Title page:

Impact of developmental temperatures on the repeatability of thermal plasticity in metabolic rate

Fonti Kar[1], Shinichi Nakagawa[1], Daniel W.A. Noble[2]

1 *School of Biological Earth and Environmental Sciences, Ecology and Evolution Research Centre, University of New South Wales, Sydney, NSW, Australia*

2 *Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT, Australia*

Corresponding author: Fonti Kar

Correspondence email: fonti.kar@gmail.com

Keywords

reaction norm, repeatability, metabolic rate, incubation temperature, thermal performance curve, thermal sensitivity, phenotypic flexibility

Total word count (excluding references, tables and figures): 5269

Number of figures: 3

Number of tables: 1

Items for online appendix: Supplementary Materials (2 Figures, 14 Tables)

Abstract

Phenotypic plasticity is an important mechanism that allows populations to adjust to changing environments. Plastic responses induced by early life experiences can have lasting impacts on how individuals respond to environmental variation later in life (i.e., reversible plasticity). Developmental environments can also influence repeatability of plastic responses thereby altering the capacity for reaction norms to respond to selection. Here, we compared metabolic thermal reaction norms in lizards (*Lampropholis delicata*) that were incubated at two developmental temperatures (n_{cold} = 26, n_{hot} = 25). We repeatedly measured individual reaction norms across six acute temperatures 10 times over ~3.5 months (n_{obs} = 3,818) to estimate the repeatability of average metabolic rate (intercept) and thermal plasticity (slope). The intercept and the slope of the population-level thermal reaction norm did not change with developmental temperatures. Repeatability of average metabolic rate was, on average, 10% lower in hot incubated lizards and was stable across acute temperatures. The slope of the reaction norm was moderately repeatable (R = 0.44, 95% CI = 0.035 – 0.93) suggesting that individual metabolic rate changed consistently with acute temperature, although credible intervals were quite broad. Importantly, reaction norm repeatability did not depend on early developmental temperature. Our work implies that thermal plasticity has the capacity to evolve, despite there being less consistent variation in metabolic rate under hot environments. This capacity for thermal plasticity to evolve will be increasingly more important for terrestrial ectotherms living in changing climate.

Introduction

A substantial amount of variation in an individual's phenotype is determined by formative processes experienced throughout embryonic development. Environmental perturbations during this critical period can have persistent effects on an individual's physiology, morphology, behaviour and life history (Noble et al. 2018; Eyck et al. 2019; O'Dea et al. 2019). Developmental shifts in phenotypes may be adaptative if it allows organisms to better cope in similar environments later in life (Beldade et al. 2011). However, environment-phenotype mismatches can occur when developmental cues fail to predict later life conditions (Auld et al. 2010; Bonamour et al. 2019). A multitude of traits throughout an animal's life are labile; reversibly responding to environmental change. Reversible plasticity in phenotypic traits allows individuals to adjust to acute changes in their surroundings (Piersma and Drent 2003), and can broadly be classified into two categories, acclimation and phenotypic flexibility (Piersma and Drent 2003; Havird et al. 2020). Acclimation is generally a slower form of reversible plasticity that involves remodelling of physiological systems from chronic exposure to a particular environment (Seebacher 2005). Phenotypic flexibility, in contrast, describes short-term changes in traits that are induced by acute environmental exposure, such as changes in metabolic rate in response to acute temperature (Piersma and Lindström 1997; Piersma and Drent 2003).

Reversible plasticity may be able to alleviate the costs associated with phenotype mismatches induced by early life environments (Angilletta Jr et al. 2003; Ghalambor et al. 2007). When environments shift predictably, flexibility in the phenotype would be advantageous because individuals can compensate for the effects of prevailing conditions to avoid discrepancies between the environment and the phenotype (Botero et al. 2015).

However, reversible plasticity can change depending on early environmental conditions and might alter phenotypic responses to environmental variation (Beaman et al. 2016). The interaction between early- and late life plasticity has been supported by a few studies that show developmental differences in plasticity for a variety of traits including mitochondrial function (Shama et al. 2014), metabolic rate (Seebacher et al. 2014) and locomotor performance (Kazerouni et al. 2016). However, these studies solely focus on the developmental effects on acclimation, whereas the influence on phenotypic flexibility and variability of plastic responses is poorly known.

It has long been recognised that individuals vary in their plasticity, with some responding more flexibly than others (Nussey et al. 2007; Dingemanse and Wolf 2013). Consistent among individual variation in plasticity may be heritable, but nonetheless, provides the phenotypic substrate for selective forces to act upon (Nussey et al. 2007; Araya-Ajoy and Dingemanse 2017). Developmental environments, however, can influence phenotypic variation available for selection (Sultan and Stearns 2005). For example, zebra finches (*Taeniopygia guttata*) that experience nutritional stress as nestlings weigh less and have reduced growth rates contributing to increases in the repeatability of metabolism and behavioural traits (Careau et al. 2014a). Consistent among individual variation in plasticity has also been reported in other labile traits including aggressiveness in great tits (*Parsus major*) (Araya-Ajoy and Dingemanse 2017), explorative behaviour in chickadees (Thompson et al. 2018) and metabolic rate in amphipods (Réveillon et al. 2019). Whether developmental environments affect consistent variation in plasticity *per se* is still not well understood. Identifying the factors that impact repeatability is necessary for understanding the evolution of plasticity in changing environments.

Energy metabolism is a key fitness related trait that is both consistently different among individuals and highly labile within individuals (Nespolo and Franco 2007; Norin and Metcalfe 2019). All organisms require energy for growth, maintenance and reproduction (Careau et al. 2014c). Numerous studies have investigated the influence of various developmental environments, such as temperature (Gangloff et al. 2015; Noble et al. 2018), ultra-violet (UV) exposure (Kazerouni et al. 2016), and dietary restriction (Careau et al. 2014a) on metabolic rate, however, the impacts on plasticity of metabolic rate is not well established (but see Seebacher et al. 2014). Developmental environments are expected to influence metabolic plasticity, possibly through modifications in metabolic enzymes or cellular membrane structure that influence their function in different environments (Angilletta Jr 2016). Such changes imply that tolerance to environmental perturbations may be determined by the developmental environment a given cohort experiences. Furthermore, if repeatability of metabolic plasticity is also affected, then the capacity to respond to selection might also depend on early life conditions. Understanding how early life environments shape metabolic plasticity will be important for ectotherms where metabolic rate is closely intertwined with prevailing environmental conditions.

Here we employed a 'reaction norm approach'(sensu Via et al. 1995) to examine the impact of developmental temperature on metabolic rate plasticity in an oviparous skink (*Lampropholis delicata*). Specifically, we were interested in testing whether developmental temperature affects the shape and repeatability of metabolic thermal reaction norms. Over 3.5 months, we repeatedly measured routine metabolic rate at six temperatures for lizards (n_{obs} = 3,818) that hatched from two incubation treatments (total individuals: n_{hot} = 25, n_{cold} = 26) to address the following key questions: (1) How does developmental temperature change the intercept and slope of the thermal reaction norm?; (2) How does the repeatability of

metabolic plasticity (i.e. slope of the reaction norm) change with developmental temperature? (3) Do developmental temperature treatments differ in their repeatability of metabolic rate (intercept) at each acute temperature (i.e. temperature-specific repeatability)? Our experimental approach provides important insights into how development environments mediate the capacity for ectotherms to respond to thermal variation during early stages of life and the energetic consequences of such effects.

