Thermal Plasticity and Mass-Scaling Variability: A Physiological Perspective on Metabolic Adaptation and Health Outcomes

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Abstract

1. Physiological processes of individuals can be highly variable and there is mounting evidence that individuals can differ in how they respond to environmental change. The ability for individuals to reversibly adjust their metabolic rate in response to temperature (i.e., metabolic thermal plasticity) may affect mass-scaling at the population level. This process has rarely been investigated before.
2. This study characterised the repeatability of metabolic thermal plasticity and tested how mass-scaling exponents change at different temperatures in the delicate skink (*Lampropholis delicata*). We repeatedly measured standard metabolic rate of forty-two individuals at six temperatures over the course of three months ($N_{[measurement]}$ = 2418). We explicitly accounted for multi-level variation in our data in order to quantify more precise estimates of mass-scaling exponents at different environmental temperatures.
3. Making use of two analytical frameworks, we found that metabolic thermal plasticity was significantly repeatable. Average standard metabolic rate increased as a function of temperature, which was associated with individuals responding more predictably (a decrease in within-individual variance) at higher temperatures. Interpretation of repeatability estimates and cross-temperature correlations varied slightly between the analytic approaches, but they were mostly in agreement.

4. After taking into account within- and among-individual level variation in our data, our estimates for mass-scaling did not change with temperature and were in line with published values for snakes and lizards. This suggests that repeatable plastic responses may contribute to thermal stability of scaling exponents.
5. Our work contributes to our understanding of whether phenotypic plasticity has the capacity to respond to selection which is particularly important for animals coping with rapid environmental change. Acknowledging multi-level variation in body mass and metabolic rate is not only important for comparative studies interested in mass-scaling across the animal kingdom, but also to theoretical research interested using the predictive power of mass-scaling.

Keywords

Phenotypic plasticity, reaction norm, thermal sensitivity, repeatability, thermal performance curves

Introduction

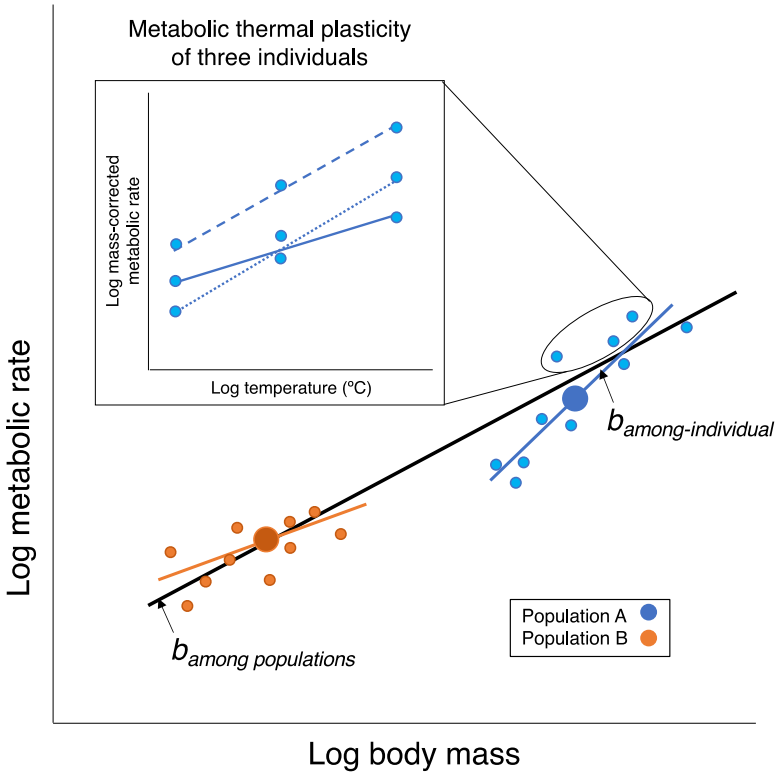
All biological processes hinge on the availability of energy (Allen, Gillooly & Brown 2005). In ectotherms, standard metabolic rate (SMR) represents the ‘idling cost of living’ at a specific temperature, and may govern how energy is allocated to competing processes such as growth, reproduction and maintenance (De Jong & Van Noordwijk 1992; Brown *et al.* 2004; Biro & Stamps 2008). SMR is predicted to be critical to fitness due to its functional link to performance, behaviour and life-history (Réale *et al.* 2010; Friesen, Johansson & Olsson 2017; Malishev, Bull & Kearney 2017; Biro & Stamps 2010). For example, common lizards that show high SMR and low exploratory behaviour and low SMR and high exploratory behaviour have the best survival during the first year of their life (Le Galliard *et al.* 2013). Indeed, numerous studies have demonstrated that SMR can vary several-fold among

individuals (reviewed in Biro & Stamps 2010), likely driven by the strong relationship between body mass and metabolic rate (i.e., mass-scaling relationships) (Glazier 2005; Bartheld *et al.* 2015). Nonetheless, there is growing interest in understanding the impact of environmental factors, such as temperature, on mass-scaling relationships given that energy expenditure can change for a given body size under varying environmental conditions (Barneche et al. 2017).

