OIKOS

Research

Individual variation in thermal plasticity and its impact on mass-scaling

Fonti Kar, Shinichi Nakagawa, Christopher R. Friesen and Daniel W. A. Noble

F. Kar (https://orcid.org/0000-0002-2760-3974) ✉ (fonti.kar@gmail.com), S. Nakagawa (https://orcid.org/0000-0002-7765-5182) and D. W. A. Noble (https://orcid.org/0000-0001-9460-8743), School of Biological Earth and Environmental Sciences, Ecology and Evolution Research Centre, Univ. of New South Wales, Sydney, NSW, Australia. SN and DWAN also at: Diabetes and Metabolism Division, Garvan Inst. of Medical Research, Darlinghurst, Sydney, NSW, Australia. – C. R. Friesen (https://orcid.org/0000-0001-5338-7454), School of Earth, Atmospheric and Life Sciences, Faculty of Science, Medicine and Health, Univ. of Wollongong, Wollongong, NSW, Australia. DWAN, Division of Ecology and Evolution, Research School of Biology, The Australian National Univ., Canberra, ACT, Australia.

Oikos
00: 1–12, 2021
doi: 10.1111/oik.08122

Subject Editor: Gregor Kalinkat
Editor-in-Chief: Dries Bonte
Accepted 26 March 2021

Physiological processes vary widely across individuals and can influence how individuals respond to environmental change. Repeatability in how metabolic rate changes across temperatures (i.e. metabolic thermal plasticity) can influence mass-scaling exponents in different thermal environments. Moreover, repeatable plastic responses are necessary for reaction norms to respond to selective forces which is important for populations living in fluctuating environments. Nonetheless, only a small number of studies have explicitly quantified repeatability in metabolic plasticity, and fewer have explored how it can impact mass-scaling. We repeatedly measured standard metabolic rate of n = 42 delicate skinks *Lampropholis delicata* at six temperatures over the course of four months ($N_{[observations]} = 4952$). Using hierarchical statistical techniques, we accounted for multi-level variation and measurement error in our data in order to obtain more precise estimates of reaction norm repeatability and mass-scaling exponents at different acute temperatures. Our results show that individual differences in metabolic thermal plasticity were somewhat consistent over time ($R_{slope} = 0.25$, 95% CI = 2.48×10^{-8} – 0.67), however estimates were associated with a large degree of error. After accounting for measurement error, which decreased steadily with temperature, we show that among individual variance remained consistent across all temperatures. Congruently, temperature specific repeatability of average metabolic rate was stable across temperatures. Cross-temperature correlations were positive but were not uniform across the reaction norm. After taking into account multiple sources of variation, our estimates for mass-scaling did not change with temperature and were in line with published values for snakes and lizards. This implies that repeatable plastic responses may promote thermal stability of scaling exponents. Our work contributes to understanding how energy expenditure scales with abiotic and biotic factors and the capacity for reaction norms to respond to selection.

Keywords: phenotypic plasticity, reaction norm, repeatability, thermal performance curves, thermal sensitivity

NORDIC SOCIETY OIKOS

www.oikosjournal.org

Introduction

All biological processes hinge on the availability of energy (Allen et al. 2005). Metabolic rate (MR) governs how much energy is available to be allocated to competing processes such as growth, reproduction and maintenance (De Jong and Van Noordwijk 1992, Brown et al. 2004, Biro and Stamps 2008). MR is thought to be critical to fitness due to its functional links to morphology, behaviour and life-history promoting the integration of these traits (Biro and Stamps 2010, Réale et al. 2010, Friesen et al. 2017, Malishev et al. 2017). For example, short-lived ecotypic garter snakes *Thamnophis elegans* tend to have much higher mass-specific metabolic rates, larger body sizes, faster growth rates and invest more heavily into reproduction compared their long lived ecotypic counterparts (Bronikowski and Vleck 2010). The integration of these traits may be due to the close association between body mass and metabolic rate. Body mass and metabolic rate typically show a power relationship with an scaling exponent ranging from 0.64 to 0.88 (White et al. 2006). Scaling exponents less than one indicate that energy expenditure scales disproportionately with mass, such that small organisms tend to have higher energy expenditure after controlling for body mass. Metabolic scaling exponents are incredibly heterogenous among (White et al. 2006, Uyeda et al. 2017) and within taxa (Burton et al. 2011, Norin and Gamperl 2018), yet the drivers of such variation are not well understood.

One powerful application of mass-scaling relationships is its ability to explain and predict ecological processes across levels of biological organisation (Brown et al. 2004, Allen et al. 2005, Barneche and Allen 2018). In theoretical studies, among and within individual variation in energy consumption is assumed to be the same, however, few empirical studies have actually tested this assumption. Indeed, individuals can vary in their relative organ mass and body composition yielding very disparate energetic demands in different environments (Scott et al. 1996, Steyermark 2005). Additionally, variation in mitochondrial efficiency in fish underpins stark differences in MR despite mass remaining the same (Salin et al. 2016). Ignoring individual variability in physiological processes may be problematic for comparative studies as individual effects can be erroneously absorbed into higher levels of biological organisation (van de Pol and Wright 2009). This may bias mass-scaling exponents and increase heterogeneity among studies. Furthermore, mass-scaling exponents may be susceptible to sampling variability because metabolic rate and body mass tend to be measured once per individual and then averaged across a population. Understanding the consistency of metabolism at the individual level may help explain interspecific variation in mass-scaling exponents (Uyeda et al. 2017).

Temperature fluctuates extensively within the lifetime of ectothermic organisms and this has a profound impact on metabolic rate. Numerous studies have found that scaling exponents show temperature dependence in a multitude of ways, however patterns are highly species-specific (Glazier 2005, Barneche et al. 2016). For example, mass-scaling exponents increased with temperature in teleost fish (Killen et al. 2010), but decreased with temperature in crustaceans (Ivleva 1980). In contrast, mass-scaling exponents were stable across temperatures in tegu lizards (Toledo et al. 2008). Temperature dependence of mass-scaling relationships imply that metabolic costs for individuals of varying body sizes depend on the thermal environment (Barneche et al. 2016). However, individuals can also vary in their metabolic thermal plasticity, that is, their capacity to adjust their metabolic rate in response to temperature (Individual × Temperature, Nussey et al. 2007). Individual thermal plasticity can be important for understanding temperature dependence of mass-scaling and how selection might shape metabolic plasticity, however, this has rarely been considered (Piersma and Drent 2003, Barneche et al. 2016). Low consistency in individual thermal plasticity can introduce variability in metabolic rate across temperatures which can give rise to spurious patterns of temperature dependence. If individuals respond to temperature consistently though, mass-scaling is expected to be robust to temperature changes (Clarke 2004). Consistent variation in metabolic thermal plasticity is also the minimum requirement for plasticity to evolve as it represents the raw material for selection to act on (Wilson 2018). Despite studies on a range of taxa recognising that individuals differ in their metabolic thermal plasticity, its repeatability has rarely been formally estimated (but see Briga and Verhulst 2017, Réveillon et al. 2019).