Materials and Methods

Lizard collection and Husbandry

We established a breeding colony of adult *L. delicata* ($n_{females}$ = 144, n_{males} = 50) using wild individuals collected across three sites throughout the Sydney region between 28 August and 8 September 2015 (UNSW Kensington Campus: -33.92, 151.24; Sydney Park: -33.91, 151.18, Macquarie Park: -33.77, 151.10). Three females were housed with a single male in opaque plastic enclosures measuring 35cm × 25cm × 15cm (L × W × H). Enclosures were kept under UV lights on a 12 hours light : 12 hours dark cycle in a temperature-controlled room set to 24ºC. Lizards had access to a heat lamp that elevated temperatures on one side of the enclosure to 32 ºC. Each enclosure was lined with newspaper and lizards had constant access to water and tree bark was used as refuge. Adult lizards were fed medium sized crickets (*Acheta domestica*) *ad libitum* dusted with calcium powder and multi-vitamin every two days. From the beginning of the egg laying season (October of each year), we replaced the newspaper 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 and vermiculite boxes were sprayed gently with water every other day to maintain a relatively humid environment. From October to November, vermiculite boxes were checked every day for eggs. 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 Manipulations

Eggs were collected between October 2017 – March 2018. When eggs were discovered, 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 0.01 mm. Following measurements, each egg was placed in a plastic cup (80 ml) containing 3 g of vermiculite and 4 g of water and covered using cling wrap which was secured by an elastic band. Eggs from each clutch were pseudo-randomly assigned to one of two fluctuating incubation 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. Both incubators fluctuated +/- 3ºC the mean temperature over a 24-hour period. 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 hatchlings. On average, the incubation duration for the 'hot' treatment was 30 days (SD = 1.40, range = 27 - 33) days and 47.7days (SD = 5.90, range = 25 - 53) for the 'cold' treatment.

Planned Missing Data and Metabolic Rate at Different Temperatures

Metabolic measurements commenced in April 2018 and continued until August 2018. At the beginning of measurements, hatchlings were on average 88.68 days old (SD = 23.75, range = 26 - 131). Due to the scale of our experiment, we used closed-system respirometry instead of flow-through respirometry. We quantified routine metabolic rate (hereafter referred to as metabolic rate [MR]) as our measurements likely included the energetic costs of random movements (Withers 1992; Mathot and Dingemanse 2015). MR was measured as the volume

of CO_2 production per unit time ($\dot{V}_{CO_2}$ mL min^{-1}) as CO_2 production is less susceptible to fluctuations in water vapour and more feasible to detect in smaller organisms (Lighton 2008; Tomlinson et al. 2018). Nonetheless, CO_2 production was strongly correlated with O_2 consumption (r =0.81, $p < 2.2e^{-16}$) with RQ values averaging 0.77 (SD = 0.41, n_{obs} = 198). Due to logistical constraints, lizards were randomly assigned to one of two blocks for MR measurements (block 1: n =26, block 2: n = 25). Each block was measured two days apart. We sampled lizards once a week for two-weeks consecutively and then allowed them to rest for one week before the next set of measurements. Each week of measurements was considered a sampling session (ten sampling sessions in total over the course of 14 weeks). We used the same incubators described above to precisely control the temperature at which MR measurements were taken (+/- 1ºC).

Metabolic rate was measured at 24ºC, 26ºC, 28ºC, 30ºC, 32ºC and 34ºC in a randomised order. However, at each sampling session we intentionally missed measurements at two randomly selected temperatures using a planned missing data design (Nakagawa 2015; Noble and Nakagawa 2018). Missing data was imputed during analysis (see Statistical Analyses). At ~06:00, lizards were gently encouraged into an opaque respiratory chamber and then weighed. After which, chambers were placed inside preheated incubators set at the randomised temperature for 30 minutes to allow body temperatures to equilibrate. The lids of the chambers were left ajar during this time to minimise CO_2 build up. After 30 minutes, each chamber was flushed with fresh air and sealed. A 3 mL 'control' air sample was immediately taken via a two-way valve to account for any residual CO_2 that was not flushed from the chambers. The chambers were left in the incubator at the set temperature for lizards to respire for 90 minutes. After this time, two replicate air samples (3 mL) were taken from each chamber in order to estimate the change in CO_2 relative to the control sample. Two samples

were taken so we could explicitly estimate measurement error (see Statistical Analyses, Ponzi et al. 2018). Chambers were then reopened and flushed with fresh air before being placed back into the incubator for the second measurement temperature (2 temperatures / day) following the same procedure approximately two hours later. Overall, this sampling design enabled us to characterise the thermal reaction norm (four out of six temperatures for our planned missing data design) for each lizard 10 times while accounting for measurement error. This resulted in n = 4,080 measurements of MR ([2 air samples x 4 temperatures] × 10 sampling sessions = 80 samples per lizard). However, additional missing data from equipment malfunction or human error meant that our total sample size was n = 3,818.

All air samples were injected into the inlet line of a Sables System FMS (Las Vegas NV, USA) with the flow rate set to 200 mL min^{-1} to measure $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$. Water vapour was scrubbed from the inlet air with Drierite. Output peaks were processed using the R package 'metabR' (https://github.com/daniel1noble/metabR). The rate of CO_2 produced by an individual was calculated following (Lighton, 2008):

$$\dot{V}_{CO_2}\text{mL}\,min^{-1} = \frac{\Delta\%CO_2 \times (V_{chamber} - V_{lizard})}{t}$$

where $\Delta\%CO_2$ is the maximum percentage of CO_2 in air sample above baseline, which was corrected by subtracting any 'residual' CO_2 from the initial flush from the larger of the two air samples; $V_{chamber}$ is the volume of the chamber (70 mL) and V_{lizard} is the volume of the lizard. We used the mass of the lizard as a proxy for its volume (1 g = 1 ml) because of their high correlation and increased accuracy and precision in mass measurements (Friesen et al. 2017; Kar et al. 2021), and *t* is the duration of time in minutes after where the chamber has been sealed and the first air sample was taken (90 minutes).

Statistical Analyses

We fitted Bayesian linear mixed effect models in *R* (Core Team 2013) using the package 'brm*s'* (Bürkner 2017). Metabolic rate was log transformed then body mass was log transformed and then z-transformed. Age and temperature were z-transformed so parameter estimates of main effects and interaction terms were more interpretable (Schielzeth 2010). Our planned missing data design resulted in random missingness across temperatures (36% missingness in MR and body mass) The 'brms' package is capable of performing model-based data imputation. As such, we performed imputation during model fitting in all of our analyses. Model-based imputation not only retains the hierarchical structure of the dataset but also increases statistical power (P. Bürkner, personal communication 25 October 2020, Nakagawa, 2015). Sensitivity analyses suggest that models with imputed data resulted in similar conclusions to complete case analyses. However, we present results from the imputation analysis in the main text as parameter estimates were more precise (Supplementary Materials). For all models we used default priors which are presented in Table S1. We ran four Markov Chain Monte Carlo (MCMC) chains; taking 800 samples from the posterior distribution after discarding the first 1,500 iterations. This gave a total of 3,200 samples from the posterior distribution across all chains. We ensured chains were mixing by inspecting trace plots and checked that scale reduction factors were less than 1.01, which indicates that all chains had converged. Throughout we report posterior means and 95% credible intervals for all parameters. All data and code to reproduce our results are provided (see Data Accessibility).