For decades, the relationship between SMR and body mass has sparked intense debate (Brown *et al.* 2004; Glazier 2015). SMR is predicted to follow an 'universal' allometric relationship with an exponent of 0.75. The commonality of the ¾ power law has been reported across a diversity of taxonomic groups, including mammals, fish and reptiles (White, Phillips & Seymour 2006), but its universality remains contentious. One problem with the ¾ power law is that it assumes the effects of temperature on metabolism is based on laws of thermodynamics. This assumption has been challenged because individuals can adaptively adjust biological rates to prevailing temperatures (reviewed in Clarke 2004; Glazier 2015). A recent phylogenetic comparative analysis in vertebrates showed that mass-scaling exponents have evolved and diverged substantially across the tree of life with exponents ranging from 0.65 to greater than 0.80 (Uyeda *et al.* 2017). Additionally, studies have shown that scaling exponents are inconsistent when applied to finer scales, such as across different populations of the same species (Burton *et al.* 2011), and even within individuals (Norin & Gamperl 2018). These findings suggest that the ¾ power law may not be as widely applicable as previously thought. Scaling relationships may depend on the environment experienced by the population, and physiological adjustments to these

conditions by individuals within the population (Killen, Atkinson & Glazier 2010; Barneche, White & Marshall 2016) (Fig.1).

Figure 1 –The scaling relationship between log body mass and log metabolic rate of two hypothetical populations where log(metabolic rate) = log(a) + b log(mass). b is the mass-scaling exponent which describes the change in log metabolic rate with log body mass and can vary depending on the level of interest. In this study, $b_{among\text{-}individual}$ is our primary interest, but can be affected by individual responses to environmental conditions. The large dots represent the mean log metabolic rate and mean log body mass of each population. The small dots represent the mean log metabolic rate and mean log body mass for one individual in the sample of each population. The black line represents the metabolic scaling relationship across the populations, the coloured lines represent the scaling relationship within each population. The top



inset shows the temperature-induced response in metabolic rate of three individuals (metabolic thermal plasticity). Metabolic thermal plasticity is represented by a ‘reaction norm’.

Environments fluctuate extensively within the lifetime of organisms. The ability for individuals to reversibly adjust their SMR in response to temperature (i.e., metabolic thermal plasticity) may be adaptive, particularly for ectotherms inhabiting variable environments (Piersma & Drent 2003). Metabolic thermal plasticity can be represented by a reaction norm where the slope between metabolic rate and temperature describes the degree to which SMR changes (Schlichting & Pigliucci 2010). Consistency in individual reaction norms (i.e., repeatability) is important for understanding both how selection might shape plastic responses and in explaining variation in mass-scaling at different temperatures (Nussey, Wilson & Brommer 2007). If individuals respond to temperature consistently, one might expect mass-scaling to be robust to changing ambient temperatures (Clarke 2004). Despite studies on a range of taxa recognising that individuals should vary in their metabolic thermal plasticity, its repeatability has rarely been formally estimated (Careau, Gifford & Biro 2014; Boratyński, Jefimow & Wojciechowski 2017; Briga & Verhulst 2017)}.

Part of the challenge in quantifying individual consistency of plastic traits is that there are multiple ways to estimate repeatability (Arnold, Kruuk & Nicotra 2019). Character-state approaches model phenotypic variation in a set of environments as discrete ‘characters’ (Via *et al.* 1995; Hunt 2014). For example, activity rate measured at 25ºC and 35ºC are considered separate traits that are correlated through shared physiological underpinnings. Repeatability can thus be derived in each environment and in turn the reaction norm can show greater malleability across environments. In contrast, function-valued approaches model the entire reaction norm by describing plastic responses across an environmental range by a set of parameters (Via *et al.* 1995). For example, the intercept of a linear reaction norm represents the average population trait value in a given environment when $x = 0$ and the slope represents plasticity. In this scenario, repeatability can be quantified for each parameter, but the shape of reaction norm is somewhat constrained by the nature of the mathematical function used to

model the reaction norm. While the conceptual differences between the modelling approaches have sparked debates (Via *et al.* 1995), both approaches can contribute to our understanding on how the shape of reaction norms can evolve

Here we examine how individuals vary in their SMR in relation to body size and acute temperature changes using an ectotherm model, the delicate skink (*Lampropholis delicata*). We take advantage of two modelling frameworks that differ in their assumptions to address four key questions. (1) Does metabolic thermal plasticity (i.e., metabolic reaction norms) consistently differ among individuals?; (2) How does repeatability of SMR change at a given temperature over short and long-time frames? (3) Do different approaches to modelling plasticity impact our conclusions, and if so, how? (4) Do population mass-scaling exponents change with temperature when accounting for among- and within-individual variation in SMR? Unravelling the complexities of individual physiological processes will have important consequences for understanding how populations respond in new or challenging environments.

Materials and Methods

Lizard collection and husbandry

Between 28 August and 8 September 2015, forty-two male *L. delicata* were collected from two 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 26ºC and were provided with a thermal gradient to allow for thermoregulation. Each lizard was kept individually in an opaque plastic enclosure measuring 35cm x 25cm x 15cm (L x W x 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). 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. 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).

Measuring standard metabolic rate at different temperatures

Closed-system respirometry was used to measure SMR of lizards between 26 December 2016 - 19 March 2017. Given the scale of our experiment, it was not possible to use intermittent-flow through respirometry methods. We measured SMR as CO_2 production per unit time ($\dot{V}_{CO_2}$ mL min^{-1}). SMR is the metabolic rate of animals at a given temperature in a resting, post-absorptive state but also includes energetic costs of random activity that we were not able to completely control for (Withers 1992; Mathot & Dingemanse 2015). Due to logistical constraints, lizards were randomly assigned to one of two blocks for metabolism measurements (block 1: n = 23, block 2: n = 22). We used two incubators (LabWit, ZXSD-R1090) to precisely control the ambient temperature at which measurements were taken (+/- 1ºC). Measurements were taken between 22ºC – 32ºC at 2ºC increments over a three day periods (measurements at two temperatures per day). Each animal was repeatedly measured across these temperatures every 10 days (10 sampling sessions in total). In order to account for carry over effects of extreme temperatures experienced by an individual on subsequent metabolic measurements, the temperature order was randomly allocated to the incubators across the three days, for each sampling session. We also statistically accounted for temperature order in our analyses (see below).

