# Title

# Abstract

# Keywords

# Introduction

For decades, early life experiences have been recognised as an important evolutionary process as they can have a lasting effect on an organism’s phenotype. Theoretical and experimental work suggests that developmental plasticity is adaptive when early life cues predict later life environments. The ability for embryos to pre-emptively match themselves to the environment will be important for ectotherms as mean temperatures as well as temperature variability continue to increase. Oviparous reptiles typically deposit their eggs in an untended nest, leaving embryos to develop in the microhabitat chosen by the mother. The incubation conditions will go on and shape the hatchling’s phenotype and impact its fitness and survival.

There is a wealth of studies that illustrate that incubation temperatures can have a profound influence on the hatchling’s phenotype.

Growth rate is a key life history trait that can influence an individual’s fitness and survival. Fast growth rates enable individuals to reach sexual maturity earlier but may also compromise their lifespan compared to those with a slow growth rate (ref). The temperature-size rule describes the widespread phenomenon that individuals or species that experience cold environments tend to grow more slowly and mature at the larger size compare to those exposure to hot environments. In order to attain a large body size in cool environments, individual must prolong growth and. Thermal plasticity in body size has been documented in

[What are the effects of incubation temperature on growth rate? Temperature-size rule, examples, are they all mean sizes? Need to know how trajectories are altered because responses are not linear, comparisons/differences may be time/age specific which has implications on…?]

Evolutionary changes in growth can occur if it is heritable and there is sufficient additive genetic variation for selection to act on. [Examples]

Despite there being some evidence that suggests that growth can evolve, we do not currently know how heritability and additive genetic variance of growth varies with environment. [What are the theoretical expectations? How does temperature/environment changeAdditive genetic variance?]. Moreover, our understanding on how heritability and genetic variance changes with age is limited.

Here we investigated how incubation temperatures affects growth trajectories and the additive genetic variance of growth using an oviparous skink (*Lampropholis delicata*). Using 8,433 SNP markers, we derived a genomic relatedness matrix to [novel stuff] address the following questions 1) How does developmental temperature affect growth trajectories? (initial mass, linear growth rate and curvature of trajectory) 2) Does developmental temperature affect the relative contributions of growth variance? 3) How does developmental temperature change the relative contributions of growth variance as individuals age?

# 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).

## Incubation treatment

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 incubation treatments. We used two incubators to precisely control the temperature of eggs (LabWit, ZXSD-R1090). The ‘hot’ incubation treatment was exposed to a mean temperature of 29ºC whereas the ‘cold’ incubation 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. We were unable to balance sex across enclosures as hatchlings were still too young to determine their sex, however sex was later determined using SNP markers and accounted for in statistical analyses (see below).

## 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.

DNA was extracted from tissue samples using a Qiagen DNeasy Blood and Tissue Kits following the manufacturer’s instructions. Diversity Arrays Technology (DArT) combines next generation sequencing platforms and genome complexity reduction methods (Kilian et al., 2012) to select the most appropriate method for *L.delicata.*

[Insert section from DArT here on complexity reduction methods, DArT tested four methods in *Gambusia* so it’s unclear what method was used for us].

Sequences from all lanes were then processed using DArT specific pipelines. The main pipeline filters our ‘poor’ quality sequences [Details needed from DArT]. For our samples, this filtering process by DArT resulted in a total of 185,963 SNPs. ]

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 generalised mixed effects models in ‘MCMCglmm’ (Hadfield, 2010). In all models, mass was log-transformed and age was z-transformed.

## Missing data augmentation using MCMCglmm

We wanted to maximise the number of individuals in the dataset and not exclude lizards died before we attained enough data for their full growth curves (n = 165). We made use of the built-it capabilities of missing data augmentation ‘MCMCglmm’. We achieved this by creating empty rows of data for lizards that we had < 16 measurements (n = 165). First, we determined how many growth measurements a given lizard is missing (mean missing = 6.32). Based on this information, we then generated additional rows of data for each lizard. For each additional row of data, we set a 35-day interval for ‘days since hatch/age’ relative to the last known growth measurement of a given lizard. Mass, SVL, lnMass and lnSVL are set as NA in these additional rows. A total of 608 empty rows were added to this dataset. We fitted the final models with the augmented dataset. See ESM and provided code for more details.

## Model fitting and selection

Given that we have no *a priori* knowledge of how the relative contributions of growth variance changes with age, we fitted six intercept only models with varying complexity in their random effects and compared their DIC values to select the most appropriate random effects structure for our analyses. Lizard identity was included twice to partition out permanent environmental effects (*PE*) as we had repeated measures of the same individuals (Wilson et al., 2010). The best supported model for our data included a random quadratic slope for lizard identity *G*) and dam identity (*M)* and a random intercept for *PE*. (Table S1). We therefore used this random effect structure for subsequent analyses. For model details see ESM and provided code.