To test whether developmental temperatures changed the shape of reaction norms, we fitted a full model with MR as the response and temperature, treatment and an interaction between treatment and temperature as predictors. The model also included a random intercept for

lizard identity and sampling session. We wanted to account for measurement error in all our models as it may conflate parameter estimates (Ponzi et al., 2018). Using the two replicate air samples, we estimated measurement error variance by including a nested random effect of lizard identity, sampling session and temperature in all our models (e.g. ID001_session1_temp24). This nested random effect (hereafter referred to as measurement error) estimates the variance attributed to differences among replicates. While we show in a previous study that measurement error can vary by temperature (Kar et al. 2021), here we assumed that measurement error was constant across temperatures by fitting it as a random intercept as estimating a random slope resulted in model convergence issues. Heterogeneous residual variance across temperatures can also influence parameter estimates (Careau et al. 2014a). However, WAIC values indicated that a heterogeneous residual variance model was not well supported, therefore homogenous variance was used in all models (Table S2). Acclimation can influence metabolic plasticity and its effects can take place throughout the course of our study. Unfortunately, it was not possible to measure MR at hatching. However, we still tested whether there were treatment differences in thermal reaction norms in the first sampling session (~2.5 months of age) where acclimation effects were likely to have the weakest effect.

We estimated adjusted repeatability of the reaction norm slope (R_{slope}) in each developmental temperature treatment by fitting separate models for each treatment group. MR was fitted as the response and temperature, body mass and age (days since hatching) as predictors. We included lizard identity, measurement error and a nested random effect of individual identity and sampling session (hereafter referred to as series, Araya-Ajoy et al. 2015). Lizard identity estimates among individual variance, whereas series partitions variance within individual across all sampling sessions which allows the estimation of slope repeatability. A random

temperature slope was estimated for lizard identity and series. The repeatability of the slope is calculated as the proportion of total variance in slopes explained by among individual differences (Araya-Ajoy et al., 2015):

$$R_{slope} = \frac{V_{I,slope}}{(V_{I,slope} + V_{series,slope})}$$

where: $V_{I,slope}$ is the among-individual variance in the temperature slope term and the $V_{series,slope}$ is the within-individual variance in the temperature slope.

We estimated adjusted repeatability of average metabolic rate (i.e. intercept of the reaction norm) at each acute temperature by fitting separate models for each treatment group. Similar to above, MR was included as the response and temperature, body mass and age as predictors. We included lizard identity, sampling session and measurement error as random intercepts and temperature as a random slope for lizard identity. We calculated among individual variance in metabolic rate at each temperature I_t following Schielzeth and Nakagawa (2020):

$$I_t = V_I + (t^2.V_S) + (2t.Cov_{I,S})$$

where V_I is the among individual variance in intercepts, t is the specific temperature at which repeatability is calculated for, V_S is the among individual variance in slope and $COV_{I,S}$ is the covariance between the intercept and slope at the among individual level. Temperature specific repeatability (R_t) is then calculated as follows:

$$R_t = \frac{I_t}{(I_t + V_{session} + V_e)}$$

where: $V_{session}$ is the variance due to sampling session and V_e is residual variance.

We also wanted to estimate overall repeatability of average metabolic rate across all acute temperatures. We therefore fitted the same model as above for each treatment, but we omitted

290 the random temperature slope for lizard identity, this estimates an average among individual
291 variance across all acute temperatures. Similarly, we calculated repeatability as per the
292 equation above but using just the single estimate of among individual variance.
293
294 In order to test for differences in repeatability among the two developmental temperatures,
295 we calculated contrasts by subtracting the posterior distributions of repeatability estimates of
296 the cold treatment from the hot treatment (Hot – Cold). To test whether the magnitude of
297 differences among treatments were significant by chance, we calculated probabilities of
298 direction (*pd*) using the package 'bayestestR'(Makowski et al. 2019b). The probability of
299 direction is calculated relative to the posterior median and ranges from 50 -100%. The value
300 of *pd* describes whether an effect is either positive or negative as it is always relative to the
301 sign of the median (Makowski et al. 2019a). If the median is positive, then *pd* describes the
302 proportion of the posterior distribution that is also positive (Makowski et al. 2019a). A *pd*
303 value of 95% can be interpreted as the effect is positive with a probability of 95%.
304

Results

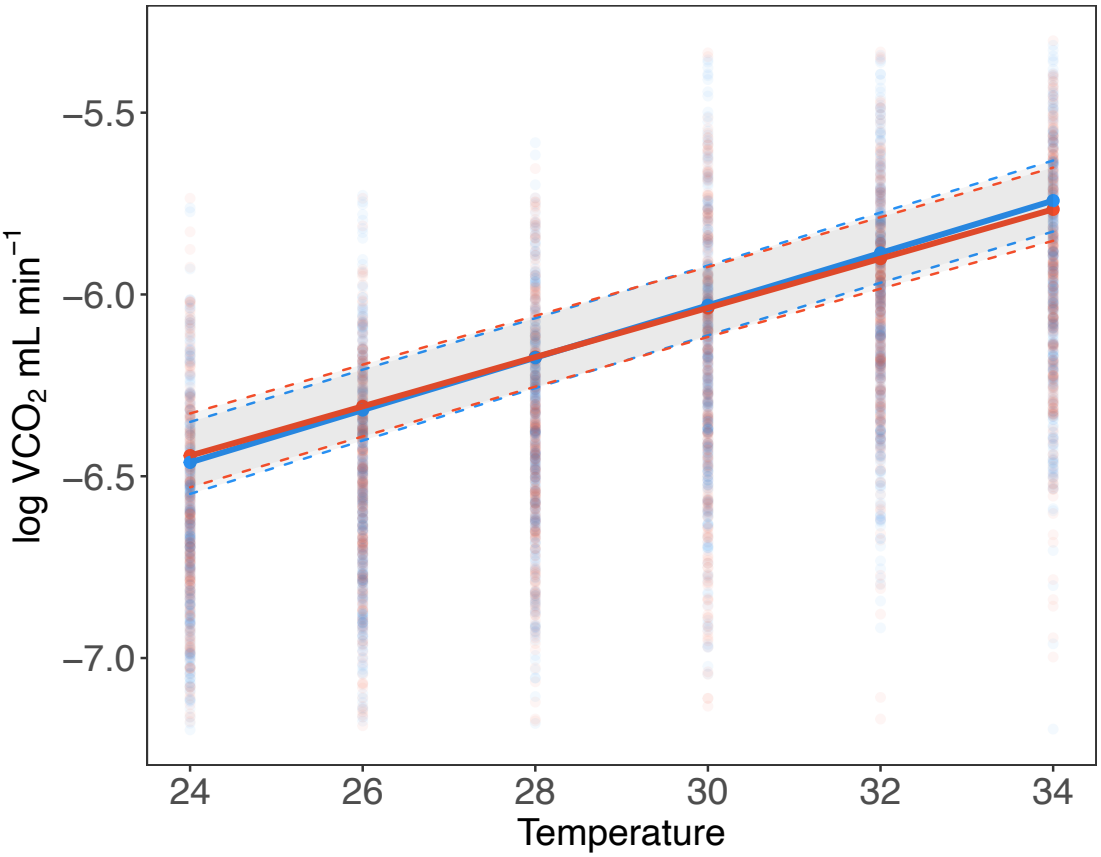


Figure 1 Predicted thermal reaction norm of metabolic rate (VCO_2 min^{-1} g^{-1}) for the 'cold' developmental temperature group (blue line, $n_{lizards}$ = 26) and the 'hot' developmental temperature group (red line, $n_{lizards}$ = 25) Points are raw data and are coloured according to treatment groups, n_{obs} = 3,818. Dashed lines represent the upper and lower bounds of 95% credible intervals.