After a 24 hour fast, 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 gently encouraged into their 146mL opaque chamber and then weighed using a digital scale to the nearest 0.01g (Ohaus SP-202). The chambers were placed inside the incubators in the dark at a predetermined temperature for 30 minutes. The lids of the chambers were left ajar during this habituation period to minimise CO_2 build up. After 30 minutes, each chamber was flushed with fresh air and sealed. A 3 mL 'control/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 animals to respire. After 90 minutes, two 3mL air samples were taken from each chamber before they were reopened to flush with fresh air and placed back into the incubator for the next measurement temperature following the same procedure.

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 $\dot{V}_{CO_2}$. Water vapour was scrubbed from the inlet air with Drierite. Output peaks were integrated using WartHog Systems LabAnalyst software to calculate the percentage of CO_2 above baseline (http://www.warthog.ucr.edu). The rate of CO_2 produced by an individual was calculated following (Lighton 2008):

Equation: 1

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

where $\%CO_2$ is the cumulative 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 (146 mL); V_{lizard} is the volume of the lizard, assuming that the mass of the lizard is the same as its volume, 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

All statistical analyses were conducted using the R environment, version 3.4.2 (Core Team 2013). We used a dataset of 2418 observations. For details on data cleaning see electronic supplementary materials (ESM). All data and code with which to generate our results are openly available via the Open Science Framework (see Data Accessibility). Initial analyses showed that there were no differences in $\log\dot{V}_{CO_2}$ between blocks of lizards or incubators, therefore these parameters were not included in our final models (see ESM). Although lizards were kept in a temperature-controlled room, there may still have been temperature differences between enclosures that had carry-over effects on metabolic rate. We therefore tested whether the previous measurement temperature or the body temperature measured in the home enclosure before the first measurement 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) = -4.73 and Δ loo = -4.73), we therefore included 'previous temperature experience' in all subsequent analyses (Table S1). Collinearity between our predictor variables was checked using a scatterplot matrix (Fig. S1) and Pearson correlation coefficients are presented in Table S2.

Function-valued and character-state approaches in modelling plasticity

We used Bayesian generalised linear mixed models from either the package 'brms' (Bürkner 2017) or 'MCMCglmm' (Hadfield 2010). For logistical and feasibility reasons, all function-valued models were run using the package 'MCMCglmm', while the one character-state

model was run using the package ‘brms’. We verified using a subset of simpler models that the overall results did not change depending on what package was used. Details on model priors and set up are presented in the ESM. For every model, we pooled the posterior estimates from multiple chains and presented posterior means and their 95% credible intervals. In all repeatability models, metabolic rate and temperature were log-transformed and body mass was first log-transformed and then z-transformed to improve the estimation of adjusted repeatabilities (Nakagawa & Schielzeth 2010), hereafter referred to as repeatability). Repeatability is a statistical measure used to summarise consistent individual differences, it is comprised of among-individual variation and within-individual variation (Nakagawa & Schielzeth 2010). Among-individual variation is variability in SMR attributed to differences among individuals, whereas within-individual variation, also referred to as ‘predictability’, constitutes the deviation of an individual relative it its own mean (Westneat, Wright & Dingemanse 2014; Cleasby, Nakagawa & Schielzeth 2014). The relative sizes of both sources of variation can shed light on the processes that promote repeatable traits (Dingemanse & Dochtermann 2013).

The function-valued approach

Repeatability of metabolic thermal plasticity

One important benefit of the function-valued approach is the ability to assess whether individual plastic responses (i.e., slope of the reaction norm) are repeatable or not. To calculate the repeatability of metabolic thermal plasticity we fitted a model where:

$$\log\dot{V}_{CO_2} \sim \text{logTemp} + \text{zlogBodyMass} + \text{logPriorTemp} + (1+ \text{logTemp} \mid \text{ID}) + (1+ \text{logTemp} \mid \text{seriesID})$$

where: $\log\dot{V}_{CO_2}$ is log-transformed $\dot{V}_{CO_2}$; logTemp is the log transformed temperature in degrees Celsius; zlogBodyMass is log-transformed body mass that is then subsequently z-

transformed; logPriorTemp is log-transformed previous temperature. Individual ID was included as a random intercept. Given that metabolic rate of the same individual can change throughout the ten sampling sessions of the experiment, we also included ‘series ID’ as a random intercept. This categorical variable denotes a unique combination of individual IDs and the sampling session IDs and is assigned to every measurement that pertains to a given individual in a given sampling session. For example, if ID001 was measured at six temperatures in session five, then its six measurements would be assigned the series ID of ID005_session5. For further explanation, see provided code and (Araya-Ajoy, Mathot & Dingemanse 2015). Log transformed temperature was fitted as a random slope for both Individual ID and series ID. The repeatability of the slope is calculated following equation 5 in the ESM (see also Araya-Ajoy *et al.* 2015). The above model was also used to derive cross-temperature correlations (see below).