Here we examine how individuals differ in energy expenditure in relation to body size and acute temperature changes in male delicate skinks *Lampropholis delicata*. While sex differences in metabolic rate have been reported in some lizard species (Orrell et al. 2004), we only used males in our study because the extended period over which metabolism measurements took place meant that females would be gravid and ovipositing – characteristics that can drastically influence metabolic rate (Patterson and Davies 1984, Angilletta and Sears 2000). We repeatedly measured routine metabolic rate over four months to address three key questions. 1) Does metabolic thermal plasticity consistently differ among individuals? 2) How does repeatability of MR change with temperature? 3) Do population mass-scaling exponents change with temperature when accounting for among- and within-individual variation in MR? Unravelling the complexities of individual physiological processes will have important consequences for understanding how populations respond in warming environments.

Material and methods

Lizard collection and husbandry

Lampropholis delicata is a small oviparous, skink found throughout eastern Australia (Chapple et al. 2011). They have a short lifespan (2–4 years in the wild) and their reproductive season is from September to February (Chapple et al. 2014). Between 28 August and 8 September 2015, 42 male *L.*

delicata were collected from four sites near Sydney, Australia. Lizards were caught by hand or by mealworm fishing and were transported individually in calico bags in an ice-cooler to Macquarie University. Lizards were housed in a temperature-controlled room set at 26°C and were provided with a thermal gradient (24–34°C) to allow for thermoregulation. Each lizard was kept individually in an opaque plastic enclosure measuring 35 × 25 × 15 cm (L × W × H). Each enclosure was lined with newspaper and lizards were given access to a water bowl and tree bark as a refuge. Enclosures were placed under UV light (11L:13D light–dark cycle). Lizards were fed three to four small crickets *Acheta domestica* dusted with calcium powder and multi-vitamin every two days when metabolism measurements were not taking place.

Measuring metabolic rate at different temperatures

Given the scale of our experiment, we used closed-system respirometry instead of intermittent-flow through respirometry. We measured routine metabolic rate (hereafter referred to as metabolic rate [MR]) as we could not control for additional energy expenditure resulting from small amounts of activity within chambers (although it was likely to be small) (Withers 1992, Mathot and Dingemanse 2015). Metabolic rate was measured as the volume of CO_2 production per unit time ($\dot{V}_{CO_2}$ ml min^{-1}) for animals in a post-absorptive state because CO_2 production is easier to detect in smaller organisms, and is less susceptible to fluctuations in water vapour than O_2 consumption (Artacho et al. 2013). Our preliminary data showed that CO_2 production was strongly correlated with O_2 consumption nonetheless ($r = 0.94$, $p \leq 0.05$). Measurements took place a year after collection, between 26 November 2016 and 19 March 2017 as lizards were also part of ongoing breeding experiments. While lizards were predicted to have acclimated to lab conditions, our measurements should have still captured any individual variation in MR attributed to genetic and developmental differences among individuals (Dingemanse and Wolf 2013). Due to logistical constraints, lizards were randomly assigned to one of two blocks for MR measurements. Each block was measured three days apart (block 1: n = 23, block 2: n = 22). We used two incubators to control the acute temperature at which measurements were taken (± 1°C). Measurements were taken in a random order at 22, 24, 26, 28, 30 and 32°C over three days (measurements at two temperatures per day). We also statistically accounted for the order of temperatures animals experienced in our analyses to control for any possible carry over effects of higher temperatures on individuals' subsequent MR measurements.

After a 24-h fasting period, the body temperature of each individual inside their enclosure was taken using an infrared laser gun (Stanley stht0-77365) in the morning (~06:00). Each lizard was then gently encouraged into their 146 ml opaque chamber and weighed using a digital scale to the nearest 0.01 g. Chambers were placed inside the incubators in the dark at a randomised temperature for 30 min. The lids of the chambers were left ajar during this time to minimise CO_2 build up. After 30 min, each chamber was flushed with fresh air and sealed. A 3 ml 'baseline' 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 min. After this time, two 3 ml air samples were taken from each chamber. Chambers were then reopened and flushed with fresh air before being placed back into the incubator for the second measurement temperature (two temperatures per day) following the same procedure.

All air samples were injected into the inlet line of a Sable System FMS 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 after the injection point using Drierite (Fig. 4.1, Lighton 2008). 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 Eq. 4.21 in Lighton 2008:

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

where $\%CO_2$ is the maximum percentage of CO_2 in air sample above baseline, which was corrected by subtracting any 'baseline' CO_2 from the initial flush from the two air samples; $V_{chamber}$ is the volume of the chamber (146 ml); 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), and t is the duration of time in minutes after the chamber had been sealed and the first air sample was taken (90 min). Overall, our protocol allowed us to characterise the full reaction norm for each lizard 10 times while accounting for measurement error. As such, our design resulted in n = 5040 measurements of metabolic rate across the 42 lizards ([2 air samples × 6 temperatures] × 10 sampling sessions = 120 samples per lizard). However, missing data, resulting from equipment malfunction, meant that our total sample size was n = 4952.

Statistical analyses

All statistical analyses were conducted using the R environment, ver. 3.6.1 (<www.r-project.org>). Details on data cleaning are presented in the Supporting information, and all data and code with which to generate our results are openly available via the Open Science Framework (doi: 10.17605/OSF.IO/TZ2H5, url: https://osf.io/tz2h5/).

Initial analyses showed that there were no differences in $\log\dot{V}_{CO_2}$ between collection sites or statistical blocks of lizards therefore these grouping variables were not included in our final models (Supporting information). Body temperatures experienced by lizards prior to measurements were different depending on both 1) their home enclosure temperature and 2) the first temperature experienced on the day of measurement. Differences in body temperature may result

in carry-over effects of the previous temperature on a lizard's MR measurement at a given point in time. We therefore tested whether the body temperature measured in the home enclosure before the first measurement or the previous measurement temperature (if MR measurements were underway) influenced $\log\dot{V}_{CO_2}$ at subsequent temperatures. We found that a model containing 'previous temperature experience' as a covariate was better supported compared to a model without it (ΔWAIC (full model–reduced model = −8.39), we therefore included 'previous temperature experience' in all subsequent analyses (Supporting information). Pearson correlation coefficients and scatterplots showed that predictor variables were not strongly collinear (Supporting information).

We used Bayesian linear mixed effect models from either the package 'brms' (Bürkner 2017) or 'MCMCglmm' (Hadfield 2010). For logistical reasons, we fitted the random slope model using 'MCMCglmm', and a multivariate response model using 'brms'. Details on model priors and set up are presented in the Supporting information. For every model, we pooled the posterior estimates from multiple chains and presented posterior means and their 95% credible intervals.