We found no evidence to suggest that metabolic rate or its response to acute temperature was influenced by early developmental temperature (Fig. 1, Table 1, Supplementary Materials Section 1 Table S3-5). Congruently, there were no treatment differences in thermal reaction norms at the first sampling session when acclimation effects are likely to have the least effect

(Supplementary Materials, Section 1). We therefore refitted the model with just the main effects (Supplementary Materials, Section 1, Table S4-S5). Across all models, temperature and body mass had positive effects on metabolic rate (Table 1, Supplementary Materials, Section 1, Table S3-5). Nonetheless, reaction norm slopes were significantly repeatable, albeit estimated with a large degree of error. Repeatability of slopes (R_{slope}) did not depend on developmental temperature treatments (Hot: R_{slope} = 0.42, 95% CI: 0.04 – 0.91; Cold: R_{slope} = 0.46, 95% CI: 0.03 – 0.95; *pd* = 53.5%, Fig. 2, Supplementary Materials, Section 2). A *pd* value of 53.5% indicates that there is roughly equal probability that the difference in R_{slope} is positive or negative, indicating little difference among treatment groups.

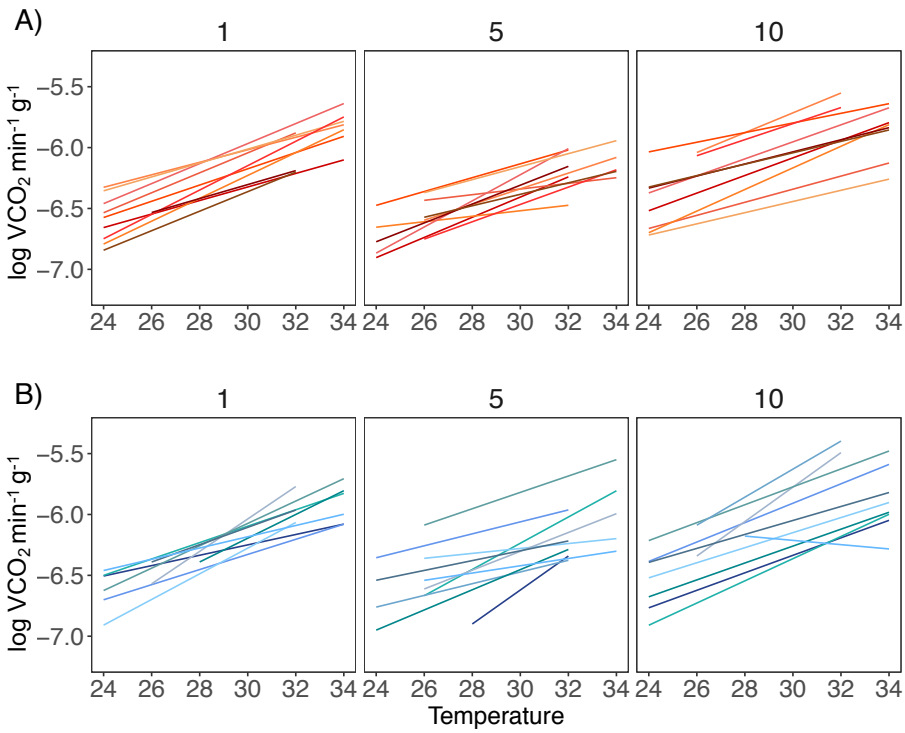


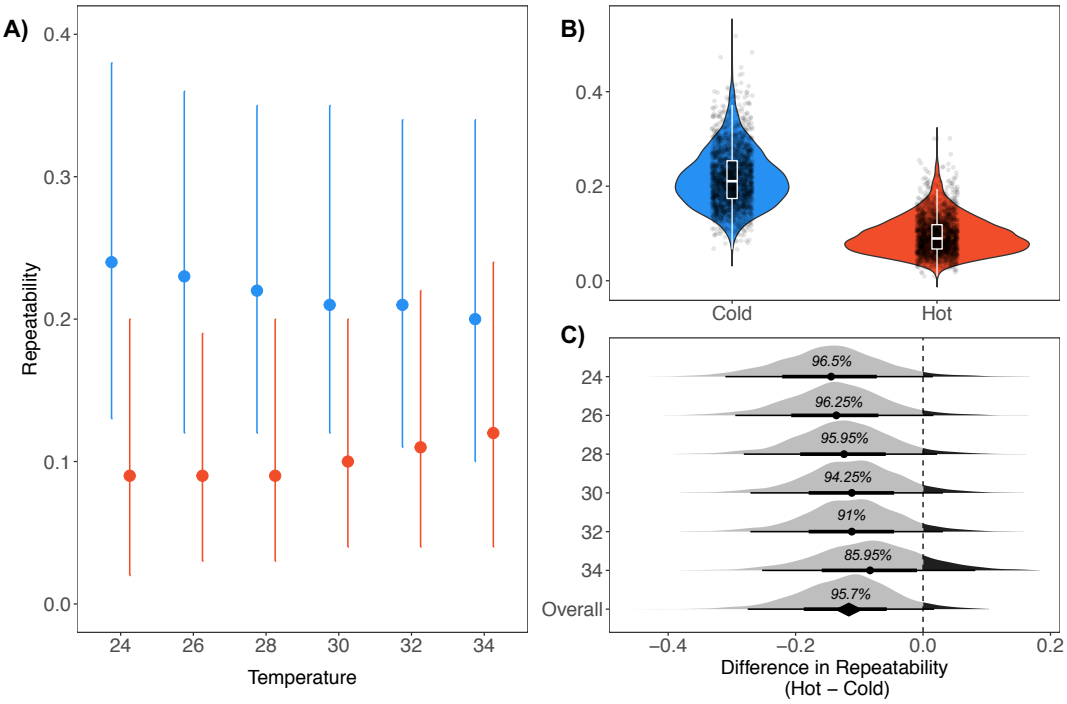
Figure 2 Thermal reaction norms of mass-adjusted metabolic rate for lizards reared at **A)** 'hot' developmental temperatures (top, red lines, $n_{lizards}$ = 25) and **B)** 'cold' developmental temperatures (bottom, blue lines, $n_{lizards}$ = 26) at session number one, five and ten. Each uniquely coloured line represents an individual reaction norm. There is a random subset of 10 individuals from each treatment.