Repeatability of the average SMR at each temperature

After assessing whether individuals differ in their metabolic thermal plasticity, we were interested in knowing whether consistent among-individual differences in average SMR (i.e. intercept of reaction norm) change at each temperature. There are two function-valued methods with which to quantify repeatability of intercepts at each temperature. The first method involves centering log temperature (x axis) so that the intercept (where $x = 0$) represents the average SMR at each measurement temperature. The second function-valued method requires deriving ‘conditional’ repeatability of intercepts at each temperature from the covariance of the intercept and slope (Singer & Willett 2003 and Briffa, Bridger & Biro 2013). Results from both methods were congruent, therefore we only presented on results from the first method. Details and results from the second method can be found in the ESM (Fig S3).

We fitted six models all with the same structure as the model above. The only difference is that log transformed temperature was centered on one of the six temperatures in order for us to estimate repeatability of intercepts (see provided code for more details). The equations used to calculate repeatability of the intercept $\dot{V}_{CO_2}$ at each measurement temperature are presented in ESM (equation 2). To assess whether repeatability changed over the course of our study, we quantified 'short term' and 'long term' repeatability of intercepts (ESM equation 3 - 4 Araya-Ajoy *et al.* 2015). 'Short-term' repeatability can be interpreted as among-individual variation that includes both intrinsic differences among individuals as well as the effects of the sampling session. In contrast, 'long-term' repeatability is a more conservative measure where variation due to sampling session is part of the total pool of variation in the data (i.e. in the denominator of the calculation, Nakagawa & Schielzeth 2010).

Cross-temperature correlations of metabolic rate

Standard metabolic rate recorded at one temperature will be inherently correlated with SMR recorded at a higher temperature. Phenotypic correlations between different environments may illuminate their underlying genetic correlations (Falconer 1952; Roff 2017) and hence any evolutionary constraint in metabolic thermal plasticity. One additional benefit in estimating repeatability and variance components of reaction norms is the ability to also determine cross-environment correlations of the phenotypic trait, which can also be useful in elucidating trade-offs in performance between temperatures (Angilletta *et al.* 2003; Brommer 2013).

We derived cross-temperature correlations from the same model that was used to calculate the repeatability of the slope using matrix algebra. Following (Brommer 2013), we obtained among-individuals and within-individual-among-sampling-session correlations from

their respective intercept and slope variance-covariance matrices. The specific details and equations of this method is presented in the provided code and ESM (ESM equation 6 - 9).

Character-state approach

Character-state models represent phenotypic plasticity as distinct 'characters' of individuals measured in different environments. In other words, character-state models estimate intercepts in each environment as opposed to fitting a slope. We were interested in comparing repeatability estimates as well as cross-temperature correlations between character-state and function-valued approaches. Following methods described by Hunt 2014 (see also Houslay 2017), we fitted one multivariate response model where we treated $\log\dot{V}_{CO_2}$ measured at each temperature as a 6 x 6 response matrix.

$$\begin{bmatrix} VCO_{2_{1,1,22^{o}C}} & VCO_{2_{1,1,24^{o}C}} & \dots & VCO_{2_{1,1,32^{o}C}} \\ VCO_{2_{1,2,22^{o}C}} & VCO_{2_{1,2,24^{o}C}} & \dots & VCO_{2_{1,2,32^{o}C}} \\ \vdots & \vdots & \ddots & \vdots \\ VCO_{2_{1,10,22^{o}C}} & VCO_{2_{1,10,24^{o}C}} & \dots & VCO_{2_{1,10,32^{o}C}} \end{bmatrix} \sim \text{zlogBodyMass} + \text{logPriorTemp} + (1|\text{ID}) + (1|\text{sessionID})$$

Where, $VCO_{2_{1,1,22^{o}C}}$ is the metabolic rate for individual 1 in sampling session 1 at 22ºC and $VCO_{2_{1,10,22^{o}C}}$ is the metabolic rate for individual 1 in sampling session 10 at 22ºC so forth. Note that temperature is no longer a predictor or a random slope as temperature is now part of the response matrix. We included Individual ID and session ID were as random intercepts and estimated their variance-covariance matrices. We calculated temperature specific repeatability following Equation 10 in the ESM. Cross-temperature correlations were conveniently estimated by the model

Estimating mass-scaling exponents at different temperatures

Population estimates of scaling exponents can be affected by the different contributions of within- and among-individual variation (van de Pol & Wright 2009). We therefore wanted to partition out within-individual effects in order to obtain more precise population 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 (i.e. within-individual centering, see van de Pol & Wright 2009). These mass effects were included in two models fitted in 'brms'. The first model (interaction model) had the following structure,

$$\log\dot{V}_{CO_2} \sim \text{Temp} * \text{AmongIDMass} + \text{Temp} * \text{WithinIDMass} + (1 + \text{WithinIDMass}|\text{ID})$$

where: Temp * AmongIDMass is the interaction term between temperature and the among individual mass effect; Temp * WithinIDMass is the interaction term between temperature and the within individual mass effect. We also included individual ID as a random intercept and WithinIDMass as a random slope given that individuals masses change at different rates through the study (see Fig S6). 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 information criterions (wAIC and loo values) between model one and two. We also presented in the ESM (Fig. S5, Table S4) an analysis that compared the within- and among individual scaling exponents with exponents from a model that represents the typical analysis of a metabolic scaling study model (i.e. does not account for the multi-level variation in the data).

Results

Repeatability of metabolic thermal plasticity

Individual slopes were significantly repeatable (R_{slope} = 0.48, Lower CI = 0.06, Upper CI = 0.91) indicating a significant individual by environment interaction (I x E) that was consistent over time (Fig. 2). This suggests that individuals' metabolic rate show different capacities to adjust to temeperature.