Measurement error and repeatability of metabolic thermal plasticity

Repeatability is a ratio of among-individual and residual variance components ($R = \sigma_A/(\sigma_A + \sigma_R)$) and represents the proportion of phenotypic variance attributed to among-individual differences (Nakagawa and Schielzeth 2010). The relative contribution to each variance component can shed light on the processes that promote repeatable traits (Dingemanse and Dochtermann 2013). Measurement error, however, can bias the estimation of variance components and affect repeatability estimates (Ponzi et al. 2018). Given that we took two air samples for each MR measurement, we were able to estimate measurement error by including a nested random effect of individual ID, sampling session and temperature (Individual_ID:Session_ID:Temp, hereafter referred to as measurement error) in our models. This term partitions out variance attributed to measurement error among replicates from the residual variance, which includes other sources of within individual variance.

We fitted the following random slope model in 'MCMCglmm' ($n_{obs} = 4952$) in order to quantify the repeatability of metabolic thermal plasticity (i.e. slopes for each individual):

$$\begin{aligned}\log\dot{V}_{CO_2} \sim\ & \text{Temp} + \text{zlogBodyMass} + \text{PriorTemp} + (1 + \text{Temp} \mid \text{Individual\_ID}) \\ & + (1 + \text{Temp} \mid \text{Individual\_ID} : \text{Session\_ID}) \\ & + (1 \mid \text{Individual\_ID} : \text{Session\_ID} : \text{Temp})\end{aligned}$$

where: $\log\dot{V}_{CO_2}$ is log-transformed $\dot{V}_{CO_2}$; Temp is the temperature in degrees Celsius; zlogBodyMass is log-transformed body mass that is then subsequently z-transformed; PriorTemp is previous temperature experienced by the lizard (enclosure temperature or the previous treatment temperature). Individual ID, series (Individual_ID:Session_ID) and measurement error were included as random intercepts. Series is a nested random effect of individual ID and sampling session (see Araya-Ajoy et al. 2015 for further explanation) which allowed us to estimate the slope repeatability. This term estmates between individual variance in slopes and takes into consideration of any changes in MR over the course of our four months of study. Temperature was included as a random slope for both individual ID and series to estimate among and within individual slopes. The repeatability of the slope is then calculated following Eq. 1 in the Supporting information (Araya-Ajoy et al. 2015).

Repeatability of MR at each temperature and cross-temperature correlations

After assessing whether individuals differ in their metabolic thermal plasticity, we were interested in knowing whether consistent among-individual differences in average MR change across temperatures. To achieve this, we fitted a multivariate response model by treating MR measurements for each of the six temperatures as separate traits ($n_{obs} = 840$) in a 6×6 response matrix:

$$\begin{aligned}&\begin{bmatrix} \log VCO_{2_{1,1,22^{\circ}C}} & \log VCO_{2_{1,1,24^{\circ}C}} & \dots & \log VCO_{2_{1,1,32^{\circ}C}} \\ \log VCO_{2_{1,2,22^{\circ}C}} & \log VCO_{2_{1,2,24^{\circ}C}} & \dots & \log VCO_{2_{1,2,32^{\circ}C}} \\ \vdots & \vdots & \ddots & \vdots \\ \log VCO_{2_{1,10,22^{\circ}C}} & \log VCO_{2_{1,10,24^{\circ}C}} & \dots & \log VCO_{2_{1,10,32^{\circ}C}} \end{bmatrix} \sim \text{zlogBodyMass} \\ &+ \text{PriorTemp} + (1 \mid \text{Individual\_ID}) + (1 \mid \text{Individual\_ID} : \text{Session\_ID})\end{aligned}$$

where, $\log VCO_{2_{1,1,22^{\circ}C}}$ is the metabolic rate for individual 1 in sampling session 1 at 22°C and $\log VCO_{2_{1,10,22^{\circ}C}}$ is the metabolic rate for individual 1 in sampling session 10 at 22°C and so forth. Similar to the random slope models, we included zlogBodyMass and PriorTemp as fixed effects. Note that temperature is no longer a predictor or a random slope term as temperature is now part of the response matrix. In some instances, mechanical errors occurred during air collection. Given that 'brms' requires complete data in the response matrix, we used the 'mi' function to impute the missing samples at each temperature as this prevented us from excluding 607 rows of data. We included individual ID and series as random intercepts. In this model, series is responsible for partitioning out measurement error from the residuals. We calculated temperature specific repeatability following Eq. 2 in the Supporting information.

We were also interested in the extent to which MR was correlated across all temperatures as this may illuminate tradeoffs in physiological function at different temperatures. We obtained cross-temperature correlations at the among-individual level using the variance–covariance matrix obtained from the multivariate response model.

Mass-scaling exponents at different temperatures

Population estimates of scaling exponents can be affected by within and among individual variation (van de Pol and Wright 2009). We therefore wanted to partition out within individual effects in order to obtain more precise estimates

of mass-scaling across temperatures. To achieve this, we calculated the mean mass across all sampling sessions for each individual (among individual effect), and subtracted an individual's mass from its own mean to account for within individual effects (also known as within-individual centering, van de Pol and Wright 2009). These mass effects were log-transformed and included in two models fitted in 'brms'. The total number of observations for this analysis was $n_{obs} = 3933$ because 'brms' requires no missing values in $\log\dot{V}_{CO_2}$ in order for this model to run. The first model (interaction model) had the following structure,

$$\log\dot{V}_{CO_2} \sim \text{Temp} \times \text{logAmongIDMass} + \text{Temp} \times \text{logWithinIDMass} + (1 + \text{logWithinIDMass} \mid \text{Individual\_ID}) + (1 \mid \text{Individual\_ID} : \text{Session\_ID} : \text{Temp})$$

where: Temp × logAmongIDMass is the interaction term between temperature and the log transformed among individual mass effect; Temp × logWithinIDMass is the interaction term between temperature and the log transformed within individual mass effect. Individual ID was fitted a random intercept with logWithinIDMass as a random slope as individuals masses changed at different rates through the study (Supporting information). We also included the measurement error term. The second model (main effects model) only had the main effects of temperature, the among individual mass effect and the within-individual mass effect and the same random effects structure as the interaction model. We tested whether population mass-scaling exponents (i.e. the among individual mass effects) changed with temperature by comparing WAIC and loo values between the interaction and main effects model. We report expected log predictive density (ELPD) values and standard error for the difference between the two models (Vehtari et al. 2017). We also present in the Supporting information an analysis that compared the mass scaling exponents with estimates, i.e. a model that represents the typical analysis of a metabolic scaling study from a model that did not account for the multi-level variation in the data.