Table 1 Model coefficients of the full model testing whether developmental temperature affects the elevation (intercept) and slope of the thermal reaction norm of metabolic rate. This model used an imputed dataset of n_{obs} = 6,000, 36% of observations were imputed. The intercept is the cold developmental temperature. MR was log transformed and mass, age and temperature were z-transformed. Bolded estimates are significantly different from zero. Lower and upper bound of estimates represent 95% credible intervals. COV represents covariance. Main effects model is presented in Table S4

Parameter	**Estimate**	**Lower**	**Upper**
Fixed effects			
Intercept MR	**-6.292**	**-6.372**	**-6.218**
Treatment 29	-0.003	-0.062	0.058
Acute Temperature	**0.262**	**0.246**	**0.278**
Treatment 29 × Acute Temperature	-0.016	-0.039	0.007
Age	-0.035	-0.079	0.006
Mass	**0.128**	**0.105**	**0.151**
Random Effects			
Lizard Identity			
Intercept	**0.009**	**0.006**	**0.015**

Temperature Slope	$9.53e^{-5}$	$1.54e^{-7}$	0.000479
$COV_{Intercept - Slope}$	-0.00018	-0.00122	0.000599
Sampling Session			
Intercept	**0.01**	**0.003**	**0.026**
Measurement Error			
Intercept	**0.044**	**0.04**	**0.049**
Residual	**0.041**	**0.038**	**0.043**

Overall, temperature-specific repeatability was relatively low, with the cold developmental treatment tending to have higher repeatability estimates compared to the hot developmental treatment (Fig. 3, Fig S1, Supplementary Materials, Section 3 Table S10). Irrespective of acute temperature, repeatability of average metabolic rate was on average 10% higher in cold incubated lizards (*pd* = 95.7%, Fig. 3B, C). There was a 95.7% probability that the difference in overall repeatability was negative, indicating that lizards from the cold treatment are more likely to have higher repeatability. Higher repeatability in the cold treatment was associated with significant increases in among individual and residual variance (Fig. S2).



353

354 **Figure 3** (A) Temperature-specific adjusted repeatability for average metabolic rate for the
355 'cold' developmental temperature group (blue, $n_{lizards}$ = 26) and the 'hot' developmental
356 temperature group (red, $n_{lizards}$ = 25). Error bars represent 95% credible intervals. (**B)** Violin
357 and boxplot showing the posterior distribution of overall adjusted repeatability of each
358 treatment group irrespective of acute temperature. **(C)** Posterior distribution of the difference
359 in repeatability (Hot – Cold) overall and at each acute temperature. Point represents the
360 median; thicker lines represent the interquartile range and thin lines represent the 95%
361 credible intervals. The probability of direction is presented on each distribution and describes
362 the probability that the difference in repeatability is either positive or negative. Grey regions
363 of the distribution represent negative estimates indicating repeatability was greater in the cold
364 treatment, whereas black regions represent positive estimates which indicates that
365 repeatability was greater in the hot treatment. All values were calculated from imputation
366 models (Supplementary Materials, Section 3 Table 11-14). Contrasts are presented in Table
367 S10.

Discussion

Early developmental temperature did not change the intercept or slope of the population reaction norm in delicate skinks. Thermal plasticity of metabolic rate was unaffected by developmental temperature, however; variation in slope had relatively high repeatable ($R > 0.4$). Temperature-specific repeatability of metabolic rate (i.e., intercept) was lower among lizards that were reared in hot developmental temperatures. Our results suggest that, while individuals displayed consistent variation in their plasticity (Individual x Environment), how metabolic rate responds to acute temperature variation later in life was robust to thermal extremes of natural nest sites. Developmental temperatures did not have an impact on average metabolic rate but rather it changed the amount of consistent individual variation in average metabolic rate. Below we discuss the implications of our results for the evolution of thermal reaction norms.

Thermal reaction norms of metabolic rate are robust to developmental temperature

Developmental environments that affect later life plasticity may affect how individuals and populations respond to environmental fluctuations (Beaman et al. 2016). Epigenetic modifications during development that influence the physiological system are likely responsible for shaping plastic responses in complex ways (Hu and Barrett 2017; McCaw et al. 2020). However, our results suggest instead that thermal reaction norms for metabolic rate were robust to changes in incubation temperature. Results have been mixed among the few studies that have investigated the effects of pre- and post-hatching temperature on the plasticity of metabolic rate (Table 1, Beaman et al., 2016). For example, wild caught mosquitofish (*Gambusia holbrooki*) developing under more variable spring conditions exhibited steeper thermal reaction norms for metabolic scope compared to fish

392 born in summer (Seebacher et al., 2014). In contrast, incubation temperature did not affect
393 plasticity in metabolic rate of striped marsh frog tadpoles (Seebacher and Grigaltchik 2014).
394 Given that our lizards were reared in a common environment post hatching, the lack of
395 difference we observed may be the result of reversible plasticity from acclimation in
396 metabolic rate to the laboratory conditions. It is possible that acclimation capacities may have
397 overwhelmed any developmental differences in thermal reaction norms. Generally,
398 acclimation of physiological function takes approximately three to four weeks to complete, so
399 it is likely that acclimation had already taken place by the time we began the study when
400 lizards were about ~2.5 months old (Seebacher, 2005). Nonetheless, it is clear that whether
401 acclimation homogenised possible developmental effects, developmental environments may
402 have little long-term impacts on reaction norms. Future studies should employ cross factorial
403 designs where post-hatch environments are deliberately matched and mismatched with early
404 environmental conditions to disassociate acclimation effects (Schnurr et al. 2014; Kazerouni
405 et al. 2016).
406
407 Stable thermal reaction norms of metabolic rate across both developmental
408 temperatures have key evolutionary implications. Our results imply that population reaction
409 norms may be robust to temperature variation within the thermal range of natural nests
410 (Cheetham et al., 2011). Past thermal regimes encountered by ancestors may have canalized
411 population responses so that they are less sensitive to fluctuations in developmental
412 temperature (Liefting et al. 2009). Canalization may reduce the costs of phenotypic plasticity
413 during development if environmental variation is predictable across generations (Aubret and
414 Shine 2010). In support of this, damselflies undergoing range expansions exhibit geographic
415 variation in thermal reaction norms that align with past climatic conditions (Lancaster et al.
416 2015). Population comparisons across environmental gradients might reveal whether local

417 adaptation shapes developmental plasticity of population reaction norms that lead to
418 canalisation (Toftegaard et al. 2015). Developmental environments may play a stronger role
419 in shaping population plastic responses in areas that experience greater thermal variability,
420 such as those in temperate or high elevation regions (Bonamour et al. 2019). While our
421 incubation treatments represent thermal extremes of natural nest sites, they may not have
422 been severe enough to induce changes in the thermal reaction norms, particularly given that
423 we used more realistic fluctuating incubation temperatures. Developmental stress is thought
424 to lead to the recruitment of heat shock proteins thereby changing reversible plasticity later in
425 life (Beaman et al. 2016; Chevin and Hoffmann 2017). Recent work has shown lizard
426 embryos exposed to extreme heat produce higher levels of heat shock proteins and have
427 greater thermal tolerance as juveniles, however this subsequently reduces thermal tolerance
428 later in life (Gao et al. 2014). This implies there may be constraints in how thermal responses
429 can be shaped by extreme developmental environments.
430

431 *Developmental temperatures and repeatable thermal plasticity of metabolic rate*

432 Repeatability of reaction norm slopes did not change with developmental temperature,
433 but lizards reared in hot temperatures had reduced repeatability in metabolic rate (intercept).
434 Variation in developmental time has important consequences on hatching condition and may
435 contribute to differences in consistent variation in hatchling phenotypes. Developmental time
436 exhibits a negative nonlinear relationship with temperature, such that development times are
437 considerably shorter at hotter temperatures (Noble et al. 2018; Marshall et al. 2020).
438 Consequently, eggs reared in warmer environments are expected to be more constrained in
439 their developmental rates, thus hatching phenotypes are more likely to be less variable
440 compared to eggs reared in cooler environments (Pettersen et al. 2019). Indeed, incubation
441 duration was short and less variable in our hot developmental treatment (Hot: 30 days, SD =

1.40, range = 27 - 33; Cold: 47.7 days, SD = 5.90, range = 25 – 53). Shortened development times may restrict embryo yolk assimilation that is needed for growth (Oufiero and Angilletta 2006; Storm and Angilletta 2007). Elevated mitochondrial proton leak at hot developmental temperatures may also lead to less efficient energy production and may explain why metabolic rate did not differ among treatments despite changes in repeatability (Chamberlin 2004). Lower repeatability under hot nest temperatures may be problematic as global temperatures continue to rise (Botero et al. 2015). Provided that some of the repeatable phenotypic differences in metabolic rate are heritable (Dohm 2002; Falconer and Mackay 2009), our results suggest that the evolutionary potential of metabolic rate may be dampened for populations incubating in warming environments. However, metabolic plasticity may still be able to evolve under rising temperatures (Ghalambor et al., 2007).