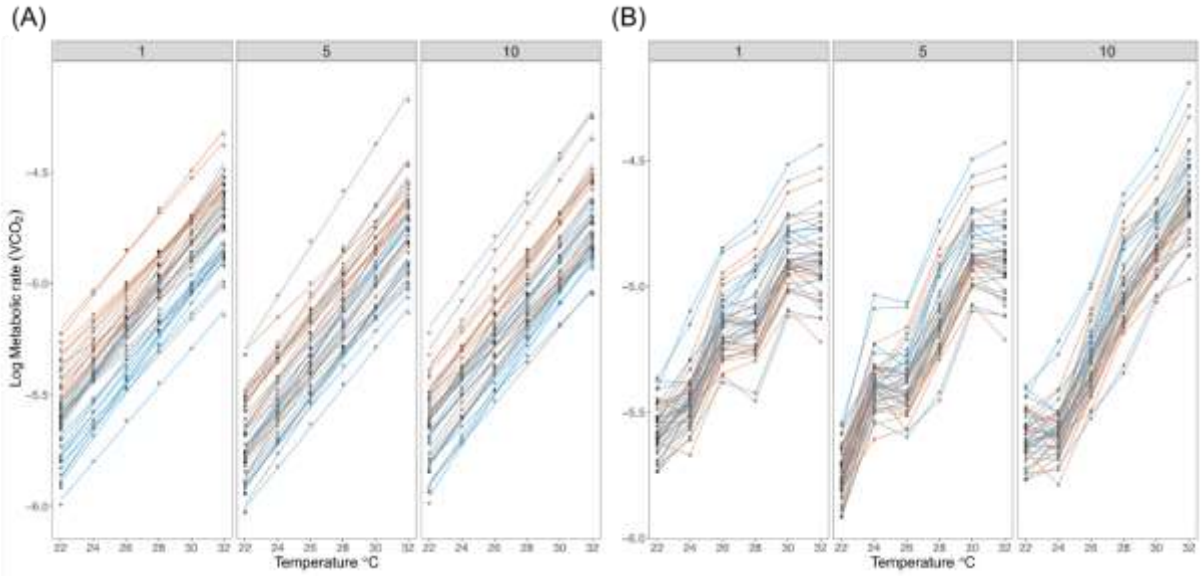


Figure 3 – Predicted individual reaction norms for forty-two individuals of log $\dot{V}_{CO_2}$ (mL) at six measurement temperatures at an average log mass at sampling session one (left panel), five (middle panel) and ten (right panel) reaction norms were estimated using (A) the function-valued approach and (B) the character-state approach. Points represent predicted values for log$\dot{V}_{CO_2}$. Each line represents a unique individual (n = 42). Note the lines are just connecting individual points in the character-state approach

Repeatability of average standard metabolic rate across temperatures

In agreement with significant repeatability in metabolic thermal plasticity, our results showed that repeatability of intercepts (i.e. average $\dot{V}_{CO_2}$) increased with temperature (Fig. 3A). Although there was a trend for SMR and body mass to decrease across the sampling sessions

(Fig. S6), average SMR was significantly repeatable at all temperatures, over short and long temporal scales (Fig. 2A ,Table S1). The function-valued method showed highest repeatability at 32ºC. On the other hand, the character-state approach found repeatability highest at 30ºC (Fig. 2A). Upon closer inspection of the variance components at each temperature, within individual variation decreased over the temperature gradient, whereas among individual variation remained relatively consistent and only increased slightly with temperature (Fig 2B). In other words, individuals were responding more consistently as temperatures became hotter and while variation among individuals increased slightly.