Results

Repeatability of metabolic thermal plasticity

Individual slopes describing the effect of temperature on MR were significantly repeatable, albeit with wide credible intervals ($R_{slope} = 0.25$, 95% CI = $2.48 \times 10^{-8} - 0.67$), suggesting individuals consistently varied in how their metabolic rate changed with temperature (Fig. 1). However, repeatability of the slope was relatively low.

Repeatability of metabolic rate at each temperature

We found that the repeatability of MR (i.e. individual intercepts) was stable across acute temperatures (Fig. 2). Temperature-specific repeatability was greatest at

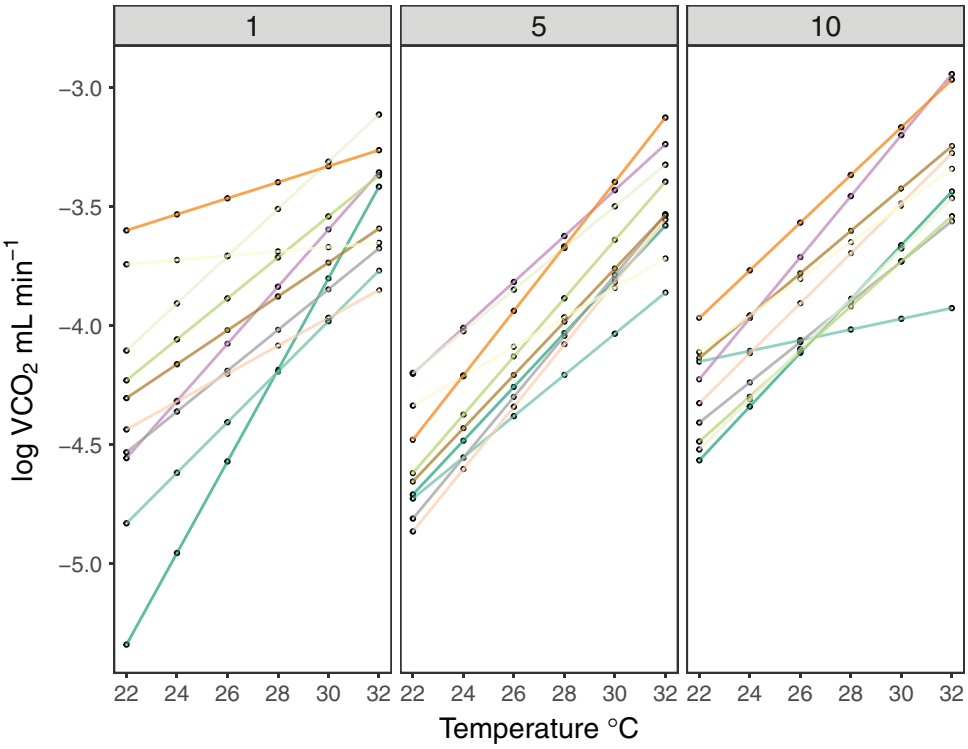

Figure 1. A subset of ten random individual reaction norms of mass-corrected log metabolic rate ($\dot{V}_{CO_2}$ ml min^{-1}) at six measurement temperatures for sampling session one (left panel), five (middle panel) and ten (right panel) reaction norms. Points are predicted values from a random slope model. Each line represents a unique individual (n = 10).

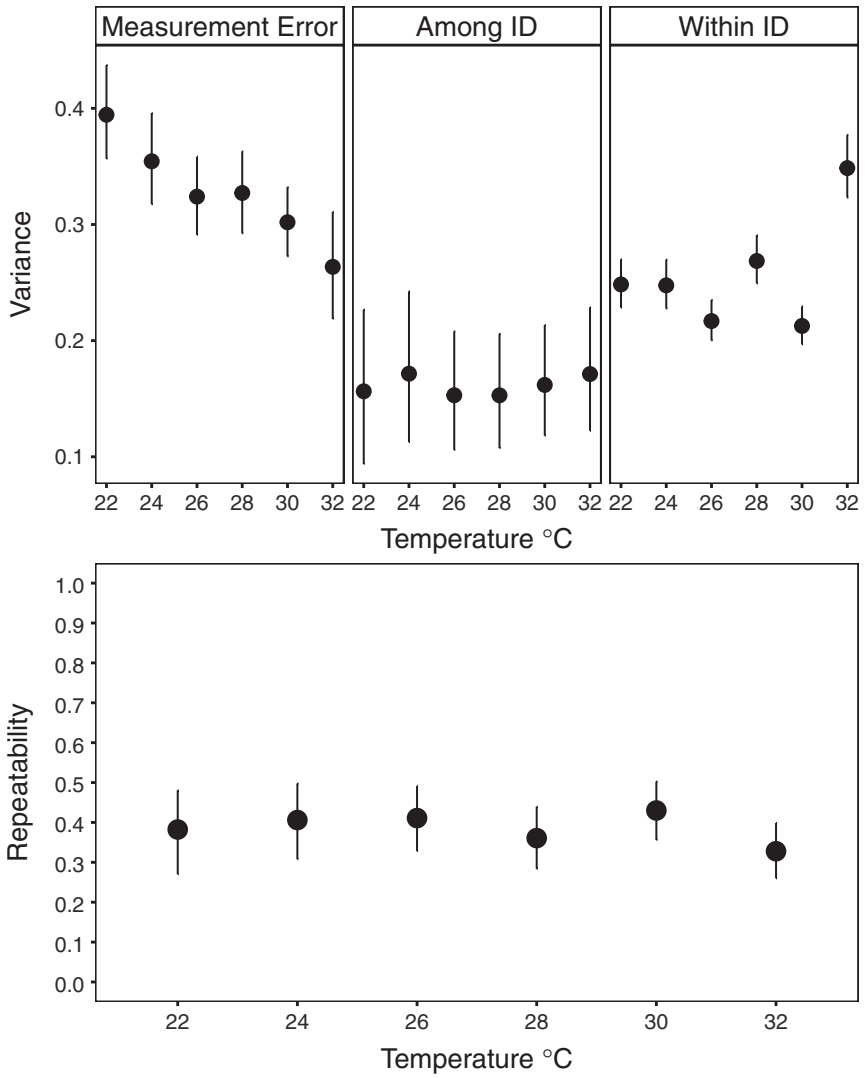

Figure 2. Posterior mean of variance components and repeatability of log metabolic rate ($\dot{V}_{CO_2}$ ml min^{-1}) at six measurement temperatures estimated over four-month period across n = 42 individuals. Error bars represent 95% credible intervals.

24°C, however credible intervals overlapped with estimates at other temperatures (Fig. 2, Supporting information). Upon closer inspection of the variance components at each temperature, we show that measurement error decreased steadily with increasing temperature, whereas among individual variation remained relatively consistent with temperature (Fig. 2B). In contrast, within individual variance showed no consistent pattern with temperature, however it was highest in 32°C. In other words, individuals were responding more variably as 32°C while among individual differences were relatively stable (Fig. 2).