We found that individuals consistently vary in metabolic plasticity in response to acute temperatures to a certain extent. While several studies have reported significant among individual variation in thermal plasticity slopes (Careau et al. 2014b; Briga and Verhulst 2017), its repeatability is rarely estimated as it requires a study design that allows partitioning of within and between individual variance of slopes (Araya-Ajoy et al., 2015). Our repeatability estimates for reaction norm slopes were consistent with a previous study of the same species (R = 0.23, Kar et al. 2021). Similarly, moderate repeatability of thermal sensitivity of metabolic rate has also been observed in amphipods (R = 0.38, Réveillon et al. 2019). Assuming that repeatable reaction norm slopes have a heritable basis (Driessen et al. 2007), our work implies that thermal plasticity has the potential to be selected upon and evolve (Falconer, 1952; but see Dohm, 2002).

Consistent individual differences in metabolic rate were stable across acute temperatures. This result demonstrates that temperatures within the operable range of *L. delicata* maintains consistent individual differences in MR (Matthews et al. 2016). Repeatability in metabolic rate may be an important mechanism that promotes consistent variation in thermoregulation, behaviour and life history (Sæther 1987; Réale et al. 2010; Goulet et al. 2017). Overall, our estimates for the repeatability of MR ranged from 0.09 – 0.22. Our results are in line with a meta-analysis showing that repeatability decreases with time between repeated measurements (White et al. 2013). Specifically, the average repeatability of MR in ectotherms from studies that had a measurement interval that was equal or larger than our study ($\geq$ 8.5 days) was R = 0.33 (SD = 0.21, n = 18). Interestingly, repeatability of average MR in wild caught adult *L. delicata* (R = 0.3 – 0.5, Kar et al. 2021) was comparatively larger relative to our results. This is likely due to life stage differences in environmental effects that shape phenotypic variation. As individuals mature, their experiences in different microhabitats (diet, thermal preferences) can promote among-individual variation in traits (Kruuk and Hadfield 2007). Such common (micro) environment effects could further increase repeatability and may explain differences between lab and wild studies (Auer et al. 2016).

Conclusion

The role of developmental temperature on phenotypic plasticity exhibited later in life is complex. At the population level, thermal plasticity of metabolic rate was robust to changes in temperature during embryonic development suggesting that thermal reaction norms may be canalised. In contrast, the impact of developmental temperature manifested as a change in the repeatability of temperature-specific metabolic rate. This has important evolutionary implications. Reduced among individual variation in hot temperatures may alter a

population's ability to respond to selection under warming climate. However, population thermal reaction norms could still respond to selective processes to some extent (assuming they are heritable), allowing populations to persist. Elucidating the role of developmental environments on shaping plastic responses may require more stressful incubation conditions and cross-factorial experimental designs to disassociate the effects of acclimation from developmental plasticity.

Data Accessibility

Datasets and code used to generate results of this study is accessible via Open Science Framework (https://bit.ly/38IzTsp)

Acknowledgements

We would like to acknowledge and pay immense respect to the Wallumedegal people, as well as the Cadigal and Wangal people – the traditional custodians of the land where this study took place. We would like to extend this respect to the Gadigal people of the greater Eora Nation. We would also like to thank Martin Whiting for the use of his facilities at Macquarie University. We are grateful for the assistance of numerous Lizard Lab members and interns with husbandry duties. Special thanks to Christine Wilson for her commitment to caring for our animals. We thank Timothee Bonnet for his advice on partitioning measurement error from our models. This study would not have been possible without the support of the Australian Research Council (ARC) Discovery Early Career Research Award to DWAN (DE150101774); also, SN was supported by an ARC Future Fellowship (FT13010026). We recognise The Office of Environment and Heritage, New South Wales for our wildlife collection permit and the animal ethics committee from University of New South Wales and Macquarie University for our animal ethics permit.

References

Angilletta Jr MJ (2016) Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press

Angilletta Jr MJ, Wilson RS, Navas CA, James RS (2003) Tradeoffs and the evolution of thermal reaction norms. Trends Ecol Evol 18:234–240. https://doi.org/10.1016/S0169-5347(03)00087-9

Araya-Ajoy YG, Dingemanse NJ (2017) Repeatability, heritability, and age-dependence of seasonal plasticity in aggressiveness in a wild passerine bird. J Anim Ecol 86:227–238. https://doi.org/10.1111/1365-2656.12621

Araya-Ajoy YG, Mathot KJ, Dingemanse NJ (2015) An approach to estimate short-term, long-term and reaction norm repeatability. J Anim Ecol 6:1462–1473. https://doi.org/10.1111/2041-210X.12430

Aubret F, Shine R (2010) Fitness costs may explain the post-colonisation erosion of phenotypic plasticity. J Exp Biol 213:735–739. https://doi.org/10.1242/jeb.040576

Auer SK, Bassar RD, Salin K, Metcalfe NB (2016) Repeatability of metabolic rate is lower for animals living under field versus laboratory conditions. J Exp Biol 219:631–634. https://doi.org/10.1242/jeb.133678

Auld JR, Agrawal AA, Relyea RA (2010) Re-evaluating the costs and limits of adaptive phenotypic plasticity. Proc R Soc B Biol Sci 277:503–511. https://doi.org/10.1098/rspb.2009.1355

534 Beaman JE, White CR, Seebacher F (2016) Evolution of plasticity: Mechanistic link between
535 development and reversible acclimation. Trends Ecol Evol 31:237–249.
536 https://doi.org/10.1016/j.tree.2016.01.004

537 Beldade P, Mateus ARA, Keller RA (2011) Evolution and molecular mechanisms of adaptive
538 developmental plasticity. Mol Ecol 20:1347–1363. https://doi.org/10.1111/j.1365-
539 294X.2011.05016.x

540 Bonamour S, Chevin L-M, Charmantier A, Teplitsky C (2019) Phenotypic plasticity in
541 response to climate change: the importance of cue variation. Philos Trans R Soc B
542 Biol Sci 374:20180178–12. https://doi.org/10.1098/rstb.2018.0178

543 Botero CA, Weissing FJ, Wright J, Rubenstein DR (2015) Evolutionary tipping points in the
544 capacity to adapt to environmental change. Proc Natl Acad Sci 112:184–189.
545 https://doi.org/10.1073/pnas.1408589111

546 Briga M, Verhulst S (2017) Individual variation in metabolic reaction norms over ambient
547 temperature causes low correlation between basal and standard metabolic rate. J Exp
548 Biol 220:3280–3289. https://doi.org/10.1242/jeb.160069