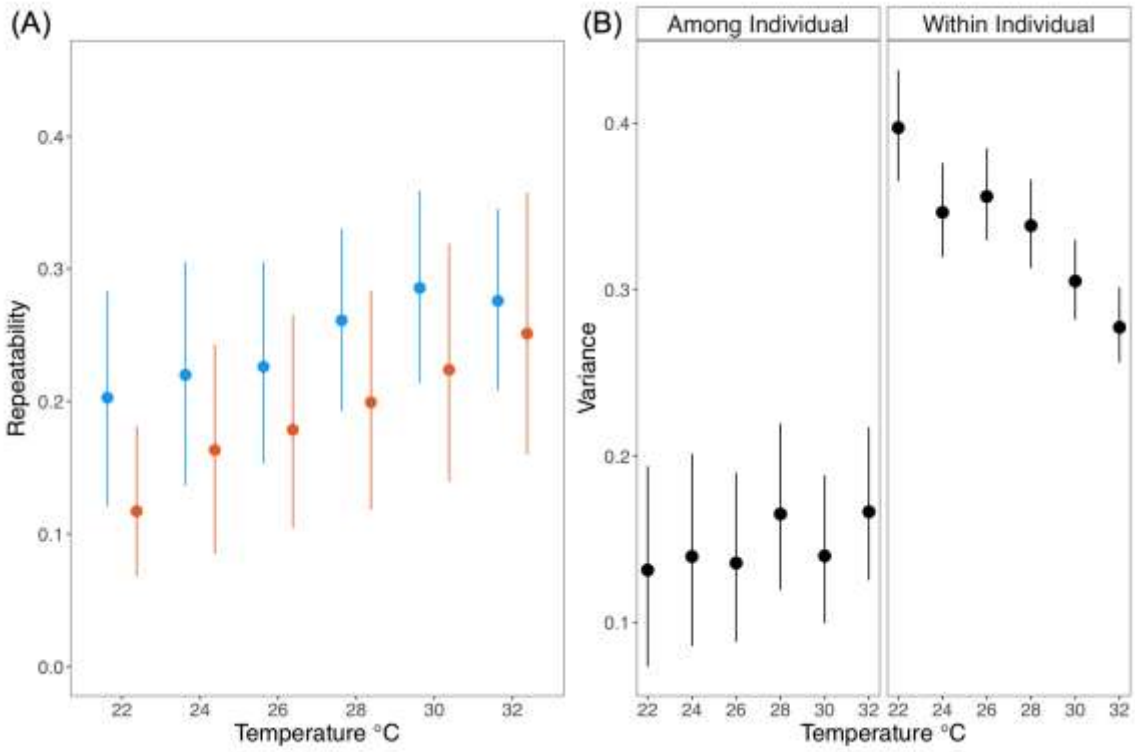


Figure 3 – A) Posterior mean of repeatability of log $\dot{V}_{CO_2}$(mL) at six measurement temperatures. Orange circles represent function-valued approaches in modelling metabolic thermal plasticity, orange filled circles represent the function-valued 'long' term repeatability method (ESM equation 3), Blue filled circles represent the character state approach in estimating repeatability (ESM equation 10). See Statistical analyses and ESM for more details. B) Posterior mean of variance of log $\dot{V}_{CO_2}$ (mL) at the among (right

panel) and within (left panel) individual level across six measurement temperatures estimated by the character-state approach. Error bars represent 95% credible intervals.

Cross-temperature correlations in metabolic rate

Metabolic rate across temperatures were positively correlated at both the among-individual and within-individual level (Fig. 4, Table S2). Although, the magnitude of correlations were weaker at the within-individual level. Certain individuals maintained a high metabolic rate relative to other individuals, while others had a relatively low metabolic rate across all temperatures (Fig. S4). This created a positive relationship between metabolic rate at the among individual-level. Metabolic rate measured at neighbouring temperatures (e.g. 22ºC and 24ºC) were strongly correlated, but the strength of this correlation decreased with increasing differences between the two temperatures (Fig. 4). Overall, cross-temperature correlations estimated using function-valued approaches are congruent with the character-state approach. Compared to the character-state approach, correlation estimates from the function-value approach were generally larger (i.e. stronger correlation) and credible intervals were very narrow and in some cases resulted in estimation issues (Table S2).

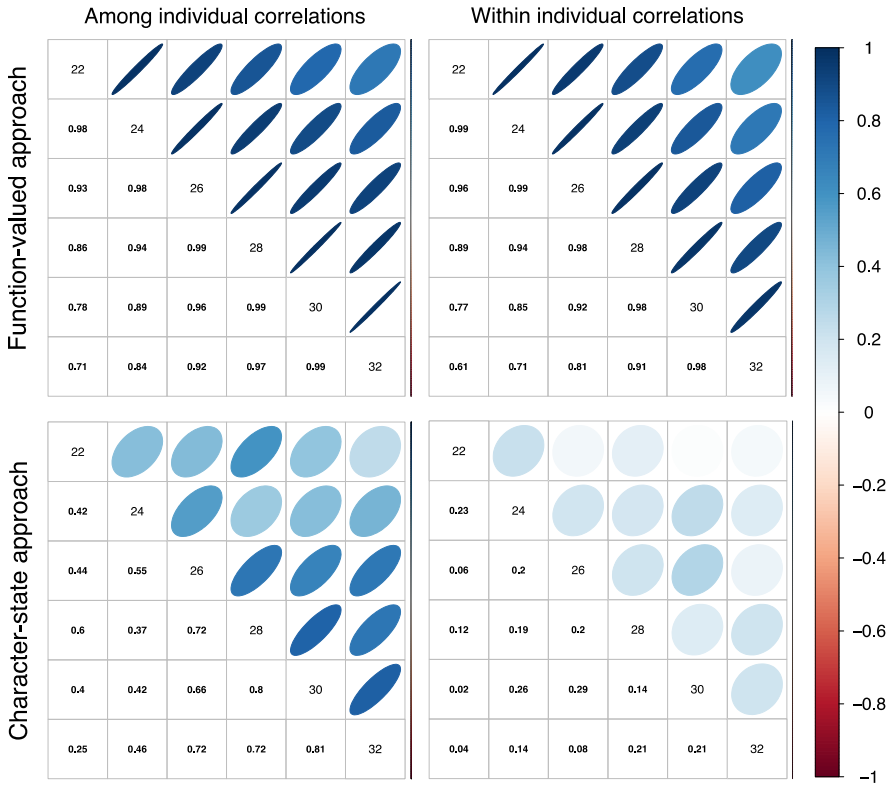


Figure 4 – Cross-temperature correlations of log $\dot{V}_{CO_2}$(mL) estimated using the function-valued approach (top) and the character-state approach using 'brms' (bottom) at the among-individual level (left) and at the within-individual level (right). Lower triangle represents posterior mean estimates, width and colour of the ellipse represents the strength of the correlation.

Temperature dependence of population mass-scaling exponents

The model containing only the main effects of temperature and the among- and within-individual mass effects was better supported than a model that included the interaction terms (Main effects model: WAIC = 1868.53, loo = 1869.02, Interaction model: WAIC = 1876.58, loo = 1877.34). This suggests a lack of temperature dependence in mass scaling (Fig. 5, Table S3). Overall, our estimated scaling exponents are in line with values reported for Squamates and credible intervals overlap 0.75 for all temperatures measured (Uyeda *et al.* 2017). There was a trend for within-individual exponents to be larger than among-individual exponents (Fig. S4, Table S4). Consequently, population estimates of mass-scaling exponents tended to

be spurious and estimated with a larger degree of error when the within- and among-individual effects were not statistically accounted for (Fig S5, Table S5).

