 2.17, range = 15 - 49) days and 48.48 days (SD = 4.18, range = 25 - 56) for the ‘cold’ treatment.

## Quantifying Growth Rate

Newly emerged hatchlings were weighed to the nearest 0.01g and a small tail tip clipping (~2mm) was taken for genetic analyses (see below). Ventral photographs were taken for digital measurement (Nikon Coolpix A900). For the first two months, photographs of hatchlings were taken approximately every 14 days. After which, hatchlings were photographed at approximately a 35-day interval. From approximately six months onwards, we manually measured hatchling SVL using a clear ruler. Growth measurements continued until we had approximately 16 measures per individual (mean = 11.5 , range = 1 - 18). From the photographs, we extracted snout-vent-length (SVL; from tip of snout to the beginning of the cloaca opening) using ImageJ software (Rueden et al., 2017). For the first initial nine months, hatchlings were housed individually in opaque plastic enclosures (32.3cm x 18.5cm x 6cm) lined with newspaper. Hatchlings were fed the same number of crickets every second day and had constant access to a tree bark refuge and water. Hatchling enclosures were placed in a temperature control room under the same conditions as described above for the adult colony. For logistical reasons, at approximately nine months, hatchlings were housed in groups of five in opaque bins with the same measurements as the adult enclosures. We pseudo-randomised individuals to each shared enclosure while maintaining a similar number of individuals from each treatment.

Pedigree and Genomic Relatedness

Tail tissue samples from adults and hatchlings were used to determine parentage of offspring. DNA extraction and single nucleotide polymorphism (SNP) genotyping was performed by Diversity Arrays Technology, a commercial company which utilises a technique called DArTseq™. For more details on DNA extraction and SNP genotyping see ESM.

In order to estimate quantitative genetic parameters, we derived a genomic relatedness matrix (GRM) using our SNP dataset for 261 offspring growth data (132 putative parents; nfemales = 69, nmales = 63). While our half-sib breeding design allowed us to assign parentage to derive a pedigree, high levels of sperm storage and low levels of multiple paternity (94% of females had been sired by a single male) meant our pedigree had low resolution to effectively estimate additive genetic variation. Recent studies have shown that GRM derived from SNPs have low error rates (<0.3%) and are able to reconstruct pedigree relationships when at least 200 SNP loci are used (Bérénos et al., 2014; Huisman, 2017). Moreover, both relatedness and heritability values estimated from a GRM are strongly correlated to those inferred using a pedigree (Bérénos et al., 2014; Huisman, 2017).

Prior to deriving our GRM, we filtered our SNPs using the R package *dartR* (Gruber et al., 2018). Using the in-built functions of *dartR*, we filtered loci based on various metrics in the following order: 1) read depth (8 – 40); reproducibility (> 0.996); call rate by loci (> 0.97) and then by individual (> 0.80); monomorphic loci; minor allele frequencies (> 0.02); Hamming Distance among loci (> 0.25) and Hardy Weinberg Equilibrium. This clean-up process resulted in a dataset of 8,438 loci with an average call rate of 98.5% (see ESM and provided code). Using these 8,438 loci we derived a GRM, which describes the proportion of the genome that is identical by descent (VanRaden, 2008). We calculated a GRM for all hatchlings using the *snpReady* R package (Granato et al., 2018) following methods described by VanRaden, 2008:

where *Z* is the centered matrix of SNP genotypes of all individuals. This is calculated from a matrix of SNP genotypes coded as -1, 0, 1 for homozygote for the reference allele, heterozygote and homozygote for the SNP allele. *pi*is the frequency of the second locus at position locus *i.* The denominator scales the GRM matrix so that the values approximates to a relatedness matrix derived from a pedigree. The GRM was then inverted for modelling fitting (see ESM and provided code).

# Statistical Analyses

All analyses were performed using *R* (Core Team, 2013). We checked the data for potential input errors using histograms, scatterplots and Cleveland plots. We fitted Bayesian linear mixed effects models (LMM) in *MCMCglmm* (Hadfield, 2010). Mass was log-transformed, and age was z-transformed. Due to natural mortality, missing measurement occasions and equipment malfunctions, we were not able to get all 16 complete measurements across age for each lizard. In total, N = 165/261 lizards had missing data. As such, missing measurement occasions were assumed as NA and we used data augmentation with *MCMCglmm* to deal with missing data (REFS). Data augmentation deals with missing data extremely well and can counter the loss of statistical power when using complete case approaches (REFS). For all models we used noninformative priors with 253,000 MCMC iterations with a burn in of 3000 and sampling the posterior every100 iterations. We ensured proper mixing by inspecting trace plots and checked samples were not strongly auto-correlated using the *autocorr* function from *coda* (Plummer et al., 2006). We report posterior means and 95% credible intervals for all parameters throughout.