549 Bürkner PC (2017) brms: An R package for Bayesian multilevel models using Stan. J Stat
550 Softw 80:1–28. https://doi.org/10.18637/jss.v080.i01

551 Careau V, Buttemer WA, Buchanan KL (2014a) Developmental stress can uncouple
552 relationships between physiology and behaviour. Biol Lett 10:20140834.
553 https://doi.org/10.1098/rsbl.2014.0834

554 Careau V, Gifford ME, Biro PA (2014b) Individual (co)variation in thermal reaction norms
555 of standard and maximal metabolic rates in wild-caught slimy salamanders. Funct
556 Ecol 28:1175–1186. https://doi.org/10.1111/1365-2435.12259

557 Careau V, Killen SS, Metcalfe NB (2014c) Adding Fuel To The “Fire Of Life”: Energy
558 Budgets Across Levels Of Variation In Ectotherms And Endotherms. In: Integrative
559 Organismal Biology, 1st edn. Wiley-Blackwell, p 17

560 Chamberlin ME (2004) Top-down control analysis of the effect of temperature on ectotherm
561 oxidative phosphorylation. Am J Physiol-Regul Integr Comp Physiol 287:R794–
562 R800. https://doi.org/10.1152/ajpregu.00240.2004

563 Cheetham E, Doody JS, Stewart B, Harlow P (2011) Embryonic mortality as a cost of
564 communal nesting in the delicate skink. J Zool 283:234–242.
565 https://doi.org/10.1111/j.1469-7998.2010.00764.x

566 Chevin L-M, Hoffmann AA (2017) Evolution of phenotypic plasticity in extreme
567 environments. Philos Trans R Soc Lond B Biol Sci 372:20160138.
568 https://doi.org/10.1098/rstb.2016.0138

569 Core Team R (2013) Team (2012). R: A language and environment for statistical computing.
570 R Foundation for Statistical Computing, Vienna, Austria

571 Dingemanse NJ, Wolf M (2013) Between-individual differences in behavioural plasticity
572 within populations: causes and consequences. Anim Behav 85:1031–1039.
573 https://doi.org/10.1016/j.anbehav.2012.12.032

574 Dohm MR (2002) Repeatability estimates do not always set an upper limit to heritability.
575 Funct Ecol 16:273–280. https://doi.org/10.1046/j.1365-2435.2002.00621.x

576 Driessen G, Ellers J, Van Straalen NM (2007) Variation, selection and heritability of thermal
577 reaction norms for juvenile growth in *Orchesella cincta* Collembola: Entomobryidae).
578 Eur J Entomol 104:39–46. https://doi.org/10.14411/eje.2007.006

579 Eyck HJF, Buchanan KL, Crino OL, Jessop TS (2019) Effects of developmental stress on
580 animal phenotype and performance: a quantitative review. Biol Rev 94:1143–1160.
581 https://doi.org/10.1111/brv.12496

582 Falconer DS, Mackay TFC (2009) Introduction to Quantitative Genetics, 4th edn. Pearson,
583 Prentice Hall, Harlow

584 Friesen CR, Johansson R, Olsson M (2017) Morph-specific metabolic rate and the timing of
585 reproductive senescence in a color polymorphic dragon. J Exp Zool Part -Ecol Integr
586 Physiol 327:433–443. https://doi.org/10.1002/jez.2118

587 Gangloff EJ, Vleck D, Bronikowski AM (2015) Developmental and immediate thermal
588 environments shape energetic trade-offs, growth efficiency, and metabolic rate in
589 divergent life-history ecotypes of the garter snake *Thamnophis Elegans*. Physiol
590 Biochem Zool 88:550–563. https://doi.org/10.1086/682239

591 Gao J, Zhang W, Dang W, et al (2014) Heat shock protein expression enhances heat
592 tolerance of reptile embryos. Proc R Soc B Biol Sci 281:20141135.
593 https://doi.org/10.1098/rspb.2014.1135

594 Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive
595 phenotypic plasticity and the potential for contemporary adaptation in new
596 environments. Funct Ecol 21:394–407. https://doi.org/10.1111/j.1365-
597 2435.2007.01283.x

598 Goulet CT, Thompson MB, Michelangeli M, et al (2017) Thermal physiology: A new
599 dimension of the pace-of-life syndrome. J Anim Ecol 86:1269–1280.
600 https://doi.org/10.1111/1365-2656.12718

601 Havird JC, Neuwald JL, Shah AA, et al (2020) Distinguishing between active plasticity due
602 to thermal acclimation and passive plasticity due to Q10 effects: Why methodology
603 matters. Funct Ecol 34:1015–1028. https://doi.org/10.1111/1365-2435.13534

604 Hu J, Barrett RDH (2017) Epigenetics in natural animal populations. J Evol Biol 30:1612–
605 1632. https://doi.org/10.1111/jeb.13130

606 Kar F, Nakagawa S, Friesen CR, Noble DWA (2021) Individual variation in thermal
607 plasticity and its impact on mass-scaling. Oikos 130:1131–1142.
608 https://doi.org/10.1111/oik.08122

609 Kazerouni EG, Franklin CE, Seebacher F (2016) UV-B radiation interacts with temperature
610 to determine animal performance. Funct Ecol 30:584–595.
611 https://doi.org/10.1111/1365-2435.12520

612 Kruuk LEB, Hadfield JD (2007) How to separate genetic and environmental causes of
613 similarity between relatives. J Evol Biol 20:1890–1903.
614 https://doi.org/10.1111/j.1420-9101.2007.01377.x

615 Lancaster LT, Dudaniec RY, Hansson B, Svensson EI (2015) Latitudinal shift in thermal
616 niche breadth results from thermal release during a climate-mediated range expansion.
617 J Biogeogr 42:1953–1963. https://doi.org/10.1111/jbi.12553

618 Liefting M, Hoffmann AA, Ellers J (2009) Plasticity versus environmental canalization:
619 population differences in thermal responses along a latitudinal gradient in *Drosophila*
620 *serrata*. Evolution 63:1954–1963. https://doi.org/10.1111/j.1558-5646.2009.00683.x

621 Lighton JRB (2008) Measuring Metabolic Rates. Oxford University Press, New York, USW

622 Makowski D, Ben-Shachar MS, Chen SHA, Lüdecke D (2019a) Indices of effect existence
623 and significance in the Bayesian framework. Front Psychol 10:.
624 https://doi.org/10.3389/fpsyg.2019.02767

625 Makowski D, Ben-Shachar MS, Lüdecke D (2019b) bayestestR: Describing effects and their
626 uncertainty, existence and significance within the Bayesian framework. J Open
627 Source Softw 4:1541. https://doi.org/10.21105/joss.01541

628 Marshall DJ, Pettersen AK, Bode M, White CR (2020) Developmental cost theory predicts
629 thermal environment and vulnerability to global warming. Nat Ecol Evol 4:406–411.
630 https://doi.org/10.1038/s41559-020-1114-9

631 Mathot KJ, Dingemanse NJ (2015) Energetics and behavior: unrequited needs and new
632 directions. Trends Ecol Evol 30:199–206. https://doi.org/10.1016/j.tree.2015.01.010

633 Matthews G, Goulet CT, Delhey K, Chapple DG (2016) The effect of skin reflectance on
634 thermal traits in a small heliothermic ectotherm. J Therm Biol 60:109–124.
635 https://doi.org/10.1016/j.jtherbio.2016.06.013