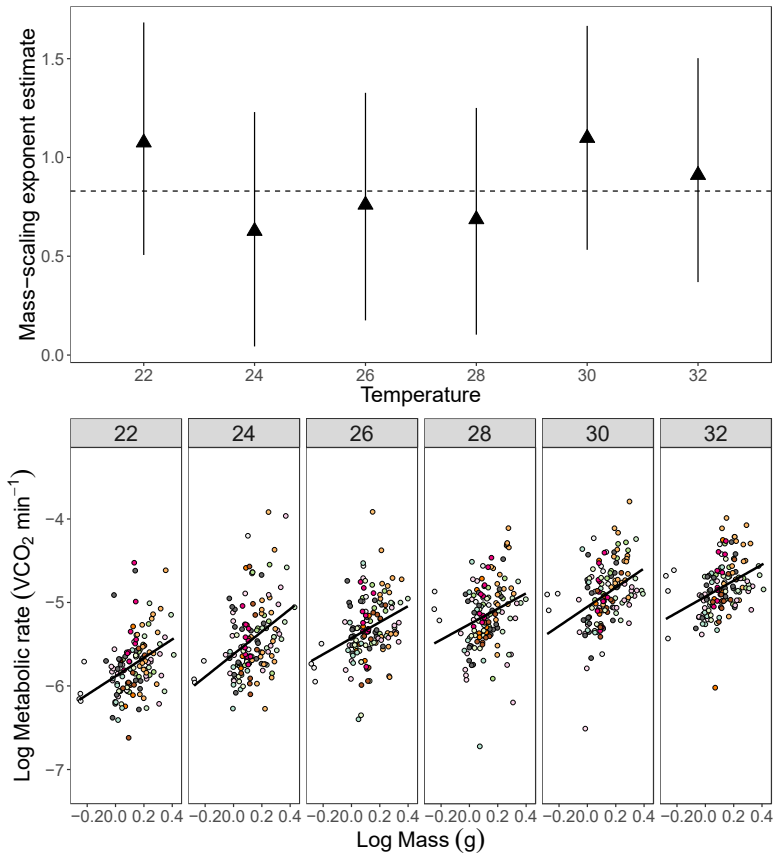


Figure 5 – (A) Posterior mean estimates of population mass scaling exponents (i.e. among individuals) of $\dot{V}_{CO_2}$(mL) across six measurement temperatures when within individual variation in mass over time has been properly partitioned (see Statistical Analyses). The dashed line represents the mass-scaling exponent of 0.83 estimated for squamates from Uyeda (2017). Error bars represent 95% credible intervals. (B) Raw log $\dot{V}_{CO_2}$plotted against log body mass for a random subset of 20 individuals across six measurement temperatures. Each uniquely coloured point represents one individual. Parameter estimates and credible intervals are presented in Table 4. Thick bold line represents the change in log $\dot{V}_{CO_2}$ over log body mass across all individuals. Faint grey lines represent the change in log $\dot{V}_{CO_2}$over log body mass within an individual.

Discussion

Our results show that metabolic thermal plasticity (individual slopes) were significantly repeatable over the 4 months of study. Moreover, the repeatability of average SMR (individual intercepts) increased with temperature which was largely due to a decrease in within-individual variance. Cross-temperature correlations of SMR were all positive at the among- and within-individual level. However, the strength of these correlations was not uniform across all temperatures and differed between the character-state and function-valued approach. Population mass scaling exponents were not strongly affected by temperature. They were also more precise and in line with values reported for squamates when within-individual variation was partitioned out. Below we discuss the implications of our results for understanding how plasticity may evolve, and how SMR scales at different hierarchical levels.

Consistent variation in metabolic thermal plasticity

Consistent among-individual variation is a key prerequisite for any trait to evolve and sets the 'upper limit of heritability' because it is the raw material that natural selection acts on (Falconer 1952, c.f. Dohm 2002). Our findings show individual slopes were significantly repeatable over time, in other words, there was consistent among-individual differences in metabolic thermal plasticity. As temperatures became hotter, an individual's SMR had greater predictability (reduced within-individual variance). Macronutrient breakdown and the production of ATP may be at homeostatic balance at warmer temperatures promoting consistent metabolic rate within individuals (Somero 1978). In support of this, 32ºC is well within the range of preferred temperatures of this species where biochemical activities are likely to be operating optimally (Merritt, Matthews & White 2013; Goulet, Thompson & Chapple 2016). The compounding effects of high among-individual and low within-

individual variation at warmer temperatures may mean that, not only is there a greater opportunity for selection in hot environments, but selection can operate more effectively (Cleasby *et al.* 2014; Nakagawa *et al.* 2015). Assuming metabolic thermal plasticity is heritable, this may facilitate adaptive evolutionary change in population metabolic reaction norms (Ghalambor *et al.* 2007).