Impact of Developmental Temperature on Additive Genetic Variance and Maternal Effects Across Age

First, we tested whether developmental temperature influenced the overall heritability of mass and the relative contributions of variance irrespective of age. To do this, we fitted intercept only models for each treatment group with random intercepts for additive genetic variance (*G*), maternal effects (*M*) and permanent environmental effects (*PE*) as we had repeated measures of the same individuals (Wilson et al., 2010). The model also estimated residual variance (*R*). Overall. Heritability (*h2*) of mass using this intercept (*I*) model was calculated as:

To then test how *G*, *M* and *h2* change across age, we used model selection to determine the most appropriate random effects structure for our data as we had no *a priori* knowledge of how the variance components change with age. We fitted six intercept only models with varying complexity in their random effects and compared their DIC values (Table S1). We fitted random intercepts and random slopes by including either a linear age term or both linear and quadratic age terms to partition variance across age. The best supported model included a random linear and quadratic slope for *G* and *M* and a random intercept for *PE*. (Table S1).

We therefore fitted an intercept only model using this random effect structure for each treatment group. We estimated a genetic variance-covariance matrix for each treatment (), where the diagonal elements represent the additive genetic variances for the intercept (), slope () and the quadratic () across age. The off-diagonal elements are the additive genetic covariances between the growth curve parameters, for example, is the additive genetic variance between the intercept and the quadratic slope.

Similarly, the variance-covariance matrix for dams () can be decomposed in the same manner as .

For each treatment group, we then calculated additive genetic variance at a given age using the random slope terms and their covariances as follows,

where is a specific age. Age-specific maternal effect was calculated using the same formula structure but with the relevant variance components from . Age-specific heritability is thus a ratio of all variance components at a given age . The proportion of variance explained by maternal effects (*m2*) is calculated in the same manner.

The Influence of Developmental Temperature on Growth Trajectories

We fitted three models that varied in their fixed effect structure to determine how developmental temperatures affect 1) initial mass (intercept of curve), 2) linear rate of growth (linear slope) and 3) curvature of the growth tracjetory (quadratic term). We also wanted to test for treatment differences in age at which lizards reach their maximum mass (maxima of quadratic curve). We fitted mass as the response and the most supported random effect structure we identified above. The first model included the main effect of developmental temperature and the linear and quadratic term for age (Table S2). The other two models differed in their interaction terms between developmental temperature with age and age2 (Table 2, S3). We then compared DIC values to select the best model for our data that explained changes in mass across age between the two developmental temperature treatments (Table 1).

# Results

Over two years, we collected 3002 observations of mass data for a total of 261 individuals ( = 125, = 136). The average age for ‘hot’ incubated lizards was 335.82 days (range: 0 – 711) and for ‘cold’ incubated lizards it was 384.8 days (range: 0 – 707). On average, a lizard had 11.5 measurements.

Developmental plasticity in growth trajectories in response to temperature

While a model containing an interaction between treatment and age (linear and quadratic) was best supported (Table 1; see also Table S2–3) the improvement was marginal, given DIC has a tendency to favour slightly more complex models (REFS). Linear growth rate and curvature of the growth trajectory did not differ significantly between the two developmental temperature treatments (Table 2, Table S3). Irrespective of treatment, a lizard mass increased by 1.65g for every SD units increase in age. Developmental temperature did however impact initial mass (Table 2, Fig. 1). Lizards from the ‘cold’ treatment were on average 0.030 g (0.018g – 0.041g) heavier compared to lizards from the ‘hot’ treatment (Table. 2). Larger initial masses meant that lizards from the ‘cold’ treatment reached their maximum mass slightly earlier (378.53 days, 95% CI: 353.4 – 407.16) compared to lizards from ‘hot’ treatment (408.1 days, 95% CI: 374.73 – 448.26). The population *G* and *M* matrices and other variance components are presented in Table S4.