636 McCaw BA, Stevenson TJ, Lancaster LT (2020) Epigenetic responses to temperature and
637 climate. Integr Comp Biol 60:1–12. https://doi.org/10.1093/icb/icaa049

638 Nakagawa S (2015) Missing data: mechanisms, methods and messages. In: Fox GA, Negrete-
639 Yankelevich S, Sosa VJ (eds) Ecological Statistics: Contemporary theory and
640 application. Oxford University Press, USA, pp 81–105

641 Nespolo RF, Franco M (2007) Whole-animal metabolic rate is a repeatable trait: a meta-
642 analysis. J Exp Biol 210:3877–3878. https://doi.org/10.1242/jeb.013110

643 Noble DWA, Nakagawa S (2018) Planned missing data design: stronger inferences, increased
644 research efficiency and improved animal welfare in ecology and evolution. BioRxiv
645 247064 8:257–32. https://doi.org/10.1101/247064

646 Noble DWA, Stenhouse V, Schwanz LE (2018) Developmental temperatures and phenotypic
647 plasticity in reptiles: a systematic review and meta-analysis. Biol Rev 93:72–97.
648 https://doi.org/10.1111/brv.12333

649 Norin T, Metcalfe NB (2019) Ecological and evolutionary consequences of metabolic rate
650 plasticity in response to environmental change. Philos Trans R Soc B Biol Sci
651 374:20180180–9. https://doi.org/10.1098/rstb.2018.0180

652 Nussey DH, Wilson AJ, Brommer JE (2007) The evolutionary ecology of individual
653 phenotypic plasticity in wild populations. J Evol Biol 20:831–844.
654 https://doi.org/10.1111/j.1420-9101.2007.01300.x

655 O'Dea RE, Lagisz M, Hendry AP, Nakagawa S (2019) Developmental temperature affects
656 phenotypic means and variability: A meta-analysis of fish data. Fish Fish 20:1005–
657 1022. https://doi.org/10.1111/faf.12394

658 Oufiero CE, Angilletta MJ (2006) Convergent evolution of embryonic growth and
659 development in the eastern fence lizard(*Sceloporus undulatus*). Evolution 60:1066–
660 1075. https://doi.org/10.1111/j.0014-3820.2006.tb01183.x

661 Pettersen AK, White CR, Bryson-Richardson RJ, Marshall DJ (2019) Linking life-history
662 theory and metabolic theory explains the offspring size-temperature relationship. Ecol
663 Lett 22:518–526. https://doi.org/10.1111/ele.13213

664 Piersma T, Drent J (2003) Phenotypic flexibility and the evolution of organismal design.
665 Trends Ecol Evol 18:228–233. https://doi.org/10.1016/S0169-5347(03)00036-3

666 Piersma T, Lindström Å (1997) Rapid reversible changes in organ size as a component of
667 adaptive behaviour. Trends Ecol Evol 12:134–138. https://doi.org/10.1016/S0169-
668 5347(97)01003-3

669 Ponzi E, Keller LF, Bonnet T, Muff S (2018) Heritability, selection, and the response to
670 selection in the presence of phenotypic measurement error: Effects, cures, and the role
671 of repeated measurements. Evolution 72:1992–2004.
672 https://doi.org/10.1111/evo.13573

673 Réale D, Garant D, Humphries MM, et al (2010) Personality and the emergence of the pace-
674 of-life syndrome concept at the population level. Philos Trans R Soc Lond B Biol Sci
675 365:4051–4063. https://doi.org/10.1098/rstb.2010.0208

676 Réveillon T, Rota T, Chauvet É, et al (2019) Repeatable inter-individual variation in the
677 thermal sensitivity of metabolic rate. Oikos 85:935–8.
678 https://doi.org/10.1111/oik.06392

679 Sæther B-E (1987) The influence of body weight on the covariation between reproductive
680 traits in European birds. Oikos 48:79–88. https://doi.org/10.2307/3565691

681 Schielzeth H (2010) Simple means to improve the interpretability of regression coefficients.
682 Methods Ecol 1:103–113. https://doi.org/10.1111/j.2041-210X.2010.00012.x

683 Schielzeth H, Nakagawa S (2020) Conditional repeatability and the variance explained by
684 reaction norm variation in random slope models. bioRxiv 2020.03.11.987073.
685 https://doi.org/10.1101/2020.03.11.987073

686 Schnurr ME, Yin Y, Scott GR (2014) Temperature during embryonic development has
687 persistent effects on metabolic enzymes in the muscle of zebrafish. J Exp Biol
688 217:1370–1380. https://doi.org/10.1242/jeb.094037

689 Seebacher F (2005) A review of thermoregulation and physiological performance in reptiles:
690 what is the role of phenotypic flexibility? J Comp Physiol B 175:453–461.
691 https://doi.org/10.1007/s00360-005-0010-6

692 Seebacher F, Beaman J, Little AG (2014) Regulation of thermal acclimation varies between
693 generations of the short-lived mosquitofish that developed in different environmental
694 conditions. Funct Ecol 28:137–148. https://doi.org/10.1111/1365-2435.12156

695 Seebacher F, Grigaltchik VS (2014) Embryonic developmental temperatures modulate
696 thermal acclimation of performance curves in tadpoles of the frog *Limnodynastes*
697 *peronii*. PLoS ONE 9:e106492. https://doi.org/10.1371/journal.pone.0106492

698 Shama LNS, Strobel A, Mark FC, Wegner KM (2014) Transgenerational plasticity in marine
699 sticklebacks: maternal effects mediate impacts of a warming ocean. Funct Ecol
700 28:1482–1493. https://doi.org/10.1111/1365-2435.12280

Storm MA, Angilletta MJ (2007) Rapid assimilation of yolk enhances growth and development of lizard embryos from a cold environment. J Exp Biol 210:3415–3421. https://doi.org/10.1242/jeb.005652

Sultan SE, Stearns SC (2005) Environmentally Contingent Variation: Phenotypic Plasticity and Norms of Reaction. In: Hallgrímsson B, Hall BK (eds) Variation. Academic Press, Burlington, pp 303–332

Thompson MJ, Evans JC, Parsons S, Morand-Ferron J (2018) Urbanization and individual differences in exploration and plasticity. Behav Ecol 29:1415–1425. https://doi.org/10.1093/beheco/ary103

Toftegaard T, Posledovich D, Navarro-Cano JA, et al (2015) Variation in plant thermal reaction norms along a latitudinal gradient - more than adaptation to season length. Oikos 125:622–628. https://doi.org/10.1111/oik.02323

Tomlinson S, Dalziell EL, Withers PC, et al (2018) Measuring metabolic rates of small terrestrial organisms by fluorescence-based closed-system respirometry. J Exp Biol 221:jeb172874. https://doi.org/10.1242/jeb.172874

Via S, Gomulkiewicz R, De Jong G, et al (1995) Adaptive phenotypic plasticity: consensus and controversy. Trends Ecol Evol 10:212–217. https://doi.org/10.1016/S0169-5347(00)89061-8

White CR, Schimpf NG, Cassey P (2013) The repeatability of metabolic rate declines with time. J Exp Biol 216:1763–1765. https://doi.org/10.1242/jeb.076562

Withers PC (1992) Comparative animal physiology. Philadelphia: Saunders College Pub