Cross-temperature correlations in metabolic rate

Metabolic rate across temperatures were positively correlated at the among-individual level (Fig. 3, Supporting information). Positive correlations indicate that some individuals maintained a consistently high metabolic rate relative to other individuals, while others had a relatively low metabolic rate, across all temperatures. Metabolic rate measured at neighbouring temperatures (e.g. 22°C and 24°C) were strongly correlated, but the strength of this correlation decreased with increasing differences between the two temperatures (Fig. 3).

Temperature dependence of population mass-scaling exponents

According to the WAIC values, there was equal support for both models ([Interaction model - main effects model] WAIC: ELPD = -4.54, SE = 3.69), whereas loo values preferred the interaction model ([Interaction model - main effects model] loo: ELPD = -18.19, SE = 6.44). Nonetheless, the credible intervals for the mass scaling exponent at each temperature overlapped substantially, suggesting a small overall effect size and weak evidence for temperature dependence (Fig. 4). Across all temperatures, the average mass scaling

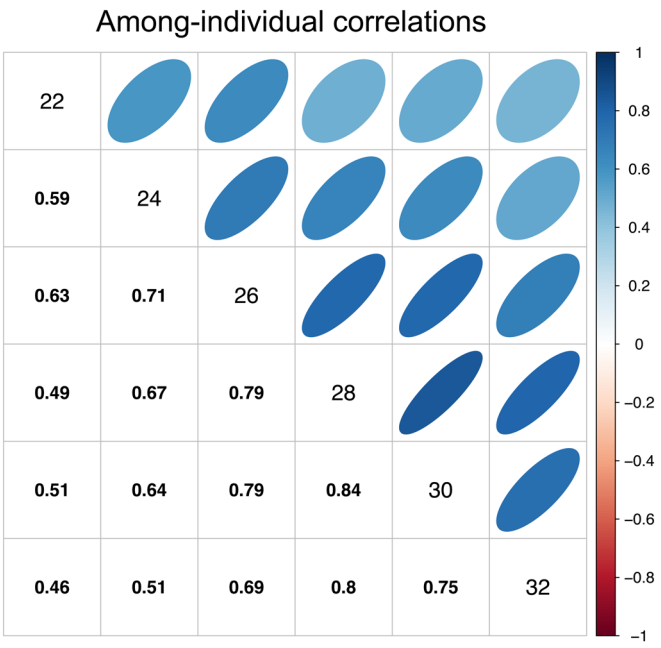

Figure 3. Cross-temperature correlations of log metabolic rate (V_{CO_2} ml min^{-1}) at the among-individual level estimated from n = 42 individuals. Diagonal values are each measurement temperatures. Lower triangle represents posterior mean estimates of correlations. Width and colour of the ellipse in the upper triangle represents the strength of the correlation.

exponent was 0.96 (95% CI = 0.39–1.52) which is in line with values reported for squamates (0.84, 95% CI = 0.70–0.97, Uyeda et al. 2017). Mass-scaling exponents tended to be spurious and estimated with a larger degree of error when the within individual effects and measurement error were not statistically accounted for, see the Supporting information.

Discussion

Our results show that metabolic thermal plasticity was weakly repeatable over the four months of study in delicate skinks. Moreover, the repeatability of average MR was also not susceptible to acute temperature changes. Cross-temperature correlations of MR were all positive at the among-individual level. However, the strength of these correlations was not uniform across all temperatures. Mass scaling exponents were not strongly affected by temperature and in line with values reported for squamates when other sources of variation were partitioned out. Below we discuss the implications of our results for understanding how plasticity may evolve, and how MR scales at different hierarchical levels.

Consistent variation in metabolic thermal plasticity

Natural selection acts on phenotypic variation among individuals. Consistent among individual variation is therefore a key prerequisite for any trait to evolve and sets the 'upper limit of heritability' (Falconer 1952, cf. Dohm 2002). Our findings show that individuals differ consistently in how their MR responds to acute temperature changes over an ecologically relevant time period, although overall repeatability of the slope was relatively low and imprecise. It is important to note that repeatability estimates capture variation due to both genetic and environmental effects, such as different developmental environments experienced by animals (Careau et al. 2014, Nørgaard et al. 2021). However, assuming a proportion of among-individual differences is the result of heritable variation our findings suggests that metabolic thermal plasticity may be capable of evolutionary change allowing shifts in population-level metabolic reaction norms (Ghalambor et al. 2007). Average metabolic rate was also repeatable and stable across temperatures and suggests that the operable range of temperature in *L. delicata* promotes consistency in physiological traits (Matthews et al. 2016, Goulet et al. 2017). Measurement error declined with increasing temperature presumably because individuals were respiring at a higher rate making it easier to detect changes in CO_2 production. Measurement error can inflate repeatability estimates if it is not accounted for statistically (Ponzi et al. 2018). Indeed, we found a significant increase in repeatability and among individual variance when we took averages between the two replicate air samples (Supporting information). Consequently, one would mistakenly conclude that the capacity for selection to act on MR would increase at hotter temperatures. We stress the importance of considering confounding sources of variances such as measurement error or shared environmental effects to ascertain the potential for physiological traits to undergo selection. Quantifying the repeatability of reaction norm slopes is incredibly labour intensive and future studies should be aware that the accuracy of slope repeatability will be highly dependent on experimental design and sample size (for guidelines see van de Pol 2012 and Araya-Ajoy et al. 2015).

Cross-temperature correlations

Metabolic rate was positively correlated across all temperatures at the among individual level. This suggests that individuals with high MR at one temperature also tend to exhibit high MR at other temperatures (and vice versa for individuals with low MR). Individuals could vary in their acquisition or allocation of resources enabling certain individuals to maintain a consistently high MR across all temperatures (De Jong and Van Noordwijk 1992, Angilletta Jr. et al. 2003). Consistent individual differences in MR, irrespective of the thermal environment, may be functionally linked with other aspects of the phenotype (Biro and Stamps 2010). Our results give precedence to 'pace-of-life' theory, specifically, the assumption that consistent individual differences in energetic expenditure underly individual differences in behaviour and life-history within the same population (Careau et al. 2008, Biro and Stamps 2010). For metabolism to be a causal driver of other phenotypic traits, it must be repeatable and correlated across different temperatures, as we have shown.