**Table 1** Comparisons of DIC of three models with different combinations of treatment interactions with age parameters. = 3982

|  |  |  |
| --- | --- | --- |
| Formula | DIC | DIC |
| Treatment \* Age + Treatment\*Age2 | -3332.981 | 0 |
| Treatment \* Age + Age2 | -3329.495 | -3.486 |
| Treatment + Age + Age2 | -3329.845 | -3.136 |

**Table 2** Coefficient estimates from full model testing the effects of developmental treatment on mass and how mass changes with age. Bolded estimates are significantly different from zero. \* indicates that value is above zero prior to rounding. = 3982

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Estimate | Lower | Upper |
| Intercept | **-0.987** | **-1.008** | **-0.965** |
| Treatment | **-0.083** | **-0.115** | **-0.051** |
| Age | **0.501** | **0.476** | **0.526** |
| Age2 | **-0.2** | **-0.221** | **-0.18** |
| Treatment\*Age | 0.008 | -0.023 | 0.039 |
| Treatment\*Age2 | 0.023 | -0.009 | 0.056 |



**Figure 1** Model predictions of log-transformed mass over age for a random subset of 40 lizards from the two developmental temperatures. Points represent mean estimates for each lizard from the hot developmental treatment (hot) and the cold developmental treatment (blue). Thick lines represent average growth curve for each treatment. Faint grey lines are each individual’s growth curve.

The influence of developmental temperature on additive genetic variance and maternal effects across age

Overall, additive genetic variance, permanent environmental variance and heritability of growth appears to be higher in the hot developmental temperature treatment (Fig. 2, Table S4). However, there were no significant differences among treatment groups (Table S5). Treatment groups did not differ in how the relative contributions of *G* and *M* changed with age as the 95% credible intervals overlapped (Fig. 3). Initially, additive genetic variance was very low and remained constant until approximately nine months of age, after which it increased steadily (Fig. S1). Maternal effects decreased sharply upon hatching and dropped to the minimum at approximately six months (Fig. S1). From six months onwards, *M* gradually increased until lizards were approximately one year of age. Consequently, *h2* peaked at approximately six months and slowly decreased until nine months where it showed a gradual increase again (Fig. 3A). In contrast, maternal effects explained a majority of the total phenotypic variance upon emergence (*M2*) and at approximately six months of age (Fig. 3B). The *G* and *M* matrices and other variance components for each treatment group are presented in Table S6-S7.



**Figure 2** Pie charts depicting the overall relative contributions of mass variance for the hot (n = 126) and cold (n =136) developmental treatment group irrespective of age. Point estimates and 95% credible intervals are presented in Table S4. There were no significant differences in variance components between developmental temperature treatments.



**Figure 3** Scatterplot showing the relationship of the how heritability (*h2*) and the proportion of total variance explained by maternal effects (*M2*) changed with age for the hot developmental treatment (n = 125, red) and the cold developmental treatment (n = 136, blue). Points represent estimates generated from the posterior distribution of the variance-covariance matrix, thin lines represent the 95% credible intervals, thick lines represent the mean for each treatment group.

# Discussion

Developmental temperature did not affect growth rate or the shape of the growth trajectory. Nonetheless, lizards incubated at a hot temperature emerged weighing less than lizards incubated at a cold temperature. Irrespective of age, the relative genetic and maternal contributions to variance did not differ between temperature treatments. Heritability first peaked at six months owing to a drop in maternal effects then it gradually increased due to a rise in additive genetic variance around nine months of age. We predicted that maternal contributions to phenotypic variance should dissipate after hatching as lizards experience the environment on their own. This prediction was partially supported. Indeed, the proportion of variance explained by maternal effects continued to decrease upon hatching but subsequently rose again at approximately six months. Our study suggests that variation in mass is largely driven by environmental sources and both genetic and non-genetic sources of maternal effects could influence the evolutionary potential of body mass over time.

1. Developmental temp did not affect growth trajectory
   1. Hot smaller than cold but not evidence of compensatory growth
   2. Little evidence that by being larger at hatching, you reach sexual maturity earlier
   3. Discuss implications on life history if environment determines size and timing of sexual maturity
2. Overall very little additive genetic variation across both treatments, environmental sources contributed most to variance in body size
   1. Genetic variation may only increase outside of constant lab conditions
   2. From 9 months onwards individuals were in shared enclosures and may have to compete for resources and coincided with an increase of Va
   3. Need long term studies in semi natural conditions
3. Maternal effects did indeed decline upon hatching but increased later in life
   1. Acknowledge that we don’t know what is driving this pattern
   2. Genetic and non-genetic contributions of maternal effects

# Conclusion

# Author contributions

# Data accessibility

Datasets and code used to generate results of this study is accessible via Open Science Framework (DOI: XXXXXXXXXXX)

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