Implications of different modelling approaches for understanding metabolic plasticity

Metabolic rate was positively correlated across all temperatures at both the within- and among-individual level. This suggests that individuals differ in their plastic responses but their rank order in SMR is maintained across different thermal environments. It has been hypothesised that trade-offs may be an important mechanism in shaping reaction norms. We found that at the within-individual level, cross-temperature correlations were all weakly positive. This does not support the generalist-specialist trade-off, where enhanced physiological performance in one environment diminishes performance in another environment, giving rise to a negative cross-temperature correlation (Angilletta *et al.* 2003). Instead, our results are more congruent with the hypothesis that individuals vary in their acquisition or allocation of resources to their physiological system because certain individuals were able to maintain a consistent SMR across all temperatures, while others did not (De Jong & Van Noordwijk 1992; Angilletta *et al.* 2003). Moreover, consistent individual differences in SMR, irrespective of the thermal environment, may be functionally linked with consistent differences in behaviour and life-history. Our results give precedence to ‘pace-of-life’ theory where individual differences in energetic expenditure may drive lead to consistent differences in behaviour and life-history within the same population (Biro & Stamps 2008; Careau *et al.* 2009).

Assuming phenotypic cross-temperature correlations reflect the underlying genetic cross-temperature correlations (Roff 1995), our results may also have important implications in understanding constraints on the evolution of metabolic thermal plasticity. The strength of correlation in metabolic rate across temperatures can dictate how strongly selection acting on one component of the reaction norm will result in indirect selection on another (Via *et al.* 1995). We found that the strength of cross-temperature correlations between neighbouring temperatures (e.g., 28°C vs. 32°C) were stronger compared to correlations at more distant temperatures (e.g., 22°C vs. 32°C) when modelling with the character-state approach. Greater measurement error at cooler temperatures could explain this change in correlation as lizards' CO_2 production was more difficult to detect compared to warmer temperatures. While there were estimation issues with the function-valued approach, correlations across all temperatures remained strong and were in agreement with the character-state approach. Estimation issues and high correlation estimates may be a result of deriving correlations from the covariance between the intercept and slope, which is a key attribute of function-valued approaches. When modelling using the character-state approach, the shape of reaction norms may evolve with weaker constraints and greater malleability. Although differences between statistical approaches may be ameliorated when curvature is properly modelled in non-linear reaction norms. However, we were unable to test this because our measurement temperatures spanned the normal operative temperature of the species where the reaction norm is mostly linear (Doody 2009).

Population mass scaling across different temperatures

Our mass-scaling exponents did not change with temperature, which is in disagreement with the growing number of studies that show temperature dependence of mass scaling exponents (Glazier 2005; Killen *et al.* 2010; Price *et al.* 2012; Glazier 2015; Barneche *et al.* 2016).

Generally, these studies demonstrate that mass scaling exponents increased with temperature and vary among species of different ecology (e.g. benthic or pelagic lifestyle, Killen *et al.* 2010). Disparity between our results and these studies may be due to the method with which we quantified mass scaling exponents. In our study, we sampled sexual mature adults repeatedly in order to estimate a static mass scaling relationship, while other studies tend to measure ontogenetic allometry (i.e. measure body mass and metabolic rate throughout development, Glazier 2009). The energetic demands of growth during ontogeny may be more sensitive to changes to temperature and therefore result in temperature-dependence in ontogenetic mass scaling exponents (Hirst, Glazier & Atkinson 2014; Barneche & Allen 2018). In support of this, a recent comparative analysis has shown that development (passing through life stages) shows stronger temperature dependence than growth (increase in mass) (Forster, Hirst & Woodward 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 variation among individuals (Dingemanse & Wolf 2013). While fluctuations in the internal environment, such as circulating hormones and body composition, can affect the predictability of an individual's response (Scott, Mitchell & Evans 1996; McCue 2010; Dupoué, Brischoux, Lourdais, Angelier 2013). We show that mass scaling exponents were generally estimated with improved precision and were slightly higher than 0.75, when accounting for within individual level effects. Our estimates were more in line with mass-scaling exponents reported from a phylogenetically informed analyses in squamates (Uyeda *et al.* 2017). This result has important implications for current designs of metabolic scaling studies as SMR and body mass tend to only be measured once, making them sensitive to sampling error and within-individual 'noise'. Moreover, predictive models that make use of metabolic scaling 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 needed to investigate the degree to which intra-individual variance in SMR and body mass impact scaling exponents as this has largely been neglected and yet may help elucidate why mass scaling exponents are variable at higher levels of biological organisation (Glazier, 2005).

Conclusion

From our study, it is apparent that metabolic thermal plasticity is indeed repeatable over ecologically relevant time scales and could be subjected to natural selection to shape population reaction norms in the face of a warming climate. Given that within-individual variance declined with increasing temperatures, this may allow selection to operate more efficiently at higher temperatures. Our results show that metabolic reaction norms may not be strictly linear and may even have the capacity to evolve more malleable forms. The conclusion was, to a certain extent, dependent on what statistical approach we used. Our study highlights the importance of considering individual variation in metabolic thermal plasticity and how it may affect mass-scaling. Individual variation (among and within) in metabolic rate and body mass can impact estimates of population mass-scaling exponents. If multi-level variation is not corrected for, population mass-scaling exponents may be composite of among- and within individual effects. This may be problematic particularly in theoretical models that utilise mass-scaling to predict ecological system dynamics. We illustrated how differences in assumptions between the function-valued and character-state approach can influence the evolutionary inferences we draw from them. The implications of such methodical differences for experimental evolution and molecular studies elucidating the mechanisms of metabolic plasticity would be an illuminating avenue to pursue.

Author contributions

All authors conceived the ideas and designed the study; FK and CF collected the data; FK, DN, SN analysed the data; FK wrote the first draft and all authors contributed to revising the manuscript.

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Data accessibility

Datasets and code used to generate results of this study will be made accessible via Open Science Framework via a public DOI link. For reviewing purposes, a 'reviewer's only' DOI link has been generated

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