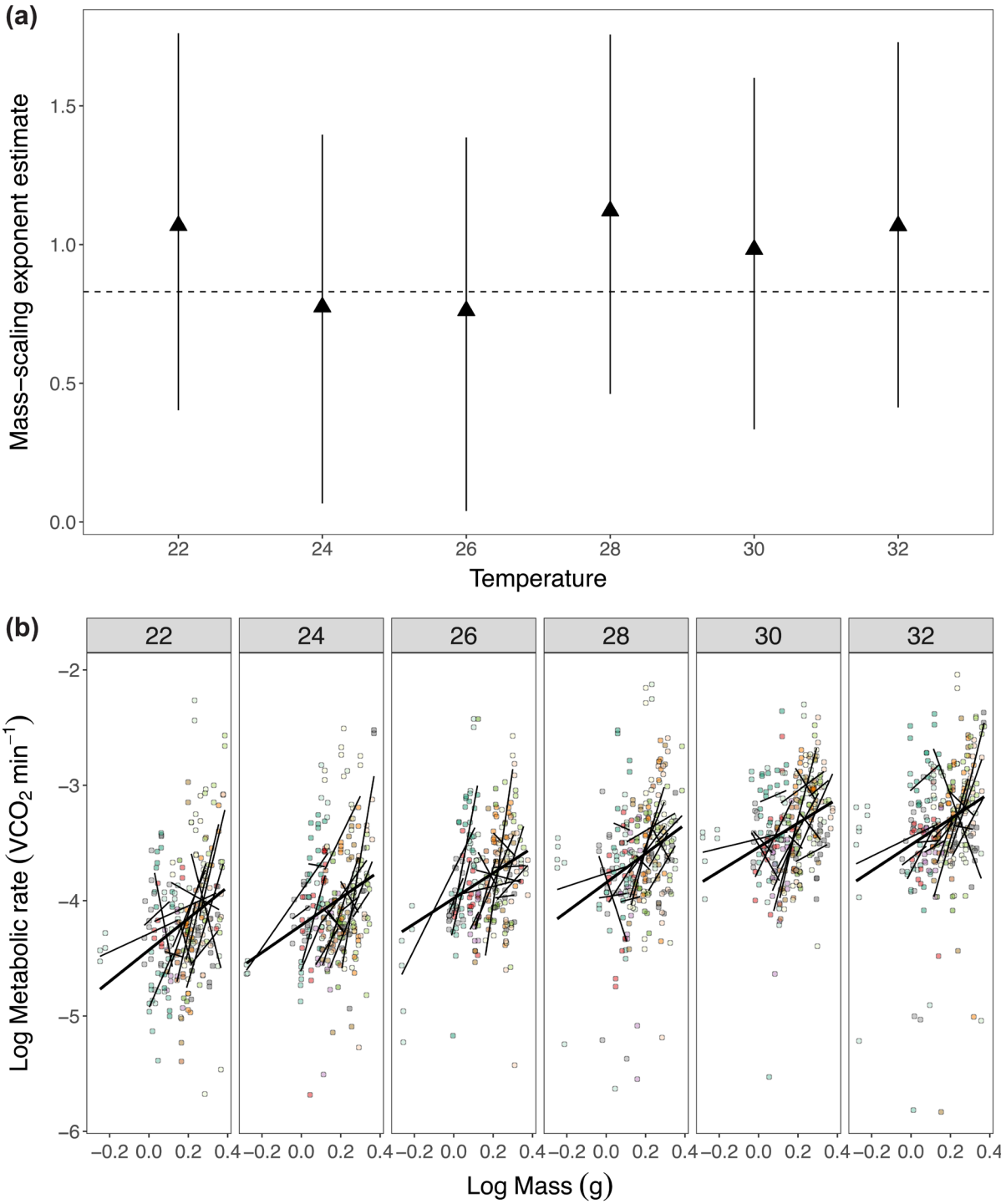

Figure 4. (Top) Posterior mean estimates of population mass scaling exponents (i.e. among individuals) of log metabolic rate ($\dot{V}_{CO_2}$ ml min^{-1}) across six measurement temperatures when within individual variation in mass and measurement error in metabolic rate has been statistically accounted for. The dashed line represents the mass-scaling exponent of 0.84 estimated for squamates from Uyeda et al. (2017). Error bars represent 95% credible intervals. (Bottom) Raw log metabolic rate plotted against log body mass for a random subset of n = 20 individuals at six measurement temperatures. Each uniquely coloured point represents one individual. Thick bold line represents the change in log metabolic rate over log body mass across all individuals (among individual mass scaling slope). Thin lines represent the change in log MR over log body mass within an individual (within individual mass scaling slopes).

The strength of cross-temperature correlations can help identify tradeoffs in physiological processes across different thermal environments. Such tradeoffs have been hypothesised to be important mechanisms in shaping reaction norms (Angilletta Jr. et al. 2003). Generalist–specialist tradeoffs occur when some individuals have enhanced physiological function in one environment but diminished function in another environment, manifesting as a negative cross environment correlation (Berger et al. 2014). We show that across different temperatures, correlations were all positive, providing no support for tradeoffs between temperatures in energy expenditure. While our temperatures fell within the normal temperature range experienced by animals in the wild, tradeoffs may exist in other parts of the thermal performance curve (Angilletta Jr. et al. 2003). Assuming phenotypic cross-temperature correlations reflect the underlying genetic architecture of metabolic rate (Roff 1995), the strength of correlation can dictate how strongly selection acting on one component of the reaction norm will result in indirect selection on another (Via et al. 1995). This implies that response to selection would be stronger between neighbouring temperatures (e.g. 28°C versus 32°C) compared to more distant temperatures (e.g. 22°C versus 32°C) which might be important for the evolution of non-linear reaction norms (Berger et al. 2013). It is possible that low precision in incubator temperatures (± 1°C) may have contributed to positive cross-temperature correlations between adjacent temperatures, however this does not explain why correlations were stronger at hot temperatures compared to cool temperatures.

Population mass-scaling across different temperatures

Mass-scaling exponents were robust to acute temperature changes, which disagrees with a growing number of studies that show temperature dependence of mass scaling exponents (Glazier 2005, 2015, Killen et al. 2010, Price et al. 2012, Barneche et al. 2016). Discrepancies may be due to the method by which we quantified mass scaling exponents. For example, we used mass as a proxy of body volume in our calculation of MR which can confound body mass with other aspects of body composition such as water content, lean mass and fat mass and in turn affect mass scaling coefficients (Daan et al. 1990, McLean and Speakman 2000). Furthermore, in our study, we sampled sexually mature adults repeatedly over four months in order to estimate a static mass scaling relationship, while other studies tend to measure ontogenetic allometry (change in body mass and metabolic rate throughout development, Glazier 2009). The energetic demands of growth during ontogeny may be more sensitive to temperature change and therefore result in temperature-dependence in ontogenetic mass scaling exponents (Hirst et al. 2014, Barneche and Allen 2018). In support of this, a recent comparative analysis has shown that development (passing through life stages) shows stronger temperature dependence than increases in mass (Forster et al. 2011).