We tested whether developmental temperatures impacted growth trajectories by fitting mass as the response and included an interaction between age and age2 with incubation treatment as fixed effects (full model). We removed any non-significant interactions in a stepwise manner and compared DIC values with the full model (Table 1).

To test whether incubation treatment influence the overall heritability of mass and the relative contributions of variance (regardless of age), we fitted intercept only models for each incubation treatment with random intercepts for lizard identity, dam identity and *PE* effects. Heritability of mass was calculated as:

where is the additive genetic variance for all lizards, is the variance attributed to dams, is the permenent environmental variance and is the residual variance.

We were also interested in quantifying the changes in heritability and the relative contributions of growth variance with age for each incubation treatment. To address this, we fitted an intercept only model for each treatment group with a random quadratic slope for lizard identity and dam identity and a random intercept for *PE* effects. We estimated a genetic variance-covariance matrix for each treatment (), where the diagonal elements are the additive genetic variances for the intercept (), slope () and the curvature () of the growth curve. 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 curvature.

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

We expected that the relative contributions of variance to change with age and treatment differences could manifest at specific ages. For each treatment group, we calculated additive genetic variance at a given age using the random slope terms and their covariances as follows,

where is an age value that is substituted throughout the equation. Age-specific dam variance is 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 ,

For all models we used uninformative priors which ran for 253,000 iterations with a burn in of 3000 and a thinning interval of 100 (see provided code). 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 represent posterior means and 95% credible intervals in our results below.

# 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 (range: 0 – 711) and for cold incubated lizards it was 384.8 (range: 0 – 707). On average, a lizard had 11.5 measurements (range: 1 – 18).

## Do growth rate curves differ between incubation treatments?

The model containing interactions between treatment and the linear and quadratic components of the growth curve was best supported by DIC values (Table. 1). Model coefficients for the other two models are presented in Table S2-3. Incubation temperature did not affect the linear or the curvature component of growth curve (Table 2, Table S2). Incubation temperature did however influence initial mass (intercept of growth curve) (Table 2, Fig. 1). With all else held equal, the initial mass of lizards from the cold treatment were on 0.030 g (0.018g – 0.041g) heavier compared to lizards from the hot treatment (Table. 2).

We calculated the age at which lizards reach their maximum mass for both treatments and found that there was a trend for cold incubated lizards to their max mass at an earlier age, however credible intervals for both treatment groups overlap (cold treatment age at maximum mass: 378.53 [353.4 – 407.16], hot treatment age at maximum mass: 408.1 [374.73 – 448.26]. There were no treatment differences in maximum mass (cold treatment maximum mass: 0.51 [0.49 – 0.54], hot treatment maximum mass: 0.5 [0.46 – 0.54]).

**Table 1** Comparisons of DIC of three models with different combinations of treatment interactions with growth curve parameters.

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

**Table 2** Coefficient estimates from full model testing the effects of incubation 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. = 3002

|  |  |  |  |
| --- | --- | --- | --- |
| 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 |
| Vadditive genetic intercept | **0.01** | **0.004** | **0.017** |
| Vadditive genetic slope | **0.015** | **0.011** | **0.02** |
| Vadditive genetic curvature | **0.015** | **0.011** | **0.02** |
| Covadditive genetic intercept - slope | 0\* | -0.004 | 0.003 |
| Covadditive genetic intercept - curvature | **-0.007** | **-0.011** | **-0.004** |
| Covadditive genetic slope - curvature | **0.011** | **0.007** | **0.015** |
| Vmaternal intercept | **0.002** | **0\*** | **0.006** |
| Vmaternal slope | **0.005** | **0.001** | **0.01** |
| Vmaternal curvature | **0.001** | **0\*** | **0.003** |
| Covmaternal intercept - slope | -0.001 | -0.004 | 0.002 |
| Covmaternal intercept - curvature | -0.001 | -0.004 | 0 |
| Covmaternal slope - curvature | 0\* | -0.002 | 0.003 |
| Vpe | **0.004** | **0.001** | **0.008** |
| Vresiduals | **0.016** | **0.015** | **0.017** |

A screenshot of a cell phone

Description automatically generated

**Figure 1** Model predictions of log-transformed mass over age for a random subset of 40 lizards from our experiment. Orange points represent mean estimates for lizards from the hot incubation treatment, blue points represent mean estimates for lizards from the cold incubation treatment (blue). Thick lines represent average growth curve. for each treatment. Faint grey lines are each individual’s growth curve.

## How does incubation treatment impact the relative contributions of mass variance and heritability of mass? How does these variance components change with age?