The magnitude and precision of mass scaling exponents may be affected by processes occurring at different hierarchical levels. Genetic and developmental differences that impact the physiological system can maintain permanent differences among individuals (Dingemanse and Wolf 2013). While fluctuations in the internal environment, such as circulating hormones and body composition, can affect the within individual responses (Scott et al. 1996, McCue 2010, Dupoué et al. 2013). After accounting for within individual effects and measurement error, our mass-scaling exponent estimates were congruent with values reported from a phylogenetically informed analyses in squamates (Uyeda et al. 2017). This result may have important implications for current designs of metabolic scaling studies as MR and body mass tend to only be measured once, making them sensitive to sampling error and within individual 'noise'. Theoretical studies that make use of the predictive relationship between body mass and metabolism should be more aware of the different sources of variation when trying to extrapolate individual level processes to higher levels of biological organisation. Future work is warranted to investigate the degree to which intra-individual variance in MR and body mass impact scaling exponents as this has largely been neglected, yet may help elucidate why mass scaling exponents are variable at higher levels of biological organisation (McLean and Speakman 2000, Maxwell et al. 2003, Glazier 2005).

Conclusion

In this study, we found that metabolic thermal plasticity was repeatable to a certain extent, with individuals showing moderately low consistency in their reaction norms to acute temperatures. Such individual consistency is difficult to quantify with high precision but may be important in promoting stability in mass-scaling across temperature. Our work implies that selective processes have a potential in shaping metabolic reaction norms. Quantitative genetic and experimental evolution studies are necessary to truly understand the evolutionary potential of metabolic thermal plasticity. However, our work also emphasises that future work should consider important hierarchical and methodological factors that can impact upon variation (e.g. measurement error). Neglecting to consider individual variation resulting from different levels of biological organisation altogether may misguide predictions about ecological processes (Botero et al. 2015) and misconstrue the evolutionary relevance of phenotypic variability in physiological traits (Ponzi et al. 2018).

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 express gratitude for all the members of the Lizard Lab at Macquarie University for assistance and support throughout this study. Especially, A/Prof. Martin Whiting for the use of his facilities. We are in debt to Christine Wilson for her assistance with animal husbandry. We

really appreciated the help of Stephan Klopper in the construction of our metabolic chambers. We would also like to acknowledge Martin Thompson at the Division of Research, University of New South Wales for his technical aid with our models. Finally, we thank David Mitchell and Tobias Uller insightful discussions and Rose O'Dea for her comments on an earlier draft of this manuscript.
Funding – 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).
Permits – 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. Animal collection was approved by the New South Wales National Parks and Wildlife Service (SL101549) and 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).
Conflicts of interest – All authors declare no conflict of interest.

Author contributions

Fonti Kar: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (lead); Software (lead); Supervision (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Shinichi Nakagawa**: Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Funding acquisition (supporting); Investigation (supporting); Methodology (supporting); Resources (supporting); Software (supporting); Supervision (supporting); Visualization (supporting); Writing – original draft (supporting); Writing – review and editing (supporting). **Christopher R. Friesen**: Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing – original draft (supporting); Writing – review and editing (supporting). **Daniel W. A. Noble**: Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Resources (lead); Supervision (supporting); Writing – original draft (supporting); Writing – review and editing (supporting).

Data accessibility

Datasets and code used to generate results of this study is accessible via Open Science Framework (doi: 10.17605/OSF.IO/TZ2H5, <https://osf.io/tz2h5/>).

References

Allen, A. P. et al. 2005. Linking the global carbon cycle to individual metabolism. – Funct. Ecol. 19: 202–213.
Angilletta Jr., M. J. et al. 2003. Tradeoffs and the evolution of thermal reaction norms. – Trends Ecol. Evol. 18: 234–240.
Angilletta, M. J. and Sears, M. W. 2000. The metabolic cost of reproduction in an oviparous lizard. – Funct. Ecol. 14: 39–45.
Araya-Ajoy, Y. G. et al. 2015. An approach to estimate short-term, long-term and reaction norm repeatability. – J. Anim. Ecol. 6: 1462–1473.
Artacho, P. et al. 2013. Interindividual variation in thermal sensitivity of maximal sprint speed, thermal behavior and resting metabolic rate in a lizard. – Physiol. Biochem. Zool. 86: 458–469.
Barneche, D. R. and Allen, A. P. 2018. The energetics of fish growth and how it constrains food-web trophic structure. – Ecol. Lett. 21: 836–844.
Barneche, D. R. et al. 2016. Temperature effects on mass-scaling exponents in colonial animals: a manipulative test. – Ecology 98: 103–111.
Berger, D. et al. 2013. Quantitative genetic divergence and standing genetic (co)variance in thermal reaction norms along latitude. – Evolution 67: 2385–2399.
Berger, D. et al. 2014. Experimental evolution for generalists and specialists reveals multivariate genetic constraints on thermal reaction norms. – J. Evol. Biol. 27: 1975–1989.
Biro, P. A. and Stamps, J. A. 2008. Are animal personality traits linked to life-history productivity? – Trends Ecol. Evol. 23: 361–368.
Biro, P. A. and Stamps, J. A. 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? – Trends Ecol. Evol. 25: 653–659.
Botero, C. A. et al. 2015. Evolutionary tipping points in the capacity to adapt to environmental change. – Proc. Natl Acad. Sci. USA 112: 184–189.
Briga, M. and Verhulst, S. 2017. Individual variation in metabolic reaction norms over ambient temperature causes low correlation between basal and standard metabolic rate. – J. Exp. Biol. 220: 3280–3289.
Bronikowski, A. M. and Vleck, D. 2010. Metabolism, body size and life span: a case study in evolutionarily divergent populations of the garter snake *Thamnophis elegans*. – Integr. Comp. Biol. 50: 880–887.
Brown, J. H. et al. 2004. Toward a metabolic theory of ecology. – Ecology 85: 1771–1789.
Bürkner, P. C. 2017. brms: an R package for Bayesian multilevel models using Stan. – <https://cran.r-project.org/web/packages/brms/index.html>.
Burton, T. et al. 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? – Proc. R. Soc. B 278: 3465–3473.
Careau, V. et al. 2008. Energy metabolism and animal personality. Oikos 117: 641–653.
Careau, V. et al. 2014. Early-developmental stress, repeatability and canalization in a suite of physiological and behavioral traits in female zebra finches. – Integr. Comp. Biol. 54: 539–554.
Chapple, D. G. et al. 2011. Phylogeographic divergence in the widespread delicate skink (*Lampropholis delicata*) corresponds to dry habitat barriers in eastern Australia. – BMC Evol. Biol. 11: 191.
Chapple, D. G. et al. 2014. Biology of the invasive delicate skink *Lampropholis delicata* on Lord Howe Island. – Aust. J. Zool. 62: 498–506.
Clarke, A. 2004. Is there a universal temperature dependence of metabolism? – Funct. Ecol. 18: 252–256.
Daan, S. et al. 1990. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. – Am. J. Physiol. Regul. Integr. Comp. Physiol. 259: R333–R340.
De Jong, G. and Van Noordwijk, A. J. 1992. Acquisition and allocation of resources: genetic (co)variances, selection and life histories. – Am. Nat. 139: 749–770.

Dingemanse, N. J. and Dochtermann, N. A. 2013. Quantifying individual variation in behaviour: mixed-effect modelling approaches. – J. Anim. Ecol. 82: 39–54.
Dingemanse, N. J. and Wolf, M. 2013. Between-individual differences in behavioural plasticity within populations: causes and consequences. – Anim. Behav. 85: 1031–1039.
Dupoué, A. et al. 2013. Influence of temperature on the corticosterone stress-response: an experiment in the Children's python *Antaresia childreni*. – Gen. Comp. Endocrinol. 193: 178–184.
Falconer, D. S. 1952. The problem of environment and selection. – Am. Nat. 86: 293–298.
Forster, J. et al. 2011. Growth and development rates have different thermal responses. – Am. Nat. 178: 668–678.
Friesen, C. R. et al. 2017. Morph-specific metabolic rate and the timing of reproductive senescence in a color polymorphic dragon. – J. Exp. Zool. Part Ecol. Integr. Physiol. 327: 433–443.
Ghalambor, C. K. et al. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. – Funct. Ecol. 21: 394–407.
Glazier, D. S. 2005. Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. – Biol. Rev. 80: 611–662.
Glazier, D. S. 2009. Ontogenetic body-mass scaling of resting metabolic rate covaries with species-specific metabolic level and body size in spiders and snakes. – Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 153: 403–407.
Glazier, D. S. 2015. Is metabolic rate a universal "pacemaker" for biological processes? – Biol. Rev. 90: 377–407.
Goulet, C. T. et al. 2017. Thermal physiology: a new dimension of the pace-of-life syndrome. – J. Anim. Ecol. 86: 1269–1280.
Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. – <https://cran.r-project.org/web/packages/MCMCglmm/index.html>.
Hirst, A. G. et al. 2014. Body shape shifting during growth permits tests that distinguish between competing geometric theories of metabolic scaling. – Ecol Lett 17: 1274–1281.
Ivleva, I. V. 1980. The dependence of crustacean respiration rate on body mass and habitat temperature. – Int. Rev. Gesamten Hydrobiol. Hydrogr. 65: 1–47.
Killen, S. S. et al. 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. – Ecol. Lett. 13: 184–193.
Lighton, J. R. B. 2008. Measuring metabolic rates. – Oxford Univ. Press.
Malishev, M. et al. 2017. An individual-based model of ectotherm movement integrating metabolic and microclimatic constraints. – Methods Ecol. Evol. 9: 472–489.
Mathot, K. J. and Dingemanse, N. J. 2015. Energetics and behavior: unrequited needs and new directions. – Trends Ecol. Evol. 30: 199–206.
Matthews, G. et al. 2016. The effect of skin reflectance on thermal traits in a small heliothermic ectotherm. – J. Therm. Biol. 60: 109–124.
Maxwell, L. K. et al. 2003. Intraspecific allometry of standard metabolic rate in green iguanas, *Iguana iguana*. – Comp. Biochem. Physiol. A Mol. Integr. Physiol. 136: 301–310.
McCue, M. D. 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. – Comp. Biochem. Physiol. A Mol. Integr. Physiol. 156: 1–18.
McLean, J. A. and Speakman, J. R. 2000. Effects of body mass and reproduction on the basal metabolic rate of brown long-eared bats *Plecotus auritus*. – Physiol. Biochem. Zool. 73: 112–121.
Nakagawa, S. and Schielzeth, H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. – Biol. Rev. 85: 935–956.
Nørgaard, L. S. et al. 2021. Energetic scaling across different host densities and its consequences for pathogen proliferation. – Funct. Ecol. 35: 475–484.
Norin, T. and Gamperl, A. K. 2018. Metabolic scaling of individuals vs populations: evidence for variation in scaling exponents at different hierarchical levels. – Funct. Ecol. 32: 379–388.
Nussey, D. H. et al. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. – J. Evol. Biol. 20: 831–844.
Orrell, K. S. et al. 2004. Intersexual differences in energy expenditure of *Anolis carolinensis* lizards during breeding and postbreeding seasons. – Physiol. Biochem. Zool. 77: 50–64.
Patterson, J. W. and Davies, P. M. C. 1984. The influence of temperature, sexual condition and season on the metabolic rate of the lizard *Psammodromus hispanicus*. – J. Comp. Physiol. B 154: 311–316.
Piersma, T. and Drent, J. 2003. Phenotypic flexibility and the evolution of organismal design. – Trends Ecol. Evol. 18: 228–233.
Ponzi, E. et al. 2018. Heritability, selection and the response to selection in the presence of phenotypic measurement error: effects, cures and the role of repeated measurements. – Evolution 72: 1992–2004.
Price, C. A. et al. 2012. Testing the metabolic theory of ecology. – Ecol. Lett. 15: 1465–1474.
Réale, D. et al. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. – Phil. Trans. R. Soc. B 365: 4051–4063.
Réveillon, T. et al. 2019. Repeatable inter-individual variation in the thermal sensitivity of metabolic rate. – Oikos 85: 935–938.
Roff, D. A. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. Heredity 74: 481–490.
Salin, K. et al. 2016. Variation in metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak respiration. – Physiol. Biochem. Zool. 89: 511–523.
Scott, I. et al. 1996. How does variation body composition affect the basal metabolic rates of birds of birds? – Funct. Ecol. 10: 307.
Steyermark, A. C. 2005. Physiological and morphological correlates of among-individual variation in standard metabolic rate in the leopard frog *Rana pipiens*. – J. Exp. Biol. 208: 1201–1208.
Toledo, L. F. et al. 2008. Effects of season, temperature and body mass on the standard metabolic rate of tegu lizards *Tupinambis merianae*. – Physiol. Biochem. Zool. 81: 158–164.
Uyeda, J. C. et al. 2017. The evolution of energetic scaling across the vertebrate tree of life. – Am. Nat. 190: 185–199.
van de Pol, M. 2012. Quantifying individual variation in reaction norms: how study design affects the accuracy, precision and power of random regression models. – Methods Ecol. Evol. 3: 268–280.
van de Pol, M. and Wright, J. 2009. A simple method for distinguishing within- versus between-subject effects using mixed models. – Anim. Behav. 77: 753–758.
Vehtari, A. et al. 2017. Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. – Stat. Comput. 27: 1413–1432.

Via, S. et al. 1995. Adaptive phenotypic plasticity: consensus and controversy. – Trends Ecol. Evol. 10: 212–217.
White, C. R. et al. 2006. The scaling and temperature dependence of vertebrate metabolism. – Biol. Lett. 2: 125–127.
Wilson, A. J. 2018. How should we interpret estimates of individual repeatability? – Evol. Lett. 219: 631–635.
Withers, P. C. 1992. Comparative animal physiology. – Saunders College Publ., Philadelphia, PA.