Overall, additive genetic variance, permanent environmental variance and heritability of growth appears to be higher in the hot incubation treatment (Fig. 2A). However, there were no significant differences among incubation treatment groups (Table 3-5). Both treatment groups showed similar patterns in how the relative contributions of growth variance changed with age (Fig. 2B). Additive genetic variance was relatively constant until approximately nine months of age, after which it increased steadily (Fig. 2B). Maternal variance and total phenotypic variance (which includes permanent environmental variance) decreased sharply upon hatchling emergence and drops to the minimum at approximately six months. Maternal variance and total phenotypic variance rapidly increased until lizards were approximately one year of age. Consequently, heritability peaked at approximate six months and slowly decreased until approximately nine months where it showed a gradual increase

**Figure 2** A) Pie charts depicting the overall relative contributions of mass variance for both treatment groups. B) Scatterplot showing the relationship of the how variance component and heritability changed with age for lizards from the hot incubation treatment (n = 125, orange) and cold incubation 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.

**Table 3** Treatment comparisons of additive genetic variance, maternal variance, permanent environmental variance and heritability. Bolded estimates are significantly different from zero. \* indicates that value is above zero prior to rounding.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variance component | Hot incubation treatment | | | Cold incubation treatment | | |
|  | Estimate | Lower | Upper | Estimate | Lower | Upper |
| Vadditive genetic | **0.014** | **0\*** | **0.054** | **0.008** | **0\*** | **0.026** |
| Vmaternal | **0.004** | **0\*** | **0.017** | **0.004** | **0\*** | **0.014** |
| Vpe | **0.027** | **0\*** | **0.058** | **0.008** | **0\*** | **0.024** |
| Vresidual | **0.251** | **0.232** | **0.272** | **0.275** | **0.255** | **0.294** |
| h2 | **0.048** | **0\*** | **0.18** | **0.027** | **0\*** | **0.085** |

**Table 4** Model coefficients from an intercept only animal model of hot incubation treatment group (n = 125) to estimate variance components of mass. Bolded estimates are significantly different from zero.

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Estimate | Lower | Upper |
| Intercept | **-1.337** | **-1.519** | **-1.168** |
| Vadditive genetic intercept | **0.009** | **0.002** | **0.02** |
| Vadditive genetic slope | **0.015** | **0.008** | **0.026** |
| Vadditive genetic curvature | **0.011** | **0.005** | **0.018** |
| Covadditive genetic intercept - slope | -0.001 | -0.007 | 0.005 |
| Covadditive genetic intercept - curvature | **-0.007** | **-0.014** | **-0.002** |
| Covadditive genetic slope - curvature | **0.008** | **0.003** | **0.015** |
| Vmaternal intercept | **0.089** | **0.011** | **0.242** |
| Vmaternal slope | **0.295** | **0.201** | **0.426** |
| Vmaternal curvature | **0.031** | **0.019** | **0.047** |
| Covmaternal intercept - slope | **0.151** | **0.051** | **0.293** |
| Covmaternal intercept - curvature | **-0.048** | **-0.094** | **-0.016** |
| Covmaternal slope - curvature | **-0.093** | **-0.137** | **-0.063** |
| Vpe | **0.006** | **0.001** | **0.01** |
| Vresiduals | **0.019** | **0.017** | **0.02** |

**Table 5** Model coefficients from an intercept only animal model of the cold incubation treatment group (n = 136). Bolded estimates are significantly different from zero.

|  |  |  |  |
| --- | --- | --- | --- |
| Variable | Estimate | Lower | Upper |
| Intercept | **-1.405** | **-1.539** | **-1.259** |
| Vadditive genetic intercept | **0.008** | **0.002** | **0.017** |
| Vadditive genetic slope | **0.015** | **0.009** | **0.022** |
| Vadditive genetic curvature | **0.013** | **0.009** | **0.019** |
| Covadditive genetic intercept - slope | 0 | -0.004 | 0.006 |
| Covadditive genetic intercept - curvature | **-0.006** | **-0.012** | **-0.002** |
| Covadditive genetic slope - curvature | **0.01** | **0.005** | **0.015** |
| Vmaternal intercept | **0.2** | **0.077** | **0.361** |
| Vmaternal slope | **0.275** | **0.191** | **0.393** |
| Vmaternal curvature | **0.049** | **0.033** | **0.072** |
| Covmaternal intercept - slope | **0.23** | **0.131** | **0.358** |
| Covmaternal intercept - curvature | **-0.096** | **-0.15** | **-0.055** |
| Covmaternal slope - curvature | **-0.114** | **-0.163** | **-0.079** |
| Vpe | **0.006** | **0.002** | **0.009** |
| Vresiduals | **0.014** | **0.013** | **0.015** |

# Discussion

# Conclusion

# Author contributions

# Data accessibility

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

# Acknowledgements